

# Design, Prototyping, and Testing of Devices for Electrical and Mechanical Stimulation During Recellularization of Facial Composite Tissue Grafts

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for obtaining the Master's degree in  
**Electro-mechanical Engineering**

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Academic year 2016-2017





# Acknowledgements

We wish to extend our deepest appreciation to Mr. Benoît Herman, Dr. Jérôme Duisit and Pr. Benoît Raucent for having proposed us such an interesting master thesis subject, and for having inspired us to continue research work in the biomedical field.

More particularly, we would like to thank Mr. Benoît Herman for his availability, technical monitoring and valuable advice as part of this master thesis, as well as in the context of our future career plans, to Dr. Jérôme Duisit for his help and the rigour he brought us in the medical field.

Our thanks must also be extended to Pr. Phillippe Gailly, who agreed to be a reader of this master thesis.

We thank the whole experimental surgery lab team (CHEX) for their welcome, and especially, to Mr. Gwen Beaurin, Mrs. Adeline Dedriche and Mr. Martial Vergauwen, for their availability, advice and help throughout this work. Their interest in our work has been particularly inspiring for us.

We thank Mr. Axel Jottard, Mr. André Lengelé and Mr. Alban Maton, as well as the cyclotron fabrication shop (UCL) for the machining of complex parts of the created device.

Our thanks goes to Atelier Legrand firm for the lending of equipment and the help provided in the manufacturing of device pieces.

We thank Pr. David Bol, Mr. Thierry Daras and Pr. Claude Oestges for their advice concerning the electrical design of the device, as well as Mr. Rémi Renard and Mr. Guy Siman from Nyxoah, for their expertise concerning electrodes.

We thank Mr. David Spote and Mr. Souley Djadjandi for their help and the lending of equipment in the implementation of electronic boards.

We thank our parents for the rereading of this master thesis, for their support throughout these five years of studies, and the years to come.

Finally, we want to express our very warm thanks to the "Ecole Polytechnique de Louvain" in its whole entity, for the quality education we received.



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# Terminological Glossary

|                  |                                                                                                                                                                                                                                                                                                                       |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| apoptosis        | Process of programmed cell death that occurs in multicellular organisms. 8                                                                                                                                                                                                                                            |
| collagen         | Main structural protein in the extracellular space in various connective tissues. 4, 8, 10                                                                                                                                                                                                                            |
| connexin-43      | Component of gap junctions, which allow for gap junction intercellular communication between cells to regulate cell death, proliferation, and differentiation. 7                                                                                                                                                      |
| cytoplasm        | Material within a living cell, excluding the cell nucleus. 6                                                                                                                                                                                                                                                          |
| cytosol          | Liquid found inside cells. 6                                                                                                                                                                                                                                                                                          |
| elastin          | Highly elastic protein in connective tissue allowing many tissues in the body to resume their shape after stretching or contracting. 4                                                                                                                                                                                |
| epithelial       | Adjective of one of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue. 2                                                                                                                                                                                          |
| fibrin           | A fibrous, non-globular protein involved in the clotting of blood. 8, 10                                                                                                                                                                                                                                              |
| fibronectin      | High-molecular weight glycoprotein of the extracellular matrix that binds extracellular matrix components such as collagen and fibrin. 4                                                                                                                                                                              |
| I <sup>2</sup> C | Multi-master, multi-slave, packet switched, single-ended, serial computer bus invented by Philips Semiconductor (now NXP Semiconductors). It is typically used for attaching lower-speed peripheral ICs to processors and microcontrollers in short-distance, intra-board communication.. 27, 39, 46, 48, 55, 92, 117 |
| ischaemia        | Restriction in blood supply to tissues, causing a shortage of oxygen and glucose needed for cellular metabolism . 3                                                                                                                                                                                                   |
| laminin          | High-molecular weight proteins of the extracellular matrix and biologically active regarding cell differentiation, migration, and adhesion. 4                                                                                                                                                                         |

|                     |                                                                                                                                                                                                                                                               |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| masticatory muscles | Active muscles during mastication. 6                                                                                                                                                                                                                          |
| Matrigel            | Trade name for a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. 8, 10                                                                                                                                                |
| phenotype           | Composite of an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties, behavior, and products of behavior. 6                                                                          |
| pO <sub>2</sub>     | Oxygen partial pressure. 5                                                                                                                                                                                                                                    |
| rheobase            | A measure of membrane excitability. Rheobase is the minimal current amplitude of infinite duration that results in the depolarization threshold of the cell membranes being reached, such as an action potential or the contraction of a muscle. 7, 8, 16, 36 |
| scaffold            | A framework or structural element that holds cells or tissues together. 1, 4, 5, 7–11, 14                                                                                                                                                                     |

# Acronyms

|         |                                                                                 |
|---------|---------------------------------------------------------------------------------|
| CM      | Culture Medium. 19                                                              |
| CSST    | Cell-on-Scaffold-Seeding Technology. I, 1, 4, 5                                 |
| DCT     | Dense Connective Tissue. 19                                                     |
| DMEM    | Dulbecco's Modified Eagle Medium. 19, 20, 27, 28, 31, 37                        |
| ECM     | Extracellular Matrix. 4                                                         |
| EPDM    | Ethylene Propylene Diene Monomer (M-class). 44                                  |
| LCT     | Loose Connective Tissue. 19                                                     |
| PCR     | Polymerase Chain Reaction. 10                                                   |
| PGA     | Poly Glycolic Acid. 8                                                           |
| PGS     | Poly Glycerol Sebacate. 8                                                       |
| POM-c   | Copolymer Polyoxymethylene. 32                                                  |
| PWM     | Pulse Width Modulation. 23, 24, 48, 55, 92, 117                                 |
| SERCA I | Sarco/Endoplasmic Reticulum $\text{Ca}^{2+}$ -ATPase I (Calcium ATPase-type). 8 |





# Introduction

The face is an essential identity element. It allows people identification, and it is also an essential vector in social interactions. Indeed, the face is important to the non-verbal communication, and especially to the expression of emotions thanks to its great mobility allowed by the orofacial muscles. However, when this one is injured, it is extremely difficult to hide or repair the wounds. Allograft transplantation techniques have been introduced, but the current cost-benefit ratio of these procedures is low due to the extensive postoperative treatments, including an immunosuppressive therapy.

A recent technique, called cell-on-scaffold-seeding technology (CSST), has emerged as a response to the problems related to standard transplantation. The basis of this technique is to cleanse an organ from its cells, and reseed it with the patient's own cells. The technique has already been tested by Dr. Jérôme Duisit and his team [1] [2] on different facial subunits, and has shown encouraging results. Moreover, a device has been designed and assembled by Alexia Springuel [3] in order to ease the technique application, and to optimize the graft physiological conditions for a proper recellularization. However, regarding grafts containing muscles, a mechanical and/or electrical stimulation could favour muscle cell growth, maturation and proper organization during the recellularization stage. It is with this background that a device capable of mechanical and electrical stimulation has been developed. This device also ensure all necessary physiological conditions for a graft optimal recellularization.

The objectives of this present work are numerous. The first one is to deeply understand what is a facial composite tissue grafts. Its composition, its mechanical and electrical features. Then, improvement suggestions regarding the device implemented by Alexia Springuel can be provided. Also, a portable electrical stimulation device has to be implemented and tested for occasional use on different facial subunit graft. Finally, a complete device including mechanical and electrical stimulation, and ensuring necessary physiological conditions for an graft optimal recellularization, has to be designed, assembled and tested.

It is important to note that the present work fits into the research process of Jérôme Duisit and his team, that is still ongoing. This work strongly depends on their research advancement. This is why the report is structured as research steps.

The 1st chapter proposes a brief contextual setting and a state of the art concerning mechanical and electrical stimulation. In the 2nd chapter, manipulations of the perfusion bioreactor assembled by Alexia Springuel are performed, and avenues for enhancing the device are suggested. In the 3rd chapter, the anatomy and features of a peculiar graft, namely a half superior lip, will be exposed. This graft will be used as a basis for all the implemented devices. Based on the state of the art discussed in the first chapter, as well as on the electrical features of the graft, the end of the chapter will present the design, the prototyping and the testing of an electrical stimulation device. Chapter 4 draws lessons from the experiments realized on the Alexia Springuel's bioreactor and from the electrical stimulation device detailed in the previous chapter, in order to design and assemble a complete perfusion bioreactor with mechanical and electrical stimulation included. The end of the 4th chapter is dedicated to experiments realized on the final device and feasible enhancements and avenues to perfect the apparatus.

# Chapter 1

## Contextual Setting & State of the Art

A disease or trauma can lead to tissues or organs damage in the human body. Treatments are needed to ease their replacement and regeneration. Traditional procedures, typically the simple tissue or organ transfer from one site to another [4], face some issues. Peculiarly, for a comfort grafting as facial graft transplantation, ethic issues are even more tangible. Tissue engineering techniques have therefore been introduced consequently. These challenges as well as a state of the art of the proposed alternative techniques for facial application are detailed in this section.

### 1.1 Facial application of grafting and transplantation

The face is our first communication tool, it plays a key role in the establishment of social links. When this one is injured, it is extremely difficult to hide or repair the wounds. Only 36 facial transplantations have been achieved since 2005 due to their major side effects [5]. Indeed, Isabelle Dinoire, the first face-grafted patient in the world, died in April 2016 due to a tumour linked to the immunosuppression therapy [5]. This therapy is needed after a traditional grafting operation. Compared to vital organ transplantation, facial transplantation is a comfort procedure. The current cost-benefit ratio is low due to the side effects linked to the operation and the low biological quality of the implanted graft. In order to understand the challenges behind facial transplantation, it is first necessary to highlight the composition of facial tissues.

The face is in fact a *composite tissue*. A tissue is a group or layer of cells that perform specific functions [6]. We can find in the body epithelial, connective, muscle and nervous tissues. For example, the pig ear can be decomposed in different tissue types: on figure 1.1, from the left to the right: fat (connective tissue), muscle, cartilage (connective tissue) and skin (epithelial tissue). Facial transplantation relates in fact to a face part and a definite number of skin layers. Isabelle Dinoire was, for example, transplanted of the nose, lips and chin, from the epidermis to the hypodermis. It is important to mention the different face parts have different functions. Parts integrating cartilage have a morphological function, as parts containing muscles have a motor function. The face muscles are skin muscles. Unlike skeletal muscle, skin muscles have at least one cutaneous attachment and don't allow bones motion [3].

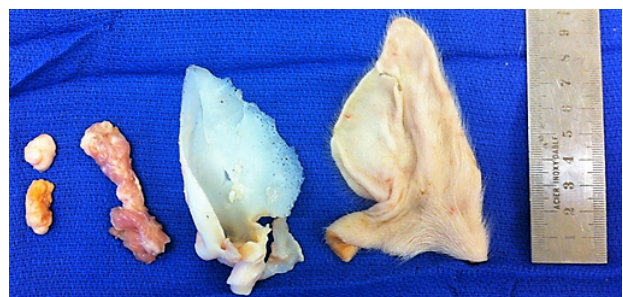


Figure 1.1: Pig ear decomposition in different tissues types [3]. From the left to the right: fat, muscle, cartilage and skin.

The face is a complex system. Besides, its grafting have to face the same problems as organ transplantation when traditional transplantation techniques are used.

## 1.2 Allotransplantation of composite tissues: Overview and encountered problems

We term *autograft* or *autotransplantation* the transfer of a graft procured from the receiver himself. The autograft reduces the graft rejection risk, but can cause postoperative pain due to the second surgical autograft site. Moreover, graft sizing is a variable that must be managed in addition to the time and the potential complexity of the procedure [7].

The *allograft* or *allotransplantation* is the transplantation between two individuals of the same species. This technique is the most common grafting technique. As the genetic heritage of both individuals are different, the receiver will therefore undergo a life long immunosuppressive therapy.

At the present time, allograft encounters three major issues. The first one is to face the shortage of transplantable organs and grafts. In 2016, a total of 1,259 patients were waiting for an organ in Belgium, but 1,077 organs were transplanted from deceased donors [8]. The graft procurement from donors who have been declared brain-dead allows to avoid graft damages caused by a prolonged ischaemia. Even if this number increased over the last four years [9] due to the implementation of techniques for a better use of available organs (by expanding, for example, the eligibility criteria for organs which would not have been chosen *prima facie*), the success of graft transplants and the ageing of the population result in an expansion of the national list of people waiting for transplants.

The second challenge is to improve the preservation conditions of the graft during the different transplantation stages. Indeed, the removed organ is brutally cut off from the bloodstream, and therefore receives neither oxygen nor nutrients from the blood circulation. The transplanted organ is then subjected to stress when the circulation resumes [10].

Finally, the last point on which great scientific advances need to be done is the immune tolerance regarding the graft. Compatibility between donors and patients is essential to graft survival rates. We can highlight four compatibility criteria [11]:

- Blood Type Matching: the ABO blood group compatibility is checked but the Rhesus blood group antigen is not taken into account.
- Histocompatibility: a match for HLA (Human Leukocyte Antigen) is performed.
- Size matching.
- Infectious status: an infected donor with HIV or hepatitis B/C is excluded.

Despite all these criteria, a graft rejection remains possible and could result in a graft destruction. The immunosuppressant drug intake drastically reduces the rejection risk but induces major side effects like fever, chills or urinating trouble [12]. The development of cancers risk is also a mentioned long term side effect by medical researchers.

### 1.3 Tissue engineering advances in order to face grafting and transplantation problems

In order to address the three major transplantation and grafting problems, some tissue engineering techniques have been introduced. One of them consists in using porous 3D scaffolds to provide an appropriate environment for organs and tissues regeneration [4]. As depicted in figure 1.2, donor cells are collected from tissue and expended in culture. Combined with bioactive factors, the cells are cultivated *in vitro* on a porous 3D scaffold. The resulting tissue can then be grafted on the patient. This method allows the elimination of the shortage of transplantable grafts issue. Also, the immune tolerance is guaranteed as the individual's own cells are used in the process. Although tissue engineering was found successful for the graft of bladders, small arteries, skin grafts, cartilage, and even trachea, the procedure is still experimental and costly [13]. Moreover, the lack of vascularity in scaffolds and tissue-engineered constructs remains a limiting factor [4].

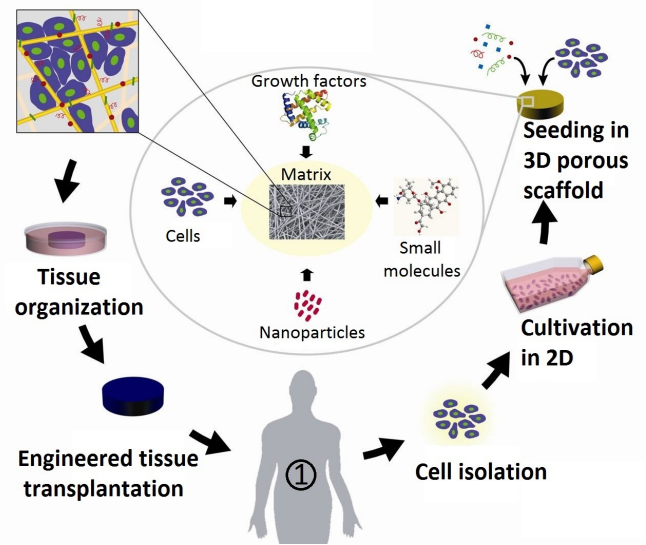


Figure 1.2: Tissue engineering: stepwise procedure schematic, beginning at step 1. (adapted from [14]).

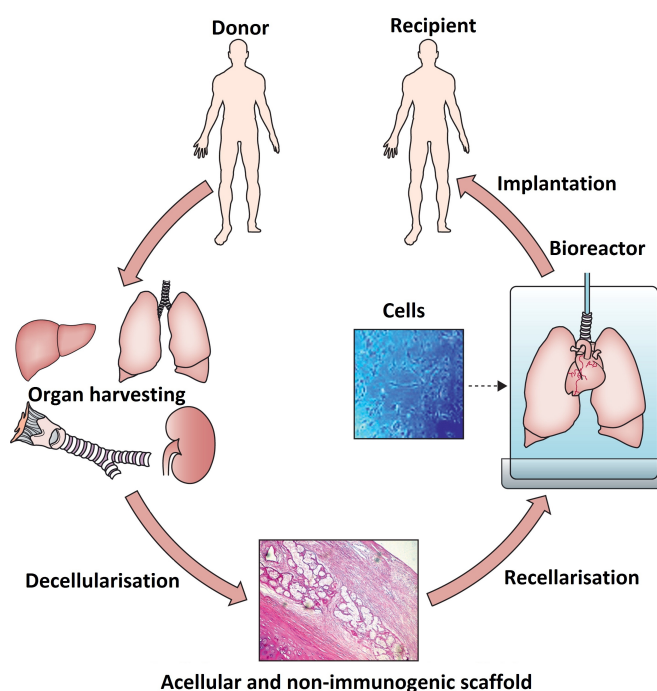


Figure 1.3: CSST: stepwise procedure schematic (adapted from [17]).

of washing away the donor cells from an organ. To do so, a detergent-based solution is injected in the vascular system in order to only preserve the architecture, namely a biocompatible immunogenic ECM scaffold, consisting of collagen, elastin, fibronectin, laminin and calcium. This scaffold preserves its vascular network and can be perfused in a bioreactor allowing the supply of nutrients and oxygen which would

Another scientific improvement to address transplantation issues is the cell-on-scaffold-seeding technology (CSST). The principle of this method is to cleanse a mammalian organ from its cells in order to produce an acellular extracellular matrix (ECM) scaffold. This resulting three-dimensional geometry and vasculature is then reseeded with the patient's own cells using a perfusion culture system that mimics the *in vivo* environment [15] [16]. CSST theoretically solves the shortage of organs problem by allowing a wider choice among donors potentially morphologically compatible with the receiver. But it also allows to avoid the intake of immunosuppressant drugs by the patient as the organ is reseeded with the individual's own cells. As the organ is conserved in an *in vivo* environment, it can be transplanted at any time. On the figure 1.3, the stepwise procedure is depicted. The first step, the decellularization, consists

be insufficient by simple diffusion at the graft external surface [3]. A bioreactor is an apparatus used to mimic the native graft physiological conditions. The scaffold and bioreactor have to be sterilized prior to reseeded in order to prevent contaminants from growing within the scaffold [15]. During the second step, the recellularization, one or several cell types will be administered through perfusion in order to recolonize the scaffold. The cells structural arrangement enable them to recover some native functions. The last step before implantation is to maintain the recellularized scaffold in optimal conditions favouring cell growth, maturation and differentiation [3]. The CSST has been tested by Jérôme Duisit and his team on different facial subunits, showing encouraging results [1] [2]. Therefore, the design and assembly of an apparatus capable of optimizing the graft physiological conditions during the CSST for an optimal recellularization makes sense.

## 1.4 Perfusion bioreactor for the CSST application

Recellularization using perfusion consists in disseminating culture medium combine to cells though the graft vascular network. This method allows a substantial supply of oxygen and nutrients which boost the propagation and differentiation of the cells. The fluid dynamic forces induced by the perfusion also results in cells organization and development.

Many different perfusion bioreactor devices have been developed, but they all consist of a similar basic design containing a medium reservoir (or the bioreactor, strictly speaking) in which the graft will be placed, pumps, a tubing circuit, and a gaz exchanger (see figure 1.4) [15]. Some perfusion bioreactor can also be composed of sensors in order to control some bioreactor parameters as the pH and the  $pO_2$ . An example of such a bioreactor is the Harvard Apparatus Bioreactor illustrated on the figure 1.5. The medium reservoir made of clear materials, which allows the visibility of the organ, is fully autoclavable. Chamber access ports are built in for a removal and addition of culture medium. The built-in windows allow for a manual access to the organ [18]. The graft is statically maintained in the perfusion chamber and biosensors are used in order to obtain pH measurements,  $O_2$  and  $CO_2$  rates. Although this device is well suited for grafts containing skin, fat and cartilage, as these tissues can recover their native function by static recellularization, it is less usable for grafts involving muscles.

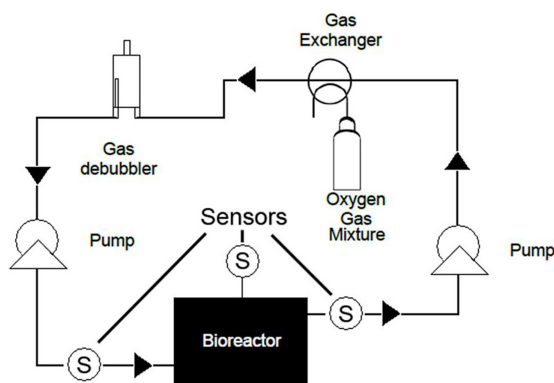


Figure 1.4: Typical process flow diagram for a bioreactor circuit [15].



Figure 1.5: Harvard Apparatus Bioreactor [19].

## 1.5 Muscular recellularization case

A muscle is a complex structure including cells and tissues of many types in addition to myocytes [20]. Myocytes, or muscle fibres, are polynucleated cells of very long length oriented to the direction of muscle stretching and compression.



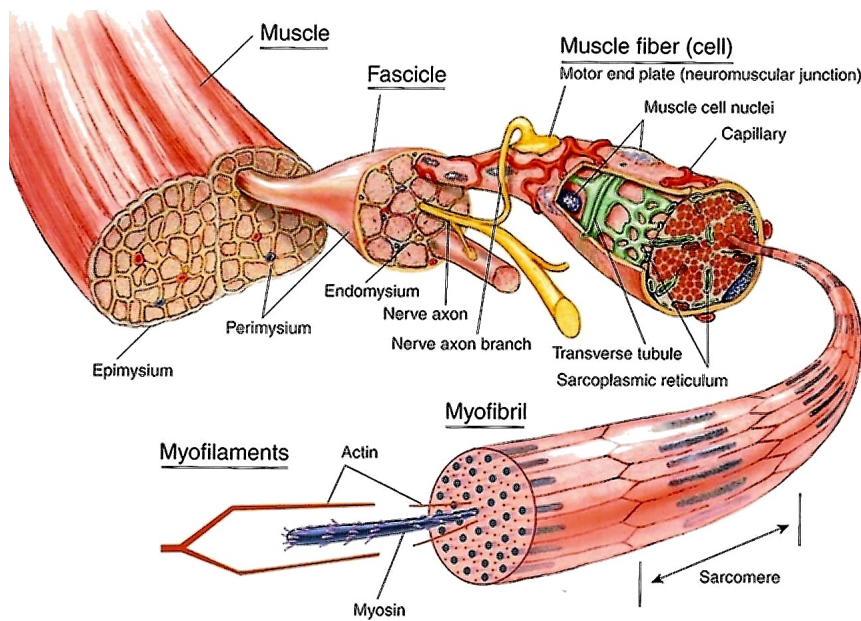


Figure 1.6: Muscle structure [22].

The fine filaments present in the myocytes consist essentially of actin and thick filaments of myosin (see figure 1.6). A muscle action potential causes a calcium ions release into the cytosol. This increase of calcium-ion concentration in the cytoplasm results in changes in the actin and myosin molecules structure causing the slide of the myofilaments (actin) on the thick myofilaments (myosin). This phenomenon induces a shortening of the fibres and therefore, of the muscle [21]. Studies have

shown that graft electrical stimulation aims to mimic the electrical environment of electro-active muscle cells by regulating cell-to-cell and cell-to-extracellular matrix interactions. As a result, the electrical stimulation can be used to enhance the differentiation and alignment of muscle cells [23]. Also, mechanical stimulation in the fibre direction could favour the muscular fibre alignment and produce longer fibres with higher fibre density [24]. The motor units in the facial muscles are largely composed of histochemical type II (*fast-twitch*) fibres, but the masticatory muscles are composed of substantial numbers of fibres between type I (*slow-twitch*) and type II [25]. Hence, it will be important to take into account the stimulation speed according to the graft fibres type during the regeneration.

## 1.6 Electromechanical stimulation during recellularization in perfusion bioreactors: state of the art

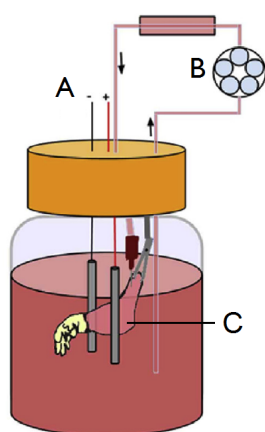


Figure 1.7: Schema of the functional bioreactor with electrical stimulation. A: Electrical stimulation system; B: Pump, C: Rat forearm (adapted from [26])

A perfusion bioreactor using electrical stimulation was designed and constructed by Bernhard J. Jank and his team (2015) [26]. After decellularization of rat forearms by perfusion of a detergent solution, they repopulated the tissues with cells of appropriate phenotypes, and matured them in the perfusion bioreactor under electrical stimulation (see figure 1.7). They initiated the electrical stimulation on day 6 by applying 6 ms pulses of 20 V and 1 Hz with a Grass S48 square pulse stimulator (Grass Technologies). An isometric force measurement was performed on days 14–16. The detected force represented approximately 80% of the specific force of a neonatal muscle [26]. Although the results of this study are promising, it is important to emphasize that researchers performed a dissection of the skin of the whole upper limb before perfusion. Thereby, we are not really dealing with grafts full of composite tissues as it remains only muscles, tendons, bones, ligaments, nerves, and blood vessels.

Another study could approach our research subject. The recellularization under electrical stimulation of a perfused rat heart after decellularization was performed by Harald C Ott and his research team (2008) [27]. The heart is exposed to a physiological preload by pulsating medium flows through the left atrium, and after-load by pulsating medium flows through the ascending aorta. Electrical stimulation (pacing) was provided through epicardial leads (1 Hz, 5–20 V, 2 ms) at day 8, and served as contractile test [27]. Good contractile properties were observed, but again, a heart is composed largely of muscles and blood vessels and thus not represent a complete composite tissue as it is the case for the face.

As no scientific research has been achieved on electromechanical stimulation of perfused composite tissue graft, we are going to focus on the electromechanical stimulation on non-perfused single tissue scaffolds during recellularization. We will then implement these techniques on facial grafts, by adding perfusion through main arteries and veins.

### Electrical stimulation on non-perfused scaffolds

Different studies have been investigated on the electrical stimulation. Details of these researches are presented in the table 1.1. Used with proper parameters, the electrical simulation increases myoblasts number and density [28]. It should be noted all these studies use synthetic scaffolds. The process of biological graft decellularization is therefore not applied here. Even though the used tissues vary across the studies, the electrical stimulation takes place in the culture medium, and not directly on the graft in all of them. Also, they all describe a scaffold completely submerged in the culture medium. Regarding the electrical stimulation itself, various electrodes materials and shapes are used like, stainless steel wire (Donnelly), carbon rods (Lasher, Ito, Maidhof), platinum wire (Thelen, Radisic) or platinum plates (Pedrotty). The graft clamping system is also a bit different among the articles. In Pedrotty's review [28], the scaffold is suspended in the culture medium as in Donnelly's and Lasher's, the constructs are fixed at their end points on two vertical rods in the medium (see figure 1.8). The duration, amplitude and frequency of the stimulation pulses contained in table 1.1, diverge completely from one study to another. These differences can be explained by the fact that all scaffolds do not have the same dimensions. If the scaffold volume increases, its resistivity (which is linked to its geometry) increases, and allows the apply of a larger voltage before cells damage. As the culture media also presents a low but non-negligible resistivity, the distance between the electrodes and the scaffold surface is also a parameter that must be taken into account. As this distance increases, the resistivity of the medium located between the scaffold and the electrodes increases. The important voltage applied in Donnelly's paper can be explained by the use of silk sutures as a scaffold structure. Indeed, the silk electrical resistance ( $R_s$  in  $\Omega kg/m^2$ ) is 9.8 ( $\log(R_s)$  value), which is extremely high compared to other materials [30].



Figure 1.8: Donnelly's 3D muscle-stimulating bioreactor. Used tissues: C2C12 cells in fibrin between two silk sutures [29].

Essential assessments are performed after a specific test duration like force, excitability measurements, or morphological assessments at the cellular level. In Radisic's study, the initiation delay of the electrical stimulation is discussed. If the electrical stimulation is applied early (at day 1), the accumulation of cardiac proteins yields poor contractile behaviour. But if applied late, it produces fewer effects because of the reduce amounts of connexin-43 and contractile proteins present in the cells. The stimulation amplitude is discussed in Donnelly's article, and it is reported that stimulation at 1.25V/mm increases force production and decreases rheobase, as at 2.5V/mm, both force and rheobase are improved. At 5V/mm, however, both force and rheobase are negatively affected. But again, the amplitude will strongly depends on the seeded scaffold geometry and type, as well as the distance between the electrodes and the scaffold surface.

Table 1.1: State of the Art: Electrical Stimulation.

| Article            | Used tissue                                                                                                             | Stimulation location | Tissue fixation                                                                  | Pulses (duration, ampl., freq.)    | Duration            | Assessments                                                                                                                       |
|--------------------|-------------------------------------------------------------------------------------------------------------------------|----------------------|----------------------------------------------------------------------------------|------------------------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Thelen 1997 [31]   | C2C12 myotubes (skeletal muscle behaviour) grown to confluence in 0.1% gelatin-coated six-well multidishes              | Culture medium       | -                                                                                | (6 ms, 3 V/cm <sup>2</sup> , 2 Hz) | from day 3 to day 5 | Myotubes morphology, cell death, SERCA I.                                                                                         |
| Radisic 2004 [32]  | Rat ventricular myocytes seeded onto ultrafoam collagen sponges using Matrigel                                          | Culture medium       | -                                                                                | (2 ms, 5 V/cm, 1 Hz)               | 5 days              | Molecular, contractile activity (video microscopy), electrical response to an electrical stimulus (electrode).                    |
| Pedrotty 2005 [28] | Skeletal myoblasts on PGA mesh scaffolding (absorbing scaffold) (3.0 X 1.0 X 0.1cm)                                     | Culture medium       | Suspended in the medium                                                          | (0.5-250 ms, 4μA-3 mA, 0.5-10 Hz)  | 14 days             | Cell number, cellular proliferation and apoptosis, differentiation.                                                               |
| Donnelly 2010 [29] | C2C12 cell lines in fibrin engineered between two long silk sutures (length:12mm thickness: 4mm)                        | Culture medium       | On one fixed anchor and a force transducer                                       | (0.1 ms, 1.5-2.5-5 V/mm, 10 Hz)    | 7 days              | Force and rheobase.                                                                                                               |
| Lasher 2012 [33]   | Fibrin-based engineered tissue samples containing ventricular cardiac cells (20 X 4.8 X 3mm)                            | Culture medium       | Fixed in the medium                                                              | (2 ms, 4 V/cm, 1 Hz)               | 9 days from day 3   | Cross-sectional area, density and orientation of myocytes, fibroblasts and nuclei, excitation threshold and maximum capture rate. |
| Maidhof 2012 [34]  | Cardiac cells on a porous PGS scaffolds (8 X 8 X 1mm)                                                                   | Culture medium       | Fixed in the medium                                                              | (3 V/cm, 3 Hz)                     | 5 days from day 3   | Motion and deformation, cell distribution, DNA quantification.                                                                    |
| Ito 2014 [35]      | C2C12 cells concentrated by magnetic force to form a structure surrounded by collagenous membranes (thickness = 350 μm) | Culture medium       | One pin attached to a force transducer and the other fixed on culture plate well | (2–10 ms, 0.1-0.5 V/mm, 0.5-2 Hz)  | day 4 to day 14     | Peak twitch force, excitability, protein content and number of nuclei.                                                            |



As an example of this dependence, Akira Ito and his team predict electrical damage above 2.5 V/mm and at frequencies over 3 Hz. But this study cannot be directly compared with Donnelly's, which predict the opposite, as the type and geometry of the used tissues are different.

Among all these studies, only those made by Donnelly, Lasher and Ito approach our research subject which is to stimulate an perfused facial composite-tissue graft. As a matter of fact, the three mentioned articles seed muscular cells in the scaffold depth, producing a full seeded scaffold. The other articles are content with a cell seed at the scaffold surface.

### Mechanical stimulation on non-perfused scaffolds

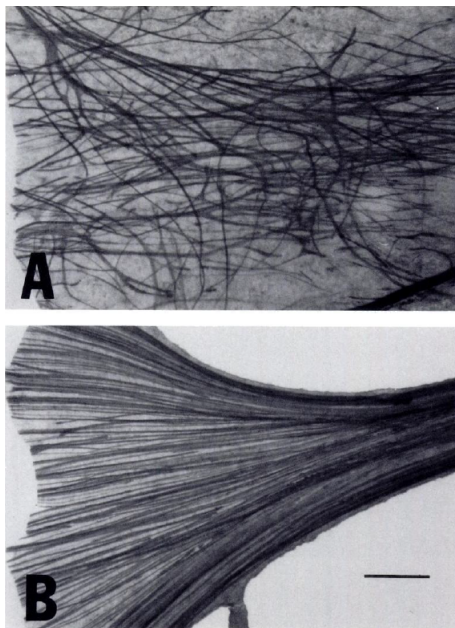


Figure 1.9: Orientation and growth of skeletal myofibers when using mechanical stimulation during the seeding operation. A: Un-stretched muscles, B: mechanically stimulated muscles [36].

On table 1.2, different studies are presented concerning the mechanical stimulation. When using appropriate parameters, mechanical stimulation in the fibre direction causes the proper orientation and growth of the skeletal myofibers [36] (see figure 1.9). Just like for the electrical stimulation, a wide range of tissues have been used as scaffolds (see table 1.2). For the vast majority of the studies, muscle constructs are developed with gel-based scaffolds. These constructs can support the contractile portion of the muscle tissue, and can be maintained in culture for several months [37]. But this approach still has significant limitations for clinical purposes. Tissue engineered muscle constructs need to be of relevant size and mechanical strength in order to be suitable to the rigour of the surgical procedures. But clearly, gel-based constructs are currently too small and too fragile for such surgical manipulations [37]. Regarding the scaffolds fixation systems, a relative similarity can be noticed. Indeed, all constructs are attached at each end and one connection moves linearly, creating a periodic back-and-forth motion. A strain of at least 5% and of maximum 20% of the construct total length is applied to follow a precise stimulation schedule (see the stimulation column in the table 1.2) during a certain amount of time varying from 4 days to 3 weeks depending on the study. After that period of time, some assessments are performed like

the contractile response to electrical field stimulation, or the generated passive force. Some histologic examination can be performed in order to check the correct organization of the fibres.

In Powell's article, the mean myofiber diameter significantly increased, as well as myofiber area percentage after eight days of repetitive stimulation. The elastic modulus of the seeded scaffold increased as well [38]. In Hussain's, histologic examination reveals a better fibre alignment in the stimulated tissue as well as longer fibres with higher fibre density after four days of stimulation [24].

### Electromechanical stimulation on non-perfused Scaffolds

Very few articles mention a true electromechanical stimulation during a cellularization step. In some mechanical stimulation experiments listed in the table 1.2, electrical stimulation is used as an assessment to test and quantify the construct contractility. Nevertheless, I-Chien Liao and his team [39] worked on a true electromechanical stimulation. Skeletal myoblasts cultured on aligned microfibres were wrapped around silicone tubes that were connected to a 95% O<sub>2</sub>/5% CO<sub>2</sub> tank.

Table 1.2: State of the art: mechanical stimulation.

| Article               | Used tissue                                                                                                         | Tissue fixation                                                          | Stimulation                                                                                                                                        | Duration                            | Assessments                                                    |
|-----------------------|---------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----------------------------------------------------------------|
| Vanderburgh 1991 [36] | Embryonic mono nucleated avian muscle cells attached to a collagen-coated elastic substratum                        | Attached at each end to stainless steel wire mesh (one end is stretched) | Strain of 5–20% substratum str/rel during 20 sec, 10 sec rest, repeated 3 times followed by a 30 min rest period                                   | After 7–8 days and during 2–3 weeks | Glucose uptake and mechanical activity                         |
| Powell 2002 [38]      | Cryopreserved primary human skeletal muscle                                                                         | At each end by two suspended pins, one pin attached to a stepper motor   | 5% strain for 2 days, 10% strain for 2 days, and 15% strain for 4 days. 5 str/rel separated from 30 sec every 28 min.                              | From day 8 to day 16                | Measurements of cell-generated passive force.                  |
| Yoo 2009 [37]         | Primary human skeletal muscle seeded onto collagen-based muscle scaffold strips derived from porcine bladder tissue | Using stitches at both ends with suture threads                          | 10% of the initial length of the cell seeded scaffolds, 3 times per minute for the first 5 minutes of every hour                                   | 3 weeks                             | Contractile response to electrical field stimulation           |
| Kensah 2011 [40]      | Neonatal rat cardiomyocytes mixed with a liquid matrix based on rat tail collagen type I and Matrigel               | Using two titanium rods at each end                                      | 10% cyclic longitudinal strain with frequency of 1 Hz                                                                                              | From day 7 to day 14                | The quantitative real-time PCR data and the force measurements |
| Hussain 2015 [24]     | C2C12 myoblasts in fibrin matrix                                                                                    | Fixed around two posts                                                   | 5% positive strain, cyclically (1 cycle per minute) for 15 minutes. The strain rate was increased over the course of several days to 5–15% strain. | From day 4 to day 8                 | Muscle strain and histologic comparison                        |

Two solenoid valves regulated the tubing pressure and stretched the scaffold wrapped around the tubing. Electrodes were wrapped around the ends of the tubes, in the culture medium, in order to electrically stimulate the construct. Different methods and schedules of stimulation were performed, and their effect could be derived [39]:

- 5% and 10% cyclic strain at 1 Hz starting at 2 days post-differentiation.
  - Very few myoblasts fuse to form myotubes on the scaffolds.
  - Short myoblasts and lack of sarcomeric structures.
  - Very little contractile proteins are present.
- 5% cyclic strain at 1 Hz starting at 2 days post-differentiation, with a constant 5 pre-stretch applied.
  - Increase fraction of striated myotubes which are distinctive of skeletal muscles.
- Electrical stimulation applied at 20 V at 1 Hz starting on day 7 post differentiation.
  - Increase of the percentage of myotubes showing striations.
- Synchronized stimulation consisted of pre-stretched mechanical stimulation with electrical stimulation starting on day 7.
  - Increase in percent of myotubes striations.

This study proves the benefits of an electrical and mechanical stimulation combination on a seeded artificial scaffold and encourage to apply such stimulation on biological scaffolds during recellularization.

Based on the presented state of the art, a perfusion bioreactor including electrical and mechanical stimulation will be designed for a facial composite-tissue graft. The range of the stimulation parameters will be based on the state of the art and the remarks concerning the graft geometry and consistence above mentioned. It could allow the surgeon to find perfect parameters for an ideal recellularization leading to a native-like graft. The muscle fibre type of the considered graft (fast-twitch or slow-twitch) is also a key factor of the stimulation, and need to be considered.

## Chapter 2

# Experimental Bioreactor for Vascularized Composite Tissue

As a technological advance in the perfusion bioreactor field handling facial grafts, we can mention the device of Alexia Springuel (the V1 bioreactor) [3]. As depicted on the figure 2.1, the medium reservoir is composed of two chambers. The graft is fixed on the support (part 6 on figure 2.1), so that the facial graft external side, which is normally exposed to ambient air, is in contact with the upper chamber containing sterile air. The facial graft internal side, which is in touch with the body internal parts, is therefore in contact with the culture medium contained in the lower chamber of the bioreactor. Two different vents containing a filter (parts 3 and 4 on figure 2.1) ensure gaseous exchanges between the bioreactor and the external environment. Ten connectors (part 5 on figure 2.1) can be used to plug the perfusion circuit or ensure the draining of the lower chamber. Such a connector is present on the upper chamber in order to drain the chamber in case of culture medium presence. A window can be open on the lower chamber allowing the user to connect tubes from the connectors to the cannulas of the cannulated graft veins and arteries once the graft is fixed on its support. It's important to notice that the two chambers, once the graft fixed, are totally isolated and sealed from each other and from the outside medium. This peculiar design makes the independent control of each chamber possible, and de facto, enables a large number of experiments in order to find the perfect conditions for an optimal regeneration of the graft.

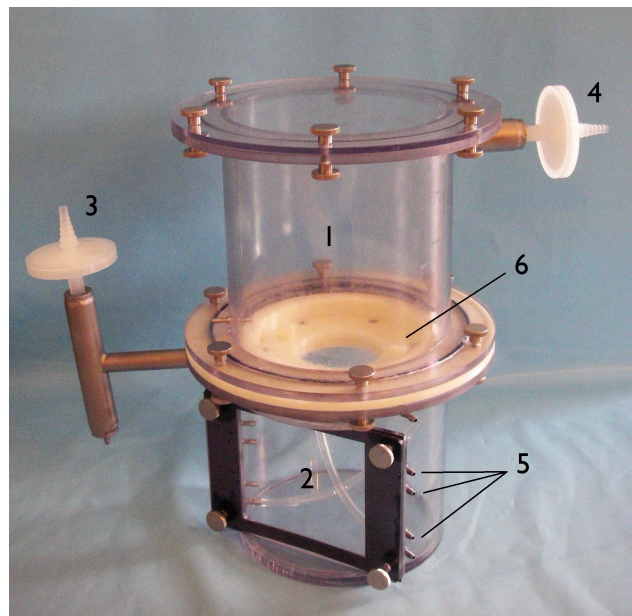


Figure 2.1: Medium reservoir of Alexia Springuel's perfusion bioreactor for recellularization. 1. Upper chamber; 2. Lower chamber; 3. Lower chamber vent; 4. Upper chamber vent; 5. Perfusion circuit connectors; 6. Graft support. (adapted from [3])

The perfusion circuit contains an external tank, a pump, a pulse reducer, an oxygenator and an air-inlet, all connected in series with the culture chamber. It should also be noted that no physiological parameters regulators are involved in the bioreactor. In fact, the bioreactor is placed in an incubator (the INCO246med incubator) allowing a temperature, humidity and CO<sub>2</sub> rate control.

The study of this device will serve as a basis for the design of a perfusion bioreactor with electrical and mechanical stimulation included. Some issues have been encountered throughout its use. Therefore,

some modifications and repairs have been made and a list of some improvements that should be made on the device has been established. The entire observations and reflections about this device will be taken into account in the final design of the stimulation bioreactor.

## 2.1 V1 bioreactor modifications and repairs after laboratory experiments

A preliminary test has been realized in order to verify the sealing and the correct assembly of the device.

Regarding the assembly, three major issues were faced. The first one relates to the depth of the grooves present on the collars. The depth is too important for the expected o-ring seal. We therefore used larger flat seals and placed them beyond the grooves.

The need to disengage the upper chamber from the rest of the device has also been reported. The upper chamber has to be removed as the support and the lower chamber still joined and held tightly. The precise assembly protocol is available in the appendix D. Both chambers are henceforth assembled as follows: we place the support assembly and the flat seal on the lower tank, then we screw the six long hex screws M4 in the six knurled nuts M4 through one hole out of two. The lower chamber and the support assembly are therefore attached and held tightly. After placing the big flat seal on support surface, we place the tank upper part on the support part in such a way that the long hex screws heads fit in the collar holes that are bigger than the screws head. Finally, we can screw the six knurled screws in the six knurled nuts through the remaining holes. The knurled screws head are bigger than the collar holes. Therefore, the latter clamp the upper chamber collar, the support and the lower chamber collar. Using this technique, the user just has to unscrew the six knurled screws in order to take off the upper chamber.

The last encountered issue was the shape of the tube connectors fixed on the V1 bioreactor chambers. The sharp edge located on the conical section (see figure 2.2) makes the tube removal quite difficult. Also, the fact that these stainless steel connections are fixed on the chambers using polymerizing glue on their cylindrical part of larger diameter (see figure 2.2) favour the ungluing of these pieces once we desire to unplug tubes from the connections. Indeed, these connections don't contain stop edges. Such connections will be used in our final device in order to connect graft arteries and veins to the recellularization circuit perfused by culture medium and cells, or just to circulate the culture medium in the bioreactor. It will therefore be essential to redesign these tube connectors in order to avoid the mentioned issues.

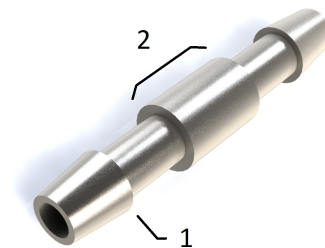


Figure 2.2: Tube connectors on the V1 bioreactor [3]. 1. Sharp edge; 2. Section glued to the chamber.

After being assembled using a pig breast graft, as mentioned in the appendix D, the upper chamber has been filled with colour water as the lower chamber was filled with clear water using a pump (see figure 2.3). Leaks were observed at the junction between the upper chamber collar and the polycarbonate cylinder. In fact, a polymerisable glue [41] has been used to fix these two parts. A bad polymerization of the glue was suggested, and some glue was added and re-polymerized using a polymerizing light with the proper wavelength. However, it has been reported after being autoclaved, that some parts of the V1 bioreactor have been found unglued. The polymerizing glue didn't endure the high temperature and moisture of the autoclave. Both chambers will therefore be made of stainless steel. The collars will be welded to the cylinders composing the chambers in order to avoid leak problems at that specific location. For our bioreactor including electrical and mechanical stimulation, this glue will be avoided so that the device will endure proper autoclave.



As depicted on the figure 2.3, the important volume of the V1 bioreactor imposes to use a large amount of culture medium. As this culture medium is very expensive, it is important to find a way to reduce the fluid quantity used in the bioreactor. It will therefore be crucial to take this fluid quantity limit into account throughout the final stimulation bioreactor conception phase. The observations and reflections gathered during this test session will serve as indications for the forthcoming design.

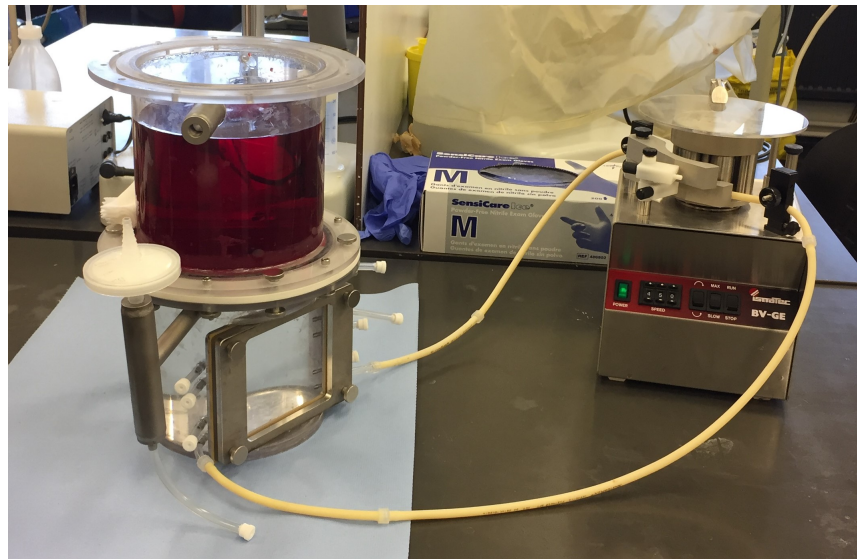


Figure 2.3: Leak test during the filling of both V1 bioreactor chambers. On the left: the medium reservoir. On the right: the pump.

## 2.2 Improvement suggestions for the V1 bioreactor

Additionally, we can suggest some modifications to the V1 bioreactor design. These modifications could enhance its usability and address the issues above-mentioned. Table 2.1 outlines these propositions.

| Suggested improvements                                                                                                        | Proposed solution                                                                                                                                                                                                                                                                                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Support the scaffold from underneath in order to prevent its sagging due to gravity and the fluid column in the upper chamber | Support adaptation: a plastic piece containing a wire mesh can be clipped on the lower side of the current support after graft fixation on the latter. The wire mesh is therefore in contact with the graft, supporting it, as the holes of the wire mesh allow the arteries and vessels passage to the connectors placed on the chamber surface. |
| Reduce the V1 bioreactor volume in order to limit the culture medium volume used, while keeping a hand access to the graft.   | A balloon placed in the lower chamber of the bioreactor. It can be filled with water through one of the connectors and therefore occupy an important volume in the lower tank. Empty, it allows a hand access to the graft.                                                                                                                       |
| Drain the V1 bioreactor upper chamber entirely by only using the pump.                                                        | Locate an I / O connection as low as possible in the upper tank. Add a pipe inside the bioreactor on that connection in order to pump out the liquid at the support centre.                                                                                                                                                                       |

| <b>Suggested improvements</b>                                                      | <b>Proposed solution</b>                                                                                 |
|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Simplify the attachment of the graft to the support.                               | Support adaptation with a series of plastic needles in order to simply prick the graft on these needles. |
| Modify the stainless steel connectors for an easier and safe removal of the tubes. | See section 4.1 for a detailed design of these connectors. See appendix J for their 2D drawing.          |

Table 2.1: Suggestions of improvement for the V1 bioreactor.

On the basis of the V1 bioreactor design and assembly, we were able to check the good or bad functioning of some specific pieces. All these observations will be taken into account in the final bioreactor with mechanical and electrical stimulation included. The efficient parts of the V1 bioreactor will inspire us, while the parts presenting some functioning or manufacturing problems will be re-engineered.

## Chapter 3

# Design, Prototyping and Testing of a Device for Electrical Stimulation of Facial Composite Tissue Grafts

As mentioned in the state of the art, an electrical stimulation of the graft muscle could increase the production force and rheobase of the muscle if proper stimulation parameters are chosen. As a first step, the task will therefore be to design, assemble and test a device capable of electrical stimulation. The choice of stimulation parameters will be based on those mentioned in the state of the art but also on some preliminary electrical stimulation tests on native muscles. In order to limit the design difficulty, the latter need to be adapted for a peculiar graft, a complete characterization of this chosen graft is first proposed.

### 3.1 Anatomy and features of the chosen graft

The first step before designing either the electrical stimulation device or the complete bioreactor with electrical and mechanical stimulation integrated is to select a graft. All the features of the chosen graft will be taken into account in the devices as it will constitute its centrepiece. The selected graft will fix the level of design complexity but also the final devices limitations.

The chosen graft is a human half superior lip. Specifically, the lip is cut in two along the sagittal plane. As shown on figure 3.1, the half-lip is composed of a single muscle, the orbicularis oris. In order to keep the entire section of this muscle, the surgeon will incise at the columella-labial junction (see figure 3.2) and will continue to one side or another while circumventing the orbicularis oris. Then, the lip will be detached by incising along the sagittal plane at the level of the philtrum and the commissure.

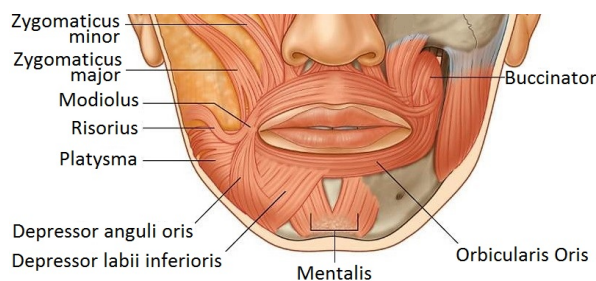


Figure 3.1: Coronal view of the lower face muscles (adapted from [42]).

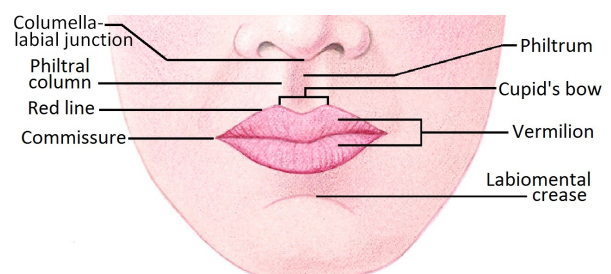


Figure 3.2: Lip anatomy (adapted from [43]).

During the graft mechanical stimulation, the latter will be anchored at its extremities, typically at the level of the philtral dimple and the commissure in order to perform the mechanical stimulation in the fibres direction



as mentioned in the state of the art. An extra length will therefore be considered on the total length of the half-lip in order to allow the anchoring of the graft. Using the modiolus (see figure 3.1) as an anchor point has been considered, but this chiasma of facial muscles presents bad mechanical properties and cannot be used as an anchor point according to Dr. Jérôme Duisit and Pr. Benoît Lengelé (specialist in reconstructive microsurgery and facial allografts).

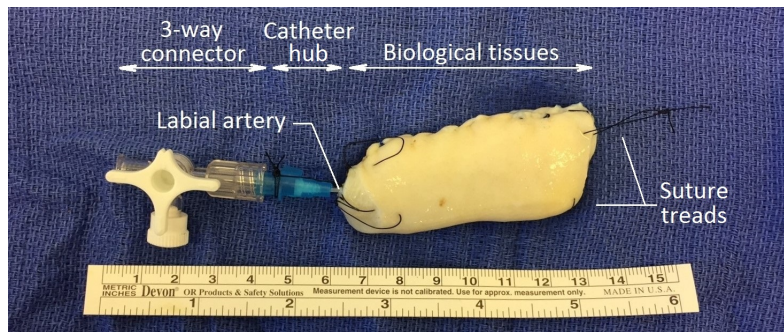


Figure 3.3: Used graft for the devices design: decellularized human half superior lip.

The figure 3.3 depicts the graft which will serve as a work basis. We can notice the presence of an artery, namely the labial artery. The considered graft can be decomposed in three different parts with different lengths. The first part is the graft strictly speaking, namely, all biological tissues (skin, fat, muscle and artery). This part will be mechanically and/or electrically stimulated by our device. The

artery being extremely fragile, no pressure, stress or strain should be applied on it. Then we can consider separately the catheter hub and the 3-way connector. The catheter is used to allow the perfusion of culture medium and cells through the artery, as the 3-way connector is used to connect the catheter hub to the recellularization circuit. The screw-on-female end of the 3-way connector allows a fixation on the catheter hub without twisting the artery. The volume of these three parts composing the graft will be taken into account in the final design, as well as the specification of unleashing at maximum any mechanical constraints on the artery.

The table 3.1 synthesizes the main graft dimensions. These measurements have been obtained using a graduated ruler, the precision is thus limited. We also test its maximum stretching by pulling the two suture threads (see figure 3.3) on one side of the graft, while keeping the two other threads anchored to the work surface. We can stretch the graft by eight millimetres. The corresponding traction force has also been measured and evaluated to 0.27 N. We have also been interested in the measurements of the different layers composing the lip. As the half-lip has been decellularised, the graft layers are hardly distinguishable due to their similar pale colour. Moreover, we didn't want to damage the human half-lip. For these reasons, we used the study of Nancy J.M. van Hees about the ultrasound imaging of healthy lips [44] in order to quantify the thickness of these layers. In this study, the thickness is evaluated on three different coronal parts of the lip; the left, the centre (at the philtrum) and the right. As the considered graft is a left-side half-lip, we will calculate the thickness mean between the left and the central measurements. The sagittal view on the figure 3.4 highlights the different layers thickness, as the table 3.2 quantifies them.

| Graft part                | Dimension [mm] |
|---------------------------|----------------|
| Tissue width              | 26             |
| Tissue thickness          | 9              |
| Tissue length             | 71             |
| Catheter hub              | 24             |
| 3-way connector           | 50             |
| <b>Graft total length</b> | <b>145</b>     |

Table 3.1: Graft main dimensions.

The total thickness calculated in the table 3.2 matches with the measurements obtained on the human half superior lip in the table 3.1, but the 9.2 mm value will be kept in the following report calculations.

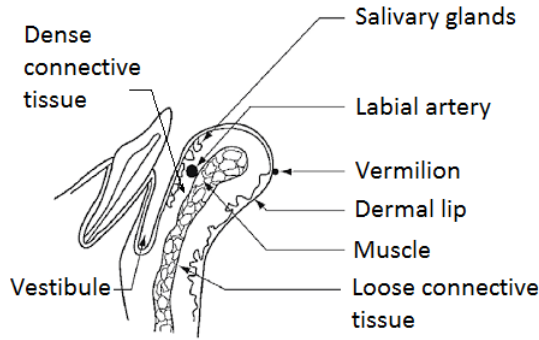


Figure 3.4: Sagittal view of the inferior lip (adapted from [45]).

| Lip layer                    | Thickness [mm] |
|------------------------------|----------------|
| Loose connective tissue      | 4.95           |
| Muscle                       | 2.05           |
| Dense connective tissue      | 2.2            |
| <b>Graft total thickness</b> | <b>9.2</b>     |

Table 3.2: Lip layer thickness [44].

## 3.2 Stimulation parameters: discussion and preliminary tests

In terms of geometry and consistency, the selected graft is nothing like the used grafts for the electrical stimulation in the state of the art. We can't therefore directly use the stimulation parameters stated in these research papers. An electrical model of the half-lip as well as a parameter identification is proposed below in order to obtain a second information about the voltage amplitude range we could apply to the graft.

### Electrical model of the selected graft

According to the study of Nina Tandon and her team [46], muscle myofibers align orthogonal to the direction the electrical field. Two stimulation options can therefore be used for the selected graft. The figures 3.5 and 3.6 depict both options. The direction of the electrical field, orthogonal to the muscular fibres, is represented by a dotted arrow.

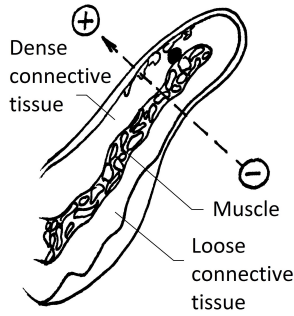


Figure 3.5: Sagittal view of a half-lip.

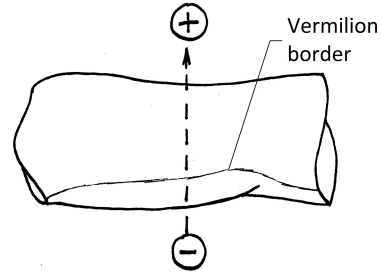


Figure 3.6: Coronal view of a half-lip.

On the basis of the figure 3.5 and 3.6 schematics, an electrical equivalent model of the graft can be formulated. Each tissue layer can be modelled by a resistor as the culture medium, which is the location of the stimulation as described in the state of the art, is modelled by an impedance composed of a resistor and a capacitor connected in parallel (figure 3.7 and 3.8).

It has been reported that an electrical field of 6V/mm may cause electroporation injury to the muscle [47]. On a different scale, the initially spherical myoblasts form their perpendicular alignment to an applied electrical field of  $\sim 0.3\text{mV/cell diameter}$  (threshold value) [48]. As the myoblast diameter is evaluated to  $10\mu\text{m}$  [49], the perpendicular alignment appears when a  $0.03\text{V/mm}$  electrical field is applied. Two orders of magnitude separate both extreme values. We can suppose the electrical field causing muscle contraction is situated in the intermediate order of magnitude. We therefore impose an electrical field of  $0.45\text{V/mm}$

across the resistor modelling the muscle:  $R_{muscle}$ .

We can find the voltage difference applied by the electrodes,  $(V_+ - V_-)$ , on the figures 3.7 and 3.8 using the geometrical measurements obtained on the graft and the conductivity table 3.3. For the following calculations, the conductivity will be noted as  $\sigma$ , as the acronyms DCT, LCT and CM are respectively the dense connective tissue, the loose connective tissue and the culture medium. The formula 3.1 will be used in order to calculate the impedance of each tissue layer.

| Graft layer                | Conductivity [S/m] |
|----------------------------|--------------------|
| Muscle [50]                | 0.35               |
| Skin [50]                  | 0.17               |
| Culture medium (DMEM) [51] | 1.5                |

Table 3.3: Conductivity table.

$$Z = \frac{L}{\sigma S} \quad (3.1)$$

With  $Z$ , the electrical impedance of the tissue layer,  $L$ , the layer length, and  $S$ , the cross-sectional area of the layer. In the first case, namely the electrical model in the sagittal plan (figure 3.7), we can calculate the following impedances.

$$\begin{aligned} R_{muscle} &= \frac{2.05e-3}{0.35 \times 71e-3 \times 26e-3} = 3.13\Omega \\ R_{DCT} &= \frac{2.2e-3}{0.17 \times 71e-3 \times 26e-3} = 7.01\Omega \\ R_{LCT} &= \frac{4.95e-3}{0.17 \times 71e-3 \times 26e-3} = 15.77\Omega \\ Z_{CM} &= \frac{x}{1.5 \times 71e-3 \times 26e-3} = 361.12x\Omega \end{aligned}$$

We can set arbitrarily the distance between one electrode and the graft to 5mm, so that  $x = 5e-3$  and  $Z_{CM} = 1.81\Omega$ . By imposing an electrical field of 0.45V/mm across  $R_{muscle}$ , we can find the current flowing in the circuit and therefore, the maximum voltage difference we can apply across the electrodes.

$$(V_+ - V_-)_{max} = V_{muscle} + V_{DCT} + V_{LCT} + 2V_{CM} = 0.92 + 2.07 + 4.65 + 1.07 = 8.7V$$

The voltage we could apply across the electrode to contract the muscle is 8.7V. It corresponds to an electrical field of 0.45V/mm. Compared to the majority of studies about electrical stimulation, namely Radisic's [32], Lasher's [33], Maidhof's [34] and Ito's [35], we obtain a 0.45V/mm amplitude stimulation which consistent with their stimulation values. As an indication, the mean of the applied electrical fields among these four studies is 0.38V/mm.

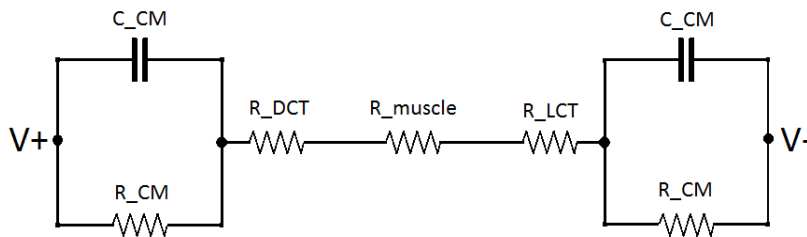


Figure 3.7: Electrical model of the stimulation in the sagittal plan.

We can follow the same approach in the second case, the electrical model in the coronal plan (figure 3.8).

$$\begin{aligned}
 R_{muscle} &= \frac{26E^{-3}}{0.35 \times 71e^{-3} \times 2.05e^{-3}} = 510.38\Omega \\
 R_{DCT} &= \frac{26e^{-3}}{0.17 \times 71e^{-3} \times 2.2e^{-3}} = 979.14\Omega \\
 R_{LCT} &= \frac{26e^{-3}}{0.17 \times 71e^{-3} \times 4.95e^{-3}} = 435.17\Omega \\
 Z_{CM} &= \frac{x}{1.5 \times 71e^{-3} \times 9.2Ee^{-3}} = 1020.62x\Omega
 \end{aligned}$$

Once again, the distance between one electrode and the graft is fixed to 5mm. Therefore,  $Z_{CM} = 5.1\Omega$ . By imposing an electrical field of 0.45V/mm across  $R_{muscle}$ , we can obtain the voltage difference we can apply across the electrodes.

$$(V_+ - V_-)_{max} = V_{DCT//muscle//LCT} + 2V_{CM} = 11.7 + 0.63 = 12.33V$$

The maximum voltage is 12.33V. It corresponds to an electrical field of 0.34 V/mm, which again matches with the majority of the studies listed in the state of the art.

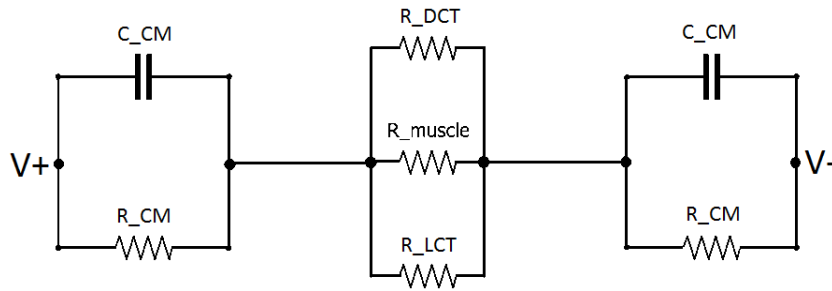


Figure 3.8: Electrical model of the stimulation in the coronal plan.

### Preliminary electrical stimulation tests on a native graft

We have analytically defined the potential voltage amplitude we can apply in order for the graft muscle to show contractions. However, experiments have to be performed on a graft containing a native muscle to verify these obtained amplitude values.

A function generator (Aim TTI Pulse Generator) was used to generate the electrical pulses and catheter needle guides (Versatus I.V. Catheters), made of stainless steel were used as electrodes. A half superior lip has been procured from a piglet and electrically stimulated using stimulation in the sagittal plan, which is the configuration where the voltage amplitude to contract the muscle is theoretically the lowest (see figure 3.9). As described in the state of the art, we stimulated in the culture medium, mainly composed of DMEM (Dulbecco's Modified Eagle Medium). The graft is laid on the bottom electrode, as the second electrode is placed at approximately 5mm above the graft. A first test was realized at 3.2V (displayed voltage by the function generator) and 1 Hz for one minute. The muscle contracted (as well as the entire graft, since it is composed of a skin muscle which presents skin insertions) at the same rate as the transmitted pulses during the entirety of the test. A second test was then performed at 10.4V and 2 Hz for one minute. Again, the graft contracted at the rate of the transmitted pulses, but we could see the contraction magnitudes diminish over time. This decrease of contraction magnitude is presumably due to a muscle tetany resulting from an over-stimulation. During this second experiment, we could also notice the appearance of a white foam around the electrode. This phenomenon, depicted on the figure 3.10, is due to a chemical reaction

between the electrode and the culture medium, called electrolysis.

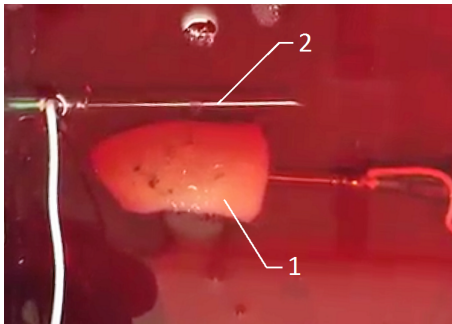


Figure 3.9: Electrical stimulation in the culture medium of a piglet graft using catheter needles. Stimulation in the sagittal plan. 1. Piglet graft; 2. Catheter needle guide.

