

# Multifunctional Bioreactor for *in vitro* Tissue Engineering of Cardiac Structures

by

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## Abstract

In this paper, some preliminary results related to the development of a multifunctional Bioreactor (BR) are presented and discussed. The work is part of a collaborative tissue-engineering project, undertaken by IRIS and the School of Engineering Science. The overall aim of this project is to grow a total tissue engineered heart valve (TEHV) *in vitro* out of a biodegradable scaffold and cardiovascular cells derived from animals. Here we discuss the concept, design and development of a prototype BR which after optimization can result in an *in vivo* environment capable for long term *in vitro* cultivation of TEHVs. This system is capable of providing the correct physiological pressure and flow of nutrient medium for both arteries and heart valves. The developed system is compact and can be placed in an incubator for contamination resistance. Moreover, the proposed reactor design is highly flexible, allowing the culture of different types of tissues under various hemodynamic stresses. Initial verification and hemodynamic testing on fluid flow indicated that this prototype BR performed well and is producing the correct physics expected. The results of the *in vitro* will assist greatly in the *in vivo* trials currently being undertaken in collaboration with Howard Florey Institute, University of Melbourne.

## 1. Introduction

The tissue engineering concept is relatively new and has significant potential for medical applications. In 1993, Langer and Vacanti <sup>[1]</sup> summarized the early developments in this field and defined tissue engineering as “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue or organ function.” The goal of tissue engineering research is the day when bio-artificial organs are grown in a laboratory and subsequently transplanted into people, potentially providing a permanent and specific cure. Tissue engineering has now emerged as a potential alternative to tissue or organ transplantation. To grow TEHVs *in vitro* three basic elements are needed; cells, scaffold and an *in vivo* environment. Especially in the field of BR-design, optimization of mass transport following cell transplantation, understanding of biomechanical requirements of engineered tissues, and using electrical/mechanical stimulation to promote desired development of TE-constructs shows little progress anywhere around the world. <sup>[2]</sup>

Although several groups have reported encouraging results from the *in vivo* testing of TE heart valve constructs<sup>[3,4]</sup>, a completely bio-mimetic heart valve remains elusive, with current attempts falling short of producing valves which are indistinguishable from native tissue. One key issue yet to be resolved is the optimal conditions under which TE constructs should be developed. The conditions under which the TE constructs are cultured and developed are integral determinants of the quality of the final product. Indeed, research shows that pulsatile flow applied during culturing leads to the improved development of TE constructs.

Therefore, many groups have employed the use of ‘bioreactors’, chambers which provide pulsatile flow of nutrient media for the development and culture of TE construct<sup>[5,6]</sup>. The ultimate aim of a bioreactor is to provide an environment which as closely as possible mimics the natural *in vivo* conditions under which TE construct would be subject to. These bioreactors have been largely designed from first principals, with little effort being directed into mimicking *in vivo* conditions. For example, in most bioreactors the pulsatile flow is driven by a pulsatile pump, which leads to the exertion of only a positive pressure. However, this is not the case *in vivo*, as during the cardiac cycle the positive pressure exerted by fluid force is slightly counterbalanced by a little vacuum. Furthermore, most currently described bioreactors contain dead angles in the lower compartments of the fluid chamber. These dead angles result in a non-uniform fluid-flow, leading to possible fluid clots and sub-optimal culturing conditions. In addition, most bioreactors are designed to be solely used for a single type of TE construct, therefore limiting their usefulness.

