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Clinical Status of Tissue Engineering and Regenerative Medicine in Cardiovascular Disease

*Miyachi H[1], Shoji T[1], Sugiura T[1], Miyamoto S[1], Breuer KC[1] and Shinoka T[1,2]**

[1]The Tissue Engineering Program and Center for Cardiovascular and Pulmonary Research, Nationwide Children's Hospital, USA

[2]Department of Cardiothoracic Surgery, The Heart Center, Nationwide Children's Hospital, USA

Abstract

Cardiovascular diseases, such as coronary artery disease, aortic disease, peripheral vascular disease, and heart failure, contribute to be significant causes of worldwide mortality despite modern advances in medicine and surgery. Current vascular grafts and prosthetic heart valves used to treat CVD have limitations such as the lack of growth capacity and risks of thrombosis, stenosis, and calcification. Similarly, left ventricular (LV) reconstruction surgery can reduce the dilated LV volume after cardiac remodeling, but is incapable of regenerating myocardium. Theoretically, cardiovascular tissue engineering and cardiac regenerative medicine have the potential to address the limitations of current grafts and prosthetic heart valves. An ideal tissue engineered vascular graft (TEVG) and heart valve (TEHV) is thrombus free, easily handled, biocompatible, durable, and maintains mechanical integrity as the scaffold degrades and remodels into native tissue. However, small-diameter (<6 mm) TEVGs have not yet shown clinical effectiveness, and TEHVs still have limitations for clinical use. Cell injection therapies, which induce myocardial regeneration, are promising approaches for myocardial repair. However, the beneficial effects on current cell injection therapies are mainly associated with the secretion of paracrine factors rather than direct differentiation of cardiac cells. Here we will review various advanced devices, approaches, and strategies to address current drawbacks, focusing on current clinical studies and ongoing clinical trials for TEVG, TEHV, and myocardial regeneration based on cardiovascular medicine.

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Correspondence:
Toshiharu Shinoka, The Tissue Engineering Program and Department of Cardiothoracic Surgery, The Heart Center, Nationwide Children's Hospital, 700 Children's Drive, T2294, Columbus, OH 43205, USA, Tel: 614-722-3103; E-mail: Toshiharu.shinoka@nationwidechildrens.org

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Introduction

Cardiovascular diseases (CVDs), such as coronary artery disease (CAD), aortic disease, peripheral artery diseases (PAD), heart valvular disease, and heart failure are leading causes of death worldwide [1]. The use of expanded polytetrafluoroethylene (ePTFE, Goretex) or polyethylene terephthalate (PET, Dacron) for vascular graft implantation is common to treat patients with PADs and aortic disease [2,3]. However, synthetic materials show limitations associated with thrombosis, lack of durability, and inability to regenerate native tissue [4]. To treat three vessel disease, patients typically undergo coronary artery bypass grafting (CABG), where autologous tissue are harvested from either the internal mammary artery or the saphenous vein. However, autologous tissues may be in short supply, thus making it difficult to perform multiple or repeat operations. Heart valve replacement procedures are commonly performed to treat patients with heart valvular disease. There are two categories of prosthetic heart valves; mechanical and biological. Unfortunately, mechanical hearts valves have a significant risk of thromboembolic complication, resulting in the need for anticoagulation therapy, whereas, and biological heart valves have their own drawbacks such as poor long-term durability and ectopic calcification. Ischemic heart disease mortalities have decreased due to recent advances in medical device therapies and surgeries. However, the prevalence of congestive heart failure secondary to ischemic heart diseases increasing. In efforts to restore cardiac function after ischemic damage, LV reconstruction surgery and cell injection therapy have been proposed as myocardial repair approaches. Though vascular, valvular, and myocardial devices are readily available and have demonstrated clinical efficacy, they are not without limitations. Several cellular therapeutic approaches for cardiac repair have been evaluated in small and large animal models. Additionally, TEVGs and TEHVs have been created to address cardiovascular challenges, and have demonstrated long-term safety and efficacy in humans. However, the current cardiovascular tissue engineered devices and regenerative medicine therapeutic approaches are far from providing ideal, off-the-shelf clinical treatment. In this article, we will discuss therapeutic applications of tissue engineered vascular grafts (TEVGs), tissue engineered heart valves (TEHVs), and regenerative

Table 1: Clinical trials of TEVG.

