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HEART DECELLULARIZATION: DESIGN AND DEVELOPMENT OF PERFUSION EQUIPMENT

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Abstract. *Develop a decellularization equipment for perfusion is rather relevant to the success of decellularization of the whole heart, owing the diverse needs as fixation, cannulation and perfusion control and temperature. Perfusion was conceived to occur by the action of a positive displacement pump with rollers, which provides continuous flow with little vibration and no direct contact of any part of the machine with the pumped solution. The fixation of the heart will be made through structures which will cannulate its two large vessels, connecting it to the product cover, and also allowing the perfusion which will occur mainly via these vessels; following the perfusion method of Langendorff. The aim of this study was to design and development of perfusion equipment, and considering, during development, the possibility of controlling the pressure and flow of perfusion, whereas they are determined by the pipe diameter and the rotational speed of the rollers. The flow capacity ranges from 30 to 8000 ml/ min. Moreover, before starting the circulation in the organ, the solution will go through a heat exchanger, enabling the temperature control. Thus, this equipment is able to perform many existing decellularization protocols, facilitating the researches to characterize and to improve this process.*

Keywords: *cardiac bioengineering, perfusion process, decellularization, tissue engineering, whole organ engineering.*

1. INTRODUCTION

Heart disease are main causes of death in developed countries (Roger and Go, 2012; Ptaszek, et al., 2012). After myocardial infarction, for example, native cardiomyocytes are not capable of restore the loss of cells, since cells are terminally differentiated, they do not possess the reproductive capacity (Leor, et al., 2005; Eschenhagen, et al., 2002), and the myocardial tissue are poor in muscle stem cells (Jawad, et al., 2008). The total organ transplantation is most commonly used procedure for end-stage of heart failure. However, the treatment is limited by the shortage of donors and the antigenicity of the transplanted organ, which may lead to rejection frames (Morimoto, et al., 2010).

Therefore, the field of tissue engineering has great potential in the research for new transplant strategies for a better quality of life (Angiolillo and Goto, 2010; Limbourg, et al., 2009; Shlechta, et al., 2010).A serious difficulty of tissue

engineering is the creation of scaffolds that can be used for the development of complex three-dimensional tissue or even whole organs (Gálvez-Mónton, et al., 2013).One solution would be to use the extracellular matrix (ECM) from own organ that you want to transplant performing its decellularization. In this process, occurs the withdrawal and lysis of the cellular materials, creating a bioartificial scaffold (Gilbert, 2012)

Bioartificial scaffolds composed of ECM are commonly used for a variety of reconstructive surgical applications, and being increasingly employed in regenerative medicine strategies and even for organ replacement. These scaffolds are able to provide physical support to cells and a structural and biochemical recommendation adapted to promote cell adhesion, migration, proliferation and differentiation (Lyons, et al., 2008; Grayson, et al., 2009).

Among the challenges of decellularization is to engineer an equipment capable of allowing the complete removal of cellular debris with perfusion trials covering all regions of the organ without affecting the structural capacity of the ECM (Weymann, et al., 2011).

The aim of this work is to design and develop the decellularization equipment to provide the necessary conditions for this process, reviewing the necessary parameters for its development.

2. DEVELOPMENT

In order to properly perform the complete process of decellularization by the perfusion method, there are several published and tested protocols (Akhyari, et al., 2011; Momtahan, et al., 2014; Ott, et al., 2008), for the vast majority some care is necessary with regard to the procedure adopted and the equipment that will enable the achievement of the whole process. The most required needs of such equipment are related to the organ perfusion, its fixation in the system, and the process temperature control.

2.1 Perfusion

The retrograde perfusion presented by Langendorff was used (Langendorff, 1895; Robert, et al., 2011) because its simplicity, low cost of implementation, and facilitate the reproducibility of the experiment. The retrograde perfusion method can also be applied to a wide variety of species and has been extensively used in studies of various mammals such as mice, rats, rabbits, pigs, primates and even human (Robert, et al., 2011). Chicken heart was used in this study.

