State of the Art: Tissue Engineering in Congenital Heart Surgery

Rebekah Boyd, BSN, Frank Parisi, BS, and David Kalfa, MD, PHD

Congenital heart disease is the leading cause of death secondary to congenital abnormalities in the United States and the incidence has increased significantly over the last 50 years. For those defects requiring surgical repair, bioprosthetic xenografts, allografts, and synthetic materials have traditionally been used. However, none of these modalities offer the potential for growth and accommodation within the pediatric population. Tissue engineering has been an area of great interest in a variety of cardiac applications as an innovative solution to create a product that can grow and regenerate within the body over time. Over the last 30 years, the original tissue engineering paradigm of a scaffold seeded with cells and cultured in a bioreactor has been expanded upon to include innovative methods of decellularization and production of "off-the-shelf" tissue-engineered products capable of in situ host cell repopulation. Despite progress in conceptual design and promising clinical results, widespread use of tissue-engineered products remains limited due to both regulatory and ongoing scientific challenges. Here, we describe the current state of the art with regards to in vitro, in vivo, and in situ tissue engineering as applicable within the field of congenital heart surgery and provide a brief overview of challenges and future directions.

Semin Thoracic Surg 31:807–817

Keywords: Tissue engineering, Congenital heart surgery

David Kalfa, MD, PHD.

Central Message

Considerable advances in tissue engineering for cardiac applications have been made; however, widespread clinical use of these constructs remains limited and presents opportunity for improvement.

INTRODUCTION

Congenital heart disease (CHD) is recognized as the leading cause of death due to congenital abnormalities in the United States.[1,2] The documented incidence of CHD has grown significantly over the past 5 decades secondarily to improvements in diagnostic methods and accuracy of registries.[3,4] Current estimates of prevalence are 8 per 1000 live-born full-term births in the United States, and defects may range from small holes between chambers to major malformations requiring multiple surgical interventions.[2–5] Of this population, 25% will require invasive surgical intervention within the first year of life.[2,4,5] Biocompatible materials are required for repair; traditionally those materials have been synthetic polymers, however, complications of stenosis, calcium deposition, thrombogenesis, and infection risk render them less than ideal.[5,6] Alternatively, allografts, xenografts, and autologous tissues may be used; however, they are susceptible to similar complications.[6] Most importantly in the pediatric population, none of the traditional patches and conduits have growth potential, necessitating reoperation which is linked to significantly increased morbidity and mortality.[5,6]

This lack of growth potential has been one of the driving factors in searching for new, innovative ways to generate materials for CHD repair through tissue engineering (TE). The concept of TE involves using biodegradable scaffolds that will degrade over time while simultaneously being replaced with autologous tissue, capable of growth and repair.[5] As such, the ideal tissue-engineered patch or conduit would be resistant to stenosis, calcification, immunogenic response activation, infection, and thrombosis in addition to being cost effective, readily available, and easily handled.[2] The classical paradigm of TE was introduced in 1993 by Langer and Vacanti: a scaffold seeded with autologous cells, in vitro tissue formation in a bioreactor, and further in vivo growth upon implantation.[7,8] Since that time, interest in the field has exploded, leading to multiple innovations and discoveries. The critical components that have been researched extensively include cell source, materials, fabrication and maturation processes, manufacturing and scalability, and navigating regulatory

Division of Cardiac, Thoracic and Vascular Surgery, Section of Pediatric and Congenital Cardiac Surgery, New-York Presbyterian Morgan Stanley Children's Hospital, Columbia University Medical Center, New York, New York

Address reprint requests to David Kalfa, MD, PHD, Pediatric Cardiac Surgery, New-York Presbyterian Morgan Stanley Children's Hospital, Columbia University Medical Center, New York, NY 10027. E-mail: dk2757@cumc.columbia.edu

1043-0679/$–see front matter

https://doi.org/10.1053/j.semtcvs.2019.05.023

challenges.[9] Currently, TE processes can be separated into 3 main categories: in vitro, in vivo, and in situ. The in vitro approach mirrors the classical paradigm: biodegradable scaffolds are seeded with cells, cultured in bioreactors, and surgically implanted for continued growth. The in vivo approach utilizes the human body as the bioreactor; the scaffold is placed subcutaneously for creation of a fibrotic matrix that can subsequently be removed and implanted. Finally, in the in situ approach, the regenerative capacity of the body is used to grow new tissue upon implantation of a cell-free scaffold in the cardiovascular system via recruitment of endogenous cells.[8] Here, we will describe the current state of the art in each of these categories as applicable to tissue-engineered heart valves (TEHV), tissue-engineered vascular grafts (TEVG), tissue-engineered myocardial patches (TEMP), and tissue-engineered tracheal grafts (TETG) applied to the field of congenital heart surgery.