Trial	Material + Cells	Site	Results	Year	Reference
Synthetic-based materials					
Shinoka et al.	PLCL, PGA + BM-MNC	Vein (TCPC)	24% stensosis (mean 5.8 years)	2001	[7]
Biological-based materials					
L'Heureux et al.	ECM derived from fibroblast + EC	AV shunt	2/3 occluted (11 months)	2009	[24]
(Cytograft tissue engineering, inc.)					
Artegraft	decellularized bovine carotid artery		60.5% patency (1 year)	2011	[26]
Combined materials					
Omniflow II	crosslinked ovine collagen + polyester mesh	AV shunt	77% patency (1 year)	1996	[27,28]
Humacyte	SMC + PGA	AV shunt	89% secondary patency (1 year)	2016	[30]

PLCL: L-lactide and ε-caprolactone; PGA: Polyglycolic Acid; BM-MNC: Bone Marrow-mononuclear Cell; TCPC: Total Cavopulonary Connections; ECM: Extracellular Matrix; EC: Endothelial Cell; SMC: Smooth Muscle Cell; AV shunt: Arteriovenous Shunt.

cardiac repair approaches that appear to be particularly promising effects in clinical studies and ongoing clinical trials.

Tissue Engineered Vascular Graft and Heart Valve

The concept of tissue engineering was first proposed during the mid-1980s in an effort to overcome the shortage of suitable donor organs for transplantation. Tissue engineering is defined as the fabrication of alternative materials for the purpose of restoring biological and physiologic function at the site injury or defect, and eventually integrating with a patient's native tissue [5,6]. The ideal tissue-engineered construct will mimic host tissue in that it consists of cells, the extracellular matrix (ECM), and a signaling system. The general concept of tissue engineering has three main components; 1) a scaffold materials, 2) cells, and 3) biochemical and physiochemical signaling [6].

Tissue engineered vascular graft

To date, hundreds of synthetic and/or biological TEVGs have been developed and evaluated in studies involving a multitude of models (Table 1). In 2001, we started a human clinical trial implanting venous TEVGs for extra cardiac total cavo-pulmonary connections. The alternative material scaffold was composed of a woven fabric made of a 50:50 mixture of L-lactide and ε-caprolactone (PLCL) reinforced with polyglycolic acid (PGA), and seeded with autologous bone marrow mononuclear cells (BM-MNCs). Long-term results demonstrated that the TEVG was clinically viable, as there was no graft-related mortality or evidence of graft rupture, aneurysm, infection, or ectopic calcification (Figure 1). Approximately 24% of patients had graft stenosis, but all underwent successful percutaneous angioplasties [7]. In animal models, various combinations of scaffold materials, cells and chemical substances have been suggested. Of synthetic degradable polymers, PGA, Polylactic acid (PLA), and poly (ε-caprolactone) (PCL) have been the most widely used [8-10]. Several groups have combined/ blended multiple materials to take advantage of the best characteristics of individual materials. The most well known of these co-polymers are PLCL, PLGA; Poly (L-lactic-co-glycolide), PHA; Polyhydroxyalkanoates, and MPEG-PDHA; polyethylene glycol and dihydrozyacetone polycarbonate [11-14]. Biological scaffolds are another approach to constructing TEVGs. The group of evaluated biological scaffolds includes collagen, fibrin, hydrogels, xenogenicsmall intestinal submucosa (SIS), and decellularized vessels from both allogenic and xenogenicsources [15-17]. As for cell sources to seed TEVGs, endothelial cells (ECs),

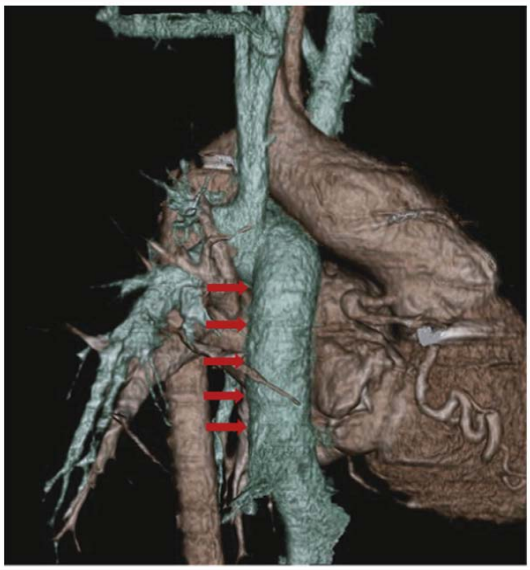

Figure 1: 3-Dimensional CT imaging 1 year after TEVG implantation. The TEVG was implanted into a patient with single ventricle physiology as extra cardiac total cavo-pulmonary conduit. Red arrows show the TEVG.

smooth muscle cells (SMCs), BM-MNCs, mesenchymal stem cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells have been investigated [7,18-21].

