Tissue Engineering in Congenital Heart Disease

Leda Klouda¹, PhD, Christopher Tsao², MS, and Jeffrey G. Jacot* ², ³, PhD

Abstract—Congenital heart disease is one of the most prevalent birth defects. Approximately 1% of babies are born with a structural heart defect. Components of the cardiovascular system that may be affected by congenital heart disease include the cardiac wall, septum, heart valves and blood vessels. Many of these cardiac anomalies require surgical correction; however current methods have some limitations. Pediatric patients often require multiple operations as they grow to replace their grafts, which may be either failing or becoming too small in size. Additionally, current materials for organ reconstruction can still not fully integrate with the host tissue and lack regenerative properties. Tissue engineering potentially offers a solution to the pressing problem of tissue damage and shortage of transplant organs. This review article provides an overview of congenital heart defects and outlines the unique needs of children as a patient population. Advances in tissue engineering of cardiac patches, heart valves and blood vessels relevant to congenital heart disease are described. Furthermore, this report outlines the challenges in the field that need to be overcome before tissue engineered myocardium, septum, valves and vessels can become clinical reality for the treatment of pediatric cardiac patients.

Keywords — Biomaterial, cardiac defect, congenital heart disease, regenerative medicine, tissue engineering

1. INTRODUCTION

Congenital heart defects (CHDs) affect approximately 1 in 100 children born in the United States, which equates to nearly 40,000 births per year [1, 2]. These statistics are a conservative estimate, and can vary depending on the classification and detection of the defect [1]. As a result, CHDs are the leading cause of infant illness and death [3]. From 1999 to 2006, the US census reported nearly 41,500 CHD-related deaths with almost 28,000 deaths resulting directly from CHDs [4]. While the exact mechanisms that cause each CHD are unclear, it is estimated that at least 15% are related to certain genetic disorders [5-7]. The type and severity of defects are vast and often occur as a combination of defects [8]. The spectrum of CHDs is very broad, with some minor defects not requiring any form of therapy, to very complex cases, where time-sensitive surgical interventions are needed to sustain the life of the child. The cost of healthcare for CHDs is massive, with nearly $1.4 billion reported in the United States in 2004 [9]. This is more than half of the entire hospital costs for birth defects reported that year. Therefore early detection and strategies to repair CHDs and prevent long-term therapy and multiple operations are important areas of research that would have significant return.

In recent years, tissue engineering has emerged as a major field of research for the regeneration and restoration of injured or defective native tissue. Where some regenerative success in years prior has come from autografts and homografts [10, 11] restoring structure similar to native tissue, the majority of approaches lack the restoration of native function. In the case of CHDs, especially those involving cardiac wall repair, the ability to contract synchronously is essential for proper blood circulation. Tissue engineering is an appealing alternative that can overcome the shortfalls of grafts in terms of availability and rejection if used in conjunction with autologous cells. Tissue engineered constructs for CHDs would not only provide structural support, but also restore native function. Therefore classical tissue engineering strategies [12, 13] to accomplish this goal are typically composed of the following key elements: a scaffold for mechanical support, mechanical stimuli to provide conditioning, biochemical stimuli to promote cellular signaling and function, and cells to assist in new tissue formation as well as signaling. Ideally, the construct would also be biodegradable with the degradation rate in tune with the new tissue ingrowth.

The developing cardiopulmonary system is unique and dynamic. This system is essential to sustaining life, and does so by means of its structural and functional complexity. Cardiac tissue is unique in itself, where cardiac muscle is distinct from skeletal and smooth muscle. Components of the cardiovascular system are regionally and functionally different, containing different cells and extracellular matrix molecules. Understanding the complexity of this system is the first step towards designing regenerative techniques. In the case of pediatric patients, CHDs pose further unique issues compared to adult chronic cardiovascular diseases, such as atherosclerosis, and therefore one must also consider the distinctive needs of children as a patient population.

Many congenital heart defects are detected during prenatal testing [14], or diagnosed during the first days of life. The most severe lesions may require treatment very soon after birth. The timing of intervention is a critical step, both in terms of surgical decision-making, but also because of the availability of tissue grafts. Sometimes a procedure cannot be undertaken until a donor tissue of the right size is found, or because a surgeon elects to postpone treatment until the patient grows and can receive a larger implant so that multiple repeat surgical procedures can be avoided. Tissue engineering could

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circumvent these concerns by providing a made-to-order solution that can grow with the child. In the case that cells will be included in the tissue engineered construct, prenatally harvested cells could be expanded and be ready for implantation quickly after the baby is born. Also, considering the young age of patients receiving a graft, it is important to stress the unique need for the implant to remodel and grow during the child’s stages of development. A child undergoes incremental growth phases, and the increase in size of most organs follows that of the general body size [15]. Ideally, a construct should be fully integrated with the host and be durable and functional for life. Studies are presently underway to determine the life span of some solutions employed for the treatment of congenital cardiovascular disease, and in many cases, reoperations and replacements are necessary. For example, existing surgical prostheses for heart valve disease have a limited functional life, which may be adequate for a middle-aged person, but often prove problematic for young recipients. Moreover, differences in the immune system of infants, children, and adults must be carefully evaluated [16-18]. Previous studies have suggested that the performance of bioprostheses is linked to the immune response which can vary between age groups, particularly infants [19-21]. The latter age category is important due to the large volume of congenital cardiac surgeries performed in early infancy. Therefore the use of immunosuppressants in pediatric patients needing bioprostheses becomes a delicate balance of implant rejection, side effects of long term exposure, and risk of infection. When designing treatment strategies, one must consider the detrimental effects of certain compounds or medications on the developing systems of young children, and also their active lifestyles, which make anticoagulant drugs for example often dangerous. Overall, these are some of the factors that may differentiate management of pediatric versus adult patients, and have potential implications in tissue engineering.

This review provides an overview of the most common congenital heart defects, as well as highlight current tissue engineering research strategies used to correct these defects. From this literature examination, current challenges in the field are elucidated and therefore give insight into future research directions for more successful treatment strategies.

2. OVERVIEW OF CONGENITAL HEART DEFECTS

Based on clinical studies, CHD is believed to affect around 1% of live births [1]. Ultrasound procedures allow for approximately 39% of major CHDs, such as tetralogy of Fallot or hypoplastic left heart syndrome, to be diagnosed in utero during the second trimester [14], while the diagnosis of acute CHDs, such as small atrial septal defects (ASDs) or ventricular septal defects (VSDs), may not occur until birth or later. Typically a trivial cardiac lesion that has eluded detection will naturally close during infancy [22]. Also, many CHDs do not manifest themselves until there is mixing of oxygenated and deoxygenated blood after birth. This results from incomplete closure of fetal circulatory vessels, such as the ductus arteriosus. Therefore the actual reported percentage of births with CHDs is likely to be slightly higher than reported [1].