TISSUE-ENGINEERED HEART VALVES

Valvular heart disease (VHD) is among the most common defects seen in CHD; worldwide the burden is dramatically increased among newborns and children in developing nations due to the high incidence of rheumatic fever.[8,10] There are no current medical therapies for VHD; surgical repair utilizing a mechanical or bioprosthetic valve is the standard of care. Mechanical valves are subject to high rates of thromboembolic events and require life-long anticoagulation, posing a significant bleeding risk. Bioprosthetics, conversely, have increased immunogenic and infectious risk, are prone to calcification, and are contraindicated in pediatric populations.[8,10] These issues, coupled with the need for growth and accommodation make TE a particularly appealing approach to combat VHD. TEHVs have been studied extensively for over 3 decades with the primary goal of developing a valve capable of mimicking native valve tissue in terms of anisotropic arrangement, strength, durability, flexibility, and the ability to open and close billions of times while withstanding transvalvular pressures, and resisting thrombogenesis and calcification.[8,10,11] Researchers have studied TEHV in in vitro, in vivo, and in situ modalities (Fig. 1).

Here, we provide a brief review of the current state of TEHV based on the specific TE approach utilized (Table 1).

In vitro approaches to TEHV have utilized multiple types of scaffolds from allogenic and xenogenic donor valves to natural polymers, synthetic biodegradable polymers, and hybrid scaffolds.[8,10,11] Allogenic and xenogenic donor valves were historically treated with glutaraldehyde to decrease the immunogenic response. However, this process was found to limit cell infiltration, thus mitigating their usefulness in TEHV applications until decellularization was introduced.[8] Decellularization refers to the removal of cells from a tissue or organ while maintaining the underlying extracellular matrix (ECM) organization, and has been shown to eradicate IgG response in decellularized porcine and bovine xenografts.[10,13] Clinical applications of decellularized allograft-based TEHV have been used successfully in the Ross procedure; however, the complications of leaflet thickening and limited availability of donor valve allografts continue to hamper their widespread acceptance.[8,14] Biodegradable scaffolds have the advantage of less risk of disease, acute or chronic rejection, and are not limited by supply.[8] Natural materials such as gelatin, collagen, and fibrin have been utilized with varying measures of success; promisingly, dynamic conditioning was found to improve cell seeding capacity and ECM remodeling in an autologous fibrin-based heart valve by Flanagan et al in 2007. However, rapid degradation and significant leaflet contraction remain problematic with these scaffold types.[8,10,15] A more recent approach to generate a completely biologic TEHV utilizes sheets of dermal fibroblasts (FBs) stacked into a thick tissue that can then be molded into a valvular conduit[16] or sutured to a ring similarly to current bioprostheses.[17] Biodegradable synthetic use was pioneered in 1995 by Shinoka et al, who successfully implanted a TE single pulmonary leaflet based on a polyglycolic acid (PGA) scaffold in a sheep model.[8,10,18] In 2000, Hoerstrup et al implanted a complete TEHV in the pulmonary position, and in 2010 progressed to transapical delivery of TE pulmonary valves in ovine models.[19,20] The primary complications observed were leaflet thickening, retraction, and subsequent regurgitation attributed to the contractile nature of the transplanted seeded cells in the classic in vitro approach.[8,14,21]

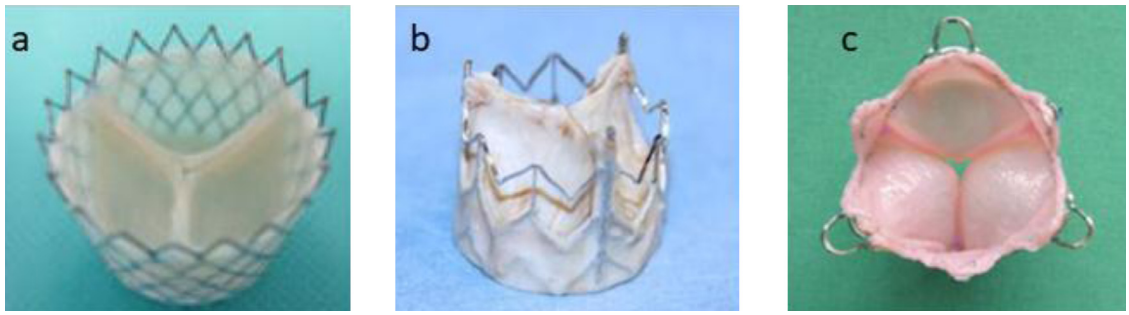

Figure 1. (a) In vitro TEHV (courtesy of B. Sanders by Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)), (b) In vivo TEHV (reprinted with permission from ref. 23), and (c) In situ TEHV (reprinted with permission from ref. 33).