There are greater challenges when designing and constructing an arterial TEVG versus a venous TEVG because it must be durable enough to withstand high arterial pressures. Thus, it is logical that many investigations have focused on creating arteriovenous (AV) shunt TEVGs as the next progression from venous scaffolds. L'Heureux et al. developed a Tissue Engineering by Self-Assembly (TESA) design approach, utilizing a new production method to form strong tissue without the use of synthetic biomaterials [22]. This patient-specific graft required autologous fibroblasts to be cultured for 6-9 months to produce sheets of tissue. The tissue sheets are then fused together around a stainless steel mandrel, dehydrated, and subsequently luminally seeded with autologous ECs [23]. Lifeline grafts (Cytograft Tissue Engineering. Novato, CA) were investigated clinically and of 3 clinical trial patients, 2 patients required interventions for stenosis (both eventually failed) within a year and 1 patient died due to infection [24]. These grafts are limited by their high production costs (>$15,000 per graft), long production times, and technically

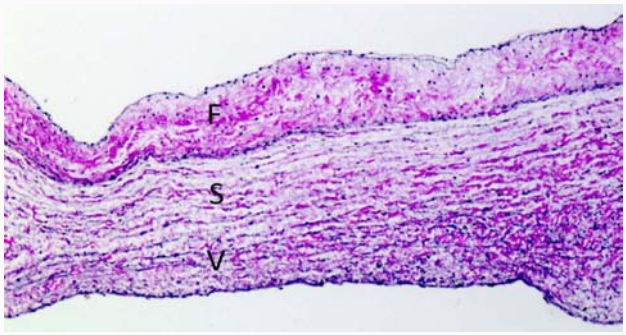

Figure 2: Tissue image of tri-layered structure of an aortic leaflet in sheep. The three layers consist of fibrosa (F), spongiosa (S), and ventricularis (V).

complicated constructions [25]. Therefore, major improvements with this approach are needed before this type of TEVG is translated clinically and commercially marketable. Artegraft is the commercial name for an AV shunt TEVG that took a biological design approach. The graft is composed of decellularized bovine carotid artery graft and favorably required fewer interventions than ePTFE grafts to maintain patency [26]. Omni flow II is a biosynthetic graft that is widely used in many European countries, but fewer South American and Asian nations. However, it is not approved for sale in the U.S., France, and Japan. This graft, composed of cross linked ovine collagen with a polyester mesh endoskeleton, has shown favorable long-term results for hemodialysis use in several studies [27,28]. The Humacyte graft, developed by Dahl et al. [29] is a promising TEVG. It is perhaps the closest to being clinically translated and serving as a readily available off-the-shelf conduit to be used in large and small diameter graft applications. The graft is constructed by culturing cadaveric human SMCs on tubular PGA scaffolds in a bioreactor that delivers cyclic radial strain. During the culture, SMCs secrete ECM proteins, and deposits collagen as the PGA degrades. The resulting tissue is then subsequently decellularized with detergents, which leaves behind a collage nous matrix. The Humacyte TEVG has shown good patency (7/8 in baboon AV shunt and 5/6 canine CABG models) [2]. Recently, two phase 2 clinical trials (n=60) investigating AV shunt TEVGs revealed a higher secondary patency of 89% at 1 year versus PTFE (55-65% at 1 year)as reported in a multicenter study [30]. The Humacyte TEVG is also currently undergoing clinical trials for peripheral artery bypass applications. Despite several TEVGs displaying promising animal results [31-33], arterial TEVGs are not yet commercially available. The ideal arterial TEVG needs to be both technically and economically viable. Additionally, many cardiovascular diseases are time sensitive and considerations must be made in terms of how long it takes for a given TEVG to be produced.