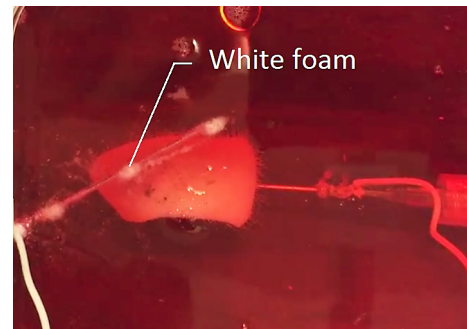


Figure 3.10: Development of a white foam around the electrode during electrical stimulation at 10V and 1 Hz.

From these experiments, we could learn that a stimulation as low as 10V (displayed voltage by the function generator) at a frequency of 2 Hz could cause muscle tetany. Also, the electrode needs to be sized and its material needs to be properly chosen in order to avoid electrolysis. Similar stimulation in the coronal plan couldn't be correctly performed because of the graft non-attachment. The latter moved in the culture medium leading to a very imprecise stimulation.

It is important to note that the function generator displayed voltage is not the real voltage sensed by the graft. In fact, the culture medium presents a very low resistance. A drastic drop of voltage is therefore noticed once the electrodes are placed in the culture medium. Using an oscilloscope, we could quantify these drops of voltage in function of the nominal voltage transmitted by the function generator. The tests have been realized three times for integer voltage values, and a mean has been calculated for each of them. We can see on the figure 3.11, that when stimulating at a voltage amplitude of 10V with an inter-electrode distance of 2cm, 8.2V is sensed by the graft. None of the studies listed in the state-of-the-art mention if the applied voltage is the voltage actually sensed by the graft, or the applied voltage by the generator. As a voltage of 8.2V sensed by the graft already cause muscle tetany, we fixed the maximum voltage our device will apply to the graft at 7V. From its effect on native tissue, such a stimulation amplitude is already considered as high for recellularization purposes according to Dr. Jérôme Duisit.

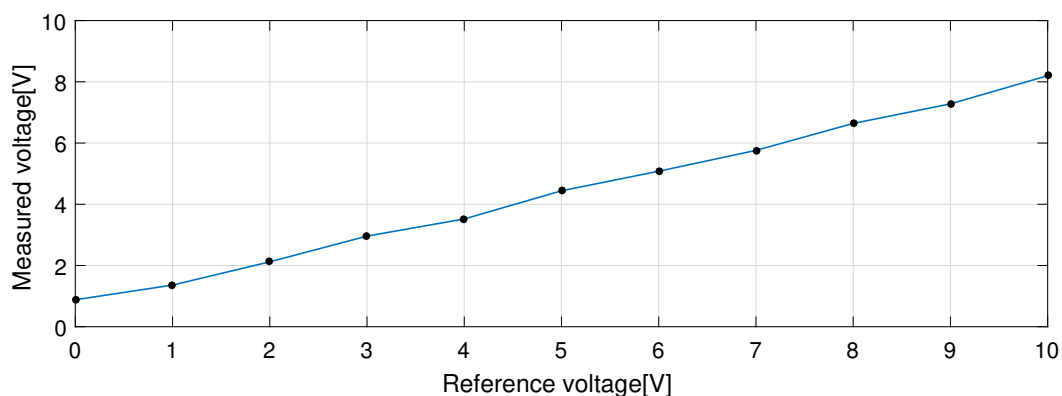


Figure 3.11: Measured voltage sensed by the graft in function of the generator transmitted voltage.

### 3.3 Design and prototyping of the electrical stimulation device.

Based on the preliminary experiments, we could begin the design and the assembly of the electrical stimulation device. At the request of Dr. Jérôme Duisit, a complete list of specifications has been completed. The device has to be easily manipulated, the stimulation parameters have to be modifiable, and an interface has to be implemented in order to visualize the selected parameters. The latest version of the entire specification list is available in appendix A. As explained in the previous section, the maximum applied voltage has been set to 7V. Other parameters, namely, the pulse duration and the frequency have been fixed to values comparable to the stimulation values listed in the state of the art (table 1.1). The table 3.4 summarizes the stimulation parameter ranges the electrical stimulation device will provide.

| Parameter     | Range    |
|---------------|----------|
| Frequency     | 0.5-10Hz |
| Amplitude     | 0-7V     |
| Peak duration | 1-20ms   |

Table 3.4: Stimulation parameter ranges the electrical stimulation device shall provide.

#### Sizing and choice of material for the electrodes

In order to avoid electrodes corrosion, alteration of the culture medium composition, and graft damages, it is necessary to size the electrodes as well as to chose the proper material.

With regard to the tissue safety, we need to ensure there is no net charge being transferred per stimulation phase. This requires a charge balanced system, meaning that there is a need of a reversal charge flow, so that overall, no net charge is being left in the tissues. A biphasic signal similar to the signal depicted on figure 3.12 will be generated by the electrical stimulation device. Regarding the electrodes safety considerations, the factor that comes into play when trying to avoid electrolysis is the charge injection capacity. This factor is related to the material of the electrodes and the aperture size (the exposed area of the electrode). We therefore need to check that the amount of charge delivered during stimulation does not exceed the allowable amount, fixed by the chosen material, when multiplying the charge injection capacity by the aperture size. For platinum electrodes, the maximum charge injection density is  $D_{plat} = 50\mu C/cm^2$  [53], as for 316L stainless steel,  $D_{316Lss} = 40\mu C/cm^2$  [53]. As using stainless steel is cheaper and easier to implement than using a platinum coating, we will choose this material, and size the electrodes consequently.

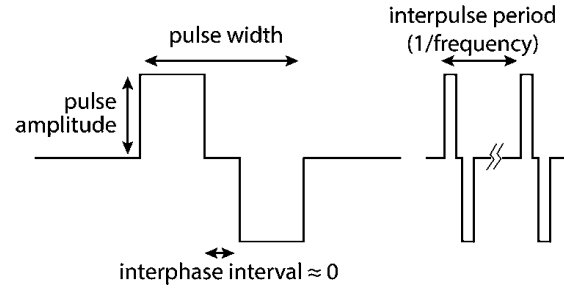


Figure 3.12: Electrical signal shape the stimulation device shall generate (adapted from [52]).

In order to create the electrical pulses, the micro-controller of an Arduino will be used as this one is relatively easy to program. The maximum current an Arduino can deliver is 200 mA if the 12V entry is used as a source. We can therefore calculate the electrode minimum surface in contact with the culture medium as the maximum pulse duration has been fixed to 20ms in the specifications. By using the definition of the electrical current as the charge flow rate, we could isolate the maximum charge quantity involved in the stimulation for one electrode (equation system 3.2).

$$\begin{aligned}
 I_{max} &= \frac{dQ_{max}}{dt_{max}} \\
 Q_{max} &= \int I_{max} dt_{max} \\
 Q_{max} &= 200e - 3 \times 10e - 3 \\
 Q_{max} &= 2e - 3[C]
 \end{aligned} \tag{3.2}$$

We can notice that  $dt_{max}$  has been fixed to  $10e-3$  in the equation system 3.2, because as depicted in the figure 3.12, only one electrode will involve charges in the system during the first half of the pulse width. The other electrode will take over during the second half of the pulse. In order to avoid electrolysis, the following equation has to be fulfilled:  $D_{316Lss} > \frac{Q_{max}}{S} \cdot S$ , representing the minimum surface in contact with the culture medium, is therefore evaluated to at least  $50cm^2$ .

## Implemented electrical circuit for the electrical stimulation

In order to fulfil the requirements from the specification list, the electrical signal generated by the device has to be modulated (see appendix A for further details). Six parameters have therefore been considered. The first three parameters are the electrical pulse width, the frequency and the number of pulses during one stimulation set. The set of pulses has to be generated periodically. This period of time defines the fourth parameter. The fifth parameter is the amplitude of the generated pulses. The last one allows the proper application of the desired output amplitude considering the imposed distance between the electrodes.

The parameters adjustment is performed using six rotating potentiometers which are modelled in the *buttons and switch* block of the figure 3.19. The Arduino will use these potentiometers output voltages (of range 0-5V) in order to deliver the desired stimulation.

The first step in the creation of the pulses is to generate the desired pulse amplitude. This voltage amplitude is produced by the *DC signal* block in the schematic of the figure 3.19. In order to provide an output amplitude between 0 and 7V, the 12V output of the Arduino has to be chopped using a PWM (Pulse Width Modulation). The PWM is created using a NMOS transistor. The source of this transistor is connected to the ground and its gate is controlled by a PWM signal (PWM<sub>0</sub> on figure 3.19) of 5V with a frequency of 980 Hz. The PWM signal is generated by the Arduino. When 5V/0V is applied to the gate, the transistor is open/blocked and the drain is connected to the ground/12V. Then, the 12V PWM signal generated at point A on the figure 3.19 passes through a first order low-pass filter turning the square signal into a DC voltage between 0V and 12V depending of the duty cycle of the PWM signal. The low-pass filter is designed to attenuate frequencies higher than 0.5Hz. This cutoff frequency of 0.5Hz has been chosen by performing some tests. It was noticed that a higher cutoff frequency resulted in a ripple of the output signal. This cutoff frequency is determined by the formula 3.3.

$$f_c = \frac{1}{2\pi R_2 C_1} \quad (3.3)$$

A relevant combination for  $R_2$  and  $C_1$  are  $R_2 = 18k\Omega$  and  $C_1 = 20\mu F$ . Because the time constant  $\tau = RC$  is quite large, the voltage measured across the capacitor rises and decreases slowly when the input PWM<sub>0</sub> changes as represented on the figure 3.13. Therefore, generating a pulse before the stabilization of the amplitude signal leads to an improper output signal.

The figure 3.14 represent the PWM signal, in blue, with a duty cycle of 60%. This signal is measured at the point A on the figure 3.19. The red signal, probed at point B, depicts the output of the low-pass filter. As expected, this signal has almost a value of 7.2V ( $12 \times 60\% = 7.2V$ ). The deviation from the ideal value of this signal is caused by the resistor  $R_1$ . This resistor allows the control of the current flow when the transistor is open. A high/low  $R_1$  will decrease/increase the current but also increase/decrease the voltage drop in the low-pass filter.  $R_1 = 1k\Omega$  is a good compromise between both options. The last element composing the *DC signal* block is an operational amplifier used as a voltage follower. The input impedance of the voltage follower is very high, causing the input voltage replication at the output. This component is



important, it prevents the low-pass filter to have a cutting frequency depending on the impedance of the circuit following parts (it is called the "loading effect").

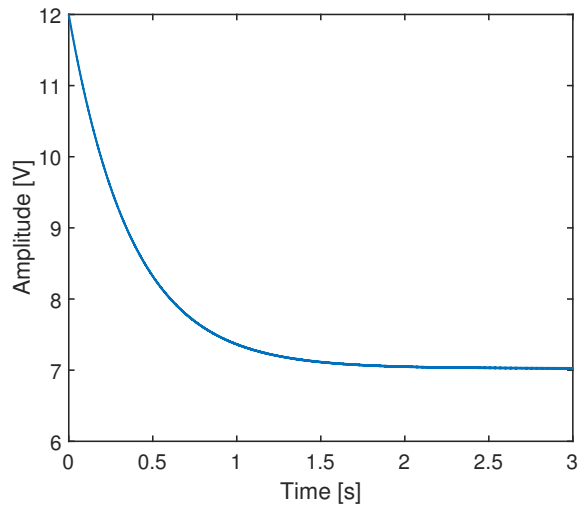


Figure 3.13: Based on the figure 3.19: measured signal at point B (LTspice simulation).

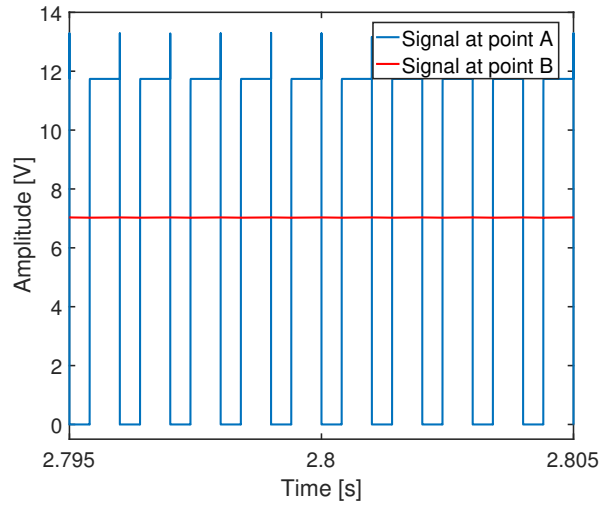


Figure 3.14: Based on the figure 3.19: PWM signal with a duty cycle of 60% and resulting signal at point B (LTspice simulation).

The second step in pulses generation is to accept the DC voltage only when a pulse has to be created. This circuit part corresponds to *Pulses and current amplification* block of the figure 3.19. To generate a pulse, a NMOS transistor is used. Its source is connected to the ground as the gate is linked the Arduino. Ordinarily, the gate signal is set to 5V, in order to connect the drain (the point C of the figure 3.19) to the ground, so that the DC signal is set to 0V. When a pulse is requested, the gate signal switches to 0V for the duration of the pulse, and the DC voltage is applied to point C. Figure 3.17 illustrates the pulse signal and the signal sent by the Arduino to the transistor gate. The brief peaks, or glitches present in the signal at point C of the figure 3.17 are due to the transistors non-idealities.

At this stage, the maximum current delivered by the circuit is not sufficient to generate the desired voltage in the culture medium. In fact, the current amplitude is limited by the LM308N8 voltage follower (LM308N8 datasheet available in the appendix K). In order to amplify this current, the signal is supplied to a LT1010 buffer. This buffer can provide a current of 150 mA (LT1010 datasheet available in the appendix K).

The last step ensures there is no net charge transferred per stimulation phase as discussed in the previous section. The stimulation will therefore be biphasic. During half the duration of the pulse, the current flows from one electrode, as during the second half of the pulse width, it flows from the other electrode. In order to create this biphasic signal, an H-bridge is used. This one is composed of four NMOS transistors. As depicted on the figures 3.15 and 3.16, when two specific transistors are on, and the 2 others are off, one electrode is supplied allowing the current to flow through the load to the other electrode. Switching the states of all four transistors will reverse the signal. Two complementary PWM

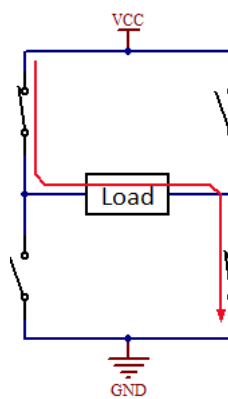


Figure 3.15: Load connections during one half of the pulse width.

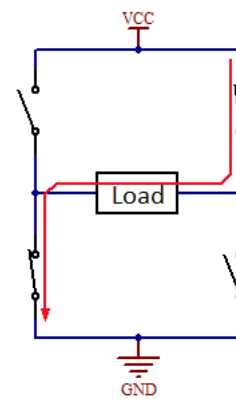


Figure 3.16: Load connections during the other half of the pulse width.



signals are used to control the four transistors. In order to switch on a transistor, the gate to source voltage ( $V_{GS}$ ) has to have a higher value than the threshold voltage ( $V_{th}$ ). Transistors T7 and T8 on the figure 3.19 need a gate voltage higher to  $V_{th}$  because the source is connected to the drain. However, the transistors T6 and T7 present at their source the pulse voltage. Therefore, we need a gate voltage higher than the pulse voltage to switch these transistors on. Because the Arduino can only generate PWM signal with a 5V amplitude, two other transistors with drain connected to the 12V signal are used (transistor T3 and T4 on figure 3.19). The 5V PWM signals generated by the Arduino are used by the transistors to produce two complementary 12V PWM. All the resistors present in the *pulses inversion* block of the circuit are used to limit the current flowing through the transistors. The figure 3.18 shows the behaviour of the signals at the output. The states of the four transistors are inverted when the complementary PWM signals change (points G and F on the figure 3.19). The voltage of the first half of the pulse width is therefore generated in the opposite direction. From the load point of view, the voltage is therefore positive during one half of the pulse width, and negative during the other half.

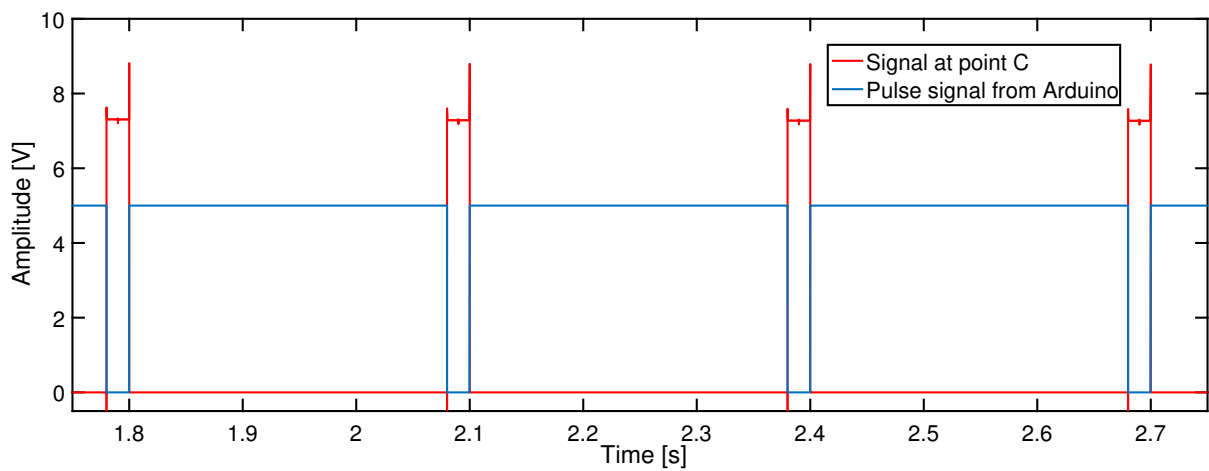


Figure 3.17: Based on the figure 3.19: Signals generated in the *pulses and current amplification* block of the circuit (LTspice simulation).

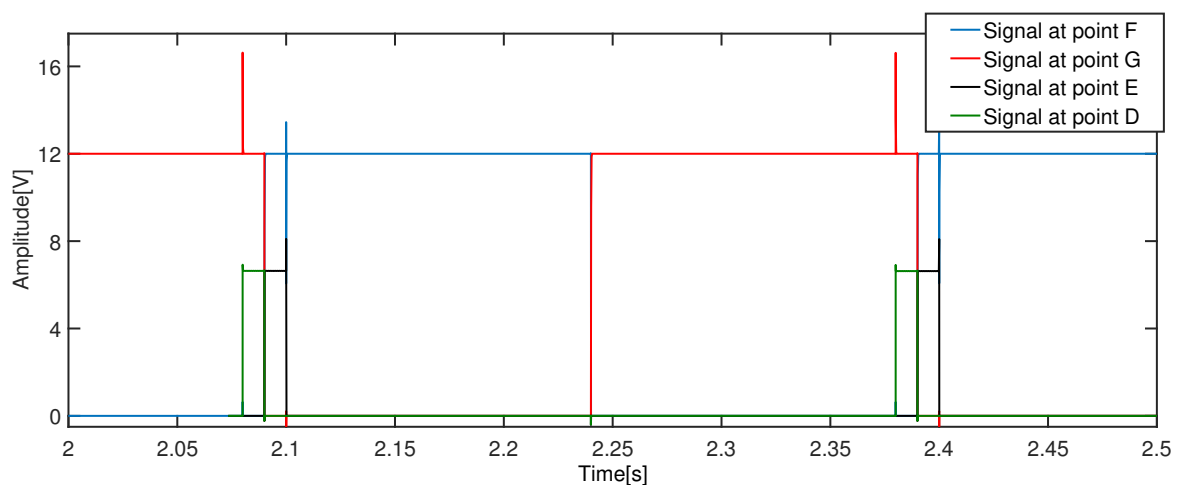


Figure 3.18: Based on the figure 3.19: Signals generated in the *pulses inversion* block of the circuit (LTspice simulation).

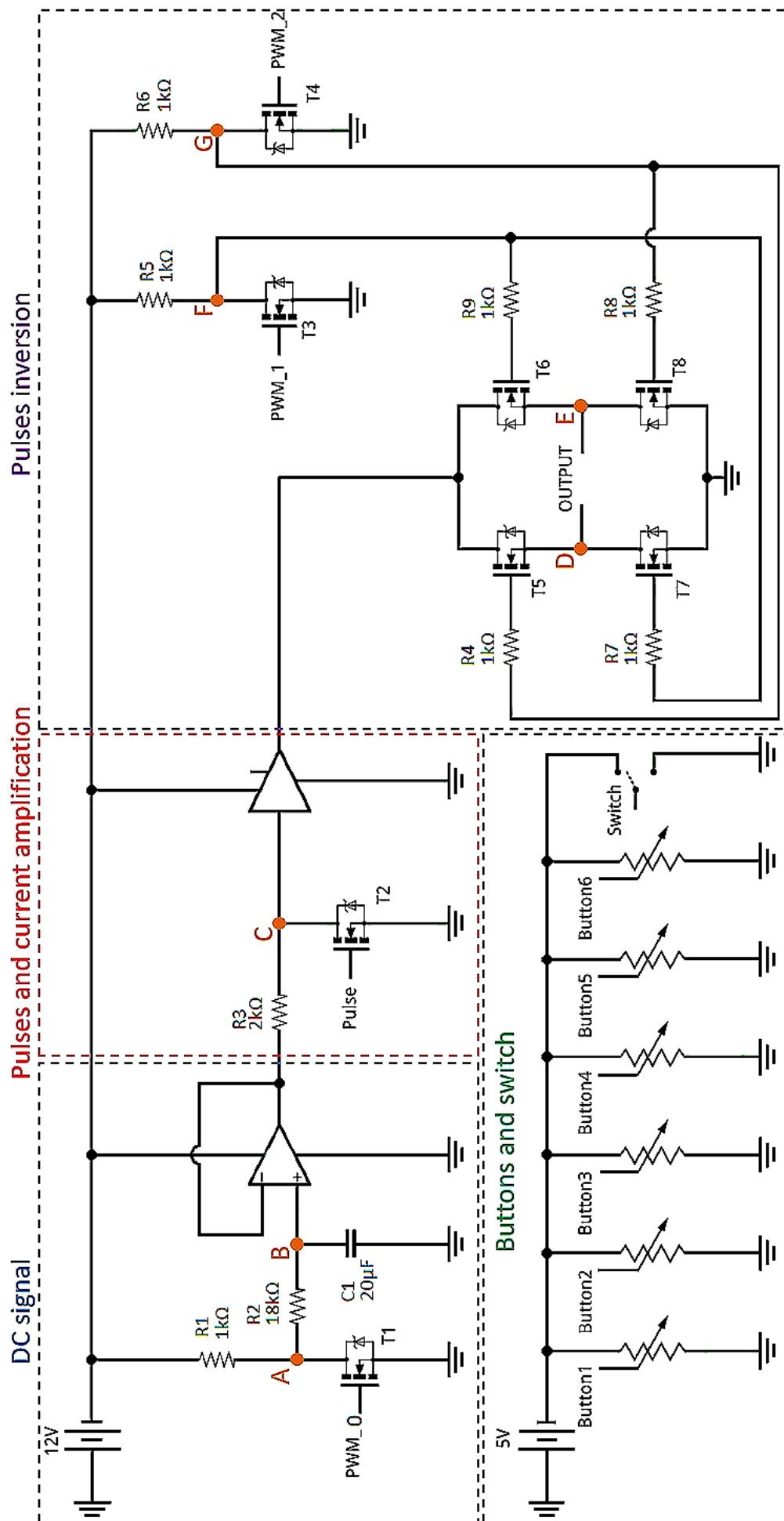


Figure 3.19: Implemented electrical circuit for the electrical stimulation.

The figure 3.20 represents both output voltage signals when actually testing the circuit. The data collected are measured using an oscilloscope. No ostensible difference between the LTspice simulation and the real obtained signals were noted. In fact, the LTspice simulation already takes into account the non-ideality of the used components. The biphasic signal produced by the circuit on the figure 3.20 has the desired amplitude of 7V ( $\pm 0.2$ V) and the duration of 20ms as expected.

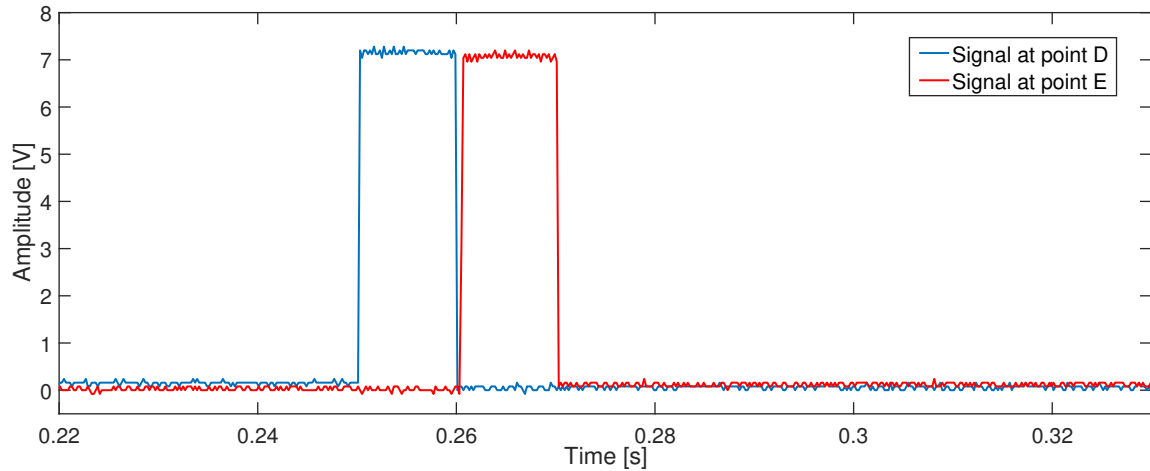


Figure 3.20: Based on the figure 3.19: Output signals obtained when performing a real test on the circuit.

### Software implementation of the interface

Another important specification was the visualization of the selected stimulation parameters. The interface is realized using a screen (see figure 3.24). This screen is linked to the Arduino which uses the potentiometers values set by the user. The implemented Arduino code, which makes the link between the electrical circuit (detailed on the figure 3.19) by wire connections and the screen by I<sup>2</sup>C bus, can be separated into two phases. During the first phase, the set up phase, the Arduino calculates all the signals parameters in function of the data entered by the user. During the second phase, the electrical stimulation is launched, and the Arduino generates all the signals the electrical circuit needs. A switch is used in order to make the transition between both phases (see figure 3.24). During the first phase, when the switch is off, the Arduino reads the voltage from the switching buttons that returns ranges of values between 0 and 1023. These ranges of values are mapped in order to match with the minimum and maximum values for each specific parameter. However, there is an exception for the amplitude parameter. In fact, as already mentioned in the section 3.2, we observed a drop of voltage once the electrodes are immersed in the culture medium. Moreover, this measured voltage is changing with the imposed distance be-

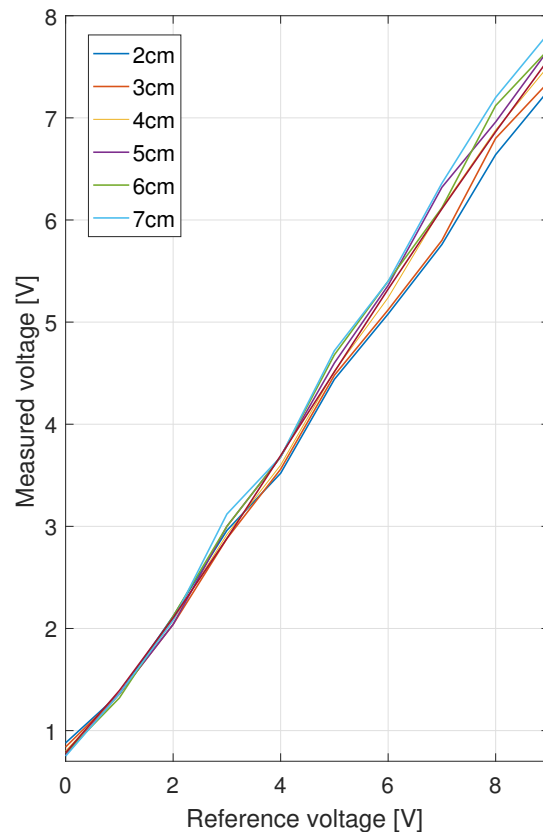


Figure 3.21: Measured voltage in the culture medium (DMEM) in function of the nominal voltage for each integer inter-electrode distance.

tween the electrodes. In order to compensate this drop, we performed some measurements in the culture medium (mainly composed of DMEM). The circuit output has been characterized according to the desired input voltage and the fixed distance between the electrodes. The characterization curves are plotted on the figure 3.21. The obtained values have been averaged over three test measurements for all integer voltage values and all integer distances. By way of information, the maximum measured standard deviation over these values is  $\sigma=0.13V$ . We then performed a linear regression for each curve. Using the slopes and origins of the linear regressions for each integer inter-electrode distance, two fourth order regressions of these two varying parameters have been calculated as depicted on the figures 3.22 and 3.23. We encoded in the Arduino the five polynomial coefficients of each regression polynomial so that it can compute the slope and the origin based on a chosen distance between the electrodes. For non-integer values of the distance, a weighted mean between both nearest integer values is calculated. Using these data, the Arduino can compute the needed output voltage in function of the desired input voltage. The amplitude displayed on the screen is the desired input voltage which will be really applied in the culture medium using this correction factor. Without taking into account the variability of the measured voltage with the inter-electrode distance, an maximum error of 0.6V has been measured with respect to the desired voltage. After using regressions, we reduced the maximum error to 0.2V.

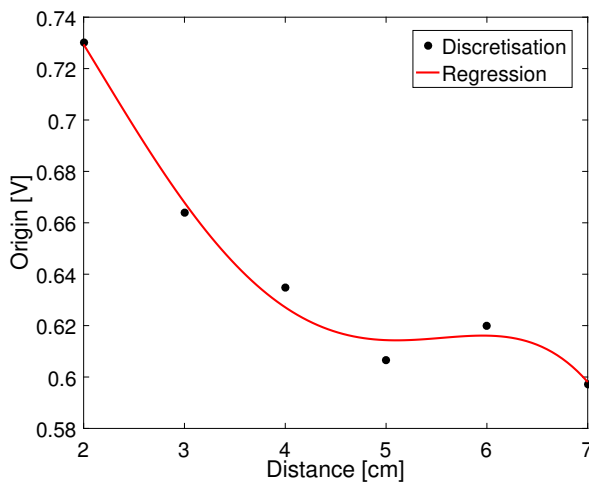


Figure 3.22: Polynomial regression of the different voltage regression lines origins.

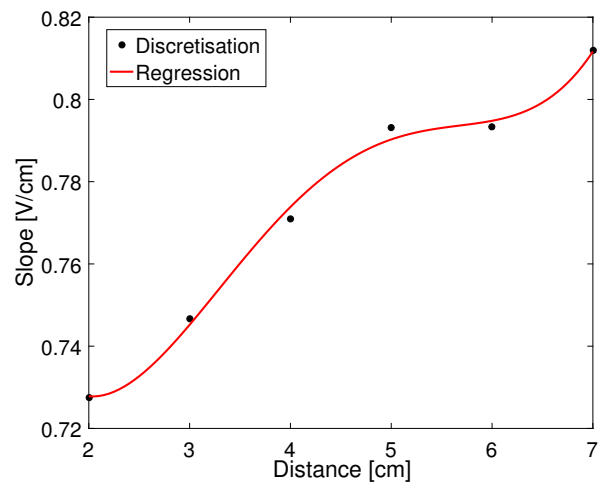


Figure 3.23: Polynomial regression of the different voltage regression lines slopes.

In the second phase, when the switch is turned on, the user can't change the stimulation values. The screen of the pulse generator depicted on the figure 3.24 turns black, and the Arduino generates the signals the electrical circuit needs to perform proper electrical stimulation.

All the electric boards have been gathered in a box for their safety. The box has also been equipped of the screen, the switch of stimulation launch and the rotating buttons. This box is in fact the so-called pulse generator pictured on the figure 3.24. The pulse generator can't endure the autoclave and placement in the incubator, but can survive splashes. The screen allows a clear visualization of the selected parameters during the set up phase, but shut down once the stimulation launched for economic reasons. Indeed, in order to cancel the noticed drop of voltage when the electrodes are immersed in the culture medium, the electrical circuit

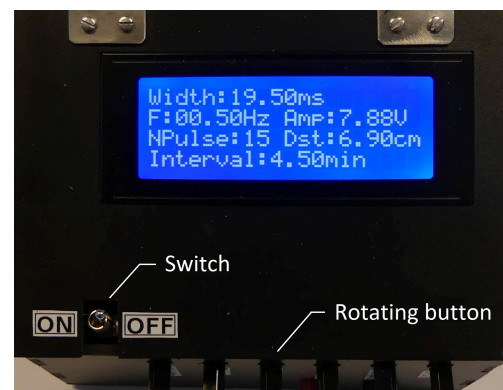


Figure 3.24: Screen and switch composing the pulse generator.

needs sufficient power. The LED's composing the screen are simply shut down, but a visualization of the parameters is still possible. The details of the Arduino wire connections and the value ranges of the different parameters are available in the appendix E.

## Device prototyping

From the specifications list (appendix A), the device has to be easily manipulated. The parts immersed in the culture medium also have to be autoclaved and placed in the ICO246med incubator. Therefore, these parts have to resist the physical and chemical conditions of the incubator ( 37° C and 5% CO<sub>2</sub>). Moreover, the distance between both electrodes should be variable in order to allow the electrical stimulation of several size grafts.

A device has therefore been designed and assemble in order to support the electrodes. The figure 3.25 details its components. All pieces are made of nylon except for the electrodes and the screws which are made of 316L stainless steel. Nylon is a biocompatible and autoclavable material. These plastic pieces have been 3D printed using the Markforged (Mark two) printer. However, as some device surfaces had to be smooth, like the internal surface of the cables passage (figure 3.25), support material has been avoided during the printing because of the bad surface finish it causes. The design is therefore engineered for an entire self-carried printing. The minimum slope ensuring self-carrying using the Markforged with nylon is 60 °.

Electrical banana cables are used to create the electrical connection between the electrodes and the pulse generator. We cut and remove the cable insulation of one side of the cable. The copper bare end of the cable is slid into the handle and comes into the slide by the two passages mentioned in the figure 3.25. Once the cable bare end in the slide, we place the electrode against the copper wire. We tight the sub-assembly by fixing a lid on the slide using two M2 screws. The contact between the copper of the cable bare end is ensured by screwing a pressure spindle through the slide. The pressure spindle will press the copper on the electrode part situated in the slide. Unscrewing the M4 clamping screw allows the adjustment of the distance between the electrodes. The exploded view on the figure 3.26 allows a clearer overview of the device assembly.

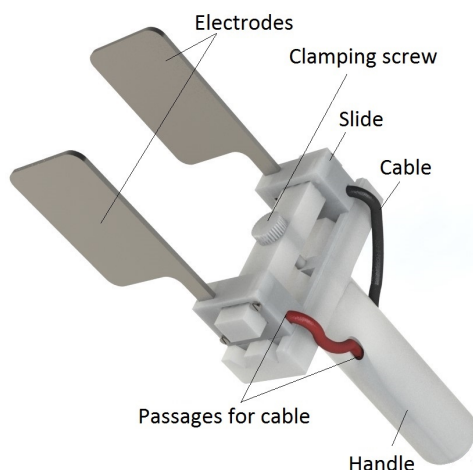


Figure 3.25: Glimpse of the portable electrode support.

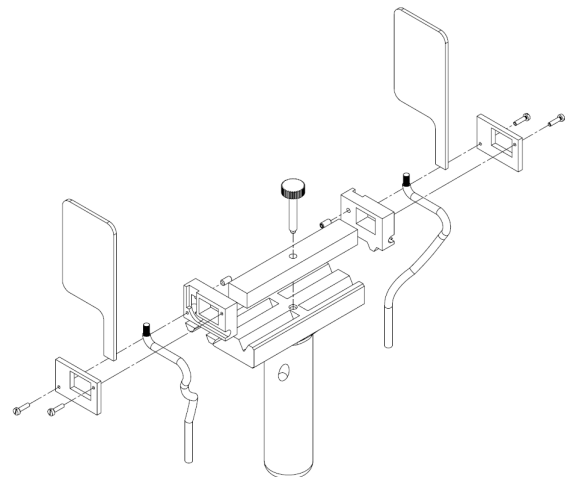


Figure 3.26: Exploded view of the portable electrode support.

In order to avoid the corrosion of the copper wire during autoclave, we sealed the slide slots using a polymerizing glue [41]. The electrical cables [55] are therefore fixed in the slide, and will endure autoclave

as the rest of the device. The sheath of the cable is made of silicon, as the plug surface material is made of nickel. These have been autoclaved five times before their placement in the device, and presented no sign of damage. The finish product is therefore portable, easily manipulated, theoretically entirely autoclavable and pluggable in the pulse generator. As depicted on the figure 3.27, a graduated rule has been added to the device, in order to ease the setting of the inter-electrode distance, and render it more accurate.

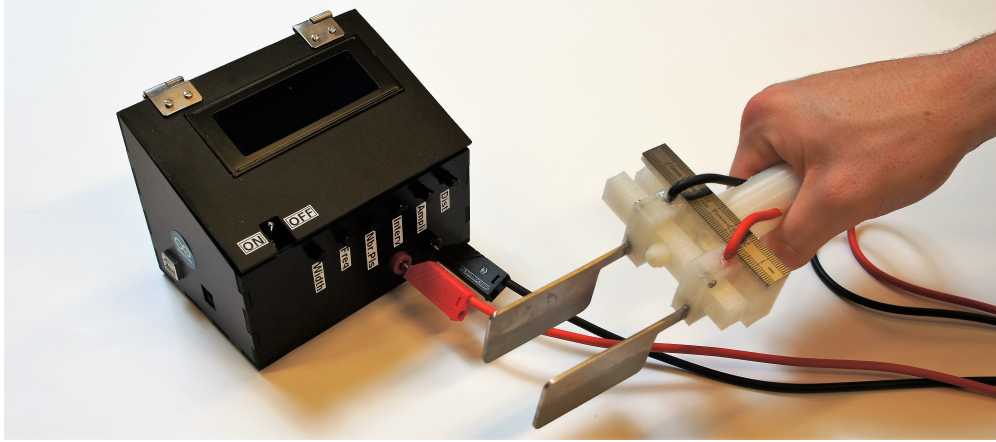


Figure 3.27: Electrical stimulation device composed of the pulse generator and the portable electrode support.

### Interface for the calculation of the submersible electrode surface

The size of the electrodes composing the portable support has been calculated in order to avoid any chemical reaction between these electrodes and the culture medium. However, if the user doesn't have the possibility to immerse the electrodes entirely, the parameter ranges of the device cannot be used in their entirety for the safety of the electrodes. A software has been implemented in Java, in order to calculate the minimum immersible height that will ensure no chemical reaction with the culture medium. The user-friendly interface invites the user to enter the desired stimulation parameters, namely, the amplitude, the pulse duration and the distance between the electrodes (see figure 3.28). Then, the program gives back two heights corresponding to the immersible electrode heights from both electrode edges (see figure 3.29).

Figure 3.28: Glimpse of the software data entry page.

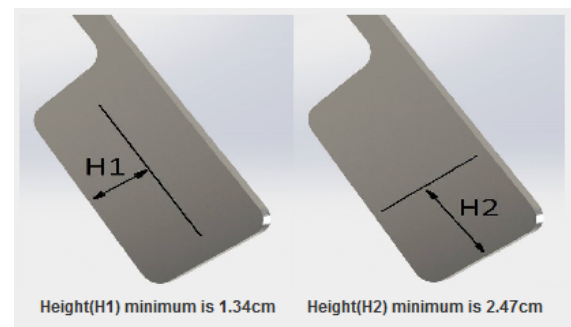


Figure 3.29: Software response page.

Behind this interface, the already mentioned maximum charge injection equation is used,  $D > \frac{I_{max} \cdot T_{max}}{S}$ , with;  $D$ , maximum charge injection density linked to the chosen material;  $I_{max}$ , the maximum current flowing through the culture medium;  $T_{max}$ , the pulse duration; and  $S$  the immersible surface. The variable  $D$  is fixed by the chosen material. For 316L stainless-steel,  $D_{316Lss} = 40 \mu C/cm^2$  [53].  $T_{max}$  is set by the user as a program input, as  $I_{max}$  is calculated on the basis of the stimulation amplitude entered by the user. Indeed, the culture medium used is a complex entity. When plotting the graphic of the induced

current in function of the applied voltage, we can notice a culture medium impedance that is not constant (see figure 3.30). Moreover, this impedance varies according to the inter-electrodes distance. A fourth degree polynomial regression has been used to model these curves, as a weighted mean between these curves has been calculated in case of non-rounded entered distance. Once  $I_{max}$  calculated using the regression and the immersible surface obtained, a simple geometry calculation is used to return the user the minimum immersible height from both edges. A security margin of 10% on the immersible height has been considered. The complete code used for the creation of this interface is available in the appendix H.

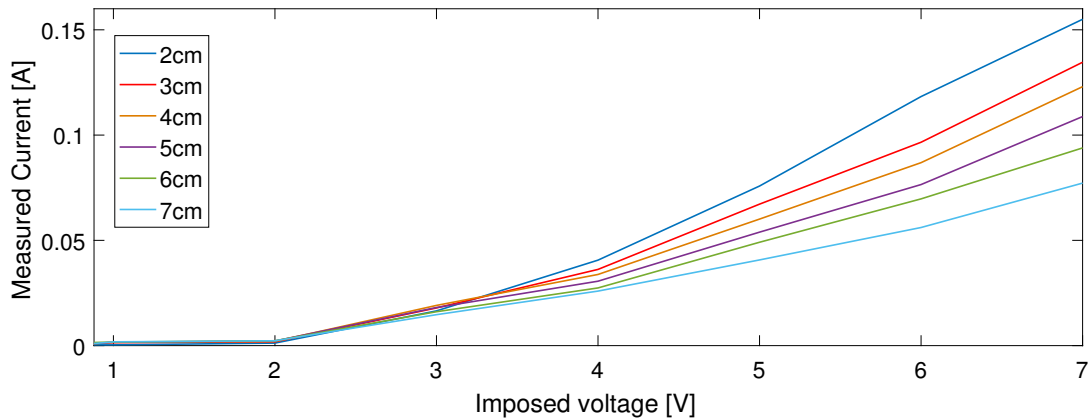


Figure 3.30: Measured Current in function of the sensed voltage in the culture medium (DMEM) for each integer inter-electrode distance.

Some tests have been performed on the culture medium in order to explain the phenomena of impedance variation in function of the applied voltage. PH tests as well as temperature tests have been performed, but the obtained results weren't convincing. No further paths for reflection were investigated due to timing reasons, but it would be interesting to go deeper into the subject.

### 3.4 Device testing in the CHEX laboratory

Experiments have been realized at the CHEX to test the proper functioning of the electrical stimulation device. Two kinds of grafts have been used; a native pig half-lip and a recellularised human half-lip. These experiments allowed to highlight some issues and possible improvements regarding the electrical stimulation device and the used technique to quantify the muscle contractions. The results of these tests will be taken into account in the design and the implementation of the final bioreactor with mechanical and electrical stimulation included.

#### Electrical stimulation on a pig half-lip

The electrical stimulation device has been tested on a pig half-lip. The graft has been procured from a pig deceased by exsanguination and conserved in a phosphate-buffered saline (PBS) solution. It is important to note that the deceased pig has not been only used for our experiment. The sacrifice allows lots of researchers at the CHEX to perform their surgical experiments. This experience was conducted in accordance with the EU Directive 2010/63/EU for animal experiments. A complete test bed has been realized. The latter is pictured and detailed on the figure 3.31.

The portable electrode support and the pulse generator are used to stimulate the pig half-lip. An oscilloscope collects the stimulation signals in order to check if the proper electrical signals are sent. A container, filled with culture medium (mainly composed of DMEM), receives the graft and the electrodes. In order to



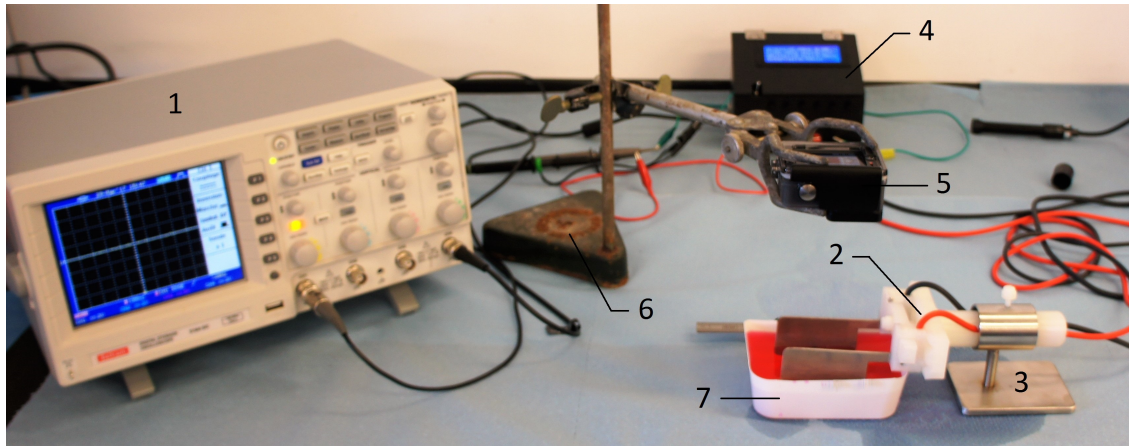


Figure 3.31: Test bench of the electrical stimulation of a pig half-lip. 1. Oscilloscope; 2. Portable electrode support; 3. Electrode support fixation; 4. Pulse generator; 5. GoPro; 6. GoPro support; 7. Graft and culture medium container.

fix the graft, we used a holed cylindrical piece made of the copolymer polyoxymethylene (POM-c) which is a biocompatible but non-implantable material. The surgeon is therefore able to stitch the graft at its centre to the plastic support as depicted on figure 3.32. Regarding the method for the check and quantification of the muscle contraction, we used small blue balls fixed at some precise locations on the graft and a GoPro to film the graft during stimulation. The recorded images are then processed using a program able to locate the blue spots on the image at any moment. The figure 3.33 illustrates the fixation of the graft on its support as well as the fixation of the blue balls on the graft. In total, four blue balls are used and fixed in the midst of each graft edge. The complete experimental protocol is available in appendix F.

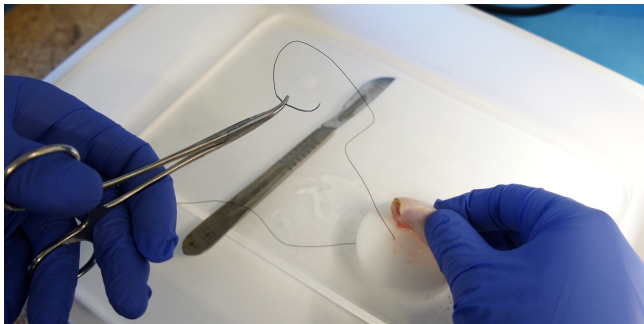


Figure 3.32: Surgeon fixing the graft on the POM-c support.

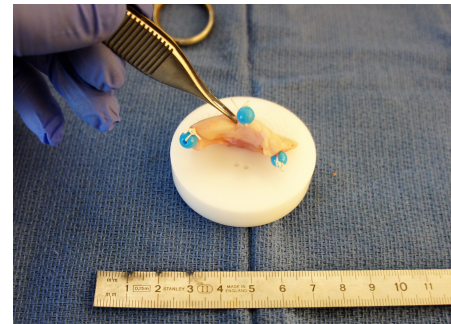


Figure 3.33: Fixation of the blue balls on the graft.

Electrical pulses at a frequency of 0.5Hz, with a duration of 20ms and amplitude varying between 1V and 7V with a step of 1V were applied to the graft. It was not until 2V that the contractions were visible to the naked eye. A pH test has also been performed before and after the experimental session. No pH change was noticed after the stimulation, meaning that no major chemical reaction happened.



In order to collect the precise data on the elongation and shortening of the graft during electrical stimulation, we adapted a Matlab code created by Lise Coleman [56] and modified by Lena Vanthourhout (UCL). This program partition the video recorded by the Go-Pro into frames. Each frame is analysed and the blue balls positions are found by detecting their circular or elliptic contours. The figure 3.34 shows the detection of the blue balls contours (in blue) and their centre (the red points) by the program. The centre positions of the six balls are registered.

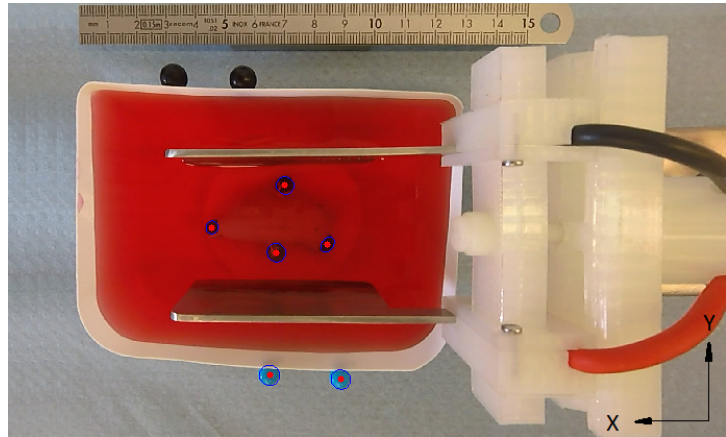


Figure 3.34: Detection of the six blue balls by the Matlab program.

The two blue balls fixed on the container (see figure 3.34) are used as a reference. Indeed, the distance between these two blue balls is well known and allows to compute the dimension of one pixel in the image. As the program can give us the number of pixels separating one ball centre to a defined reference point, we could therefore compute and quantify the displacements in millimetres of the four balls fixed on the graft. These displacements are based on the balls positions at the very first frame. The figure 3.35 shows the displacement of the balls in the x axis direction as the figure 3.36 shows the displacement in the y axis direction during electrical stimulation. The amplitude stimulation was fixed to 6V, and the frequency to 0.5Hz. The pulse generator generated 10 pulses of 20 ms width. The inter-electrode distance has been set to 4cm. We clearly see on the figures 3.35 and 3.36 the graft enduring this specific electrical stimulation. We can distinguish the ten stimulation peaks at the imposed stimulation frequency.

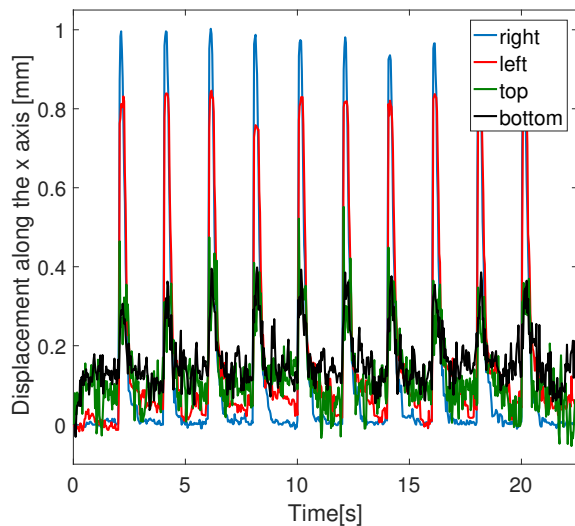


Figure 3.35: Displacements of the four balls along the x direction over time.

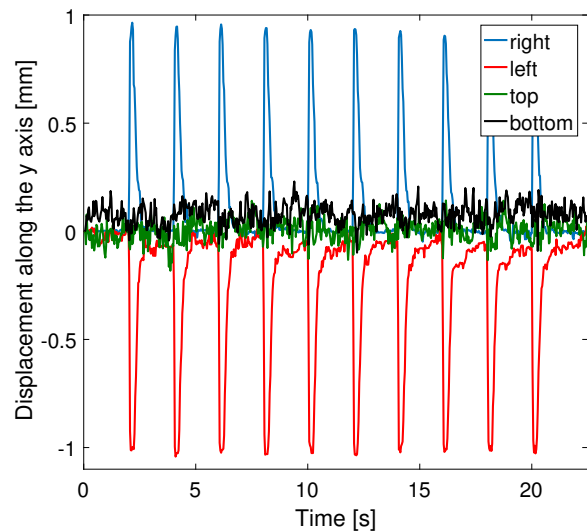


Figure 3.36: Displacements of the four balls along the y direction over time.

The figure 3.37 shows the distance along the x axis between the left and right balls. During the electrical stimulation, the x inter-balls distance is increasing. This phenomenon can be explained by the fact that the balls are attached to the skin of the graft and not at the muscle extremities. Also, the graft is not guided during the stimulation and therefore, contracts in random directions. The figure 3.38 however shows the distance along the y axis between the bottom and top balls. This distance doesn't change in a significant way during the whole stimulation period.

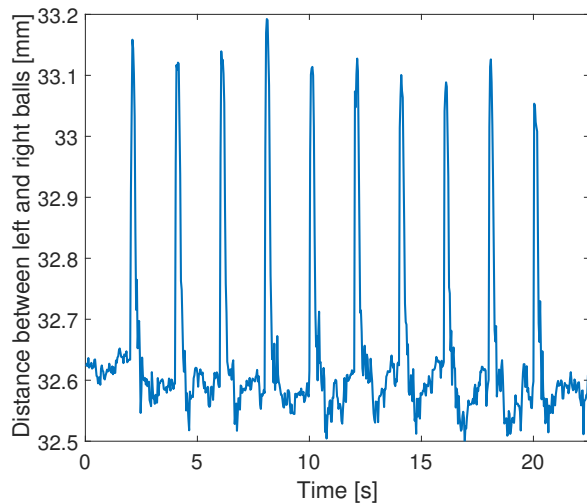


Figure 3.37: Distance between the left and right balls over time.

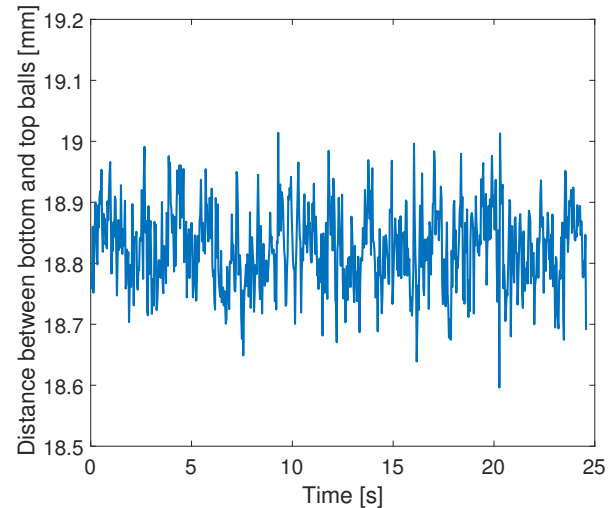


Figure 3.38: Distance between the bottom and top balls over time.

Experiments on native muscles are essential to quantify their shortening and elongation. A comparison with the contractions of recellularized muscles can therefore be made in order to check the good development and differentiation of the muscle cells. The surgeon can have a reference in terms of native contractibility. However, the data we obtained only proof the good contractibility of the native graft and the good functioning of the electrical stimulation device. As the graft is not guided during the stimulation, we can't precisely quantify the elongation and shortening of the muscle. A guidance will therefore be implemented in the final stimulation bioreactor.

### Electrical stimulation on recellularised human half-lip

Using the same methods of stimulation and quantification of muscle contractions than the test on a native pig half-lip, the stimulation of a recellularised human half-lip has been performed. The half lip has been perfused with C2C12 cells for 15 days. We can notice on the figure 3.39 that the graft presents a bright pink colour specific to the perfused culture medium. The complete experimental protocol is available in appendix F. Electrical pulses at a frequency of 0.5Hz, with a duration of 20ms and an amplitude varying between 1V and 7V with a step of 1V were applied to the graft. No contractions were visible on the naked eye. No balls ostensible movements were noticed neither when using the ball detection program. After histologic study of the graft, it has emerged that not enough cells were injected. The cells present in the graft exhibited good maturation and differentiation, but they were not so dense to show visible contractibility under electrical stimulation. The contraction detection system needs to be changed in order to sense micro-contractions. A pressure sensor sensitive to micro-contractions will therefore be used in the final stimulation bioreactor. Moreover, a mechanical stimulation during the recellularization step could help the myofibers densification and growth. The creation of bioreactor including mechanical stimulation is therefore relevant.

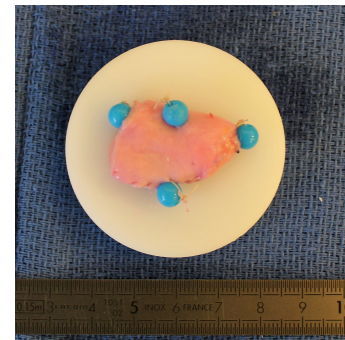


Figure 3.39: Recellularised human half-lip fixed to the support.

## 3.5 Feasible improvements on the electrical stimulation device

When confronting the electrical stimulation device with its initial specifications (see appendix A), it can be noticed that it doesn't fulfil two of them.

After autoclave of the experimental bioreactor V1 [3], some parts were found unglued. In fact, the polymerizing glue was unable to withstand the autoclave conditions. As our portable electrodes support has been sealed with this polymerizing glue, it is possible it could not resist to the autoclave temperature and moisture conditions. If the slide parts of the electrodes support debonds, the bare end of the banana cable made of copper will oxidize leading to a poor electrical connection between the electrodes and the pulse generator. It is however possible to change the design of the electrodes support slightly in order to avoid the use of glue. The solution is to extend the electrode profile in the slide, and use complete banana cables. As illustrated on the figure 3.40, we simply have to plug the banana cable in the slide in order to make the electrical connection. The figure 3.41 details the layout of the device different parts. As no oxidizable materials are used, the portable electrode support can be entirely autoclaved.

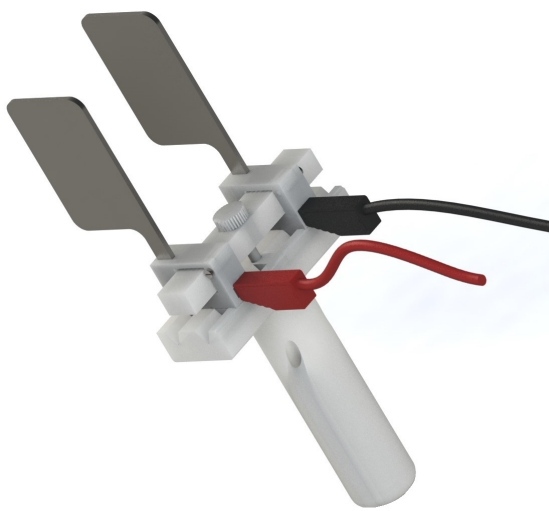


Figure 3.40: Glimpse of the modified portable electrode support.

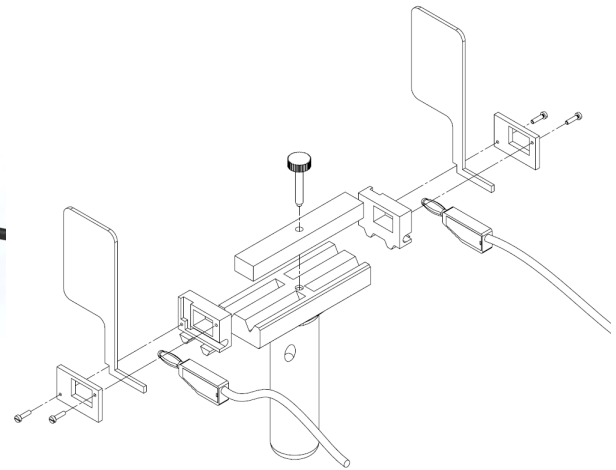


Figure 3.41: Exploded view of the modified portable electrode support.

The second encountered issue is the variability of the impedance of the culture medium. Indeed, the software used to calculate the submersible electrode surface uses the curves of the figure 3.30. The problem is that these curves are based on measurements that we can't explain. However, one track is still unexplored which is the bad quality of the multimeter used to measure the current. The impedance variation could be explained by the fact that the multimeter underestimates the current when this one is small. Better measurements could be obtained with a more sophisticated and precise current measurement device. Even if a security margin of 10% has been taken on the electrode immersible height, more accurate measurements could guarantee the safeness of the electrodes as well as the graft during a stimulation using this software.

Based on the developed electrical stimulation system, a bioreactor capable of electrical and mechanical stimulation will be designed and assembled. Some observations made on the electrical stimulation system will be taken into account in the design following steps. We noticed during our experiments in the culture medium that a variation of the inter-electrode distance can be compensated by an increase (or decrease) of the applied voltage (see figure 3.21). Therefore, the inter-electrode distance will be fixed in the final design. Also, no polymerizing glue will be used in the bioreactor assembly, and a guidance system for the graft will be implemented in order to ensure contractions parallel to the muscle fibres. Finally, a force sensor will be used in order to quantify micro-contractions during the graft recellularization.

## Chapter 4

# Design, Prototyping and Testing of a Bioreactor Capable of Electrical and Mechanical Stimulation of Facial Composite Tissue Grafts

When using appropriate parameters, a mechanical stimulation in the fibre direction can orient and develop myofibers [24]. Electrical stimulation, for its part, can increase the production force and rheobase [29], a combination of electrical and mechanical stimulation could therefore favour a good maturation and differentiation of the muscle cells during the recellularization step. With the creation of a bioreactor with electrical and mechanical stimulation included, we want to recreate all the physiological conditions a native muscle experiences in the human body. The synthesizing of previous scientific works on the choice of ideal mechanical parameters for a proper muscle cell development will guide us in the implementation of the mechanical stimulation. The design and assembly of the electrical stimulation device will serve as basis for the creation of an electrical nature stimulation. Regarding the environment in which the graft will evolve during the recellularization, the V1 bioreactor designed by Alexia Springuel [3] will inspire us during the conception of the final device. In this chapter, the choices and sizing of the main parts of the device are explained and confronted with the initial specifications. The final bioreactor is then presented and its mechanisms are detailed. Finally, a test bench created in order to verify the good functioning of the final device is presented, and the results of these experiments are exposed.

### 4.1 Design of the bioreactor chamber with electrical and mechanical stimulation integrated

The design of the final bioreactor chamber has been conducted in three stages. First, a specification list has been formulated, and the bioreactor main operation modes have been highlighted. Then, a morphological graph has been realized and used in order to choose the most suitable components regarding each operation mode. Finally, the rest of the design has been achieved in accordance with the specifications and the previously chosen components.

#### Device objectives and tasks to be performed

In the following design steps, the same graft as for the electrical stimulation device is used as a basis, namely, a human half-lip. The device we need to develop has to stimulate the graft electrically and mechanically, but not only. Indeed, the device also needs to ensure optimal physiological conditions for a proper recellularization. More specifically, the device needs to maintain the graft in the culture medium while allowing its visibility. Also, it has to allow the graft perfusion in the best conditions. As a remainder, no pressure, stress or strain have to be applied on the artery. Moreover, the device needs to ensure gaseous exchanges between the outside environment and the culture medium. The temperature, the humidity and

the CO<sub>2</sub> rate need to be regulated in the chamber. To do so, all the parts in contact with the graft and the needed media for a proper recellularization (namely, the culture medium and an air layer) have to be placed in an incubator and resist its physical and chemical conditions. The INCO246med incubator can be used for this purpose. However, the parts immersed in the culture medium or in contact with the air layer ensuring gaseous exchanges with the culture medium, have to be sterilizable using autoclave and stay sterile during the entire recellularization process. Regarding the stimulations, the mechanical one will be the main stimulation as the electrical one will serve either as a contraction control after recellularization or as a complementary stimulation. As an example of electrical complementary stimulation, we could imagine a stimulation where the graft contraction is caused by electrical stimulation as the relaxation of the muscle is induced by a mechanical stretching. About the functioning of these stimulations, four operation modes need to be implemented:

- A position control of the mechanical stretching and relaxation of the graft while measuring the traction force undergone by the graft.
- A force control of the mechanical stretching and relaxation of the graft while measuring the distance between the graft ends in the stimulation direction.
- An electrical stimulation of the graft while measuring the distance between the graft ends in the stimulation direction.
- An electrical stimulation of the graft while measuring the traction force undergone by the graft.

Two complete lists of specifications are available in the appendix B and C. These two documents are complementary, one detailing the electrical stimulation specifications, as the other details the mechanical stimulation and the bioreactor chamber specifications the device needs to fulfil.

### **Sizing and choice of the main device parts**

The design of the final bioreactor chamber is based on the four operation modes above mentioned. These operation modes are in fact the specifications that impose the greatest limits on the design. Once an implementation is found for all the operating modes, the design choices for the rest of the bioreactor components will be clearer.

The table 4.1 groups all the potential implementation ideas for each operation mode with an assessment regarding their feasibility and cost. The solutions including immersible components in the culture medium have been discarded from the outset. Indeed, these solutions are expensive due to their additional sealing system. Some of them could also increase the internal volume of the bioreactor, which we try to reduce at most as possible in order to avoid using the culture medium in large quantities. As a reminder, the used culture medium, mainly composed of DMEM, is quite expensive.

From the assessment of the table 4.1, the solution of using a linear actuator with position control included as well as an external force sensor has been chosen. The measurement of the position during electrical stimulation will be achieved using a zero force control of the linear actuator. In order to measure the tension force during the electrical and mechanical stimulation, we will simply use the output data from the external force sensor.

The actuator and the force sensor have to be selected before designing the rest of the device. Indeed, cheap force sensors with the required precision of 0.01 N cannot be found easily. Let us recall that the maximum traction force measured on the half-lip graft is 0.27N. The chosen sensor is a SingleTact pressure sensor, pictured on the figure 4.1 (the force sensor datasheet is available in the appendix K).