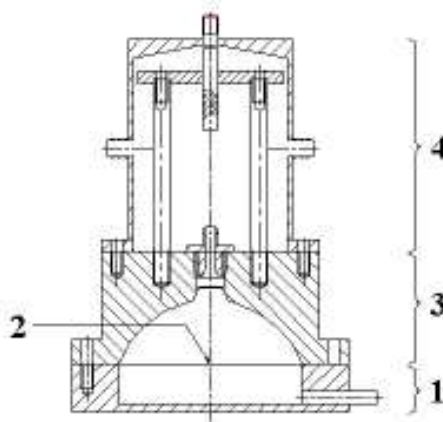
In this section we describe the design and development of a new and novel bioreactor. The described bioreactor (I) contains no dead-angles, (II) provides a good hemodynamic profile, (III) has a slight amount of vacuum as part of the back-pressure exerted from fluid flow, therefore mimicking *in vivo* conditions and (IV) has a multi-functional design (i.e. allows for the culture of various types of TE constructs). In addition, no previous attempts have been made to analyze the fluid forces and hemodynamic conditions created by existing bioreactors. We describe here the use of Laser Doppler Anemometry (LDA) to analyze the forces which are produced by the pulsatile perfusion system. Therefore, we describe a multi-functional system which eliminates certain limitations in existing bioreactor designs and provides a model system under which TE constructs may be optimally cultured (see Figure 1)



**Figure 1 - Set-up of bioreactor (left) in a standard humidified incubator at 37°C and 5% CO<sub>2</sub>, connected to a reservoir (right) for CO<sub>2</sub>-exchange.**

## 2. Bioreactor design

The proposed bioreactor (Figure 2) is versatile in its use, though it is very compact in design. The approximate dimensions of the bioreactor are 17-cm diameter and 23-cm height. The material of construction is acrylic-plastic. It is inexpensive, strong and easy to sterilize using ethylene oxide. The bioreactor itself (Figure 1) is comprised of three main compartments. The air-chamber (compartment 1) is filled with air by the pulsatile air pump, which mimics the beat of the cardiac cycle. The pressure differential will fibrillate the silicon rubber diaphragm (2) like a heartbeat. The diaphragm is located between chamber number 1 and 2 and attached like a drum-skin. The pressure-chamber (compartment 3) is filled with blood or a nutrient (Dulbecco's modified Eagle's medium) which flows to chamber 3 due to the fibrillating membrane. The tangential position of the two inlets as well as the sphere-shape of the pressure-chamber leads to a good mixture of the growth medium. In this chamber there are no dead angles and the chamber has a volume of 220 ml. The TE construct is secured and grown in the tissue growth-chamber (compartment 4). The design of this chamber can be altered to suit multiple types of TE constructs, making this bioreactor multi-functional.

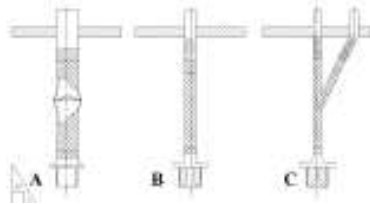


*Figure 2 - Layout of the Multifunctional Bioreactor.*

To enhance the circulation of growth-medium throughout the TE construct, outlets are placed halfway along the perfusion-chamber. Following pulsatile flow the silicon diaphragm will return to equilibrium, therefore transferring the growth-medium from the medium-reservoir through the two inlets on the outside of the pressure-chamber. A one-way valve, located between the pressure chamber and perfusion chamber, prevents back-flow from the perfusion-chamber to the pressure-chamber. To maintain a reasonable pulsatile flow a bi-leaflet aortic valve is used. This type of valve has a low interference with good hemodynamic characteristics.

### 3. Multifunctional Design Parameters

As stated above, the major advancement in design of this bioreactor over existing bio reactors is the incorporation of a multifunctional design feature. Various types of TE constructs may be cultured in this single bioreactor by simple exchange of a connection parts in the perfusion chamber. (See Figure 3) These exchangeable parts are divided into two major areas (1) the artery-parts are subdivided for different diameters of arteries and (2) and heart-valves. These connection parts (see Figure 4) can be easily screwed at the top of the pressure chamber. Moreover, it is possible the grow arteries in vertical position, from 5 up to 12 mm in inside diameter. Valves, on the other hand will be sutured inside a TE construct or silicon tube which is attached on a valve connection part, ranging in diameter from 20 – 28 mm inside. The top-part of the TE constructs will be attached to a acrylic-plastic disk. Different acrylic-plastic hollow tubes on this disk corresponding to the size of the connection parts will secure a uniform fluid flow though the whole construct. More tubes on the disk surface allow attaching branches of the construct.



