Regenerative Therapy for Patients with Congenital Heart Disease

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Congenital heart disease (CHD) is the most common birth defect, affecting 1 in 100 babies. Among CHDs, single ventricle (SV) physiologies, such as hypoplastic left heart syndrome and tricuspid atresia, are particularly severe conditions that require multiple palliative surgeries, including the Fontan procedure. Although the management strategies for SV patients have markedly improved, the prevalence of ventricular dysfunction continues to increase over time, especially after the Fontan procedure. At present, the final treatment for SV patients who develop heart failure is heart transplantation; however, transplantation is difficult to achieve because of severe donor shortages. Recently, various regenerative therapies for heart failure have been developed that increase cardiomyocytes and restore cardiac function, with promising results in adults. The clinical application of various forms of regenerative medicine for CHD patients with heart failure is highly anticipated, and the latest research in this field is reviewed here. In addition, regenerative therapy is important for children with CHD because of their natural growth. The ideal pediatric cardiovascular device would have the potential to adapt to a child’s growth. Therefore, if a device that increases in size in accordance with the patient’s growth could be developed using regenerative medicine, it would be highly beneficial. This review provides an overview of the available regenerative technologies for CHD patients.

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Introduction

Heart failure is one of the most common causes of death in heart disease patients worldwide. Many studies have focused on pharmacological therapy, non-pharmacological therapy, the use of ventricular assist devices, and heart transplantation for ameliorating heart failure and treating advanced heart failure. However, it is important to recognize that ameliorating heart failure does not mean the recovery of viable functional cardiomyocytes. From this viewpoint, much attention has been given to regenerative therapy. Recently, stem cell biology has made remarkable progress, and many basic studies have been conducted on stem cells such as mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs). Moreover, several clinical studies have been performed in the adult cardiac field which have shown promising results, despite the many obstacles that remain to be overcome.1–3 Heart failure is an important condition not only in adults but also in children with congenital heart disease (CHD). However, the advancements in regenerative therapy for adults have yet to be applied to children with CHD. As in the case of adult patients, treatments for end-stage heart failure in children for whom medical therapies have not been effective involve device therapies and, ultimately, heart transplantation. With respect to device therapies, the technology of ventricular assist devices has steadily advanced, and various improvements and miniaturization of devices have already been implemented. However, devices that can be used in small children remain limited: only the Berlin Heart EXCOR device is available at present. For heart transplantation, the donor shortage is a more seri-
CHDs are diagnosed in approximately 1 in 100 births and are the most frequent pathological congenital condition. The anatomical and pathological conditions of patients may vary greatly. Among CHDs, single-ventricle (SV) physiologies, such as hypoplastic left heart syndrome (HLHS) and tricuspid atresia, were previously considered to be severe and often lethal conditions (Fig. 1). In 1984, it was reported that only 30% of SV patients not undergoing surgical treatment were alive by the age of 16 years. To survive and grow, a baby born with SV usually requires three stages of palliations (i.e., systemic to pulmonary shunt/pulmonary artery banding, the Glenn procedure, and the Fontan procedure). As the management strategies for these kinds of patients have improved gradually and significantly, many SV patients survive using the Fontan procedure. However, the prevalence of ventricular dysfunction continues to increase over time after the Fontan procedure, and ventricular dysfunction has been reported as an independent risk factor for an adverse outcome after the Fontan procedure. A patient with SV, particularly the right-ventricle dominant type, may suffer from ventricular dysfunction at any time because of volume overload, atrioventricular valve regurgitation, or arrhythmia, among others. At present, the final treatment for SV patients who develop heart failure is heart transplantation. The revised organ transplant law enabling pediatric heart transplantation in Japan was implemented in July 2010; however, only 17 pediatric patients had undergone heart transplantation by the end of June 2016 after the law came into effect.