Table 1. Overview of Select TEHV Studies

	Group	Scaffold	Cell Type	Results
Preclinical	Flanagan et al (2007)	Fibrin	Ovine carotid artery cells	Dynamic conditioning enhances cell attachment and ECM remodeling
	Dijkman et al (2012)	Nonwoven PGA mesh	Decellularized "off-the-shelf"	Able to be stored for 18 months and reseeded
	Tremblay et al (2014)	Scaffold-free	Fibroblasts	Remained populated with cells and ECM; dense leaflets
	Dubé et al (2014)	Scaffold-free	Fibroblasts	Resistant tissue, able to sustain multiple duty cycles
	Reimer et al (2015)	Decellularized collagenous matrix	Decellularized	Excellent initial pulse duplicator and fatigue testing
Clinical	Shinoka et al (1995)	PGA	Ovine fibroblasts and endothelial cells	No evidence stenosis, trivial regurgitation, thickening and in
	Schmidt et al (2010)	Nonwoven PGA mesh	Autologous myofibroblasts and endothelial cells	Functional with mobile but thickened leaflets
	Emmert et al (2014)	Nonwoven PGA mesh	Autologous bone marrow mononuclear cells	Intact, mobile leaflets. Paravalvular leakage and aortic regurgitation occurred
	Driessen-Mol et al (2014)	Nonwoven PGA mesh	Decellularized—in situ host cell repopulation	At 24 weeks follow-up, moderate central regurgitation, significant matrix remodeling, and host cell population
	Kishimoto et al (2015)	Scaffold-free	In body tissue architecture—connective tissue mold	Less than mild aortic regurgitation, no detectable leakage
	Kluin et al (2017)	Bis-urea-modified polycarbonate	Decellularized—in situ host cell repopulation	12 months follow-up showed mild central regurgitation, no stenosis, and almost complete endothelialization
	Lintas et al (2018)	Human-derived ECM	Decellularized—in situ host cell repopulation	Normal leaflet motion, absence stenosis, or paravalvular leak
In human	da Costa et al (2010)	Decellularized aortic valve allograft	In situ host cell repopulation	Up to 53-month follow-up, no reoperation, 7% overall mortality rate
	Dohmen et al (2011)	Cryopreserved pulmonary allograft	Autologous vascular endothelial cells	10-year follow-up, all recipients' valves functional with NYHA class I symptomology
	Brown et al (2011)	Decellularized pulmonary valve allograft	In situ host cell repopulation	Up to 9-year follow-up; good functionality, no reoperation
	Sarikouch et al (2016)	Decellularized pulmonary valve allograft	In situ host cell repopulation	10-year follow-up; good functionality no reoperation

ECM, extracellular matrix; PGA, polyglycolic acid.

A single-step interventional approach has also been utilized to deliver both pulmonic and aortic TEHV in a minimally invasive manner to primates and adult sheep; the TEHV are seeded with autologous marrow stromal cells at the time of TEHV implantation.[22] Hybrid scaffolds combining collagen and a nondegradable nitinol mesh surrounded by layers of smooth muscle cells (SMCs), FBs, myofibroblasts, and endothelial cells (ECs) are currently awaiting preclinical studies.[11]

In vivo methods relying on the natural wound-healing capacity of the body have been used to a lesser degree in TEHV manufacturing.[8,10] This process involves subcutaneous implantation of a molded foreign body. Fibroblasts surround the foreign matter and form a fibrotic capsule by actively producing a collagen-rich matrix. The resulting matrix is then removed and used as an autologous, nontoxic, nonimmunogenic valve conduit; in 2015, this procedure was successfully used for an aortic replacement with balloon-expandable stent in a goat model, however, thrombogenicity, lack of elastin and inability to control the thickness of the resulting matrix limit widespread use.[8,23]

In situ methods of TEHV fabrication show potential in being less complex and potentially more cost effective. The possibility for off-the-shelf availability is also appealing; however, controlling the recruitment and tissue formation in vivo are challenges to this approach.[8,11] Early attempts at in situ use of a decellularized porcine graft had catastrophic results, leading to the death of 3 pediatric patients due to immunogenicity, stenosis, and severe thickening.[8,24] Use of in situ decellularized

pulmonary valve homografts for pulmonary valve replacement and the Ross procedure, however, have been shown to convey good functionality and freedom from reoperation.[25,26] Decellularized aortic valve homografts have also been used clinically in the aortic root position, and demonstrated adequate functionality, freedom from reoperation related to the TEHV, and limited in vivo host cell repopulation with fibroblastic-appearing cells.[27] In 2012, Dijkman et al introduced a decellularized living TEHV—the first homologous off-the-shelf available TEHV—that was found to rapidly repopulate in vivo with host cells in primate studies. The advantages of this model included suitability for transcatheter implantation, and the ability to use screened and standardized homologous cell lines to create the TEHV. No significant leaflet thickening was observed; however, leaflet retraction persisted.[28–30] To combat this problem, Sanders et al used a constraining bioreactor insert during culture that demonstrated reduced propensity for leaflet retraction in a preclinical trial.[12] One approach to limit progressive leaflet shortening is the creation of a decellularized engineered tissue tube by Syedain et al in 2013. In this model, the tube is mounted on a frame with 3 struts, and back pressure causes the tube to collapse into 3 leaflet-like structures. Recently, the concept of a "tube within a tube" has been used in which 2 engineered tubes are sewn together with degradable sutures to create a completely biological valve.[31] Some of the most recent developments in in situ TEHV include a cell-free synthetic bioresorbable microporous scaffold TEHV created from an electrospun tube of bis-urea-modified polycarbonate followed for 12 months in a sheep model,[32] a TEHV suitable for transcatheter aortic valve replacement based on a human cell-derived ECM,[33] and an off-the-shelf TE sinus valve suitable for transcatheter pulmonary valve replacement,[34] all of which show promise for future clinical studies.