In this method, the fluid is perfused through the aorta with enough pressure to also feed the coronary and this way perfuse whole heart using the own vascular network. The perfusate entered the heart through a cannula positioned into the aorta, which means the opposite direction of the natural physiological flow. The aortic valve closes under the pressure, so the coronary vessels are filled in by the two coronary ostias (right and left ostium, Fig. 1) in their respective cavities situated in the aortic root. The perfusate then passed through the vascular bed, before being removed through the coronary veins into the coronary sinus in the right atrium.

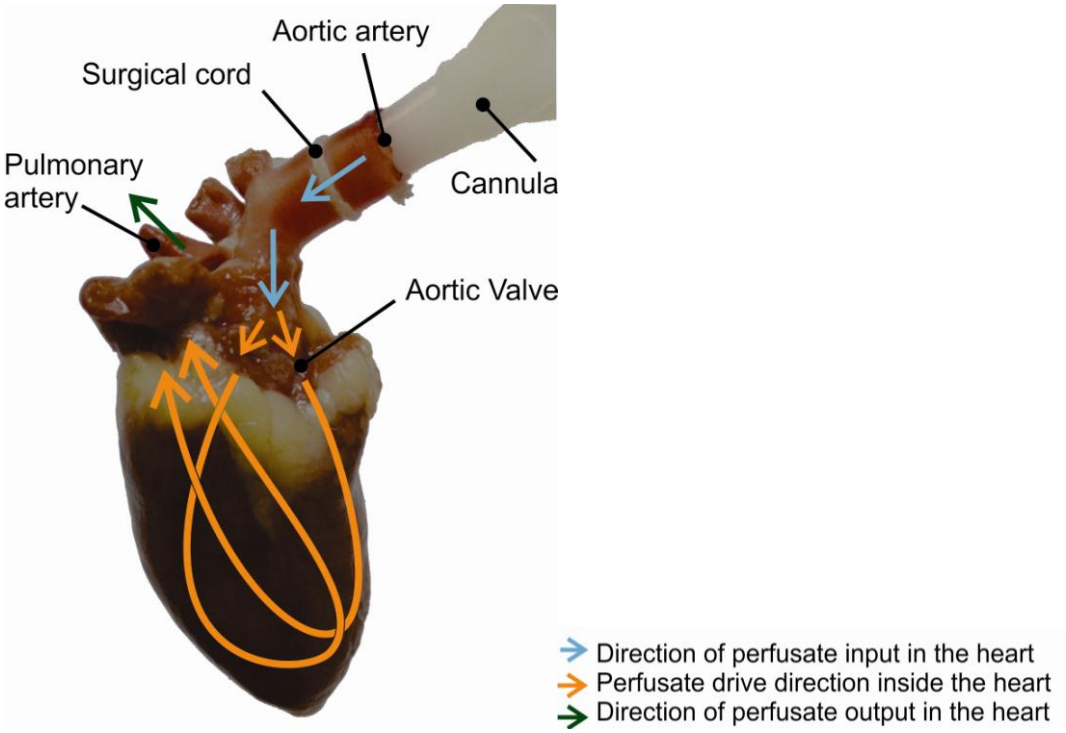

Figure 1.Heart circulation by coronary

There are two main ways to control the retrograde perfusion to the heart, the constant pressure and constant flow controls. The constant pressure can easily be achieved cheaply maintaining a constant hydrostatic pressure, by the weight of the fluid column positioning the vessel at a known height on the perfusion cannula. This is the simplest solution, but certainly not the only solution to reach the constant pressure. It can also be obtained and preserved adding a pressure transducer in the system, the transducer should work as a negative feeder of a pressure control system, for example using a peristaltic pump like actuator. This setting is preferable when there is limited availability or expensive decellularization fluid. However, the constant pressure mode is not the most appointed to perform the decellularization; it is very common when using retrograde perfusion Langendorff is used to study the self-regularization of coronary tone and myocardial ischemia studies (Robert, et al., 2011).

For decellularization the constant flow mode becomes more attractive due to its simplicity and does not require a control system using only a calibrated pump in its rotation to provide the constant flow. The positive displacement rollers pumps are one of the most used for perfusion of both organ and patients, particularly because they are simpler and there is no direct contact between any of the parts of the pump and the fluid. Other advantages are the accuracy relative to the flow, durability, versatility, the absence of contamination (since the fluid remains inside the hose throughout pump circuit), and the ability to provide continuous flow with low vibration.