For problems such as those mentioned above, the clinical application of various forms of regenerative medicine, such as stem cell therapy and patch therapy, for CHD patients (particularly SV patients) with heart failure is highly anticipated and desired as the ultimate therapy or “bridge to transplantation” therapy. Although it is thought that cardiomyocytes in normal hearts hardly regenerate, basic research using mice has shown that cardiomyocytes have regenerative ability, particularly during the neonatal period immediately after birth, but not in adulthood. Therefore, regenerative therapy in children, particularly in newborns, could be more effective than regenerative therapy in adults.

Importantly, growth is something that cannot be ignored in pediatric patients. Children with CHDs who undergo blood vessel replacement or heart valve replacement often require an increase in the size of the implanted devices as they grow (Fig. 2). Unfortunately, they have to suffer from repeated interventions, including multiple redo surgeries, that are a major burden and a cause of pain. If, thanks to progress in regenerative medicine, a device that increases in size in accordance with a patient’s growth could be developed, such a device would be of great value to children with CHDs.

In this review, I focus on the advancements and latest findings in the treatment of CHDs from stem cell therapy to cardiovascular regenerative therapy, particularly in the field of pediatric cardiac surgery (Fig. 3). I also discuss the future direction of regenerative medicine for children with CHDs.

Regenerative Therapy for Heart Failure

Various cell types have been used in cell therapy, mainly for ischemic heart disease (Table 1). MSCs are believed to reside in the perivascular regions of all tissues, e.g., adipose tissue, bone marrow, skeletal muscle, and myocardium. MSCs have proangiogenic ability, are able to secrete numerous angiogenic factors, and can promote myocardial angiogenesis to improve ventricular function. Moreover, MSCs differentiate into cardiomyocytes after exposure to 5-azacytidine in vitro. Cardiac progenitor cells (CPCs) are believed to reside in the myocardium and have the potential to differentiate into various types of cells, such as endothelial cells, smooth muscle cells, and cardiomyocytes. Several basic studies have reported the validity of CPC injection for inducing angiogenesis and stimulating endogenous CPCs to promote regeneration. Endothelial progenitor cells (EPCs) are found in the peripheral circulation and are believed to be derived from the bone marrow. EPCs differentiate into endothelial cells to maintain endothelial homeostasis and promote angiogenesis. Embryonic stem cells (ESCs) have the potential to differentiate into the derivatives of all three germ layers and can grow and divide indefinitely. Although these cells spontaneously differentiate into cardiomyocytes at a certain rate under appropriate conditions, various improvements have been made to efficiently differentiate them into cardiomyocytes with high purity for cell therapy. Thus far, animal studies of transplantation with cardiomyocytes derived from ESCs have shown promising results, although some
inevitable obstacles such as electrophysiological issues and immune rejection remain. In 2006, iPSCs were generated from adult human fibroblasts reprogrammed using Yamanaka factors. These cells have the same characteristics as ESCs, including gene expression and the capacity to differentiate into the derivatives of all three germ layers. Patient-specific iPSCs could provide a solution to immune rejection and could be transplanted without causing rejection. Recent studies have shown that direct lineage reprogramming can develop several cell types, including cardiomyocytes, and further research is expected to make progress in this field. The delivery system for cell transplantation is important in that it has to be safe, effective, and efficient. Several approaches have been reported, namely, direct or percutaneous intramyocardial injection, percutaneous transvenous injection, and percutaneous transarterial injection. Each approach has its own advantages and disadvantages (Table 2), and the best method remains controversial. Another method of cardiomyocyte transplantation is the use of tissue-engineered cardiac patches (Table 2). Okano’s group first reported cardiac patches consisting of several sheets made from neonatal cardiomyocytes. They layered the cardiac sheets three-dimensionally, which led to the generation of a pulse, and demonstrated multiple vascularization in the patch after implantation into nude rats. Sawa’s group performed scaffold-free autologous skeletal-muscle-derived cell sheet implantation for ischemic cardiomyopathy in a multicenter study that demonstrated the feasibility and safety of the approach. Treatment with autologous skeletal muscle-derived cell sheets is presently covered by health insurance in Japan, and clinical application has commenced. Scaffold-free, viable, and contractile three-dimensional cardiac tissues have also been created using new technology based on the self-assembling nature of cells, and the clinical application of this technology for heart failure may be feasible in the future.