**Figure 3 - Various types of TE constructs:**

**A: Aortic Valve**

**B: Artery**

**C: Coronary artery**



**Figure 4- Connection parts**

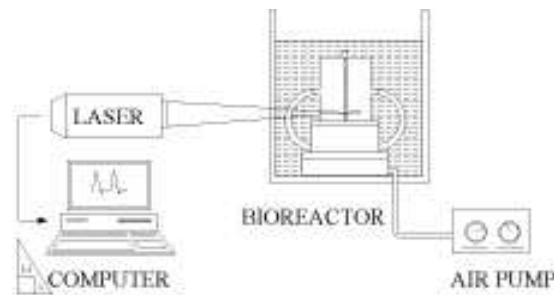
### 4. Experimental evaluation

Laser Doppler Anemometry (2D LDA) available at Swinburne University <sup>[7]</sup> has been used to determine correct physiological hemodynamic characteristics in the model under pulsatile conditions (Figure 5). For this experiment, the measurements are subjected to a transparent silicon tube (ID; 10mm, OD; 12mm) which was used as substitute for a TE construct and located in the tissue growth chamber.

The described bioreactor is placed in a tank of water. In the set-up for these LDA tests we connected the bioreactor as a *shortcut closed loop system*, which means the bioreactor outlets are directly connected to the inlets. This because the culture medium reservoir used in the TE phase will not affect the measurements inside the TE construct. A schematic of the set-up for the flow testing is shown in Figure 4a.

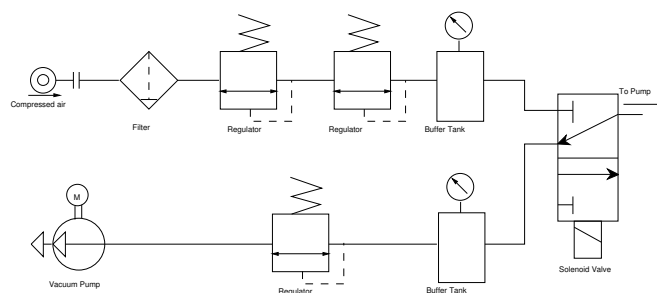
The fluid flow tests were carried out to verify the pulsatile flow-condition on this bioreactor. Moreover, to determine the fluid flow through the silicon tube, Laser

Doppler Anemometry (LDA) was applied. The bioreactor was placed in a tank, which contains water, necessary for measurements with LDA.



**Figure 5 - Set-up used for the LDA measurements.**

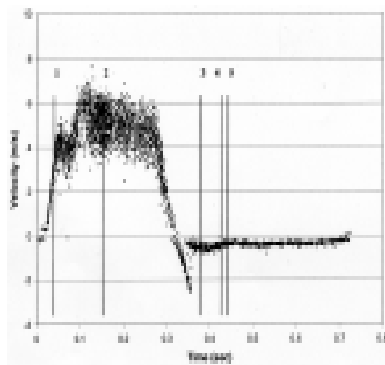
Water (containing small particles) was used as circulating fluid inside the bioreactor, this because of their high ratio of refractive indices. During the systolic phase, circulation water with the particles leaves the pressure-chamber of the bioreactor and enters the tissue growth-chamber with the TE construct. During the diastolic phase, circulation fluid from this tissue growth chamber directly re-enters the pressure-chamber and closes the fluid circle. The pulsatile flow generated in the bioreactor, is activated by a pneumatic pulsatile pump (adjustable vacuum and positive pressure, designed at Waseda university, Japan 1994), which is placed outside the tank and connected to the bioreactor by an air hose. This pneumatic pulsatile pump mainly consists of a vacuum pump, buffer tanks, regulators and a solenoid valve. A block diagram of the pneumatic circuits of this pump is presented in Figure 6. By adjusting stroke volume, stroke rate (BPM) and the inspiration/expiration time of this pump, various pulsatile flows and hemodynamic stresses can be subjected to the TE construct. Throughout this experiment the pump rate was maintained at 80 beats/min and a systolic duration of 35%.