TISSUE-ENGINEERED VASCULAR GRAFTS

While relatively rare in comparison to other critical congenital heart defects, univentricular anomalies such as tricuspid atresia or hypoplastic left heart syndrome provide a model to study TEVG inside the framework of the Fontan procedure.[35] The extracardiac Fontan, a modification of the original procedure, uses a vascular graft as a cavopulmonary conduit to route blood from either the superior or inferior vena cava directly to the pulmonary artery, allowing the one functional ventricle to supply the systemic circulation with oxygenated blood.[35,36] The extracardiac Fontan procedure has been performed using both autologous tissue and synthetic polymers, offering an approach that decreases the incidence of arrhythmias and streamlines venous laminar flow patterns.[35,37] Both options, however, do not offer growth potential in addition to presenting other obstacles.[35,37] Synthetic grafts, such as polytetrafluoroethylene, are vulnerable to thromboembolism, stenosis, ectopic calcification, intimal hyperplasia, atherosclerosis, and infection.[35,36,38] Autologous tissues, as well as allografts and xenografts, often fail for similar reasons and may be difficult to harvest or unsuitable for use.[35,38] TEVGs offer a potential solution to these problems by creating a vessel that can grow, remodel, and repair in vivo.[35,39] Here, we present an overview of studies within the field (Table 2).

The most common in vitro method for TEVG implementation is the use of a scaffold-based template for construction of the graft.[38] Scaffold-based methods can be further subdivided based on the use of synthetic, natural, or hybrid polymers.[38] In 2001, Shinoka et al began a clinical trial using a bioresorbable synthetic polymer scaffold.[36,38] The scaffold contained woven poly-l-lactic acid (PLA) or PGA covered with a 50:50 copolymer sealant solution of poly (l-lactic-co-ε-caprolactone) (PLCL).[36] This biodegradable scaffold was seeded with autologous bone marrow-derived mononuclear cells (MNCs) in vitro prior to implantation.[35,36,38] After seeding with bone marrow cells, the graft remained patent and a neovessel composed of functional endothelial and smooth muscle cells formed, demonstrating growth capacity.[35,38] These grafts were implanted in 25 patients between 2001 and 2004, and with an average follow-up of 11.1 years, no patients have died from graft-related mortality.[36] Shinoka et al continued their work with the first US Food and Drug Administration (FDA)-approved clinical trial using these TEVGs in 2011 with implantation in 6 pediatric patients at Yale; currently, FDA approval is in process for a second trial staring in September 2019 at Nationwide Children's Hospital for implantation in up to 24 pediatric patients (unpublished data). While the TEVG placed in the initial clinical trial by Shinoka et al utilized grafts of large internal diameter (12–24 mm) in high-flow low-pressure flow vessels, Niklason and Langer have demonstrated success developing small diameter TEVGs using a synthetic polymer scaffold in high-pressure arterial flow vessels.[38,40] They created PGA scaffolds seeded with bovine aortic ECs and SMCs; however, instead of growing them under static conditions, they applied mechanical stimulation in the form of pulsatile flow via bioreactors.[40] This led to improved scaffold physical strength and allowed it to withstand the high-pressure flow of the small diameter vessels.[38,40,41]

Natural polymer scaffolds make use of fibrin, silk, collagen, and chitosan, with fibrin offering substantial successes.[35,38] Huynh and Tranquillo assembled TEVGs using fibrin gel to capture neonatal human dermal FBs.[42] After creating the conduits from a mold, the tubes were layered concentrically to allow them to fuse together.[38,42] However, due to inadequate burst pressures, these scaffolds were then cultured in a perfusion bioreactor, and mechanical stimulation was applied to generate high-pressure flow, resulting in comparable burst pressures to human veins.[38,42]

Decellularization makes use of synthetic and natural polymer scaffolds, but uses a process to remove antigenic material from the graft and avoid detrimental consequences of immunologic processes.[38] In a human trial, Olausson et al decellularized an allogeneic human iliac vein and seeded it with ECs and SMCs differentiated from autologous stem cells from the patient's bone marrow.[43] The graft was implanted in a 10-year-old girl suffering from extrahepatic portal vein obstruction.[38,43] While patency had decreased after 9 months, a second surgery

Table 2. Overview of Selected TEVG Studies

	Group	Scaffold	Cell Type	Results
Preclinical	Huynh and Tranquillo (2010)	Fibrin gel	Human dermal fibroblasts	Comparable burst pressure to human veins
	Niklason and Langer (1997)	PGA mesh	Bovine aortic endothelial & smooth muscle cells	High pressure, small diameter vessel patency
Clinical	Yokota et al (2008)	PGA and PLA with collagen microsponge	In situ tissue regeneration	Implanted in canine carotid arteries. All patent with no signs of thrombosis or aneurysm. Elastin and collagen reconstruction
	Quint et al (2012)	Decellularized PGA mesh with collagen matrix derived from human SMCs	In situ tissue regeneration	83% patency with no evidence of rupture or aneurysm in aortic position in rats. Confluent endothelialization found after 6 weeks
	Syedain et al (2014)	Decellularized fibrin gel derived from ovine FBs	In situ tissue regeneration	No evidence of dilatation or mineralization after 8 weeks as femoral arterial graft in ovine model. Complete endothelialization by 24 weeks
In human	Peck et al (2011)	Scaffold free	Autologous fibroblasts and endothelial cells	40% patency up to 20 months as AV shunts for hemodialysis
	Chalajour et al (2012)	Decellularized porcine SIS	In situ recellularization	Single human study. Recellularization with endothelial cells. Removed due to inflammation.
	Olausson et al (2012)	Decellularized human iliac vein	Autologous ECs and SMCs	Restored patency with second graft. No immunosuppression required
	Bockeria et al (2017)	Bioabsorbable supramolecular polyester	In situ recellularization	Implanted in 5 pediatric patients; 12-month follow-up showed graft patency and functional stability
	Sugiura et al (2018)	PGA and PLA with PLCL copolymer	Autologous bone marrow-derived MNCs	Patency up to 11 years. Asymptomatic graft stenosis in 28%