Several case reports have been published regarding the potential benefit of stem cell therapy in the pediatric population. Oh’s group reported the world’s first clinical trial of cell therapy for children with CHD using transcoronary infusion of cardiac progenitor cells (TICAP) in patients with SV physiology. This phase I trial was started in 2011 and aimed to assess the feasibility, safety, and efficacy of intracoronary delivery of autologous cardiosphere-derived cells (CDCs) in patients with HLHS. CDCs appear to have the greatest therapeutic potential to alleviate ventricular remodeling through differentiation and paracrine factor secretion in situ. The subjects were 14 children with HLHS, of which 7 underwent intracoronary infusion of CDCs 1 month after their second-stage or third-stage palliation, and the remaining 7 underwent standard care only as the control group. The outcomes were promising and there were no complications or tumor formation during the 3-year follow-up. Clinical improvement of the right ventricular ejection fraction and a decrease in brain natriuretic peptide levels have been reported. Based on these results, a phase II trial (PERSEUS, cardiac progenitor cell infusion to treat univentricular heart disease) was conducted, and the results were published recently. This trial was a randomized-controlled study, and a total of 34

Fig. 2 Artificial devices that are widely used in cardiac surgery. (A) Gore-tex patch, (B) various sizes of synthetic vascular grafts made of Gore-tex, and (C) prosthetic valves.
patients with SV physiology were enrolled. The efficacy of the intracoronary delivery of autologous CDCs after second-stage or third-stage palliation was evaluated. The primary outcome measure was to assess improvement in cardiac function at 3-month follow-up. The results of this trial were promising. The intracoronary delivery of autologous CDCs favorably affected cardiac function (including reduced ventricular volumes and fibrosis), heart failure status, somatic growth, and health-related quality of life from baseline to 3-month and 12-month analyses. Based on the results of these clinical cell therapy trials, an industry-sponsored, multicenter, randomized phase III trial (APOLLON, cardiac stem/progenitor cell infusion in univentricular physiology) is ongoing at three children's hospitals to confirm the risks/benefits of the intracoronary delivery of autologous CDCs favorably affected cardiac function (including reduced ventricular volumes and fibrosis), heart failure status, somatic growth, and health-related quality of life from baseline to 3-month and 12-month analyses. Based on the results of these clinical cell therapy trials, an industry-sponsored, multicenter, randomized phase III trial (APOLLON, cardiac stem/progenitor cell infusion in univentricular physiology) is ongoing at three children's hospitals to confirm the risks/benefits of the intracoronary delivery of autologous CDCs. Hopefully, these novel regenerative strategies in the field of CHD will become clinically applicable in the near future.

**Cardiovascular Patch**

Cardiovascular patch is one of the most utilized devices in congenital cardiac surgeries. The ideal cardiovascular patch is non-thrombogenic, has good compliance and strength, and has the potential to grow. The patches used for congenital cardiac surgeries are made of various materials, such as Dacron (polyethylene terephthalate), Gore-tex (expanded-polytetrafluoroethylene) (Fig. 2A), autologous pericardium, or bovine pericardium; however, none of these patches has the capacity to grow. CorMatrix (CorMatrix Cardiovascular, Inc., Atlanta, GA, USA) is decellularized extracellular matrix (ECM) made from porcine small intestine submucosa (SIS) and is commercially available in the USA for repairing cardiac defects, although it is not yet approved in Japan. Small intestine submucosa–extracellular matrix (SIS-ECM) is thought to act as a bioscaffold in the repaired lesion and allows the ingrowth of site-specific native cells (Fig. 4). SIS-ECM may be transformed into native tissue, i.e., regenerated native tissue.