The heart is a complex organ and myriad diseases can result from lesions or malfunctions. As suggested by the statistical occurrence of CHDs, the degree of severity in CHDs is very diverse. There are a number of risk factors that can lead to CHD, including but not limited to, maternal diabetes and maternal lithium, phenytoin and alcohol use. In cases of maternal diabetes, the risk of having a newborn with a structural heart defect increases by 30% [23]. Family history of CHD can also play a role, about 1-4% of babies born to parents with CHDs are affected [1]. The exact causes for the majority of CHDs are unknown, but are proposed to be linked to causative genes in developing fetuses [7]. From the perspective of tissue engineering for CHDs, corrective action attempts to treat different lesions associated with a given congenital heart disease. A brief overview of clinically relevant lesion specific CHDs will help to better understand the engineering challenges in creating a viable construct. Table 1 summarizes some specific CHDs with associated causes and current treatment options.
### Table 1: Congenital Heart Defects

<table>
<thead>
<tr>
<th>Defect</th>
<th>Estimated % of Total Congenital Heart Defects in the US [1, 2]</th>
<th>Causes</th>
<th>Diagnosis</th>
<th>Treatment Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular septal defects</td>
<td>20%</td>
<td>Hole in ventricular septum</td>
<td>Cardiac auscultation, chest x-ray, echocardiogram</td>
<td>Occlusion devices [24-26], patch closure materials [27]</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>10%</td>
<td>1. Large ventricular defect 2. Severe right ventricular outflow tract obstruction 3. Overriding of the aorta 4. Right ventricular hypertrophy</td>
<td>Echocardiogram, chest x-ray, pulse oximetry, MRI</td>
<td>Right ventricular outflow tract reconstructio n [28] VSD closure device [29], [30]</td>
</tr>
<tr>
<td>Coarctation of the aorta</td>
<td>10%</td>
<td>Mild-complete obstruction of aorta</td>
<td>Magnetic resonance angiography, echocardiogram</td>
<td>Patch for aortoplasty [31]</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>10%</td>
<td>Ductus arteriosus remains open after birth</td>
<td>Chest x-ray, echocardiogram</td>
<td>PDA occlusion device [32], occlusion spring coil [33]</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>5%</td>
<td>Hole in atrial septum</td>
<td>Cardiac auscultation, chest x-ray,</td>
<td>Occlusion devices [34, 35], patch closure materials [36, 37], Arterial switch operation [38], septal occlusion device</td>
</tr>
<tr>
<td>Transposition of the great vessels</td>
<td>5%</td>
<td>Abnormal spatial arrangement of great vessels</td>
<td>Echocardiogram, X-ray</td>
<td>Arterial switch operation [38], septal occlusion device</td>
</tr>
<tr>
<td>Aortic valve stenosis</td>
<td>5%</td>
<td>Narrowing across aortic valve</td>
<td>Echocardiogram</td>
<td>Surgical or balloon valvuloplast y [39], bioprosthes e s [40]</td>
</tr>
<tr>
<td>Ebstein anomaly</td>
<td>1%</td>
<td>Abnormal tricuspid valve and atrialization of ventricle</td>
<td>Echocardiogram, ECG, chest x-ray</td>
<td>Valve reconstructio n, Bioprosthetic or mechanical valve, surgical or transcatheter placement [41, 42]</td>
</tr>
<tr>
<td>Anomalies of the</td>
<td>1%</td>
<td>Mild-complete obstruction of</td>
<td>Pulse oximetry,</td>
<td>Venous connection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Defect</th>
<th>Causes</th>
<th>Diagnosis</th>
<th>Treatment Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricuspid atresia</td>
<td>Absence of tricuspid valve</td>
<td>Echocardiogram, chest x-ray, ECG</td>
<td>Valve reconstructio n, Bioprosthetic or mechanical valve, surgical or transcatheter placement [42, 45]</td>
</tr>
<tr>
<td>Interrupted aortic arch</td>
<td>Underdevelop ed aorta</td>
<td>Echocardiogram, loss of appetite, pale</td>
<td>Patch for aortoplasty [31]</td>
</tr>
<tr>
<td>Hypoplastic left heart syndrome</td>
<td>Varying degrees of underdevelopment of aorta, aortic valve, left ventricle, mitral valve, and left atrium</td>
<td>Echocardiogram</td>
<td>Left heart total repair, shunts [46], aortoplasty [31]</td>
</tr>
</tbody>
</table>

3. **Tissue Engineering Strategies for Cardiac Wall Repair in Congenital Heart Disease**

In congenital heart disease, common abnormalities requiring surgical intervention involve a lesion or hole in septal heart tissue requiring closure. Small defects (<5mm) often do not require surgical intervention and will spontaneously close [54]. However, when the hole is too large, surgeons will place patches or occlusion materials in a patient to help bridge the native heart tissue and allow for restored structure. In terms of an ideal tissue engineering construct, restoring structural support is only part of the challenge. A biomaterial may meet the mechanical properties needed to withstand the fatigue of a contracting heart, but if the material is inert it will not completely restore native function. Vascularization is also a limiting factor in current cardiac patches [55]. Unless the cardiac patch is prevascularized or able to have neovascular ingrowth, the patch will be diffusion limited and therefore limited to the overall thickness [56]. Another requirement in restoring native function is the synchronized beating of an implanted cardiac patch with the native heart pace [57]. Following the restoration of natural function is the ability of living tissue to grow and expand. This is particularly important in pediatric patients, where their continued growth can lead to implant failure, requiring reoperations [22]. This necessity to grow and expand presents a unique engineering challenge which is not as relevant in adult applications. A number of CHDs require cardiac wall repair, the most common being ventricular/atrial septal wall repairs using occlusion devices termed “baffles”, which are defined as materials that direct the flow of blood between desired chambers while sealing blood from other chambers [58]. This is contrary to full-thickness cardiac wall patches where the goal is to augment free wall structures. Examples of full-thickness cardiac patches needed during surgical intervention include: ventricular outflow patch...
for tetralogy of Fallot, complete left heart remodeling in hypoplastic left heart syndrome, and right ventricular patch in Ebstein anomaly. To date, no commercially available patch can completely restore native structure and function after implantation.

Naturally derived cardiac patches

A number of strategies involve the use of naturally derived materials as a scaffold for a cardiac tissue engineering construct. The major advantage of naturally derived materials is that they are composed of molecules found in vivo and will degrade into natural metabolic products. Also cellular response can be favorable compared to other synthetic materials in that adhesion molecules which enhance bioactivity are already present within the natural material. In CHDs, many surgical procedures have used autologous pericardium as patch material [59]. The advantages to this technique are that the material is immediately available, nonimmunogenic, and free of cost. Pericardium has mechanical properties that are inferior to native cardiac tissue and therefore in order to enhance the mechanical properties, some surgical procedures involve glutaraldehyde fixation of the material. With this method, aldehyde groups will crosslink at the amine groups on the lysine and hydroxylsine residues of pericardium collagen. Pericardium is typically crosslinked for 15 to 30 minutes, where the duration of crosslinking can affect the resulting mechanical properties [60]. Glutaraldehyde is a toxic solution and therefore adequate washing of the patch material is needed prior to implantation [61]. Other crosslinking agents, such as genipin [62] and acyl azide [63], have been tested as well. One of the main drawbacks in the use of autologous pericardium as a patch material is the inability of the material to expand or grow in pediatric patients. Also, crosslinking procedures will lyse any native cells, as well as create a chemically different material resulting in possible calcification and fibrous encapsulation [64].