Table 4.1: Comparison table of solutions for each operation mode.

| Operation mode                                                                      | Proposed solution                                                                                                                                                                                       | Potential issues                                                                                                                                                                                                                                                                 | Assesment (Feasibility; Cost; Reliability) |
|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| Position control and force measurements/<br>Force control and position measurements | Linear actuator/motor, guide rail, with position and current control included, external to the bioreactor                                                                                               | Expensive and current control imposes not to use traditional joint at the outer-inner bioreactor interface                                                                                                                                                                       | (0; 0; 0)                                  |
|                                                                                     | Linear actuator/motor with included guidance and with position and current control included, external to the bioreactor                                                                                 | Expensive and current control imposes not to use traditional joint at the outer-inner bioreactor interface                                                                                                                                                                       | (+; 0; 0)                                  |
|                                                                                     | Linear actuator/motor with included guidance and position control included, external to the bioreactor. Addition of an external force sensor                                                            | -                                                                                                                                                                                                                                                                                | (0; ++; 0)                                 |
|                                                                                     | Rotative motor, external to the bioreactor, with cable and restoring element (spring or load). Addition of an external force sensor and external position measurements (via laser, or video processing) | External position measurements via laser or video processing is not enough accurate for control. However a sophisticated instruments can be used, but are more expensive.                                                                                                        | (-; 0; --)                                 |
|                                                                                     | Rotative actuator with force control included, external to the bioreactor, with cable and restoring element (spring or load). External position measurements (via laser, or video processing)           | External position measurements via laser or video processing is not enough accurate for control. However a sophisticated instruments can be used, but are more expensive. Moreover, current control imposes not to use traditional joint at the outer-inner bioreactor interface | (-; 0; --)                                 |
| Electrical stimulation and position measurements                                    | Using the signal generator and the position feed-back given by the position controlled motor                                                                                                            | The use of the motor feed-back imposes not to use traditional joint at the outer-inner bioreactor interface. This solution requires a linear motor, which is more expensive than a linear actuator                                                                               | (0; 0; 0)                                  |
|                                                                                     | Using the signal generator and an external position measurements (via laser, or video processing)                                                                                                       | External position measurements via laser or video processing is not enough accurate for control. However sophisticated instruments can be used, but are more expensive                                                                                                           | (-; 0; -)                                  |
|                                                                                     | Using the signal generator and zero force control of a linear actuator with position feed-back integrated                                                                                               | The zero force control need a quick data refreshment of the force sensor and a rapid actuator response time                                                                                                                                                                      | (-; +; +)                                  |
| Electrical stimulation and force measurements                                       | Using the signal generator and the force feed-back from the actuator/motor                                                                                                                              | The force feed-back is incorrect if using traditional joint at the outer-inner bioreactor interface                                                                                                                                                                              | (0; 0; 0)                                  |
|                                                                                     | Using the signal generator and an external force sensor                                                                                                                                                 | -                                                                                                                                                                                                                                                                                | (0; +; +)                                  |

It provides errors less than 0.01N over a range of 0 to 3N. A data acquisition board and an I<sup>2</sup>C interface is integrated, allowing an easy communication with an Arduino.

Also, the actuator has to measure the elongation with a 0.5 mm precision. The maximum elongation measured on the half-lip graft is 8mm. The chosen linear actuator fulfilling these specifications is a Firgelli L12-50-6I, depicted on the figure 4.2 (the actuator datasheet is available in the appendix K). This linear actuator is in fact an electric cylinder. It has a 30mm stroke and a backdrive force of 43N. A position control is included and a position feed-back is available. This actuator can be easily used with an Arduino. However, as its power voltage is fixed to 6V, a buck converter is needed in order to supply the actuator with the 12V pin of the Arduino. Only one actuator will be used in the bioreactor allowing the stretching of one side of the graft, as the other side is fixed to the bioreactor frame. Moreover, in order to be able to use the actuator position controller, the graft has to be rigidly connected to the actuator shaft.



Figure 4.1: SingleTact pressure sensor [57].

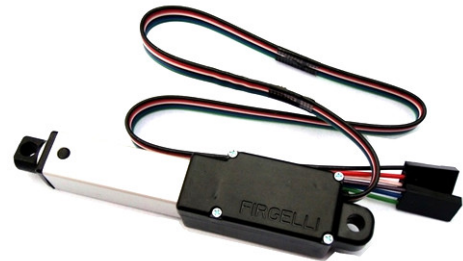


Figure 4.2: Firgelli linear actuator L12-50-6I [58].

## Design of the main sealing systems

The whole sealing system of the bioreactor chamber, now that the used components for the four operation modes are fixed, can be designed. It is important to note that the bioreactor chamber has to be air and water tight in order to ensure the sterility inside the bioreactor chamber during the entire recellularization step. Additionally, as for the V1 bioreactor, an air layer has to be present in the bioreactor in order to ensure gaseous exchanges with the culture medium. Two different sealing mechanisms have to be developed. One for the actuator, and another for the sensor, as we choose not to select immersible components.

For the actuator, a system similar to a protection below is used. This tight sheath, schematized on the figure 4.3 is fixed at one end to the bioreactor chamber internal wall, and to the part of the shaft internal to the bioreactor at the other end. The shaft passage way through the bioreactor wall is located in the air layer. In this way, no additional culture medium movements to the one caused by the linear movement of the graft attachment system is created. Limiting turbulent culture medium movements allows a better visual of the stimulated graft during the recellularization. Regarding the sensor part of the system, the pressure sensor has been placed at the other extremity of the device. This one will be indirectly linked to the graft end opposed to the one rigidly connected to the motor shaft. The difficulty is to avoid any friction in the sealing system under penalty of collecting skewed data from the sensor. The implemented system is in fact a flexible membrane (see figure 4.4). The membrane is fixed to the bioreactor wall and to a shaft at which is attached one graft extremity. The pressure sensor is placed between the bioreactor frame and a planar piece rigidly fixed to the shaft. The movements involved in the assembly are in fact shaft micro-movements. As the deformable membrane is fixed to the shaft, this one will warp and follow the shaft micro-movements when a load is applied axially. The pressure sensor will therefore only sense the pressure applied by the load, no friction force is perceived by the latter.



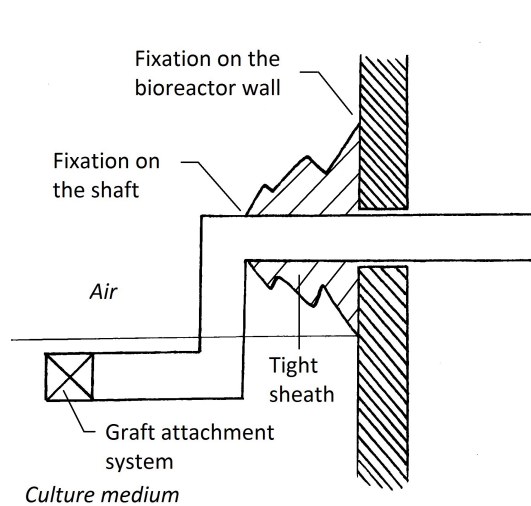


Figure 4.3: Schematic of the motor shaft sealing system (cross-sectional view).

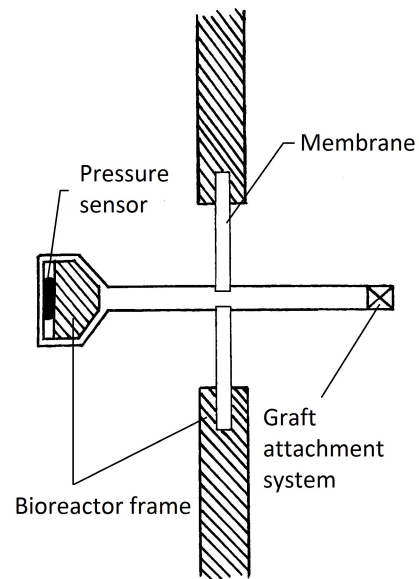


Figure 4.4: Schematic of sensor sealing system (cross-sectional view).

In the specifications, an easy access to the graft by the top of the device as well as a visual monitoring of the stimulation was required. The lid of the bioreactor will be made of polycarbonate, which is a translucent material. The lid will be fixed on the top surface of the bioreactor using bolts. A large o-ring seal placed at the interface between the top surface of the bioreactor and the lid will ensure the sealing. The groove in which the o-ring seal is placed is sized using the sizing tables of Eriks [59].

### Design of the tube connectors

As explained in the chapter 2, the stainless-steel tube connectors of the V1 bioreactor needed design modifications for an easier and safer removal of the tubes. Instead of glue, we use a thread-nut system. The tube connector is composed of a collar that serves as a shoulder and a contact surface for the o-ring seal. The collar and the seal are situated inside the bioreactor. Outside, a nut is used to tighten and seal the assembly, as depicted on the figure 4.5. Using this design, removing a

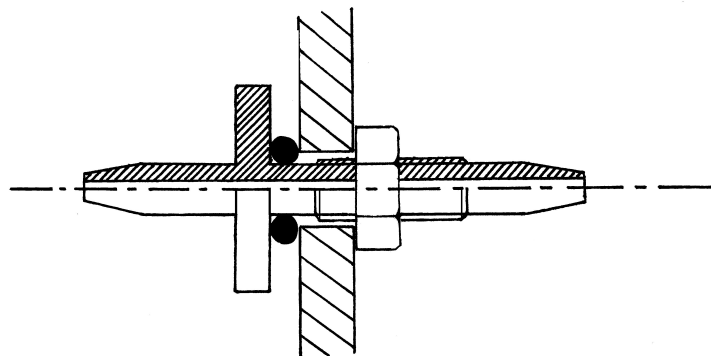


Figure 4.5: Technical drawing of the tube connectors fixation system (cross-sectional view).

tube from one side or another of the tube connector is safe and will not result in a disassembly of the system. Respectively, the collar of the tube connector and the nut serve as bearing surfaces when a tube is removed either at the outside or the inside of the bioreactor chamber.



## Design of the connection between the electrodes and the pulse generator

In order to produce the electrical stimulation, the already implemented pulse generator will be used. As an increase/decrease of voltage amplitude can compensate an inter-electrode distance increase/decrease, the distance between both electrodes will be fixed in the bioreactor. The connection system between the electrodes and the pulse generator involves a fastening component with an integrated nut (element 2 in the figure 4.6). The electrode (element 1 in the figure 4.6) is composed of a metallic plate fixed to a threaded pin. The pin is composed of a collar on which an o-ring seal can be placed. The fastening component with an integrated nut is used to tighten and seal the electrode-bioreactor wall assembly. In order to create the electrical contact between the pulse generator and the electrode, a banana plug receiver can be screwed in the fastening component until its metallic tip touches the electrode pin. Then, plugging the banana plug in its receiver will ensure the connection.

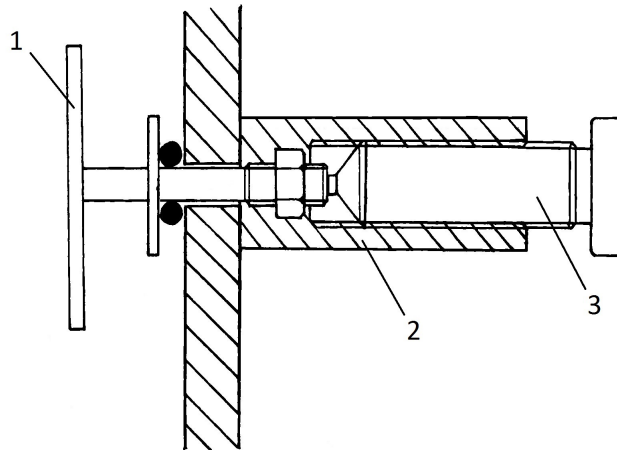


Figure 4.6: Technical scheme of the connection system between the electrodes and the signal generator (cross-sectional view). 1. Electrode; 2. Fastening component with integrated nut ; 3. Banana plug receiver.

## Sizing of the bioreactor main dimensions

As a visual monitoring and an easy access is required on the top of the device, the electrodes will be placed perpendicularly to the lid of the bioreactor as the graft will be oriented parallel to the lid plan. In this way, the larger surface of the graft can be observed through the polycarbonate lid.

The bioreactor has to contain the entire graft, namely, the biological tissues, the catheter hub and the 3-way connector. Also, the graft will be stretched of maximum 8mm. A security margin has been taken on the total length of the bioreactor in order for the surgeon to be able to manipulate the tubes and to perform the fixation of the 3-way connector to a stainless-steel tube connector. The total inner length of the bioreactor is therefore fixed to 210mm. The inner bioreactor width takes into account the width of the graft and the electrodes dimensions, and is fixed to 70mm. The height has also been fixed to 70mm ensuring a minimum but sufficient volume of culture medium and air for proper recellularization conditions.

## 4.2 Prototyping and assembly of the stimulation bioreactor

The assembly of the final bioreactor presents three sides, the first one is the prototyping and assembly of the mechanical parts of the bioreactor chamber. The second is the implementation of the actuator and sensor control system in order to achieve the four operation modes. Finally, a complete assembly of the stimulation bioreactor is proposed.

### Bioreactor chamber prototyping and assembly

Based on the design of the mechanisms mentioned in the previous section, as well as the lessons learned from the V1 bioreactor and the portative electrode support issues, we could prototype and assemble the whole bioreactor chamber with electrical and mechanical stimulation integrated. The figure 4.7 shows

an overview of the bioreactor assembly. The bioreactor walls have been 3D printed in nylon as the lid has been machined in polycarbonate. A 2D plan view of the bioreactor lid is available in the appendix J. The large seal on which the lid is placed is a flat seal which has been molded in silicon due to its non-standard dimensions. The used silicone has been autoclaved five times and presented no damages. Each bioreactor part will be detailed in order to deeply understand its functioning. Additional global and sectional views are available in the appendix I.

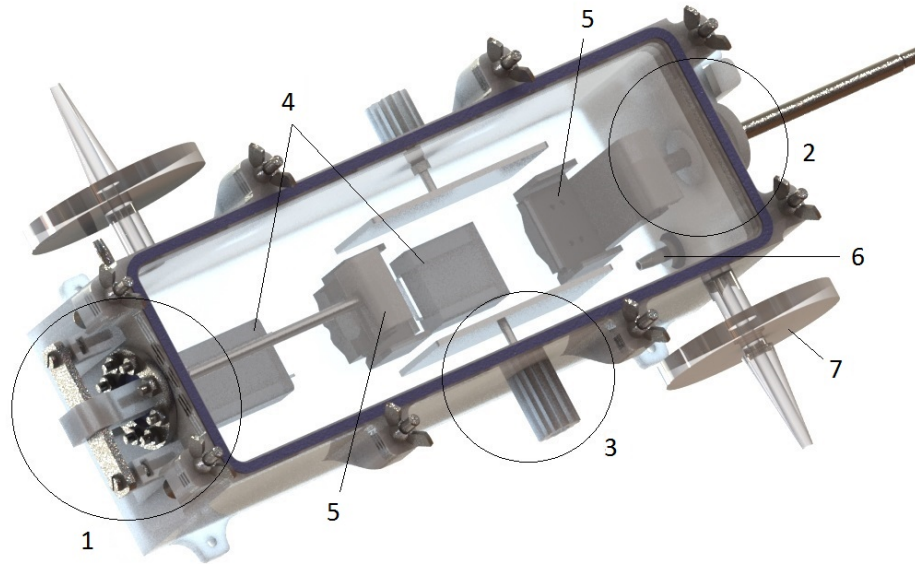


Figure 4.7: Overview of the bioreactor chamber with mechanical and electrical stimulation included. 1. Pressure sensor mechanism and sealing; 2. Motor shaft sealing mechanism; 3. Connection mechanism between the electrode and the pulse generator; 4. Graft support; 5. Graft attachment system; 6. Tube connector; 7. Air vent.

### 1. Pressure sensor mechanism and sealing

As explained in the design section, a deformable membrane is used to ensure the bioreactor sealing while avoiding any friction force when using the pressure sensor. Figures 4.8 and 4.9 show respectively the external and the internal part of the bioreactor at the location of the flexible membrane. The membrane, casting in silicone, is placed on the external surface of the bioreactor. A flat M18 washer, drilled with eight holes for M3 screws and nuts allows the membrane external border compression (see appendix J for a 2D plan view of the drilled washer). The shaft at which one graft extremity is attached, passes through the membrane and is fixed to the latter using, at the inner side, a washer and nut, and at the external side, a plastic threaded piece, 3D printed in nylon. The tightening of the external plastic piece and the nut ensure the sealing of this part of the bioreactor chamber. A flat metallic piece, made of stainless-steel, is slid through the nylon piece fixed to the shaft. This flat metallic piece is in fact rigidly fixed to the bioreactor frame and represents a flat and fix contact surface for the pressure sensor. The sensor is, as explained in the design section, placed between the parts 3 and 4 of the figure 4.8. The part 3, namely the nylon piece fixed to the shaft, is laid on the pressure sensor in such a way that the sensor senses a zero force when no load is applied to the shaft, but a positive pressure force when the graft is stretched or contracts. This part of the bioreactor chamber is therefore static, only micro-movements from the sensor deformations during graft stretching or contractions can be noted.

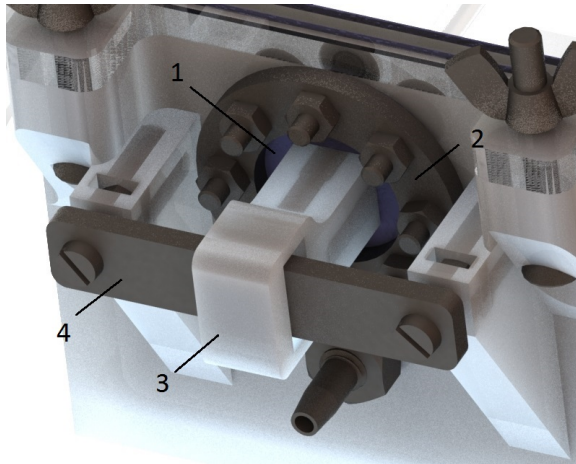


Figure 4.8: Sensor sealing mechanism (bioreactor chamber outside view). 1. Flexible membrane; 2. Drilled washer; 3. Nylon threaded piece; 4. Flat metallic piece.

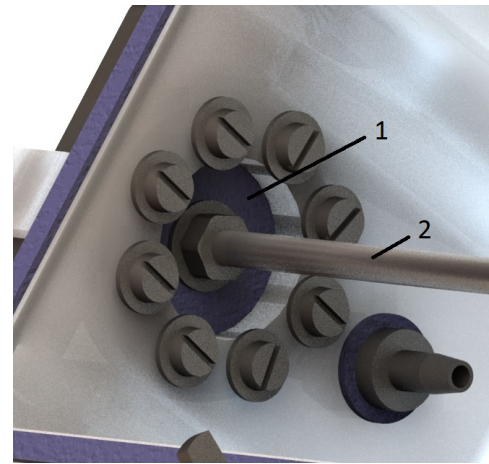


Figure 4.9: Sensor sealing mechanism (bioreactor chamber inside view). 1. Flexible membrane; 2. Shaft.

In order to set the pressure induced by the nylon threaded piece (piece 1 on the figure 4.10) on the sensor to zero, the flat metallic piece is made adjustable. Two M3 nuts are integrated to the bioreactor frame (piece 5 on the figure 4.10). The flat metallic piece is screwed in these nuts. The fact that the screws are more or less screwed allows a pressure adjustment on the sensor. The flat metallic piece is then clamped using two other M3 nuts screwed against the flat piece (piece 4 on the figure 4.10).

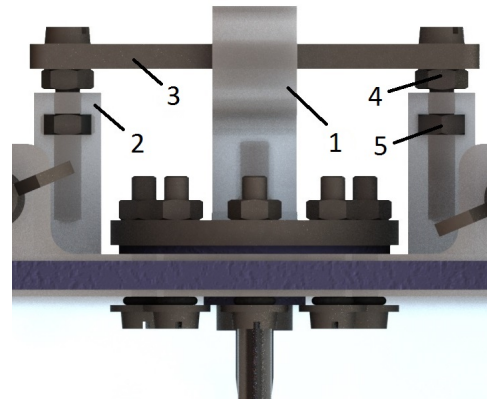


Figure 4.10: Top view of the sensor parts of the bioreactor. 1. Nylon threaded piece; 2. Bioreactor frame; 3. Flat metallic piece; 4. Clamping nut; 5. Adjustment nut.

## 2. Motor shaft sealing mechanism

The motor shaft is in fact a long screw. The screw head is slid through a nylon piece on which the graft end that will be stretched during the mechanical stimulation is fixed (part 4 on the figure 4.12). The sealing of the motor shaft is ensured by using a tight sheath fixed on the shaft at one side and to the bioreactor frame at the other side. In fact, one finger from a surgeon glove is used as tight sheath. The long screw is placed in the finger glove and is then slid into the graft attachment piece placed inside the bioreactor chamber (see figure 4.12). The cut extremity of the finger glove is then put on a conical nylon part at the outside of the bioreactor in which the shaft moves linearly during the mechanical stimulation (part 1 on the figure 4.11). The tightening of the conical part covered with the finger glove in the corresponding conical hole in the bioreactor wall allows the sealing of the assembly. A prismatic pin ensure the tightening of the assembly at the outside of the bioreactor chamber (see figure 4.11).

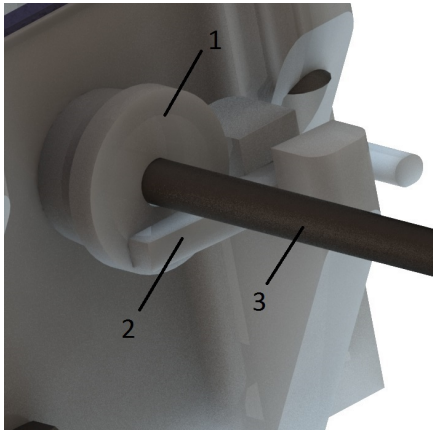


Figure 4.11: Motor shaft sealing mechanism (bioreactor chamber outside view). 1. Conical nylon piece; 2. Prismatic pin; 3. Motor shaft.

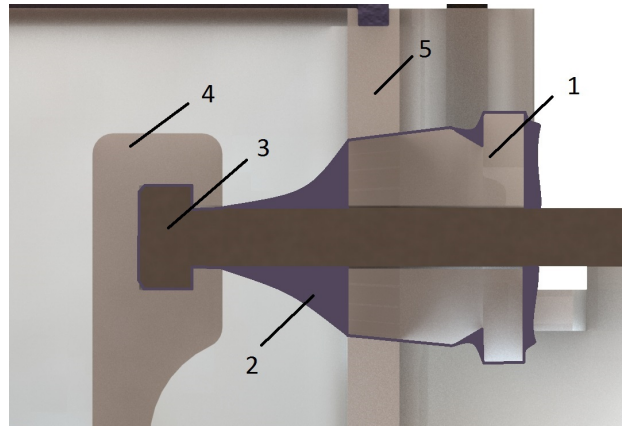


Figure 4.12: Motor shaft sealing mechanism (bioreactor lengthwise sectional view). 1. Conical nylon piece; 2. Tight sheath (Finger glove); 3. Head screw; 4. Graft attachment piece; 5. Bioreactor wall.

### 3. Connection mechanism between the electrode and the pulse generator

As the design of this bioreactor part has been detailed in the design section, only the materials and the used manufacturing techniques will be explained. The electrodes, 316L stainless steel plates of 2mm thickness, are welded to a stainless steel pin. This pin is composed of a collar on which an EPDM rubber o-ring is placed inside the bioreactor (see elements 2 and 3 on figure 4.13). A 2D plan view of the electrode pin is available in the appendix J. At the outside of the bioreactor, a nylon piece in which a nut has been included during its 3D printing allows the tightening and the sealing of the assembly. In order to create the electrical connection with the pulse generator via banana plugs, a banana plug receiver can be screwed inside the 3D printed clamping part until its metallic tip touches the end of the electrode pin. A thread is therefore manufactured in the banana plug receiver passage (part 5 of the figure 4.13).

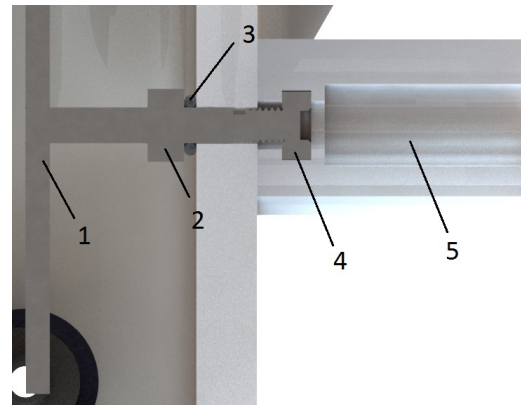


Figure 4.13: Electrode connection mechanism (bioreactor widthwise sectional view). 1. Electrode plate; 2. Tenon collar; 3. O-ring seal; 4. M3 Nut; 5. Banana plug receiver passage.

### 4. Graft supports

In order to avoid the graft subsidence during the electrical and mechanical stimulation, a nylon support has been 3D printed and fixed between both graft attachment system inside the bioreactor (parts 1, 2 and 3 on the figure 4.15). In order to ease the mechanical installation, the support can be clipped at the bottom of the bioreactor chamber. The support contains a dovetail groove complementary to the groove at the bottom of the bioreactor (part 2 on the figure 4.14). We use in fact the nylon flexibility in order to clip the two parts. The assembly is tighten due to the low backlash between both nylon pieces. A second nylon piece is used to support the tubing linked to the perfusion system of the graft (part 4 on the figure 4.15). Indeed, in order to avoid any stress or strain on the graft artery, the tubing needs to be placed in the same axis as the artery. The same fixating technique as for the other support is used.



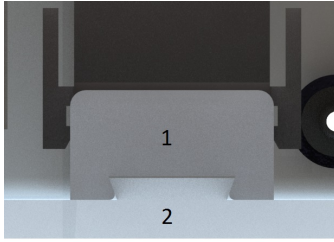


Figure 4.14: Graft support piece (bioreactor widthwise sectional view). 1. Graft support piece; 2. Bottom of the bioreactor.

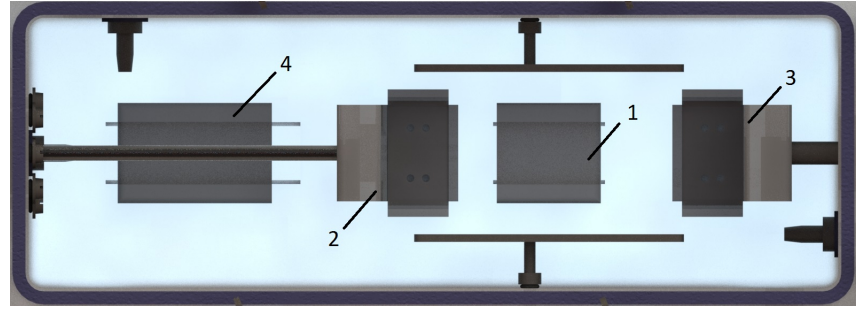


Figure 4.15: Top view of the bioreactor chamber. 1. Graft support; 2. Graft attachment system (sensor part); 3. Graft attachment system (motor part); 4. Tubing support.

## 5. Graft attachment system

The graft is attached at one end to the shaft that applies pressure on the sensor, and at the other end to the shaft linked to the linear actuator. In order to make the graft attachment easier, the surgeon can fix the graft to two removable graft attachment pieces depicted in the figure 4.16 using suture thread. This operation can be done at the outside of the bioreactor. Once the graft fixed at each end to the nylon holed pieces, these pieces can be clipped by pinching their fins on the intermediate piece (part 2 on the figure 4.17) that makes the link between the removable graft attachment piece and the shaft. Due to the flexibility of the membrane, a stainless-steel ball has been added at the bottom of the bioreactor in order to support the graft attachment system at the sensor side. Indeed, in order to avoid receiving skewed data from the sensor, the applied force on the latter only has to be oriented in the graft axis. The intermediate attachment piece at the sensor side is therefore composed of a triangular groove allowing two tangent support points with the ball (see figure 4.18).



Figure 4.16: Removable graft attachment piece made of nylon.

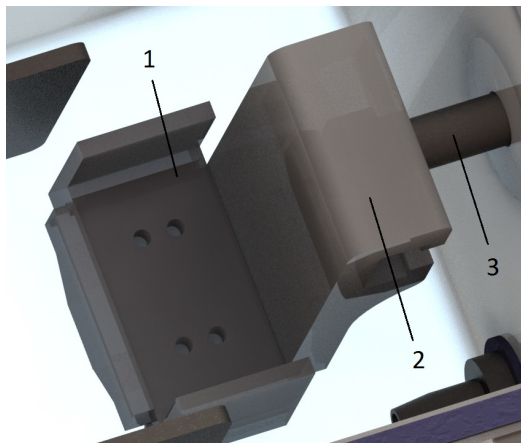


Figure 4.17: Zoom on the graft attachment system inside the bioreactor chamber. 1. Removable graft attachment piece; 2. Intermediate attachment piece; 3. Motor shaft.

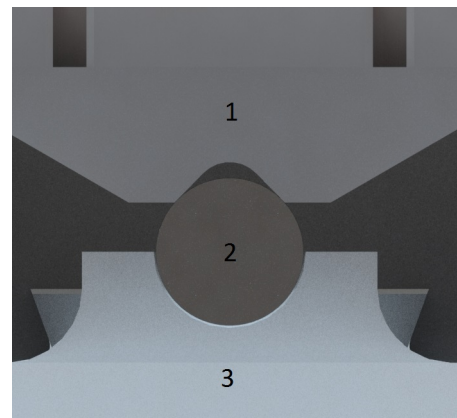


Figure 4.18: Support ball (bioreactor widthwise sectional view). 1. Intermediate attachment piece; 2. Ball; 3. Bottom of the bioreactor chamber.

## 6. Tube connector

The tube connectors made of stainless steel has been machined based on the design detailed in the previous section. A 2D plan view of these tube connectors is available in the appendix J. However, due to the smaller section of the part designed to allow the output of the threading tool, the o-ring joint originally used wasn't properly driven resulting in poor sealing. A flat seal, silicone molded, has therefore been used in order to ensure the sealing of the tube connectors. The bioreactor contains three tube connectors. One is placed in the axis of the graft artery, allowing the graft perfusion. The other two, opposite one another, allow a culture medium circulation when one of them is connected to the pump, or the drain of the bioreactor chamber.

## 7. Air vent

Air vents similar to the vents found on the V1 bioreactor (Millex Vent filter 50 mm) have been used in order to ensure gaseous exchange between the external medium in the inside of the bioreactor chamber. The simple screwing of the polypropylene vent in the bioreactor can ensure an air-tightness. Indeed, the threaded end of the vent is conical, allowing an adequate tightness when screwing the latter in the bioreactor chamber which is also made of a supple material. The threaded end of the vent is a 1/8 gas thread. Two air vents are used in the bioreactor, one at each end, in order to maximize the gaseous exchanges.

## Electrical and software implementation of the sensor and actuator control system

In order to achieve the four operation modes, namely:

- A position control of the actuator while measuring the induced traction force.
- A force control of the actuator while measuring its position.
- Performing electrical stimulation while measuring the actuator shaft position.
- Performing electrical stimulation while measuring the induced force due to the graft contraction.

a micro-controller needs to be programmed. The micro-controller used to control the sensor and the actuator is, as used in the pulse generator, an Arduino.

The main code of the Arduino is separated into four parts. The block diagram of the program is depicted on the figure 4.20. The first part is only executed once when the Arduino is turned on. In this part of the block diagram, all the local variables used in the program are allocated. The Arduino is linked to two screens and the sensor via I<sup>2</sup>C bus. A 2 lines, 16 characters LCD screen is used to display the force and position measurements at any time (see figure 4.19), and a 4 lines, 20 characters LCD screen is used to display the different selection mode parameters (see figure 4.21). The digital and analogue pins of the Arduino are configured as inputs or outputs. When the first part of the program is executed, it enters a continuous loop. The loop executes sequentially the three other parts of the program.



Figure 4.19: Display of the force and position on the 2x16 LCD screen.

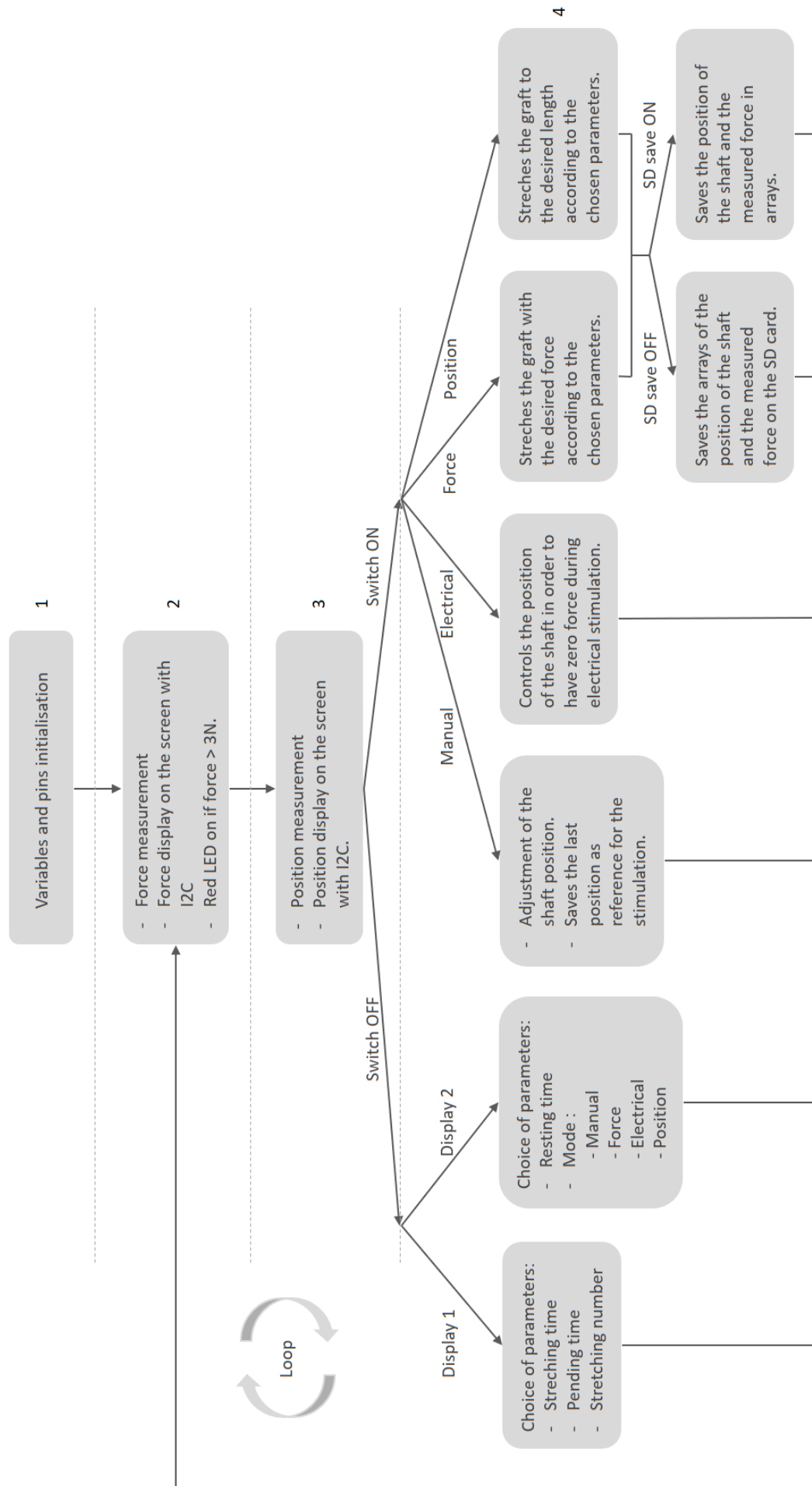


Figure 4.20: Bloc diagram of the implementation of the sensor and actuator control system.

The second part of the block diagram computes the force applied on the sensor. The sensor writes in its internal registers the measured force. On its part, the Arduino receives the data bytes by the I<sup>2</sup>C bus communication, and deducts a force expressed in Newtons. Again using the I<sup>2</sup>C bus, this force is sent to the screen as shown in the figure 4.19. The screen is refreshed with a new value of the force and length every 200ms. If the force measured by the sensor is greater than 3N, a red led lights up to warn the user he exceeded the sensor acceptable range. Indeed, exerting higher force on the sensor could disturb its calibration.

The third part of the program, schematized on the figure 4.20, measures the position of the actuator shaft. A feed-back of the actuator position is available and expressed by an analogue voltage between 0 and 3.3V. The position is therefore computed by mapping this range of voltage with the elongation range of the shaft (0-30mm). Using the I<sup>2</sup>C bus communication, the inferred position is sent to the screen as depicted on the figure 4.19.

The fourth part of the block diagram executes one of the six options presented at stage 4 on the figure 4.20. One option is selected depending on the status of the switch, the screen displays parameter (1 or 2) and the mode parameter. If the switch is OFF, the user can select the mode and the stimulation parameters. When the switch is turned ON, the system executes the mode chosen by the user (manual control, force control, position control or electrical control). It is important to note that these functioning modes are different from the operation modes previously introduced. The operation modes are conceptual while the functioning modes are actually implemented.

More precisely, when the switch is OFF, the user can choose several parameters and see its parameter choice on the 4x20 LCD screen. The screen is composed of two screen windows. The user can switch to one window or another using the display button. When the display parameter equals to 1, the user can set the first three parameters, namely:

- The duration of the graft stretch (Stretching time).
- The time between two stretches (Pending time).
- The number of stretches (Stretching number)

The figure 4.21 depicts the display of these three parameters. However, when the display parameter equal to 2, the user can set the three last parameters, namely:

- The time between two set of stretches (Resting time).
- The control mode of the actuator (Mode).
- The parameter linked the chosen mode.

The figure 4.22 allows a glimpse of the display of the last three parameters when using the force mode (the force control), and imposing a 0.05N force. As already mentioned, the program offers four functioning modes in order to control the actuator:

- The manual control and its parameter: the position of the shaft, which allows a manual control of the actuator position. The chosen position is in fact sent to the actuator via a PWM signal. As the chosen actuator (Firgelli linear actuator L12-50-6) is provided with an internal position controller, the simple sending of the PWM signal allows the control of the shaft position. By way of information, the PWM signal duty cycle (0-100%) determines the position (0-30mm).
- The position control and its parameter: the stretch length of the graft, which allows a mechanical stimulation by controlling the stretching. As for the manual control, the internal position controller



of the actuator handles the control of the shaft position during the stimulation. However, before launching the stimulation, the user has to move manually (using the manual control) the shaft until zero force is applied on the sensor. The position of the shaft is then registered as the reference position. During the mechanical stimulation, two position instructions are therefore sent alternatively to the actuator, namely, the reference position and the chosen stretch length, creating the desired mechanical stimulation.

- The force control and its parameter: the applied traction force on the graft, which allows a mechanical stimulation by controlling the force exerted on the graft. Before launching this stimulation, the user has to move manually (using the manual control) the shaft until zero force is applied on the sensor. The reached position will therefore be the reference position when relaxing the graft. The force controlled stretching is performed using a P-controller. In order to find the proportional term of this controller, we use the Hooke's law:  $F = Kx$ , with  $F$ , the tension force applied on the graft,  $x$ , the induced stretching distance and  $K$ , the stiffness of the graft. The stiffness is obtained by stretching the graft by a known force while measuring the induced stretching distance. The proportional term of the controller is therefore  $1/K$  since this factor will multiply the deduced force error (which is the difference between the target force and the measured one) in order to give back the shaft setpoint position at each time increment.
- The electrical control having no specific parameter, allows the acquisition of the shaft position during electrical stimulation, and therefore the quantification of the graft shortening due to its contraction. Again, before launching this stimulation, the user has to move the shaft manually until zero force is applied on the sensor. The reached position will be used as a reference position when relaxing the graft. In this mode, a zero-force control is applied to the actuator using the same P-controller as for the force control mode.

When the switch is ON, one of these four functioning mode is executed.

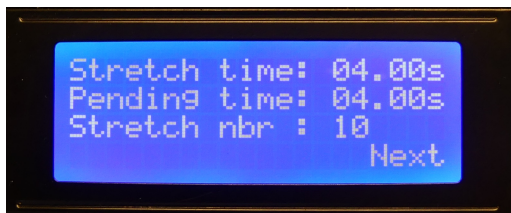


Figure 4.21: Display of first three parameters on the 4x20 LCD screen.

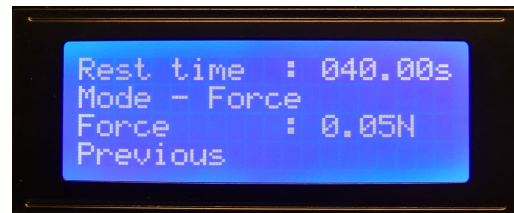


Figure 4.22: Display of the last three parameters on the 4x20 LCD screen.

The 2x16 LCD screen (see figure 4.19) displayed the force sensed by the sensor and the absolute position of the shaft at any time. The device also contained an SD card in order record the obtained data. More precisely, the data are saved in arrays during the stimulation, and copied on the SD card during the pause cycles. In this way, the stimulation is not disturbed by the data saving.

The last operation mode which performs electrical stimulation while measuring the induced force is realized by first, setting the shaft to a position inducing zero force on the sensor (using the manual control). As the measured force by the sensor is available at any time, the simple launch of the electrical stimulation as the shaft is fixed to the manually chosen position allows to obtain the data of interest.

As for the electrical device, all the electric boards have been gathered in a box for their safety (see figure 4.23). The box has also been equipped of the two screens, the stimulation launch switch and the rotating buttons. The box can't endure the autoclave and placement in the incubator. Therefore, we used two 2m cables in order to transmit signals to the pressure sensor and the actuator. These cables present a low capacitance in order to avoid signal disruptions. The cables are connected to the actuator and the sensor with

a connector allowing an easy connection as shown in the figure 4.24. The "M" cable has to be connected to the actuator, as the "S" cable has to be plugged into the sensor socket. The detail of the wire connections in the Arduino and the ranges of values of the different parameters can be found in the appendix G.



Figure 4.23: Mechanical stimulation control box.

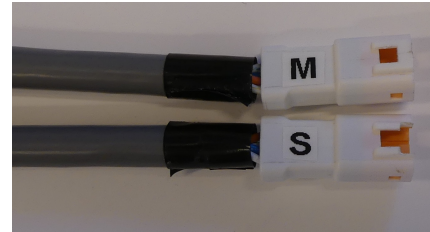


Figure 4.24: Actuator and sensor plugs.

In order to stimulate the graft mechanically, we use the Firgelli linear actuator L12-50-6I (available datasheet in the appendix K). This actuator has to be powered up with a 6V voltage. However, the Arduino can only supply voltages of 3.3V, 5V and 12V. In order to obtain a 6V power supply, a buck converter has been made. The buck reduces the 12 V voltage provided by the Arduino, and increases the current. This converter provides greater power efficiency than linear regulators, namely, a voltage divider. The figure 4.25 shows the electrical schematic of the hand-made buck converter.

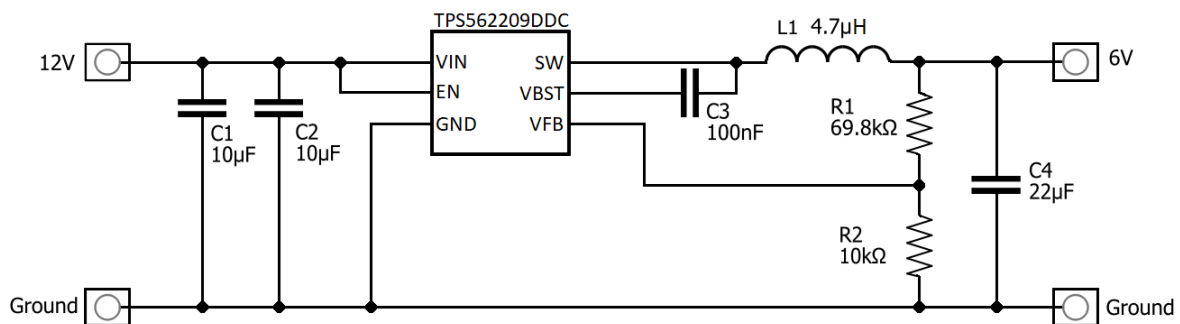


Figure 4.25: 12V to 6V buck-converter used to supply the actuator.

## Complete assembly of the bioreactor

The complete assembly of the bioreactor includes the bioreactor chamber, a pump, the mechanical stimulation control box, the pulse generator and a frame on which the sensor, the actuator and the bioreactor chamber can be fixed. All these components (except for both control box) will be placed in the INCO246med incubator allowing a control of the temperature, the humidity and the CO<sub>2</sub> rate. The complete assembly has been realized for the testing of the bioreactor as depicted on the figure 4.28.

More precisely, the actuator is squeezed between 3D printed nylon parts as depicted on the figure 4.26. The lower part can slide in the graft axis direction allowing an actuator position adjustment. This support slide piece can be fixed to the frame once the adjustment realized, by using two bolts. An upper clamping part is then screwed to the lower one tightening the actuator. An intermediate dive part is used to connect

the actuator shaft to long screw entering the bioreactor chamber (part 3 on the figure 4.26).

Regarding the sensor fixation, the small PCB in which is plugged the sensor is placed in the cavity of a support slide piece (part 1 on the figure 4.27). The PCB is then blocked by the screwing of a small blocking piece in the support part (part 2 on the figure 4.27). As for the actuator, the slide piece allows a sensor position adjustment. The adjustment is, in this case, linear (in the graft axis direction) and rotational.

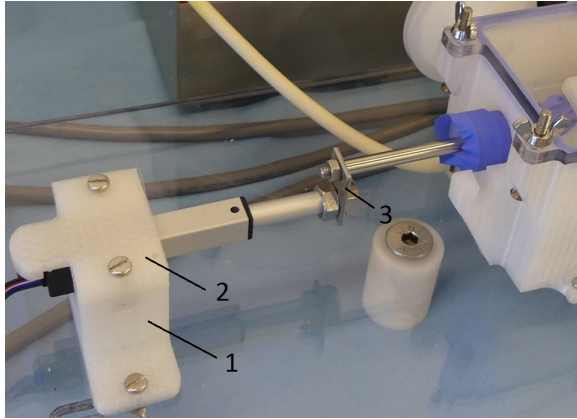


Figure 4.26: Actuator fixation system. 1. Support slide piece; 2. Clamping part; 3. Intermediate drive part.

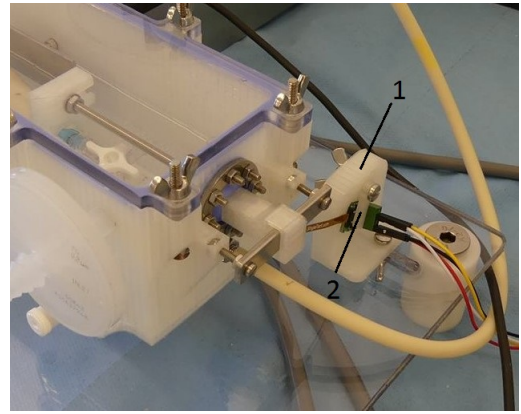


Figure 4.27: Sensor fixation system. 1. Support slide piece; 2. Blocking piece.

### 4.3 Testing of the stimulation bioreactor

In order to test the proper functioning of the bioreactor with electrical and mechanical stimulation included, a complete test bed was realized. The latter is depicted on the figure 4.28. The graft has been attached at both ends using suture threads on each removable attachment piece at the outside of the bioreactor chamber. The material used for this operation is shown on the figure 4.29. Both removable attachment pieces have then been fixed in the bioreactor chamber as depicted on the figure 4.30. The chamber has been filled with water, and the pump was used to create a medium circulation. The complete use protocol is available in the appendix G.

Regarding the sealing of the bioreactor chamber, we noticed a leak through the chamber wall. Indeed, the chamber walls have been 3D printed using nylon. We can see on the bioreactor walls that the printer missed one nylon layer. This layer is the cause of the noticed leak. Nevertheless, a complete air and water tightness test needs to be performed on the bioreactor in order to guarantee its sealing.



Figure 4.29: Graft and both removable graft attachment pieces.

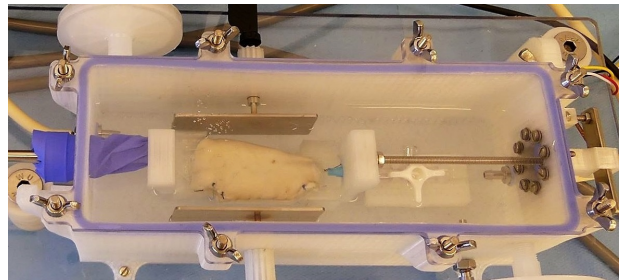


Figure 4.30: Glimpse of the top of the bioreactor chamber during mechanical stimulation.

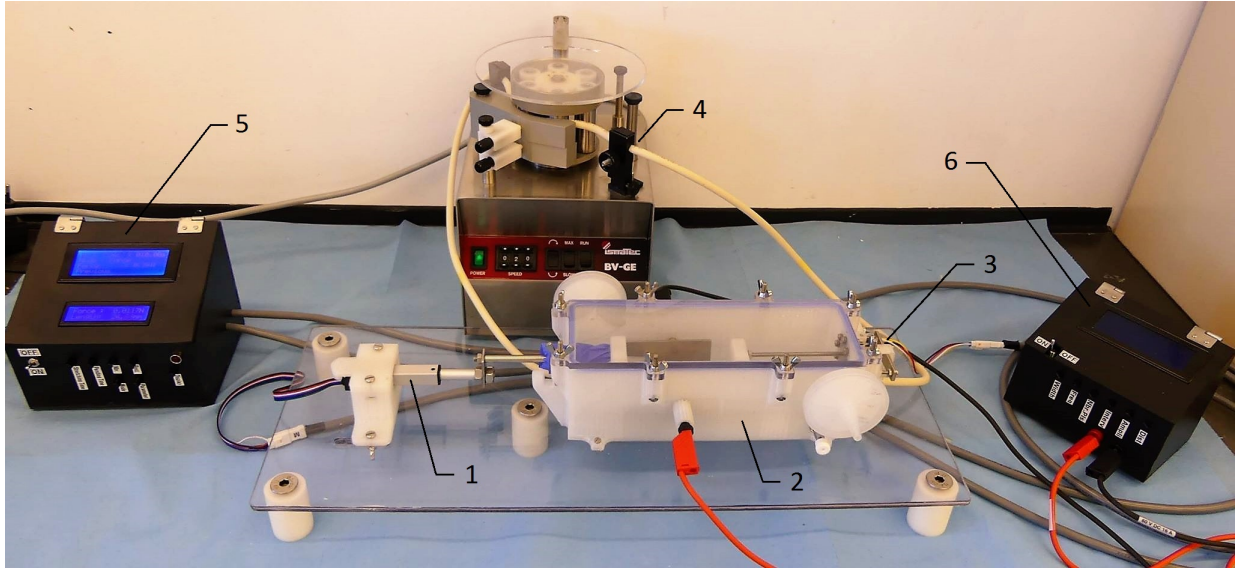


Figure 4.28: Test bench of the stimulation bioreactor. 1. Linear actuator; 2. Bioreactor chamber; 3. Pressure sensor; 4. Pump; 5. Mechanical stimulation control box; 6. Pulse generator.

The first operating mode, namely, the position control of the actuator has first been tested. As explained in the section 4.2 (Electrical and software implementation of the sensor and actuator control system), we begin with an initialization phase. During this stage, the shaft is positioned manually in such a way that the graft is at rest. This position is recorded as being the reference position. The stretching, position controlled, can therefore begin from that point. We imposed ten 12mm stretches, as depicted on the figure 4.31. The stretching we performed is much more important than the mentioned one in the specifications. In fact, the graft has been left in a conservation medium for a long time. Its mechanical properties changed since the first measurements were performed on the graft.

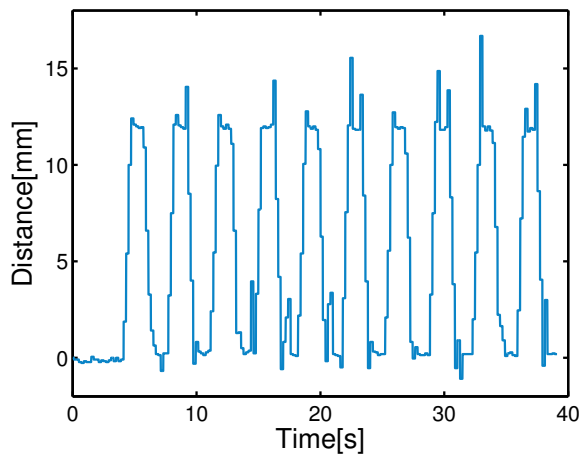


Figure 4.31: Stretching distance during mechanical stimulation (ten 12mm stretches).

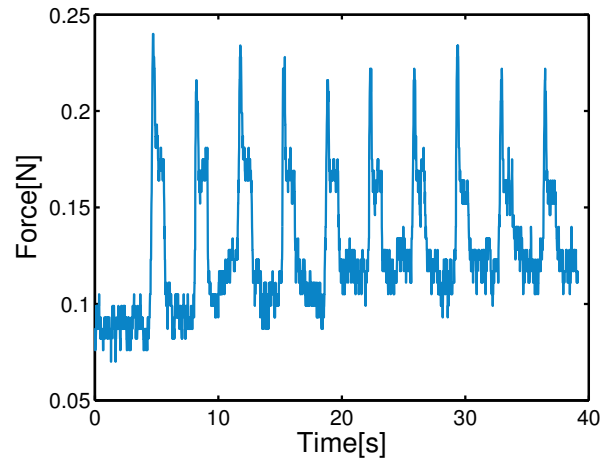


Figure 4.32: Tension force during mechanical stimulation (ten 12mm stretches).

The tension force sensed by the sensor has been recorded during the stimulation and plotted on the figure 4.32. On this force graph, we can notice we don't have zero force when the graft is a rest. Indeed, adjusting the system offload by positioning the flat metallic piece (see figure 4.10) to obtain exactly zero force is not easy. It is, however, possible to save this initial non-zero force and take it as a reference for the whole experiment. The forces obtained are situated in the range we initially defined, namely,  $[0 - 0.27\text{N}]$ . We can



notice on the figure 4.31 that the internal position controller of the actuator is not accurate. Indeed, we can perceive overshoots and oscillations once the actuator reaches a goal position. The parameters of this position controller can't be changed as these are internal to the actuator. The actuator we received is therefore damaged or poorly designed.

In order to test the force control of the actuator, we used the test bench pictured on the figure 4.33. As we don't wanted to damage the graft during our tests, we assumed the graft behaves as a spring, so we attached both sides of a spring to the intermediate graft attachment pieces (see figure 4.33). The used spring presents a stiffness of 0.14 N/mm. We therefore set the controller proportional term to 7.14 mm/N, as explained in the previous section.

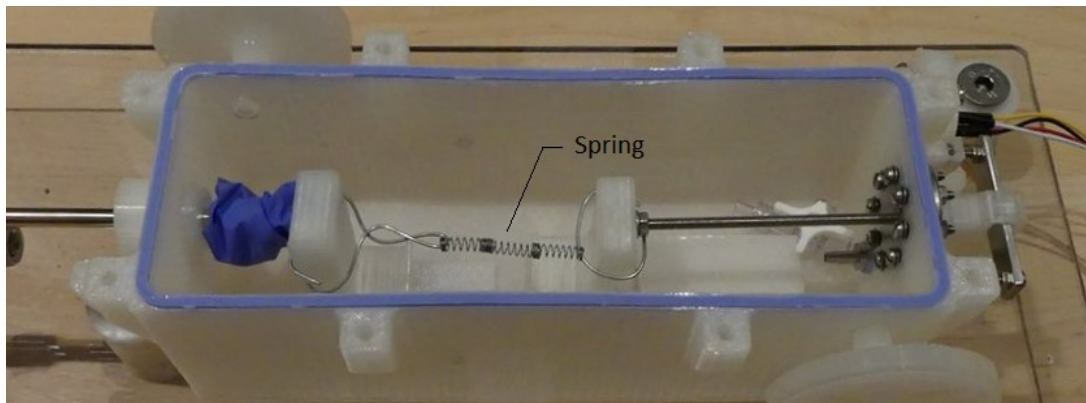


Figure 4.33: Test bench used to test the implemented force controller.

We launched the mechanical stimulation by imposing ten stretches with a 1.43N maximum tension force. The results of this stimulation are plotted on the figures 4.34 and 4.35. We clearly see that the system reaches its force limit of 1.43N during the entire stimulation set. Imposing a tension force of 1.43N to the spring corresponds to a 9 mm stretching on the figure 4.35. However, according to the Hooke's law, the stretching should reach 10 mm. This error can be explained by the fact that it is extremely difficult to tune manually the system in order to obtain zero force when the spring (or the graft) is at rest, and a small force increment as soon as the spring begin to be stretched. In this case, it seems that the spring was already a little bit stretched during the initialization phase.

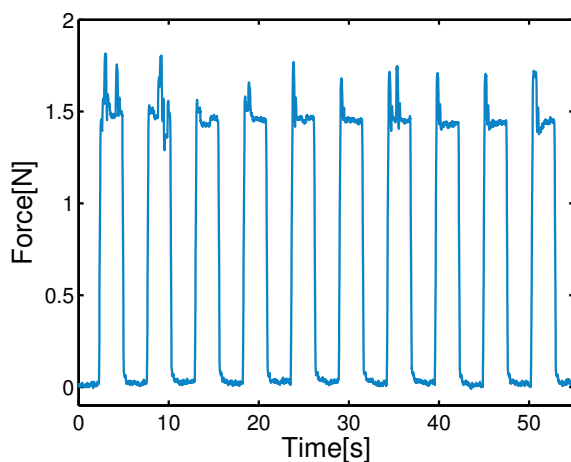


Figure 4.34: Tension force during mechanical stimulation (Maximum force of 1.43N).

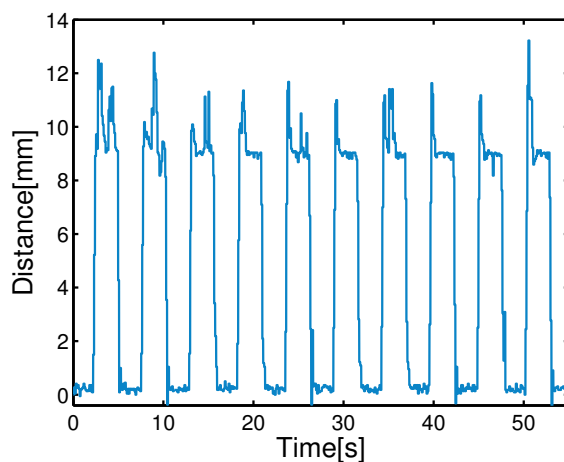


Figure 4.35: Stretching distance during mechanical stimulation (Maximum force of 1.43N).

Also, as depicted on both figures 4.34 and 4.35, significant oscillations are noticed once the actuator reaches a goal position. In fact, the actuator uses its poor position controller any time a position input is sent to it. As the position controller is of poor quality, a potential static error in its feedback loop could also explain the distance static error made by the force controller. The last two operation modes involving electrical stimulation couldn't be tested due to repetitive postponements of pork sacrifices. Nevertheless, with the already performed tests, we can deduce performing electrical stimulation while measuring the force can be achieved properly by the device. Indeed, the system used to execute this operation mode is exactly the same as for the first experiment, namely, a position control of the actuator while measuring the force. However, the zero force control used during the electrical stimulation in order to measure the shaft position needs its own test bench. The challenge will then be to check if the feedback loop is quick enough to ensure proper zero force control during the electrical stimulation. It is important to note that the electrical connection between the pulse generator and the bioreactor chamber has been tested and works as expected.

A bioreactor with electrical and mechanical stimulation included has been designed, assembled and tested. By confronting the final device with the initial specifications, we notice some additional experiments need to be performed in the operational conditions in order to fully characterize the stimulation bioreactor. Indeed, a full-sealing test on the 3D bioreactor chamber needs to be performed. Also, the force controller has to be tested on a graft. This controller has already been evaluated by using a spring with a stiffness of 0.14N/mm, but not using a half-lip for which the stiffness has been evaluated to 0.03N/mm. Finally, the electrical stimulation needs to be tested on a native muscle in order to check the good functioning of the zero force controller.

## **4.4 Avenues for Enhancing the Stimulation Bioreactor**

Based on the previous experiments, we can suggest some improvements regarding the assembled bioreactor. These improvements can be separated into two parts, first, the improvements concerning the prototyping and assembly of the bioreactor chamber, and second, the improvements regarding the electrical and software implementation of the actuator and sensor control system. As previously explained, some tests still need to be performed in order to assess all the bioreactor aspects. However, it is strongly recommended to, first, fix the few remaining problems by following the tracks listed below.

### **Improvements regarding the design, prototyping and assembly of the bioreactor chamber**

The major encountered problem is the leak presents at the bioreactor wall due to the bad printing of the piece. The bioreactor frame has been 3D printed in nylon using a fill factor of 50%, and a layer thickness of 0.2mm. In order to avoid future leaks, we suggest printing the frame with a fill factor of 100%, and a layer thickness of 0.1mm. Another solution could be to machine or mold it in a non-conductive, autoclavable and biocompatible material.

Regarding the assembly of the bioreactor, the long screw linked to the actuator shaft is difficult to tighten on the intermediate drive part (see figure 4.26). A solution could be to create two flattened portions on the long screw in order to insert a key and ease the clamping.

As explained in the design part of the chapter, the sensor shaft is fixed to the membrane using on one side a nut, and on the other, a nylon threaded piece (see figure 4.9). The tighten of this nut is quite difficult due to the narrow space of this specific bioreactor part. A solution could be to replace the nut by a long threaded spacer allowing the tightening of the assembly a bit further on the shaft, where the space allows a hand tightening.

During the experiments realized using the mechanical stimulation, we noticed that the graft was caught

between the intermediate attachment piece and the graft support at the actuator side of the bioreactor (see figure 4.36). A solution could be to remove the graft support, and design a new intermediate attachment piece acting like a support as well.

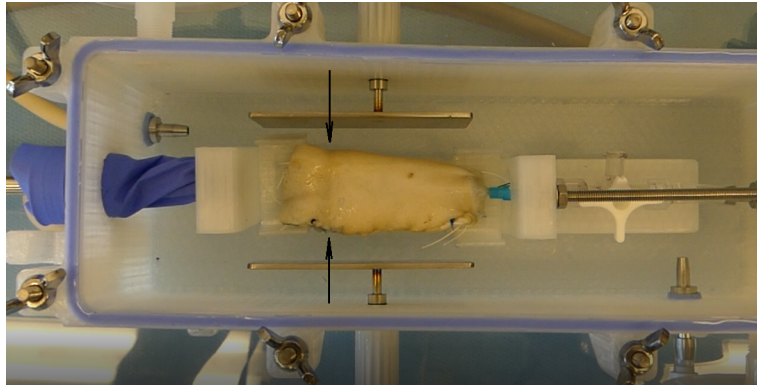


Figure 4.36: Top view of the bioreactor chamber: both arrows indicate the graft entrapment.

As observed in the testing part of this chapter, the actuator is either damaged or poorly designed. We suggest replacing it with an equivalent system of better quality.

### **Improvements regarding the electrical and software implementation of the actuator and sensor control system**

An issue regarding the software implementation is the precision of the sent and received signals by the Arduino. For example, the position of the actuator is sent using a PWM signal. The duty cycle of this signal is mapped with the actual elongation of the shaft. The output pins of the Arduino generating the PWM signals, works on 8 bits. 8 bits only allow to send 256 different PWM signal values. Therefore, with a shaft allowing a maximum elongation of 30mm, the actuator can only move by steps of 0,117mm. The same problem occurs with the reading of the shaft position. The solution is to extend the bit number of the Arduino output pins. An specific order sent to the Arduino should solve the problem.

At this stage, the electrical and mechanical stimulations don't work together. The electrical mode only ensures a zero force control of the actuator, but doesn't contract the graft. In order to make both stimulations complementary, a fifth mode should be created. Cables should link both control boxes (the pulse generator and the mechanical control box) and an I<sup>2</sup>C bus should be used in order to create a communication between both boxes. The mechanical control box could be set as the master, as the electrical control box could behave as a slave. The user could fix the parameters on both control boxes. During the resting time, the master Arduino could be able to send signals to the slave. The slave will therefore generate the pulses according to the chosen parameters.

As for now, the proportional term of the controller is fixed to a specific value corresponding to a specific graft. However, this term may vary according to the stimulated graft. This parameter could be automatically computed each time a new graft is stimulated. In fact, at the beginning of the force controlled stimulation, an initial phase could use the position controller to stretch the graft at a known distance and measure the force applied on the sensor. Using the Hooke's law, the stiffness of the graft could therefore be calculated, as well as the proportional term of the controller. A proper force control, corresponding to the used graft, could therefore be achieved by the system.

The last important improvement regarding the software implementation is the automation of the initialization phase. At the present time, the tuning of the shaft initial position before stimulation is done manually,



leading to poor stretching distance results when using the force controller. Obtaining zero force when the graft is at rest, and a small force increment as soon as the graft begins to be stretched can be automated. The shaft could be fully deployed at the begin of the phase, and could retract slowly until reaching a very small force increment (0.01N for example). To do so, the sensor needs to be calibrated before each experiment.

Through this chapter, the main design and manufacturing method choices as well as the electrical and software architecture of the stimulation bioreactor have been detailed. The steps followed throughout the bioreactor design have been determined according to the limiting degree of the specifications. Experiments have been realized on the final device, and showed defects linked to the used components and the manufacturing techniques. Finally, some ways for improving the assembled device are proposed in order for the designed bioreactor to reach the initial specifications.

# Conclusion

In the present work, a complete bioreactor with mechanical and electrical stimulation included has been designed, assembled and tested. To do so, scientific papers about mechanical and electrical stimulation have been dissected, and the V1 bioreactor designed by Alexia Springuel [3] has been studied. The efficient parts of the V1 bioreactor inspired us in the design of our final device, as the parts presenting failures have been redesigned.

An electrical equivalent model of the used graft, namely a half-lip, has then been proposed in order to confront the model analysis conclusions with the results obtained in several scientific papers. Based on these both sources of information and on an experiment realized on a piglet graft (containing a native muscle), we could fix plausible electrical stimulation parameter ranges.

A first electrical stimulation prototype was therefore implemented and tested. To do so, a complete test bench was built and the electrical stimulation device was tested on pork grafts (containing a native muscle) and on a recellularized human half-lip. From these experiments, we could prove the good functioning of our apparatus, but we could notice our contractions detection system wasn't adapted for recellularized grafts. The test results also suggest the relevance of a mechanical stimulation during the recellularization step.

Based on the state of the art, the V1 bioreactor study and on the electrical stimulation prototype, we could design, assemble and test a complete perfusion bioreactor with mechanical and electrical stimulation included. All elements in contact with the graft are autoclavable and the complete device can be placed in an incubator for complete recellularization experiments. The stimulation bioreactor has four operation modes including a graft stretching control and a tension force control.

As for the electrical stimulation prototype, a test bench was build in order to test the final stimulation bioreactor. From the experiment results, we could notice a leak in the bioreactor chamber frame. Also, it has emerged that the used actuator presents some manufacturing defects leading to a poor position control of the shaft allowing the stretching and relaxation of the graft. However, some tracks have been proposed in the last chapter in order fix these issues and enhance the global functioning of the device.