2.2 Fixation

The cannula has the function of allowing the perfusion of the heart by the proposed method, along with the achievement of the organ fixing in the decellularization chamber. The aortic perfusion cannula can be built up by a variety of materials such as glass, plastic or stainless steel. The external diameter of the cannula must usually be similar or slightly larger than the diameter of the aorta. Figure 2 shows examples of different cannulas designed using SolidWorks® Ver. 2010, which can be used according to the desired technique. The presence of few small barbs in the cannula can improve the security of attachment of the aorta.

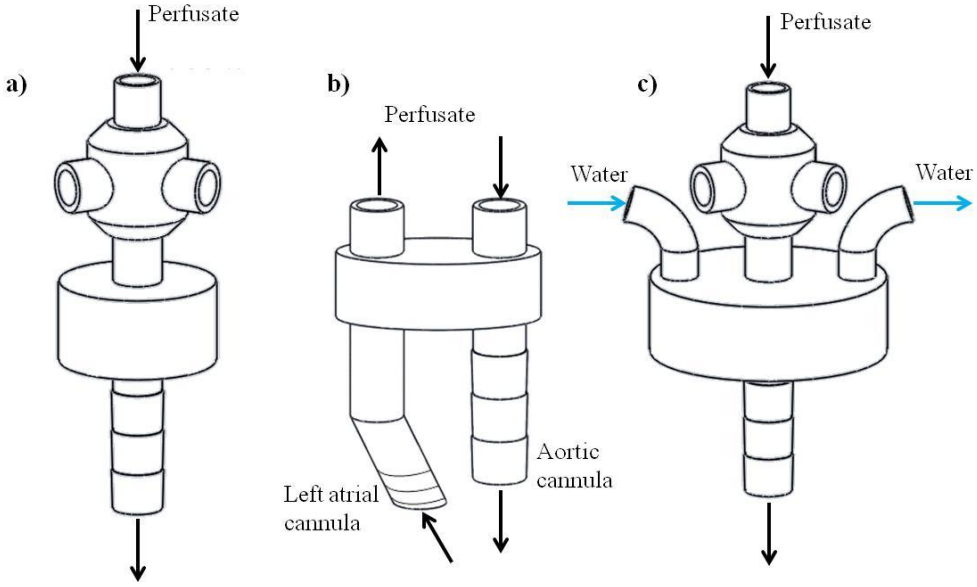

Figure 2. Langendorff perfusion cannula: a) Standard cannula b) Cannula which may also cannulate the pulmonary artery. c) Heated cannula set in a chamber through which water with controlled temperature is circulated. Note the circumferential grooves at the outflow end of the cannula which assist in securing the heart to the cannula.

To perform the cannulation is advisable to have the perfusate gently dripping of the aortic cannula at the time of cannulation, to minimize the potential issues caused by air embolism in the heart. The cannulation may be assisted doing a section along the aortic arch to afford a greater area of cannulation. The heart may be insured by carefully using thin tweezers, avoiding tearing or stretching the aortic wall. At this point, the cannula is softly inserted through the aorta, with care to not insert the cannula too far into the aorta which could cause obstruction of the coronary ostia or damaging the aortic valve.

Once fixed, a ligature is quickly strapped around the aorta, holding between the barbs of the cannula. To do this may be used the same cord that helps cannulation in cardiac surgery. The cannula must be connected to the perfusion set and fixed in the decellularization chamber cover, and then the decellularization process should be started as fast as possible.

The drainage of the perfusate from the right side of the heart, which occurs naturally through the pulmonary artery, must not be prevented. Thus to avoid that, for some accidental reason, the pulmonary artery is obstructed is

recommended to produce a small incision at the base of the pulmonary artery, to facilitate adequate drainage (Sutherland andand Hearse, 2000).

Some researchers, who perform retrograde perfusion, choose cannulate also the pulmonary artery (Fig. 2.b), in this way after its passage through the coronary vasculature the perfusate can be discarded or collected for analysis. If the fluid is used in recirculating it can then be returned to the reservoir.