The use of decellularized matrix as a scaffold is appealing because the construct was originally functional tissue. Depending on the decellularization process, key ECM molecules can be preserved and therefore may help in promoting new tissue remodeling. Currently there are three decellularized patches approved for cardiovascular patch applications: a pulmonary artery patch material (MatrACELLTM , Virginia Beach, VA), a pericardial patch (CryoPatch®, CryoLife Inc, Kennesaw, GA), and a decellularized porcine intestinal submucosa (CorMatrix®, Alpharetta, GA) [65]. CorMatrix® however is approved only for use on artery, valve and pericardial tissue repair. Some disadvantages associated with decellularized matrices are immunogenicity, risk of disease transmission, and donor availability [66]. Recent studies by Rajabi-Zeleti et al. [67] attempt to renew the use of pericardium-derived patches using an alternative approach to glutaraldehyde fixation. Their group used decellularized pericardium that was enzymatically digested and then reformed into pericardium gels. They showed that after one month of subcutaneous implantation in rats, the pericardium gel based scaffold had low immunological response, enhanced angiogenesis, and cardiomyocyte differentiation compared to control collagen and plain decellularized pericardium. Crapo et al. [68] show that a small intestinal submucosa (SIS) based gel seeded with neonatal rat cardiac cells created tissue closer to physiological function compared to that of cells seeded on Matrigel. Function was measured in terms of contraction rate and normalized troponin T expression, both higher in the SIS gel group. This difference is attributed to the differing components of each gel. While both gels contain a variety of different ECM components, the main difference is that the SIS based gel contains high concentrations of collagen type I and III, similar to that of myocardium. At a minimum this study proves that myocardial cell attachment and resulting function is complex and must be considered carefully when choosing biomaterials for cardiac tissue engineering.

Since native cardiac tissue extracellular matrix is predominately composed of collagen, many studies aim to manipulate collagen based scaffolds using growth factors and/or altering properties such as alignment and microstructure [69]. Collagen has good cellular attachment and proliferation [70]. The main drawbacks to collagen based scaffolds in tissue engineering applications are inferior mechanical and degradation properties. A major requirement for cardiac tissue engineering is the ability to withstand contractile forces of a beating heart. Also as a patch material, collagen patches can be difficult to suture due to their mechanical weakness. However, since collagen is still the main component of cardiac ECM, there is relevance to its use as a scaffold. Miyagi et al [71] produced a collagen based patch that contained covalently immobilized vascular endothelial growth factor (VEGF). This patch was implanted into the right ventricular walls of rat hearts for up to 28 days, showing improved neotissue formation in terms of cell recruitment, proliferation, and blood vessel density when compared to scaffolds without VEGF. A higher density of VEGF immobilization was also shown to have a greater resulting blood vessel density compared to lower VEGF concentrations. In another study, Serpooshan et al. [72] used compressed collagen type I as scaffolds for myocardial infarction repair. While this is not a congenital disorder, this patch may have applications for remodeling hearts with tetralogy of Fallot. Their results after four weeks of implantation exhibited limited fibrosis, diminished dilation of the left ventricle, as well as neo-angiogenesis within the patch when compared to the control.

Because decellularized matrices and purely collagen based scaffolds both have disadvantages in creating a fully functioning cardiac patch, other naturally derived polymers such as alginate, chitosan, and silk fibroin continue to have research interest [73]. Each of these polymers is extracted from living organisms and has shown to have applications for tissue engineering. Studies have shown that alginate, a natural polymer found in cell walls of seaweed [74], combined with RGD peptide can be formed into scaffolds for cardiac tissue engineering. The RGD is important in cellular attachment to the scaffold, and is immobilized by carbodiimide chemistry. Shachar et al. [75] show the maintenance of key cardiac markers, α-actinin, N-cadherin, and connexin-43, suggesting that alginate-RGD immobilized scaffolds alleviate the need for the addition of ECM proteins or Matrigel into scaffolds. Chitosan is also a promising natural polymer investigated as a
component for cardiac patch tissue engineering. It is a linear polysaccharide derived from chitin, which is found in shellfish exoskeletons. Alone, chitosan has been successfully applied to the wound healing market as a clotting agent accelerating hemostasis [76]. In tissue engineering, when chitosan is combined with collagen, the resulting material has mechanical properties superior than collagen alone. Kathuria et al. [77] developed and characterized an elastic chitosan-gelatin cryogel that could be used for potential tissue engineering applications. This material was able to withstand cyclic deformations up to 40% without significant deformation with a Young’s modulus ranging from 36-39 kPa.

Silk fibroin is investigated in a number of tissue engineering applications because it is mechanically strong, noncytotoxic, presents low immunogenicity, and is biodegradable. An investigation into silk fibroin from A. mylitta silk worms shows better cardiomyocyte attachment and functional beating after seeding for up to 20 days [78]. These results are superior than similar scaffolds using silk fibroin derived from mulberry B. mori silk worms. This is proposed to be a result of A. mylitta having RGD domains. Chi et al. [79] investigated a chitosan-hyaluronan/silk fibroin cardiac patch implanted into the left ventricles of rats. This patch was developed creating an aqueous silk fibroin/chitosan/hyaluronan solution in a 10:1:1 ratio, and then spray-dried into patch form. Their implanted patch showed reduced dilation of left ventricular diameter (4.27 ± 0.29mm), increased wall thickness (1.5 ± 0.13mm), and improved left ventricular fractional shortening (42.8 ± 2.4%).

**Synthetic based cardiac patches**

The use of synthetic polymers in surgical correction of CHDs is limited to bioinert materials which can often illicit an inflammatory response and fibrosis. The appeal of synthetic polymers stem from their tunable mechanical, structural and degradation properties. Fabrication of synthetic polymer based scaffolds is very diverse and can involve techniques such as UV polymerization [80], electrospinning [81], or laser sintering [82], to name a few. Polymers such as polyethylene terephthalate and expanded polytetrafluoroethylene (ePTFE) have been used as cardiac patch materials in areas of lower cyclic mechanical stress such as the septal wall. However both polymers are not biodegradable and present issues in terms of tissue ingrowth and remodeling. Ideally, corrections for CHDs will only require one surgical procedure. In the case of cardiac patches, this requires a delicate balance of new tissue ingrowth aligned with scaffold degradation. Therefore much research has focused on using biodegradable synthetic polymers such as poly(lactic-co-glycolic acid) (PLGA), poly(plycerol sebacate) (PGS), polyurethanes (PU), and polycaprolactone (PCL) as scaffolds for cardiac tissue applications. The main issue when using synthetic polymers as a scaffold material is inferior cellular adhesion compared to that of natural polymers. Therefore some strategies involve coating synthetic polymers with other naturally derived materials, as discussed later in the chapter.

Dacron® (polyethylene terephthalate) grafts are sometimes used as septal defect patch material. Dacron® is a strong, stable polymer that exhibits minimal degradation in vivo. Once implanted, Dacron® elicits an inflammatory reaction and subsequent fibrosis occurs. This is a structural solution for defective cardiac tissue as applied to septal repairs, but the material remains inert [83]. Another synthetic polymer, ePTFE, is also currently used in repairing CHDs. The patch materials developed from ePTFE are arranged spatially to have pores ranging from 20-30 μm, which has shown to inhibit cellular ingrowth [84]. Also, ePTFE does not induce as much of a fibrous reaction as Dacron®, and therefore can be used as a patch in areas of blood flow, such as a right ventricular outflow reconstruction [85]. Both of these patch materials are far from an ideal engineered cardiac tissue. Since they are not remodeled and incorporated into existing tissue and elicit an inflammatory response [86], they result in deficient mechanical properties as well as hemodynamic changes. These polymers will not degrade after implantation and will not promote complete tissue remodeling. This is important in pediatric patients that will continue to grow and therefore an inert patch will likely require subsequent surgical operations.

