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Current State of the Art in Biological Fontan Pumps

Ravi K. Birla* | Chief Science Officer, BIOLIFE4D, Houston, TX, USA

Abstract

During the Fontan procedure in congenital heart surgery, an inert passive tubular graft is used as a flow conduit between the inferior vena cava and pulmonary artery. This tubular graft does not provide any functional support. Recent advances in the field of tissue engineering has led to the development of biological Fontan pumps, by coupling contractile cardiomyocytes surrounding a tubular graft. These biological Fontan pumps are pulsatile and can lead to functional support within the Fontan circuit. This will be a significant advancement in congenital heart surgery. This review article will provide insight into the development of biological Fontan pumps, along with scientific and technological hurdles that need to be overcome to facilitate the use of these pumps clinically.

Introduction to Cardiac Tissue Engineering

Heart failure remains a major cause of mortality globally and in the case of end-stage heart failure, a heart transplantation is the only real solution. While heart transplantation is used in case of chronic heart failure, there are other treatment modalities that are implemented during earlier stages of acute heart failure, including the use of pharmacological agents which are known to restore heart muscle contractility. Given the high degree of mortality associated with heart failure, there are now many different strategies being development to help this patient population, with cardiac tissue engineering being one such treatment modality. Cardiac tissue engineering strategies are focused on bioengineering contractile tissue by culturing contracting cardiomyocytes within a complex 3-dimensional (3D) extracellular matrix [1]. The resulting bioengineered tissue is then conditioned using bioreactors for electromechanical stimulation and perfusion to support functional maturation of the bioengineered tissue [2-6]. The field of cardiac tissue engineering has now expanded and now includes many different methods to bioengineer functional cardiac patches [7-15]. In addition to cardiac patches, recent advances in the field have resulted in the fabrication of biological Fontan pumps [16-26], bioengineered ventricles [26-33] and even whole hearts [34-38] (Figure 1). There have been recent reviews that cover cardiac patches [39-42] and whole hearts [43]; however, there are no review articles that cover the field of biological pumps. As a result, this review will focus exclusively on the field of biological pumps with the following objectives: 1) Describe the process to bioengineer biological pumps, 2) Discuss different methods to bioengineer biological pumps based on published literature, 3) Illustrate the potential use of biological pumps as Fontan pumps for surgical reconstruction for congenital heart patients and 4) Describe scientific and technological challenges in the field that need to be overcome for the field of Fontan pumps to move forward towards potential clinical applications.

Process to Bioengineer Biological Fontan Pumps

There is no standardized process to bioengineer biological pumps. However, there are now close to a dozen publications in the field and this collective body of work has provided a roadmap to bioengineer biological pumps. This is illustrated in Figure 2. The first step in the process in cell sourcing and many different cell types have now been used. Animal derived cells are often sourced in the form of ventricular cardiomyocytes (CMs) obtained from neonatal rat hearts. These cells have frequently been used for model developmental and validation and for initial proof of concept studies. While animal derived cells cannot be used for any clinical applications, they can prove to be advantageous for initial proof of concept studies. During initial stages of model development, there is a need to test and validate many different process variables and often times, requires a large number of cells. This requirement can easily be satisfied through the use of animal derived cells, as stem cells and other human sources are expensive to work with. After initial proof of concepts have been completed using animal derived cells, the next step is to move towards human cells and the most common cell source induced pluripotent stem cells (iPSCs) that can be converted to CMs. iPSC derived CMs have become the gold standard in the field of tissue and organ fabrication and there are now well-established protocols for the culture and expansion of iPSCs and also for the conversion of iPSCs to contracting CMs. The next step in the process is to couple the contractile CMs with a biomaterial, designed and developed to

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***Corresponding author:**

Ravi K. Birla, Ph.D.,

Chief Science Officer, BIOLIFE4D
2450 Holcombe Blvd, Houston, TX, 77204
Tel: 713-474-3449
E-mail: rkbirla2@gmail.com

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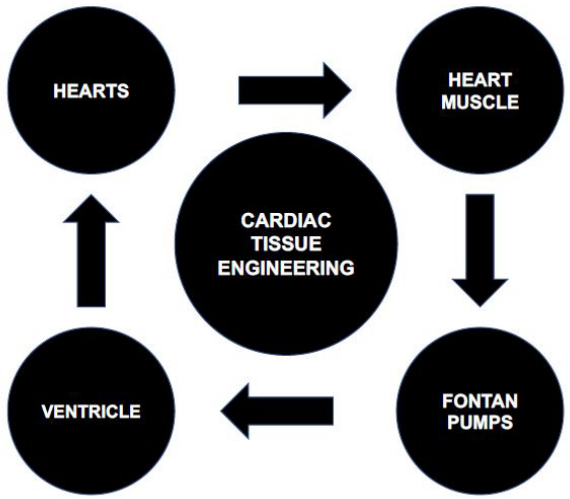

Figure 1: Overview of Cardiac Tissue Engineering – the field consists of methods to bioengineer heart muscle, Fontan pumps, ventricles and whole hearts.