ECs, endothelial cells; MNCs, mononuclear cells; PGA, polyglycolic acid; PLA, poly-l-lactic acid; PLCL, poly (l-lactic-co-ε-caprolactone); SMCs, smooth muscle cells.

was performed to remove tissue that was compressing the graft and to lengthen the vessel; using the same graft procedure from the first operation.[38,43] The procedure restored the patency of the graft while the patient had no anti-EC antibodies and did not require immunosuppression; though it is the only human study of this procedure.[38,43]

Another in vitro method for TEVGs is TE by self-assembly (TESA). TESA does not use a scaffold or support structure to create the graft, but instead uses methods such as sheet-based engineering, microtissue aggregation, and cell printing.[38,44] L'Heureux et al began investigating TESA in 1999 to avoid the negative effects of synthetic scaffolds.[38,44] Using mesenchymal stem cells (MSCs), self-assembled sheets are rolled into many discrete layers and wrapped around a mandrel, which then form the tubular structure that is the vascular graft.[38,44] Upon removal of the mandrel and fusing of the layers after 8 weeks, the graft contained numerous ECM proteins, particularly collagen, and a working endothelium, all structurally similar to the native tissue.[38,44]

In situ methods to create TEVGs are currently focused on the development of small diameter vessels without using natural or synthetic scaffolds. Yokota et al note that while biodegradable grafts have been developed and applied clinically using cell seeding and bioreactor perfusion, there has not been the development of small-caliber vascular grafts.[45] They engineered a 4-mm diameter graft using a collagen microsponge with a woven biodegradable and absorbable polymer tube constructed using PGA and PLA fibers.[45] These grafts were then implanted into the carotid arteries of mongrel dogs and evaluated at 2, 4, 6, and 12 months showing patent grafts with no signs of thrombosis or rupture, excellent in situ tissue regeneration, and a reconstructed vessel wall with elastin and collagen fibers.[45] Quint et al seeded human SMCs on a biodegradable scaffold, and then implemented a 2-step decellularization process using an amalgam of enzymes, detergents, and hypertonic solutions.[38,46] The decellularized vessels were implanted in nude rats as aortic interpositional grafts.[38,46] After 6 weeks, the graft developed confluent

endothelialization and subendothelial SMCs.[46] The vessel showed no deterioration of the collagen ECM after decellularization, the mechanical strength and burst pressure remained similar to that of a human vein, and the patency rate was 83% with no evidence of rupture or aneurysm.[38,46] Syedain et al demonstrated successful arterial implantation in an ovine model of a decellularized "off-the-shelf" graft that demonstrated complete in situ endothelialization by 24 weeks.[47] In 2017, Bockeria et al performed the first clinical experience of implantation of a decellularized bioresorbable graft as an extracardiac cavopulmonary conduit between the inferior vena cava and the pulmonary artery in 5 pediatric patients with univentricular pathologies. Follow-up at 12 months showed patent grafts with functional and anatomical stability.[48]

Nonvascular tissue has also been used to develop TEVGs. The porcine small intestinal submucosa (SIS) is a decellularized collagenous extracellular matrix that contains growth factors suitable for adhesion of endothelial cells.[49] Chalajour et al evaluated the growth potential of the Porcine SIS when it was used to supplement the pulmonary artery in a newborn patient born with total anomalous pulmonary venous return and pulmonary atresia.[49] While the patch was removed after 2 months due to abnormal wall thickening caused by inflammation, the graft showed recellularization and vascularization similar to the native pulmonary artery, though the origin of the cells could not be identified.[49]

TISSUE-ENGINEERED MYOCARDIAL PATCHES

The most prevalent congenital heart defect requiring surgical patch repair are VSDs; other common surgical procedures that use patches are aortic valvotomy, arterial switch AVSD complete repair, tetralogy repair, and isolated cortication repair.[50,51] Additionally, use of TEMPs to regenerate damaged myocardium in adult survivors of myocardial infarction has been studied; this concept would be beneficial to strengthen ventricular function in children with heart failure secondary to CHD. Possible use in single-ventricle pathologies that lead to heart failure due to ventricular overload from supplying both systemic and pulmonic circulations is also worth consideration.[52] In theory, making tissue-engineered 3-dimensional heart tissue might be considered one of the easier constructs—given the fact that immature cardiomyocytes (CMs) are known to beat spontaneously and form synapses.[53] In reality, however, this area of TE faces unique challenges such as low engraftment rates and difficulty with electrical and mechanical integration potentially leading to life-threatening arrhythmias, and problems with size and scalability to produce clinically relevant patches.[54,55]