Creating a synthetic material that is biocompatible and is able to grow and remodel with a patient is no simple task. Therefore bioresorbable polymer research into a viable patch for CHDs has much attention. Like natural polymers, synthetic bioresorbable polymers will be degraded over time in vivo resulting from hydrolysis or enzymatic cleavage. The ideal construct will provide mechanical support long enough for native or seeded cells to produce and remodel ECM as well as promote angiogenesis. In CHD patients, this allows for continued growth without the need for additional surgical interventions. Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable polymer that is used in many applications in tissue engineering from drug delivery to hard and soft tissue. The appeal of PLGA stems from the naturally metabolized degradation products of lactic and glycolic acid. Zhou et al. [55] showed that a PLGA scaffold wrapped with omentum significantly improved ventricular remodeling and cardiac function.

PGS has been studied frequently in the field of soft tissue engineering. PGS is an appealing polymer that is able to sustain and recover from deformation with minimal loss of elasticity. Therefore the viscoelastic properties of PGS are suggested to fit a mechanically dynamic environment such as the heart. Chen et al. [87] developed a cardiac patch using preconditioned PGS scaffolds in combination with human embryonic stem cell-derived cardiomyocytes. The PGS scaffolds were preconditioned for 6 days in media and cardiomyocytes were seeded and shown to attach without the need for a gelatin coating. The conditioned PGS scaffold was able to sustain beating cardiomyocytes in vitro for longer than 3 months. Recently, Rai et al. [88] created a biomimetic PGS scaffold attempting to chemically modify the surface of the material in order to enhance cardiomyocyte attachment. This was done by a process involving alkaline hydrolysis and acidification to expose surface carboxyl chemical groups. These groups could then be functionalized with peptides YIGSR and GRGDSP in order to promote cellular attachment. Their results showed that a ligand surface concentration of 10-15 mL/cm2 was sufficient to support attachment and growth of both rat and human cardiac progenitor cells. The Karp lab has expanded on the mechanical properties of PGS, creating
poly(plycerc poly 13 sebacate urethane) (PGSU). This co-polymer blend was shown to have tunable mechanics and the ability to deliver localized biomolecules when tested as a cardiac patch material [89].

Fujimoto et al. [84] developed a polyester urethane urea (PEUU) cardiac patch and implanted them into rats with infarcted left ventricular wall. Their results suggested that the scaffold promoted ingrowth of smooth muscle bundles with mature contractile phenotype. Also, 8 weeks after implantation the PEEU patch was largely resorbed, suggesting cellular migration and improved cardiac remodeling.

PCL is another synthetic polymer of interest when considering cardiac patch development. PCL is a biodegradable polymer and can be hydrolyzed at the ester linkages forming nontoxic byproducts. Yeong, et al. [82] fabricated a PCL scaffold using a computer-aided selective laser sintering technique. This fabrication technique showed proof of concept for a customizable scaffold design allowing for uniform control of pore size and patterning. By controlling the laser wattage and scanning speed, PCL particles were sintered into a disc shaped scaffold composed of small repeating square pyramid units. In this particular design, they were able to obtain a compressive stiffness of 345 kPa. Further optimization of scaffold design could lead to tensile strengths closer to native myocardium, which is on the range of 3–15 kPa. It is important for a patch to exhibit stiffness similar to native tissue as very elastic materials could form aneurysms and less elastic materials could present with high local stress areas [90].

Natural/synthetic hybrid based patches
In order to take advantage of the cellular attachment and signaling capabilities of natural polymers while maintaining a way to control and optimize mechanical properties, researchers have investigated a number of materials combining both natural and synthetic materials. Pok et al. [90, 91] developed a multilayered scaffold composed of PCL, chitosan and gelatin. This scaffold was self-assembled with the stronger PCL core sandwiched between emulsified solutions of gelatin/chitosan (Figure 1). By controlling the average molecular weight of the PCL core, the ultimate tensile strength of the scaffold could be controlled in the range of 2-4 MPa. When combined with the gelatin/chitosan, the compressive modulus of the scaffold was close to native cardiac tissue (~15 kPa). This provided for a biocompatible cardiac patch that when seeded with neonatal rat ventricular myocytes resulted in spontaneous beating in a 50 vol.% gelatin:50 vol.% chitosan blend. Aside from layering different polymers, the blending of both natural and synthetic polymers without any chemical linkages show potential in the development of viable cardiac scaffolds. Kharaziaha et al. [92] developed a scaffold composed of electrospun PGS and gelatin nanofibers, showing that a 33 wt.% PGS formulation induced optimal synchronous contractions of seeded cardiomyocytes. Recent studies by Martins et al. [93] combine chitosan and carbon nanofibers to create scaffolds that can enhance the electrical properties of a patch material as well, showing a conductivity of 0.25 0.09 S/m. This is important in cardiac tissue where conductivity is necessary to transmit signals for beating. Being able to optimize the properties of both natural and synthetic materials suggests a more adaptive and structurally sound scaffold compared to a homogenous construct.

![Figure 1](image)

Figure 1: Composite hydrogel patch with a multilayered structure. The scaffold is made of a self-assembled PCL core placed between gelatin-chitosan hydrogel layers. (A) Macroscopic structure after disk formation. (B) Scanning electron micrograph depicting a cross-section of the scaffold. Reproduced from [90] with permission.

Injectable cardiac gels
Another highly researched area is the use of injectable biomaterials for the treatment of myocardial defects. The idea is that an amorphous matrix composed of critical tissue forming components (cells, ECM proteins, small molecules) can be injected directly to the site of deficiency, aiming to preserve and promote cardiac tissue remodeling [94]. This strategy is very appealing to myocardial infarction cases where the rapid degradation of cardiac tissue is unrepairable. While CHDs do not include myocardial infarction, a number of diseases (coarctation of the aorta, anomalous origin of the left coronary artery arising from the pulmonary artery, etc) [95] run the risk of creating necrotic regions of cardiac tissue due to poor perfusion or inadequate oxygen saturation of the systemic blood [96]. As with myocardial infarction, the lack of oxygenated blood due to CHD can manifest itself immediately after birth and upon closure of the ductus arteriosus. Aside from maintaining a PDA by administration of prostaglandin E1, the use of an injectable cardiac matrix in CHD patients could at a minimum help to preserve cardiac tissue until other corrective action can be considered.

Similar to the cardiac patch studies, the variety of biomaterials used for injectable gels can be both natural and synthetic. Injectable gels are typically based on natural materials such as fibrin, collagen, alginate, but there are some gels which incorporate synthetic polymers either as hydrogel copolymers or encapsulation vessels [97, 98]. Upon injection into physiological temperatures (37°C), the gelling of the components can occur very rapidly, within seconds in most cases. The other key component of injectable materials are living cells aimed to assist in regenerating damaged tissue by paracrine delivery of different signaling molecules such as growth factors, cytokines, hormones, etc. The key advantages to an injectable strategy are: 1) they are minimally invasive and easy to administer through injection, 2) amorphous structure allows for high contourability allowing the gel to fill various defect shapes and sizes and 3) therapeutic agents are easily incorporated and delivered to the defect site.