More interestingly, the experiments realized on the final perfusion bioreactor allowed us to perceive the next steps in the achievement of facial composite tissues recellularization. Using the built stimulation bioreactor, a series of experiments can be conducted on half-lips in order to find the perfect physical and physiological conditions leading to a native-like graft. A complete recellularization could be performed by only using mechanical stimulation. The results could be compared to recellularized grafts having endured either only electrical stimulation or electrical and mechanical combined stimulation. Assuming optimal stimulation parameters can be found, a new device handling the recellularization under stimulation of several facial sub-units could be created.

The built apparatus as well as the V1 bioreactor of Alexia Springuel allow medical researchers to investigate the cell-on-scaffold-seeding technology on a larger scale and on complex tissues which are facial composite tissues. The present work can therefore allow the researchers to progress one step further towards the ultimate objective being a full face transplantation without the need of extensive postoperative treatments.

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## Appendix A

# Specifications: Portable electrical stimulation

|                                                                                                                                              |         |                                                                                                                                                          |                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                       |         | <b>Specifications of a portable electrical stimulation device</b>                                                                                        | Date : 02/03/2017<br>Version : 1.1 |
| <b>Context:</b> Jérôme Duisit required the design of a portable electrical stimulation device in order to stimulate different kind of graft. |         |                                                                                                                                                          |                                    |
| Date                                                                                                                                         | Source  | <b>Functions</b>                                                                                                                                         |                                    |
| 07/10/16                                                                                                                                     | J.D     | F1 : Stimulate the graft electrically.                                                                                                                   |                                    |
| 10/02/17                                                                                                                                     | B.H     | F2 : Set stimulation parameters.                                                                                                                         |                                    |
| 10/02/17                                                                                                                                     | B.H     | F3 : Be easily manipulated.                                                                                                                              |                                    |
| 10/02/17                                                                                                                                     | B.H     | F4 : Visualize the selected parameters.                                                                                                                  |                                    |
|                                                                                                                                              |         | <b>Functional Requirement (FR) criteria and levels</b>                                                                                                   |                                    |
| 16/11/16                                                                                                                                     | J.D     | FR 1.1 : Stimulate the culture medium.                                                                                                                   |                                    |
| 16/11/16                                                                                                                                     | C.F-J.L | FR 1.2 : The electric field has to be perpendicular to the graft muscular fibres.                                                                        |                                    |
| 08/02/17                                                                                                                                     | C.F-J.L | FR 1.3 : With a range of frequency : 0.5-10Hz.                                                                                                           |                                    |
| 02/03/17                                                                                                                                     | C.F-J.L | FR 1.4 : With a range of amplitude: 0-7V.                                                                                                                |                                    |
| 02/03/17                                                                                                                                     | C.F-J.L | FR 1.5 : With a range of peak duration: 1-20ms.                                                                                                          |                                    |
| 02/03/17                                                                                                                                     | C.F-J.L | FR 1.6 : At a distance of 5 to 20 mm from the graft.                                                                                                     |                                    |
| 02/03/17                                                                                                                                     | B.H     | FR 1.7 : With a maximum intensity of 200 mA.                                                                                                             |                                    |
| 10/02/17                                                                                                                                     | C.F-J.L | FR 2.1 : Set the frequency, the amplitude, the peak duration, the duration of a cycle and duration between two stimulation cycles.                       |                                    |
| 11/02/17                                                                                                                                     | J.D     | FR 2.2 : The interface used to set the stimulation parameters has to be an integral part of the device (no computer plug to set the variables).          |                                    |
| 10/02/17                                                                                                                                     | B.H     | FR 3.1 : Manipulation of the stimulation system part in contact with the culture medium using one hand.                                                  |                                    |
| 11/02/17                                                                                                                                     | J.D     | FR 4.1 : The interface used to visualize the selected parameters has to be an integral part of the device (no computer plug to visualize the variables). |                                    |
|                                                                                                                                              |         | <b>Constraints</b>                                                                                                                                       |                                    |
| 07/10/16                                                                                                                                     | J.D     | C1 : Parts in contact with the graft and the culture medium.                                                                                             |                                    |

|          |         |                                                                                                                                                                                                                                                              |
|----------|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 08/02/17 | J.D     | C2 : Sterility.                                                                                                                                                                                                                                              |
| 02/03/17 | C.F-J.L | C3 : Graft safety.                                                                                                                                                                                                                                           |
| 07/10/16 | J.D     | <b>Constraint Requirement (CR) criteria and levels</b><br>CR 1.1 : The parts in contact with the graft and the culture medium have to be placed in the INCO246med incubator of 640 x 640 x 600 mm and resist the incubator physical and chemical conditions. |
| 08/02/17 | J.D     | CR 2.1 : The parts immerse in the culture medium have to be sterilizable using autoclave.                                                                                                                                                                    |
| 02/03/17 | C.F-J.L | CR 3.1 : No net charge accumulated in the graft.                                                                                                                                                                                                             |
| 02/03/17 | C.F-J.L | CR 3.2 : No medium composition alteration during the electrical stimulation.                                                                                                                                                                                 |

## Appendix B

# Specifications: Bioreator with electrical stimulation

|                                                                                                                                                                                                                                        |         |                                                                                                  |                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                                 |         | <b>Specifications of an electrical stimulation device</b>                                        | Date : 17/11/2016<br>Version : 1.1 |
| <b>Context:</b> In order to familiarize ourselves with the grafts and the electrical stimulation, as well as to obtain some preliminary results, we would like to design an electrical stimulation device using some existing devices. |         |                                                                                                  |                                    |
| Date                                                                                                                                                                                                                                   | Source  | <b>Functions</b>                                                                                 |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | F1 : Stimulate the graft electrically.                                                           |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | F2 : Maintain the graft in the liquid culture.                                                   |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | F3 : Measure the graft behaviour under stimulation.                                              |                                    |
| 16/11/16                                                                                                                                                                                                                               | C.F-J.L | <b>Functional Requirement (FR) criteria and levels</b>                                           |                                    |
| 03/11/16                                                                                                                                                                                                                               | C.F-J.L | FR 1.1 : Stimulate the culture medium.                                                           |                                    |
| 03/11/16                                                                                                                                                                                                                               | C.F-J.L | FR 2.1 : Maintain the graft by two points.                                                       |                                    |
| 03/11/16                                                                                                                                                                                                                               | C.F-J.L | FR 3.1 : Measure of the contraction distance.                                                    |                                    |
| 16/11/16                                                                                                                                                                                                                               | C.F-J.L | FR 3.2 : Check if the graft remains of sufficient quality.                                       |                                    |
| 7/10/16                                                                                                                                                                                                                                | J.D     | <b>Constraints</b>                                                                               |                                    |
| 7/10/16                                                                                                                                                                                                                                | J.D     | C1 : Graft attachment.                                                                           |                                    |
| 7/10/16                                                                                                                                                                                                                                | J.D     | C2 : Size of the graft.                                                                          |                                    |
| 7/10/16                                                                                                                                                                                                                                | J.D     | C3 : Size of the whole device.                                                                   |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | <b>Constraint Requirement (CR) criteria and levels</b>                                           |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | CR 1.1 : Minimize cellular damage.                                                               |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | CR 2.1 : Graft measurements are depicted in Appendix 1.                                          |                                    |
| 07/10/16                                                                                                                                                                                                                               | J.D     | CR 3.1 : The whole device has to be placed in the INCO246med incubator of<br>640 x 640 x 600 mm. |                                    |

## Appendix 1 :



Figure B.1: Graft measurements.

|                                                                                                                                                                                                                         |         |                                                                                                                                         |                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                  |         | <b>Specifications of an electrical stimulation device</b>                                                                               | Date : 08/02/2017<br>Version : 2.1 |
| <b>Context:</b> It was asked us to design an electrical stimulation device in order to stimulate a half-lip. The electrical stimulation will be used punctually in order to test some physical properties of the graft. |         |                                                                                                                                         |                                    |
| Date                                                                                                                                                                                                                    | Source  | <b>Functions</b>                                                                                                                        |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | F1 : Stimulate the graft electrically.                                                                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | F2 : Maintain the graft in the liquid culture.                                                                                          |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | F3 : Measure the graft behaviour.                                                                                                       |                                    |
|                                                                                                                                                                                                                         |         | <b>Functional Requirement (FR) criteria and levels</b>                                                                                  |                                    |
| 16/11/16                                                                                                                                                                                                                | J.D     | FR 1.1 : Stimulate the culture medium.                                                                                                  |                                    |
| 16/11/16                                                                                                                                                                                                                | C.F-J.L | FR 1.2 : The electric field has to be perpendicular to the graft muscular fibres.                                                       |                                    |
| 08/02/17                                                                                                                                                                                                                | C.F-J.L | FR 1.3 : With a range of frequency : 0.5-10Hz.                                                                                          |                                    |
| 08/02/17                                                                                                                                                                                                                | C.F-J.L | FR 1.4 : With a range of amplitude: 0-10.5V.                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                                | C.F-J.L | FR 1.5 : With a range of peak duration: 0.1-10ms.                                                                                       |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | FR 2.1 : Maintain the graft against gravity in order to avoid deflection.                                                               |                                    |
| 03/11/16                                                                                                                                                                                                                | C.F-J.L | FR 3.1 : Measure the tensile force during contraction.                                                                                  |                                    |
| 16/11/16                                                                                                                                                                                                                | J.D     | FR 3.2 : Check if the graft remains of sufficient quality.                                                                              |                                    |
|                                                                                                                                                                                                                         |         | <b>Constraints</b>                                                                                                                      |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | C1 : Graft attachment.                                                                                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | C2 : Size of the graft.                                                                                                                 |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | C3 : Size of parts in contact with the graft and the culture medium.                                                                    |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | C4 : Sterile parts                                                                                                                      |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | C5 : Visibility of the graft.                                                                                                           |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | C6 : Access to the graft.                                                                                                               |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | C7 : Culture medium circulation.                                                                                                        |                                    |
| 08/02/17                                                                                                                                                                                                                | C.F-J.L | C8 : Chemical reactions.                                                                                                                |                                    |
| 21/12/16                                                                                                                                                                                                                | B.H     | C9 : Mechanical stimulation system.                                                                                                     |                                    |
|                                                                                                                                                                                                                         |         | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                  |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 1.1 : Using stitches                                                                                                                 |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 2.1 : Graft measurements are based on two scientific articles (see [44],[62])                                                        |                                    |
| 07/10/16                                                                                                                                                                                                                | J.D     | CR 3.1 : The parts in contact with the graft and the culture medium have to                                                             |                                    |
|                                                                                                                                                                                                                         |         | be placed in the INCO246med incubator of 640 x 640 x 600 mm.                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 4.1 : The parts immerse in the culture medium have to be sterile.                                                                    |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 5.1 : Only the graft part that shows the elongation and the relaxation needs to be visible and potentially be recorded via a camera. |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 6.1 : An access to the graft is needed at the top of the device.                                                                     |                                    |
| 08/02/17                                                                                                                                                                                                                | J.D     | CR 7.1 : An external tank linked to the device is needed.                                                                               |                                    |
| 08/02/17                                                                                                                                                                                                                | C.F-J.L | CR 8.1 : Avoid electrolysis.                                                                                                            |                                    |
| 21/12/16                                                                                                                                                                                                                | B.H     | CR 9.1 : The electrical stimulation system have to coexist with the mechanical                                                          |                                    |



|  |  |                     |
|--|--|---------------------|
|  |  | stimulation system. |
|--|--|---------------------|

|                                                                                                                                                                                                                                    |         |                                                                                                                                                                                                   |                                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                             |         | <b>Specifications of an electrical stimulation device</b>                                                                                                                                         | Date : 10/02/2017<br>Version : 2.3 |
| <b>Context:</b> Jérôme Duisit required the design of an electrical stimulation device in order to stimulate a half-lip. The electrical stimulation will be used punctually in order to test some physical properties of the graft. |         |                                                                                                                                                                                                   |                                    |
| Date                                                                                                                                                                                                                               | Source  | <b>Functions</b>                                                                                                                                                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                           | J.D     | F1 : Stimulate the graft electrically.                                                                                                                                                            |                                    |
| 10/02/17                                                                                                                                                                                                                           | B.H     | F2 : Set stimulation parameters.                                                                                                                                                                  |                                    |
| 10/02/17                                                                                                                                                                                                                           | B.H     | F3 : Visualize selected parameters.                                                                                                                                                               |                                    |
| 16/11/16                                                                                                                                                                                                                           | J.D     | <b>Functional Requirement (FR) criteria and levels</b>                                                                                                                                            |                                    |
| 16/11/16                                                                                                                                                                                                                           | C.F-J.L | FR 1.1 : Stimulate the culture medium.                                                                                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | FR 1.2 : The electric field has to be perpendicular to the graft muscular fibres.                                                                                                                 |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | FR 1.3 : With a range of frequency : 0.5-10Hz.                                                                                                                                                    |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | FR 1.4 : With a range of amplitude: 0-10.5V.                                                                                                                                                      |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | FR 1.5 : With a range of peak duration: 0.1-10ms.                                                                                                                                                 |                                    |
| 10/02/17                                                                                                                                                                                                                           | B.H     | FR 1.7 : With a maximum intensity of 20 mA.                                                                                                                                                       |                                    |
| 10/02/17                                                                                                                                                                                                                           | C.F-J.L | FR 2.1 : Set the frequency, the amplitude, the peak duration, the duration of a cycle and duration between two stimulation cycles.                                                                |                                    |
| 11/02/17                                                                                                                                                                                                                           | J.D     | FR 2.2 : The interface used to set the stimulation parameters has to be an integral part of the device (no computer plug to set the variables).                                                   |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | <b>Constraints</b>                                                                                                                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                                           | J.D     | C1 : Size of the graft.                                                                                                                                                                           |                                    |
| 08/02/17                                                                                                                                                                                                                           | J.D     | C2 : Parts in contact with the graft and the culture medium.                                                                                                                                      |                                    |
| 21/12/16                                                                                                                                                                                                                           | B.H     | C3 : Sterility.                                                                                                                                                                                   |                                    |
|                                                                                                                                                                                                                                    |         | C4 : Mechanical stimulation system.                                                                                                                                                               |                                    |
| 08/02/17                                                                                                                                                                                                                           | C.F-J.L | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                                                                            |                                    |
| 07/10/16                                                                                                                                                                                                                           | J.D     | CR 1.1 : Graft measurements are available in Appendix1                                                                                                                                            |                                    |
|                                                                                                                                                                                                                                    |         | CR 2.1 : The parts in contact with the graft and the culture medium have to be placed in the INCO246med incubator of 640 x 640 x 600 mm and resist to incubator physical and chemical conditions. |                                    |
| 08/02/17                                                                                                                                                                                                                           | J.D     | CR 3.1 : The parts immerse in the culture medium have to be sterilizable using. autoclave.                                                                                                        |                                    |
| 21/12/16                                                                                                                                                                                                                           | B.H     | CR 4.1 : The electrical stimulation system has to be able to be integrated in the mechanical stimulation system                                                                                   |                                    |

## Appendix 1 :

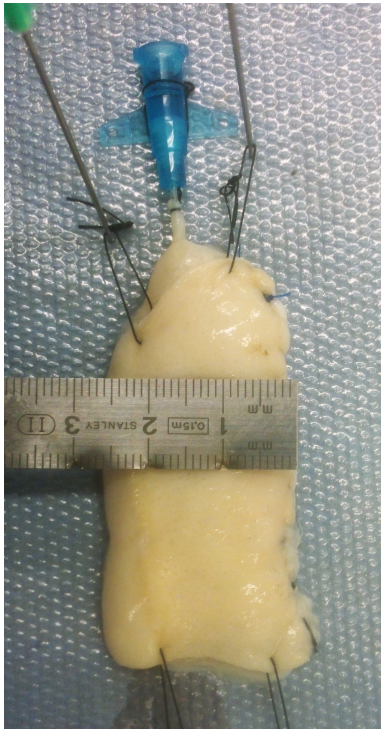


Figure B.2: Graft width.

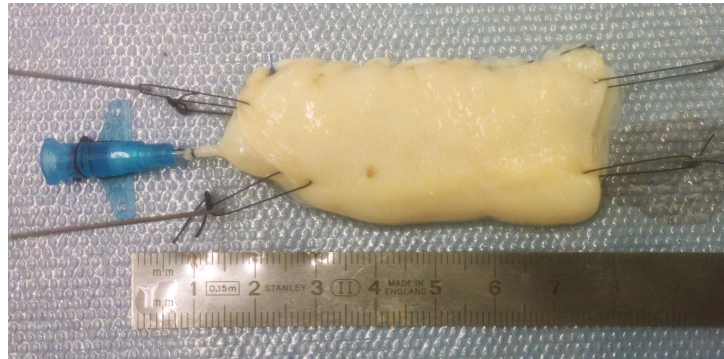


Figure B.3: Graft length.

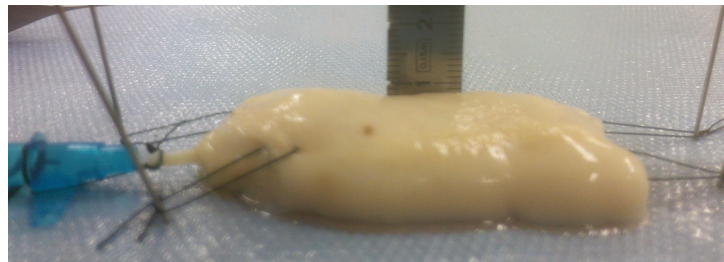


Figure B.4: Graft thickness.

We will therefore consider a graft with the following dimensions :

| Graft features                   | Dimensions[mm] |
|----------------------------------|----------------|
| Graft width (skin width)         | 26             |
| Graft thickness (skin thickness) | 9              |
| Skin length                      | 62             |
| Vessels length                   | 9              |
| Catheter hub                     | 24             |
| Total graft length               | 95             |

A 20% margin will be taken on the total length.

|                                                                                                                                                                                                                                                                                             |         |                                                                                                                                                                                                   |                                    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                                                                                      |         | <b>Specifications of an electrical stimulation device</b>                                                                                                                                         | Date : 12/03/2017<br>Version : 2.5 |
| <b>Context:</b> Jérôme Duisit required the design of an electrical stimulation device in order to stimulate a half-lip. The electrical stimulation will be used punctually in order to test some physical properties of the graft, or as a complementary stimulation to the mechanical one. |         |                                                                                                                                                                                                   |                                    |
| Date                                                                                                                                                                                                                                                                                        | Source  | <b>Functions</b>                                                                                                                                                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                                                                                    | J.D     | F1 : Stimulate the graft electrically.                                                                                                                                                            |                                    |
| 10/02/17                                                                                                                                                                                                                                                                                    | B.H     | F2 : Set stimulation parameters.                                                                                                                                                                  |                                    |
| 10/02/17                                                                                                                                                                                                                                                                                    | B.H     | F3 : Visualize selected parameters.                                                                                                                                                               |                                    |
| 16/11/16                                                                                                                                                                                                                                                                                    | J.D     | <b>Functional Requirement (FR) criteria and levels</b>                                                                                                                                            |                                    |
| 16/11/16                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 1.1 : Stimulate the culture medium.                                                                                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 1.2 : The electric field has to be perpendicular to the graft muscular fibres.                                                                                                                 |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 1.3 : With a range of frequency : 0.5-10Hz.                                                                                                                                                    |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 1.4 : With a range of amplitude: 0-7V.                                                                                                                                                         |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 1.5 : With a range of peak duration: 1-20ms.                                                                                                                                                   |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | B.H     | FR 1.6 : With a maximum intensity of 200 mA.                                                                                                                                                      |                                    |
| 10/02/17                                                                                                                                                                                                                                                                                    | C.F-J.L | FR 2.1 : Set the frequency, the amplitude, the peak duration, the duration of a cycle and duration between two stimulation cycles.                                                                |                                    |
| 11/02/17                                                                                                                                                                                                                                                                                    | J.D     | FR 2.2 : The interface used to set the stimulation parameters has to be an integral part of the device (no computer plug to set the variables).                                                   |                                    |
| 11/02/17                                                                                                                                                                                                                                                                                    | J.D     | FR 3.1 : The interface used to visualize the stimulation parameters has to be an integral part of the device (no computer plug to visualize the variables).                                       |                                    |
| 08/02/17                                                                                                                                                                                                                                                                                    | C.F-J.L | <b>Constraints</b>                                                                                                                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                                                                                                    | J.D     | C1 : Size of the graft.                                                                                                                                                                           |                                    |
| 08/02/17                                                                                                                                                                                                                                                                                    | J.D     | C2 : Parts in contact with the graft and the culture medium.                                                                                                                                      |                                    |
| 21/12/16                                                                                                                                                                                                                                                                                    | B.H     | C3 : Sterility.                                                                                                                                                                                   |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | C4 : Mechanical stimulation system.                                                                                                                                                               |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | C5 : Graft safety.                                                                                                                                                                                |                                    |
| 08/02/17                                                                                                                                                                                                                                                                                    | C.F-J.L | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                                                                            |                                    |
| 07/10/16                                                                                                                                                                                                                                                                                    | J.D     | CR 1.1 : Graft measurements are available in Appendix1                                                                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                                                                                                    | J.D     | CR 2.1 : The parts in contact with the graft and the culture medium have to be placed in the INCO246med incubator of 640 x 640 x 600 mm and resist to incubator physical and chemical conditions. |                                    |
| 21/12/16                                                                                                                                                                                                                                                                                    | B.H     | CR 3.1 : The parts immerse in the culture medium have to be sterilizable using. autoclave.                                                                                                        |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | CR 4.1 : The electrical stimulation system has to be able to be integrated in the mechanical stimulation system.                                                                                  |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | CR 5.1 : No net charge accumulated in the graft.                                                                                                                                                  |                                    |
| 02/03/17                                                                                                                                                                                                                                                                                    | C.F-J.L | CR 5.2 : No medium composition alteration during the electrical stimulation.                                                                                                                      |                                    |

## Appendix 1 :

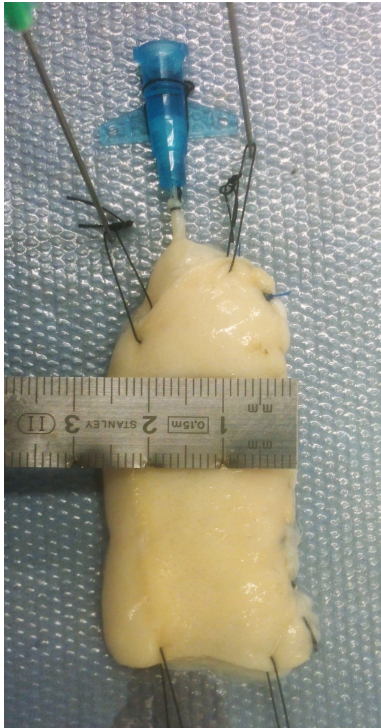


Figure B.5: Graft width.

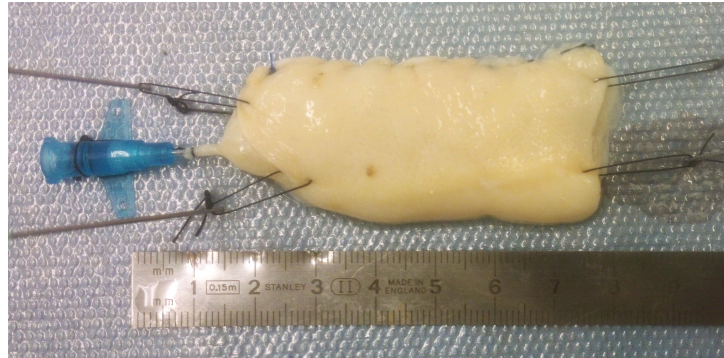


Figure B.6: Graft length.

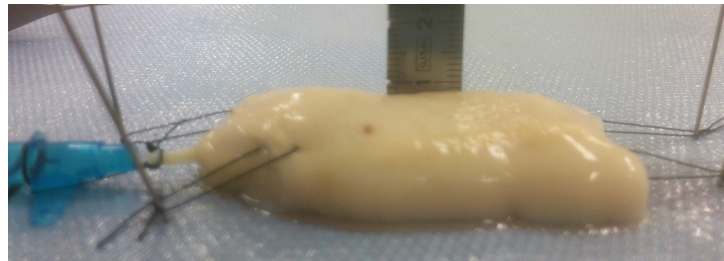


Figure B.7: Graft thickness.

We will therefore consider a graft with the following dimensions :

| Graft features                   | Dimensions[mm] |
|----------------------------------|----------------|
| Graft width (skin width)         | 26             |
| Graft thickness (skin thickness) | 9              |
| Skin length                      | 62             |
| Vessels length                   | 9              |
| Catheter hub                     | 24             |
| Total graft length               | 95             |

A 20% margin will be taken on the total length.

## Appendix C

# Specifications: Bioreactor with mechanical stimulation

|                                                                                                                                                                                                                                                 |         |                                                                                                 |                                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                                          |         | <b>Specifications of a mono axial mechanical stimulation device</b>                             | Date : 17/11/2016<br>Version : 1.1 |
| <b>Context:</b> In order to familiarize ourselves with the grafts and the mechanical stimulation, as well as to obtain some preliminary results, we would like to design a mono axial mechanical stimulation device with some existing devices. |         |                                                                                                 |                                    |
| Date                                                                                                                                                                                                                                            | Source  | <b>Functions</b>                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | F1 : Stimulate the graft mechanically.                                                          |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | F2 : Maintain the graft in the liquid culture.                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | F3 : Measure the graft behaviour under stimulation.                                             |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | <b>Functional Requirement (FR) criteria and levels</b>                                          |                                    |
| 03/11/16                                                                                                                                                                                                                                        | B.H     | FR 1.1 : Stimulate in a mono axial way .                                                        |                                    |
| 03/11/16                                                                                                                                                                                                                                        | C.F-J.L | FR 1.2 : Speed of the movements : 150 to 200 mm/s maximum .                                     |                                    |
| 03/11/16                                                                                                                                                                                                                                        | C.F-J.L | FR 2.1 : Maintain the graft by two points.                                                      |                                    |
| 03/11/16                                                                                                                                                                                                                                        | C.F-J.L | FR 3.1 : Measure the elongation in function of time                                             |                                    |
| 16/11/16                                                                                                                                                                                                                                        | C.F-J.L | FR 3.2 : Check if the graft remains of sufficient quality                                       |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | <b>Constraints</b>                                                                              |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | C1 : Graft attachment.                                                                          |                                    |
| 09/11/16                                                                                                                                                                                                                                        | J.D     | C2 : Size of the graft.                                                                         |                                    |
| 09/11/16                                                                                                                                                                                                                                        | J.D     | C3 : Size of the whole device.                                                                  |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | <b>Constraint Requirement (CR) criteria and levels</b>                                          |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | CR 1.1 : Minimize cellular damages                                                              |                                    |
| 07/10/16                                                                                                                                                                                                                                        | J.D     | CR 2.1 : The graft measurements are depicted in Appendix 1                                      |                                    |
| 09/11/16                                                                                                                                                                                                                                        | J.D     | CR 3.1 : The whole device has to be placed in the INCO246med incubator of<br>640 x 640 x 600 mm |                                    |

## Appendix 1 :



Figure C.1: Graft measurements.

|                                                                                                                                                                                                              |        |                                                                                                                                             |                                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|---------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                       |        | <b>Specifications of a mono axial mechanical stimulation device</b>                                                                         | Date : 08/02/2017<br>Version : 2.1 |
| <b>Context:</b> It was asked us to design a mono axial mechanical stimulation device in order to stimulate a half -lip. The mechanical stimulation will be used during the entire recellularization process. |        |                                                                                                                                             |                                    |
| Date                                                                                                                                                                                                         | Source | <b>Functions</b>                                                                                                                            |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | F1 : Stimulate the graft mechanically.                                                                                                      |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | F2 : Maintain the graft in the liquid culture.                                                                                              |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | F3 : Measure the graft behaviour under stimulation.                                                                                         |                                    |
|                                                                                                                                                                                                              |        | <b>Functional Requirement (FR) criteria and levels</b>                                                                                      |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | FR 1.1 : Stimulate in a mono axial way.                                                                                                     |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | FR 1.2 : Elongation: +30% from the initial graft length.                                                                                    |                                    |
| 03/11/16                                                                                                                                                                                                     | B.H    | FR 1.3 : Speed of the movements : 150 to 200 mm/s maximum.                                                                                  |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | FR 2.1 : Maintain the graft against gravity in order to avoid deflection.                                                                   |                                    |
| 03/11/16                                                                                                                                                                                                     | J.D    | FR 3.1 : Measure the force and the elongation.                                                                                              |                                    |
| 16/11/16                                                                                                                                                                                                     | J.D    | FR 3.2 : Allow to Check if the graft remains of sufficient quality                                                                          |                                    |
|                                                                                                                                                                                                              |        | <b>Constraints</b>                                                                                                                          |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | C1 : Graft attachment.                                                                                                                      |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | C2 : Size of the graft.                                                                                                                     |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | C3 : Size of parts in contact with the graft and the culture medium .                                                                       |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | C4 : Sterility.                                                                                                                             |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | C5 : Visibility of the graft.                                                                                                               |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | C6 : Access to the graft.                                                                                                                   |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | C7 : Culture medium circulation.                                                                                                            |                                    |
| 21/12/16                                                                                                                                                                                                     | B.H    | C8 : Electrical stimulation system.                                                                                                         |                                    |
|                                                                                                                                                                                                              |        | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                      |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 1.1 : Using stitches with independent suture thread.                                                                                     |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 2.1 : Graft measurements are based on two scientific articles (see [44],[62])                                                            |                                    |
| 07/10/16                                                                                                                                                                                                     | J.D    | CR 3.1 : Size of parts in contact with the graft and the culture medium have to be placed in the INCO246med incubator of 640 x 640 x 600 mm |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 4.1 : The parts immerse in the culture medium have to be sterilizable using autoclave.                                                   |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 5.1 : Only the graft part that shows the elongation and the relaxation needs to be visible and potentially be recorded via a camera.     |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 6.1 : An access to the graft is needed at the top of the device.                                                                         |                                    |
| 08/02/17                                                                                                                                                                                                     | J.D    | CR 7.1 : An external tank linked to the device is needed.                                                                                   |                                    |
| 21/12/16                                                                                                                                                                                                     | B.H    | CR 8.1 : The mechanical stimulation system have to coexist with the electrical stimulation system.                                          |                                    |



|                                                                                                                                                                                                                        |         |                                                                                                                                                 |                                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                 |         | <b>Specifications of a mono axial mechanical stimulation device</b>                                                                             | Date : 11/02/2017<br>Version : 2.2 |
| <b>Context:</b> Jérôme Duisit required the design of a mono axial mechanical stimulation device in order to stimulate a half-lip. The mechanical stimulation will be used during the entire recellularization process. |         |                                                                                                                                                 |                                    |
| Date                                                                                                                                                                                                                   | Source  | <b>Functions</b>                                                                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F1 : Stimulate the graft mechanically.                                                                                                          |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F2 : Maintain the graft in the liquid culture.                                                                                                  |                                    |
| 10/02/16                                                                                                                                                                                                               | B.H     | F3 : Set stimulation parameters.                                                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F4 : Measure the graft behaviour under stimulation.                                                                                             |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | F5 : Allow the visual monitoring of the stimulation.                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | F6 : Allow an access to the graft.                                                                                                              |                                    |
|                                                                                                                                                                                                                        |         | <b>Functional Requirement (FR) criteria and levels</b>                                                                                          |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | FR 1.1 : Stimulate in a mono axial way.                                                                                                         |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 1.2 : Maximum elongation: 8mm for the considered graft (see Appendix1).                                                                      |                                    |
| 03/11/16                                                                                                                                                                                                               | B.H     | FR 1.3 : Speed of the graft movements : 200 mm/s maximum <sub>1</sub> .                                                                         |                                    |
| 10/02/17                                                                                                                                                                                                               | B.H     | FR 1.4 : Acceleration of the graft movements: 900 mm/s <sup>2</sup> maximum <sub>1</sub> .                                                      |                                    |
| 10/02/17                                                                                                                                                                                                               | B.H     | FR 1.5 : Stimulate by imposing a force or an elongation.                                                                                        |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 2.1 : Maintain the graft against gravity in order to avoid deflection.                                                                       |                                    |
| 11/02/17                                                                                                                                                                                                               | J.D     | FR 3.1 : Set the elongation, the duration of a stimulation cycle and duration between two stimulation cycles.                                   |                                    |
| 11/02/17                                                                                                                                                                                                               | J.D     | FR 3.2 : The interface used to set the stimulation parameters has to be an integral part of the device (no computer plug to set the variables). |                                    |
| 03/11/16                                                                                                                                                                                                               | C.F-J.L | FR 4.1 : Measure force and elongation.                                                                                                          |                                    |
| 16/11/16                                                                                                                                                                                                               | C.F-J.L | FR 4.2 : Allow to check if the graft remains of sufficient quality.                                                                             |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 5.1 : Allow the visibility and the record of the graft part that shows the elongation and the relaxation.                                    |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 6.1 : Allow an access to the graft at the top of the device.                                                                                 |                                    |
|                                                                                                                                                                                                                        |         | <b>Constraints</b>                                                                                                                              |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C1 : Graft attachment.                                                                                                                          |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C2 : Device internal dimensions.                                                                                                                |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C3 : Features of parts in contact with the graft and the culture medium.                                                                        |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | C4 : Sterility.                                                                                                                                 |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | C5 : Culture medium circulation.                                                                                                                |                                    |
| 21/12/16                                                                                                                                                                                                               | B.H     | C6 : Electrical stimulation system.                                                                                                             |                                    |
|                                                                                                                                                                                                                        |         | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                          |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | CR 1.1 : Using stitches with independent suture thread.                                                                                         |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | CR 2.1 : The internal dimensions of the device have to be adapted to the size of the graft (graft measurements are available in the Appendix1). |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | CR 3.1 : The parts in contact with the graft and the culture medium have to be                                                                  |                                    |

|          |     |                                                                                                                                                                                                                 |
|----------|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 08/02/17 | J.D | placed in the INCO246med incubator of 640 x 640 x 600 mm and resist to incubator physical and chemical conditions.<br>CR 4.1 : The parts immerse in the culture medium have to be sterilizable using autoclave. |
| 08/02/17 | J.D | CR 5.1 : An external tank has to be connected to the device (if necessary) via a circulation system one input/one output.                                                                                       |
| 21/12/16 | B.H | CR 6.1 : The mechanical stimulation system has to be able to receive the electrical stimulation system.                                                                                                         |

<sub>1</sub>. Based on the master thesis of Lise Colmant : « Lip Motion Tracking System » [56].

|                                                                                                                                                                                                                        |         |                                                                                                                                                                |                                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| C.Faes,J.Legrand,<br>J.Duisit,B.Herman                                                                                                                                                                                 |         | <b>Specifications of a mono axial mechanical stimulation device</b>                                                                                            | Date : 12/03/2017<br>Version : 2.5 |
| <b>Context:</b> Jérôme Duisit required the design of a mono axial mechanical stimulation device in order to stimulate a half-lip. The mechanical stimulation will be used during the entire recellularization process. |         |                                                                                                                                                                |                                    |
| Date                                                                                                                                                                                                                   | Source  | <b>Functions</b>                                                                                                                                               |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F1 : Stimulate the graft mechanically.                                                                                                                         |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F2 : Maintain the graft in the liquid culture.                                                                                                                 |                                    |
| 10/02/16                                                                                                                                                                                                               | B.H     | F3 : Set stimulation parameters.                                                                                                                               |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | F4 : Measure the graft behaviour under stimulation.                                                                                                            |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | F5 : Allow the visual monitoring of the stimulation .                                                                                                          |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | F6 : Allow an access to the graft.                                                                                                                             |                                    |
| 10/02/16                                                                                                                                                                                                               | J.D     | F7 : Visualize selected parameters.                                                                                                                            |                                    |
| 06/03/17                                                                                                                                                                                                               | J.D     | F8 : Oxygenate the culture medium.                                                                                                                             |                                    |
|                                                                                                                                                                                                                        |         | <b>Functional Requirement (FR) criteria and levels</b>                                                                                                         |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | FR 1.1 : Stimulate in a mono axial way.                                                                                                                        |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 1.2 : Maximum elongation: 8mm for the considered graft (see Appendix1).                                                                                     |                                    |
| 03/11/16                                                                                                                                                                                                               | B.H     | FR 1.3 : Speed of the graft movements : 200 mm/s maximum <sub>1</sub> .                                                                                        |                                    |
| 10/02/17                                                                                                                                                                                                               | B.H     | FR 1.4 : Acceleration of the graft movements: 900 mm/s <sup>2</sup> maximum <sub>1</sub> .                                                                     |                                    |
| 10/02/17                                                                                                                                                                                                               | B.H     | FR 1.5 : Stimulate by imposing a force or an elongation.                                                                                                       |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 2.1 : Maintain the graft against gravity in order to avoid deflection.                                                                                      |                                    |
| 27/02/17                                                                                                                                                                                                               | J.D     | FR 3.1 : Set the elongation or the tensile force, the stimulation frequency, the duration of a stimulation cycle, and duration between two stimulation cycles. |                                    |
| 11/02/17                                                                                                                                                                                                               | J.D     | FR 3.2 : The interface used to set the stimulation parameters has to be an integral part of the device (no computer plug to set the variables).                |                                    |
| 12/03/07                                                                                                                                                                                                               | C.F-J.L | FR 4.1 : Measure force with a +/- 0.01N precision.                                                                                                             |                                    |
| 12/03/07                                                                                                                                                                                                               | C.F-J.L | FR 4.2 : Measure elongation with a +/- 0.5 mm precision.                                                                                                       |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 5.1 : Allow the visibility and the record of the graft part that shows the elongation and the relaxation.                                                   |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | FR 6.1 : Allow an access to the graft at the top of the device.                                                                                                |                                    |
| 12/03/07                                                                                                                                                                                                               | J.D     | FR 7.1 : The interface used to visualize the stimulation parameters has to be an integral part of the device (no computer plug to visualize the variables).    |                                    |
| 06/03/17                                                                                                                                                                                                               | C.F-J.L | FR 8.1 : O <sub>2</sub> flow rate: 1-10ml/min.                                                                                                                 |                                    |
|                                                                                                                                                                                                                        |         | <b>Constraints</b>                                                                                                                                             |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C1 : Graft attachment.                                                                                                                                         |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C2 : Device internal dimensions.                                                                                                                               |                                    |
| 07/10/16                                                                                                                                                                                                               | J.D     | C3 : Features of parts in contact with the graft and the culture medium.                                                                                       |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | C4 : Sterility.                                                                                                                                                |                                    |
| 08/02/17                                                                                                                                                                                                               | J.D     | C5 : Culture medium circulation.                                                                                                                               |                                    |

|          |     |                                                                                                                                                 |
|----------|-----|-------------------------------------------------------------------------------------------------------------------------------------------------|
| 21/12/16 | B.H | C6 : Electrical stimulation system.                                                                                                             |
| 12/03/07 | J.D | C7 : Graft safety.                                                                                                                              |
| 12/03/07 | J.D | <b>Constraint Requirement (CR) criteria and levels</b>                                                                                          |
| 08/02/17 | J.D | CR 1.1 : The attachment has to allow the complete recellularization of the graft.                                                               |
| 08/02/17 | J.D | CR 2.1 : The internal dimensions of the device have to be adapted to the size of the graft (graft measurements are available in the Appendix1). |
| 07/10/16 | J.D | CR 3.1 : The parts in contact with the graft and the culture medium have to be                                                                  |
| 08/02/17 | J.D | placed in the INCO246med incubator of 640 x 640 x 600 mm and resist to incubator physical and chemical conditions.                              |
| 08/02/17 | J.D | CR 4.1 : The parts immerse in the culture medium have to be sterilizable using autoclave.                                                       |
| 08/02/17 | J.D | CR 5.1 : An external tank has to be connected to the device (if necessary) via a circulation system one input/one output.                       |
| 21/12/16 | B.H | CR 6.1 : The mechanical stimulation system has to be able to receive the electrical stimulation system.                                         |
| 12/03/07 | J.D | CR 7.1 : No mechanical strain of stress applied on the artery at any time (during manipulation or recellularization).                           |

1. Based on the master thesis of Lise Colmant : « Lip Motion Tracking System » [56].

## Appendix 1 :

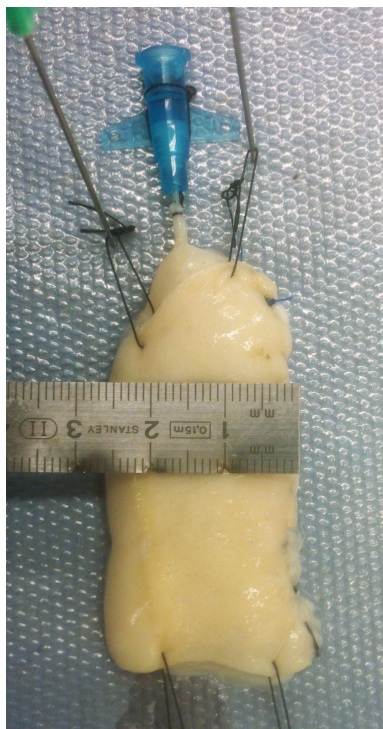


Figure C.2: Graft width.

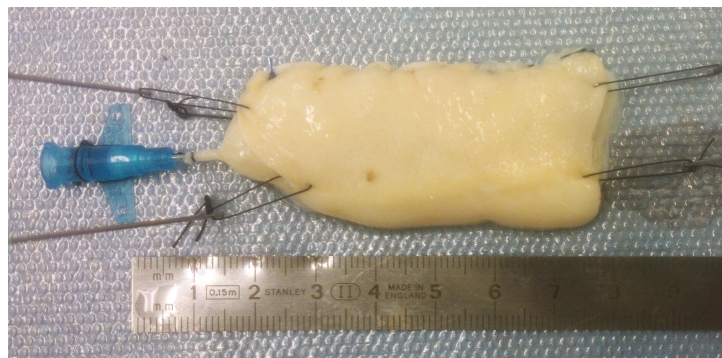


Figure C.3: Graft length.

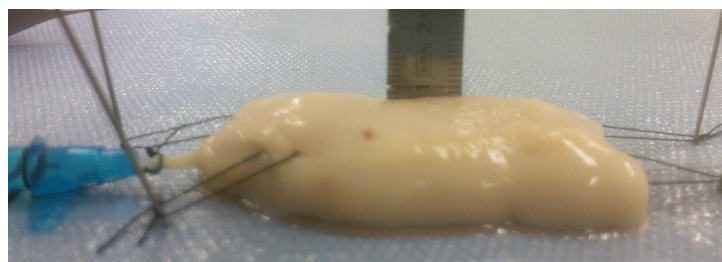


Figure C.4: Graft thickness.

We will therefore consider a graft with the following dimensions :

| Graft features                   | Dimensions[mm] |
|----------------------------------|----------------|
| Graft width (skin width)         | 26             |
| Graft thickness (skin thickness) | 9              |
| Skin length                      | 62             |
| Vessels length                   | 9              |
| Catheter hub                     | 24             |
| Total graft length               | 95             |

A 20% margin will be taken on the total length.

## Appendix D

# Assembly protocol of the experimental bioreactor for vascularized composite tissue

### Assembly of the window and the lower chamber

**Material** (figure D.1):

- Lower chamber
- Window seal
- Plastic window
- 4 long knurled screw

**Assembly** (figure D.2 and D.3):

- Place the seal on the fixed part of the lower chamber window.
- Place the plastic window on the window seal.
- Place the stainless steel window on the plastic window.
- Tighten the assembly by screwing the 4 screws.

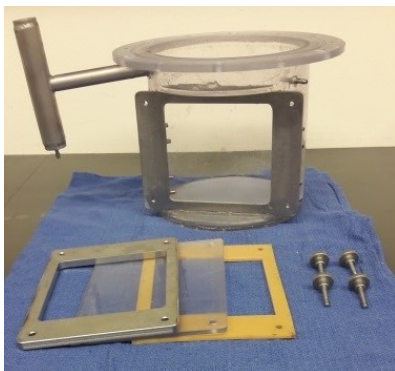


Figure D.1: Used material - window and lower chamber.

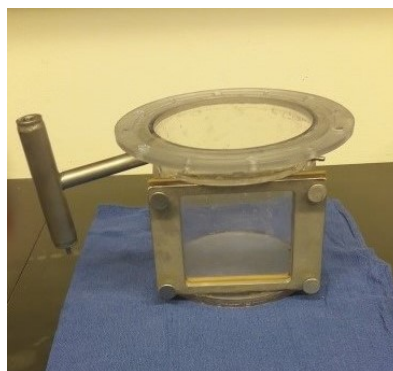


Figure D.2: Front view of the lower chamber assembly.

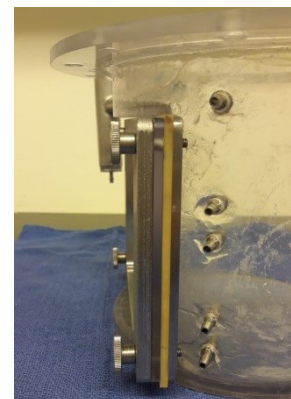


Figure D.3: Lateral view of the lower chamber assembly.

## Assembly of the support

**Material** (figure D.4):

- Graft support
- Graft compressor
- 6 small nuts
- 6 seals for small screws
- A small wrench

**Assembly** (figure D.5 and D.3):

- Put the graft compressor on the graft support.
- Put on the screws from behind (in such a way that the nuts can be visible when the compressor is placed on the graft support).
- Tighten the screws using the wrench.

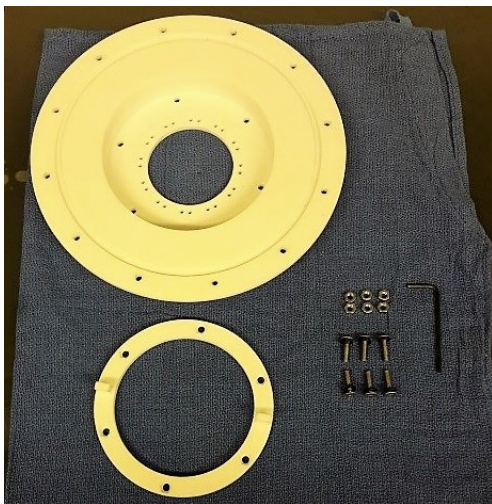


Figure D.4: Used material - support.

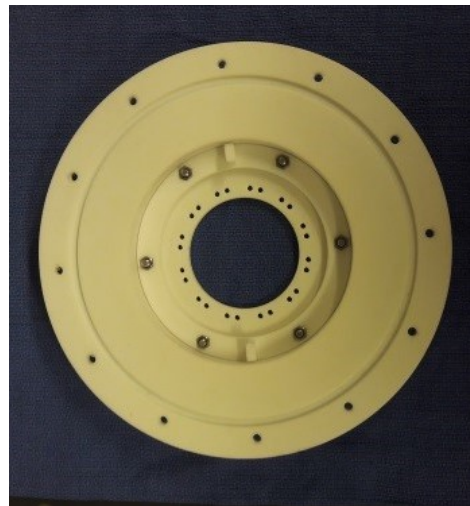


Figure D.5: Support assembly view.

## Assembly of the upper chamber

**Material** (figure D.6):

- Upper and lower chamber
- Graft support and compressor
- 6 long knurled screw
- 6 long hex screws
- 12 knurled nuts
- 2 flat black seals



### Assembly (figure D.8):

- Put a seal on the upper part of the lower chamber.
- Put the graft support with compressor on the lower chamber.
- Put a seal on the graft support.
- Put the upper chamber on the second seal. (The red arrow downwards, figure D.7)
- Tighten the assembly using, one hole out of two, the 6 long knurled screws and the 6 knurled nuts by pressing them in the plastic holes.
- Screw the 6 long hex screws in the 6 knurled through the remaining holes.

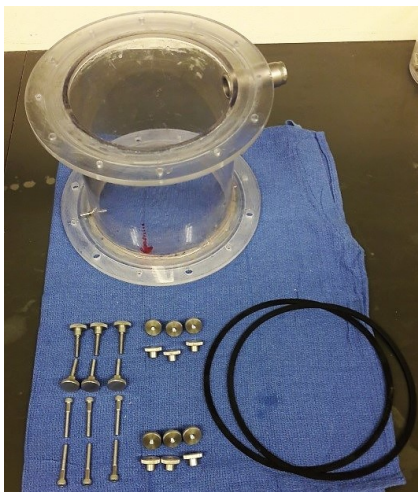


Figure D.6: Used material - upper chamber

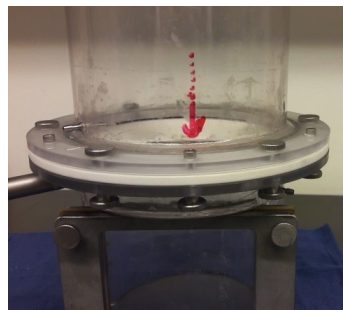


Figure D.7: Zoom on the red arrow.

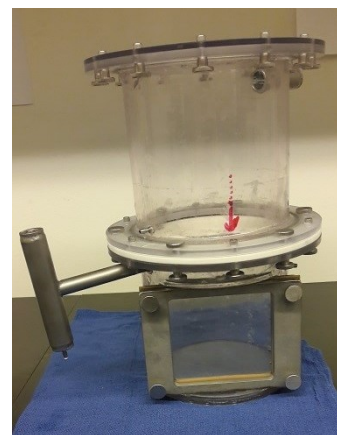


Figure D.8: Assembly of both chambers.

## Final assembly

### Material (figure D.6):

- Bioreactor
- 2 big vents
- 7 small clear tubes (3-4cm long)
- 4 medium clear tubes (+/- 15cm long)
- 9 3-way connectors
- 2 small connectors
- 1 long yellow tube (+/-50cm)
- Clamps
- Pump



**Assembly** (figure D.9):

- Put the 2 vents on the chambers of the bioreactor.
- Fix 2 clear medium tubes down the lower chamber (in/out), add clamps.
- Connect the two medium tubes to the yellow tube by using the small connector.
- Fix the small tubes at the remaining input of the lower chamber. Close with 3-way connectors.
- Place a medium clear tube below the vent of the lower chamber. Close with 3-way connectors.
- Place the yellow tube though the pump.

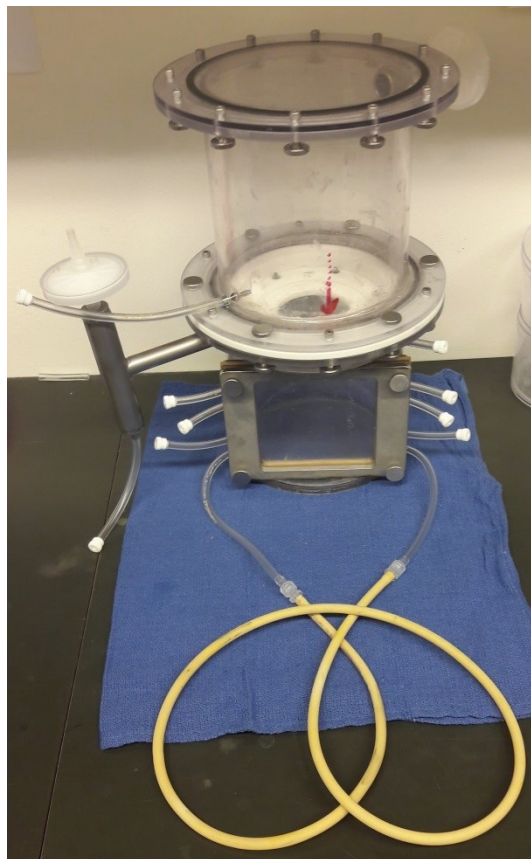


Figure D.9: Final assembly of the bioreactor.

## Appendix E

# Use protocol of the electrical stimulation device

### Update of the Arduino code

Normally, the Arduino is already programmed, and requires no additional manipulations than a simple power up using a 12V converter or a USB cable. Nevertheless, if a programming problem occurs, the following steps should be followed in order to update the Arduino code.

First, download the Arduino IDE using the following link and install the latest version: <https://www.arduino.cc/en/Main/Software>. Then, open the code.

The next step is to include the libraries: when the IDE is open, click on sketch and add the libraries by clicking on manage libraries or add .ZIP library as shown on figure E.1. Two libraries have to be added. The first one is the LiquidCristal library, and the second is the PID library.

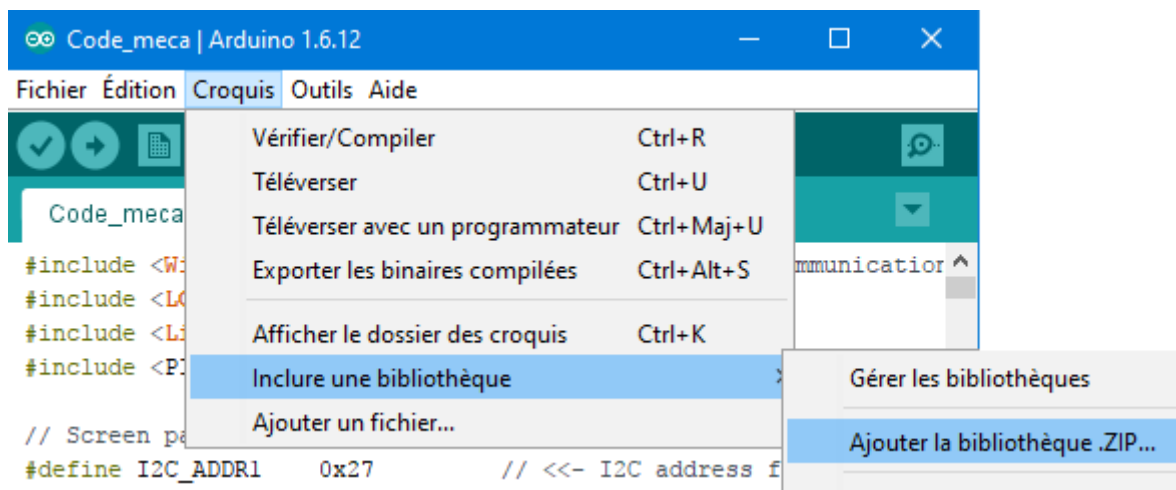


Figure E.1: Add a library on the Arduino IDE

Plug the Arduino in the computer with the USB cable and click on the Tools. Select the Arduino type used and the correct port as shown on figure E.2. Select the Arduino/Genuino Mega or Mega 2560. In order to select the port, you can look at the available ports and identify which one disappears when you unplug the USB cable.

In the top left of the Arduino IDE, click the “Upload” icon circled in red on figure E.3 in order to transfer the

program to the Arduino.

Finally, unplug the Arduino and power it with the 12V power supply, using the 12V converter, or with the USB cable.

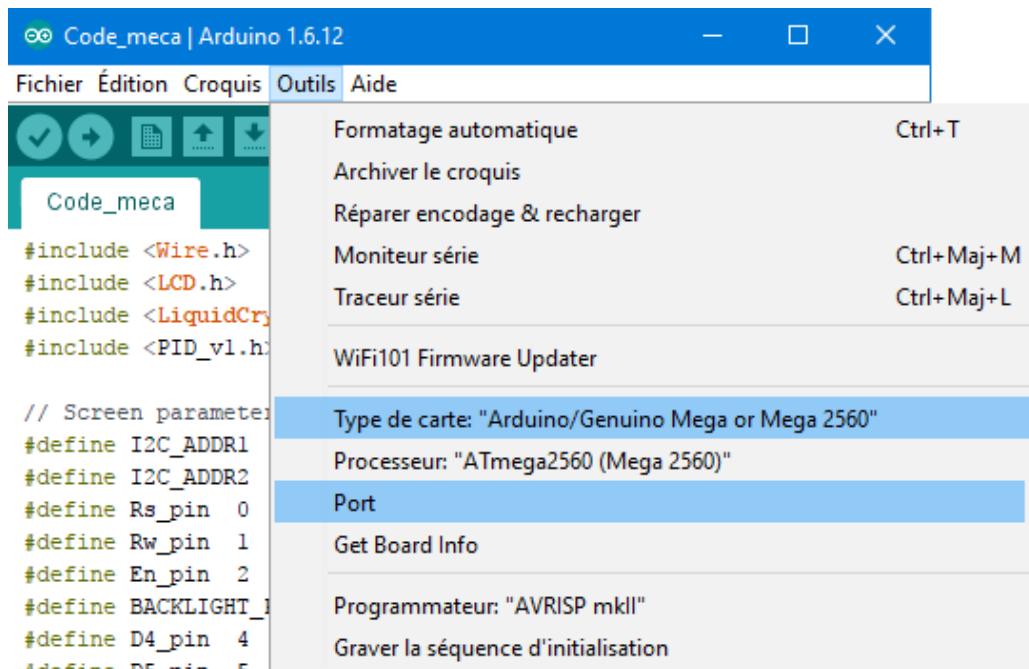


Figure E.2: Select Arduino type and port.

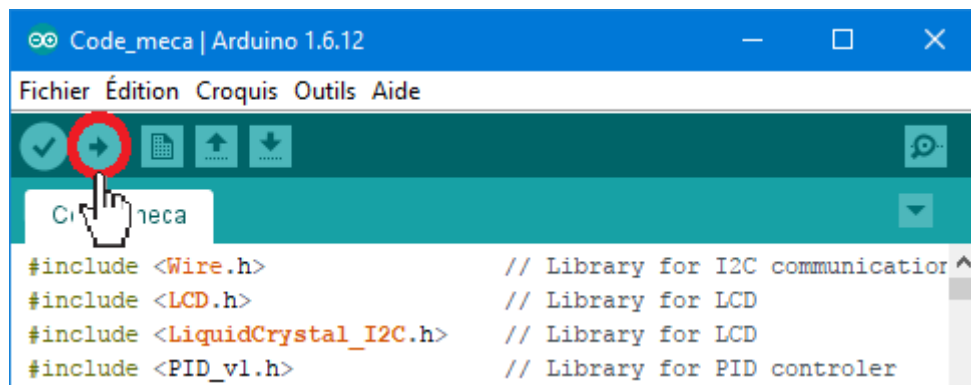


Figure E.3: Update of the Arduino code.

## Electrical stimulation device: usage

The electrical stimulation device, the black box on figure E.4 and E.5, can be used either with the portable electrodes (figure E.4) or with the bioreactor (figure E.5). Follow the subsequent steps in order to power up, select the parameters and launch the electrical stimulation.

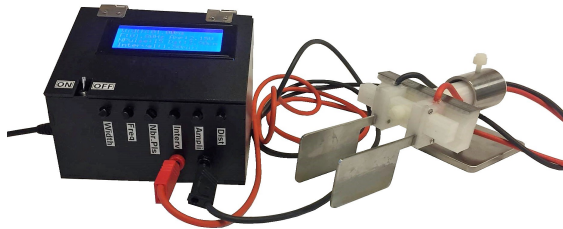


Figure E.4: Electrical stimulation device used with portable electrodes.

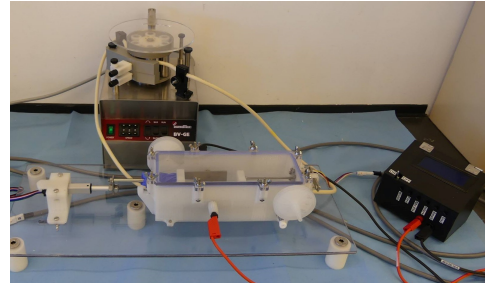


Figure E.5: Electrical stimulation device used with the perfusion bioreactor.

Before powering up the device, make sure the switch depicted on figure E.6 is OFF.

Power the device with the 12V transformer. The 5V power supply cannot be used for the stimulation because it doesn't deliver enough current. On the figure E.7, the 5V power socket is situated on the left, as the 12V power socket is placed on the right.

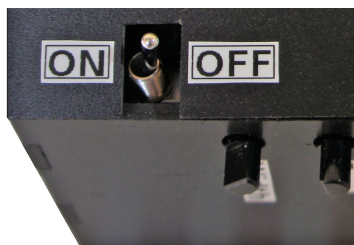


Figure E.6: On-Off switch



Figure E.7: Power supply plugs

When the switch is OFF, you can select the different stimulation parameters by rotating the black buttons (see figure E.8). The table G.2 outline the different parameters with their units, ranges and steps.

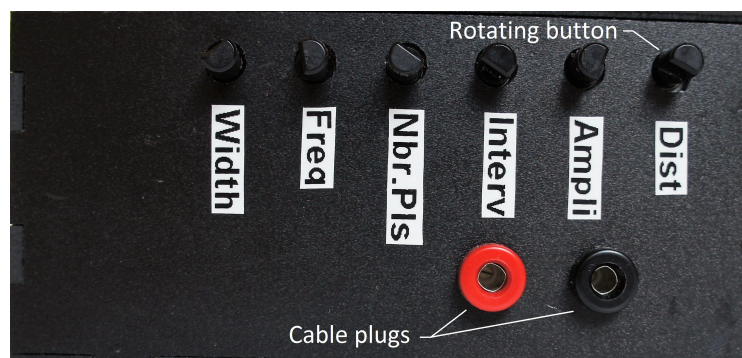


Figure E.8: Front view of the pulse generator.

| Button   | Utility                                              | Unit | Min value | Max value | Step |
|----------|------------------------------------------------------|------|-----------|-----------|------|
| Width    | Duration of the electrical pulses                    | ms   | 1         | 20        | 0.1  |
| Freq     | Frequency of the electrical pulses                   | Hz   | 0.5       | 10        | 0.1  |
| Nbr. Pls | Number of electrical pulses during a stimulation set | -    | 1         | 15        | 1    |
| Interv   | Interval of time between two stimulation sets        | s    | 5         | 120       | 1    |
| Ampli    | Amplitude of the electrical pulses                   | V    | 0.8       | 7         | 0.1  |
| Dist     | Distance between the electrode plates                | cm   | 2         | 7         | 0.1  |

Table E.1: Electrical stimulation parameters.

If you select the amplitude before the distance between the electrode, don't forget to reselect the proper amplitude afterwards (the distance button automatically adjusts the amplitude according to its value). If the electrical stimulation is used with the infused bioreactor, fix the distance between the electrodes to 4.4cm.

Then, plug the red and black cables in the corresponding plugs (see figure E.8). If you are using the portable electrodes, adapt the distance between both electrodes corresponding to the chosen distance displayed on the screen. To do so, unscrew slightly the nylon screw depicted on the figure E.9, and slide the electrodes. The graduated ruler can help you to fix the distance precisely.

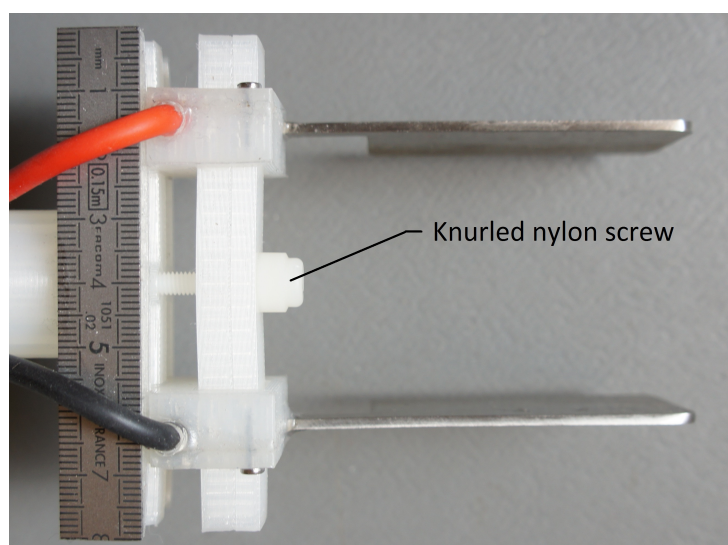


Figure E.9: Top view of the electrode portable support.

When everything is ready, place the electrodes inside the culture medium around the graft, and turn on the switch. The stimulation is running and the screen should be black.

The following example (figure E.10) allows to visualize the electrical wave generated with a pulse duration of 20ms, a frequency of 2Hz, 4 pulses during one set, 5 seconds between two sets and an amplitude of 4V. A zoom on one pulse is depicted on the figure E.11.

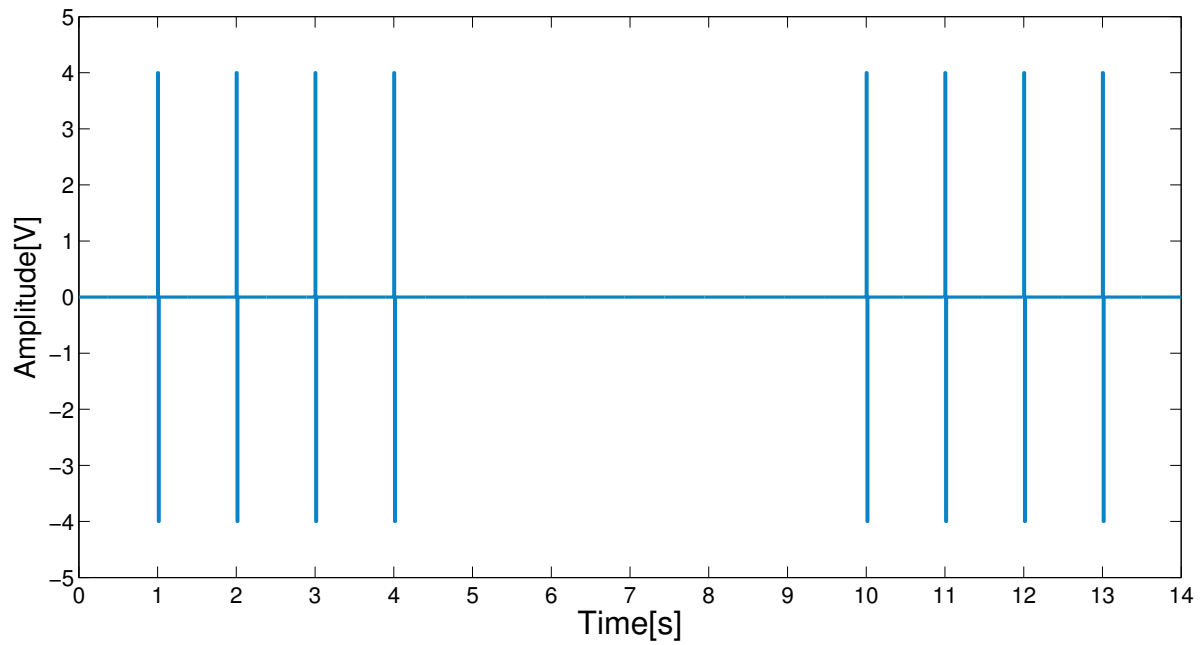


Figure E.10: Example of the generated electrical pulses.