Tissue engineered heart valve

The TEHV concept consists of an autologous cell-seeded or unseeded three-dimensional (3D) biocompatible and/or biodegradable scaffold. A scaffold provides the 3D template and environment for cells to attach and proliferate into neo tissue. Ideally, the neo tissue will eventually provide the TEHV with the proper mechanical properties as the scaffold degrades. There have been many groups that focused their TEHV research designs on mimicking native heart valve structure. Human semi lunar valves (pulmonary and aortic) consist of three semicircular leaflets (cusps) attached to a fibrous annulus called roots [34]. Valve cusp thickness is generally less than 1mm in depth, and is thicker at the base and tip. These flexible valve leaflets (cusps) are composed of three distinct layers: the fibrosa, spongiosa, and ventricular is (Figure 2) [34,35]. The fibrosa is located closest to the aorta, composed of circumferentially oriented fibrillary collagens (type I and III), and associated with a cusp's mechanical stiffness and strength [35]. The middle surface is the sponginosa, and consists of proteoglycans interspersed with collagen fibers. The layer works as a cushioned interface between the two outer layers to facilitate valve movement and integrity. The ventricular is composed of aligned elastic fibers interspersed with short collagen fibers. It enables valve extension and recoiling under diastolic and systolic pressures. In general, heart valves include a layer of ECs and valvular interstitial cells (VICs) between the ECM.A recent studies revealed that ECs play an important role in heart valve development by undergoing endocardial-to-mesenchymal transformation [36]. The VICs are differentiating into myofibroblasts [37], have features found in both SMC and the fibroblast. Thus, VICs are also thought to play a very important role in generating TEHVs neo tissue due to their ECM production. We first introduced a TEHV concept in 1995 that consisted of a polyglactin woven mesh, sandwiched between 2 non-woven PGA mesh sheets. The TEHV was seeded with myofibroblasts and ECs and then used to reconstruct right posterior pulmonary heart valve leaflets in a sheep model [38]. Subsequently, we also investigated a TEHV made of a PGA and PLA co-polymer scaffold in a lamb model [39]. Many synthetic based materials have been studied in large animal models and include poly-hydrozyalkanoates (PHAs) and poly-4-hydroxybutyrate (P4HB) [40,41], poly-hydroxyoctanoate (PHO) [42], PGA and P4HB [43], PLCL and poly(D, L-lactide-co-glycolide) (PLGA) [44], and polygllycerolsebacate (PGS) [45] (Table 2). Scaffolds blending both biological and synthetic materials have also been investigated. Porous chitosan-modified PCL scaffolds have been designed and constructed to improve fibroblast cell attachment [46]. A composite scaffold composed of PLCL, PLGA, and type 1 collagen has also been tested for TEHV efficacy. A variety of synthetic material approaches have been studied and include applying P4HA to mold PGA meshes, PGA/PLLA composite fibrous scaffolds, and PGS-PCL hybrid constructions [43,47-49]. To date however, synthetic heart valves have not yet been clinically translated. Contrastingly, decellularized biological-based TEHVs have shown promising clinical results and some fixed varieties using this approach are already commercially available (Table 2). TEHV scaffolds require space for cell attachment, migration, and proliferation. However, current commercially available non-decellularized xenogenic heart valves have this ECM space occupied with fixed cells. Therefore we excluded profiling unseeded, fixed xenogenic heart valve approaches in this review. Dohmen et al. [50,51] investigated decellularized allograft implantations seeded patient ECs for reconstruction of the right ventricular outflow tract during the Ross operation and displayed favorable long-term results [52]. Their commercially available cryopreserved pulmonary allografts (CyrolifeInc, Kennesaw, GA) are created using a proprietary process in the Auto tissue Laboratories (Auto Tissue GmbH, Berlin, Germany). Cyrolife subsequently developed a decellularized CyroValve SG pulmonary human heart valve using Syner Graft technology and demonstrated clinically acceptable outcomes when compared with conventional pulmonary allografts [53]. Matrix P plus N is another decellularized porcine pulmonary heart valve that is already clinically available in Germany and developed by Auto Tissue GmbH. It has been tested for right ventricular outflow tract (RVOT) reconstruction or pulmonary valve replacement during Ross procedures. However, several recent studies have presented controversial results. In a study involving 93 pediatric patients undergoing RVOT reconstruction using Matrix

Table 2: Large animal and clinical trials of TEHV.