Fibrin glue has been studied as an injectable material for myocardium repair. Fibrin glue is currently FDA approved as a
hemostasis sealant for use during surgical operations [99]. When applied as an endoventricular heart patch, Christman et al. [100] showed that the use of a fibrin glue increased cell transport and survival of skeletal myoblasts, decreased the size of infarcted left ventricle, and increased blood flow to the area of myocardial ischemia compared to controls. Other studies focused on the delivery of growth factors with the goal of enhancing native cardiac cells to survive and proliferate. The Christman group also developed an injectable material composed of decellularized heart matrix [101], which was shown to have endothelial and smooth muscle cell migration as well as arteriole formation after 11 days. Ruvinov et al. [102] show that an alginate based gel can be used to sequentially deliver insulin-like growth factor (IGF-1) and hepatocyte growth factor (HGF) aimed to induce myocardial regeneration. The alginate gel sufficiently prevented proteolysis of the two proteins and when injected into a rat acute myocardial infarction model, infarct expansion was attenuated and increased angiogenesis throughout affected area was observed after 4 weeks. A combination of growth factors and stem cells as a regenerative therapy is popular as well. This concept will not only deliver signaling molecules to native cardiac cells, but provide additional cells that may differentiate and aid in the remodeling process. Wang et al. [97] developed an injectable hydrogel composed of collagen type I, chondroitin sulfate, and a thermosensitive copolymer (Figure 2). This hydrogel was shown in vitro to be capable of releasing IGF-1 over a 2 week period in order to enhance the survival and growth of encapsulated mesenchymal stromal cells (MSC). The differentiation potential of the MSCs was maintained within the hydrogel, but the addition of IGF-1 was shown to significantly accelerate MSC growth. The Davies group conducted studies into the effects of injectable gels on cardiac tissue remodeling, showing that injection of PEG based gels following infarction could aid in the immediate healing and remodeling [103]. They also showed a temporal remodeling relationship, where the same gels were injected one week post-infarction had slower degradation rates compared to gels injected immediately post-infarction [104].

The major disadvantage of injectable biomaterials for the repair of infarcted cardiac tissue is that they lack sufficient stiffness compared to the tissue in chronic diseased states. Where healthy adult myocardium has a modulus of approximately 50 kPa, diseased states can range from 200-300 kPa [105]. Iffovits et. al. elucidated the effects of stiffness on ovine infarct models using injectable methacrylated hyaluronic acid hydrogels with varying moduli. The study shows that a higher modulus (~43 kPa) gel applied post-infarction resulted in less infarct expansion and reduced left ventricular dilation compared to a lower modulus (8 kPa) [106]. While these injectable gels nearly match the modulus of healthy adult myocardium, the stiffness may not be sufficient to support chronic diseased myocardium. However, injectable biomaterials may have potential in pediatric cases where years of cardiac remodeling have not altered the mechanical properties of the myocardium.

Figure 2: An injectable, flexible, thermally responsive hydrogel composite for cardiovascular tissue engineering applications. The material is comprised of type I collagen, chondroitin sulfate, and a thermosensitive copolymer based on N-isopropylacrylamide. (a) The composite is liquid at 4°C. (b) Hydrogel forms upon temperature increase to 37°C. (c) The material is injectable through a 26-gauge needle at 4°C. (d) Hydrogel strip at 37°C before stretching. (e) Hydrogel strip at 37°C after stretching. Reproduced from [97] with permission.

4. HEART VALVE TISSUE ENGINEERING IN CONGENITAL HEART DISEASE

The direction of blood flow through the heart is regulated by the heart valves (aortic, pulmonary, mitral and tricuspid valve). All four heart valves can be affected by a congenital defect. Problems include stenosis and regurgitation, and both cases impede physiological blood flow. Ebstein’s anomaly is characterized by an abnormal structure of the tricuspid valve and regurgitation. Another type of congenital heart defect is a bicuspid aortic valve where two cusps are present instead of three. This defect does not usually involve severe complications in the pediatric population and surgical correction, if deemed necessary, is performed well into adulthood [107]. Anomalies of the pulmonary valve are the most common among the four valves and are often manifested in conjunction with other congenital heart defects [108]. Also, in certain lesions, the pulmonary valve may be underdeveloped and a reconstruction of the right ventricular outflow tract (RVOT) is a beneficial option. A valved conduit is used to connect the right ventricle to the pulmonary artery [109].

When replacing a failing heart valve, a surgeon generally has the following options: A mechanical valve, or a bioprosthetic valve which can be either a homograft or a xenograft. The use of a mechanical valve is associated with increased risk for thrombosis and patients will need lifelong anticoagulant medication. Anticoagulants can prove dangerous for children due to possible hemorrhaging which is a threat in this physically active patient population. Therefore, bioprosthetic valves are usually preferred for surgical correction in pediatric patients. Human donor heart valves are commonly cryopreserved until implantation, [110] but a main drawback is immunogenicity [111]. Decellularization techniques have been more recently proposed to reduce this effect in homografts [112]. As with all homografts, availability
is a common limiting factor. In the pediatric population, this proves even more problematic as a valve of the right size needs to be found and transplanted for each child. Xenograft valves are typically bovine or porcine tissues crosslinked with glutaraldehyde. While xenografts are more readily available than homografts, the concern about immunogenicity remains [113, 114]. Bioprosthetic valves, both human and animal-derived, oftentimes suffer from early degeneration which can be detrimental to young patients, necessitating replacement operations. One of the mechanisms of bioprosthetic heart valve degeneration that can lead to failure is linked to calcification. The phenomenon of bioprosthetic heart valve calcification has been reviewed elsewhere [115].

As an alternative to tissue crosslinking, decellularization has been introduced in order to preserve the original tissue matrix to a certain extent while reducing cellular antigens. Commercially available decellularized heart valve porcine xenografts (e.g. Synergraft®, Matrix P®) and homografts (CryoValve SG®) exist that are suitable for use in a pediatric congenital heart disease population. However, failure resulting in death or need for graft replacement has been reported for porcine decellularized heart valves due to a possible immune response and limited cell migration from the host causing poor recellularization [116-118].

Another option for the surgical management of aortic valve disease in children is the Ross procedure. During this procedure, the aortic valve is replaced by the patient’s own pulmonary valve. Since an autograft is placed in the aortic position, the commonly associated problems with mechanical, homo- or xenograft valves are eliminated, but it generates the need for a suitable replacement for the pulmonary valve [119]. The autologously implanted aortic valve has been shown to grow and function satisfactorily in young children [120].

For all types of currently available options, excluding the new aortic valve after the Ross procedure, as the child grows, reoperation will be necessary to provide a new valve that matches their size. Additionally, the prosthetic tissues have limited intrinsic potential for repair. General risk factors for prosthetic heart valve complications in children have been identified, which include age and type of defect [110, 121]. Therefore, the field of tissue engineering has been faced with much anticipation for a solution that can eliminate some of the current limitations in heart valve replacement for children.

Heart valves are unique tissues considering their architecture and the biomechanical environment in which they operate. Even though all four valves show a similar pattern in cell phenotypes and extracellular matrix components, they have structural and morphological variances. These differences in structure allow valves to optimally serve their function, for example the high pressure setting of the aortic valve presents different mechanical loading compared to the lower-pressure pulmonary circulation. Moreover, heterogeneity and anisotropy is pronounced within regions of each valve [122-124]. Therefore, a profound understanding of the properties of each valve is important in order to create tissue engineered analogs. The field of heart valve tissue engineering is largely benefiting from research performed in the biomechanics, mechanobiology and cell biology of heart valves [125-128].