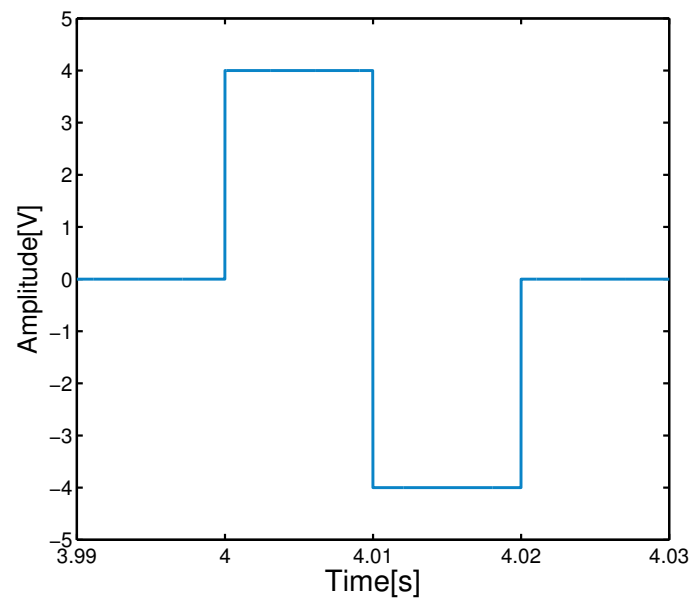


Figure E.11: Zoom on one pulse of the figure E.10.



**!** If you're not able to immerse the electrodes entirely in the culture medium, use the immersible electrode surface software "*IES.exe*" (see figure E.12) in order to be informed of the minimum immersible height in function of the chosen stimulation parameters.

Figure E.12: Software icon [63].

## Electrical schematic and connections

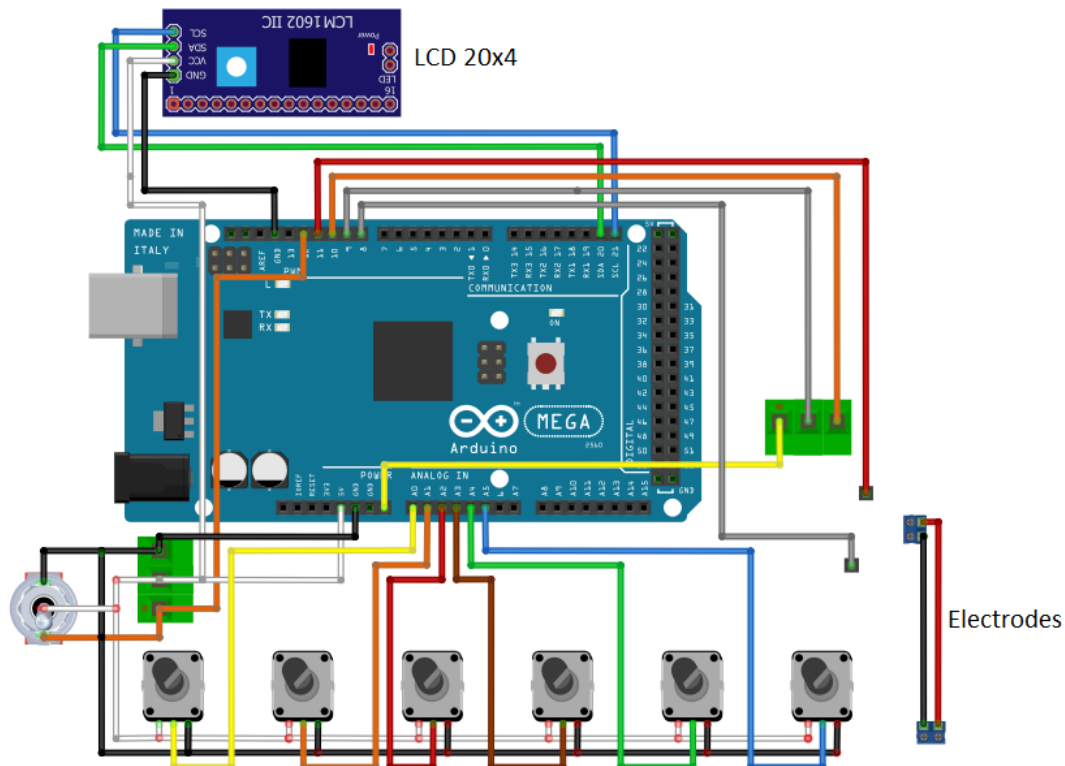


Figure E.13: Arduino connections (using Gobetwino).

| Pin | Utility                                              | Wire colour |
|-----|------------------------------------------------------|-------------|
| 8   | PWM1 for the transistors H bridge                    | Grey        |
| 9   | PWM controlling the output voltage amplitude (0-12V) | Grey        |
| 10  | Pulse signal (5V)                                    | Orange      |
| 11  | PWM2 for the transistors H bridge                    | Red         |
| 12  | State of the switch                                  | Orange      |
| 20  | SDA : I <sup>2</sup> C communication for the LCD     | Green       |
| 21  | SCL : I <sup>2</sup> C communication for the LCD     | Blue        |
| GND | Ground for the LCD and the PCB                       | Black       |
| 5V  | Power supply for the screen and the PCB              | White       |
| Vin | Power supply modulated by the PWM of pin 9           | Yellow      |
| A1  | Analog read of the pulse width potentiometer         | Yellow      |
| A2  | Analog read of the pulse frequency potentiometer     | Orange      |
| A3  | Analog read of the number of pulse potentiometer     | Red         |
| A4  | Analog read of the rest time potentiometer           | Brown       |
| A5  | Analog read of the pulse amplitude potentiometer     | Green       |
| A6  | Analog read of the electrodes distance potentiometer | Blue        |

Table E.2: Arduino connections: caption.



## **Appendix F**

# **Experimental protocol of the electrical stimulation of half lips**

- Electrical stimulation of a pig half-lip (27/03/2017)
- Electrical stimulation of a human recellularized half-lip (03/04/2017)



|                                          |                                                                   |                  |
|------------------------------------------|-------------------------------------------------------------------|------------------|
| C.Faes, J.Legrand,<br>J.Duisit, B.Herman | <b>Protocole expérimental: Stimulation électrique d'une lèvre</b> | Date: 27/03/2017 |
|------------------------------------------|-------------------------------------------------------------------|------------------|

|                                                                                                                                    |                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| Donneur: <i>Poulet</i><br>Poids: <i>5,4 kg</i><br>Médicaments injectés: <i>mort par exsanguination</i><br>Conservation: <i>PBS</i> | Protocole:<br>LS / <i>LI</i> → <i>hemi-lèvre.</i><br><i>Heure du décès: 15 h 08</i> |
|------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|

PREPARATION: (avant sacrifice et extraction du greffon)

1. Fixation et réglage de la go-pro au-dessus du dispositif (l'image capturée doit contenir le bac et la règle graduée).
2. Branchement de la « boîte stimulation » et branchement du système d'électrodes portatif.
3. Branchement de l'oscilloscope et préparation de la réception de ses données.

BACK-TABLE: (Début: *15 h 18*)

1. Mesures greffon:
  - a. Poids: *2,96 g*
  - b. Dimensions (L-l-h): *3,7 x 2 x 0,8 cm*
  - c. Masse de muscle: *0,7 g*
  - d. Dimensions du muscle (L-l-h): ... x ... x ... cm
2. Fixation du greffon (utiliser fil de suture) sur le support en 3 points.
3. Fixation des 4 perles bleues (utiliser fil de suture) aux extrémités du greffon.
4. Réglage de la distance entre les électrodes.
5. Verser le milieu de culture dans le bac.
6. Placement du support et des électrodes dans le bac.

STIMULATION: (Début stimulation: *15 h 34*)

1. Réglage des 6 boutons selon la stimulation désirée.
2. Lancer l'enregistrement de la go-pro et de l'oscilloscope en même temps.
3. Enclencher le switch pour lancer la stimulation (la led de l'écran s'éteint alors).
4. Dès que la première pulsation apparaît à l'oscilloscope, crier « TOP » afin de potentiellement pouvoir synchroniser les images de la go-pro et de l'oscilloscope en post-processing.

| Width       | Freq         | Amp          | Nbr Puls  | Interv     | Dist        | Impressions à l'œil nu |
|-------------|--------------|--------------|-----------|------------|-------------|------------------------|
| <i>20ms</i> | <i>0,542</i> | <i>0,67V</i> | <i>10</i> | <i>5 s</i> | <i>4 cm</i> | <i>Rien ...</i>        |
| <i>20ms</i> | <i>0,5</i>   | <i>1V</i>    | <i>10</i> | <i>5 s</i> | <i>4</i>    | <i>Rien ...</i>        |



|                                          |                                                                                                   |                  |
|------------------------------------------|---------------------------------------------------------------------------------------------------|------------------|
| C.Faes, J.Legrand,<br>J.Duisit, B.Herman | <b>Protocole expérimental: Stimulation électrique d'une lèvre</b><br><i>LEVRE RECELLULARISEE.</i> | Date: 03/04/2017 |
|------------------------------------------|---------------------------------------------------------------------------------------------------|------------------|

|                                                                                                                                               |                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Donneur: <i>humain.</i><br>Poids:<br><i>Arrêt de la pompe: 9h24.</i><br><i>(15 jours de recellularisation</i><br><i>avec cellules C2C12.)</i> | Protocole:<br>LS / LI |
|-----------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|

PREPARATION: (avant sacrifice et extraction du greffon)

1. Fixation et réglage de la go-pro au-dessus du dispositif (l'image capturée doit contenir le bac et la règle graduée).
2. Branchement de la « boîte stimulation » et branchement du système d'électrodes portatif.
3. Branchement de l'oscilloscope et préparation de la réception de ses données.

BACK-TABLE : (Début : *9h38*)

1. Mesures greffon :
  - a. Poids : .... g
  - b. Dimensions (L-l-h): *5 x 2 x 0,7 cm*
  - c. Masse de muscle : .... g
  - d. Dimensions du muscle (L-l-h): ... x ... x ... cm
2. Fixation du greffon (utiliser fil de suture) sur le support en 3 points.
3. Fixation des 4 perles bleues (utiliser fil de suture) aux extrémités du greffon.
4. Réglage de la distance entre les électrodes.
5. Verser le milieu de culture dans le bac.
6. Placement du support et des électrodes dans le bac.

STIMULATION : (Début stimulation : *9h59*)

1. Réglage des 6 boutons selon la stimulation désirée.
2. Lancer l'enregistrement de la go-pro et de l'oscilloscope en même temps.
3. Enclencher le switch pour lancer la stimulation (la led de l'écran s'éteint alors).
4. Dès que la première pulsation apparaît à l'oscilloscope, crier « TOP » afin de potentiellement pouvoir synchroniser les images de la go-pro et de l'oscilloscope en post-processing.

| Width        | Freq          | Amp           | Nbr Puls  | Interv     | Dist        | Impressions à l'œil nu |
|--------------|---------------|---------------|-----------|------------|-------------|------------------------|
| <i>20 ms</i> | <i>0,5 Hz</i> | <i>0,67 V</i> | <i>10</i> | <i>5 S</i> | <i>4 cm</i> | <i>rien ...</i>        |
| <i>10 ms</i> | <i>0,5 Hz</i> | <i>1 V</i>    | <i>10</i> | <i>5 S</i> | <i>4 cm</i> | <i>rien ...</i>        |





## Appendix G

# Assembly and use protocol of the mechanical stimulation device

### Material used for the bioreactor chamber assembly (Figure G.1 to G.10)

The bioreactor chamber is separated into 10 parts or sub-assemblies. All the components of these 10 sub-assemblies have to be autoclaved except for one element (which is mentioned below). Equally, a screwdriver and a pair of pliers have to be used during the bioreactor chamber assembling. Every screws and nuts must be tightened with those.

- 1 : 1x Bioreactor box.

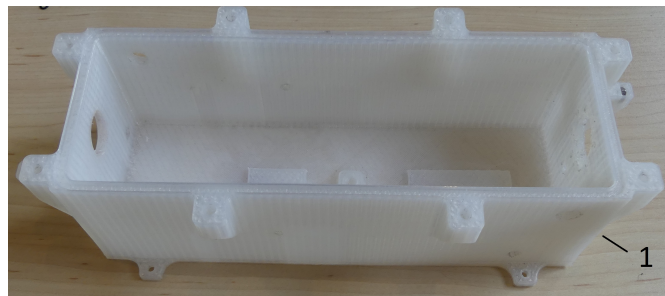


Figure G.1: 1st part : Bioreactor frame.

- 1 : 3x Tube connector.
- 2 : 3x Tube connector seal.
- 3 : 3x Tube connector nut (M6).



Figure G.2: 2nd sub-assembly : Tube connectors.

- 1 : 1x Support tubing.
- 2 : 1x Support graft.

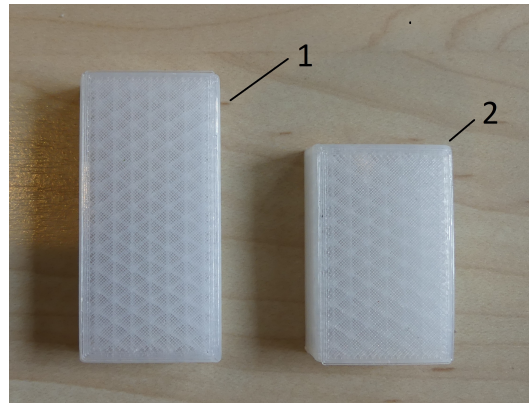


Figure G.3: 3rd part : Supports.

- 1 : 1x Actuator shaft (long screw).
- 2 : 1x Actuator nut (M4).
- 3 : 1x Cone-shaped nylon piece.
- 4 : 1x Finger glove.
- 5 : 1x Intermediate graft attachment piece.
- 6 : 1x Removable graft attachment piece.
- 7 : 1x Prismatic pin.

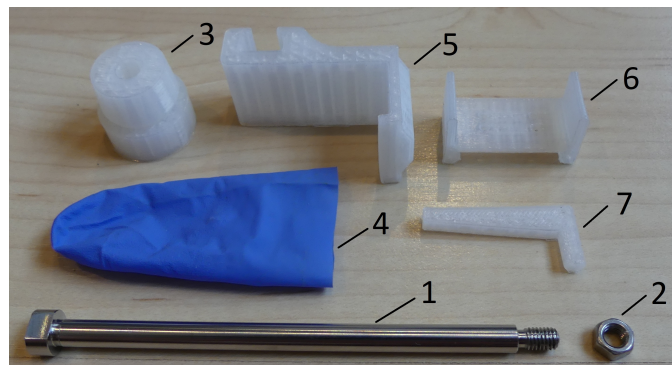


Figure G.4: 4th sub-assembly : Graft connection to the actuator shaft.

- 1 : 2x Fastening component with integrated nut.
- 2 : 2x Banana plug receiver.  
(⚠ These pieces cannot be autoclaved)
- 3 : 2x Electrode.
- 4 : 2x Electrode seal.

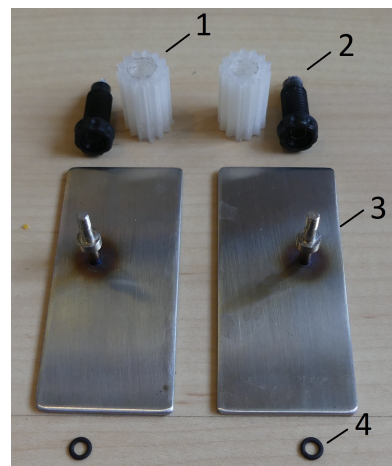


Figure G.5: 5th sub-assembly : Electrode connection.

- 1 : 1x Membrane.
- 2 : 1x Metallic ring.
- 3 : 8x Ring screw (M3).
- 4 : 8x Small ring seal.
- 5 : 8x Ring washer (M3).
- 6 : 8x Ring nut (M3).

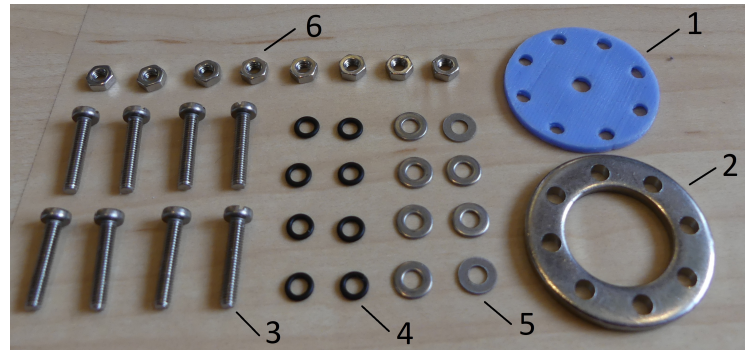


Figure G.6: 6th sub-assembly : Membrane attachment system.

- 1 : 1x Sensor shaft.
- 2 : 2x Sensor nut (M4).
- 3 : 2x Sensor washer.
- 4 : 1x Ball.
- 5 : 1x Sensor compressor.
- 6 : 1x Removable graft attachment piece
- 7 : 1x Intermediate graft attachment piece (sensor part).

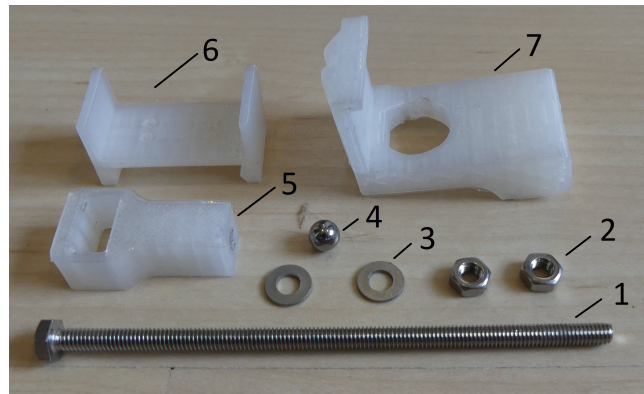


Figure G.7: 7th sub-assembly : Graft connection to the sensor shaft.

- 1 : 1x Flat metallic piece.
- 2 : 4x Flat piece nut (M3).
- 3 : 2x Flat piece screw (M3).

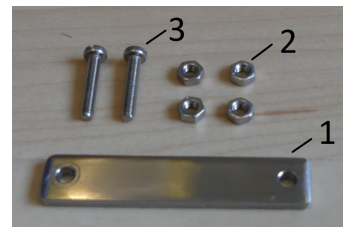


Figure G.8: 8th sub-assembly : Sensor support.

- 1 : 2x Large vents.



Figure G.9: 9th part : Vents.



- 1 : 1x Bioreactor lid.
- 2 : 1x Large seal.
- 3 : 8x Long screw (M4).
- 4 : 8x Wing nut (M4).

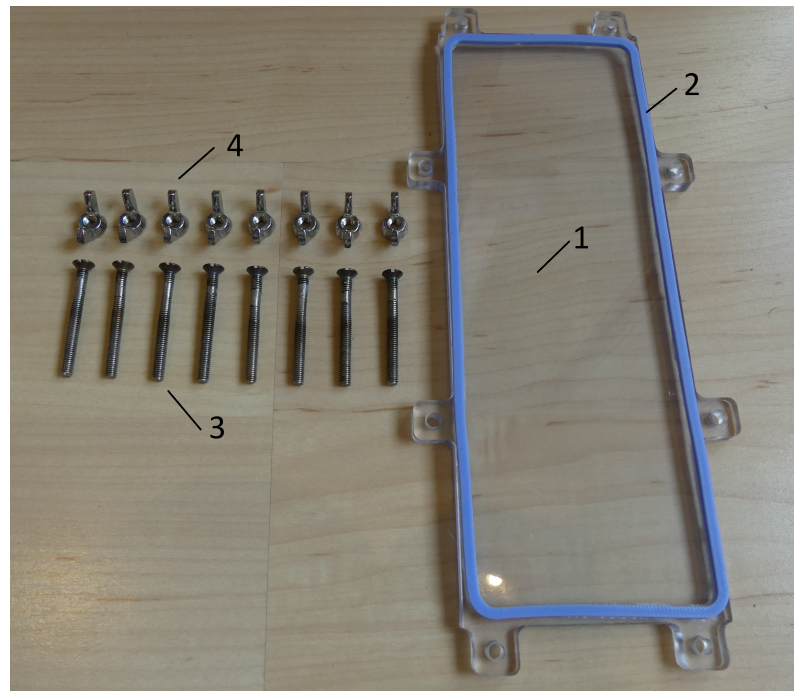


Figure G.10: 10th sub-assembly : Bioreactor lid assembly.

## Assembly of the bioreactor chamber (figure G.11 to G.34)

The assembly of the bioreactor chamber can be achieved by following the next 6 parts sequentially.

### Assembly of the 1st and 2nd sub-assembly, namely, the bioreactor frame and the tube connectors. (figure G.1 and G.2)

- Put a seal on a tube connector (figure G.11).
- Insert the tube connector and seal through the bioreactor and tighten the assembly using the M4 nut. The seal is placed inside the bioreactor chamber (figure G.12 and G.13).
- Repeat the operation for the three tube connectors (see figure G.13).



Figure G.11: Seal on the tube connector.

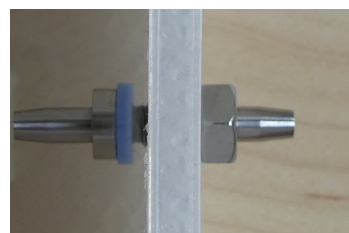


Figure G.12: Tightening of the tube connector.



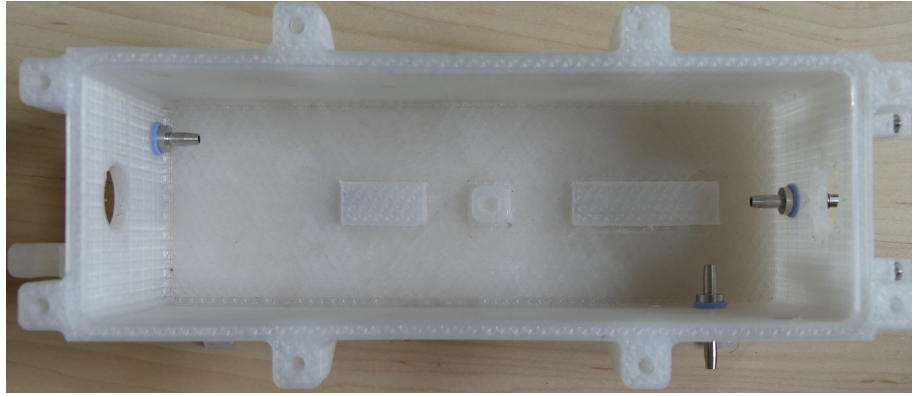


Figure G.13: Top view of the bioreactor chamber with the fixation of the three tube connectors.

**Add the 3rd part, the supports. (figure G.3)**

- Clip the lip support, the smallest one, as pictured in the figure G.14.
- Clip the catheter support as pictured in the figure G.14.

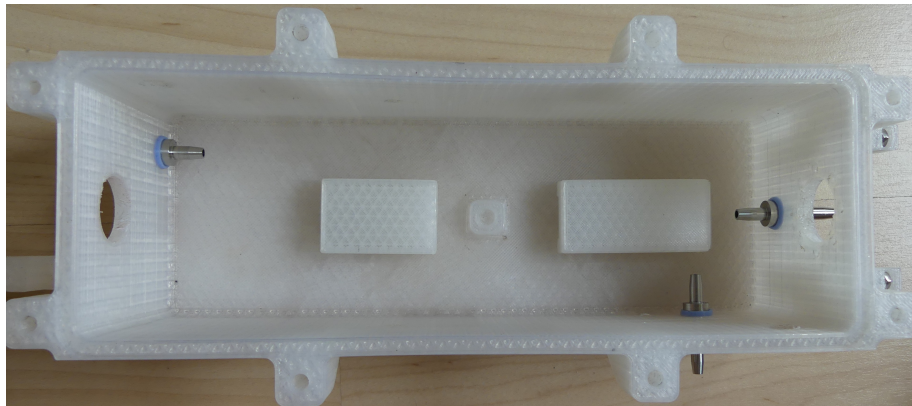


Figure G.14: Top view of the bioreactor chamber with the fixation of both supports.

**Add the 4th part, The connection system to the actuator shaft. (figure G.4)**

- Insert the cone-shaped nylon piece on the actuator shaft. (figure G.15)
- Insert the finger glove on the shaft and the cone-shaped sleeve. (figure G.16)
- Insert and compress the system through the bioreactor. Make sure avoiding creases when compressing the cone-shaped nylon piece in the bioreactor. (figure G.17)
- Block the cone-shaped nylon piece with the prismatic pin. (figure G.18)
- Insert the Intermediate graft attachment piece on the head of the actuator shaft. Use the flat spot of the head to lock these two parts together. (figure G.18)
- Put the nut on the threaded side of the shaft in order to avoid losing it.

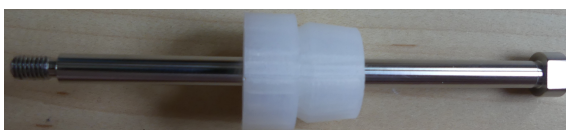


Figure G.15

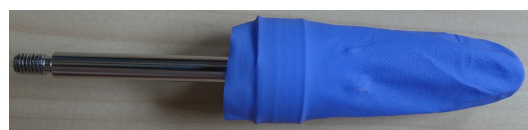


Figure G.16

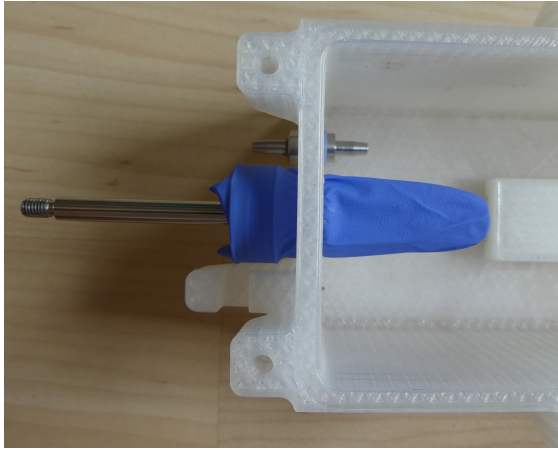


Figure G.17

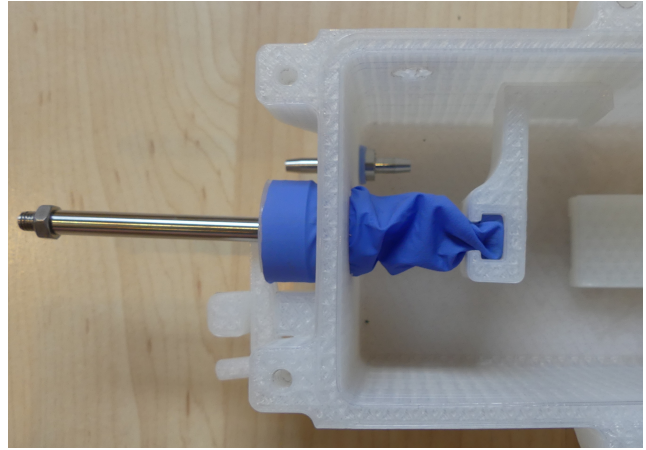


Figure G.18

**Add the 5th part, the electrodes. (figure G.5)**

- Insert a seal on each electrode. (figure G.19)
- Insert the electrodes through the bioreactor wall, and tighten the assembly using with the fastening components with integrated nut at the outside of the bioreactor wall. (figure G.20)
- The electrodes are asymmetric. the longest horizontal distance must be placed toward the linear actuator shaft. The longest vertical distance must be placed downward.

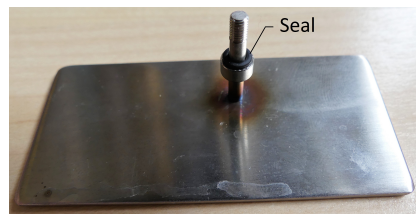


Figure G.19: Electrode with seal.

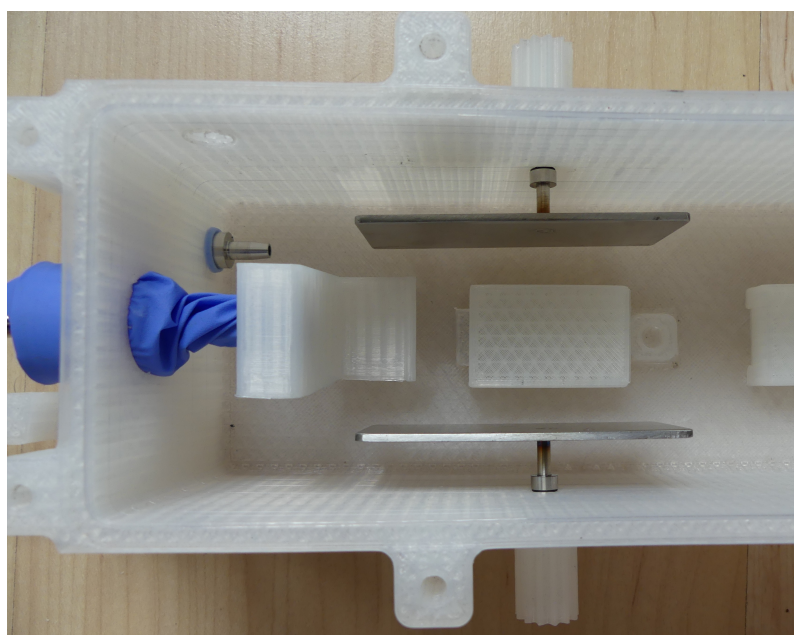


Figure G.20: Top view of the bioreactor chamber with the electrode assembly.

**Add the 6th part, the membrane attachment system. (figure G.6)**

- Insert a washer on each screw. (figure G.21)
- Insert a little seal on each screw. (figure G.21)
- Insert the 8 screws through the bioreactor wall by the inside. (figure G.22)
- Insert the membrane on the 8 screws at the outside of the bioreactor. (figure G.23)
- Insert the metallic ring on the membrane. (figure G.23)
- Tighten the assembly by using the 8 nuts. (figure G.23)



Figure G.21

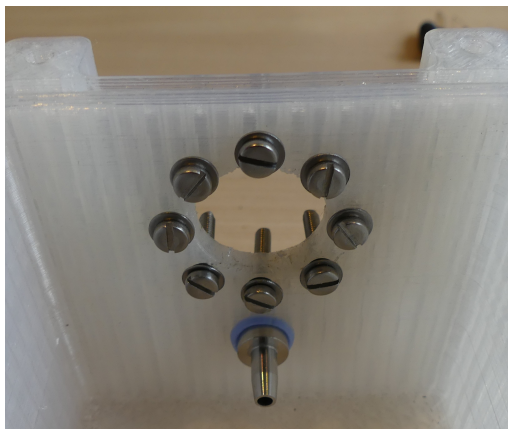


Figure G.22

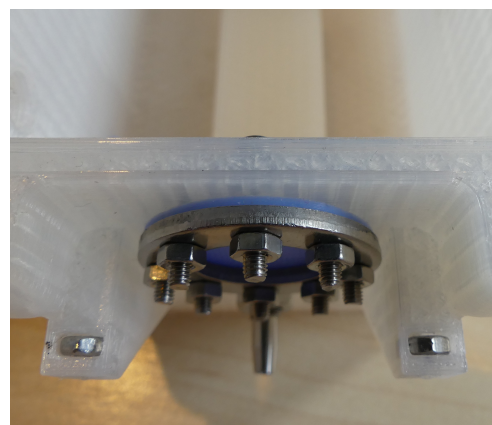


Figure G.23

**Add the 7th part, the connection system to the sensor shaft. (figure G.7)**

- Insert the ball in its location inside the bioreactor. (figure G.24)
- Insert the sensor shaft head in the intermediate graft attachment piece. (figure G.25)
- Insert a washer and a nut on the sensor shaft. (figure G.25)
- Screw the nut on the intermediate piece. (figure G.25)
- Insert a nut and then a washer on the shaft sensor. (figure G.25)
- Insert the sensor shaft through the membrane. (figure G.26)
- Screw the sensor compressor on the sensor shaft (7 to 10 turns). Do not screw it too much, it can damage the thread. (figure G.26)
- Using a pair of pliers, screw the nut on the membrane at the inside of the bioreactor. (figure G.27)
- Place the intermediate piece on the ball. If everything is well tightened, the assembly should be rigid.



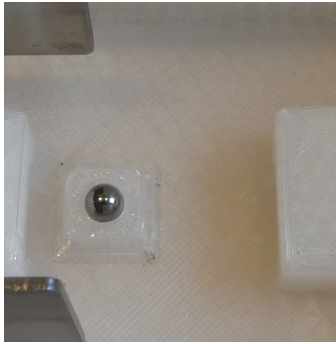


Figure G.24

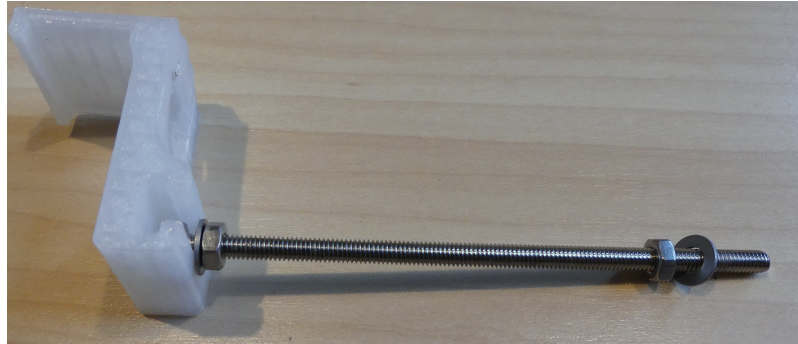


Figure G.25



Figure G.26



Figure G.27

**Add the 8th part, the sensor support. (figure G.8)**

- Insert 2 nuts in the slots of the bioreactor. (figure G.28)
- Insert the flat metallic piece through the sensor compressor. (figure G.29)
- Insert 2 screws through the holes of the flat metallic piece. (figure G.29)
- Insert the last 2 nuts on the screws. (figure G.29)
- Screw a bit the screws in the nuts placed in the bioreactor frame.

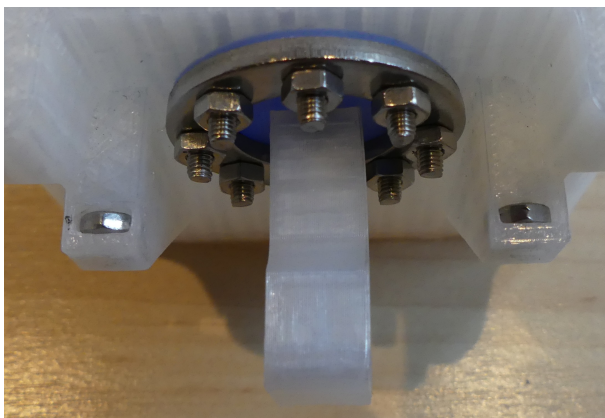


Figure G.28

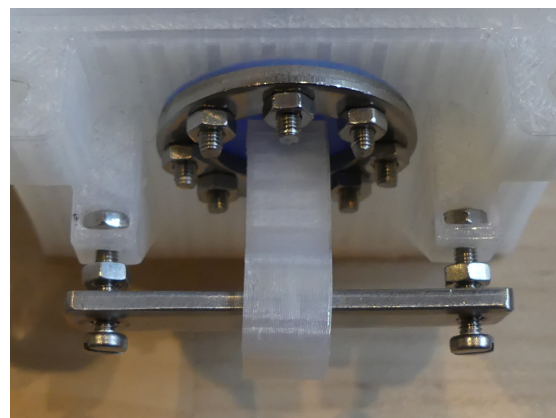


Figure G.29

**Add the 9th part, the vents. (figure G.9)**

- Screw both vents on the bioreactor. (figure G.30)

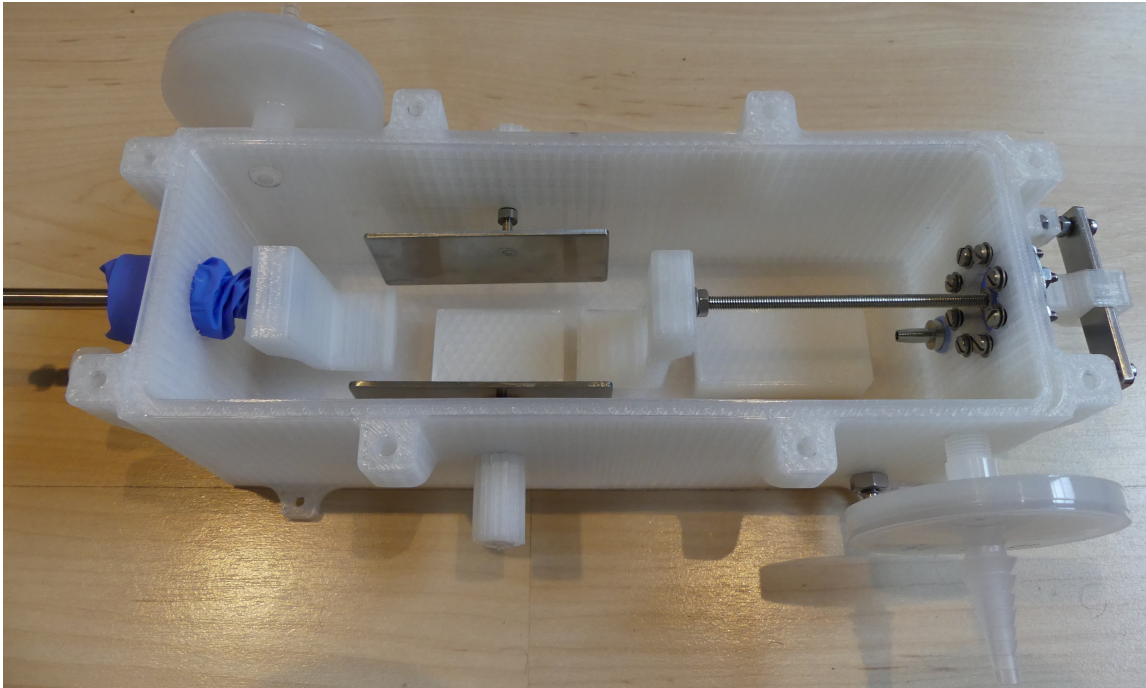


Figure G.30

**Fix the half-lip in the bioreactor chamber and connect the tubes to the tube connectors**

- Fix the half-lip on the 2 removable graft attachment piece of figure in its extremities. (figure G.31)
- Clip the removable attachment piece of the artery side to the sensor intermediate attachment piece. The artery should be placed inside the hole of the intermediate piece. (figure G.32)
- Connect the artery to the 3-way connector and then to the tube connectors. (figure G.32)
- Clip the second removable graft attachment piece to the actuator intermediate attachment piece.
- Connect all the tubes to the tube connectors.
- Check if all the screws and nuts are well tightened.
- Fill the bioreactor with the culture medium.

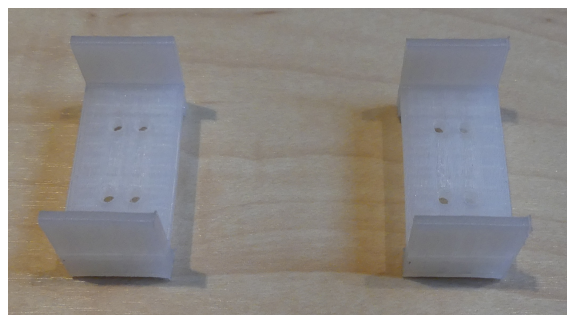


Figure G.31





Figure G.32

**Add the 10th part, the lid of the bioreactor. (figure G.9)**

- Put the large seal in its groove on the top of the bioreactor. (figure G.33)
- Put the lid on the top of the seal. (figure G.34)
- Insert the 8 screws from below and tighten the lid by using the 8 wing nuts. (figure G.34)

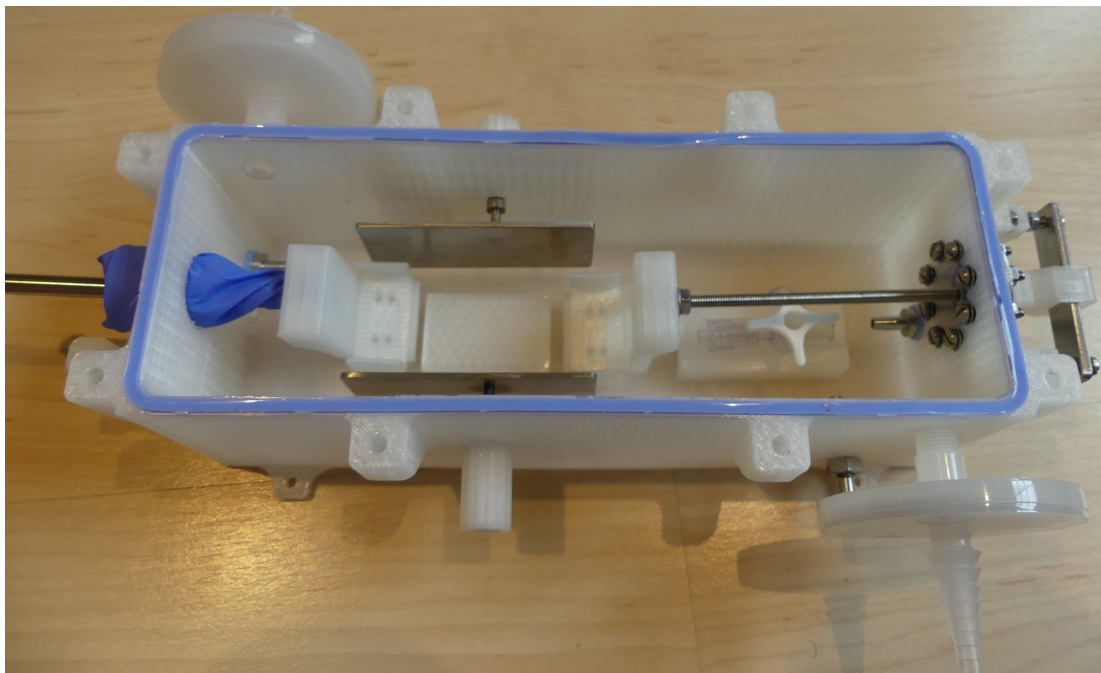


Figure G.33

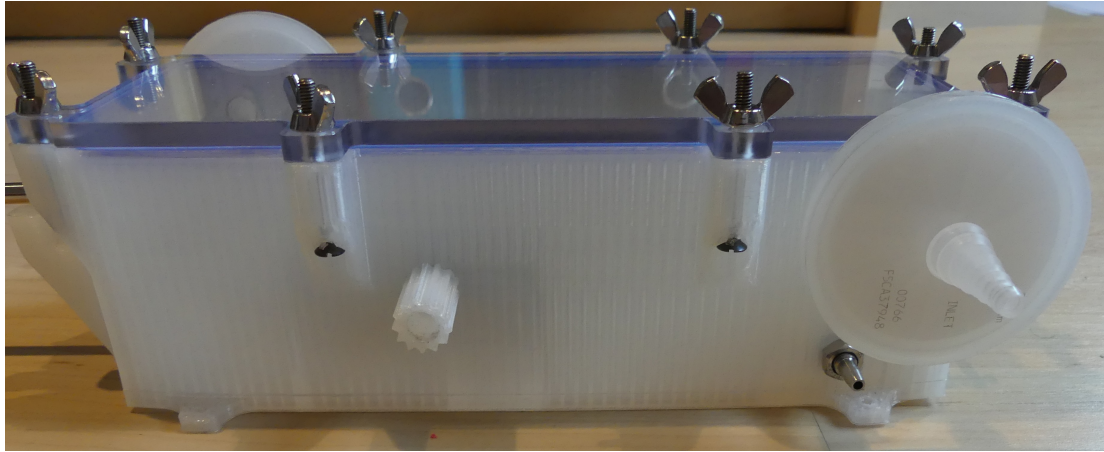


Figure G.34

### Material for the assembly of the stimulation device (Figure G.35 to G.40)

The assembly of the stimulation device is split into 6 parts. All the components of these 6 parts must not be autoclaved.

- 1 : 1x Bioreactor chamber

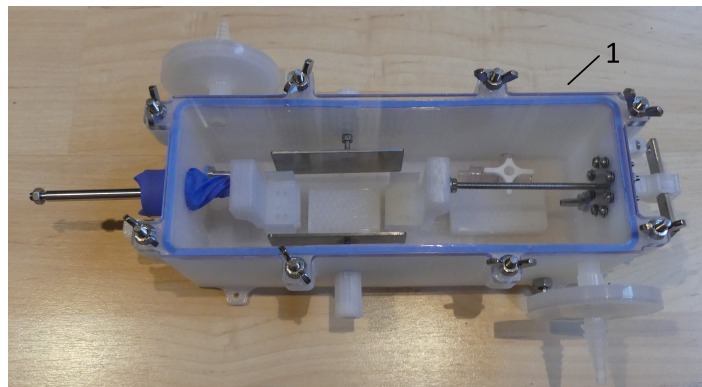


Figure G.35: 1st part : The assembled bioreactor chamber.

- 1 : 1x Plate



Figure G.36: 2nd part : plate.



- 1 : 4x Connection screw (M4)
- 1 : 4x Connection nut (M4)

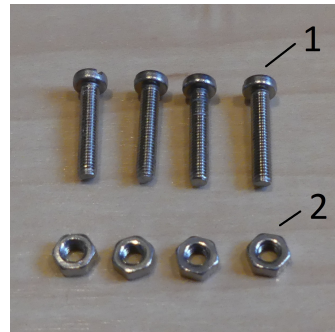


Figure G.37: 3rd sub-assembly : Fixation of the bioreactor chamber on the plate.

- 1 : 1x Sensor
- 2 : 1x Sensor support
- 3 : 2x Sensor screw
- 4 : 1x Sensor blocking piece
- 5 : 2x Sensor wing nut

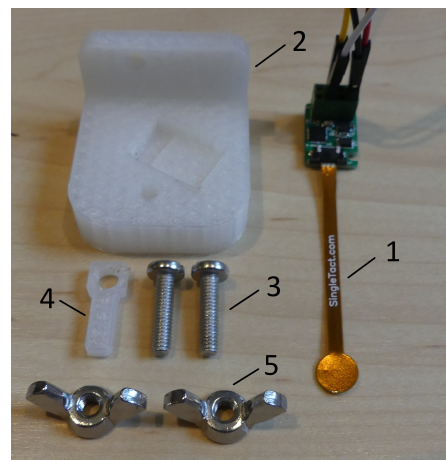


Figure G.38: 4th sub-assembly : Sensor fixation on the plate.

- 1 : 1x Linear actuator
- 2 : 1x Actuator support
- 3 : 1x Actuator blocking piece
- 4 : 4x Actuator screw
- 5 : 2x Actuator nut
- 6 : 2x Actuator wing nut
- 7 : 1x Actuator-to-shaft junction
- 8 : 2x Actuator big nut

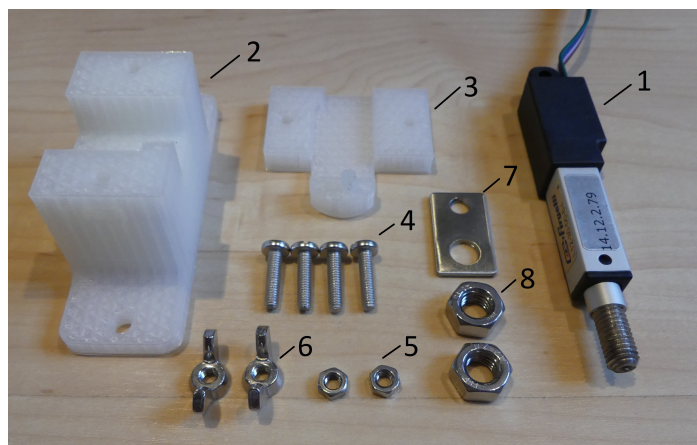


Figure G.39: 5th part : Actuator fixation on the plate.

- 1 : 2x Banana plug receiver

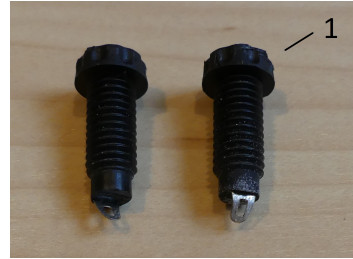


Figure G.40: 6th part : Plug receiver.

## **Assembly of the stimulation device (figure G.1 to G.10)**

The assembly will be achieved by following the next 4 parts sequentially.

### **Assembly of the first three parts, the bioreactor and the plate using the screws. (figure G.35,G.36 and G.37)**

- Put the bioreactor on the plate.
- Make sure the actuator shaft is on the left side of the platter if the platter is placed as shown on the figure G.36.
- Insert the 4 screw on the slots and tighten the bioreactor to the plate using the 4 nuts. (Figure G.41)



Figure G.41

### **Assembly and placement of the sensor elements. (Figure G.38)**

- Insert the sensor in the sensor support. (Figure G.42)
- Use the sensor blocking piece with a screw and a wing nut to fix the sensor. (Figure G.43)
- Place the sensor assembly on the plate on the side with one notch. (Figure G.44)

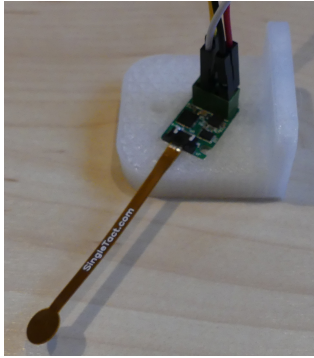


Figure G.42

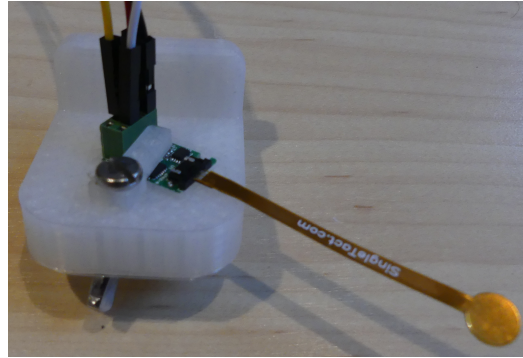


Figure G.43

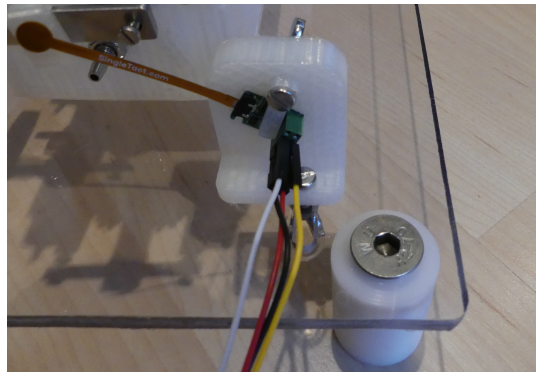


Figure G.44: Fixation of the sensor on the plate.

#### **Assembly and placement of the linear actuator elements. (Figure G.39)**

- Insert the actuator in the actuator blocking piece. (Figure G.45)
- Insert 2 actuator nuts inside the slots of the blocking piece. (Figure G.46)
- Position the assembly on the actuator support and tighten the assembly using two screws. (Figure G.46)
- Screw a big nut on the threaded part of the shaft of the actuator. (Figure G.47)
- Insert on the shaft threaded part the actuator-to-shaft junction. (Figure G.47)
- Screw the second big nut on the actuator-to-shaft junction and position this junction with the left hole to the top. (Figure G.47)
- Using the 2 screws and the 2 wing nuts, position the obtained assembly on the plate. Don't tighten them now. (Figure G.48)



Figure G.45



Figure G.46

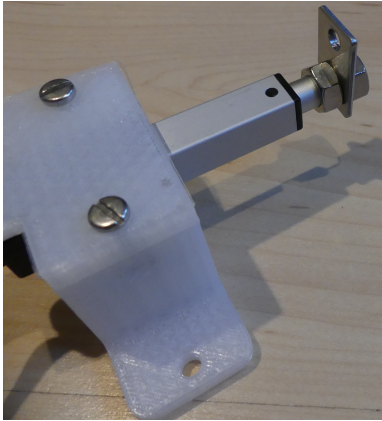


Figure G.47

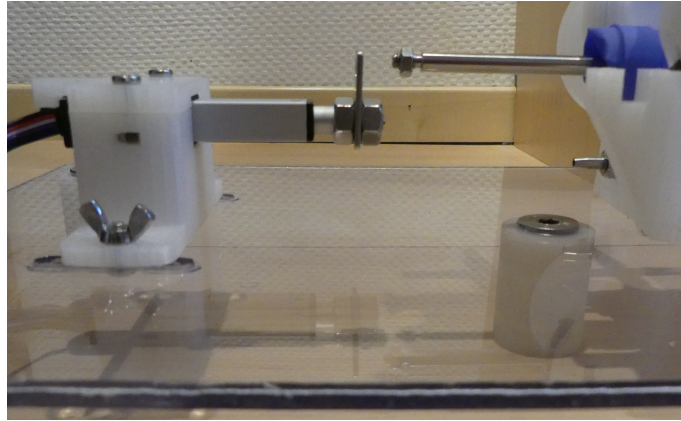


Figure G.48

**Add the banana plug receiver to build the connection with the pulse generator. (Figure G.40)**

- Screw the 2 banana plug receivers inside the fastening component with integrated nut until their tip reach the electrodes tips (Figure G.49). The electrical connection can be checked using a multimeter.

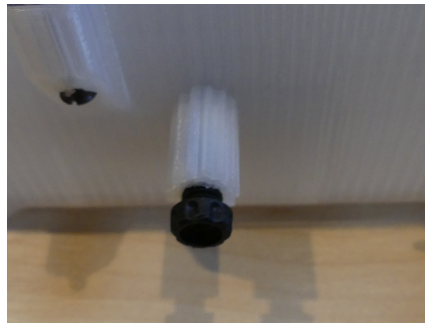


Figure G.49

The final assembly is depicted on the figure G.50.

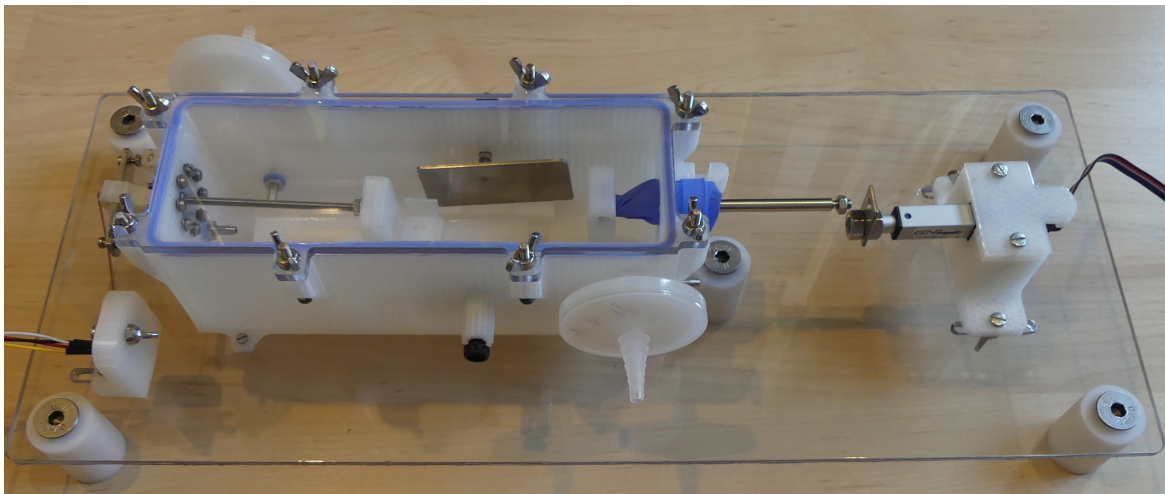


Figure G.50

**Connecting the stimulation devices and begin the stimulation**

**Material:** (figure G.51)



- 1: Plate with the bioreactor chamber, the sensor and the actuator fixed.
- 2: Mechanical control box.
- 3: 5V USB cable (only if data need to be stored on the computer).
- 4: 12V transformer.

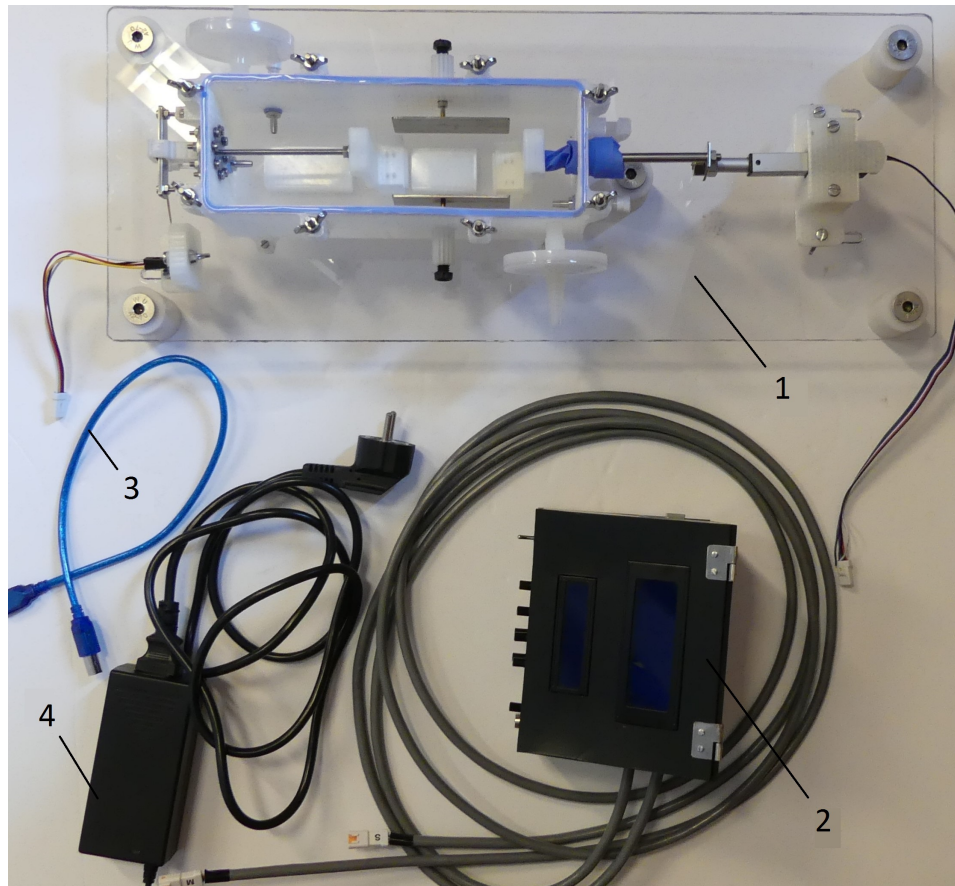


Figure G.51: Material used in order to launch the mechanical stimulation

#### Assembly:

- Connect the cables of the linear actuator and force sensor with the cables of the mechanical control box. "M" for the actuator, and "S" for the sensor.
- Set the switch button to OFF position.
- Power the device by plugging the 12V transformer in the mechanical control box.

Before powering up the device, make sure having connected the linear actuator and the sensor to proper cables! "M" for the actuator, and "S" for the sensor. Inverting these two cables will permanently damage the linear actuator and the force sensor. If the force sensor is already in contact with the bioreactor, remove it. The 5V power supply cannot be used for the stimulation because the actuator is powered with 6V obtained from the 12V transformer.

**⚠ Never plug the 5V USB cable when the stimulation is running or if the graft is attached to the linear actuator. Plugging the 5V USB cable will reset the arduino and the signals. The actuator will therefore retract entirely, causing an over-stretching of the graft.**

In order to additionally use the electrical stimulation device, refer to the appendix E.

### Parameters for the stimulation:

As pictured on the figure G.52, the mechanical stimulation control box is composed of 7 rotating potentiometers. The four potentiometers on the top are used to select the contraction parameters. The two buttons below are used to select the stimulation mode and to set the specific parameter of this mode. The last rotating button is used to switch to one or another screen window. A reminder of all the button functions as well as their ranges are listed in the table G.1.

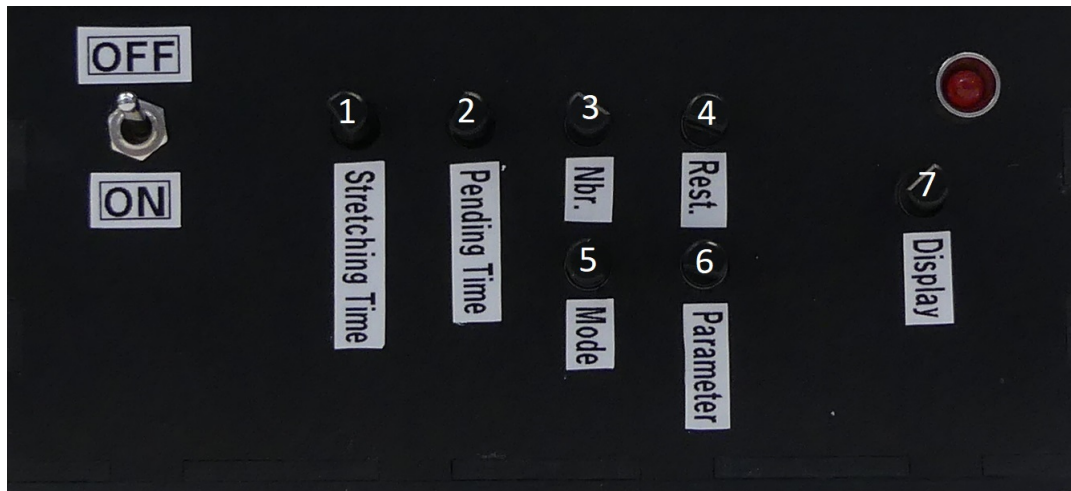


Figure G.52: Rotating buttons allowing the parameters selection.

| Button               | Utility                                                      | Unit | Min  | Max   | Step |
|----------------------|--------------------------------------------------------------|------|------|-------|------|
| ① Stretching time :  | Duration of stretch                                          | s    | 0.5  | 15    | 0.1  |
| ② Pending time :     | Duration between stretches                                   | s    | 2    | 30    | 1    |
| ③ Nbr. :             | Number of stretches per set                                  | -    | 1    | 10    | 1    |
| ④ Rest. :            | Time between two set of stretches                            | s    | 5    | 120   | 1    |
| ⑤ Mode               |                                                              |      |      |       |      |
| • Manual :           | Manual : control the position of the actuator shaft manually |      |      |       |      |
| • Electrical :       | Control to ensure zero force during electrical stimulation   |      |      |       |      |
| • Position :         | Control by imposing a stretch length                         |      |      |       |      |
| • Force :            | Control by imposing a stretch force                          |      |      |       |      |
| ⑥ Parameter          |                                                              |      |      |       |      |
| • Position :         | Position of the shaft                                        | mm   | 0    | 30    | 0.1  |
| -                    | Zero force control                                           | -    | -    | -     |      |
| • Length :           | Length to stretch from reference position                    | mm   | 0    | 15    | 0.1  |
| • Force :            | Force to apply from reference position                       | N    | 0    | 3     | 0.01 |
| ⑦ Screen parameter : | Switch the screen window                                     | -    | left | right | -    |

Table G.1: Button functions and ranges.

### Steps to launch the stimulation

- Check if the switch is on the OFF position.
- Put the head of the sensor inside the sensor compressor of figure G.7. (Figure G.54)
- Fix the first 4 stimulation parameter (1)(2)(3)(4) of table G.1.
- Turn the mode button (5) until the manual mode is selected.
- Turn the parameter button (6) to match the positions on both screens. (⚠ Important to not stretch too much the graft)
- Turn ON the switch button.
- Extend the actuator and connect the two shaft by screwing the nut. (Figure G.53)
- When they are connected, fix the support of the actuator by tightening the knurled nuts.
- Turn the parameter button (6) to ensure that almost a zero force is measured by the sensor.
- Turn OFF the switch button. The last position of the shaft is then saved as reference. After each stimulation, the shaft will go back to this position.
- Turn the mode button (5) to choose the stimulation mode.
- Turn the parameter button (6) to impose the length or the force of the stimulation.
- Check if all the parameters are correct.
- Turn ON the switch button in order to launched the desired stimulation.

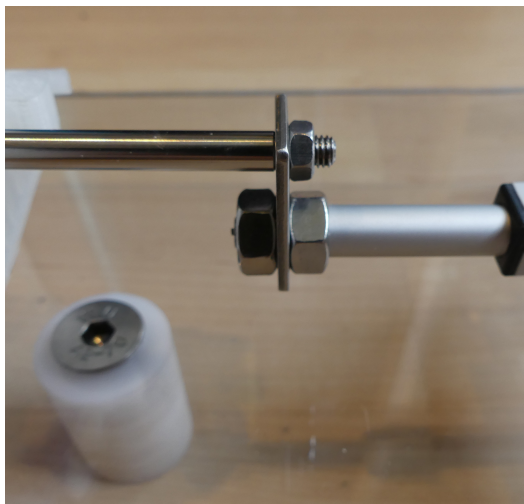


Figure G.53: Attachment of both shafts.



Figure G.54: Sensor head placement.