Cell-based therapies began in the 1990s, with rapid succession to patches being tested in rat models, in vivo repair with human CM-based patches, to most recently injectable patches allowing minimally invasive delivery.[54,56] The cells utilized in all of these techniques followed similar advancements: from skeletal myoblasts, to primary cardiac cells, mixed cultures of CMs and fibroblasts, MSCs, and most recently pluripotent stem cells (PSCs), both embryonic stem cells (ESCs) and inducible pluripotent stem cells (iPSCs).[54] Prefabricated porous matrices seeded with these cells have been expansive, including natural polymers (eg, gelatin, chitosan, and collagen), synthetic biodegradable polymers (eg PGA, and PLA), and hybrid scaffolds (via blending, coating, copolymerizing, and multilayering).[50,53,57,58] Scaffold-free assembly has also been pursued with the use of stacked sheets of CMs[59] and prevascularized human patches made from CMs, ECs, and FBs.[60] Over the course of these multiple studies, 2 notable findings were made: patches improved when CMs were cocultured with ECs and FBs for vascularization, and when receiving either electrical or mechanical stimulation.[54,61] While numerous meritorious studies have been done on this topic, here, we will highlight a few (Table 3).

In vitro approaches have been the primary modality researched in the production of TEMP. The manufacture of cardiac tissues generated from human-induced pluripotent stem cells (hiPSCs) in 2011 by Tulloch et al spurred many additional developments.[58] In 2016, Sugiura et al used hiPSC-CMs cultured on a PGA and 50:50 PLCL patch and implanted them in nude rats. At 4 weeks postimplantation, none of the seeded hiPSC-CMs were present; however, at 16 weeks, there was marked increase in the number of regenerated host CMs on seeded patches vs nonseeded patches.[57] In 2018, Bejleri et al introduced a novel patch composed of pediatric human cardiac progenitor cells seeded on a bioprinted decellularized cardiac ECM hydrogel that was found to remain highly viable up to 7 days, exhibit improved differentiation of CMs and angiogenesis, and share host vascularization after 14 days of attachment to rat hearts.[62]

One of the main challenges of TEMP has been size and scalability; Gao et al successfully engineered large cardiac patches (4 cm × 2 cm × 1.25 mm) from hiPSC-derived ECs, SMCs, and CMs in a fibrin scaffold. In a porcine model, these patches were shown to improve left ventricular function while decreasing myocardial wall stress, hypertrophy, and infarct size. One concern noted by the researchers was that conduction velocity of action potentials within the graft only reached 25% of that of the native myocardium, increasing the risk of reentry arrhythmia.[63] Shadrin et al also successfully scaled up a hiPSC TEMP to a size of 4 cm × 4 cm that was noted to have successful vascularization within 2 weeks of small animal implantation.[55]

New horizons for TEMP include instrumented patches, placement of epicardial or endocardial patches (vs standard myocardial patch/cell injection therapy), and the development of anisotropic layers mimicking native heart tissue with the potential to engineer entire ventricles.[54] A recent breakthrough by Noor et al led to the successful bioprinting of a completely autologous whole heart, complete with major blood vessels. The group induced pluripotent stem cells from autologous omental tissue which were then differentiated into CMs and ECs, while the resulting ECM was used to create a hydrogel. All 3 components were processed to generate bioinks used to

Table 3. Overview of Select TEMP Studies

	Group	Scaffold	Cell Type	Results
Preclinical	Noor et al (2019)	Bioprinted ECM hydrogel	hiPSC-derived CMs and ECs	Thick, vascularized perfusable patches and stand-alone cellularized heart with great blood vessels
	Shimizu et al (2002)	Scaffold-free	Neonatal rat CM sheets	Spontaneous beating at 3 weeks; long-term survival of pulsatile grafts up to 12 weeks in rats
	Sekine et al (2008)	Scaffold-free	Rat CMs and ECs	Preformed EC networks easily connect to host vessels, inhibit fibrosis, improve overall cardiac function in rats
Clinical	Stevens et al (2009)	Scaffold-free	hESC-derived CMs, HUVEC, mouse embryo FBs	Active contraction, able to be electrically paced, 10-fold larger cell graft with EC and FB compared to CM alone
	Tulloch et al (2011)	Collagen matrix	hESC and hiPSC-CMs	Human myocardium constructs able to survive and graft to rat host myocardium; perfused by host circulation within a week
	Sugiura et al (2016)	PGA & 50:50 poly(l-lactic-co-ε-caprolactone) copolymer	hiPSC-CMs	Seeded cells disappeared within 4 weeks of implantation in rats; host cells repopulated patch at 16 weeks postimplantation
	Shadrin et al (2017)	Hydrogel	hiPSC-CMs, SMCs, FBs	Clinically relevant size (4 cm × 4 cm), similar to adult human myocardium in terms of vascularization and maintaining electrical function in rats
	Montgomery et al (2017)	UV-crosslinkable and elastomeric polymer poly (octamethylene maleate (anhydride) citrate	rat CMs	Injectable; delivery through orifice as small as 1 mm, recovering initial shape. Significant improvement in cardiac function in rats post MI
	Bejleri et al (2018)	Bioprinted native cardiac ECM hydrogel	Human pediatric cardiac progenitor cells	Increased angiogenic potential with vascularization occurring over 14 days in vivo in rats
	Gao et al (2018)	Fibrin scaffold	hiPSC-derived CMs, SMCs, ECs	4 cm × 2 cm × 1.25 cm patches, beating within 1 day, mature within 7 days. Improved cardiac function and decreased infarct size in swine