Approaches involving naturally derived matrices

Building upon the existing surgical practice of bioprosthetic valves, heart valves have been decellularized and reseeded with cells following a tissue engineering paradigm. The goal of this effort is to combine the advantageous properties of a native matrix with the regenerative and remodeling potential of living, autologous cells. The first clinical case of this kind was described in 2002. A cryopreserved pulmonary homograft was decellularized and reseeded with endothelial cells from the patient’s forearm vein. One year after implantation, the patient was in good clinical condition with appropriate valve function [129]. The same group reported the results of clinical studies performed over ten years. Eleven adult patients, some of them with congenital heart disease, underwent the Ross procedure and a tissue engineered heart valve (TEHV) was placed in the pulmonary position. The TEHV was fabricated as above. At the ten-year follow-up, the survival rate was 100% and there were no signs of valve regurgitation, calcification or morphologic degradation. These results were encouraging, however more long-term studies are needed to evaluate the longevity of these valves, especially in a younger patient population [130]. A clinical report involving the placement of decellularized and reseeded pulmonary valves in two pediatric tetralogy of Fallot patients was reported in 2006. Autologous endothelial progenitor cells were harvested from peripheral blood and seeded on valve homografts. Both children were doing well clinically at the 3.5 year follow-up. The valves showed sufficient mobility, no degeneration or stenosis, and importantly, they were shown to grow with the children [131].

These approaches have shown promising clinical value, but certain limitations are still present. The decellularized matrix is a homograft, and the donor scarcity problem remains. The cell harvesting, often done in an invasive manner, as well as the isolation and expansion procedures, impose an additional step which is time-and effort-consuming. Adequate quality control systems must be in place. Finally, there are questions about the fate of the seeded cells and their role in the regenerative process. Work by Roh et al. [132] has shed some light on the last question and will be discussed in the next chapter. Therefore, some researchers have focused on the in situ recellularization from the host. This approach falls under the classical tissue engineering paradigm, which involves scaffolds, cells and stimuli, and where cell seeding happens in vivo. Commercially available, decellularized and not re-seeded matrices have not always been very successful clinically, and even devastating in some cases, as previously mentioned [116-118]. Factors that will stimulate and improve the active in vivo recellularization are currently being investigated. Jordan et al. decellularized porcine pulmonary valves and conjugated them with an antibody that recognized ovine endothelial progenitor cell (EPC) markers. The valves were implanted in sheep in the pulmonary position. At three months post-implantation, histological characterization showed significantly higher cell numbers in the antibody-conjugated valves compared to unconjugated, unseeded matrices or valves seeded with EPCs that served as controls. On the latter type, the initially seeded cells seemed to have disappeared within the first month (Figure 3). The mechanical properties of the conjugated valves were also found to be superior compared to
the controls [133]. Moreover, in vitro studies have shown that the anisotropic characteristics of the valve microenvironment have an effect on cell migration and subsequent recellularization [134]. These findings may prove useful towards the rational design of tissue engineering strategies for the in situ cellular repopulation of a previously decellularized matrix.

![Image](http://www.researchpub.org/journal/iphf/iphf.html)

**Figure 3**: Porcine pulmonary valves were decellularized and either (1) not treated (un-conjugated), (2) reseeded with autologous endothelial progenitor cells (cell-seeded), or (3) conjugated with a CD133 antibody specific for ovine endothelial progenitor cell markers (conjugated). A) Hematoxylin and eosin stained sections of valve leaflets after 3 months in vivo. Unconjugated and cell-seeded valves show very few cells. Conjugated valves showed staining indicating cell content throughout the valve. B) Cell content in conjugated valve leaflets after 1 week, 1 month, and 3 months in vivo. Increased recellularization is observed over time with 4,6-diamino-2-phenylindole nuclear staining. Reproduced from [133] with permission.

Scaffolds based on natural polymers have also been used in heart valve tissue engineering, albeit not yet on a clinical level. Collagen and glycosaminoglycans (GAG) are two of the main ECM components in all four native heart valves [135], and efforts have been directed towards their incorporation into tissue engineering. Collagen can be obtained from animal tissues which are in adequate supply. Tedder et al. have prepared collagen scaffolds from decellularized porcine pericardium and pulmonary arteries. Layered constructs incorporating collagen derived from those two tissues could be shaped into a heart valve with unique properties in each region. They have also suggested the use of penta-galloyl glucose, a collagen-binding agent, as an alternative to glutaraldehyde in order to stabilize collagen from premature in vivo degradation without inducing calcification [136, 137]. Collagen hydrogels have also been rendered more porous and bioactive with the addition of GAG, thereby supporting co-cultures of valvular interstitial cells (VIC) and endothelial cells [138]. Fibrin is another protein introduced to heart valve tissue engineering. It can lead to a completely autologous approach as this protein can be isolated from the patient’s own blood. Studies have shown that fibrin can be used in a mechanical loading environment and promote ECM production when seeded with cells [139-141]. Hyaluronic acid (HA) is a polysaccharide and a major GAG component of the heart valve extracellular matrix. Early work demonstrated the potential of crosslinked HA hydrogels as cell culture vehicles and assessed proliferation and ECM production [142, 143]. The molecular weight and degree of modification of HA has an effect of the resulting hydrogel properties, which in turn influence VIC phenotype and behavior [144]. Interactions between VIC and HA have been shown to have implications in mineralization [145]. This observation can be useful for HA-based tissue engineering strategies towards the prevention of heart valve calcification.

**Approaches involving synthetic matrices**

Biodegradable polyesters such as poly(glycolic acid) (PGA) and poly(lactic-co-glycolic acid) (PLGA) have been some of the first synthetic materials introduced to heart valve tissue engineering [146]. PGA, PLLA and PLGA scaffolds are still widely used by many investigators. The group of M. Sacks has been examining the effect of biomechanical stimuli on cells seeded on these types of materials towards the development of a heart valve tissue engineering equivalent. They recently reported the effects of physiological conditioning of tri-leaflet heart valve constructs in a bioreactor in which oscillatory pressure and flow conditions could be adjusted to simulate pulmonary artery hemodynamic conditions. This bioreactor allows for investigation of the effects of organ level, dynamic pulsatile culture conditions such as mean pressure, mean flow rate, beat frequency, stroke volume and shear stress [147]. Increased collagen production was observed in the biomechanical environment of an organ level, tri-leaflet valve as compared with a bioreactor system that applies flexure and flow-induced shear stress to rectangular, cell-seeded scaffolds [148]. They and others [149] have stressed the importance of biomechanical stimuli for optimal tissue engineered heart valve formation.

Poly(glycolic acid) mesh scaffolds coated with poly(4-hydroxybutyrate) (PGA/P4HB) have been used extensively by Hoerstrup and collaborators over the past decade. P4HB is a naturally derived biopolymer which is biodegradable and pliable. They first reported seeding of these scaffolds with myofibroblasts and endothelial cells and culturing them in a dynamic bioreactor system before implantation in the pulmonary position in sheep. After twenty weeks in vivo, the scaffold material was completely degraded and the neo-tissue was similar in architecture and mechanical properties to native heart valve leaflets [150]. More recently, the group reported use of these scaffolds with amniotic fluid derived stem cells (AFSC). These cells could be harvested prenatally following the detection of a heart defect in a fetus, and a tissue engineered construct could be fabricated in vitro and be ready for implantation by the time of birth. AFSC
showed growth and differentiation potential when cultured in a bioreactor system. The mechanical properties of the resulting valves did not reach physiological values, but sufficient opening and closing of the valves was observed [151]. Seeded scaffolds were implanted in sheep fetuses in the pulmonary position via a minimally invasive transapical technique (Figure 4). This work served as a first proof of concept for a single-step, fetal cardiac interventional procedure with potential implications in congenital heart disease [152]. Another approach presented by the same group involved the decellularization of a PGA/P4HB scaffold that was previously seeded with human myofibroblasts and cultured dynamically in a bioreactor. These decellularized tissue engineered heart valves showed significantly higher recellularization in vivo in a non-human primate model as compared to decellularized native homograft valves, which served as controls. The authors suggest that this in situ tissue engineering approach might facilitate the creation of “off the shelf” non-immunogenic heart valve analogs [153].