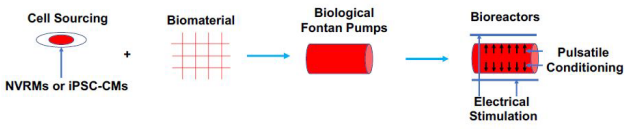

Figure 2: Process to Fabricate Biological Fontan Pumps – Contractile CMs are combined with biomaterials to form Biological Fontan Pumps that are conditioned using bioreactors for coupled electromechanical stimulation.

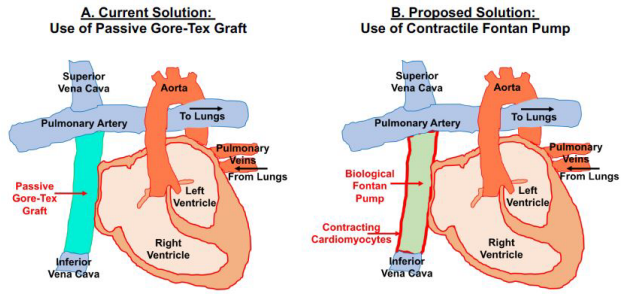

Figure 3: Biological Fontan Pumps in the Fontan Circuit

(A) Current Solution – a passive inert Gore-Tex tubular graft is used between the inferior vena cava and the pulmonary artery. This tubular graft provides a flow path, with no pulsatile support. **(B) Proposed Solution** – a biological Fontan pump can be bioengineered using patient derived autologous cells. This pump will then be positioned in between the inferior vena cava and the pulmonary artery, in place of the GoreTex graft. However, this biological pump will be contractile and provide pulsatile support in moving blood from the inferior vena cava to the pulmonary artery.

simulate the properties of the mammalian extracellular matrix (ECM). Type I collagen has been used extensively for these applications, as this is the major component of mammalian cardiac ECM. Other biomaterials have also been used an include gelatin and chitosan. Next, the contractile cells are combined with the biomaterial to form biological Fontan pumps. There are now many different published methods to achieve this and will be described in more detail in a later section; however, current tissue fabrication technologies include self-organization, cell sheet engineering and use of custom molds as scaffolds. The goal of this process is to couple the contractile CMs with the biomaterial to form a tubular graft with concentric organization of CMs, thereby supporting pulsatile function of the biological pump in response to CM contraction. The final step in the process is the use of bioreactors for electromechanical conditioning of the biological pumps. Bioreactors are devices designed to recapitulate complex in vivo signals during *in vitro* culture. This sequential process leads to the formation of biological Fontan pumps, that are capable of generating intraluminal pressure upon CM contractions. There are numerous applications of these Fontan pumps, as described in the next section.

Applications of Biological Fontan Pumps

The most important application of biological pumps is as Fontan Pumps in cases of hypoplastic left heart syndrome (HLHS) cases in pediatric patients [44]. HLHS is a congenital disorder and is associated with an underdeveloped left ventricle at the time of birth. In most cases, the condition is diagnosed in utero [44]. If left untreated, HLHS almost always results in mortality.

However, with recent advances in congenital heart surgery, a series of three surgical procedures are now performed, with a very high success rate at tertiary care centers. A series of three very delicate surgeries are performed, Norwood, Glenn and Fontan procedures [44]. In the Norwood procedure, an underdeveloped aorta is joined with the pulmonary artery, the patent ductus arterioles (PDA) is removed and a shunt is used to deliver blood to the lungs. In the Glenn procedure the superior vena cava is connected to the pulmonary artery and the Norwood shunt is removed. In the Fontan procedure, the inferior vena cava is connected to the pulmonary artery using an inert Gore-Tex graft. This tubular graft is inert and does not provide any pulsatile support to move the patient's blood from the inferior vena cava to the pulmonary artery. This results in severe challenges for the pediatric patient and is known to result in exercise fatigue and can lead to progressive heart failure over time. This is where biological Fontan pumps will prove to be beneficial. Once we can generate an autologous Fontan pump from patient derived cells, this biological Fontan pump can be used in place of the inert Gore-Tex tube and provide pulsatile support in moving blood to the pulmonary arteries. This solution will provide a more biological solution in place of the current inert Gore-Tex grafts in use and is expected to solve the problems associated with exercise fatigue and progressive heart failure. This will be a game changing solution in the field of congenital heart surgery and one that will help many patients with HLHS (Figure 3).

Strategies to Bioengineer Biological Fontan Pumps

There have been several important studies in the field of biological Fontan pumps [16-26], Table 1, though the field is still at a stage of infancy. However, these studies serve to demonstrate the feasibility of this technology and its potential applications in the field of congenital heart surgery.