Trial	Material	Cells	Animal	Value	Year	Reference
Synthetic-based materials						
Shinoka et al.	Polyglactin, PGA	Myofibroblast, endothelial cell	Lamb	PV	1995	[38]
Sodian et al.	PHA	Vascularcell	Lamb	PV	2000	[40,41]
Hoerstrup et al.	PGA, P4HB	Myofibroblast, endothelial cell	Lamb	PV	2000	[43]
Stock et al.	PHO, PGA	Endothelial cell, vascular medial cell	Sheep	PV	2000	[42]
Weber et al.	PGA, P4HB	BM-MNC	Primate	PV	2011	[47]
Biological-based materials						
Dohmen et al.	Allograft	Autologous ECs	Human	PV	2002	[50-52]
Matrix P® and P Plus®	Xenograft (porcine)	-	Human	PV	2004	[54,55]
Brown et al.	Allograft	-	Human	PV	2010	[53]
(CryoValveSyneGraft®)				(Ross AVR)		
Ozaki et al.	Autologous pericardium	-	Human	Av	2014	[56,57]

PGA; Polyglycolic Acid; PHA: Poly-hydroxyalkanoate; PHO: Polyhydroxyoctanoate; P4HB: poly-4-hydroxybutyrate; BM-MNC: Bone Marrow-mononuclear Cell; PV: Pulmonary Valve; AVR: Aortic Valve Replacement; AV: Aortic Valve.

P/P plus valves, 35.5% reported conduit failure and 29% conduit dysfunction [54]. In contrast, a different study, where Matrix P valves were implanted in 61 patients with congenital heart disease, showed favorable mid-term performance [55].

Ozaki et al. [56] recently investigated a novel aortic valve reconstruction method using autologous pericardium and had favorable mid-term results. They treated pericardium with a 0.6% glutaraldehyde solution for 10minutes, implanted the pericardium, and the tissue manually reformed into leaflets [56,57]. The advantages of allogenic materials, such as CyroValve SG pulmonary human heart valve, are their smaller immunogenic reactions and the absence of potential disease transmission when compared to xenografts. Advantageously, autologous materials, like the method demonstrated by Ozaki, may be able to overcome the general supply shortage that allogenic materials typically present, however repeated surgery is not feasible.

Cardiac Regenerative Therapy

Ischemic heart disease mortalities have decreased with advances in medical and device therapies, but congestive heart failure secondary to ischemic heart disease has increased world-wide. Acute myocardial infarction (MI) occurs when a coronary vessel occludes and, results in ischemic myocardium. Infracted myocardial are as elicits an inflammatory response which produces collage nous and/or non-contractile scar tissue. Subsequently, the ventricle will progressively dilate in a phenomenon known as ventricular remodeling, which contribute to decreased ventricular contractile function, cardiac failure, and congestive heart failure. Thus, finding a viable therapeutic strategy to treat post-infarction remodeling and progressive heart failure remains a challenge for clinicians. Several cardiac regenerative therapeutic strategies have been suggested such as; Cell-based therapeutic, biomaterial, and endogenous factor approaches (Figure 3). Several biomaterial-based strategies focused on viable cells retention and reinforcing damaged myocardial areas have been evaluated pre-clinically. Additionally, a cellular material-based strategies and endogenous factor-stimulating bioactive molecules have not yet achieved clinical translation. Strategies and approaches that have not yet reached human trials will be disregarded for the purposes of this paper, instead this review will focus on the clinical status of cell-therapeutic and a cellular material-based approach investigations.

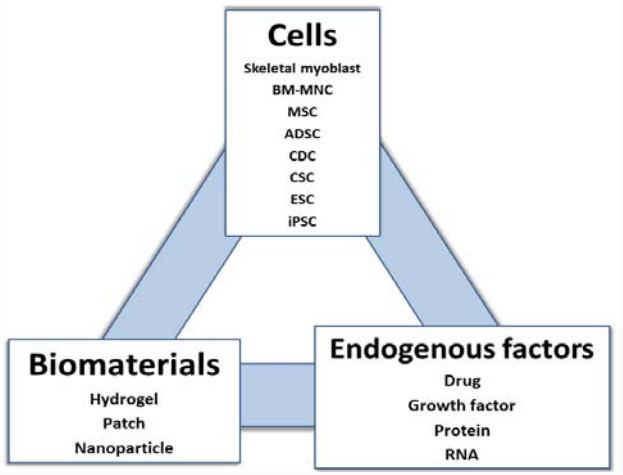

Figure 3: Three components of cardiac regenerative medicine. This schema represents three component of cardiac regenerative medicine; Cells, materials, and endogenous factors. Different combinations can be considered.