In animal experiments, it has been reported that the application of SIS-ECM resulted in remodeling of the heart and regeneration into myocardial tissue; however, disappointing results have also been observed in retrospective human studies in recent years. SIS-ECM was explanted from CHD patients who underwent surgical repair using SIS-ECM to repair cardiac defects. Histological studies showed no regeneration of native tissue in the patch. A prospective study also showed similar results of no ingrowth of native cells or native tissue regeneration in ten cases. However, other studies employing histological analyses showed that SIS-ECM used for pericardial reconstruction was remodeled to connective tissue similar to native pericardial tissue. Moreover, SIS-ECM enhanced with the controlled release of fibroblast growth factor facilitated host cell repopulation and regeneration.

<table>
<thead>
<tr>
<th>Regenerative therapy</th>
<th>Application</th>
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</table>
| 1. Stem cell therapy | Regenerate myocardium  
                          Heart failure  
                          Single ventricle physiology  
                          Congenital cardiac surgery |
| 2. Tissue-engineered cardiovascular patch | Augment vessels or stenotic site  
                                             Close septal defect  
                                             Congenital cardiac surgery |
| 3. Tissue-engineered blood vessels | Replace vessels  
                                         Create Fontan pathway  
                                         Congenital cardiac surgery |
| 4. Tissue-engineered heart valves | Replace heart valves  
                                          Congenital cardiac surgery |
| 5. Tissue-engineered heart (whole heart) | Heart transplantation  
                                           Congenital cardiac surgery |

**Fig. 3 Strategies of regenerative therapy for congenital heart disease.**
of cardiomyocytes. These are promising results, particularly in this field, and further research and improvement of SIS-ECM techniques is expected in the future.

Another approach for producing a bio-scaffold is to apply tenside (sodium dodecyl sulfate) and DNase treatment to heart tissue, remove all potential immunogenic factors from the heart, and use the remaining ECM as a cardiac patch. Ott et al. reported promising first short-term data from a heterotopic heart transplantation model using this approach.42–44

One unique approach for a regenerative cardiovascular patch is the use of “Biotube.” Biotube was developed based on the concept of tissue encapsulation of foreign materials in living bodies. The first clinical application of Biotube as a patch for a CHD patient was recently reported, with promising midterm results.45

Table 1. Advantages and disadvantages of cell sources for regenerative therapy for cardiac disease

<table>
<thead>
<tr>
<th>Origin</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchymal Stem Cells</td>
<td>Pluripotency, Easy to grow, Suppress immune rejection, No malignant transformation</td>
<td>Unknown detailed mechanism</td>
</tr>
<tr>
<td>Resident Cardiac Stem Cells (Cardiac Progenitor Cells, Cardiosphere-Derived Cells)</td>
<td>No immune rejection, No malignant transformation</td>
<td>Unknown origin (hematopoietic origin?)</td>
</tr>
<tr>
<td>Endothelial Progenitor Cells</td>
<td>No immune rejection, No malignant transformation</td>
<td>Only differentiate into endothelial cells</td>
</tr>
<tr>
<td>Human Embryonic Stem Cells</td>
<td>Pluripotency, Grow and divide indefinitely</td>
<td>Ethical issues, Immune rejection, Malignant transformation</td>
</tr>
<tr>
<td>Induced Pluripotent Stem Cells (iPS cells)</td>
<td>Pluripotency, Grow and divide indefinitely</td>
<td>Malignant transformation, Labor-intensive and time-consuming</td>
</tr>
<tr>
<td>Induced Cardiomyocytes</td>
<td>No immune rejection, No malignant transformation</td>
<td>Non-pluripotency, Labor-intensive and time-consuming (less then iPS cells)</td>
</tr>
</tbody>
</table>

Table 2. Advantages and disadvantages of delivery systems for regenerative therapy for cardiac disease