2.3 Temperature Control

The temperature control may be performed in two main ways, in the first the actuator for temperature control, which may be for example a resistor to dissipate heat, is placed directly in the fluid in a reservoir to warm up to the required temperature with help of a temperature measurer and a controller to maintain this temperature throughout the experiment.

However, to avoid contamination and direct action on the decellularization fluid that is recirculated, and to allow the use of various chemical compounds that may be necessary in the process, it is recommended to use the indirect temperature control: a fluid, which has a known and properly controlled temperature, performs the heat exchange without contact with the decellularization fluid, using for this purpose a heat exchanger (Fig. 2.c).

The heat exchanger may be defined as a device used for performing the thermal exchange between two or more fluids with different temperatures. The heat exchanger can heating or cooling a certain fluid, and are found in various forms according to the heat transfer process, the surface compaction degree, type of construction and the arrangement of currents of the fluids. Considering the type of construction, they are classified into tubular (Fig. 3), plates, and extended surface and regenerative (Incropera, 2011).

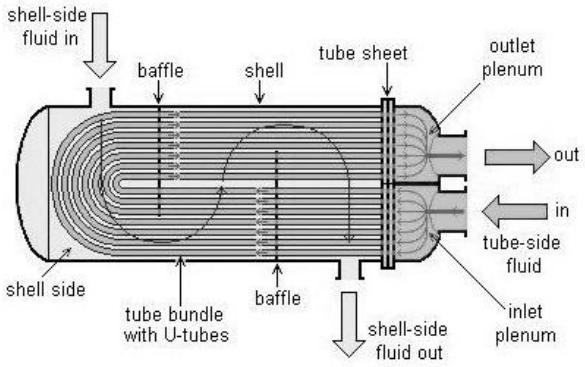

Figure 3. Shell and tube exchangers with one pass. A fluid travels through the inside of the tubes while the other fluid is forced to flow through the enclosure. Source: (Cengel, et al., 2008)

The heat transfer in a heat exchanger usually involves each fluid convection and conduction across the wall that separates the two fluids. In heat exchangers analysis is convenient to work with the overall coefficient of heat transfer, which represents the contribution of all these effects on the heat transfer (Cengel, et al., 2008). Figure 3 shows the shell-tube assembly which has been selected to perform heat exchange in the equipment, because of the good heat transfer coefficient in a reduced total volume (reducing the amount of perfusate in the system).

3. RESULTS AND DISCUSSION

The perfusion circuit was assembled as shown in the Fig. 4, it enables the exchange of perfusate, and withdrawing samples, and temperature and pressure measurements in facilitated manner. The scheme also shows the arrangement of each device in the circuit to enhance the results.

The roller pump (Ravenol Laboratories) was altered by adding a digital rotation control, using a microcontroller (PIC18F4550; Microchip Technology) and a digital potentiometer which actuates the dimmer that provides power to the engine. Therefore, the rotation drive became more accurate and easier to reproduce than the original analog drive.

At the same time a rotation meter was added via a disc encoder (SG23FF; Kodenshi Korea) attached to the shaft of the rollers. It provided accurate rotation to the microcontroller which enables controlling the rotation and consequently the flow rate, regardless of external disturbances and noises. It was considered in the development the possibility to control pressure and flow of the perfusion, these being established by the tube diameter and the rotational speed of the rollers.

Some works (Weymann, et al., 2011) have opted to use the constant pressure control that can be achieved by adding a controller and a pressure transducer in the system. This work uses constant flow control, with the pressure monitored

by a pressure gauge (Fig.4.j). This is a less accurate solution, but more simple and cheap. The flow capacity that varies according to the selection tube was 30-8000 ml/min.