Cell-based therapy

Cell-based therapies have emerged as promising treatments to regenerate dead myocardium and improve LV function. Many studies have evaluated the functional efficacy of isolated progenitor cells in preclinical large animal models. Clinical trials have demonstrated the safety and feasibility of using bone marrow-derived stem cells [BM-MNCs or mesenchymal stem cells (MSCs)], adipose derived stem cells (ADSCs), or heart-derived stem cells [cardiac stem cells (CSCs) or cardio sphere-derived cells (CDCs)] in humans. However, long-term clinical results regarding the use of aforementioned cell populations to treat patients with MI, heart failure, and refractory angina, are varied and display minimal improvement in LV function [58-60]. Additionally, the benefits associated with adult stem cell use are attributed to paracrine factor secretion as opposed to direct de novo cardiac cell differentiation [61]. By promoting growth factor and cytokine secretion, stem cell therapies reduce the scar volume associated with MI and myocyte apoptosis, while simultaneously activating endogenous cardiac stem cells to increase myocyte

Table 3: Clinical trials of cell therapy for myocardium repair.

Trial	Cells	Delivery method	Evaluation	Follow-up period	Outcome	Year	Reference
MAGIC	Skeletal myoblast	epicardial	Echocardiography	6 months	LVEF→, EDV↓	1995	[66]
BOOST	BM-MNC	intracoronary	MRI	18 months	LVEF→	2006	[59]
TOPCARE-AMI	BM-MNC	intracoronary	Echocardiography	4 months	LVEF↑, ESV↓	2002	[72]
FINCELL	BM-MNC	intracoronary	Echo, Angio	6 months	LVEF↑	2008	[70]
HEBE	BM-MNC	intracoronary	MRI	4 months	scar size↓	2011	[74]
POSEIDON	MSC	transendocardial	CT	12 months	LVEF→, EDV↓	2012	[78]
C-CURE	MSC	transendocardial	Echocardiography	6, 24 months	LVEF↑, ESV↓	2013	[79]
APOLLO	ADSC	intracoronary	MRI, SPECT	6 months	scar size↓, Perfusion defect↓	2012	[80]
PRECISE	ADSC	transendocardial	Echo, MRI, SPECT	6, 12,18 months	LVEF→, scar size→	2014	[81]
ATHENA I/II	ADSC	transendocardial			LVEF→, scar size→	2016	[82]
CADUCEUS	CDC	intracoronary	MRI	6, 12 months	LVEF→, scar size↓	2014	[85,86]
ALCARDIA	CDC	epicardial	Echo,MRI	6 months	LVEF↑, scar size↓	2012	[87]
SCIPIO	CSC	intracoronary	Echocardiography	12 months	LVEF↑, scar size↓	2011	[88]

BM-MNC: Bone marrow-mononuclear cell; MSC: Mesenchymal Stem Cell; ADSC: Adipose Derived Stem Cell; CDC: Cardiosphere-derived Cells; CSC: Cardiac Stem Cell; MRI; Magnetic Resonance Imaging; CT: Computed Tomography; SPECT: Single Photo Emission Computed Tomography; LVEF: Left Ventricular Ejection Fraction; EDV: End Diastolic Volume; ESV: End Systolic Volume.

production and proliferation. Another factor to consider is that stem cell injection approaches have had difficulties with cell retention and achieving efficiently consistent outcomes [62]. Therefore, the utilization of hydrogel and/or patches is being examined to increase cell survivability and enable better localized cell delivery without mechanical washout.

Skeletal myoblasts: In the late 1990s, several animal studies revealed that skeletal myoblasts could differentiate into new myocardium and improved post-infracted cardiac function [63-65]. However, the MAGIC trial failed to improve the cardiac function and increased the risk for ventricular arrhythmia, thereby possibly suggesting that human skeletal myoblasts are unable to differentiate into cardiac myocytes and synchronize native myocardial electrical activity [66]. Therefore, these beneficial effects may be due to paracrine secretion. Currently in the United States, the MARVEL trial investigating catheter-delivered autologous skeletal myoblasts (MyoCELL) injection therapy is ongoing [67]. Transplantation of skeletal myoblast sheets has shown to improve cardiac function in patients with heart failure due to ischemic heart disease [68]. However, the skeletal myoblast sheets are not available worldwide.

Bone marrow derived mononuclear cells (BM-MNCs): BM-MNCs have been widely investigated in preliminary clinical studies. Though studies have demonstrated the feasibility of safely injecting BM-MNCs at the site of ischemic injury, the clinical benefits of this approach remains controversial. Several clinical trials such as BOOST [59,69], FINCELL [70], REPAIR-AMI [71], and TOPCARE-AMI [72,73], have shown that BM-MNC intracoronary injections improved left ventricular ejection fraction (LVEF) when compared to controls. However, the BOOST trial did not demonstrate any tangible long-term benefit [59], and other clinical investigations, such as the ASTAMI, BONAMI, Leuven-AMI, FOCUS-CCTRN, and HEBE trials [74], resulted in negative outcomes. Additionally, the TIME and Late TIME trials revealed that time of BM-MNC injection had no correlation to inducing a clinical benefit when treating acute MI [75,76]. Several ongoing clinical studies, such as the REVITALIZE (NCT00874354), REGEN-AMI (NCT00765453), and BARI (NCT01569178) trials, are still investigating the efficacy of BM-MNC injection approaches, however the overall negative outcomes of these trials have shifted research focus toward the utilization of other cell types.