There have been several publications describing the fabrication of biological pumps using cell sheet engineering [22,25]. The idea is to culture contractile CMs as a cell monolayer on the surface that has been coated with a temperature responsive polymer, poly (Nisopropylacrylamide), (PIPAAm), which changes from hydrophilic to hydrophobic surface by reducing the temperature and this in turns, causes detachment of the cohesive cell monolayer as a sheet of cells. In this study, neonatal ventricular rat myocytes (NVRMs) were used

Cells	Matrix	Fabrication Method	Pressure (mmHg)	Bioreactor	Novelty	Ref.
NVRMs	Rat Thoracic Aorta	Cell Sheet Engineering	5.9+/-1.7	None	Use of cell sheet engineering	[22]
iPSC-CMs	None	Cell Sheet Engineering	0.2	Pulsatile Fluid Flow	Use if iPS-CMs with cell sheet engineering	[25]
NVRMs	Type I Collagen	Casting	Not Reported	None	Novel design of tissue construct	[26]
NVRMs	Chitosan	Self- Organization	0.1	None	Use of self organization technology	[18]
NVRMs	Fibrin	*In vivo* Implantation	2	None	Use of self organization technology	[17]

NVRMs – neonatal ventricular rat myocytes, iPSC – induced pluripotent stem cell, CMs - cardiomyocytes

Table 1: Strategies to Bioengineer Biological Fontan Pumps

to generate cell sheets, which were then wrapped around an adult rat thoracic aorta and then transplanted in the position of the abdominal aorta in recipient rats for a period of 4 weeks [22]. Upon explantation, the transplanted tissue had formed a highly functional pulsatile pump and shown to generate intraluminal pressures of 5.9 +/- 1.7 mmHg [22]. This same technology was later applied using iPS derived CMs, though the resulting pressures were much lower and in the order of 0.1- 0.2 mmHg [25]. The novelty of this technology lies in the use of the temperature sensitive polymer that supports the formation of cohesive cell sheets, which can then be used to bioengineer biological Fontan pumps.

In another study, engineered heart tissue, referred to as EHTs, were cast into the form of a balloon, by suspending NVRMs in type I collagen, maintained in culture for a period of time and then implanted over the left and right ventricles of uninjured rat hearts for 14 days [26]. The results of this study indicated stability of the tissue construct and viability of the cells after the 14-day implantation study [26]. The novelty of this technology lies in the generation of biological tissue constructs in the shape of a balloon that can be fitted around the external surface of a rodent heart and in theory, provide functional support.

In another study, NVRMs were cultured on the surface of carefully engineered culture surfaces coated with the adhesion protein laminin, to support the formation of a cohesive cell monolayer [18]. After *in vitro* culture, there was delamination of the cohesive cell monolayer towards the center of the tissue culture plate, where a tubular graft fabricated using chitosan, was positioned [18]. The cohesive cell monolayer anchored to the outer surface of tubular chitosan graft, supporting the formation of a functional biological pump [18]. The novelty of this method lies in the use of self-organization technology to first support the formation of a cohesive and contractile cell monolayer using NVRMs, followed by the use of this newly formed cell monolayer to fabricate biological pumps.

In another study, NVRMs were suspended in a fibrin gel, secured in a tubular chamber and implanted in the thigh region of recipient rats, with the femoral artery and vein positioned in close proximity to the implanted cells [17]. After an implantation period of 3 weeks, the tubular chamber was explanted and the tissue separated, with the isolated cells formed thick vascularized tissue with the femoral artery and vein secured within the explanted tissue [17]. Intraluminal pressure of up to 2 mmHg were demonstrated [17]. The novelty of this method was the utilization of an *in vivo* environment to support the formation of highly functional and vascularized biological pumps.

Next Steps in the Development of Biological Fontan Pumps

There is now a convincing body of literature, as described in the previous section, that outlines the development of biological Fontan pumps. Different cell sources, NVRMs and iPSCCMs have been used, different biomatrices have been tested and include type I collagen, fibrin and chitosan and different fabrication strategies have been implemented and include cell sheet engineering, self-organization, mold casting and *in vivo* implantation. The intraluminal pressure has been low in most cases, reported to be under 5 mmHg, which limits any potential clinical utilization at this stage of development. Development of this technology towards potential clinical applications will necessitate the use of iPSC-CMs and precludes any work with NVRMs. The challenges of large number of high purity and mature iPSC-CMs will be the most significant hurdle to overcome in this field. In addition, developing an optimal biomaterial that functionally integrates with iPSC-CMs, along with bioreactors for pulsatile flow conditioning and control over the microenvironment, will be critical to development of biological Fontan pumps that can be used clinically.

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