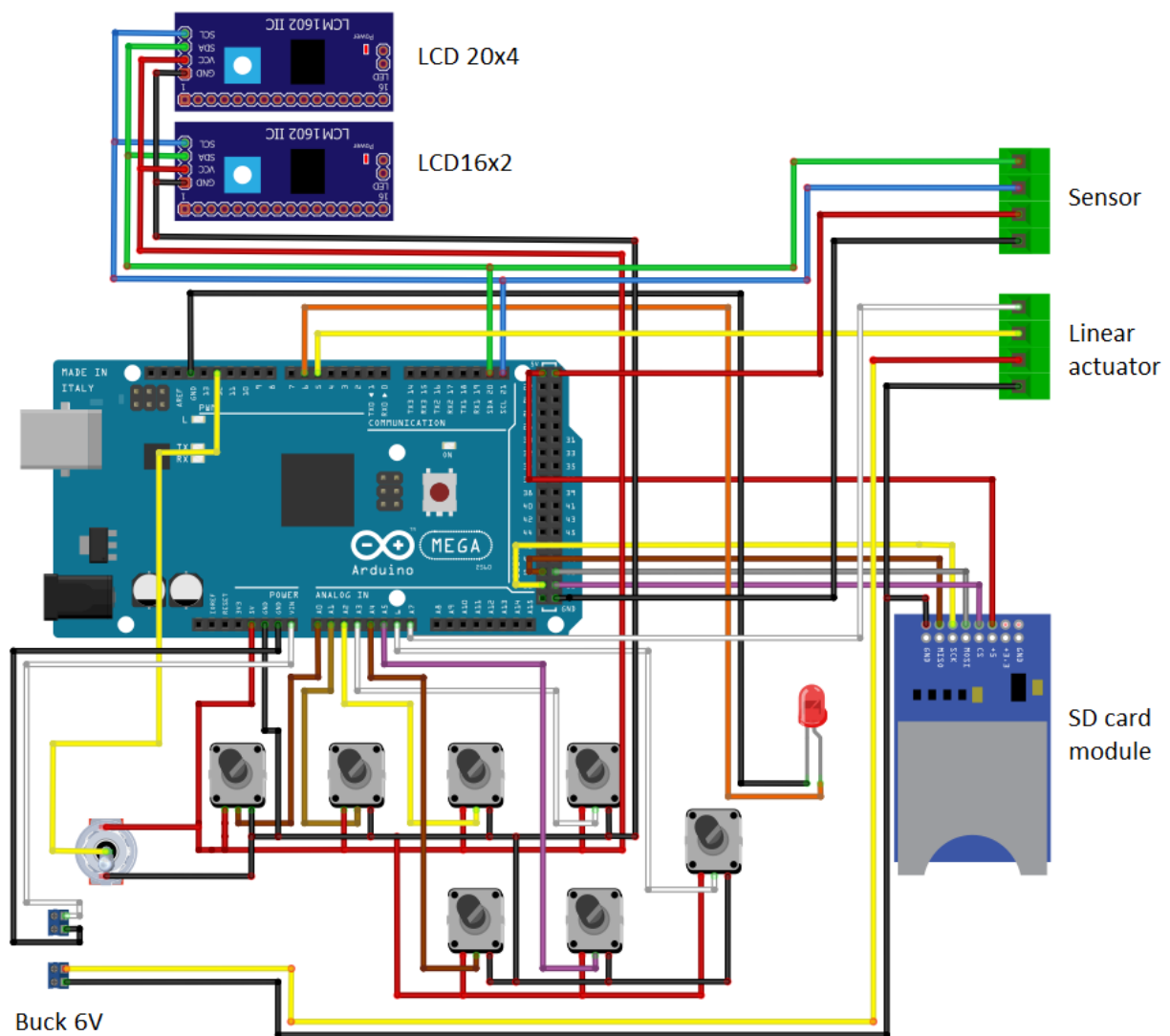


Figure G.55: Wire configuration for the mechanical stimulation (using Fritzing).

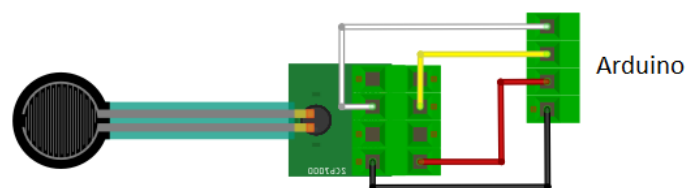


Figure G.56: Wire configuration for the sensor (using Fritzing).

| Pin | Utility                                                    | Wire colour |
|-----|------------------------------------------------------------|-------------|
| 4   | PWM controlling the motor position                         | Yellow      |
| 6   | Signal for the secure LED                                  | Orange      |
| 12  | State of the switch                                        | Yellow      |
| 20  | SDA : I <sup>2</sup> C communication for the 20x4 LCD      | Green       |
|     | SDA : I <sup>2</sup> C communication for the 16x2 LCD      | Green       |
|     | SDA : I <sup>2</sup> C communication for the force sensor  | Green       |
| 21  | SCL : I <sup>2</sup> C communication for the 20x4 LCD      | Blue        |
|     | SCL : I <sup>2</sup> C communication for the 16x2 LCD      | Blue        |
|     | SCL : I <sup>2</sup> C communication for the force sensor  | Blue        |
| 50  | MISO for the SD card module                                | Brown       |
| 51  | MOSI for the SD card module                                | Grey        |
| 52  | SCK for the SD card module                                 | Yellow      |
| 53  | CS for the SD card module                                  | Purple      |
| GND | Ground for the 20x4 LCD                                    | Black       |
|     | Ground for the 16x2 LCD                                    | Black       |
|     | Ground for the linear motor                                | Black       |
|     | Ground for the force sensor                                | Black       |
|     | Ground for the switch                                      | Black       |
|     | Ground for the PCB (all potentiometers)                    | Black       |
|     | Ground for the LED                                         | Black       |
|     | Ground for the SD card module                              | Black       |
| 5V  | Power supply for the 20x4 LCD                              | Red         |
|     | Power supply for the 16x2 LCD                              | Red         |
|     | Power supply for force sensor                              | Red         |
|     | Power supply for the switch                                | Red         |
|     | Power supply for the PCB (all potentiometers)              | Red         |
|     | Power supply for the SD card module                        | Red         |
| Vin | Power supply for the 6V buck                               | White       |
| A1  | Analog read of the contractions duration potentiometer     | Brown       |
| A2  | Analog read of the time between contractions potentiometer | Brown       |
| A3  | Analog read of the number of contractions potentiometer    | Yellow      |
| A4  | Analog read of the time between sets potentiometer         | White       |
| A5  | Analog read of the mode potentiometer                      | Brown       |
| A6  | Analog read of the value for the mode potentiometer        | Purple      |
| A7  | Analog read of the screen potentiometer                    | White       |
| A8  | Analog read of the force feedback potentiometer            | White       |

Table G.2: Wire configuration for the sensor: caption.

## Appendix H

# Java code: Interface for the calculation of the submersible electrode surface

```
// Generates the first two windows.
// On the second window, you can set the parameters of the stimulation.
import java.awt.FlowLayout;
import javax.swing.ImageIcon;
import java.awt.event.ActionEvent;
import java.awt.event.ActionListener;
import javax.swing.JButton;
import javax.swing.JFrame;
import java.awt.Label;
import javax.swing.JLabel;
import javax.swing.JOptionPane;
import java.awt.Dimension;
import java.awt.image.BufferedImage;
import javax.imageio.ImageIO;
import java.io.File;
import java.awt.Image;
import java.awt.Graphics2D;
import java.awt.RenderingHints;

public class Fenetre extends JFrame {
    private JButton bouton = new JButton("Begin calculation");
    public Fenetre(){

        this.setTitle("Calcul");
        this.setSize(500, 400);
        this.setLocationRelativeTo(null);
        this.setDefaultCloseOperation(JFrame.EXIT_ON_CLOSE);
        this.setLocationRelativeTo(null);
        this.getContentPane().setLayout(new FlowLayout());
        ImageIcon icon = new ImageIcon(new
            ImageIcon("h3.jpg").getImage().getScaledInstance(350, 300,
            Image.SCALE_DEFAULT));
        JLabel h3 = new JLabel(icon);
        this.getContentPane().add(h3);
        this.getContentPane().add(bouton);
        bouton.addActionListener(new ActionListener(){
            public void actionPerformed(ActionEvent arg0) {
                ZDialog zd = new ZDialog(null, "", true);
                ZDialogInfo zInfo = zd.showZDialog();
            }
        });
        this.setVisible(true);
    }
}
```

```

public static void main(String[] main){
    Fenetre fen = new Fenetre();
    JFrame snakeFrame = new JFrame();
    fen.setBounds(100, 200, 800, 800);
    fen.setVisible(true);
    fen.pack();
}
}

// Compute the minimum heights of the electrodes to dive in the media.
// The computation is based on the values encoded on the window of the previous
part.
public class ZDialogInfo {
    private double amplitude, pulse_duration, distance;
    public ZDialogInfo(){}
    public ZDialogInfo(double first, double second, double third){
        this.amplitude = first;
        this.pulse_duration = second;
        this.distance = third;
    }

    public String toString(){

        String str;
        str = "Minimum horizontal height is " + calcul()[0] + "cm" + "\n\n";
        str += "Minimum vertical height is " + calcul()[1] + "cm" + "\n\n";

        return str;
    }

    public String toString1(){
        // Result of the first height computation
        String str;
        str = "Height(H1) minimum is " + calcul()[0] + "cm" + "\n\n";

        return str;
    }

    public String toString2(){
        // Result of the second height computation
        String str;
        str = "      Height(H2) minimum is " + calcul()[1] + "cm" + "\n\n";

        return str;
    }

    public double[] calcul(){
        // Interpolations coefficients of the 4th (distance : 2,3,4,5,6,7 cm)
        double a21 = -0.0001341978;
        double a22 = 0.0016406296;
        double a23 = -0.0016553131;
        double a24 = -0.0017065253;
        double a25 = 0.0029438072;

        double a31 = 0.0000677430;
        double a32 = -0.0013217477;
        double a33 = 0.0119679822;
        double a34 = -0.0252243669;
        double a35 = 0.0153672621;

        double a41 = 0.0001115492;

```

```

double a42 = -0.0018869802;
double a43 = 0.0138137952;
double a44 = -0.0272007263;
double a45 = 0.0159544186;

double a51 = 0.0001301793;
double a52 = -0.0021479872;
double a53 = 0.0145345994;
double a54 = -0.0278870330;
double a55 = 0.0160209094;

double a61 = 0.0000435641;
double a62 = -0.0009119140;
double a63 = 0.0083633405;
double a64 = -0.0168309269;
double a65 = 0.0101453440;

double a71 = 0.0001405403;
double a72 = -0.0023488143;
double a73 = 0.0147781434;
double a74 = -0.0277804090;
double a75 = 0.0158030118;

double dec = 100*this.distance;
int count = 0;
while(dec > 100){
    dec = dec-100;
    count++;
}
double up = dec;
double down = 100-dec;

// Interpolations.
double I2 = a21*Math.pow(this.amplitude,4) + a22*Math.pow(this.amplitude,3) +
    a23*Math.pow(this.amplitude,2) + a24*(this.amplitude) + a25;
double I3 = a31*Math.pow(this.amplitude,4) + a32*Math.pow(this.amplitude,3) +
    a33*Math.pow(this.amplitude,2) + a34*(this.amplitude) + a35;
double I4 = a41*Math.pow(this.amplitude,4) + a42*Math.pow(this.amplitude,3) +
    a43*Math.pow(this.amplitude,2) + a44*(this.amplitude) + a45;
double I5 = a51*Math.pow(this.amplitude,4) + a52*Math.pow(this.amplitude,3) +
    a53*Math.pow(this.amplitude,2) + a54*(this.amplitude) + a55;
double I6 = a61*Math.pow(this.amplitude,4) + a62*Math.pow(this.amplitude,3) +
    a63*Math.pow(this.amplitude,2) + a64*(this.amplitude) + a65;
double I7 = a71*Math.pow(this.amplitude,4) + a72*Math.pow(this.amplitude,3) +
    a73*Math.pow(this.amplitude,2) + a74*(this.amplitude) + a75;

double[] liste ={I2, I3, I4, I5, I6, I7};

double Imax = 0;
// Imax correspond to the distance incoded. If the distance is between two
// reference distances, we compute Imax with weights
// defined by the decimal value. ( Imax[2.6cm] = 60% Imax[3cm] + 40%
// Imax[2cm] )
if(up == 100){
    Imax = liste[count-1];
}
else{
    Imax = liste[count-1]*up/100 + liste[count-2]*down/100;
}

// D316Lss = 40  $\mu$ C/cm2
double D = 0.00004;

```

```

// Computation of the areas base on the dimentions of the electrodes.
// The quarter circles are approximated by triangles if the distance is below
// the radius of the circles.
// If the heigth is higher then the radius of the circles, the are of the
// circles is computed (AreaRef)
double AreaRef1 = 0.94 + (26 + Math.sqrt(2.0))/5;
double AreaRef2 = 0.54 + (16 + Math.sqrt(2.0))/5;

double Area = Imax*this.pulse_duration/(2000*D);

double h1 = 0;
double h2 = 0;

if(Area > AreaRef1){
    h1 = ((Area-AreaRef1)/10.8) + 0.5;
}
else{
    h1 = 5*(Area-0.94)/(52 + 2*Math.sqrt(2.0));
}
h1 = Math.round(h1*1.1*100);
h1 = h1/100;

if(Area > AreaRef2){
    h2 = ((Area-AreaRef2)/5.8) + 0.5;
}
else{
    h2 = 5*(Area-0.54)/(32 + 2*Math.sqrt(2.0));
}
h2 = Math.round(h2*1.1*100);
h2 = h2/100;

double[] height = {h1,h2};
return height;
}
}

```

```

// Generate the last window with the results of the computation.

```

```

import java.awt.FlowLayout;
import javax.swing.ImageIcon;
import java.awt.event.ActionEvent;
import java.awt.event.ActionListener;
import javax.swing.JButton;
import javax.swing.JFrame;
import java.awt.Label;
import javax.swing.JLabel;
import javax.swing.JOptionPane;
import java.awt.Dimension;
import java.awt.image.BufferedImage;
import javax.imageio.ImageIO;
import java.io.File;
import java.awt.Image;
import java.awt.Graphics2D;
import java.awt.RenderingHints;

public class Fenetre2 extends JFrame {
    private JButton bouton = new JButton("Begin computation");
    public Fenetre2(){
        this.getContentPane().setLayout(null);
        this.setTitle("Result");
        this.setSize(300, 100);
        this.setDefaultCloseOperation(JFrame.EXIT_ON_CLOSE);
        this.setLocationRelativeTo(null);
    }
}

```



```

        this.setVisible(true);
    }
}

// Code linking all the parts together.
import java.awt.BorderLayout;
import java.awt.Color;
import java.awt.Dimension;
import java.awt.event.ActionEvent;
import java.awt.event.ActionListener;
import javax.swing.BorderFactory;
import javax.swing.ImageIcon;
import javax.swing.JButton;
import javax.swing.JComboBox;
import javax.swing.JDialog;
import java.awt.FlowLayout;
import javax.swing.JFrame;
import javax.swing.JLabel;
import javax.swing.JPanel;
import javax.swing.JRadioButton;
import javax.swing.ButtonGroup;
import javax.swing.JTextField;
import java.awt.Dimension;
import java.awt.image.BufferedImage;
import javax.imageio.ImageIO;
import java.io.File;
import java.awt.Graphics;
import java.awt.Image;
import java.awt.image.BufferedImage;
import java.awt.Image;
import java.awt.Graphics2D;
import java.awt.RenderingHints;

public class ZDialog extends JDialog {
    private ZDialogInfo zInfo = new ZDialogInfo();
    private boolean sendData;
    private JLabel firstLabel, secondLabel, thirdLabel, labelicon1, labelicon2;
    private JRadioButton tranche1, tranche2, tranche3, tranche4;
    private JTextField first, second, third;

    public ZDialog(JFrame parent, String title, boolean modal){
        super(parent, title, modal);
        this.setSize(500, 150);
        this.setLocationRelativeTo(null);
        this.setResizable(false);
        this.setDefaultCloseOperation(JDialog.DO_NOTHING_ON_CLOSE);
        this.initComponent();
    }

    public ZDialogInfo showZDialog(){
        this.sendData = false;
        this.setVisible(true);
        return this.zInfo;
    }

    private void initComponent(){

        //Icon
        ImageIcon icon1 = new ImageIcon(new
            ImageIcon("h1.jpg").getImage().getScaledInstance(100, 100,
                Image.SCALE_DEFAULT));
        labelicon1 = new JLabel(icon1);
        JPanel panIcon1 = new JPanel();

```

```

panIcon1.setBackground(Color.white);
panIcon1.setLayout(new BorderLayout());
panIcon1.add(labelicon1);
panIcon1.setVisible(false);

ImageIcon icon2 = new ImageIcon(new
    ImageIcon("h2.jpg").getImage().getScaledInstance(100, 100,
        Image.SCALE_DEFAULT));
labelicon1 = new JLabel(icon2);
JPanel panIcon2 = new JPanel();
panIcon2.setBackground(Color.white);
panIcon2.setLayout(new BorderLayout());
panIcon2.add(labelicon1);
panIcon2.setVisible(false);

// First parameter window
JPanel panFirst = new JPanel();
panFirst.setBackground(Color.white);
panFirst.setPreferredSize(new Dimension(120, 90));
first = new JTextField();
first.setPreferredSize(new Dimension(100, 25));
firstLabel = new JLabel("Amplitude[V]");
panFirst.add(firstLabel);
panFirst.add(first);

// Second parameter window
JPanel panSecond = new JPanel();
panSecond.setBackground(Color.white);
panSecond.setPreferredSize(new Dimension(120, 90));
second = new JTextField();
second.setPreferredSize(new Dimension(100, 25));
secondLabel = new JLabel("Pulse Duration[ms]");
panSecond.add(secondLabel);
panSecond.add(second);

// Third parameter window
JPanel panThird = new JPanel();
panThird.setBackground(Color.white);
panThird.setPreferredSize(new Dimension(120, 90));
third = new JTextField();
third.setPreferredSize(new Dimension(100, 25));
thirdLabel = new JLabel("Spacing[cm]");
panThird.add(thirdLabel);
panThird.add(third);

JPanel content = new JPanel();
content.setBackground(Color.white);
content.add(panFirst);
content.add(panSecond);
content.add(panThird);

JPanel control = new JPanel();
JButton okBouton = new JButton("OK");

okBouton.addActionListener(new ActionListener(){
    public void actionPerformed(ActionEvent arg0) {
        zInfo = new ZDialogInfo(getFirst(), getSecond(), getThird());
        setVisible(false);
        JPanel p1 = new JPanel();
        JPanel p2 = new JPanel();
        JPanel p3 = new JPanel();

        // Window with the results

```

```

Fenetre2 fen = new Fenetre2();
fen.setBounds(100, 200, 600, 300);
fen.setVisible(true);

ImageIcon icon1 = new ImageIcon(new
    ImageIcon("hh.jpg").getImage().getScaledInstance(400, 200,
        Image.SCALE_DEFAULT));
JLabel hh = new JLabel(icon1);
p1.add(hh, BorderLayout.NORTH);

String resultat1 = zInfo.toString1();
JLabel texte1 = new JLabel(resultat1);
p1.add(texte1, BorderLayout.SOUTH);
String resultat2 = zInfo.toString2();
JLabel texte2 = new JLabel(resultat2);
p1.add(texte2, BorderLayout.SOUTH);

ImageIcon icon2 = new ImageIcon(new
    ImageIcon("h2.jpg").getImage().getScaledInstance(200, 200,
        Image.SCALE_DEFAULT));
JLabel h2 = new JLabel(icon2);
p1.setBounds(50, 0, 500, 575);
fen.add(p1);
fen.add(p2);
}

public double getFirst(){
    return Double.parseDouble(first.getText());
}
public double getSecond(){
    return Double.parseDouble(second.getText());
}
public double getThird(){
    return Double.parseDouble(third.getText());
}
});

JButton cancelBouton = new JButton("Cancel");
cancelBouton.addActionListener(new ActionListener(){
    public void actionPerformed(ActionEvent arg0) {
        setVisible(false);
        labelicon1.setVisible(true);
        labelicon2.setVisible(true);
    }
});

control.add(okBouton);
control.add(cancelBouton);
this.getContentPane().add(content, BorderLayout.CENTER);
this.getContentPane().add(control, BorderLayout.SOUTH);
}
}

```

## Appendix I

### Detailed views of the bioreactor with mechanical and electrical stimulation included

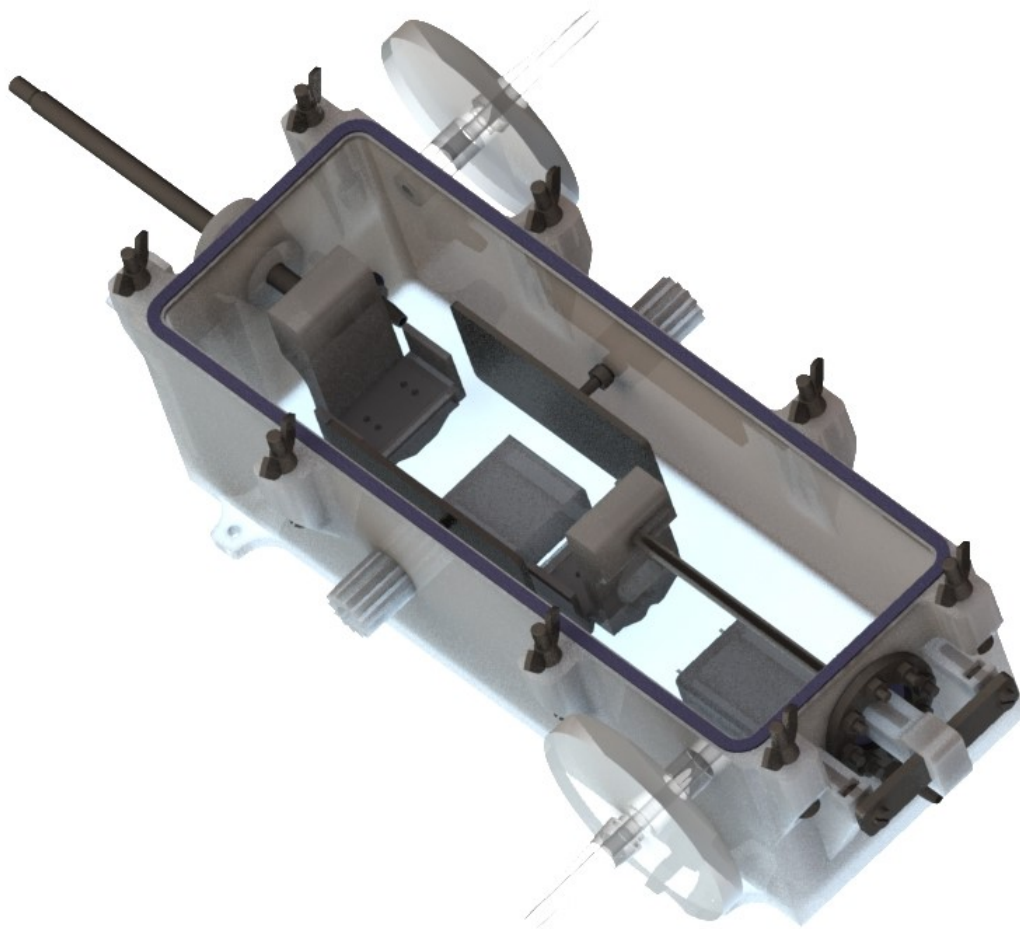


Figure I.1: Bioreactor chamber 3D view (sensor side).

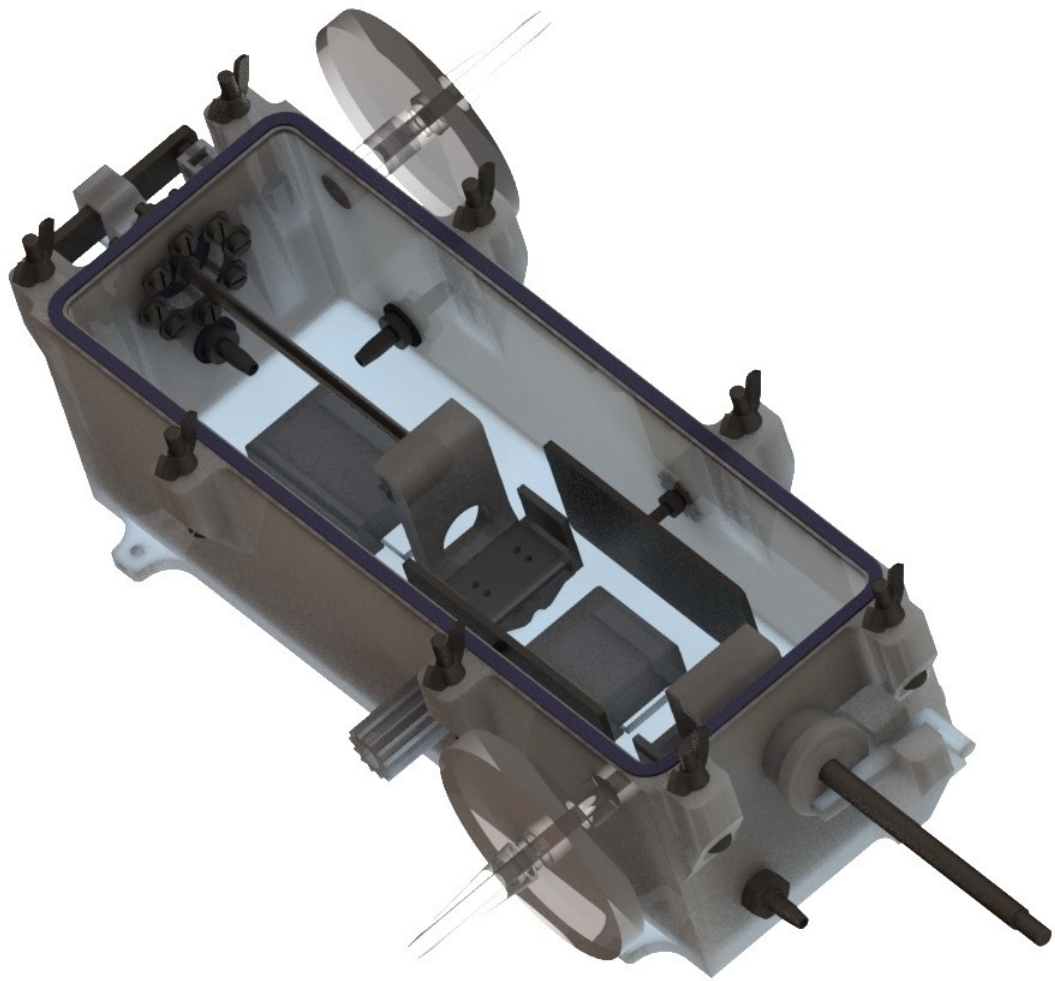


Figure I.2: Bioreactor chamber 3D view (actuator side).

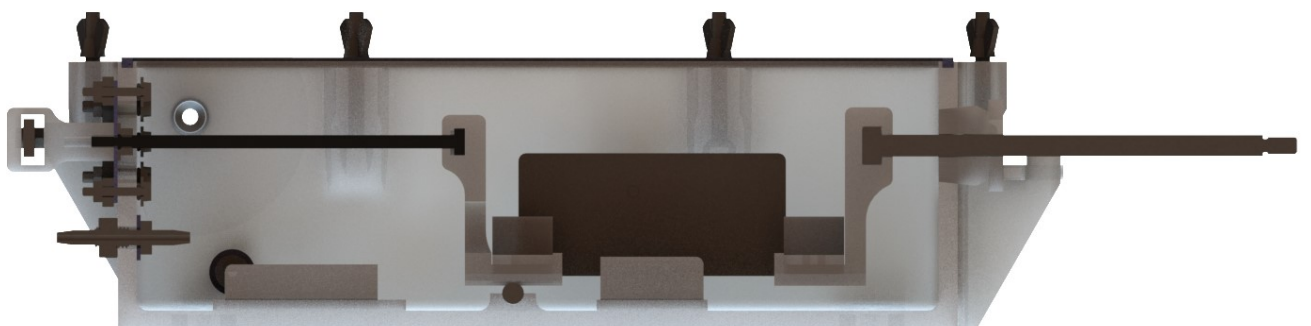


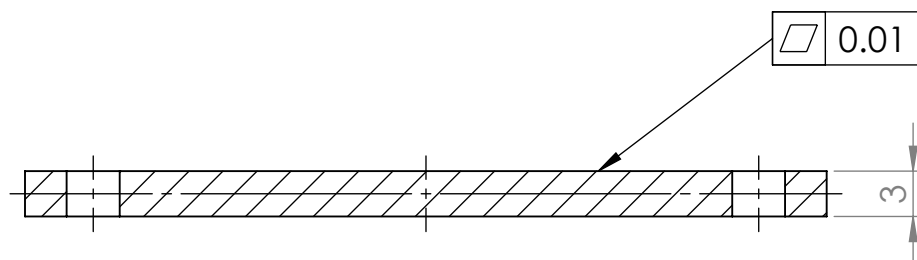
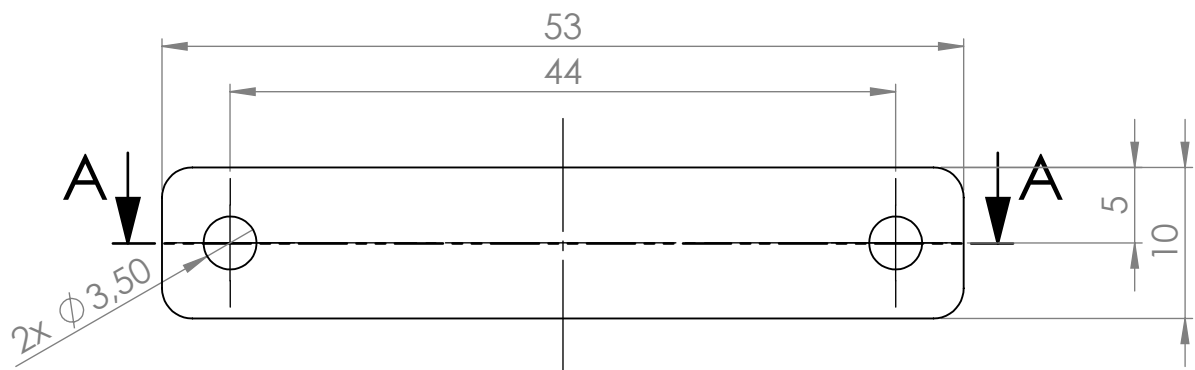
Figure I.3: Bioreactor chamber lengthwise sectional view.

## **Appendix J**

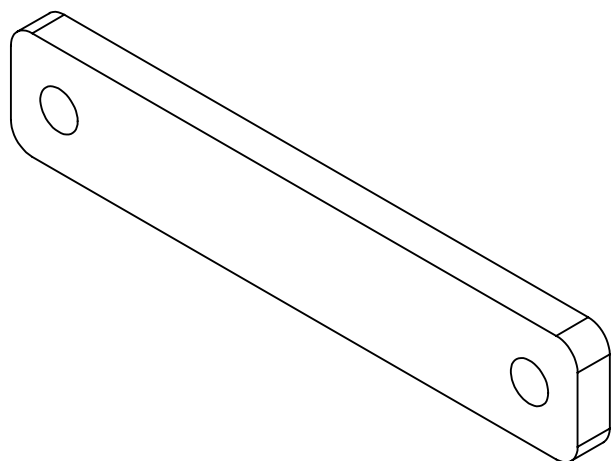
### **2D-drawings of the machined parts**

- Sensor support
- Tube connector
- Bioreactor lid
- Electrode
- Electrode pin
- M18 washer





COUPE A-A



Author: Julie Legrand

Scale: 2:1

Qty: 1

Verif. : Charles Faes

Date: 11-04-17

A4

Sensor support

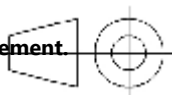
Note: Eliminate sharp angles

General tolerance: 0.1

Produit d'éducation SOLIDWORKS - À titre éducatif uniquement

Gen. surface cond. : Ra 3.2

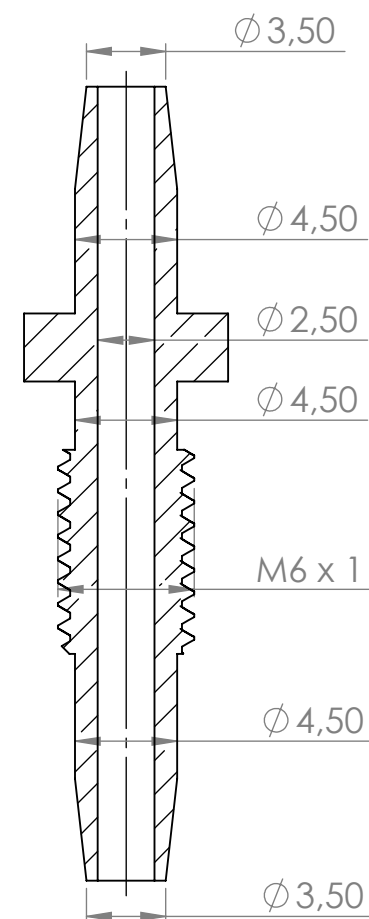
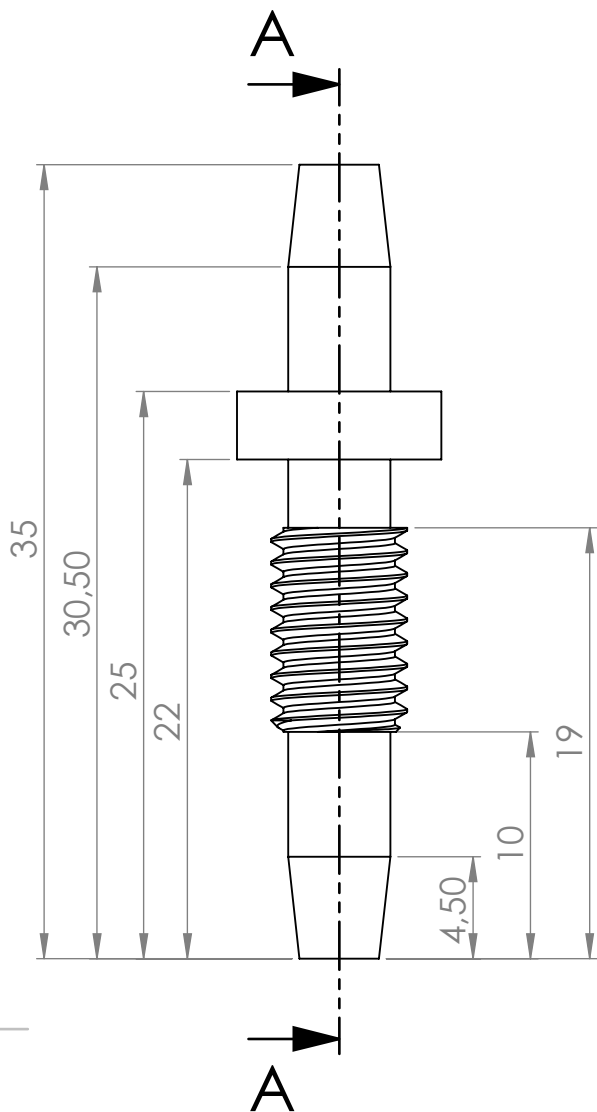
Material: Cold drawn stainless steel



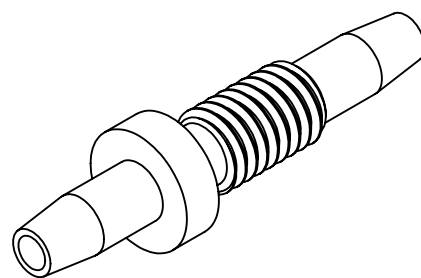
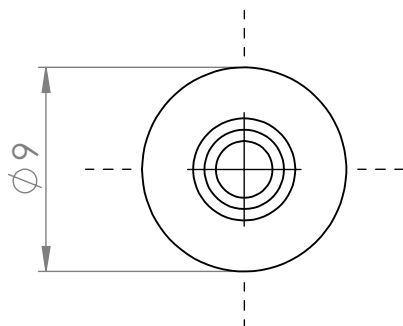
UCL  
Université  
catholique  
de Louvain

epl

IREC



COUPE A-A  
ECHELLE 3 : 1



Author: Julie Legrand

Scale: 3:1

Qty: 3

Verif. : Charles Faes

Date: 11-04-17

A4

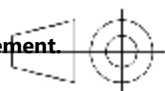
Tube connector

Note: Eliminate sharp angles

General tolerance: 0.1  
Produit d'éducation SOLIDWORKS - A titre indicatif uniquement.

Gen. surface cond. : Ra 1.6

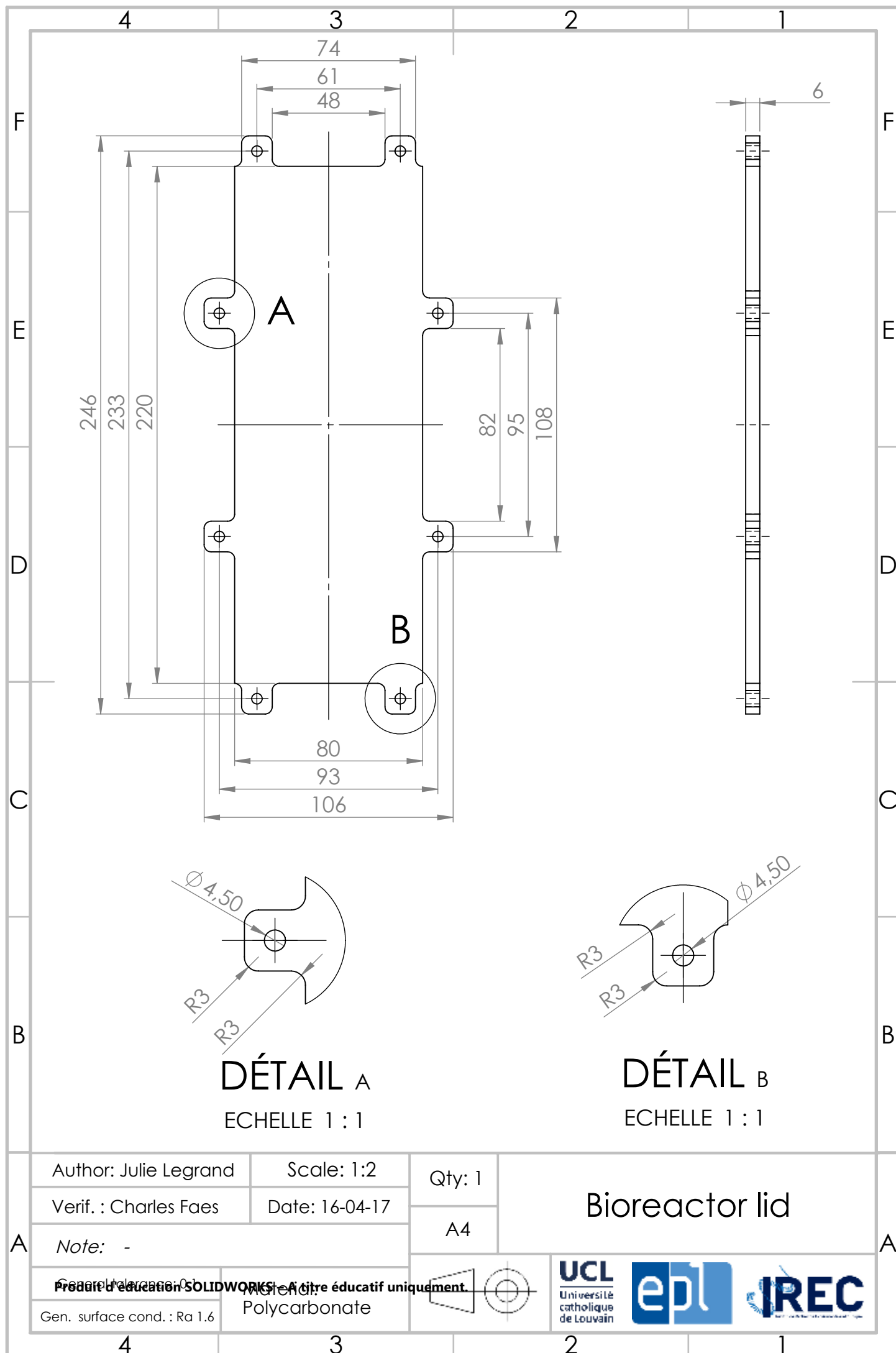
Material: Stainless steel 316L



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catholique  
de Louvain

epl

IREC



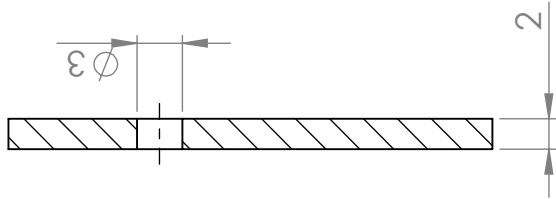
6 5 4 3 2 1

D

C

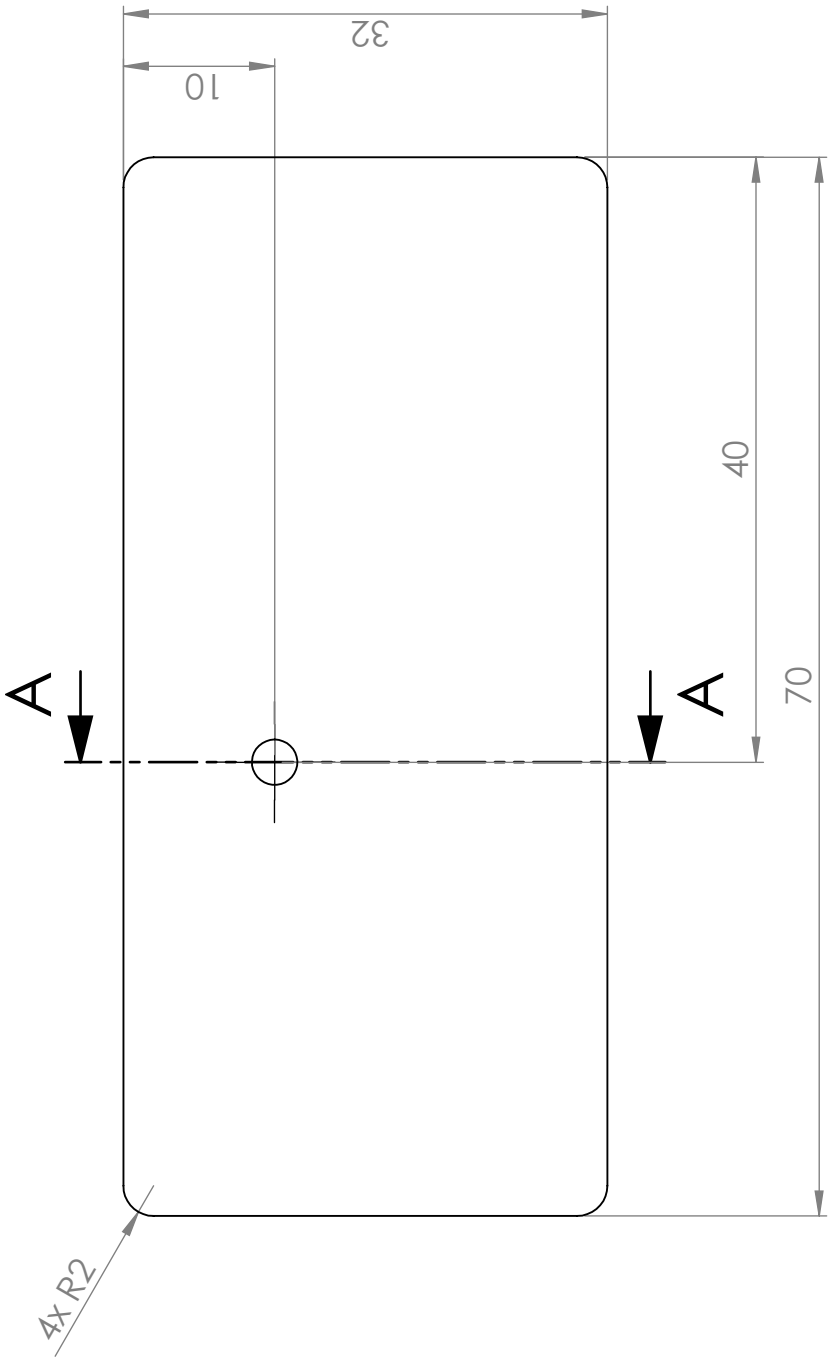
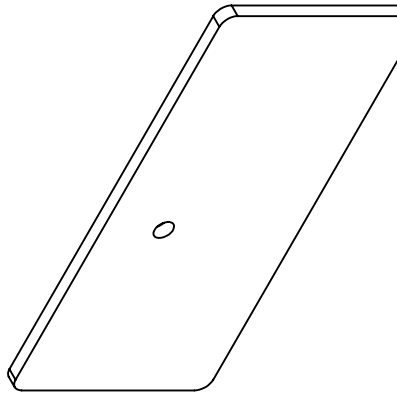
B

A



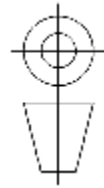
COUPE A-A

ECHELLE 2 : 1



|                                     |                |                                          |  |
|-------------------------------------|----------------|------------------------------------------|--|
| Author: Julie Legrand               | Scale: 2:1     | Qty: 2                                   |  |
| Verif. : Charles Faes               | Date: 16-04-17 | A4                                       |  |
| Note: <i>Eliminate sharp angles</i> |                | Material: Stainless steel 316L 2B finish |  |
| General tolerance: 0.1              |                | Gen. surface cond. : Ra 3.2              |  |

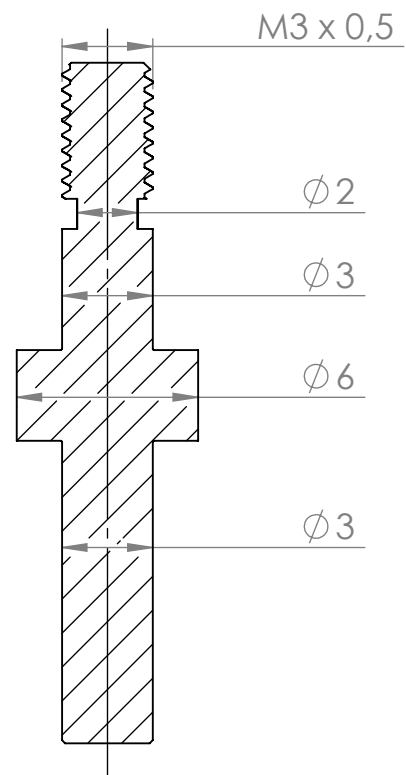
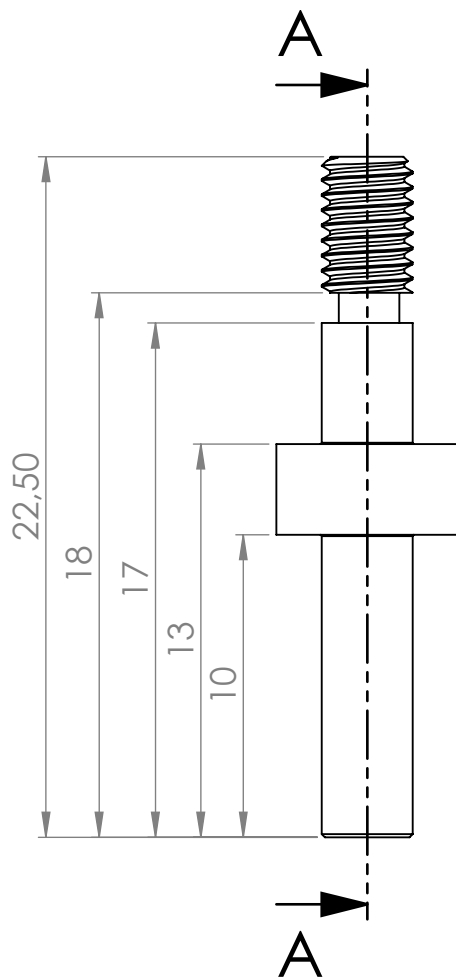
Electrode



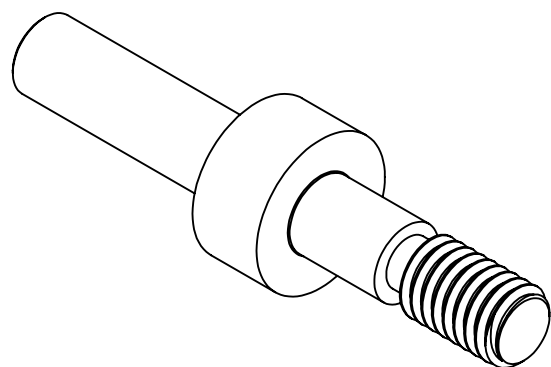
ucl  
Université  
catholique  
de Louvain



6 5 4 3 2 1



COUPE A-A  
ECHELLE 4 : 1



Author: Julie Legrand

Scale: 3:1

Qty: 2

Verif. : Charles Faes

Date: 22-04-17

A4

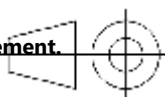
Electrode pin

Note: Eliminate sharp angles

Produit avec SOLIDWORKS - A titre éducatif uniquement.

Gen. surface cond. : Ra 1.6

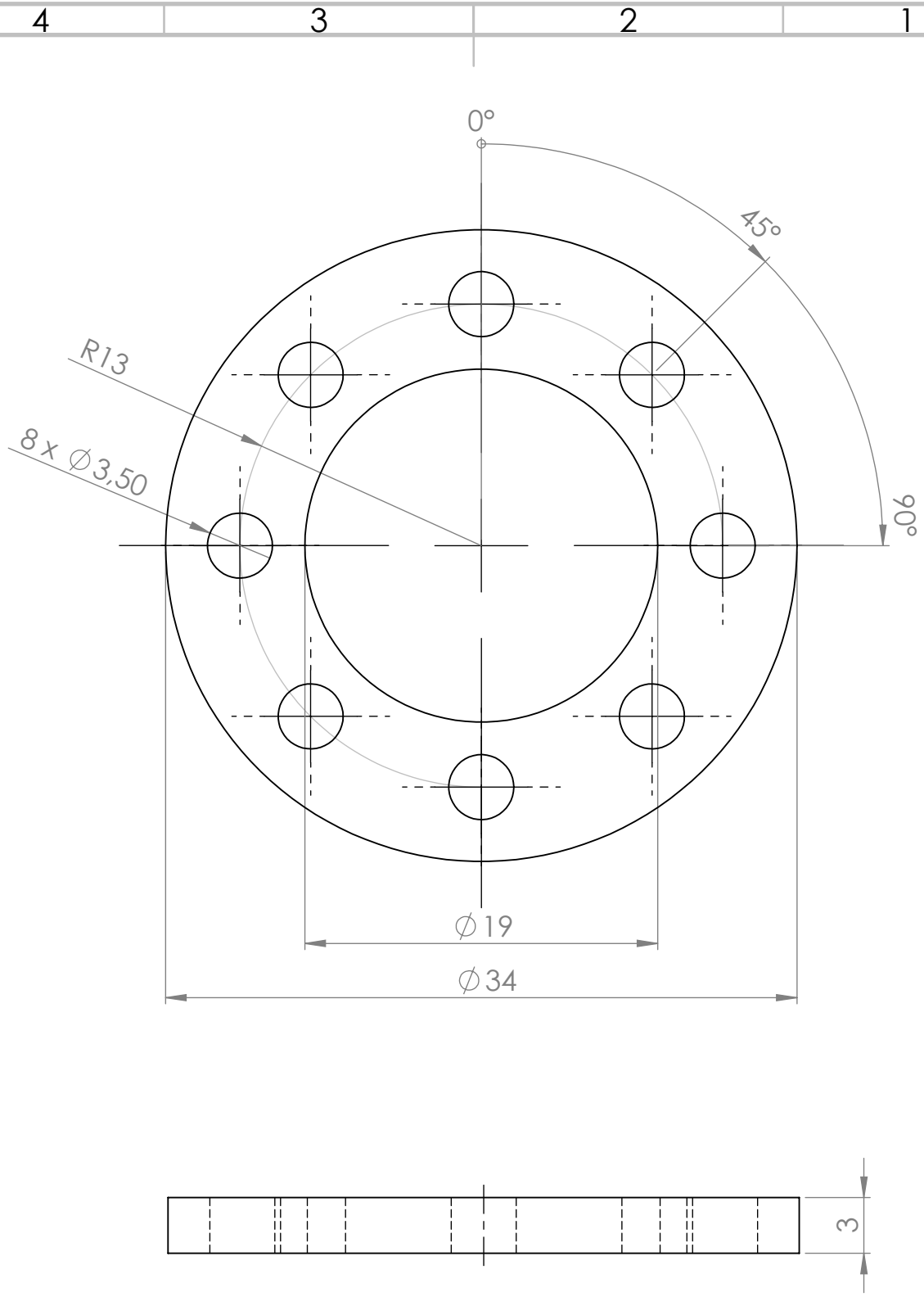
Material: Stainless steel 316L



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catholique  
de Louvain

epl

IREC



Author: Julie Legrand

Scale: 3:1

Qty: 1

# M18 Washer

(Holes drilling)

Verif. : Charles Faes

Date: 16-04-17

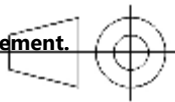
A4

Note: a M18 washer is used as base material

Produced with **SOLIDWORKS** - A titre éducatif uniquement.

Gen. surface cond. : Ra 1.6

Material: Stainless steel 316L



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catholique  
de Louvain

**edpl**

**IREC**  
Institut de Recherche en Électronique et en Catalyse

## **Appendix K**

### **Datasheets of the used components**

- LM308A
- LT1010
- SingleTact pressure sensor
- Firgelli linear actuator L12



## FEATURES

- *Guaranteed* 200pA max. input offset current
- *Guaranteed* 2nA max. input bias current
- *Guaranteed* 600 $\mu$ A max. supply current
- *Guaranteed* 0.5mV max. offset voltage
- *Guaranteed* 5 $\mu$ V/ $^{\circ}$ C max. drift
- Wide supply voltage range:  $\pm 2$ V to  $\pm 18$ V

## APPLICATIONS

- Integrators
- Transducer amplifiers
- Analog memories
- Light meters

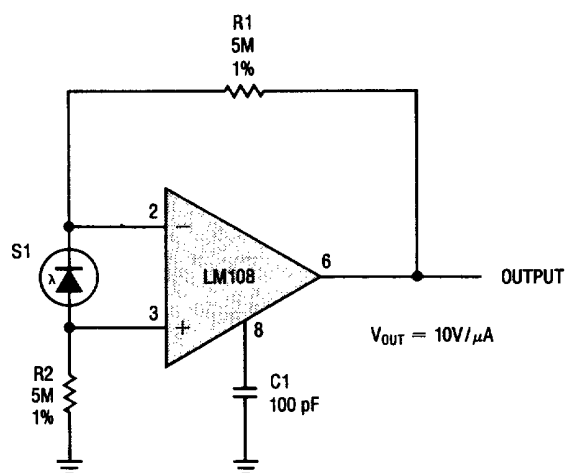
## DESCRIPTION

The LM108 series of precision operational amplifiers are particularly well-suited for high source impedance applications requiring low offset and bias currents as well as low power dissipation. Unlike FET input amplifiers, the offset and bias currents of the LM108 do not change significantly with temperature variations. Advanced design, processing and testing techniques make Linear's LM108 a superior choice over previous devices.

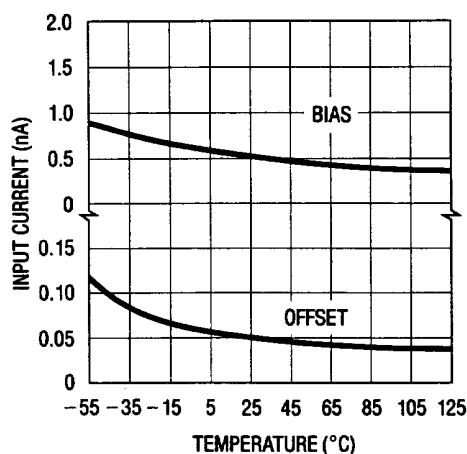
A photodiode sensor application is shown below. For applications requiring higher performance, see the LT1008, and LT1012.

**2**

Amplifier For Photodiode Sensor



Input Currents



# LM108A/LM308A LM108/LM308

## ABSOLUTE MAXIMUM RATINGS

### Supply Voltage

LM108A/LM108 .....  $\pm 20V$

LM308A/LM308 .....  $\pm 18V$

Differential Input Current (Note 1).....  $\pm 10mA$

Input Voltage (Note 2).....  $\pm 15V$

Output Short Circuit Duration..... Indefinite

### Operating Temperature Range

LM108A/LM108 .....  $-55^{\circ}C$  to  $125^{\circ}C$

LM308A/LM308 .....  $0^{\circ}C$  to  $70^{\circ}C$

### Storage Temperature Range

All Devices.....  $-65^{\circ}C$  to  $150^{\circ}C$

Lead Temperature (Soldering, 10 sec.).....  $300^{\circ}C$

## PACKAGE/ORDER INFORMATION

| TOP VIEW | ORDER PART NO.                         |
|----------|----------------------------------------|
|          | LM108AH<br>LM108H<br>LM308AH<br>LM308H |
| TOP VIEW | ORDER PART NO.                         |
|          |                                        |
|          | LM308AN8<br>LM308N8                    |

## ELECTRICAL CHARACTERISTICS $\pm 5V \leq V_s \leq \pm 20V$ and $-55^{\circ}C \leq T_A \leq 125^{\circ}C$ , unless otherwise noted.

| SYMBOL                              | PARAMETER                                               | CONDITIONS                                                                | MIN | LM108A<br>TYP | MAX        | MIN         | LM108<br>TYP | MAX | UNITS             |
|-------------------------------------|---------------------------------------------------------|---------------------------------------------------------------------------|-----|---------------|------------|-------------|--------------|-----|-------------------|
| $V_{OS}$                            | Input Offset Voltage                                    | $T_A = 25^{\circ}C$                                                       | ●   | 0.3           | 0.5<br>1.0 | 0.7         | 2.0          | 3.0 | mV<br>mV          |
| $\frac{\Delta V_{OS}}{\Delta Temp}$ | Average Temperature Coefficient of Input Offset Voltage |                                                                           | ●   | 1.0           | 5.0        | 3.0         | 15           |     | $\mu V/^{\circ}C$ |
| $I_{OS}$                            | Input Offset Current                                    | $T_A = 25^{\circ}C$                                                       | ●   | 0.05          | 0.2<br>0.4 | 0.05        | 0.2          | 0.4 | nA<br>nA          |
| $\frac{\Delta I_{OS}}{\Delta Temp}$ | Average Temperature Coefficient of Input Offset Current |                                                                           | ●   | 0.5           | 2.5        | 0.5         | 2.5          |     | $pA/^{\circ}C$    |
| $I_B$                               | Input Bias Current                                      | $T_A = 25^{\circ}C$                                                       | ●   | 0.5           | 2.0<br>3.0 | 0.5         | 2.0          | 3.0 | nA<br>nA          |
| $A_{VOL}$                           | Large Signal Voltage Gain                               | $T_A = 25^{\circ}C, V_S = \pm 15V, V_{OUT} = \pm 10V, R_L \geq 10k\Omega$ | ●   | 80<br>40      | 300        | 50<br>25    | 300          |     | V/mV<br>V/mV      |
| CMRR                                | Common Mode Rejection Ratio                             |                                                                           | ●   | 96            | 110        | 85          | 100          |     | dB                |
| PSRR                                | Power Supply Rejection Ratio                            |                                                                           | ●   | 96            | 110        | 80          | 96           |     | dB                |
|                                     | Input Voltage Range                                     | $V_S = \pm 15V$                                                           | ●   | $\pm 13.5$    |            | $\pm 13.5$  |              |     | V                 |
| $V_{OUT}$                           | Output Voltage Swing                                    | $V_S = \pm 15V, R_L = 10k\Omega$                                          | ●   | $\pm 13$      | $\pm 14$   | $\pm 13$    | $\pm 14$     |     | V                 |
| $R_{IN}$                            | Input Resistance                                        | $T_A = 25^{\circ}C$ (Note 3)                                              |     | 30            | 70         | 30          | 70           |     | M $\Omega$        |
| $I_S$                               | Supply Current                                          | $T_A = 25^{\circ}C$<br>$T_A = 125^{\circ}C$                               |     | 0.3<br>0.15   | 0.6<br>0.4 | 0.3<br>0.15 | 0.6<br>0.4   |     | mA<br>mA          |

## ELECTRICAL CHARACTERISTICS $\pm 5V \leq V_s \leq \pm 15V$ and $0^\circ C \leq T_A \leq 70^\circ C$ , unless otherwise noted.

| SYMBOL                              | PARAMETER                                               | CONDITIONS                                                             | MIN | LM308A<br>TYP | MAX         | MIN | LM308<br>TYP | MAX        | UNITS            |
|-------------------------------------|---------------------------------------------------------|------------------------------------------------------------------------|-----|---------------|-------------|-----|--------------|------------|------------------|
| $V_{OS}$                            | Input Offset Voltage                                    | $T_A = 25^\circ C$                                                     | ●   | 0.3           | 0.5<br>0.73 |     | 2.0          | 7.5<br>10  | mV<br>mV         |
| $\frac{\Delta V_{OS}}{\Delta Temp}$ | Average Temperature Coefficient of Input Offset Voltage |                                                                        | ●   | 2.0           | 5.0         |     | 6.0          | 30         | $\mu V/^\circ C$ |
| $I_{OS}$                            | Input Offset Current                                    | $T_A = 25^\circ C$                                                     | ●   | 0.2           | 1.0<br>1.5  |     | 0.2          | 1.0<br>1.5 | nA<br>nA         |
| $\frac{\Delta I_{OS}}{\Delta Temp}$ | Average Temperature Coefficient of Input Offset Current |                                                                        | ●   | 2.0           | 10          |     | 2.0          | 10         | $pA/^\circ C$    |
| $I_B$                               | Input Bias Current                                      | $T_A = 25^\circ C$                                                     | ●   | 1.5           | 7.0<br>10   |     | 1.5          | 7.0<br>10  | nA<br>nA         |
| $A_{VOL}$                           | Large Signal Voltage Gain                               | $T_A = 25^\circ C, V_s \pm 15V, V_{OUT} = \pm 10V, R_L \geq 10k\Omega$ | ●   | 80<br>60      | 300         |     | 25<br>15     | 300        | V/mV<br>V/mV     |
| CMRR                                | Common Mode Rejection Ratio                             |                                                                        | ●   | 96            | 110         |     | 80           | 100        | dB               |
| PSRR                                | Power Supply Rejection Ratio                            |                                                                        | ●   | 96            | 110         |     | 80           | 96         | dB               |
|                                     | Input Voltage Range                                     | $V_s = \pm 15V$                                                        | ●   | $\pm 14$      |             |     | $\pm 14$     |            | V                |
| $V_{OUT}$                           | Output Voltage Swing                                    | $V_s = \pm 15V, R_L = 10k\Omega$                                       | ●   | $\pm 13$      | $\pm 14$    |     | $\pm 13$     | $\pm 14$   | V                |
| $R_{IN}$                            | Input Resistance                                        | $T_A = 25^\circ C$ (Note 3)                                            |     | 10            | 40          |     | 10           | 40         | M $\Omega$       |
| $I_S$                               | Supply Current                                          | $T_A = 25^\circ C$                                                     |     | 0.3           | 0.8         |     | 0.3          | 0.8        | mA               |

The ● denotes the specifications which apply over the full operating temperature range.

For MIL-STD components, please refer to LTC 883 data sheet for test listing and parameters.

**Note 1:** Differential input voltages greater than 1V will cause excessive current to flow through the input protection diodes unless current limiting resistance is used.

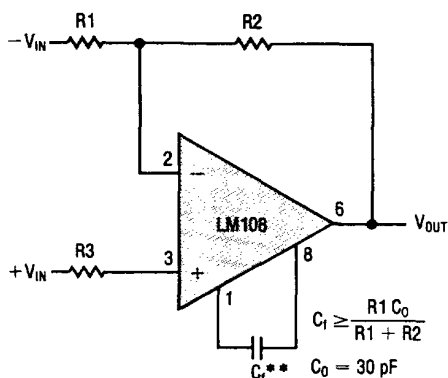
**Note 2:** For supply voltages less than  $\pm 15V$ , the maximum input voltage is equal to the supply voltage.

**Note 3:** Guaranteed by design.

## TYPICAL APPLICATIONS

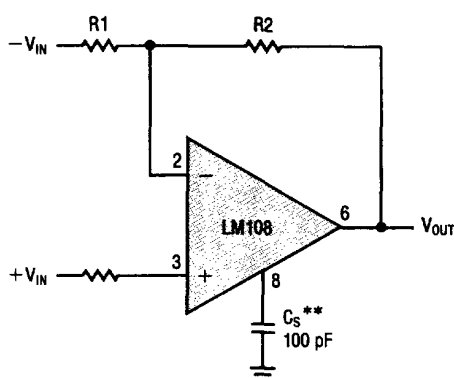
### COMPENSATION CIRCUITS

#### Standard Compensation Circuit



\*\* BANDWIDTH AND SLEW RATE ARE PROPORTIONAL TO  $1/C_1$

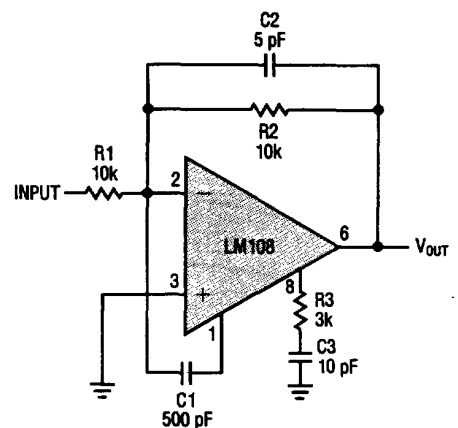
#### Alternate\* Frequency Compensation



\* IMPROVES REJECTION OF POWER SUPPLY NOISE BY A FACTOR OF TEN.