CMs, cardiomyocytes; ECM, extracellular matrix; ECs, endothelial cells; FBs, fibroblasts; hESC, human embryonic stem cells; hiPSC, human-induced pluripotent stem cells; HUVEC, human umbilical vein endothelial cell; PGA, polyglycolic acid; SMCs, smooth muscle cells; UV, ultraviolet.

bioprint fully autologous thick, vascularized patches, in addition to independent complete cardiac models.[64] Also of great interest is the successful development by Montgomery et al of an injectable flexible scaffold with shape-memory that can insert 1 cm × 1 cm patches or scaffolds through a hole as small as 1 mm while preserving CM viability and function.[56] This scaffold was created from a biodegradable polymer: poly(octamethylene maleate (anhydride) citrate. It was injected into rats and found to have equivalent vascularization and cell survival when compared to open surgical patch placement, in addition to substantially improving rat cardiac function after MI. The group was further able to place human cell-derived patches in the epicardium, aorta, and liver of large animals in a porcine liver through the keyhole approach with a surgical tool as small as 0.5 cm.[56]

TISSUE-ENGINEERED TRACHEAL GRAFTS

A pulmonary artery sling is a defect observed in CHD where the left pulmonary artery originates from the right pulmonary artery. This causes compression of the trachea and right bronchus which leads to multiple complications, including tracheobronchial stenosis.[65] Currently, there is a lack of materials suitable for repair of long-segment airway defects; anything over 30% of the airway length in the pediatric population has

no treatment except palliative care.[65–67] This has made TE a highly anticipated approach; however, the clinical performance of TETG has yet to reach acceptable levels. As it stands, the clinical use of TETG is limited to compassionate use only.[66,67] One potential explanation is the unique challenges to TE in the context of tracheal repair; the avascular nature of hyaline cartilage, the lack of sterile environment, and the significant morbidity of graft failure to include airway obstruction and death have all been posited as playing a role in the difficulty this particular TE construct faces.[67] Despite these impediments, several promising studies are in development, some of which will be described here (Table 4).

In vitro models traditionally utilized decellularized cadaveric donor tracheal allografts, which showed promising support for epithelial growth but mixed results with chondrocyte repopulation and regeneration of host cartilage prior to scaffold degradation.[67–69] The first completely TE airway replacement was performed in 2008 by Gonfiotti et al in a 30-year-old female with end-stage left main bronchus malacia. A decellularized cadaver donor tracheal allograft was seeded with autologous epithelial cells and chondrocytes of MSC origin; 6 months after implantation, a progressive cicatricial scar began to cause proximal graft stenosis that required multiple bronchoscopies and stent placements.[70] The first pediatric TETG implantation was performed in 2010 by Elliott et al with a decellularized cadaveric donor seeded with bone marrow MNCs and patches of autologous respiratory epithelium. The graft revascularized within 1 week of implantation; however, it did not obtain proximal rigidity for almost 2 years.[71] In 2017, a decellularized TETG was implanted in a female teenager with congenital tracheal stenosis with the advent of sudden airway obstruction and death 3 weeks after implantation, thought to be caused by an intrathoracic hemorrhage.[72] Initial reports of clinical implantation of a TETG with a biosynthetic scaffold have since been retracted. Preclinical trials of an electrospun polyethylene terephthalate and polyurethane scaffold with 3D-printed porous polycarbonate c-shaped rings embedded during the electrospinning process and seeded with ovine BM-MNC showed superior cell seeding and was able to mimic the biomechanics of native ovine trachea.[66] A scaffold-free approach utilizing self-assembly of human MSC-derived cartilaginous rings and structures is also under investigation.[73]

In vivo studies have had mixed outcomes as well. In a rabbit model, the fabricated sheets of cartilage were used in combination with a muscle and silicone construct, cultured heterotopically, and then orthotopically transplanted after 12–14 weeks; all of the rabbits died following implantation.[74] In 2010, however, Delaere et al successfully implanted a fresh cadaver trachea allograft into the forearm of a 55-year-old female. The allograft was then orthotopically transplanted after 4 months. After discontinuation of immunosuppressive therapies, the donor-derived epithelium was eradicated from the graft, but there was no significant impact on the cartilage matrix, speculated to be due to the lack of blood vessels in adult cartilage.[75]

In situ TE approaches have shown the least promise. In 2010, Remlinger et al used hydrated xenogeneic decellularized porcine tracheal matrices in a canine model. While development of columnar pseudostratified ciliated epithelium was supported, cartilage was found to degrade with limited new cartilage formation.[69] Kutten et al used decellularized tracheal extracellular matrices in a murine model in 2015. Their findings included epithelial restoration of the decellularized grafts and maintained patency at 8 weeks; however, they observed decellularized cartilaginous portions of the graft to remain acellular with lack of radiopaque cartilaginous rings on imaging.[76]