Mesenchymal stem cells: Mesenchymal stem cells are derived from bone marrow aspirates or adipose tissue. The former have high engraftment and can induce endogenous cardiomyogenesis. The TAC-HFT trial compared the use of MSCs and BM-MNCs in patients with heart failure. The investigation revealed that both cell types were safe to implant and favorably trended toward reverse remodeling and regional contractility [77]. The POSEIDON trial compared autologous and allogenic MSCs transplantation in patients with ischemic cardiomyopathy. The investigation revealed that allogenic cells did not induce an unwanted immune response, and while each group did not increase LVEF, both displayed improved functional capacity [78]. The cardiopoietic stem cell therapy in heart failure (C-CURE) trial tested the ability of a cardiogenic cocktail to enhance the therapeutic benefits of autologous MSCs. The trial demonstrated both feasibility and safety, while producing a 7% increase in LVEF and positive effects on exercise tolerance [79]. Clinically, two companies have focused on utilizing ADSCs as a therapeutic approach. An ADSC known commercially as Adipocell (U.S. stem cell, inc. Sunrise, FL) recently completed its ANGEL phase 1 trial, whereas Cytori cell (Cytori Therapeutics, inc. San Diego, CA) is already available for sale in Europe. The APOLLO phase I/II a trial that administrated Cytori cell to patients with ST-elevation MI resulted in a positive trend towards improved cardiac function and perfusion [80]. The PRECISE trial evaluated Cytori cell use in patients with chronic myocardial ischemia and showed no ejection fraction (EF) improvement or reductions in scar size, but resulted in better patient symptoms and exercise tolerance [81]. The ATHENA I and II trials, investigating Cytori cell use in patients with ischemic heart failure demonstrated feasibility and safety, but resulted in no differences in LVEF or volume when compared with controls [82].

Cardiac stem cells: CSCs are clonogenic, multipotent, self-renewing, and heart specific stem cells with the ability to differentiate

into cardiomyocytes, ECs, and vascular SMCs. CSCs are typically isolated via antibody selection after homogenizing large pieces of cardiac tissue. However, this method is applicable only to patients that undergo cardiac surgery. A different and less-invasive isolation approach is to culture CSCs acquired by single biopsy, but the obtained volumes are significantly smaller. Alternatively, Smith et al. [83] cultured tissue from percutaneous myocardial biopsy specimens, formed cardio spheres, and subsequently expanded them in a monolayer to isolate what is termed cardio sphere-derived cells (CDCs). CDCs are a heterogeneous cell population that not only contain adult CSCs, but also vascular cells and differentiated progenitor cells [84]. The CADUCEUS phase 1 trial examined a CDC therapeutic approach in 25 myocardial infracted patients [85]. CDCs extracted from right ventricular endomyocardial biopsies and subsequently cultured for approximately 36 days, were injected into the infracted coronary arteries6-12 weeks after acute MI. MRI analysis of patients treated with CDCs showed reductions in scar mass and increases in viable mass. However, LVEF and cardiac volume did not improve by 1 year [86]. Similarly, the phase I/II ALLSTAR trial (NCT01458405) is an ongoing study examining the safety and efficacy of intracoronary CDCs injection in MI patients. The ALCADIA (NCT00981006) trial is pilot study investigating the safety and efficacy of utilizing autologous CDCs injection to treat ischemic cardiomyopathy [87]. To compensate for poor CDCs retention, a gelatin sheet containing bFGF was placed on epicardium during CABG in conjunction with a CDC transendocardial injection. Only 6 patients were enrolled, but cardiac MRI indicated a 12.1% EF increase and 3.3% infarct size reduction. The stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO) trial is the first-in-human, phase 1, randomized, open-label trial for autologous c-kit (+) CSCs in patients with ischemic heart failure undergoing CABG [88]. In the SCIPIO trial, c-kit (+) CSCs were isolated from a right atrial appendage biopsy and administered via intracoronary infusion. Investigators reported a significant increases in LVEF and decreases in infracted size of >30%. However, the cardiogenic potential of these cells is an area of significant controversy. A recent report has shown that c-kit (+) cells can only generate cardiomyocytes at functionally insignificant levels (<0.03%), and resulting new cardiomyocyte formation is unlikely to be caused by CSC injection [89]. Additionally, a separate study revealed that c-kit (+) cardiovascular precursor cells are able to generate cardiomyocytes in neonatal mice hearts, but not adult mice hearts [90].