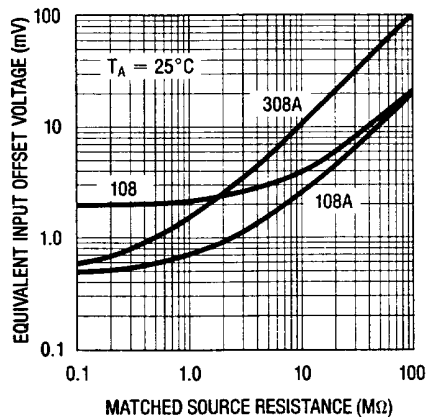
\*\* BANDWIDTH AND SLEW RATE ARE PROPORTIONAL TO  $1/C_1$

#### Feedforward Compensation

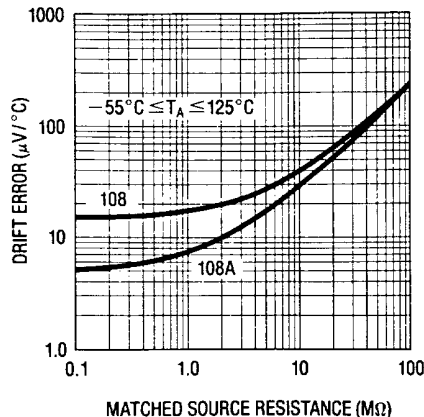


## TYPICAL PERFORMANCE CHARACTERISTICS

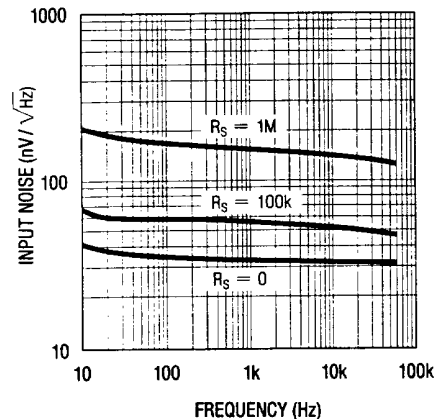
**Guaranteed Offset Error**



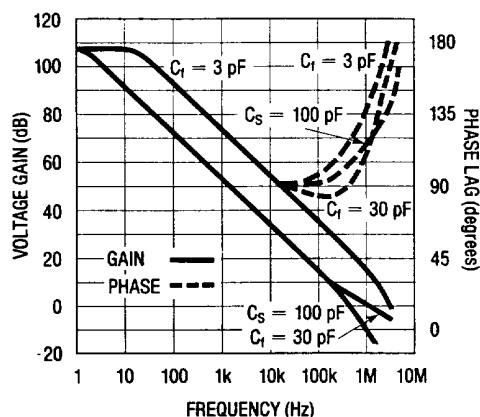
**Guaranteed Drift Error**



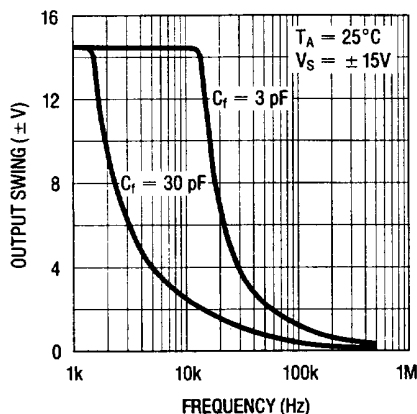
**Input Noise Voltage**



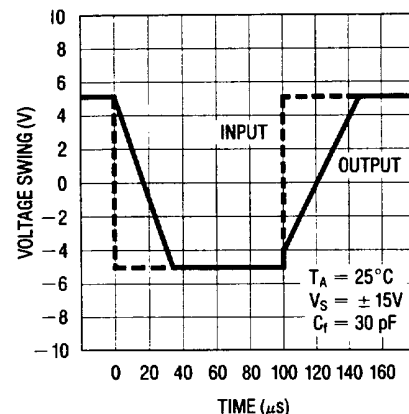
**Open Loop Frequency Response**



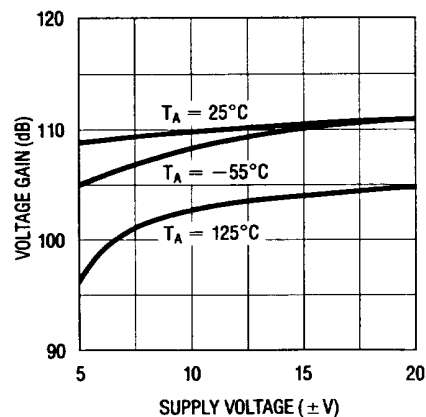
**Large Signal Frequency Response**



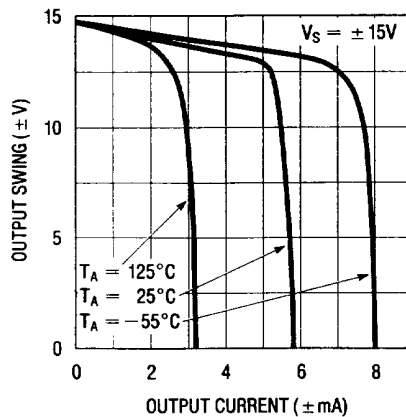
**Voltage Follower Pulse Response**



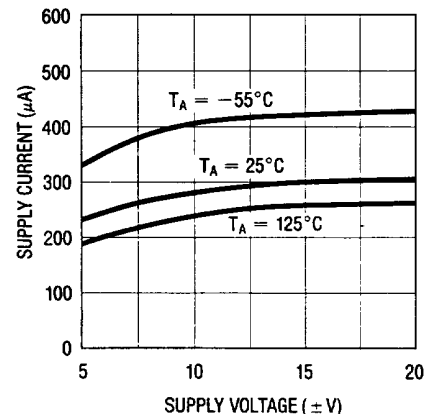
**Voltage Gain**



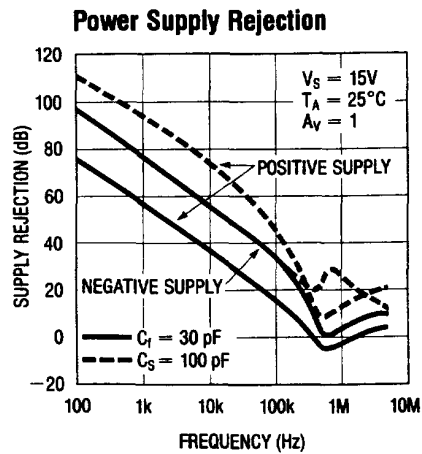
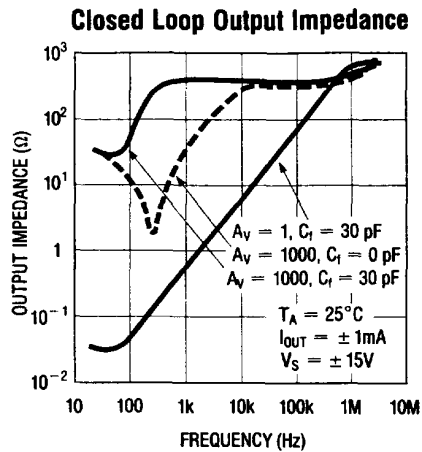
**Output Swing**



**Supply Current**



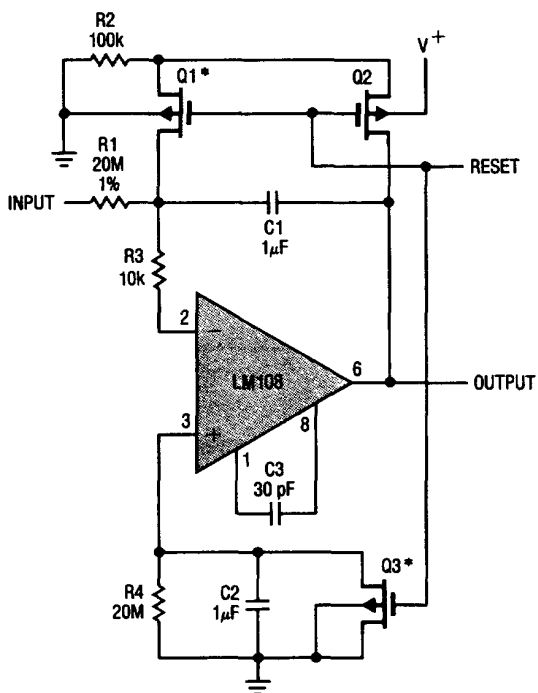
## TYPICAL PERFORMANCE CHARACTERISTICS



2

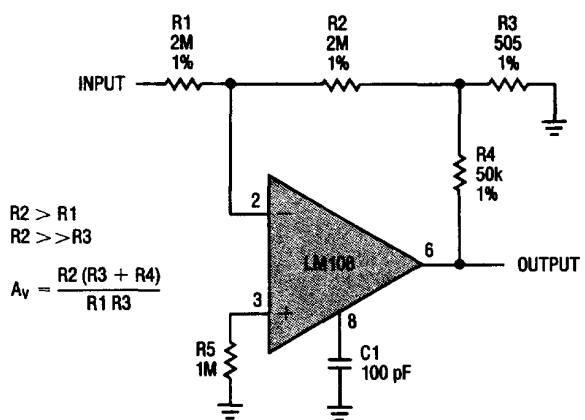
## TYPICAL APPLICATIONS

**Low Drift Integrator With Reset**



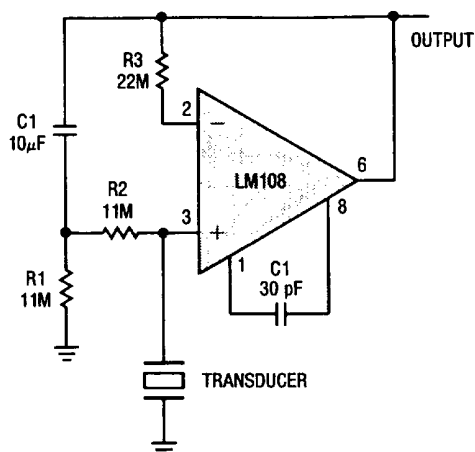
\* Q1 AND Q3 SHOULD NOT HAVE INTERNAL GATE-PROTECTION DIODES.

**Inverting Amplifier With High Input Resistance**

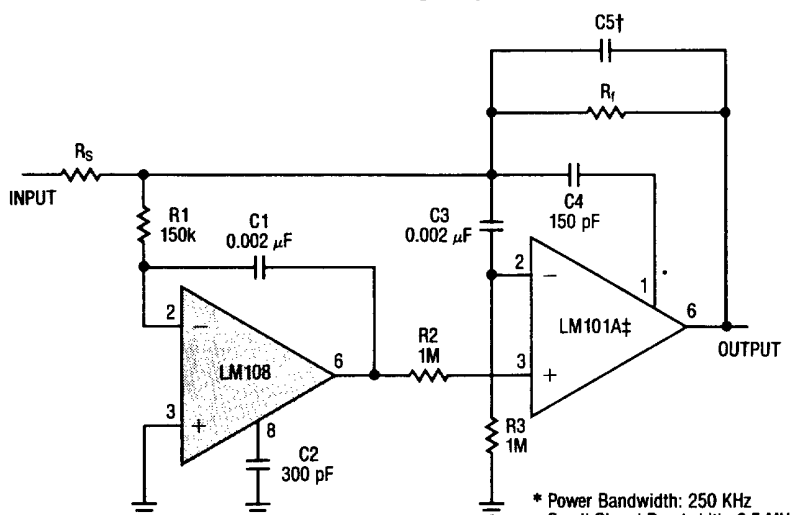


## TYPICAL APPLICATIONS

Amplifier For Piezoelectric Transducers



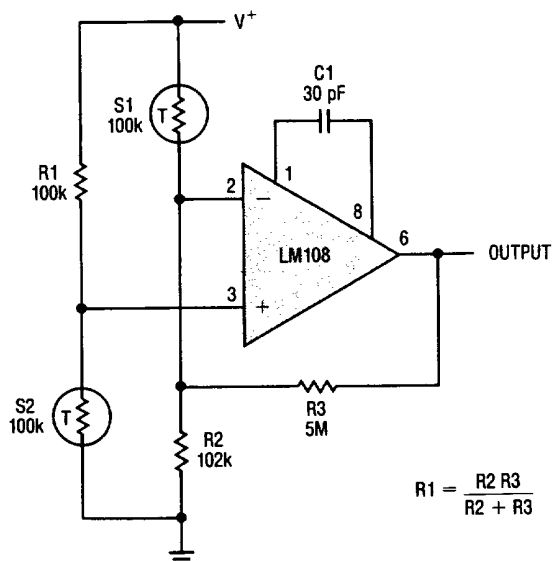
Fast\* Summing Amplifier



‡ In addition to increasing speed, the LM101A raises high and low frequency gain, increases output drive capability and eliminates thermal feedback.

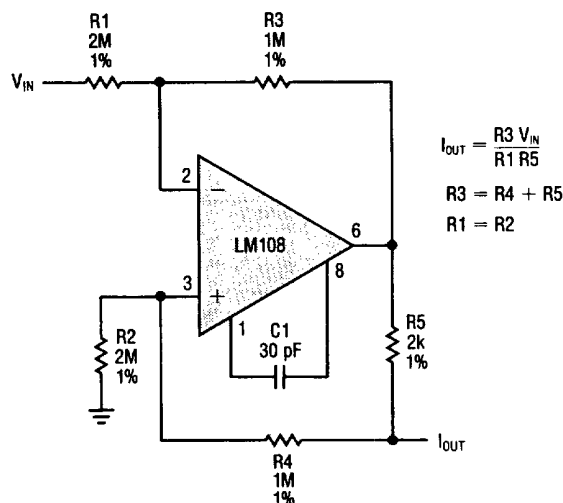
\* Power Bandwidth: 250 KHz  
Small Signal Bandwidth: 3.5 MHz  
Slew Rate: 10V/μS  
 $\dagger C5 = \frac{6 \times 10^{-8}}{R_f}$

Amplifier For Bridge Transducers



$$R1 = \frac{R2 R3}{R2 + R3}$$

Bilateral Current Source

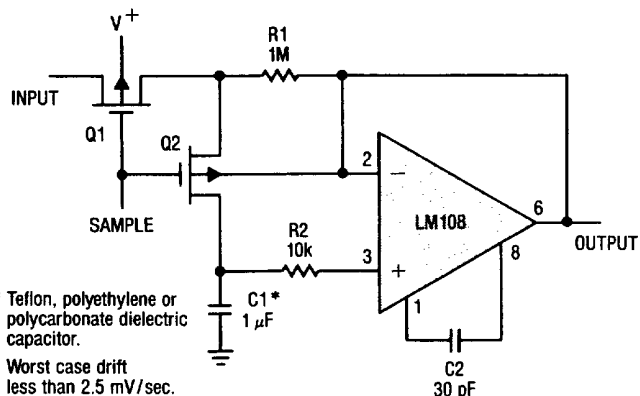


$$I_{OUT} = \frac{R3 V_{IN}}{R1 R5}$$

$$R3 = R4 + R5$$

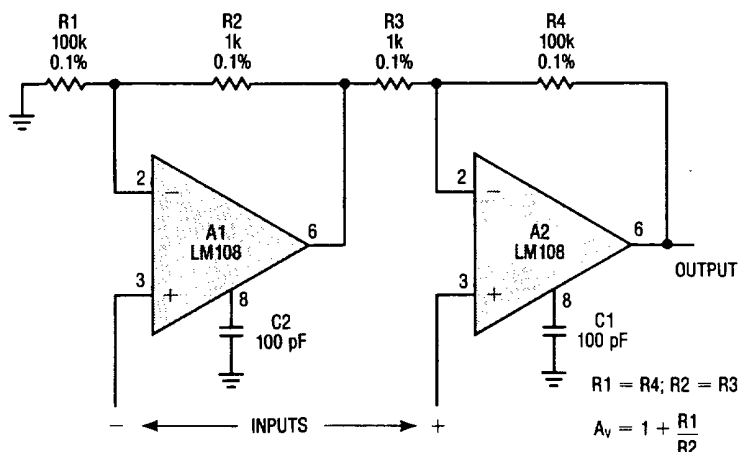
$$R1 = R2$$

Sample and Hold



\* Teflon, polyethylene or polycarbonate dielectric capacitor.  
Worst case drift less than 2.5 mV/sec.

Differential Input Instrumentation Amplifier



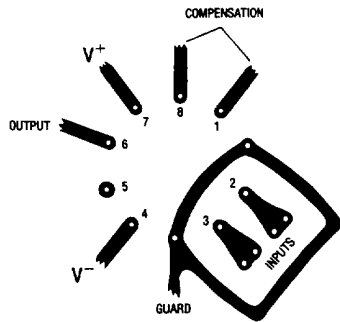
$$R1 = R4; R2 = R3$$

$$A_v = 1 + \frac{R1}{R2}$$

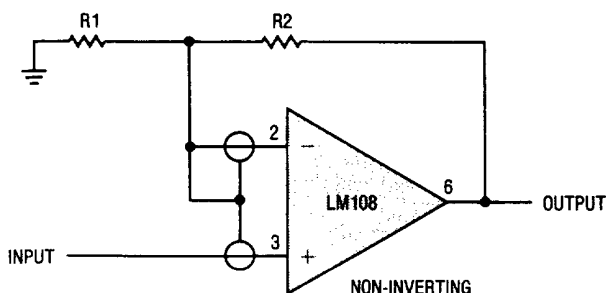
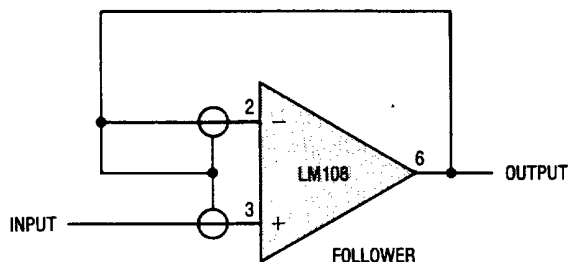
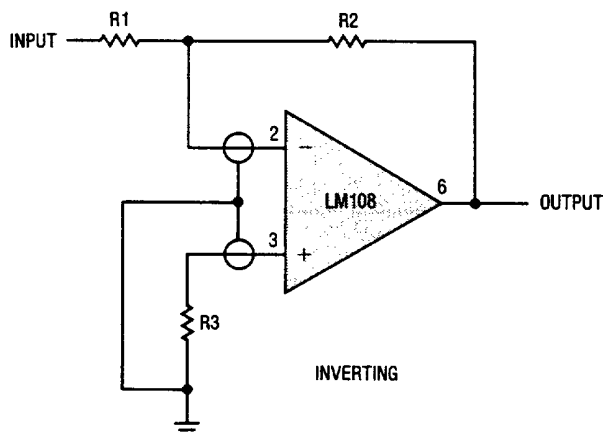
## APPLICATIONS INFORMATION

### Input guarding

Input guarding is used to reduce surface leakage. Guarding both sides of the board is required. Bulk leakage reduction is less and depends on the guard ring width.

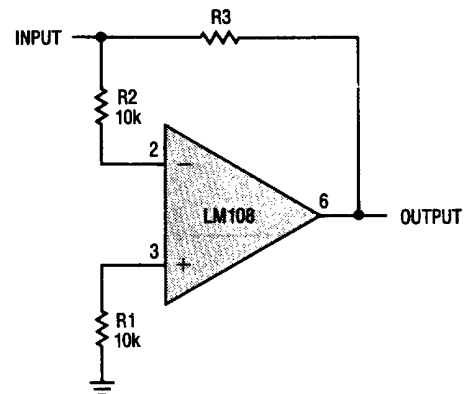


The guard ring is connected to a low impedance point at same potential as the sensitive input leads. Connections for various op amp configurations are shown below.

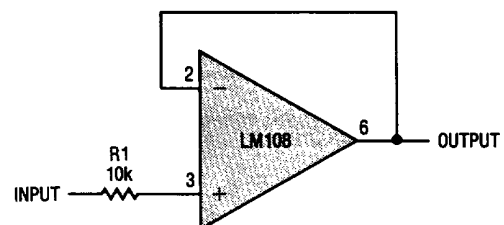


### Input protection

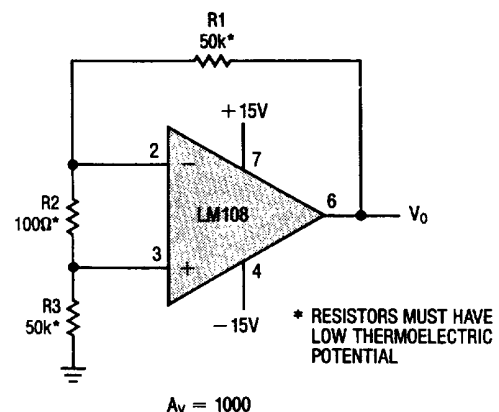
Current is limited by R2 even when input is connected to a voltage source outside the common mode range. If one supply reverses, current is controlled by R1. These resistors do not affect normal operation.



The input resistor controls the current when the input exceeds the supply voltages, when the power for the op amp is turned off, or when the output is shorted.



### Offset Voltage Test Circuit†



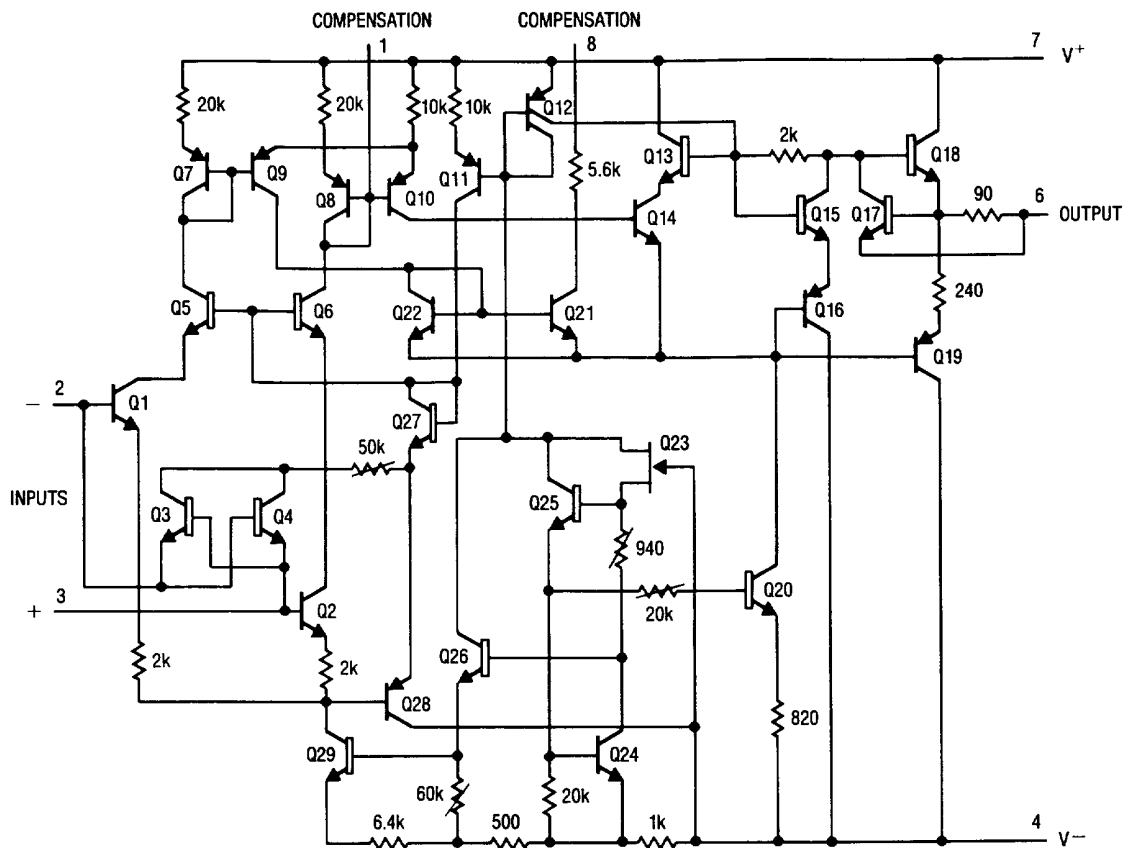
$A_V = 1000$

† THIS CIRCUIT IS ALSO USED AS THE BURN-IN CONFIGURATION WITH SUPPLY VOLTAGES EQUAL TO  $\pm 20V$ ,  $R1 = R3 = 10k$ ,  $R2 = 200k$ ,  $A_V = 100$ .



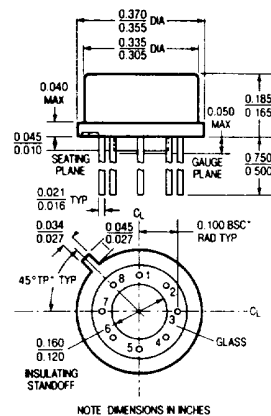
LM108A/LM308A  
LM108/LM308

SCHEMATIC DIAGRAM



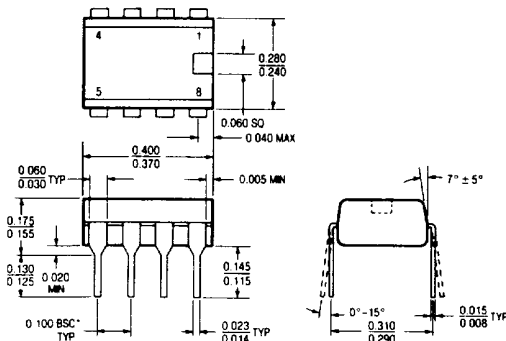
PACKAGE DESCRIPTION

H Package  
Metal Can



|           |               |               |
|-----------|---------------|---------------|
| $T_j$ max | $\theta_{ja}$ | $\theta_{jc}$ |
| 150°C     | 150°C/W       | 45°C/W        |

N8 Package  
8 Lead Plastic



|           |               |
|-----------|---------------|
| $T_j$ max | $\theta_{ja}$ |
| 100°C     | 130°C/W       |

## FEATURES

- 20MHz Bandwidth
- 75V/ $\mu\text{s}$  Slew Rate
- Drives  $\pm 10\text{V}$  Into 75 $\Omega$
- 5mA Quiescent Current
- Drives Capacitive Loads > 1 $\mu\text{F}$
- Current and Thermal Limit
- Operates from Single Supply  $\geq 4.5\text{V}$
- Very Low Distortion Operation
- Available in 8-Pin miniDIP, Plastic TO-220 and Tiny 3mm  $\times$  3mm  $\times$  0.75mm 8-Pin DFN Packages

## APPLICATIONS

- Boost Op Amp Output
- Isolate Capacitive Loads
- Drive Long Cables
- Audio Amplifiers
- Video Amplifiers
- Power Small Motors
- Operational Power Supply
- FET Driver

## DESCRIPTION

The LT<sup>®</sup>1010 is a fast, unity-gain buffer that can increase the output capability of existing IC op amps by more than an order of magnitude. This easy-to-use part makes fast amplifiers less sensitive to capacitive loading and reduces thermal feedback in precision DC amplifiers.

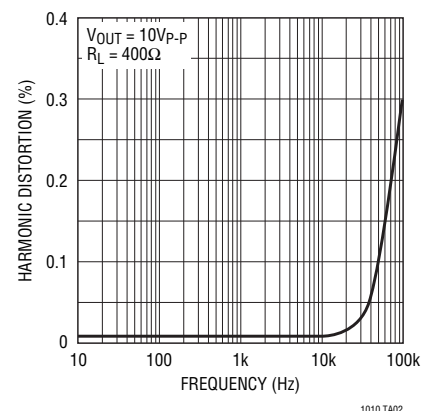
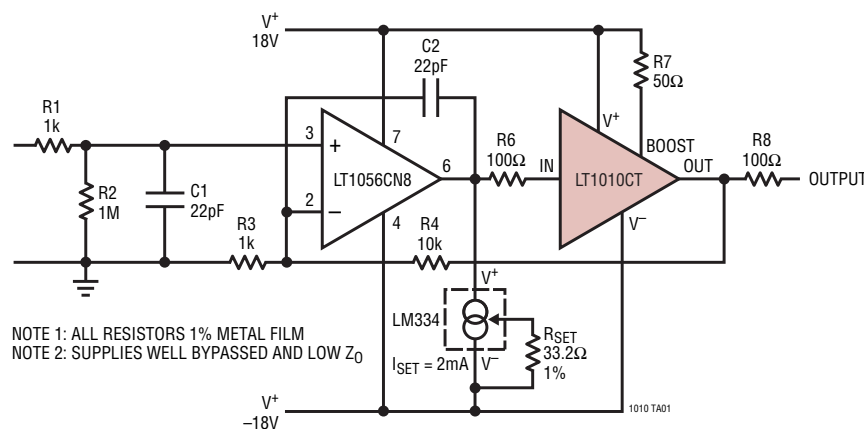
Designed to be incorporated within the feedback loop, the buffer can isolate almost any reactive load. Speed can be improved with a single external resistor. Internal operating currents are essentially unaffected by the supply voltage range. Single supply operation is also practical.

This monolithic IC is supplied in 8-pin miniDIP, plastic TO-220 and 8-pin DFN packages. The low thermal resistance power package is an aid in reducing operating junction temperatures.

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## TYPICAL APPLICATION

Very Low Distortion Buffered Preamplifier



# LT1010

## ABSOLUTE MAXIMUM RATINGS

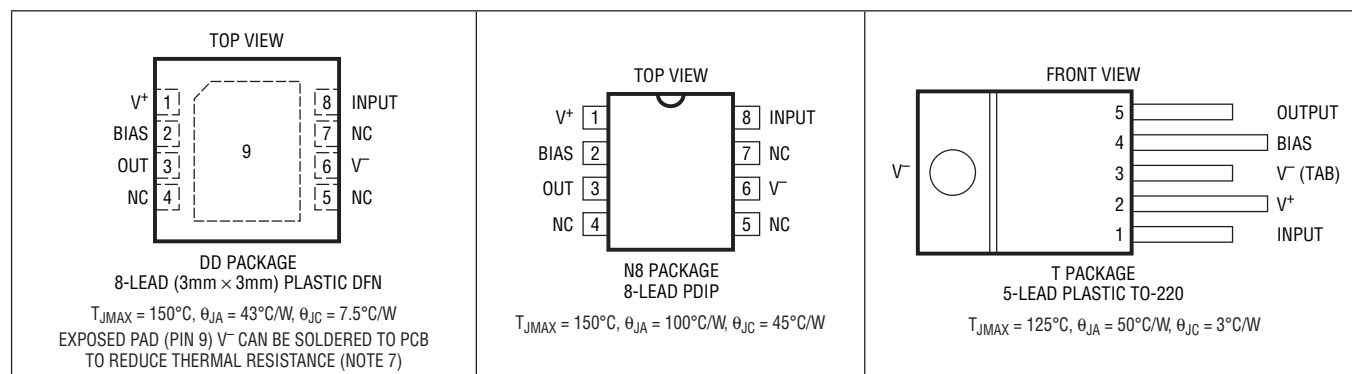
(Note 1)

Total Supply Voltage .....  $\pm 22\text{V}$   
 Continuous Output Current (Note 2) .....  $\pm 150\text{mA}$   
 Input Current (Note 3) .....  $\pm 40\text{mA}$   
 Junction Temperature Range  
     LT1010C .....  $0^{\circ}\text{C}$  to  $125^{\circ}\text{C}$   
 Storage Temperature Range .....  $-65^{\circ}\text{C}$  to  $150^{\circ}\text{C}$   
 Lead Temperature (Soldering, 10 sec) .....  $300^{\circ}\text{C}$

## PRECONDITIONING

100% Thermal Limit Burn In—LT1010CT

## PIN CONFIGURATION



## ORDER INFORMATION

| LEAD FREE FINISH        | TAPE AND REEL   | PART MARKING | PACKAGE DESCRIPTION                   | TEMPERATURE RANGE                            |
|-------------------------|-----------------|--------------|---------------------------------------|----------------------------------------------|
| LT1010CDD#PBF           | LT1010CDD#TRPBF | LBWZ         | 8-Lead (3mm $\times$ 3mm) Plastic DFN | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| LT1010CN8#PBF           | LT1010CN8#TRPBF | LTC1010CN8   | 8-Lead PDIP                           | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| LT1010CT#PBF            | LT1010CT#TRPBF  | LTC1010CT    | 5-Lead Plastic TO-220                 | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| LEAD BASED FINISH       | TAPE AND REEL   | PART MARKING | PACKAGE DESCRIPTION                   | TEMPERATURE RANGE                            |
| LT1010CDD               | LT1010CDD#TR    | LBWZ         | 8-Lead (3mm $\times$ 3mm) Plastic DFN | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| LT1010CN8               | LT1010CN8#TR    | LTC1010CN8   | 8-Lead PDIP                           | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| LT1010CT                | LT1010CT#TR     | LTC1010CT    | 5-Lead Plastic TO-220                 | $0^{\circ}\text{C}$ to $100^{\circ}\text{C}$ |
| <b>OBSOLETE PACKAGE</b> |                 |              |                                       |                                              |

Consult LTC Marketing for parts specified with wider operating temperature ranges.

For more information on lead free part marking, go to: <http://www.linear.com/leadfree/>

For more information on tape and reel specifications, go to: <http://www.linear.com/tapeandreel/>

## ELECTRICAL CHARACTERISTICS

The ● denotes the specifications which apply over the full operating temperature range, otherwise specifications are at  $T_A = 25^\circ\text{C}$ . (See Note 4. Typical values in curves.)

| SYMBOL      | PARAMETER                  | CONDITIONS (Note 4)                                                                                | MIN   | TYP | MAX  | UNITS            |
|-------------|----------------------------|----------------------------------------------------------------------------------------------------|-------|-----|------|------------------|
| $V_{OS}$    | Output Offset Voltage      | (Note 4)                                                                                           | 0     |     | 150  | mV               |
|             |                            |                                                                                                    | –20   |     | 220  | mV               |
|             |                            | $V_S = \pm 15\text{V}$ , $V_{IN} = 0\text{V}$                                                      | 20    |     | 100  | mV               |
| $I_B$       | Input Bias Current         | $I_{OUT} = 0\text{mA}$                                                                             | 0     |     | 250  | $\mu\text{A}$    |
|             |                            | $I_{OUT} \leq 150\text{mA}$                                                                        | 0     |     | 500  | $\mu\text{A}$    |
|             |                            |                                                                                                    | 0     |     | 800  | $\mu\text{A}$    |
| $A_V$       | Large-Signal Voltage Gain  |                                                                                                    | 0.995 |     | 1.00 | V/V              |
| $R_{OUT}$   | Output Resistance          | $I_{OUT} = \pm 1\text{mA}$                                                                         | 5     |     | 10   | $\Omega$         |
|             |                            | $I_{OUT} = \pm 150\text{mA}$                                                                       | 5     |     | 10   | $\Omega$         |
|             |                            |                                                                                                    |       |     | 12   | $\Omega$         |
|             | Slew Rate                  | $V_S = \pm 15\text{V}$ , $V_{IN} = \pm 10\text{V}$ , $V_{OUT} = \pm 8\text{V}$ , $R_L = 100\Omega$ | 75    |     |      | V/ $\mu\text{s}$ |
| $V_{SOS}^+$ | Positive Saturation Offset | $I_{OUT} = 0$ (Note 5)                                                                             |       |     | 1.0  | V                |
|             |                            |                                                                                                    |       |     | 1.1  | V                |
| $V_{SOS}^-$ | Negative Saturation Offset | $I_{OUT} = 0$ (Note 5)                                                                             |       |     | 0.2  | V                |
|             |                            |                                                                                                    |       |     | 0.3  | V                |
| $R_{SAT}$   | Saturation Resistance      | $I_{OUT} = \pm 150\text{mA}$ (Note 5)                                                              |       |     | 22   | $\Omega$         |
|             |                            |                                                                                                    |       |     | 28   | $\Omega$         |
| $V_{BIAS}$  | Bias Terminal Voltage      | $R_{BIAS} = 20\Omega$ (Note 6)                                                                     | 700   |     | 840  | mV               |
|             |                            |                                                                                                    | 560   |     | 880  | mV               |
| $I_S$       | Supply Current             | $I_{OUT} = 0$ , $I_{BIAS} = 0$                                                                     |       |     | 9    | mA               |
|             |                            |                                                                                                    |       |     | 10   | mA               |

**Note 1:** Stresses beyond those listed under Absolute Maximum Ratings may cause permanent damage to the device. Exposure to any Absolute Maximum Rating condition for extended periods may affect device reliability and lifetime.

**Note 2:** Dissipation must be based on a thermal resistance. See Application Information for power dissipation.

**Note 3:** In current limit or thermal limit, input current increases sharply with input-output differentials greater than 8V; so input current must be limited. Input current also rises rapidly for input voltages 8V above  $V^+$  or 0.5V below  $V^-$ .

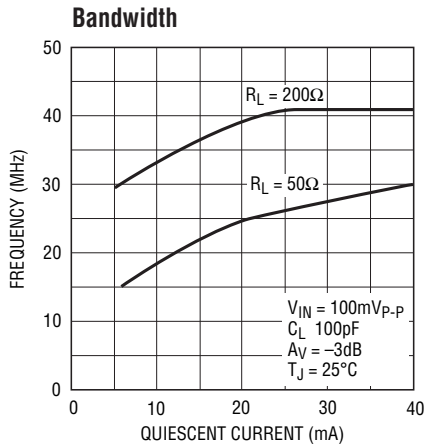
**Note 4:** Specifications apply for  $4.5\text{V} \leq V_S \leq 40\text{V}$ ,  $V^- + 0.5\text{V} \leq V_{IN} \leq V^+ - 1.5\text{V}$  and  $I_{OUT} = 0$ , unless otherwise stated. Temperature range is  $0^\circ\text{C} \leq T_C \leq 100^\circ\text{C}$ .

**Note 5:** The output saturation characteristics are measured with 100mV output clipping. See Applications Information for determining available output swing and input drive requirements for a given load.

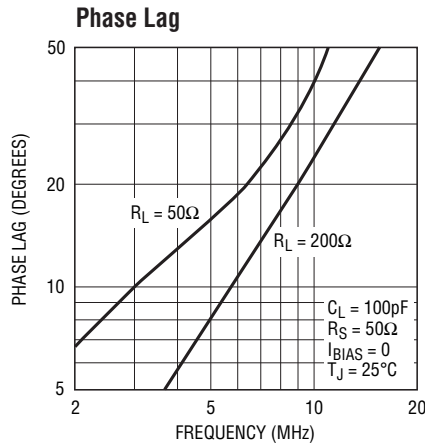
**Note 6:** The output stage quiescent current can be increased by connecting a resistor between the BIAS pin and  $V^+$ . The increase is equal to the bias terminal voltage divided by this resistance.

**Note 7:** Thermal resistance varies depending upon the amount of PC board metal attached to the pin (Pin 9) of the device.  $\theta_{JA}$  is specified for a certain amount of 1oz copper metal trace connecting to Pin 9 as described in the thermal resistance tables in the Applications Information section.

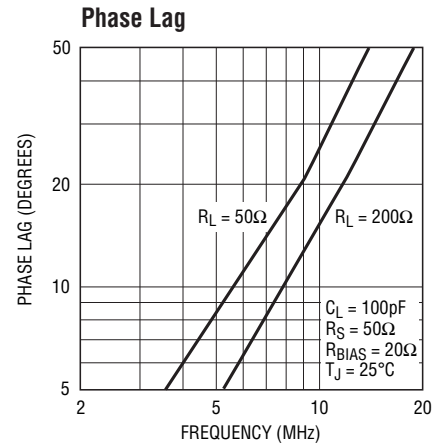
# TYPICAL PERFORMANCE CHARACTERISTICS



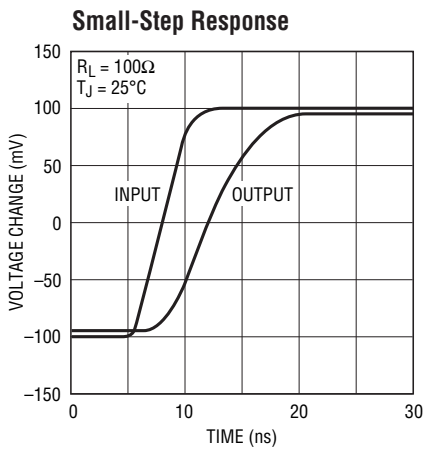
1010 G01



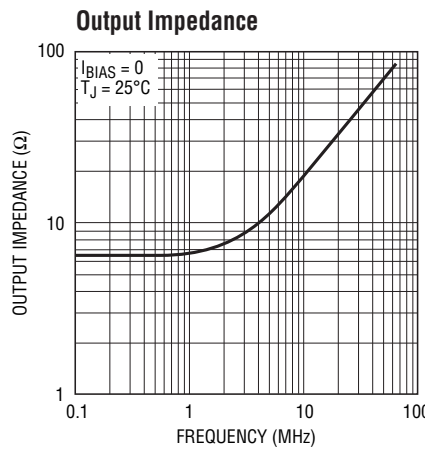
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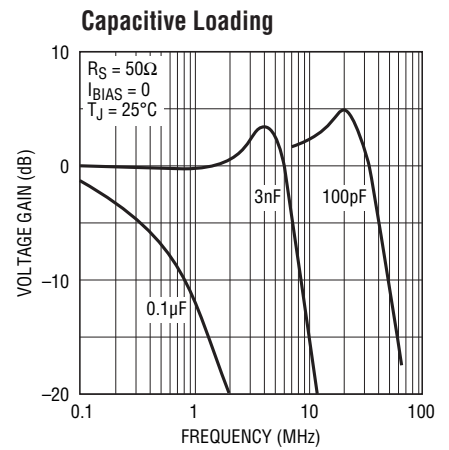
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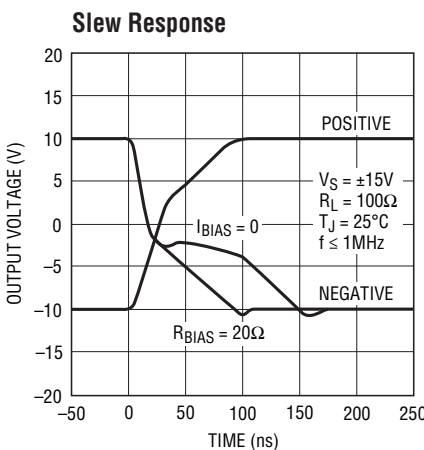
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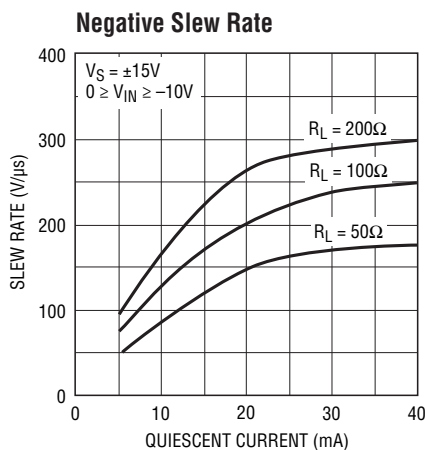
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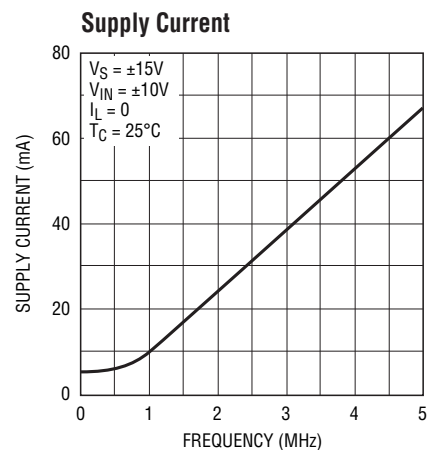
1010 G06



1010 G07



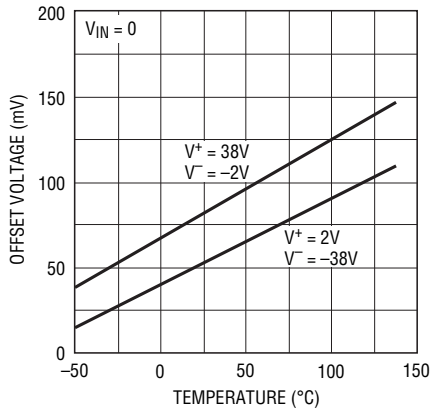
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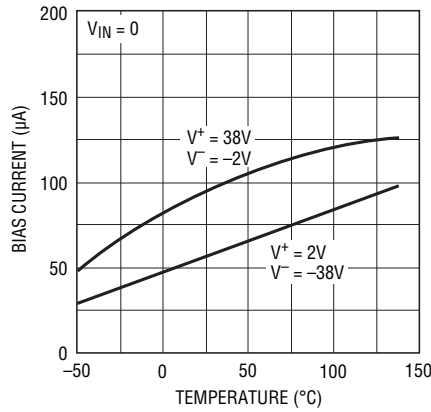
1010 G09

# TYPICAL PERFORMANCE CHARACTERISTICS

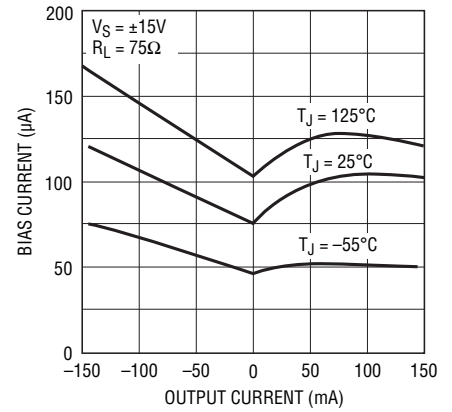
Output Offset Voltage



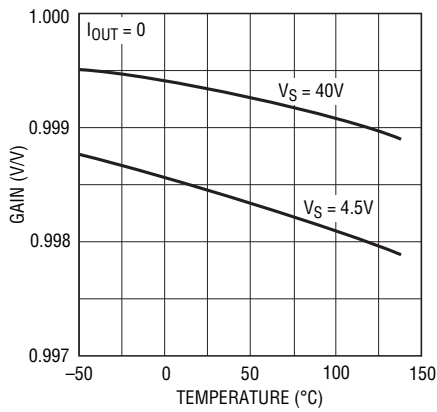
Input Bias Current



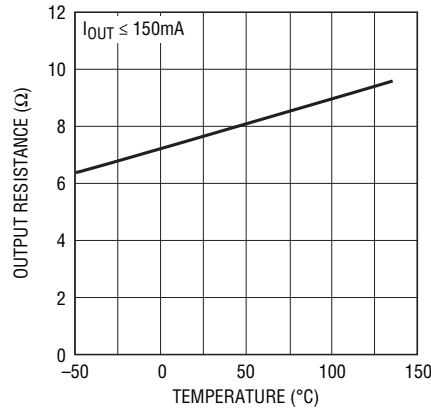
Input Bias Current



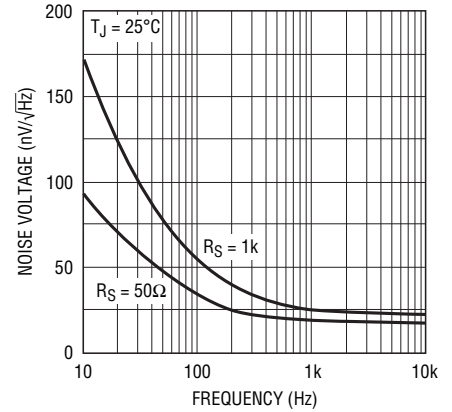
Voltage Gain



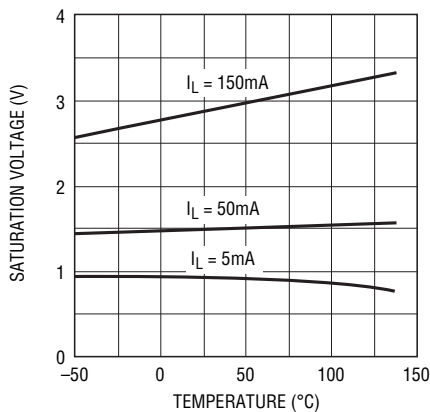
Output Resistance



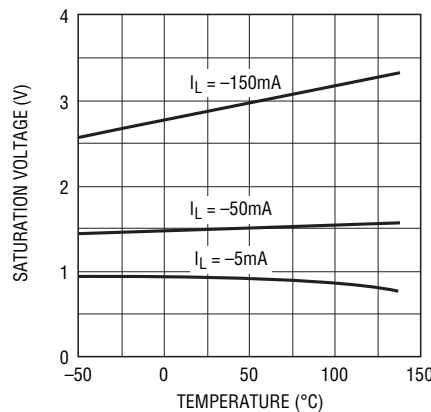
Output Noise Voltage



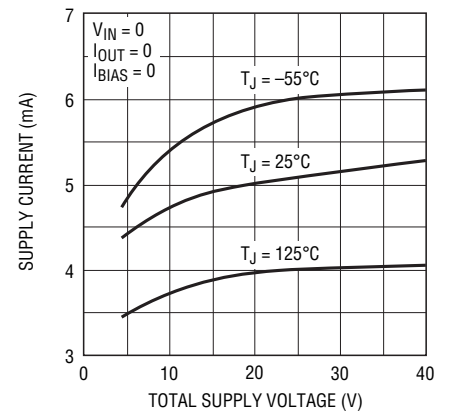
Positive Saturation Voltage



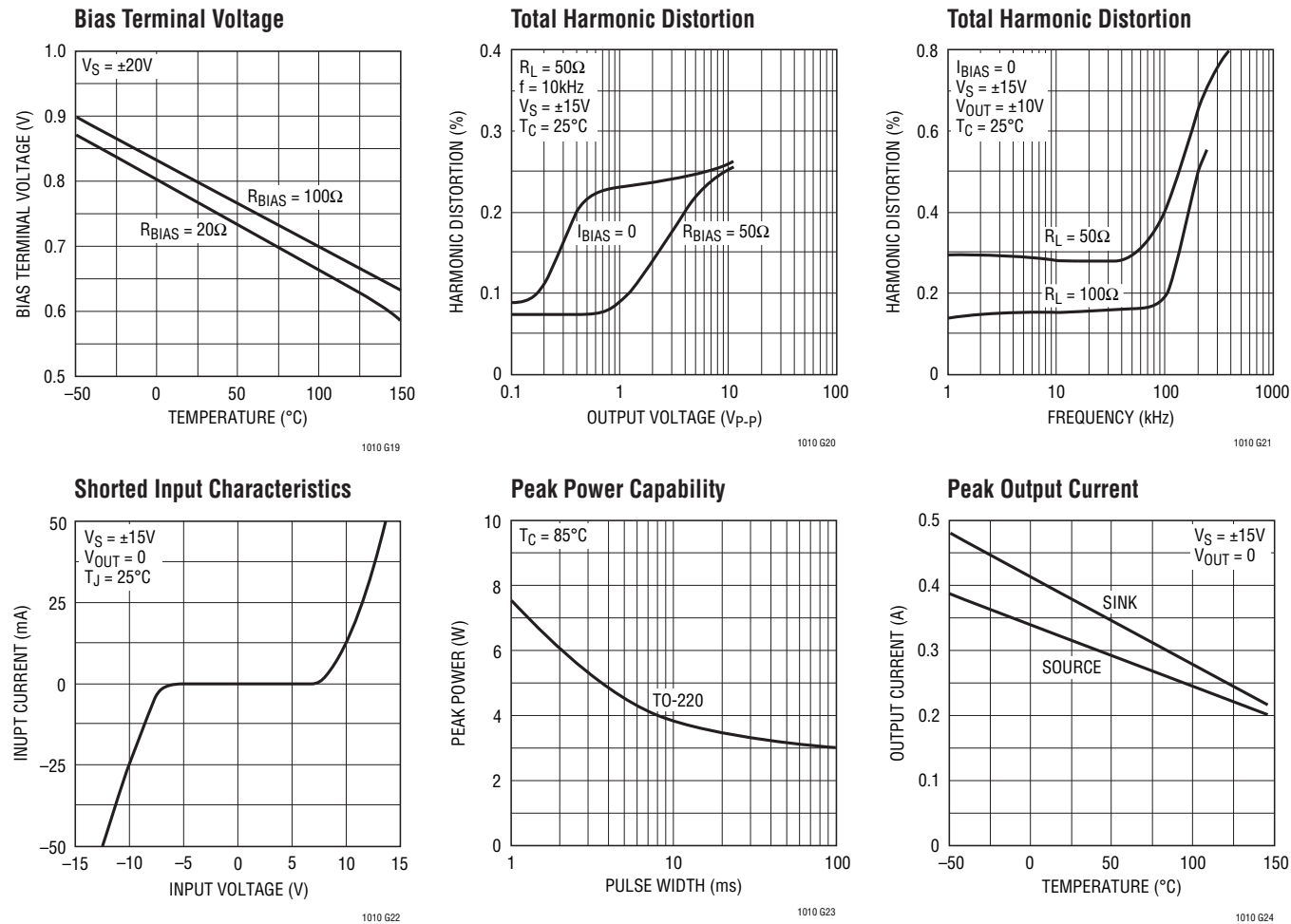
Negative Saturation Voltage



Supply Current



TYPICAL PERFORMANCE CHARACTERISTICS





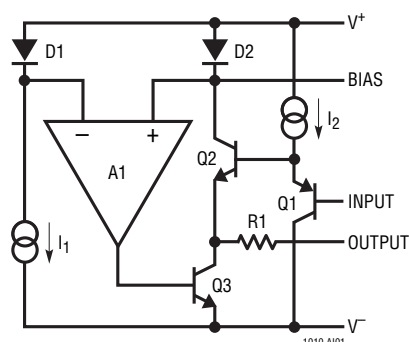
# APPLICATIONS INFORMATION

## General

These notes briefly describe the LT1010 and how it is used; a detailed explanation is given elsewhere.<sup>1</sup> Emphasis here will be on practical suggestions that have resulted from working extensively with the part over a wide range of conditions. A number of applications are also outlined that demonstrate the usefulness of the buffer beyond that of driving a heavy load.

## Design Concept

The schematic below describes the basic elements of the buffer design. The op amp drives the output sink transistor, Q3, such that the collector current of the output follower, Q2, never drops below the quiescent value (determined by  $I_1$  and the area ratio of D1 and D2). As a result, the high frequency response is essentially that of a simple follower even when Q3 is supplying the load current. The internal feedback loop is isolated from the effects of capacitive loading by a small resistor in the output lead.

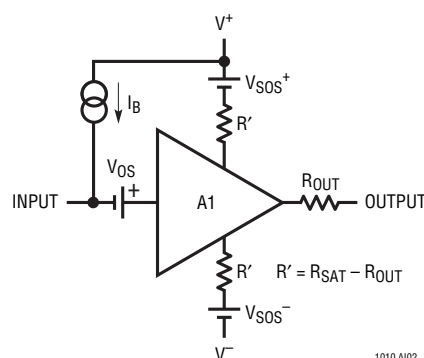


The scheme is not perfect in that the rate of rise of sink current is noticeably less than for source current. This can be mitigated by connecting a resistor between the bias terminal and  $V^+$ , raising quiescent current. A feature of the final design is that the output resistance is largely independent of the follower quiescent current or the output load current. The output will also swing to the negative rail, which is particularly useful with single supply operation.

## Equivalent Circuit

Below 1MHz, the LT1010 is quite accurately represented by the equivalent circuit shown here for both small- and large-signal operation. The internal element, A1, is an

idealized buffer with the unloaded gain specified for the LT1010. Otherwise, it has zero offset voltage, bias current and output resistance. Its output also saturates to the internal supply terminals.<sup>2</sup>



Loaded voltage gain can be determined from the unloaded gain,  $A_V$ , the output resistance,  $R_{OUT}$ , and the load resistance,  $R_L$ , using:

$$A_{VL} = \frac{A_V R_L}{R_{OUT} + R_L}$$

Maximum positive output swing is given by:

$$V_{OUT}^+ = \frac{(V^+ - V_{SOS}^+) R_L}{R_{SAT} + R_L}$$

The input swing required for this output is:

$$V_{IN}^+ = V_{OUT}^+ \left( 1 + \frac{R_{OUT}}{R_L} \right) - V_{OS} + \Delta V_{OS}$$

where  $\Delta V_{OS}$  is the 100mV clipping specified for the saturation measurements. Negative output swing and input drive requirements are similarly determined.

## Supply Bypass

The buffer is no more sensitive to supply bypassing than slower op amps as far as stability is concerned. The 0.1μF disc ceramic capacitors usually recommended for op amps are certainly adequate for low frequency work. As always, keeping the capacitor leads short and using

<sup>1</sup>R. J. Widlar, "Unique IC Buffer Enhances Op Amp Designs; Tames Fast Amplifiers," Linear Technology Corp. TP-1, April, 1984.

<sup>2</sup>See electrical characteristics section for guaranteed limits.

## APPLICATIONS INFORMATION

a ground plane is prudent, especially when operating at high frequencies.

The buffer slew rate can be reduced by inadequate supply bypass. With output current changes much above 100mA/μs, using 10μF solid tantalum capacitors on both supplies is good practice, although bypassing from the positive to the negative supply may suffice.

When used in conjunction with an op amp and heavily loaded (resistive or capacitive), the buffer can couple into supply leads common to the op amp causing stability problems with the overall loop and extended settling time. Adequate bypassing can usually be provided by 10μF solid tantalum capacitors. Alternately, smaller capacitors could be used with decoupling resistors. Sometimes the op amp has much better high frequency rejection on one supply, so bypass requirements are less on this supply.

### Power Dissipation

In many applications the LT1010 will require heat sinking. Thermal resistance, junction to still air is 50°C/W for the TO-220 package and 100°C/W for the miniDIP package. Circulating air, a heat sink or mounting the package to a printed circuit board will reduce thermal resistance.

In DC circuits, buffer dissipation is easily computed. In AC circuits, signal waveshape and the nature of the load determine dissipation. Peak dissipation can be several times average with reactive loads. It is particularly important to determine dissipation when driving large load capacitance.

With AC loading, power is divided between the two output transistors. This reduces the effective thermal resistance, junction to case is 3°C/W for the TO-220 package as long as the peak rating of neither output transistor is exceeded. The typical curves indicate the peak dissipation capabilities of one output transistor.

### Overload Protection

The LT1010 has both instantaneous current limit and thermal overload protection. Foldback current limiting has not been used, enabling the buffer to drive complex loads without limiting. Because of this, it is capable of power dissipation in excess of its continuous ratings.

Normally, thermal overload protection will limit dissipation and prevent damage. However, with more than 30V across the conducting output transistor, thermal limiting is not quick enough to ensure protection in current limit. The thermal protection is effective with 40V across the conducting output transistor as long as the load current is otherwise limited to 150mA.

### Drive Impedance

When driving capacitive loads, the LT1010 likes to be driven from a low source impedance at high frequencies. Certain low power op amps (e.g., the LM10) are marginal in this respect. Some care may be required to avoid oscillations, especially at low temperatures.

Bypassing the buffer input with more than 200pF will solve the problem. Raising the operating current also works.

### Parallel Operation

Parallel operation provides reduced output impedance, more drive capability and increased frequency response under load. Any number of buffers can be directly paralleled as long as the increased dissipation in individual units caused by mismatches of output resistance and offset voltage is taken into account.

When the inputs and outputs of two buffers are connected together, a current,  $\Delta I_{OUT}$ , flows between the outputs:

$$\Delta I_{OUT} = \frac{V_{OS1} - V_{OS2}}{R_{OUT1} + R_{OUT2}}$$

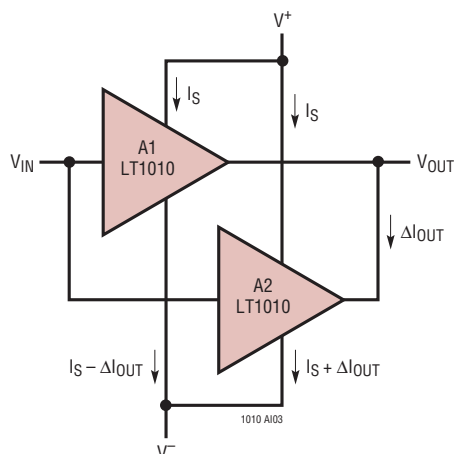
where  $V_{OS}$  and  $R_{OUT}$  are the offset voltage and output resistance of the respective buffers.

Normally, the negative supply current of one unit will increase and the other decrease, with the positive supply current staying the same. The worst-case ( $V_{IN} \rightarrow V^+$ ) increase in standby dissipation can be assumed to be  $\Delta I_{OUT} V_T$ , where  $V_T$  is the total supply voltage.

Offset voltage is specified worst case over a range of supply voltages, input voltage and temperature. It would be unrealistic to use these worst-case numbers above because paralleled units are operating under identical conditions.

## APPLICATIONS INFORMATION

The offset voltage specified for  $V_S = \pm 15V$ ,  $V_{IN} = 0V$  and  $T_A = 25^\circ C$  will suffice for a worst-case condition.



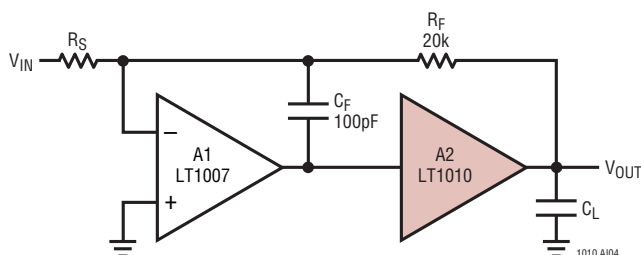
Output load current will be divided based on the output resistance of the individual buffers. Therefore, the available output current will not quite be doubled unless output resistances are matched. As for offset voltage, the  $25^\circ C$  limits should be used for worst-case calculations.

Parallel operation is not thermally unstable. Should one unit get hotter than its mates, its share of the output and its standby dissipation will decrease.

As a practical matter, parallel connection needs only some increased attention to heat sinking. In some applications, a few ohms equalization resistance in each output may be wise. Only the most demanding applications should require matching, and then just of output resistance at  $25^\circ C$ .

### Isolating Capacitive Loads

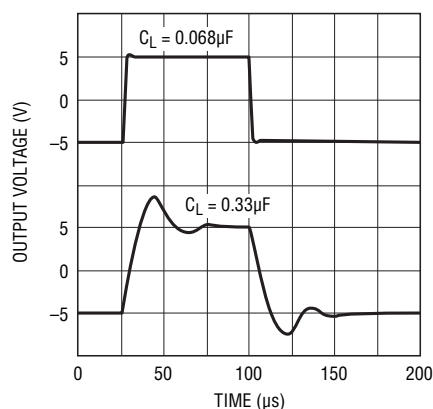
The inverting amplifier below shows the recommended method of isolating capacitive loads. Noninverting amplifiers are handled similarly.



At lower frequencies, the buffer is within the feedback loop so that its offset voltage and gain errors are negligible. At higher frequencies, feedback is through  $C_F$ , so that phase shift from the load capacitance acting against the buffer output resistance does not cause loop instability.

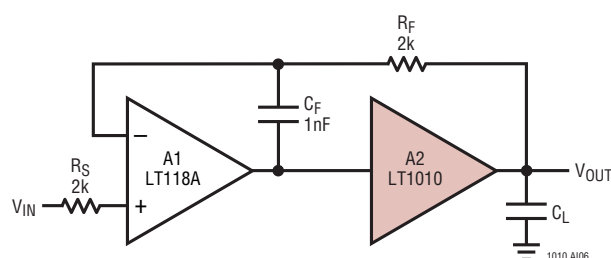
Stability depends upon the  $R_F C_F$  time constant or the closed-loop bandwidth. With an 80kHz bandwidth, ringing is negligible for  $C_L = 0.068\mu F$  and damps rapidly for  $C_L = 0.33\mu F$ . The pulse response is shown in the graph.

Pulse Response



1010 AIO5

Small-signal bandwidth is reduced by  $C_F$ , but considerable isolation can be obtained without reducing it below the power bandwidth. Often, a bandwidth reduction is desirable to filter high frequency noise or unwanted signals.



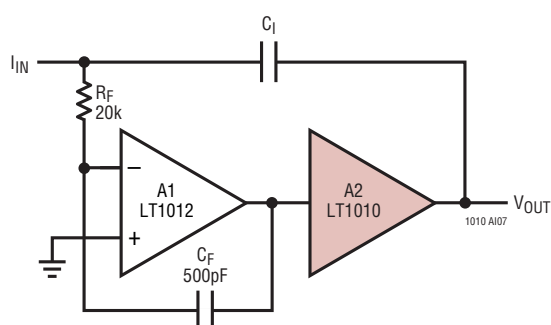
The follower configuration is unique in that capacitive load isolation is obtained without a reduction in small-signal bandwidth, although the output impedance of the buffer comes into play at high frequencies. The precision unity-gain buffer above has a 10MHz bandwidth without capacitive loading, yet it is stable for all load capacitance to over  $0.3\mu F$ , again determined by  $R_F C_F$ .

## APPLICATIONS INFORMATION

This is a good example of how fast op amps can be made quite easy to use by employing an output buffer.

### Integrator

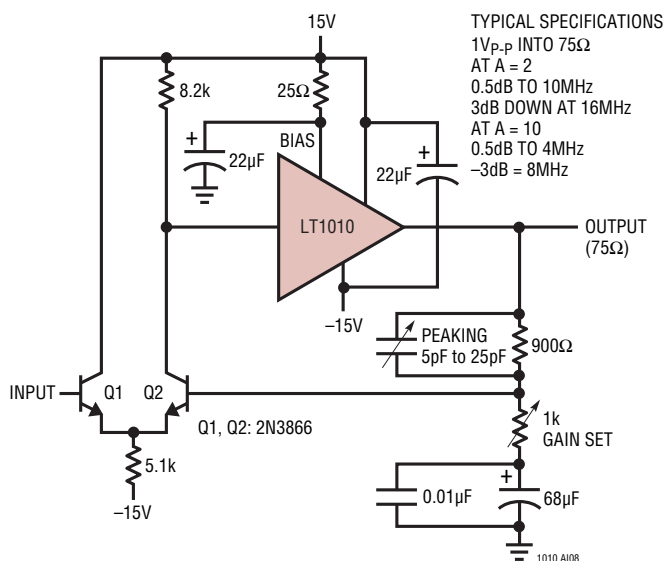
A lowpass amplifier can be formed just by using large  $C_F$  in the inverter described earlier, as long as the increasing closed-loop output impedance above the cutoff frequency is not a problem and the op amp is capable of supplying the required current at the summing junction.



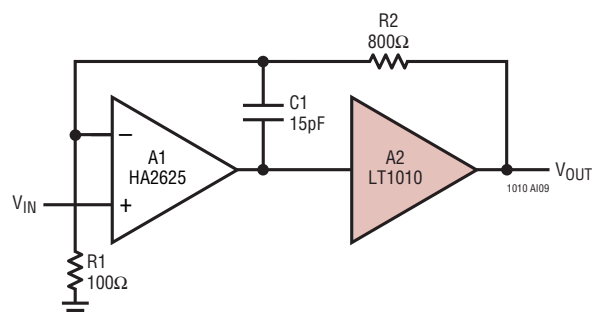
If the integrating capacitor must be driven from the buffer output, the circuit above can be used to provide capacitive load isolation. As before, the stability with large capacitive loads is determined by  $R_F C_F$ .

### Wideband Amplifiers

This simple circuit provides an adjustable gain video amplifier that will drive  $1V_{P-P}$  into  $75\Omega$ . The differential pair provides gain with the LT1010 serving as an output



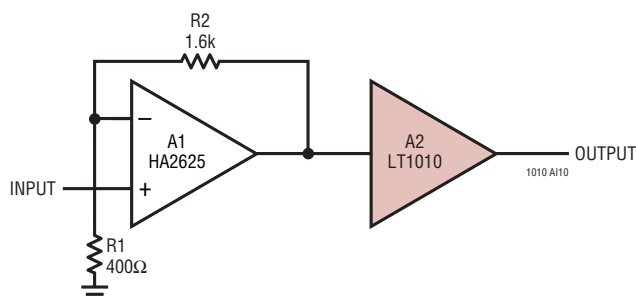
stage. Feedback is arranged in the conventional manner, although the  $68\mu F-0.01\mu F$  combination limits DC gain to unity for all gain settings. For applications sensitive to NTSC requirements, dropping the  $25\Omega$  output stage bias value will aid performance.