Table 4. Select Experiences With TETG

	Group	Scaffold	Cell Type	Results
Preclinical	Best et al (2018)	2:8 PET: PU with solid or porous polycarbonate C-shaped rings	Ovine bone marrow-derived MNC	Porous rings more closely biomimetic of native trachea
	Dikina et al (2015)	Scaffold-free	hMSC	Cartilaginous rings and successful fusion into cartilage tubes
	Kutten et al (2015)	Decellularized murine tracheal allograft	In situ endogenous recellularization	Cartilaginous rings lost structure during decellularization and did not repopulate
Clinical	Weidenbecher et al (2009)	Scaffold-free	Auricular rabbit chondrocytes	Graft stenosis
	Remlinger et al (2010)	Decellularized porcine tracheal graft	In situ endogenous recellularization	Cartilage degradation
In human	Gonfiotti et al (2008)	Decellularized cadaver allograft	Autologous epithelial cells and chondrocytes of MSC origin	Proximal graft stenosis
	Elliott et al (2010)	Decellularized cadaver allograft	Bone marrow MNC and autologous epithelium	Almost 2 years for proximal rigidity
	Delaere et al (2010)	Donor allograft	Orthotopic forearm implantation	Viable at one-year follow-up

hMSC, human mesenchymal stem cell; MNC, mononuclear cell; PET:PU, polyethylene terephthalate:polyurethane.

Regardless of the method of TE utilized, graft stenosis remains the predominant morbidity in TETG implantation; the optimal scaffold and cell source also remain unclear at this time.[67]

CHALLENGES

The challenges facing TE in CHD are multifaceted. As such, despite the promising preclinical and clinical trials highlighted here, widespread acceptance of TE constructs remains limited. One of the main hurdles for TE products is regulation. The heterogeneity of TE products in terms of scaffolds, status of cellularization, and main mode of exerting effects, makes classification cumbersome. Currently, under European regulation 1394/2007, TE products are classified as advanced therapy medicinal products; however, there is a proposal from the Medical Device Regulation to reclassify all nonliving TE products as medical devices that will become fully actionable in May 2020.[77,78] In the United States, the FDA considers TE products to be human medical products that are further classified into 3 different categories: drug, biological product, or medical device, depending on the use of living cells vs a decellularized scaffold.[77] The confusion surrounding appropriate regulation within and between countries confound the ability to focus on quality control and product consistency. This is especially important as there are no existing overarching standards to adequately regulate TE technologies, and reliance on cells and extracellular in vivo elements to produce strength and functionality is necessarily associated with far greater variation than other medical devices.[79]

Clinical opportunities for growth within the field of TE include standardization of a monitoring or surveillance program for recipients of TE products, ensuring the feasibility of use in an average clinical setting, and innovative ways to detect variability between patients that may have a profound effect on the ability to standardize these treatments.[8,77] In order to be accepted broadly in the clinical setting, TE products must be easy to handle, have off-the-shelf availability, and maintain sterility comparable to current standard of care CHD repair products.[8] As so much of this field is patient specific and results depend on comorbidities and advancement of disease and native tissue, use of technologies such as organ-on-a-chip may help predict individual regenerative capacity, allowing the identification of patients who may not be candidates for TE products.[77]

From a scientific standpoint, safety of the TE products and approximation to native tissue functionality are paramount. Currently, the goals of TE are to minimize the immune and inflammatory reactions, reduce thrombogenicity, and optimize cell infiltration for remodeling and growth.[77] Strategies to address immunogenicity and inflammation include control of the blood-material interface, use of allogenic starting materials and autologous cells, or acellular scaffolds based on decellularized or polymeric biodegradable substances.[8,77] Customized surface biofunctionalization to modulate immune cell infiltration and enhance in vivo endothelial cell recruitment and adhesion is an area of active research.[8,77,80] Surface modification with antibodies (eg, CD34), peptides (eg RGD, heparin), and growth factors have been attempted, although caution must be taken as aberrant cell recruitment in vivo may contribute to the commonly observed complications of stenosis and intimal hyperplasia.[80]

Although the challenges facing TE are numerous, the large amount of active research in the field indicates solutions will be found in the near future as momentum continues to build.

FUTURE DIRECTIONS

Despite over 3 decades of research in TE and the many preclinical and clinical studies outlined in this review, there remains a paucity of clinically applicable TE products in the field of congenital heart surgery. This becomes critically important, as we consider their position within the field in the face of active developments and improvements in other arenas. The RESILIA bovine pericardial valve by Edwards was recently released with marked improvement in anticalcification properties. Similarly, the TriFlo mechanical valve is purported to not require any anticoagulation in tested bovine models (Vesta Keep PEEK Evonik). To remain competitive options within the field, TE products must progress more rapidly.

Incorporation of minimally invasive techniques, such as transcatheter or transapical valve replacement using TEHV, injection of shape-memory scaffolds, and use of keyhole surgical approaches to place cardiac patches, is one way for TE constructs to accelerate. Another consideration is combination of TE with biostable materials to create a new generation of TE products that would incorporate growth and regeneration of host tissue with partially degradable, partially stable scaffolds to mitigate issues with strength and stability.

One of the key issues hampering this progression is lack of regulatory agreement. Regulation of living TE constructs is imperative; however, lack of consensus on classification, approvals, and appropriate surveillance programs continues to detract from moving the field forward. We call on regulatory agencies to aid us in standardization of approvals both within and between countries to facilitate safe, efficacious use of TE products.

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