Figure 4: Amniotic fluid stem cell-based heart valves. (a) Matrices were fabricated from PGA and placed within self-expandable stents. Asterisk indicates the three valve leaflets. (b) Matrices were coated with P4HB. Demonstrated here is the micro-CT image with arrows indicating the PGA-P4HB-composite matrix. (c-d) Amniotic fluid stem cells were seeded onto the valves using fibrin as a carrier. Grating interferometry showed homogeneous cell seeding throughout the scaffold. Arrows indicate fibrin matrix. (e) Macroscopic view of cell-seeded matrix. (f-h) The cell-seeded matrices were cramped and placed inside the delivery system. (i) Planar fluorescence reflectance imaging was performed to analyze cell loss during crimping. Red signal indicates autofluorescence and yellow is specific signal for CFSE-labeled cells. (j) Confocal microscopy image of cell-seeded matrix. CFSE-labeled cells appear green. Reproduced from [152] with permission.

Other synthetic materials currently used in heart valve tissue engineering include polycaprolactone [154], poly(glycerol sebacate) [155] or combinations thereof [156]. Polyurethanes have been also attractive because of their largely tunable properties [157, 158]. Poly (ethylene glycol) (PEG) hydrogels have been used as three-dimensional matrices for valvular interstitial cell encapsulation. Studies have led to a better understanding of the cells’ mechanobiology while suggesting that PEG-based hydrogels can be tailored to provide an instructive environment for the development of more mature tissues [159, 160]. Hydrogels also allow for the fabrication of layered constructs which can mimic the architecture and mechanical function of each region of the native valve. Tri-layered PEG hydrogels with two stiff outer layers and a soft inner layer as proposed by the researchers have potential of creating a multilaminate environment for cell encapsulation [161]. In another biomimetic approach, PEG-PLLA hybrid scaffolds were electrospun and each region was functionalized with biomolecules present in the extracellular matrix of native heart valves [162]. In summary, promising results have been obtained with synthetic matrices, however a better understanding of their in vivo behavior, especially with regard to cell infiltration and biomechanical performance, is necessary before clinical translation.

5. BLOOD VESSEL TISSUE ENGINEERING IN CONGENITAL HEART DISEASE

It is important to differentiate how disorders involving the blood vessels which may benefit from tissue engineering present in children as compared to adults. Whereas heart disease affecting children is mostly congenital, many forms of acquired heart disease in adults stem from atherosclerosis, which is caused by the buildup of fatty plaque inside the arteries. This results in narrowing of the arteries leading to a reduction in tissue perfusion. Coronary artery disease is a common and serious manifestation. Bypass surgery is one of the possible treatments for coronary artery disease, in which a vascular graft is required.

Congenital heart defects involving blood vessels include coronary artery anomalies [163], coarctation (narrowing) of the aorta [164], interrupted aortic arch [165], and pulmonary artery stenosis [166]. However, there are certain cases of complex congenital heart disease, in which the blood flow to and from the heart needs to be rearranged. This rearrangement allows for proper oxygenation of the blood and improvement of hemodynamic properties. In a structurally normal, biventricular heart, the systemic and pulmonary circulations are each supported by a ventricle and the circulations are in series. One particular congenital defect is a heart with only one functional ventricle. In patients born with a single ventricular chamber, the two circulations are in parallel and patients only survive because the systemic and pulmonary venous bloods mix [167]. A palliative surgical procedure that has been applied for treatment of children born with a functional single ventricle is the Fontan operation and its variations. The various Fontan-type procedures involve diverting the systemic venous blood to the pulmonary arteries, bypassing the right heart [168,
These surgeries are performed in stages and grafts are required for the vascular reconstruction.

Solutions used by pediatric cardiothoracic surgeons include synthetic and biological grafts. Synthetic polymer grafts such as polyethylene terephthalate (Dacron®) and the currently more prevalent expanded polytetrafluoroethylene (ePTFE, Gore-Tex®) have been associated with increased risk for thrombus formation, stenosis, foreign body reaction, and calcification [170]. Due to the increased risk of thrombosis, life-long use of anticoagulants is essential. Biological grafts include auto- and homografts, which have a lower tendency for thromboembolic events, but may show early degeneration due to increased calcification [171, 172]. Additionally, their supply is not always guaranteed. Vascular grafts presently employed for Fontan-type operations and their limitations are summarized in a review article by Patterson et al [173]. For pediatric surgery, a vascular graft typically has smaller diameters than those needed for similar procedures in adults, and most importantly, it needs the ability to grow with the patient and have high life expectancy. One exception where growth is not required is the case of vascular grafts used in temporary shunt systems as for example the modified Blalock-Taussig shunt. This shunt connects the systemic to the pulmonary artery and provides short-term palliation in complex cyanotic cases until further corrective or palliative surgery can be performed [174].

**General considerations for blood vessel tissue engineering**

Progress in the field of blood vessel tissue engineering has brought some initial successes, and important basic research has improved our understanding of the physiological remodeling process. Some recent review articles [175-177] summarize the advances in the general area of blood vessel tissue engineering. As with other tissue engineering paradigms, approaches include the use of cells with and without scaffolds, which can be either decellularized native matrix, or a natural/synthetic material. Some of the remaining challenges to be resolved before tissue engineered blood vessels can be translated into clinical practice have been highlighted in articles by L’Heureux et al. [178] and Udelsman et al. [179].

In the case of cell-seeded matrices, the cell source must be easily accessible and cells should be readily expandable in sufficient numbers in order not to slow down the transplantation process. Additionally, a reproducible cell seeding technique is important [180, 181]. Endothelial cells play an important role in the function of blood vessels. They serve as the interface between the blood and the vessel wall, and among other functions, increase thromboresistance. The presence of endothelial cells in tissue engineered blood vessels has been shown to impart patency and prevent the formation of thrombi [182]. Early studies by Campbell et al. [183] utilized a silastic tube placed in the peritoneal cavity of rats and rabbits which was covered in granulation tissue after two weeks in vivo. This tissue was removed from the silastic tube, which served as the inflammatory agent, and inverted. A layered structure with myofibroblasts and collagen was observed. Mesothelial cells, or migrated endothelial cells, which stained positive for von Willebrand factor, formed the innermost layer. The tube made of tissue was implanted in experimental animals and served as a patent vascular graft over a period of four months. In recent years, the quest for the elucidation of the mechanism of endothelialization in vivo along with the questions on graft stenosis as observed in early clinical trials [184] have led to the intense investigation of the molecular and cellular processes that govern the in vivo remodeling of tissue engineered blood vessels. It was demonstrated that scaffolds seeded with bone marrow mononuclear cells elicited an inflammatory response in vivo, which attracted host monocytes. The graft matured into a living blood vessel repopulated with endothelial and smooth muscle cells over six months. Interestingly, the initially seeded bone marrow mononuclear cells were not detectable a few days after implantation [132]. Further studies confirmed the adjacent blood vessel wall as the source of the endothelial and smooth muscle cells that were found in the tissue engineered blood vessel graft [185]. Moreover, macrophage infiltration was shown to be involved in the formation of stenosis in a murine model [186]. These studies proved the importance of the inflammation process in the in vivo remodeling of tissue engineered blood vessels, and have initiated a discussion on the role of seeded cells in the various tissue engineering models.