This shows the buffer being used with a wideband amplifier that is not unity-gain stable. In this case,  $C1$  cannot be used to isolate large capacitive loads. Instead, it has an optimum value for a limited range of load capacitances.

The buffer can cause stability problems in circuits like this. With the TO-220 packages, behavior can be improved by raising the quiescent current with a  $20\Omega$  resistor from the bias terminal to  $V^+$ . Alternately, devices in the miniDIP can be operated in parallel.

It is possible to improve capacitive load stability by operating the buffer class A at high frequencies. This is done by using quiescent current boost and bypassing the bias terminal to  $V^-$  with more than  $0.02\mu F$ .



Putting the buffer outside the feedback loop as shown here will give capacitive load isolation, with large output capacitors only reducing bandwidth. Buffer offset, referred to the op amp input, is divided by the gain. If the load resistance is known, gain error is determined by the output resistance tolerance. Distortion is low.

1010fe



When working with wideband amplifiers, special attention should *always* be paid to supply bypassing, stray capacitance and keeping leads short. Direct grounding of test probes, rather than the usual ground lead, is absolutely necessary for reasonable results.

The LT1010 has slew limitations that are not obvious from standard specifications. Negative slew is subject to glitching, but this can be minimized with quiescent

## Track and Hold

The 5MHz track and hold shown here has a 400kHz power bandwidth driving  $\pm 10V$ . A buffered input follower drives the hold capacitor, C4, through Q1, a low resistance FET switch. The positive hold command is supplied by TTL logic with Q3 level shifting to the switch driver, Q2. The output is buffered by A3.

When the gate is driven to  $V^-$  for HOLD, it pulls charge out of the hold capacitor. A compensating charge is put into the hold capacitor through C3. The step into hold is made independent of the input level with R7 and adjusted to zero with R10.

Since internal dissipation can be quite high when driving fast signals into a capacitive load, using a buffer in a power package is recommended. Raising buffer quiescent current to 40mA with R3 improves frequency response.

This circuit is equally useful as a fast acquisition sample and hold. An LT1056 might be used for A3 to reduce drift in hold because its lower slew rate is not usually a problem in this application.

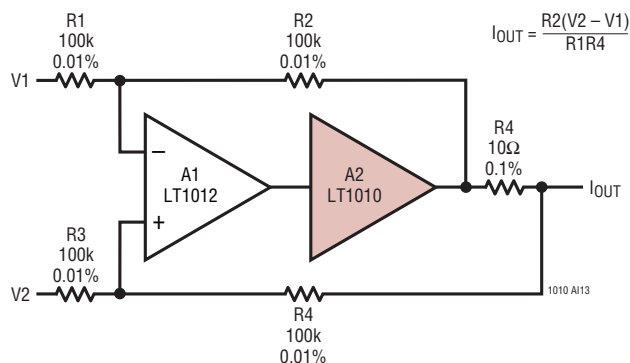
## Current Sources

A standard op amp voltage to current converter with a buffer to increase output current is shown here. As usual,

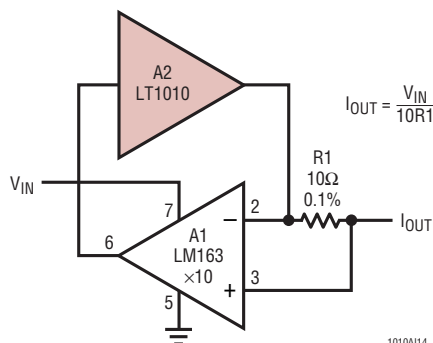


## APPLICATIONS INFORMATION

excellent matching of the feedback resistors is required to get high output resistance. Output is bidirectional.



This circuit uses an instrumentation amplifier to eliminate the matched resistors. The input is not high impedance and must be driven from a low impedance source like an op amp. Reversal of output sense can be obtained by grounding Pin 7 of the LM163 and driving Pin 5.

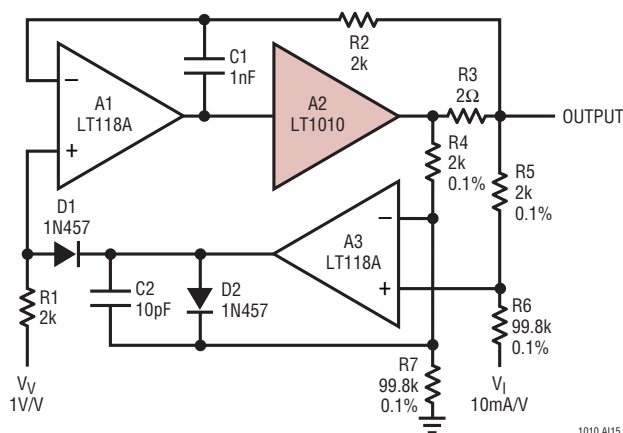


Output resistances of several megohms can be obtained with both circuits. This is impressive considering the  $\pm 150\text{mA}$  output capability. High frequency output characteristics will depend on the bandwidth and slew rate of the amplifiers. Both these circuits have an equivalent output capacitance of about 30nF.

### Voltage/Current Regulator

This circuit regulates the output voltage at  $V_V$  until the load current reaches a value programmed by  $V_I$ . For heavier loads, it is a precision current regulator.

With output currents below the current limit, the current regulator is disconnected from the loop by D1 with D2 keeping its output out of saturation. This output clamp



enables the current regulator to get control of the output current from the buffer current limit within a microsecond for an instantaneous short.

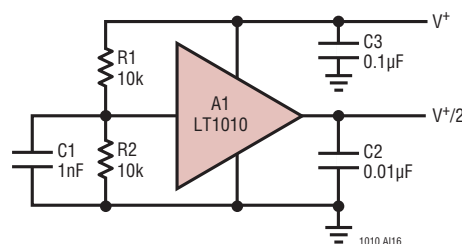
In the voltage regulation mode, A1 and A2 act as a fast voltage follower using the capacitive load isolation technique described earlier. Load transient recovery as well as capacitive load stability are determined by C1. Recovery from short circuit is clean.

Bidirectional current limit can be obtained by adding another op amp connected as a complement to A3.

### Supply Splitter

Dual supply op amps and comparators can be operated from a single supply by creating an artificial ground at half the supply voltage. The supply splitter shown here can source or sink 150mA.

The output capacitor, C2, can be made as large as necessary to absorb current transients. An input capacitor is also used on the buffer to avoid high frequency instability that can be caused by high source impedance.



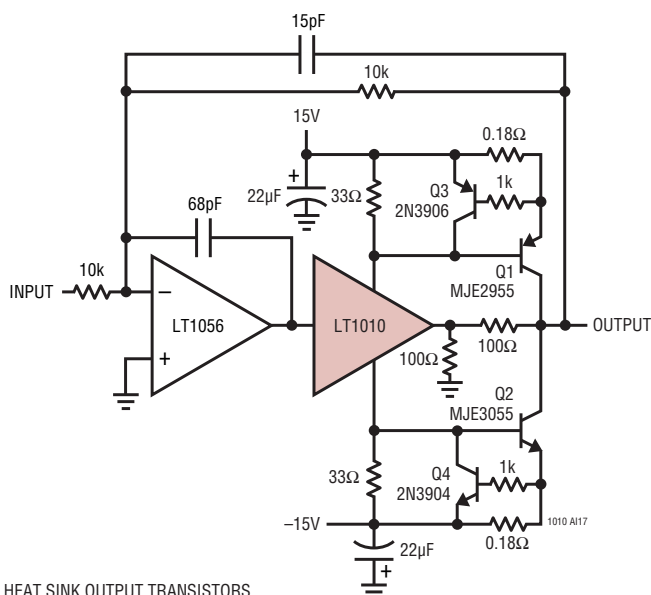


## APPLICATIONS INFORMATION

## High Current Booster

The circuit below uses a discrete stage to get 3A output capacity. The configuration shown provides a clean, quick way to increase LT1010 output power. It is useful for high current loads such as linear actuator coils in disk drives.

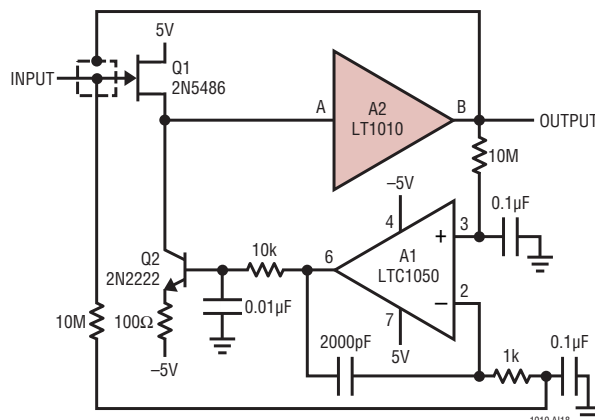
The 33Ω resistors sense the LT1010's supply current with the grounded 100Ω resistor supplying a load for the LT1010. The voltage drop across the 33Ω resistors biases Q1 and Q2. Another 100Ω value closes a local feedback loop, stabilizing the output stage. Feedback to the LT1056 control amplifier is via the 10k value. Q3 and Q4, sensing across the 0.18Ω units, furnish current limiting at about 3.3A.



HEAT SINK OUTPUT TRANSISTORS

### Wideband FET Input Stabilized Buffer

The figure below shows a highly stable unity-gain buffer with good speed and high input impedance. Q1 and Q2 constitute a simple, high speed FET input buffer. Q1 functions as a source follower with the Q2 current source load setting the drain-source channel current. The LT1010 buffer provides output drive capability for cables or whatever load is required. Normally, this open-loop configuration would be quite drifty because there is no DC feedback. The LTC®1050 contributes this function to stabilize the circuit. It does this by comparing the filtered circuit output to a



similarly filtered version of the input signal. The amplified difference between these signals is used to set Q2's bias, and hence, Q1's channel current. This forces Q1's  $V_{GS}$  to whatever voltage is required to match the circuit's input and output potentials. The 2000pF capacitor at A1 provides stable loop compensation. The RC network in A1's output prevents it from seeing high speed edges coupled through Q2's collector-base junction. A2's output is also fed back to the shield around Q1's gate lead, bootstrapping the circuit's effective input capacitance down to less than 1pF.

## Gain-Trimable Wideband FET Amplifier

A potential difficulty with the previous circuit is that the gain is not quite unity. The figure labeled A on the next page maintains high speed and low bias while achieving a true unity-gain transfer function.

This circuit is somewhat similar except that the Q2-Q3 stage takes gain. A2 DC stabilizes the input-output path and A1 provides drive capability. Feedback is to Q2's emitter from A1's output. The 1k adjustment allows the gain to be precisely set to unity. With the LT1010, output stage slew and full power bandwidth ( $1V_{P-P}$ ) are  $100V/\mu s$  and 10MHz respectively.  $-3dB$  bandwidth exceeds 35MHz. At  $A = 10$  (e.g., 1k adjustment set at  $50\Omega$ ), full power bandwidth stays at 10MHz while the  $-3dB$  point falls to 22MHz.

With the optional discrete stage, slew exceeds 1000V/ $\mu$ s and full power bandwidth (1V<sub>P-P</sub>) is 18MHz. -3dB bandwidth is 58MHz. At A=10, full power is available to 10MHz, with the -3dB point at 36MHz.



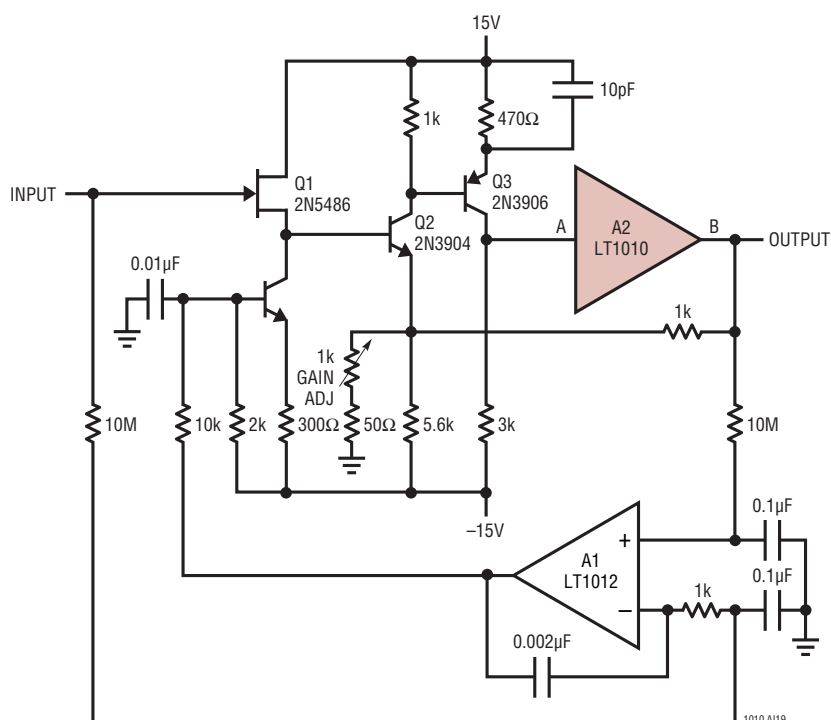
## APPLICATIONS INFORMATION

Figures A and B show response with both output stages. The LT1010 is used in Figure A (Trace A = input, Trace B = output). Figure B uses the discrete stage and is slightly faster. Either stage provides more than adequate performance for driving video cable or data converters and the LT1012 maintains DC stability under all conditions.

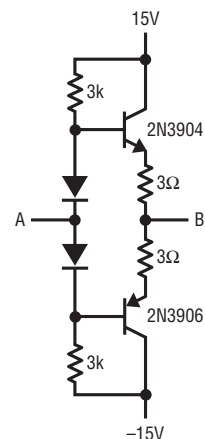
### Thermal Considerations for the MiniDIP Package

The miniDIP package requires special thermal considerations since it is not designed to dissipate much power. Be aware that for applications requiring large output currents, another package should be used.

Gain-Trimable Wideband FET Amplifier



(A)



(B)

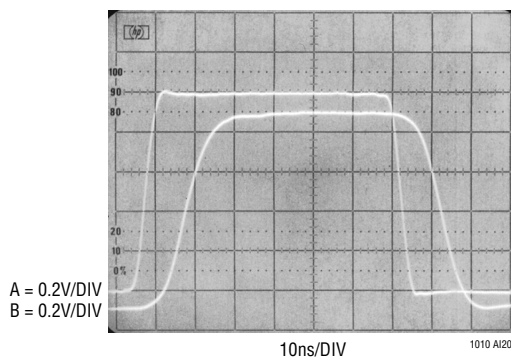


Figure A. Waveforms Using LT1010

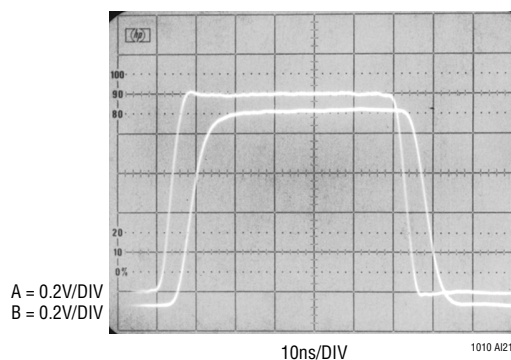


Figure B. Waveforms Using Discrete Stage

## APPLICATIONS INFORMATION

Typical thermal calculations for the miniDIP package are detailed in the following paragraphs.

For 4.8mA supply current (typical at 50°C, 30V supply voltage—see supply current graphs) to the LT1010 at  $\pm 15V$ ,  $P_D$  = power dissipated in the part is equal to:

$$(30V)(0.0048A) = 0.144W$$

The rise in junction is then:

$$(0.144W)(100^\circ C/W \text{—This is } \theta_{JA} \text{ for the N package}) \\ = 14.4^\circ C.$$

This means that the junction temperature in 50°C ambient air without driving any current into a load is:

$$14.4^\circ C + 50^\circ C = 64.4^\circ C$$

Using the LT1010 to drive 8V DC into a 200 $\Omega$  load using  $\pm 15V$  power supplies dissipates  $P_D$  in the LT1010 where:

$$P_D = \frac{(V^+ - V_{OUT})(V_{OUT})}{R_L} \\ = \frac{(15V - 8V)(8V)}{200\Omega} = 0.280W$$

This causes the LT1010 junction temperature to rise another  $(0.280W)(100^\circ C/W) = 28^\circ C$ .

This heats the junction to  $64.4^\circ C + 28^\circ C = 92.4^\circ C$ .

An example of 1MHz operation further shows the limitations of the N (or miniDIP) package. For  $\pm 15V$  operation:

$$P_D \text{ at } I_L = 0 \text{ at } 1MHz^* = (10mA)(30V) = 0.30W$$

This power dissipation causes the junction to heat from 50°C (ambient in this example) to  $50^\circ C + (0.3W)(100^\circ C/W) = 80^\circ C$ . Driving 2V<sub>RMS</sub> of 1MHz signal into a 200 $\Omega$  load causes an additional

$$P_D = \left( \frac{2V}{200\Omega} \right) \cdot (15 - 2) = 0.130W$$

to be dissipated, resulting in another  $(0.130W)(100^\circ C/W) = 13^\circ C$  rise in junction temperature to  $80^\circ C + 13^\circ C = 93^\circ C$ .

### Thermal Resistance of DFN Package

For surface mount devices, heat sinking is accomplished by using the heat spreading capabilities of the PC board and its copper traces. Copper board stiffeners and plated through-holes can also be used to spread the heat generated by power devices.

The following table lists thermal resistance for several different board sizes and copper areas. All measurements were taken in still air on 3/32" FR-4 board with one ounce copper.

**Table 1. DFN Measured Thermal Resistance**

| COPPER AREA |            | BOARD AREA | THERMAL RESISTANCE<br>(JUNCTION-TO-AMBIENT) |
|-------------|------------|------------|---------------------------------------------|
| TOPSIDE     | BACKSIDE   |            |                                             |
| 2500 sq mm  | 2500 sq mm | 2500 sq mm | 40°C/W                                      |
| 1000 sq mm  | 2500 sq mm | 2500 sq mm | 45°C/W                                      |
| 225 sq mm   | 2500 sq mm | 2500 sq mm | 50°C/W                                      |
| 100 sq mm   | 2500 sq mm | 2500 sq mm | 62°C/W                                      |

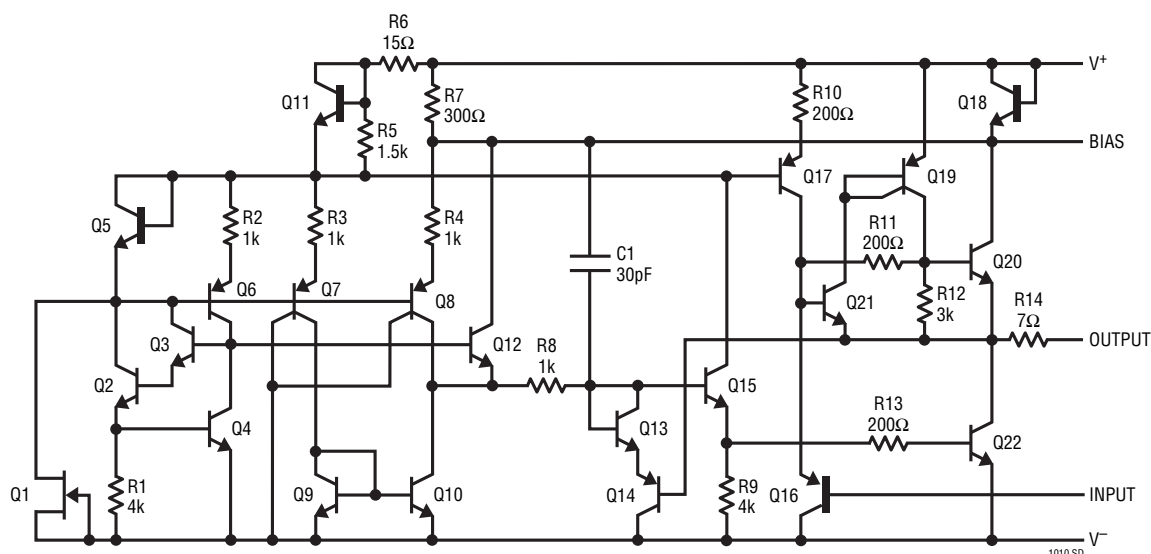
For the DFN package, the thermal resistance junction-to-case ( $\theta_{JC}$ ), measured at the Exposed Pad on the back of the die, is 7.5°C/W.

Continuous operation at the maximum supply voltage and maximum load current is not practical due to thermal limitations. Transient operation at the maximum supply is possible. The approximate thermal time constant for a 2500sq mm 3/32" FR-4 board with maximum topside and backside area for one ounce copper is 3 seconds. This time constant will increase as more thermal mass is added (i.e., vias, larger board and other components).

For an application with transient high power peaks, average power dissipation can be used for junction temperature calculations as long as the pulse period is significantly less than the thermal time constant of the device and board.

\*See Supply Current vs Frequency graph.

## SCHEMATIC DIAGRAM (Excluding protection circuits)



## DEFINITION OF TERMS

**Output Offset Voltage:** The output voltage measured with the input grounded (split supply operation).

**Input Bias Current:** The current out of the input terminal.

**Large-Signal Voltage Gain:** The ratio of the output voltage change to the input voltage change over the specified input voltage range.\*

**Output Resistance:** The ratio of the change in output voltage to the change in load current producing it.\*

**Output Saturation Voltage:** The voltage between the output and the supply rail at the limit of the output swing toward that rail.

**Saturation Offset Voltage:** The output saturation voltage with no load.

**Saturation Resistance:** The ratio of the change in output saturation voltage to the change in current producing it, going from no load to full load.\*

**Slew Rate:** The average time rate of change of output voltage over the specified output range with an input step between the specified limits.

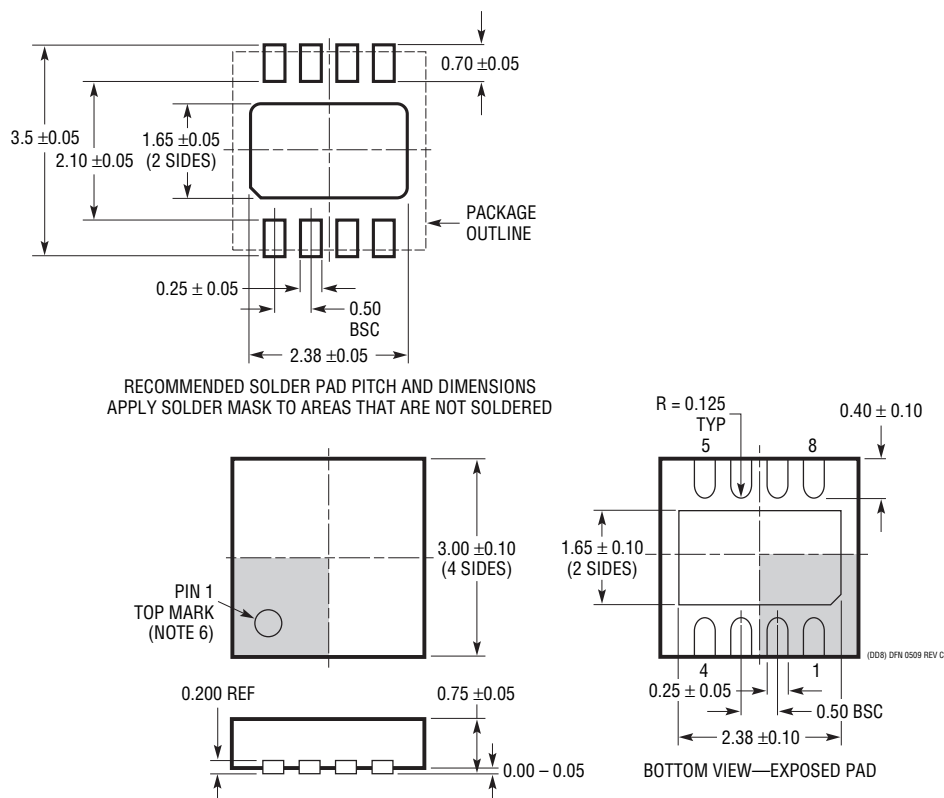
**Bias Terminal Voltage:** The voltage between the bias terminal and  $V^+$ .

**Supply Current:** The current at either supply terminal with no output loading.

\*Pulse measurements (~1ms) as required to minimize thermal effects.

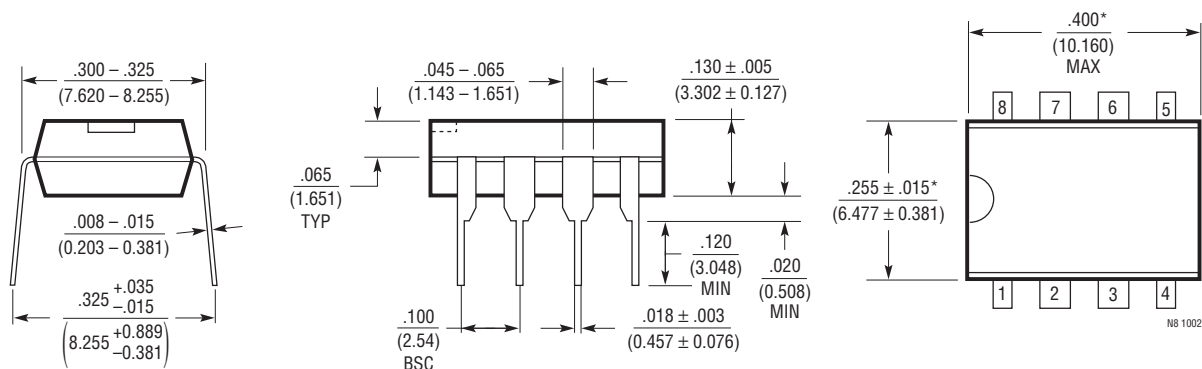
# PACKAGE DESCRIPTION

## DD Package 8-Lead Plastic DFN (3mm × 3mm) (Reference LTC DWG # 05-08-1698 Rev C)



## PACKAGE DESCRIPTION

### N8 Package 8-Lead PDIP (Narrow .300 Inch) (Reference LTC DWG # 05-08-1510)

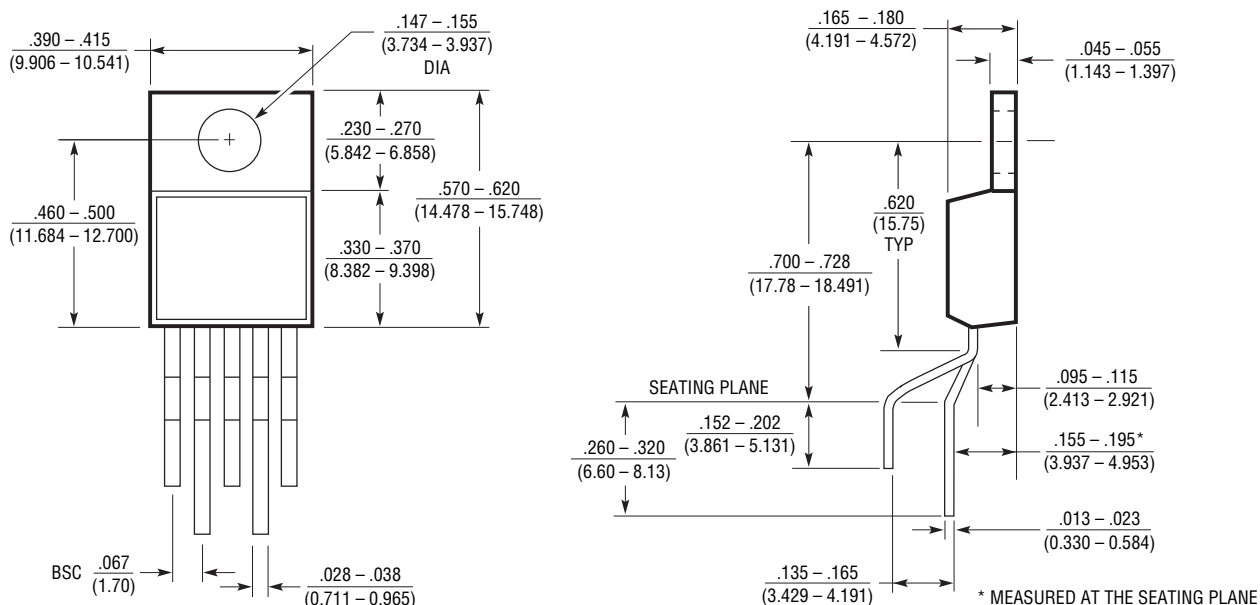


**NOTE:**

1. DIMENSIONS ARE  $\frac{\text{INCHES}}{\text{MILLIMETERS}}$

\*THESE DIMENSIONS DO NOT INCLUDE MOLD FLASH OR PROTRUSIONS.  
MOLD FLASH OR PROTRUSIONS SHALL NOT EXCEED .010 INCH (0.254mm)

### T Package 5-Lead Plastic TO-220 (Standard) (Reference LTC DWG # 05-08-1421)



\* MEASURED AT THE SEATING PLANE

T5 (TO-220) 0801

**REVISION HISTORY** (Revision history begins at Rev E)

| REV | DATE | DESCRIPTION                                                                                                                                                                             | PAGE NUMBER |
|-----|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| E   | 5/11 | Revised temperature ranges and values in Absolute Maximum Ratings and Pin Configuration sections                                                                                        | 2           |
|     |      | Revised Notes 2 and 4 in Electrical Characteristics section                                                                                                                             | 3           |
|     |      | Updated temperature values in Power Dissipation, Thermal Considerations for the MiniDIP Package, and Thermal Resistance of DFN Package sections of the Applications Information section | 8, 15       |

# LT1010

## RELATED PARTS

| PART NUMBER | DESCRIPTION                             | COMMENTS                                                    |
|-------------|-----------------------------------------|-------------------------------------------------------------|
| LT1206      | 250mA, 60MHz Current Feedback Amplifier | 900V/ $\mu$ s, Excellent Video Characteristics              |
| LT1210      | 1.1A, 35MHz Current Feedback Amplifier  | 900V/ $\mu$ s Slew Rate, Stable with Large Capacitive Loads |
| LT1795      | Dual 500mA, 50MHz CFA                   | 500mA $I_{OUT}$ ADSL Driver                                 |
| LT1886      | Dual 700MHz, 200mA Op Amp               | DSL Driver                                                  |

1010fe



SingleTact is the most refined miniature force sensor that is performance oriented. Ultra-thin, single-element capacitive sensor with industry-leading sensitivity and repeatability that accurately and reliably quantifies forces.



### REPEATABILITY

SingleTact has repeatability errors that are less than 1.0% of FSR.



### ACCURACY

SingleTact has the best accuracy among thin film force sensors.



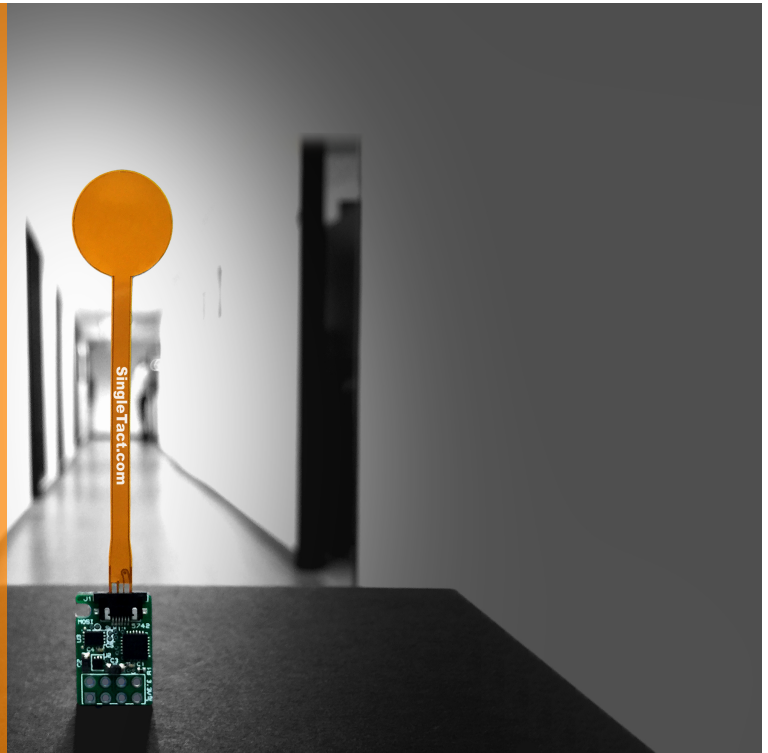
### EASY TO USE

SingleTact Sensors are designed to be versatile, but user-friendly.



### CUSTOM DESIGN

SingleTact can be customized for your OEM applications.



## SingleTact FEATURES

**Ultra-thin force sensors** come in sizes of 8mm and 15mm diameter that is only 0.35mm thick.

**Highly sensitive and repeatable sensors** provide high dynamic range and errors less than 1.0%.

**Simple Analog 3-wire interface** for immediate DAQ integration.

**I2C interface** for digital integration.

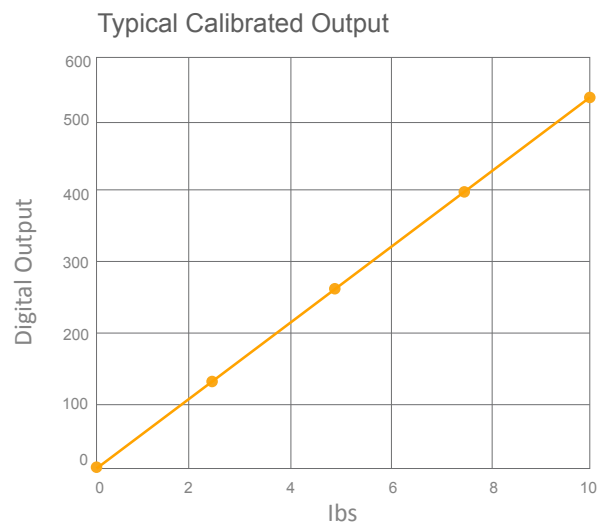
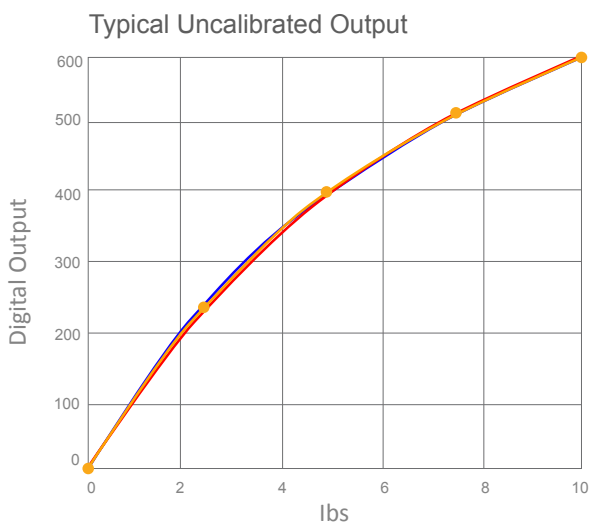
**Arduino and DAQ Software** to begin collecting data right out of the box.

**Custom designed solutions available for OEM applications.**

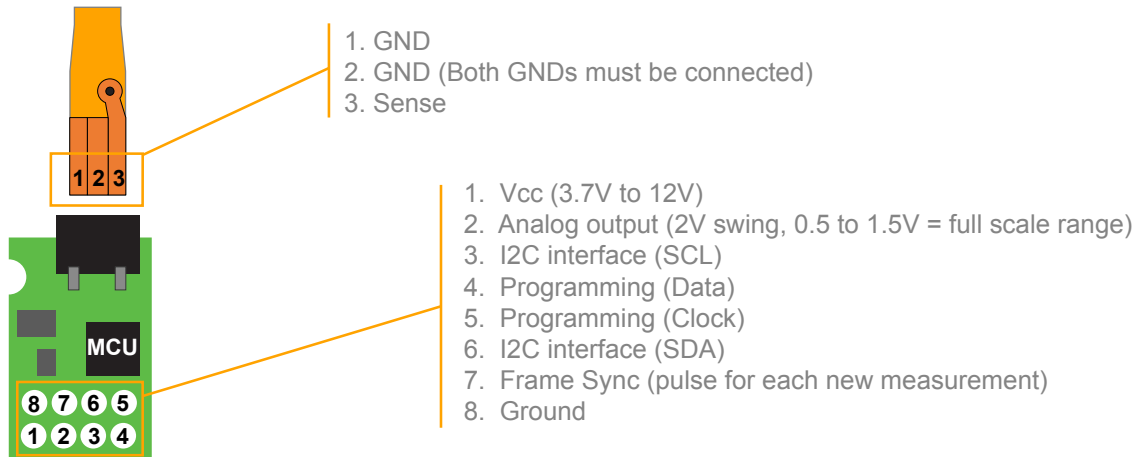
## SENSOR PERFORMANCE

|                                     |                                     |
|-------------------------------------|-------------------------------------|
| Typical Signal to Noise Ratio (SNR) | 500:1                               |
| Force Resolution                    | < 0.2% of Full Scale (FS)           |
| Maximum Force                       | 300% of FS                          |
| Typical Repeatability Error         | < 1.0% (1 sigma of FS)              |
| Operating Temperature               | -40°C – 200°C                       |
| Temperature Sensitivity             | Up to 0.2%/°C                       |
| Linearity Error                     | < 2.0%                              |
| Drift                               | < 2% per logarithmic time scale     |
| Hysteresis                          | < 4.0%                              |
| Response Time                       | < 1ms(Measured using Oscilloscope)  |
| Contact Surface Material            | Polyimide                           |
| Sensor Thickness                    | 0.35mm                              |
| Tail Length                         | 50mm                                |
| Typical Baseline Capacitance        | 8mm: 75 pF; 15mm : 230 pF @ 100kHz  |
| Typical Capacitance Change          | 8mm: 2.2 pF; 15mm : 5.5 pF @ 100kHz |
| ESD Sensitivity                     | Not sensitive to ESD                |
| Material Grade                      | UL grade 94 V-1 or better           |

## SENSOR CHARACTERISTICS



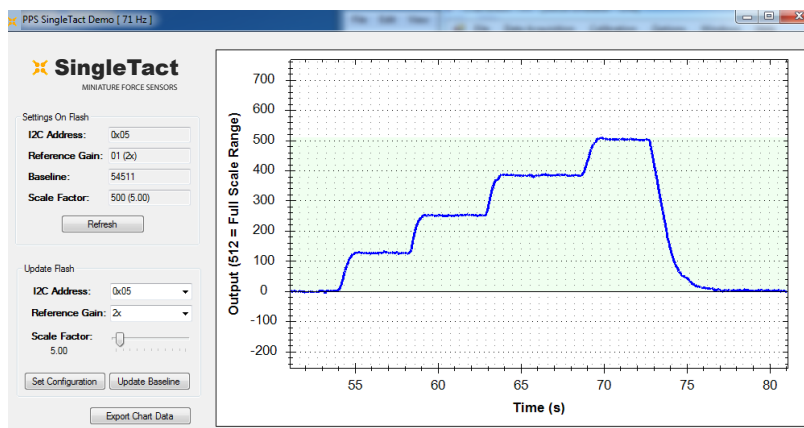
## PINOUTS DIAGRAM FOR SENSOR & I2C BOARD



## ELECTRONICS SPECIFICATIONS

|                       |                                 |
|-----------------------|---------------------------------|
| Update Rate           | >300 Hz                         |
| Analog Out            | 0.5–1.5V                        |
| Digital Interface     | I2C (100kHz), 10-bit resolution |
| IO Voltage            | 3.3V                            |
| Supply Voltage        | 3.7-12V                         |
| Input Current         | 2.5mA running at 3MHz           |
| Weight                | Sensor 0.23g/ Electronics 1.6g  |
| RoHS                  | Compliant                       |
| Operating Temperature | -40°C – 85°C                    |

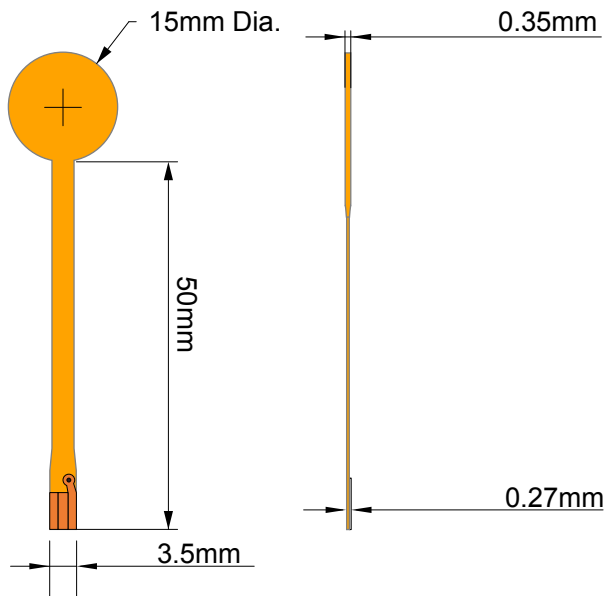
## SOFTWARE



Download SingleTact Software

[www.singletact.com/software-download](http://www.singletact.com/software-download)

## SENSOR MECHANICAL SPEC-15mm



### SENSORS

#### S15-4.5N

+Full scale Range: 0.45kg (1.0lbs)  
+Minimal Detectable Force: 0.9g

#### S15-45N

+Full scale Range: 4.5kg (10lbs)  
+Minimal Detectable Force: 9g

#### S15-450N

+Full scale Range: 45kg (100lbs)  
+Minimal Detectable Force: 90g

### CALIBRATED SENSORS

#### CS15-4.5N

+Full scale Range: 0.45kg (1.0lbs)  
+Minimal Detectable Force: 0.9g

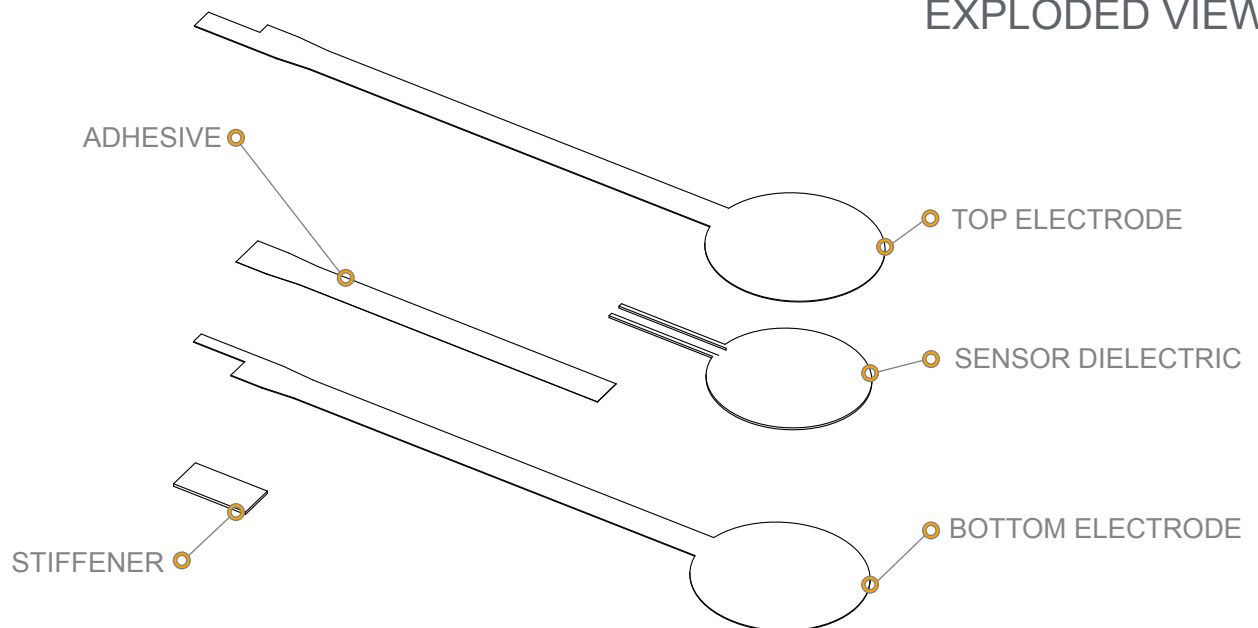
#### CS15-45N

+Full scale Range: 4.5kg (10lbs)  
+Minimal Detectable Force: 9g

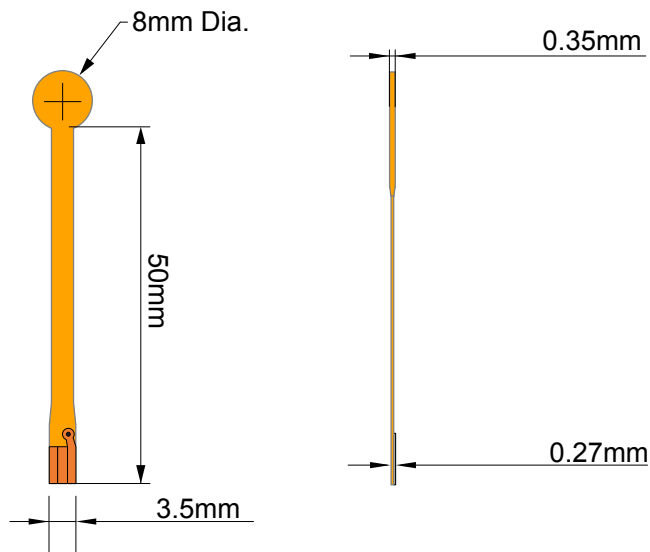
#### CS15-450N

+Full scale Range: 45kg (100lbs)  
+Minimal Detectable Force: 90g

## EXPLODED VIEW



## SENSOR MECHANICAL SPEC-8mm



### SENSORS

#### S8-1N

+Full scale Range: 100g (0.22lbs)  
+Minimal Detectable Force: 0.2g

#### S8-10N

+Full scale Range: 1.0kg (2.2lbs)  
+Minimal Detectable Force: 2g

#### S8-100N

+Full scale Range: 10kg (22lbs)  
+Minimal Detectable Force: 20g

### CALIBRATED SENSORS

#### CS8-1N

+Full scale Range: 100g (0.22lbs)  
+Minimal Detectable Force: 0.2g

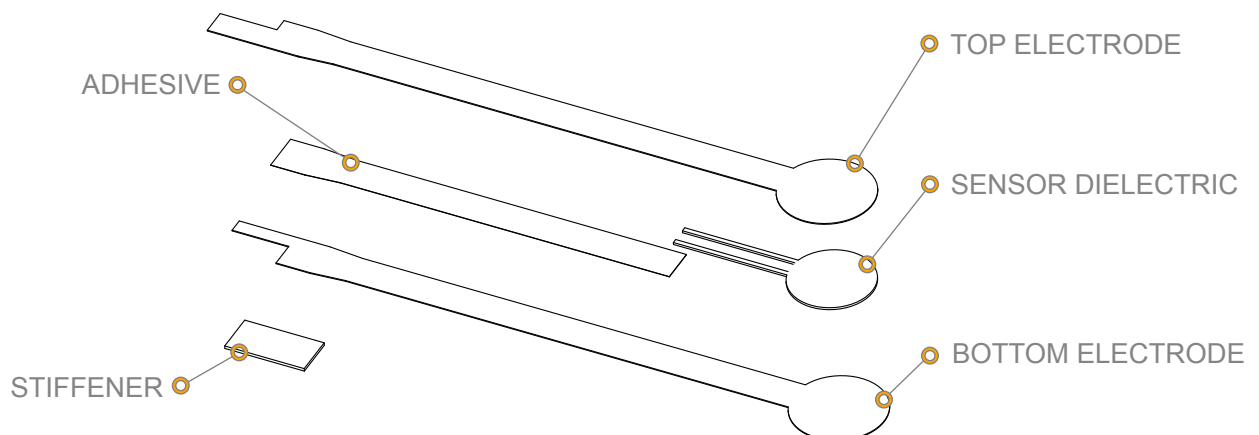
#### CS8-10N

+Full scale Range: 1.0kg (2.2lbs)  
+Minimal Detectable Force: 2g

#### CS8-100N

+Full scale Range: 10kg (22lbs)  
+Minimal Detectable Force: 20g

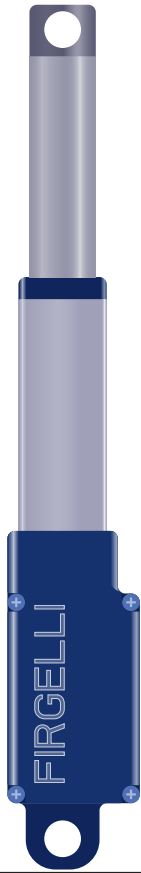
## EXPLODED VIEW



## Miniature Linear Motion Series • L12

Firgelli Technologies' unique line of Miniature Linear Actuators enables a new generation of motion-enabled product designs, with capabilities that have never before been combined in a device of this size. These small linear actuators are a superior alternative to designing with awkward gears, motors, servos and linkages.

Firgelli's L series of micro linear actuators combine the best features of our existing micro actuator families into a highly flexible, configurable and compact platform with an optional sophisticated on-board microcontroller. The first member of the L series, the L12, is an axial design with a powerful drivetrain and a rectangular cross section for increased rigidity. But by far the most attractive feature of this actuator is the broad spectrum of available configurations.



### Benefits

- Compact miniature size
- Simple control using industry standard interfaces
- Low voltage
- Equal push / pull force
- Easy mounting

### Applications

- Robotics
- Consumer appliances
- Toys
- Automotive
- Industrial automation

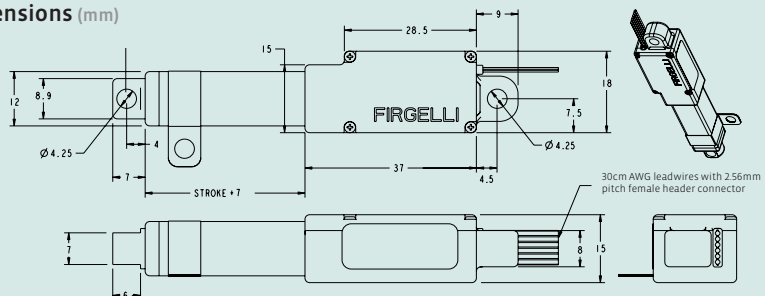
### L12 Specifications

| Gearing Option                  | 50                                  | 100           | 210             |        |
|---------------------------------|-------------------------------------|---------------|-----------------|--------|
| Peak Power Point <sup>1</sup>   | 12 N @ 11 mm/s                      | 23 N @ 6 mm/s | 45 N @ 2.5 mm/s |        |
| Peak Efficiency Point           | 6 N @ 16 mm/s                       | 12 N @ 8 mm/s | 18 N @ 4 mm/s   |        |
| Max Speed (no load)             | 23 mm/s                             | 12 mm/s       | 5 mm/s          |        |
| Backdrive Force <sup>2</sup>    | 43 N                                | 80 N          | 150 N           |        |
| Stroke Option                   | 10 mm                               | 30 mm         | 50 mm           | 100 mm |
| Weight                          | 28 g                                | 34 g          | 40 g            | 56 g   |
| Positional Accuracy             | 0.1 mm                              | 0.2 mm        | 0.2 mm          | 0.3 mm |
| Max Side Force (fully extended) | 50 N                                | 40 N          | 30 N            | 15 N   |
| Mechanical Backlash             | 0.1 mm                              |               |                 |        |
| Feedback Potentiometer          | 2.75 kΩ/mm ± 30%, 1% linearity      |               |                 |        |
| Duty Cycle                      | 20 %                                |               |                 |        |
| Lifetime                        | 1000 hours at rated duty cycle      |               |                 |        |
| Operating Temperature           | -10°C to +50°C                      |               |                 |        |
| Storage Temperature             | -30°C to +70°C                      |               |                 |        |
| Ingress Protection Rating       | IP-54                               |               |                 |        |
| Audible Noise                   | 55 dB at 45 cm                      |               |                 |        |
| Stall Current                   | 450 mA at 5 V & 6 V, 200 mA at 12 V |               |                 |        |

<sup>1</sup> 1 N (Newton) = 0.225 lb<sub>f</sub> (pound-force)

<sup>2</sup> a powered-off actuator will statically hold a force up to the Backdrive Force

### Dimensions (mm)



### Firgelli Technologies Inc.

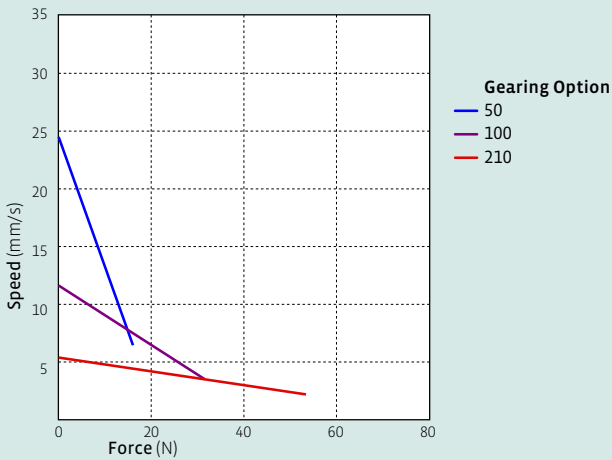
4585 Seawood Tce.  
Victoria, BC V8N 3W1  
Canada

1 (206) 347-9684 phone  
1 (888) 225-9198 toll-free  
1 (206) 347-9684 fax

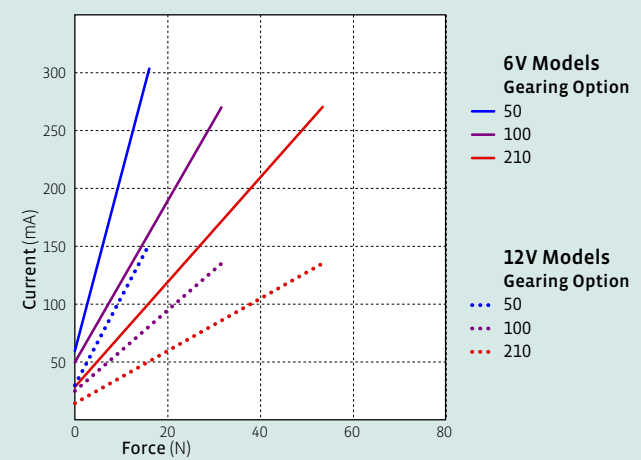
sales@firgelli.com  
www.firgelli.com

## L12 Specifications

Load Curves



Current Curves



## Model Selection

The L12 has five configurable features. L12 configurations are identified according to the following scheme:

**L12-SS-GG-VV-C-L**

| feature                                                      | options                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SS:</b> Stroke Length (in mm)                             | <b>10, 30, 50, 100</b><br>Any stroke length between 10 and 100 mm is available on custom orders, in 2 mm increments.                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>GG:</b> Gear reduction ratio (refer to force/speed plots) | <b>50, 100, 210</b><br>Other gearing options may be possible on custom orders.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>VV:</b> Voltage                                           | <b>06</b> 6V (5V power for Controller options B and P)<br><b>12</b> 12V                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| <b>C:</b> Controller                                         | <b>B</b> Basic 2-wire open-loop interface, no position feedback, control, or limit switching. Positive voltage extends, negative retracts.<br><b>S</b> 2-wire open-loop interface (like B option) with limit switching at stroke endpoints.<br><b>P</b> Simple analog position feedback signal, no on-board controller.<br><b>I</b> Integrated controller with Industrial and RC servo interfaces (see L12 Controller Options section). Not available with 10mm stroke length configurations.<br><b>R</b> RC Linear Servo. Not available with 10mm stroke or 12 volts. |
| <b>L:</b> Mechanical or electrical interface customizations  | Custom option codes will be issued by Firgelli for custom builds when applicable.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |

## Basis of Operation

The L12 actuator is designed to move push or pull loads along its full stroke length. The speed of travel is determined by the gearing of the actuator and the load or force the actuator is working against at a given point in time (see Load Curves chart on this datasheet). When power is removed, the actuator stops moving and holds its position, unless the applied load exceeds the backdrive force, in which case the actuator will backdrive. Stalling the actuator under power for short periods of time (several seconds) will not damage the actuator. Do not reverse the supply voltage polarity to actuators containing an integrated controller (I controller option).

Each L12 actuator ships with two mounting clamps, two mounting brackets and two rod end options: a clevis end and a threaded end with nut (see drawing on page 4). When changing rod ends, extend the actuator completely and hold the round shaft while unscrewing the rod end. Standard lead wires are 28 AWG, 30 cm long with 2.56 mm (0.1") pitch female header connector (Hi-Tec™ and Futaba™ compatible). Actuators are a sealed unit (IP-54 rating, resistant to dust and water ingress but not fully waterproof).

## Ordering information

Sample quantities may be ordered with a credit card directly from [www.firgelli.com](http://www.firgelli.com).

Please contact Firgelli at [sales@firgelli.com](mailto:sales@firgelli.com) for volume pricing or custom configurations.

Note that not all configuration combinations are stocked as standard products. Please refer to [www.firgelli.com/orders](http://www.firgelli.com/orders) for current inventory.



## L12 Controller options

### Option B—Basic 2-wire interface

#### WIRING:

**1** (red) **Motor V+** (5V or 12V)

**2** (black) **Motor ground**

The –B actuators offer no control or feedback mechanisms. While voltage is applied to the motor V+ and ground leads, the actuator extends. If the polarity of this voltage is reversed, the actuator retracts. The 5 V actuator is rated for 5 V but can operate at 6V.

### Option S—Basic 2-wire interface

#### WIRING:

**1** (red) **Motor V+** (5V or 12V)

**2** (black) **Motor ground**

When the actuator moves to a position within 0.5mm of its fully-retracted or fully-extended stroke endpoint, a limit switch will stop power to the motor. When this occurs, the actuator can only be reversed away from the stroke endpoint. Once the actuator is positioned away from its stroke endpoint, normal operation resumes. For custom orders, limit switch trigger positions can be modified at the time of manufacture, in 0.5mm increments.

### Option P—Position feedback signal

#### WIRING:

**1** (orange) **Feedback potentiometer negative reference rail**

**2** (purple) **Feedback potentiometer wiper** (position signal)

**3** (red) **Motor V+** (5V or 12V)

**4** (black) **Motor ground**

**5** (yellow) **Feedback potentiometer positive reference rail**

The –P actuators offer no built-in controller, but do provide an analog position feedback signal that can be input to an external controller. While voltage is applied to the motor V+ and ground leads, the actuator extends. If the polarity of this voltage is reversed, the actuator retracts. Actuator stroke position may be monitored by providing any stable low and high reference voltages on leads 1 and 5, and then reading the position signal on lead 2. The voltage on lead 2 will vary linearly between the two reference voltages in proportion to the position of the actuator stroke.

### Option I—Integrated controller with industrial and RC servo interfaces

#### WIRING:

**1** (green) **Current input signal** (used for 4–20 mA interface mode)

**2** (blue) **Voltage input signal** (used for the 0–5V interface mode and PWM interface modes)

**3** (purple) **Position Feedback signal** (0–3.3 V, linearly proportional to actuator position)

**4** (white) **RC input signal** (used for RC-servo compatible interface mode)

**5** (red) **Motor V+** (+6 Vdc for 6 V models, +12 Vdc for 12 V models)

**6** (black) **Ground**

The –I actuator models feature an on-board software-based digital microcontroller. The microcontroller is not user-programmable.

The six lead wires are split into two connectors. Leads 4, 5 and 6 terminate at a universal RC servo three-pin connector (Hi-Tec™ and Futaba™ compatible). Leads 1, 2 and 3 terminate at a separate, similarly sized connector.

When the actuator is powered up, it will repeatedly scan leads 1, 2, 4 for an input signal that is valid under any of the four supported interface modes. When a valid signal is detected, the actuator will self-configure to the corresponding interface mode, and all other interface modes and input leads are disabled until the actuator is next powered on.

**0–5V Interface Mode:** This mode allows the actuator to be controlled with just a battery, and a potentiometer to signal the desired position to the actuator – a simple interface for prototypes or home automation projects. The desired actuator position (setpoint) is input to the actuator on lead 2 as a voltage between ground and 5V. The setpoint voltage must be held on lead 2 until the desired actuator stroke position is reached. Lead 2 is a high impedance input.

**4–20 mA Interface Mode:** This mode is compatible with PLC devices typically used in industrial control applications. The desired actuator position (setpoint) is input to the actuator on lead 1 as a current between 4 mA and 20 mA. The setpoint current must be held on lead 1 until the desired actuator stroke position is reached.

**RC Servo Interface Mode:** This is a standard hobby-type remote-control digital servo interface (CMOS logic), compatible with servos and receivers from manufacturers like Futaba™ and Hi-Tec™. The desired actuator position is input to the actuator on lead 4 as a positive 5 Volt pulse width signal. A 1.0 ms pulse commands the controller to fully retract the actuator, and a 2.0 ms pulse signals full extension. If the motion of the actuator, or of other servos in your system, seems erratic, place a 1–4Ω resistor in series with the actuator's red V+ leadwire.

**PWM Mode:** This mode allows control of the actuator using a single digital output pin from an external microcontroller. The desired actuator position is encoded as the duty cycle of a 5 Volt 1 kHz square wave on actuator lead 2, where the % duty cycle sets the actuator position to the same % of full stroke extension. The waveform must be 0V to +5V in order to access the full stroke range of the actuator.

### Option R—RC Linear Servo

#### WIRING:

**1** (white) **RC input signal**

**2** (red) **Motor V+** (6VOC)

**3** (black) **Ground**

The –R actuators or 'linear servos' are a direct replacement for regular radio controlled hobby servos. Operation is as above in RC servo interface mode (option I). The –R actuators are available in 6 volt and 30, 50 and 100 mm strokes only.



# Understanding the Data Sheet

Products: **All**

This note is supplied to provide further information about the data presented in Firgelli Data sheets. The intent is to provide clarification on ratings, units, and graphs.

## Terms:

|                              |                                                                                                                                                                                     |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Peak Power Point</b>      | – Power is calculated by multiplying speed by force. The Peak Power point is the specific speed and force point that will result in the largest power output.                       |
| <b>Peak Efficiency Point</b> | – This indicates the motor speed where the actuator most efficiently converts electricity to mechanical power.                                                                      |
| <b>Max Speed (no load)</b>   | – This is the maximum speed that the actuator can achieve. This occurs when nothing is attached to the actuators piston.                                                            |
| <b>Max Force (lifted)</b>    | – The maximum force that can be reliably lifted by the actuator without causing damage.                                                                                             |
| <b>Max Side Load</b>         | – This is the maximum external force that can be applied perpendicular to the actuators shaft. If this is exceeded, the actuator may not operate correctly and life may be reduced. |
| <b>Back Drive Force</b>      | – This is the amount of force that it takes to overcome friction in an actuator with the power removed, causing the shaft to move.                                                  |
| <b>Stroke</b>                | – This is the distance that the actuator shaft can travel.                                                                                                                          |
| <b>Input Voltage</b>         | – This is the maximum recommended supply voltage for the actuator. Using a voltage lower than this will result in reduced speed and force.                                          |
| <b>Stall Current</b>         | – This is the maximum current that the actuator will draw when it is allowed to stall against a hard limit.                                                                         |
| <b>Mass</b>                  | – Weight of the actuator.                                                                                                                                                           |



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|                              |                                                                                                                                                                                                                                                                                     |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Operating Temperature</b> | – This is the ambient temperature range where the actuator will operate within its performance specs. Outside this range the actuator will continue to operate, but at different speeds, forces and currents, and with reduced life expectancy.                                     |
| <b>Positional Accuracy</b>   | – For models with potentiometer feedback this is the level of accuracy that is attainable based on the linearity of the feedback.                                                                                                                                                   |
| <b>Lifetime</b>              | – Cycle life is based on testing of unloaded actuators with one stall condition at either end of stroke. This tests potentiometer life and mechanical life. The stall condition is the worst case load for an actuator. This applies a load well in excess of the Peak Power Point. |
| <b>Audible Noise</b>         | – Maximum noise levels during normal operation measured at 45cm from the actuator.                                                                                                                                                                                                  |
| <b>Ingress Protection</b>    | – This rating indicates the amount of environmental protection the case provides. IP54 indicates “Protected against dust limited ingress (no harmful deposit).” And “Protection against water sprayed from all directions - limited ingress permitted.”                             |

## Units:

|                                       |                                                                                                                                                                                                         |
|---------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>g</b> – Grams,                     | SI Units used to indicate the weight of the actuator. Convert to ounces by multiplying by 0.035273.                                                                                                     |
| <b>N</b> – Newtons,                   | SI Units used to define forces which the actuator can push or pull. Newtons can be converted to an equivalent hung Weight in pounds by dividing by 9.8 and multiplying by 2.2.                          |
| <b>mm/s</b> – Millimeters per Second, | SI Unit used to define actuator speed. Convert to inches per second by multiplying by 0.039370078                                                                                                       |
| <b>V,VDC</b> - Volts Direct Current,  | These are used interchangeably. No Firgelli actuators accept AC voltages. An example of AC would be the power in our houses. To get DC an AC/DC transformer can be used. An example of DC is a battery. |
| <b>°C</b> – Degrees Celsius           | Convert to degrees Fahrenheit by multiplying by 1.8 and then adding 32.                                                                                                                                 |
| <b>dB</b> – Decibel,                  | A measurement of sound level. A reading of 60-75 is comparable to a normal conversation at 3-5 feet.                                                                                                    |



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## Graphs:

### **Load Curves**

This graph shows a plot of speed versus load. As the load is increased the speed of the actuator will be reduced. The multiple lines indicate different gear options that can be specified when ordering.

### **Current curves**

This graph shows a plot of current versus load. As the load is increased the amount of current drawn is increased. The multiple lines show different motor voltage and gear options.

### **Speed curves**

A graph of speed versus voltage is not shown however this is also linear. Actuator speed for a fixed load can be varied by varying the motor voltage. For integrated controllers or the CIB the minimum allowed voltage is ~4V.

## **Schematics:**

**Dimensions** - All dimensions are given in mm. To convert to inches multiply by 0.039370078.

Ø – Indicates a diameter.

**Pitch** – This is the center to center distance between two adjacent pins or pads on a connector.

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