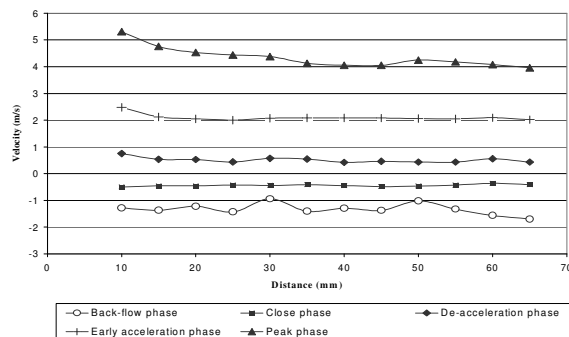
**Figure 6 - Block diagram of the pneumatic circuits for the compact driver**

## 5. Results

Under the present operating conditions, following results are obtained. As shown in Figure 7(a), there is a *pulsatile flow* inside the tube, measured from point 1 (point of origin) is 10 mm above the bottom of the tissue growth-chamber. The chart in Figure 5a is divided into five (1-5) phases namely, *the Acceleration phase (1)*, *Peak Phase (2)*, *De-acceleration phase (3)*, *Back-flow phase (4)* and *the Close phase (5)*. Each phase represents an average of measurements taken during a specific time-period (0-0.7sec). The various phases described in Figure 5a are quantitatively measured by LDA and presented in Figure 7(b). The velocity profile for each phase was obtained by measuring the velocity of the fluid flow along the height of the TE construct. The height (mm) was plotted on the x-axis and the velocity (m/s) on the y-axis. In this figure we see that the velocities during the different phases do not significantly change in height of the TE construct. This means that along the whole TE construct a pulsatile flow is obtained as well a little vacuum (negative velocity) and so will be subjected to right hemodynamic stresses. These results follow the expected pattern.



**Figure 7a - Pulsatile flow measured inside the TE construct, 10mm above point of origin.**



**Figure 7b - velocities of the different phases along the height of TE construct.**

## 6. Discussion

Research shows that development of TE constructs can be improved by the aid of pulsatile flow<sup>[8, 9]</sup>. Previous attempts to generate a bioreactor that will fulfill this purpose have been too simplistic in their design. Certain design flaws have restricted the achievement of optimal culturing conditions for TE constructs. We describe here a multi-functional bioreactor designed from an engineering standpoint. The main advantages of the described bioreactor are lack of dead angles which guarantees a uniform fluid flow though the TE construct and the supply of a slight amount of vacuum created by the respirator, therefore mimicking the *in vivo* hemodynamic stresses observed in the cardiovascular system.

Another new and novel feature of our bioreactor is its multi-functional design. The bioreactor can be easily and conveniently converted to suite various types of TE constructs (heart valves, arteries etc) by the simple adaptation of the TE construct connection parts located in the tissue growth chamber. However, what remains unclear is whether the same culturing conditions are going to be optimal for various types of TE constructs, or whether the flow, pressure and pulse rate need to be tailored to the type of tissue being engineered. To our knowledge, there have been no reports of optimization of bioreactor culturing conditions. We are currently investigating this issue.

We have described herein the design parameters of a new and novel bioreactor designed specifically for the culture of TE constructs. The bioreactor is small, light-weight and able to be sterilized by simply using ethylene oxide. Furthermore, the novel multi-functional nature of the bioreactor will allow for almost universal use in the culturing of TE constructs.

## **7. Acknowledgements**

We thank David and Aaron, at the Engineering workshop of Swinburne University of Technology for their technical input and assistance in fabricating the bioreactor. Further we thank Dr P.A Barton (Biochemist), Dr E Palombo (Molecular biologist), Oliver Vasilevski (research on cells) and Tim Moore (research on polymers) for their involvement in the Tissue Engineering project.

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