**Blood vessel tissue engineering in congenital heart disease: First clinically relevant attempts**

Besides the need for small diameter (<6mm) vascular grafts for coronary artery bypass surgeries in adults, for which no ideal and readily available substitute exists, tissue engineering has been also recognized as a potential solution for pediatric vascular grafts. One of the first reported studies geared specifically towards congenital heart defects was the work on tissue engineered pulmonary artery autografts in 1998. Shinoka et al [187] performed an in vivo study in sheep replacing a 2-cm segment of their pulmonary arteries with tissue engineered grafts. Tubular scaffolds were made of biodegradable polyglactin/ polyglycolic acid mesh. The cellular source was autologous artery or vein. Cells were separated and the endothelial cell-rich fraction was seeded in the lumen, whereas the endothelial-deficient fraction was seeded on the periphery of the scaffold. After implantation, the sheep were monitored by echocardiography and angiography, and samples were harvested in intervals up to six months. No scaffold material was detectable after 11 weeks. Seeded scaffolds were patent and did not show macroscopic signs of calcification, but evidence of ECM remodeling and diameter increase was observed.

The first successful clinical application of a tissue engineered vascular graft was reported in 2001 in Japan. A four-year old girl with single ventricle physiology received a construct made of a PLLA/PCL copolymer reinforced with PGA fibers and seeded with autologous peripheral vein cells. The cells were previously expanded over a period of eight weeks. The patient had previously undergone the Fontan procedure and a 2-cm segment of her pulmonary artery was occluded and needed replacement. The tissue engineered graft had been cultured in vitro for ten days prior to implantation, and measured 10 mm in diameter, 20 mm in length and 1 mm in thickness. The polymer was designed to degrade within eight weeks in vivo. No complications were reported immediately
after the surgery, and seven months post-operatively, the patient was doing well [188]. Soon after, the same group initiated a clinical trial in Japan involving a biodegradable scaffold composed of PGA and PLLA or PCL formed into a tubular graft. Autologous bone marrow-derived mononuclear cells were obtained and seeded onto the scaffolds. The recipient cohort comprised of twenty-five patients with single ventricle physiology with an age range from 1 to 24 years. After the surgery, patients were treated with anticoagulants for three to six months and were monitored by angiography, echocardiography and computed tomography. No adverse reactions were reported immediately postoperatively. At a late-term follow up (mean of 5.8 years after implantation) no evidence of graft rupture, aneurysm, or ectopic calcification was observed (Figure 5). No graft related mortality was reported, however one patient died 6 months post implantation of congestive heart failure, and three more within four years. The later-term deaths were related to other cardiovascular anomalies. Graft stenosis was observed in 24% of the patients. This study as the first of its kind demonstrated the feasibility and relevance of tissue engineered vascular grafts in congenital heart surgery. It also raised questions about the in vivo mechanisms of vessel remodeling and the causes of graft stenosis [184].

Figure 5: Results from the first human trial involving tissue engineered blood vessels in congenital heart disease. A tissue engineered vascular graft was fabricated by seeding mononuclear cells on a biodegradable scaffold and evaluated in pediatric patients with single ventricle physiology. Image depicts a computed tomography (CT) scan one year after the tissue engineered vascular graft was implanted. The arrows indicate the extracardiac graft. No signs of aneurysm formation and graft rupture were observed, and the graft remained patent. Reproduced from [184] with permission.

Further advances in blood vessel tissue engineering for congenital heart disease

Hoorstrup et al. [189] demonstrated the fabrication of pulmonary artery conduits using human umbilical cord cells seeded on synthetic biodegradable PGA/P4HB scaffolds and cultured in a bioreactor. Morphologic characterization of the tissue engineered pulmonary artery showed ECM formation and myofibroblast viability. Morphologic characterization of the tissue engineered pulmonary artery showed ECM formation and myofibroblast viability. The construct’s mechanical properties were comparable to human tissue. The authors proposed umbilical cord cells as a new and easily available cell source for tissue engineering with potential applications in congenital heart disease. These cells could be harvested at birth and cultured in vitro with promising growth potential. More recently, the same authors evaluated amniotic-fluid derived stem cells which is another attractive cell source for congenital applications. They were able to construct in vitro small-and large-diameter vascular grafts as a first pre-clinical attempt [153].

Another approach suggested the use of a decellularized allogeneic matrix as a scaffold for pulmonary artery tissue engineering [190]. Ovine pulmonary arteries were decellularized and seeded with autologous endothelial cells obtained from carotid arteries, and subsequently implanted in sheep. Unseeded, decellularized arteries served as controls. After six months, no calcification or thrombus formation was observed in any of the explanted blood vessels. All vessels showed an increase in diameter; however the unseeded blood vessels showed a disproportionate increase in diameter resulting in aneurysm formation. This study served as a proof of concept for the in vitro re-endothelialization of decellularized pulmonary arteries as an alternative to biodegradable synthetic scaffolds, and their short-term performance in vivo.

The field of congenital heart disease may benefit from the overall developments in the field of blood vessel tissue engineering. Some challenges that remain are the translation into high-pressure areas such as for congenital defects of the aorta, as early studies by Shinoka and colleagues have been in low-pressure systems. Compliance properties of the neo-vessel should match those of the native vessels as the child grows. Finally, more information on long-term outcomes is warranted. The first clinical trial in the United States specifically geared towards pediatric heart defects was initiated in 2009 with Dr. Christopher Breuer as the principal investigator (A Pilot Study Investigating the Clinical Use of Tissue Engineered Vascular Grafts in Congenital Heart Surgery) [191]. Results of the study had not been published at the time of writing this manuscript.

6. Conclusions

Current surgical management options for the treatment of congenital heart disease have some limitations, and outcomes could be potentially improved by employment of tissue engineering strategies. Significant progress has been made in the tissue engineering of cardiac patches, heart valves and blood vessels, with valuable implications for their applicability in congenital heart disease. Clinical studies with tissue engineered heart valves and blood vessels have generated excitement and paved the way for further research. However, a clear appreciation of the in vivo processes is warranted for the
successful clinical translation of tissue engineering strategies. Current research is broadening our knowledge about the in vivo molecular mechanisms that govern cell behavior as well as material-host interaction. Pediatric cardiac tissue engineering shares most of the challenges that the general field is facing, and some unique ones. A thorough understanding of the patients and their needs is important so that appropriate strategies can be designed. Ideally, a graft that grows with the patient and one that is durable over their entire life span is desired. In order to achieve this goal, more long term studies need to be implemented. Appropriate animal models that can parallel children’s growth and can demonstrate the long term behavior of tissue engineered implants are necessary. Many of the current studies are performed on adult animals, which may not prove sufficient indicators for pediatric cardiac tissue engineering. Scaffold materials might provoke varying inflammatory responses and exhibit different degradation profiles in younger versus adult bodies. The benefits of including autologous cells in the tissue engineering paradigm for children must be evaluated. Often, time is an extremely critical factor and cell expansion can be time-consuming, unless potent cells can be harvested prenatally in a safe manner and be ready for use by the child’s birth. Lastly, the potential of tissue engineering stems from its multidisciplinary nature. Great progress can be achieved if research projects can bring together basic scientists, engineers, and pediatric clinician specialists, to work in a concerted effort. Once these challenges are overcome, there is great opportunity for tissue engineering to provide a solution to the large number of children affected by congenital heart disease.

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