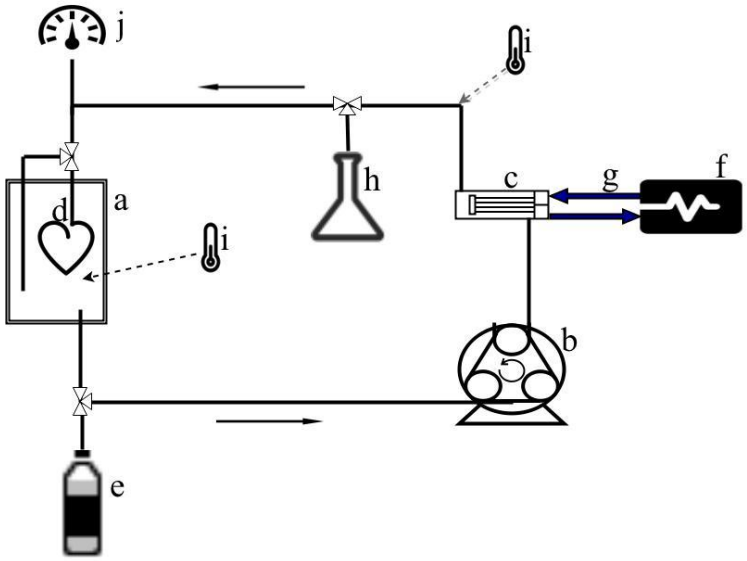

Figure 4. Complete scheme of equipment for the decellularization process: a) Chamber of decellularization b) Peristaltic Pump c) Heat exchanger d) Heart cannulated through the aorta e) Reservoir for the perfusate inlet f) Thermal bath g) Hot water at controlled temperature h) Reservoir for the perfusate outlet i) Temperature gauge j) Pressure gauge.

In order to facilitate the decellularization process, avoiding contamination, reducing the demand of decellularization solution, and to improve standardization of the decellularization process, it will occur in the decellularization chamber which was specifically designed for this functionality. The chamber was designed in acrylic, due to its transparency, capacity to thermally insulate the external environment, and the easiness of building (to machine and paste) characteristic of this material (Fig. 5).

The chamber has the duty of sustaining heart, to work as the perfusion system reservoir and also to trap air bubbles. For this, the fluid will be removed from the bottom and then goes straight to roller pump, as can be observed in Fig.5. After the fluid follows going through the heat exchanger in the direction cannula to pass through the heart and then be released back into the decellularization chamber. On the cover there are several inputs and outputs to withdraw or change the fluid without the need for manipulation of the reservoir or heart.

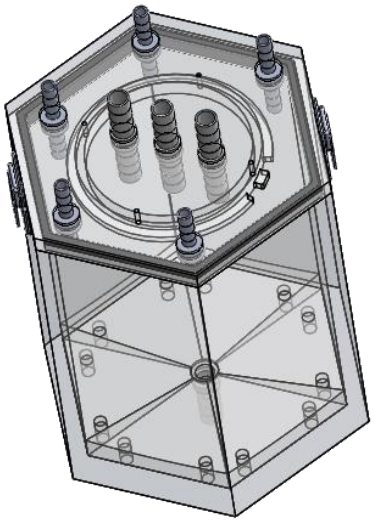

Figure 5. The decellularization chamber design with all inputs and outputs to perform the process without internal manipulation. Designed in SolidWorks® Ver. 2010.

The work of Akhyari, et al. (2011) shows a comparison between different decellularization protocols, from which we can draw the importance of temperature control for different protocols and perfusates with different active principles. The present work used a tube and shell heat exchanger (NIPRO Medical), which has a great coefficient of heat transfer for flow rates below 3000 ml/min, and operates until a maximum temperature of 40ºC. To provide the fluid with the controlled temperature to the heat exchanger will be utilized a thermostatic bath (CE-110/6; Cienlab), which is able supply a water flow with controlled temperature and 0.1°C of accuracy using an electronic controller temperature, with PID e PT100 sensor.

Moreover, there are foreseen two points for taking temperature, where the sensor can be seamlessly inserted, being the first in the decellularization chamber and the other in the heat exchanger exit (via a connector adapted for this purpose). The pressure is measured by a manometer, with the assistance of a three-way connector positioned just before the aortic cannula.

4. CONCLUSIONS

The equipment was built and is now in testing phases, further amendments can be made to improve efficiency to accomplish the full decellularization whole heart. It was designed to meet several existing decellularization protocols and may modify in a controlled manner the flow and perfusion pressure, the temperature of each procedure and cannulation technique of the organ. It is expected that the equipment can help in the study of characterization and improvement of the decellularization process, apart from allow futures adaptations to the process of recellularization and use in different organs.

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