<table>
<thead>
<tr>
<th>Approach</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Intramyocardial injection (Open-chest, epicardial)</td>
<td>Low risk of hematogenous dissemination, Direct visualization of the targeted area</td>
<td>Highly invasive approach</td>
</tr>
<tr>
<td>Intramyocardial injection (Percutaneous, epicardial)</td>
<td>Low risk of hematogenous dissemination, Less invasive approach</td>
<td>Need new technology for injection</td>
</tr>
<tr>
<td>Intramyocardial injection (Percutaneous, endocardial)</td>
<td>Less invasive approach</td>
<td>Moderate risk of hematogenous dissemination, Difficult visualization of the targeted area</td>
</tr>
<tr>
<td>Percutaneous transvenous injection (via peripheral vein)</td>
<td>Less invasive approach</td>
<td>High risk of hematogenous dissemination, Low efficiency</td>
</tr>
<tr>
<td>Percutaneous transvenous injection (via coronary sinus)</td>
<td>Less invasive approach</td>
<td>High risk of hematogenous dissemination, Low efficiency</td>
</tr>
<tr>
<td>Percutaneous transarterial injection (via coronary artery)</td>
<td>Less invasive approach</td>
<td>Moderate risk of hematogenous dissemination, Low efficiency if the targeted coronary artery is occluded</td>
</tr>
<tr>
<td>Cell sheet technology</td>
<td>No risk of hematogenous dissemination, Three-dimensional cardiac tissue</td>
<td>Highly invasive approach</td>
</tr>
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Tissue-engineered Blood Vessels

Synthetic vascular grafts are commonly used in pediatric cardiac surgeries such as the Fontan operation for SV patients and the Rastelli operation. In many cases, surgeons use synthetic vascular grafts made of Gore-tex or Dacron (Fig. 2B). However, if new vascular grafts that are resistant to thrombus and infection and have potential for growth could be developed from native tissue, these grafts would be of great benefit. Such small vascular grafts placed during childhood would grow larger with the growth of the body and would not need to be replaced.46,47
Fig. 4 Putative mechanism of small intestine submucosa-extracellular matrix (SIS-ECM) repair of heart tissue. After patching the heart defect with SIS-ECM (A), site-specific host cells (including cardiac stem cells) ingrow into the SIS-ECM (B) and regenerate native tissue (C).
Tissue-engineered vascular grafts (TEVGs) have been reported since the 1980s.\textsuperscript{48} Shinoka’s group published excellent results of their human clinical trial of TEVG implantation in 2001, the first such trial in the world.\textsuperscript{49} They explanted the patient’s peripheral vein, from which they isolated mononuclear cells. These cells were cultured and expanded, and then cultured with a biodegradable polymer conduit composed of a polycaprolactone-polyactic acid copolymer reinforced with woven polyglycolic acid. The tissue-engineered graft was transplanted to a 4-year-old girl with right pulmonary artery occlusion. The artery was successfully replaced with no evidence of graft occlusion 7 months later. They implanted a total of 25 tissue-engineered grafts as extracardiac cavopulmonary conduits in patients with SV physiology. The long-term results were suboptimal because of the stenosis of four grafts that required intervention.\textsuperscript{50,51}

Instead of using a biodegradable polymer conduit as a scaffold, a tissue-engineered vascular graft made of decellularized conduit was clinically implanted in a child with portal vein obstruction.\textsuperscript{52} A 9-cm segment of an allogeneic donor iliac vein was decellularized and then recellularized with endothelial and smooth muscle cells differentiated from bone marrow-derived MSCs of the recipient. Although this graft had to be extended by a second TEVG at 1 year because of stenosis caused by mechanical obstruction, the patient benefitted from improved exercise tolerance, improved mental function, and growth without receiving immunosuppressive drugs. It was not reported whether this TEVG had grown; however, a major advantage of this procedure is that the immunogenicity of the graft appeared to be very low, despite donor tissue being used.