The problem of cell-based therapies: Cell injection therapies have shown only partially favorable results. Generally, isolated cells are put in saline, and the suspensions are administered systemically via intravenous infusion, directly myocardium injection (epicardially or transendocardially), or perfusion into the coronary arteries (intracoronary) or coronary sinus. These methods enabled delivery of isolated cells to targeted areas of infracted myocardium. However, a key challenge facing cellular therapeutic approaches is being able to retain viable cells in targeted heart tissues and saline solutions have not proven to be adequate in this regard. To address this issue, several cell therapy techniques have been developed and investigated, such as injectable hydrogels and biomaterial patches.

Biomaterial-based strategies

Injectable hydrogels are utilized to encapsulate, deliver and retain cells to ischemic target areas for long term recovery. Hydrogels used in cellular therapeutic approaches must have mechanical properties to support the ventricular wall and also degrade without producing toxic byproducts. Hydrogels are typically injected via three routes; intracoronary, epicardially, or transendocardially. In vitro, some hydrogels have demonstrated increased cell retention [91-93]. More recently, *in vitro* and *in vivo* studies have focused on the application of bioactive drug-releasing hydrogels. Bioactive molecules enclosed in these hydrogels include prostaglandins [94], RNA [95], growth factors [96], or bone morphogenetic protein-2 [97]. However, despite promising data designing injectable cell-loaded hydrogels, there are only two clinical studies that have investigated unseeded hydrogels as a viable myocardial therapeutic approach. The first successful clinical trial was performed with an intracoronary alginiate hydrogel [98]. The hydrogel was administered alone and preserved LV function. It is hypothesized that this temporary scaffold replaces the damaged extracellular matrix, and thereby reduces wall thinning and strain. Another clinical trial consisted of 6 patients who were implanted with Algisly-LVR epicardially through concomitant CABG or valve surgery, and this strategy improved LV size and function. By applying Laplace's law to a failing dilated ventricle, this hydrogels injection could theoretically increase wall thickness and reduce the chamber diameter, resulting in decreased wall stress and improved LV function. To date, however, there have been no clinical trials investigating a combined cell therapy and hydrogel injection approach. Another cardiac repair strategy is the use of a biomaterial patch that can be applied epicardially onto a damaged heart. Biomaterial patchescan supply therapeutic effects by releasing bioactive molecules, delivering cells, providing mechanical support, and reducing dilatation. It is well known the Dor procedure, which uses a Dacron patch to minimize ventricle wall scarring, restores ventricular shape, increases LVEF, and decreases LV volume. Biomaterial patches can also provide the additional beneficial effects of cell therapy and degradable materials to the Dor procedure. Biomaterial patches must be able to provide stability, flexibility, and mechanical strength. In fact, several electrospun biomaterials, such as PLGA, PCL, and PGS, have already demonstrated beneficial results in vitro studies [99-101]. But there have been no clinical trials utilizing biomaterial patches with cells to date. To apply these cardiac regenerative therapies in a clinical setting, more time and energy dedicated to studying *in vitro and in vivo* research and surgical procedures is needed. One important key is that administered cells promote cardiomyocyte proliferation, improve cardiac function, and decreases long-term mortality.

Conclusion

This review focused on the clinical status of tissue engineering and regenerative medicine in cardiovascular disease. Currently, the development of vascular grafts and heart valves is trending towards biomaterial utilization instead of cell-based approaches. Contrastingly, investigations into cardiac repair therapies are primarily focused on cellular approaches. However, it is not essential that these trends continue, as new scientific discoveries and technological advances may warrant paradigm shifts in translational research. In order to reach clinical translation, therapeutic strategies must factor approaches that are less-invasive, cost effective, time-saving without breaching ethical concerns. Successful translation of complex multidisciplinary technologies clinically requires an active, multidisciplinary, and collaborative participation of clinicians, engineers, chemists, and biologists. Within this collaboration, promising future studies will further optimize tissue engineering technologies that will advance regenerative medicine and patient care.

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