Interesting options for creating tubular tissue as a vascular graft without using scaffolds have also been reported. One option is to use cardiomyocyte sheets to create pulsatile myocardial tubes.\textsuperscript{53} Neonatal rat cardiomyocyte sheets were harvested from temperature-responsive culture dishes and wrapped around a resected rat thoracic aorta to create functional myocardial tubes. Four weeks after implantation in place of rat abdominal aortas, the tubes demonstrated spontaneous and synchronous pulsations. Another option is to create scaffold-free, small-caliber tubular tissues made of multicellular spheroids consisting of endothelial cells, smooth muscle cells, and fibroblasts using a bio-3D printer.\textsuperscript{54} These tissues were implanted into the abdominal aortas of nude rats. Explanted tissues showed enlargement of the lumen area and thinning of the wall, and a layer of endothelial cells was confirmed. These tubes may have the potential to remodel and grow in the body. New approaches to make TEVGs using bone marrow-derived MSCs and iPSCs are being developed.\textsuperscript{30,55} In addition, the production of small-diameter TEVGs (<6 mm) using a fast-degrading material as scaffold\textsuperscript{56} and the development of patient-specific TEVGs using new 3D printing technology are evolving.\textsuperscript{57} The results are promising, and further investigation is encouraged for future clinical application.\textsuperscript{58}

**Heart Valves**

Children with CHD who need valve surgeries, such as mitral or tricuspid valve repairs, sometimes require repeated interventions including multiple redo surgeries as they grow because of the unavailability of suitable devices that accommodate growth (Fig. 2C). A novel growth-accommodating device could improve the durability of pediatric heart valve repairs while also accommodating children's growth, leading to a decrease in the number of repeated interventions. Many research results on regeneratated heart valve using a tissue engineering approach have been reported, although only a few are in the pediatric field.

SIS-ECM has been successfully used as a patch material, and some studies have reported its usefulness as a raw material for a novel growing valve. One such valve is a tubular valve (TV) made of SIS-ECM that has shown early physiological remodeling in animal experiments. Zafar et al. assessed the remodeling potential of TV by evaluating its growth, structure, and function in a growing ovine model.\textsuperscript{59} The tricuspid valves of young sheep were replaced with TVs. At the 3-month and 8-month follow-ups, an incremental increase in the annular diameter similar to that of native valve was found. TV function was maintained in seven cases, but was impaired in one case. Explanted TV resembled a native valve. Histopathological examination demonstrated migration of resident mesenchymal cells into TVs and trilaminar extracellular matrix organization without inflammation or calcification. These results demonstrated that TVs sufficiently grew along with the growth of the body. The same model was also observed over a longer period of 18 months, and the same results were obtained.\textsuperscript{60} Some young adults have already received TVs on a compassionate use basis, with promising early outcomes. Clinical trials of TV implantation in children and adults approved by U.S. Food and Drug Administration are ongoing, as are ovine studies examining TV implants in the mitral position.

Recently, a very promising result was reported by a Harvard group that developed a new growth-accommodating device for pediatric application.\textsuperscript{51} This device consists of two components: a degrading, biopolymer core and a braided, tubular sleeve that elongates over time in response to the tensile forces exerted by the surrounding growing tissue. As the inner biopolymer degrades, the tubular sleeve becomes thinner and elongates in response to native tissue growth. An annuloplasty ring made of this device was developed for heart valve surgery, and animal experiments using pigs were conducted to implant the ring to the tricuspid valve annulus. The growth of the tricuspid valve without impairing the valve function was
confirmed, and further improvements and applications are expected in the near future.

Conclusion

In recent years, regenerative therapy has received great attention and shown promising results worldwide. Many studies on regenerative therapy for cardiovascular diseases are currently ongoing. Good outcomes of clinical trials for stem cell transplantation to adult patients with severe heart failure have been reported, and perspectives on the safety and effectiveness of regenerative therapies are being established. In contrast, regenerative therapies for pediatric patients with CHD, including SV physiology, have only just started. Many future tasks involving specificity, complexity, and ethical aspects of the treatment remain. However, the biological potential of stem cells may be greater in neonates and infants than in adults, and therefore the efficacy of regenerative therapies may be higher in this population. The development of new growing patches, valves, and vascular grafts would have tremendous benefits (e.g., reduction in the need for reoperation) for children with CHD. Further advancement of this field is greatly anticipated.

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References


