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Tissue engineering: Relevance to neonatal congenital heart disease

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ABSTRACT

Congenital heart disease (CHD) represents a large clinical burden, representing the most common cause of birth defect-related death in the newborn. The mainstay of treatment for CHD remains palliative surgery using prosthetic vascular grafts and valves. These devices have limited effectiveness in pediatric patients due to thrombosis, infection, limited endothelialization, and a lack of growth potential. Tissue engineering has shown promise in providing new solutions for pediatric CHD patients through the development of tissue engineered vascular grafts, heart patches, and heart valves. In this review, we examine the current surgical treatments for congenital heart disease and the research being conducted to create tissue engineered products for these patients. While much research remains to be done before tissue engineering becomes a mainstay of clinical treatment for CHD patients, developments have been progressing rapidly towards translation of tissue engineering devices to the clinic.

1. Current status of congenital heart defects and impact on healthcare

The field of tissue engineering represents a rapidly progressing discipline which provides new tools for surgeons and clinicians to manage complex clinical diseases. A hallmark of tissue engineering, the ability of new autologous tissues that can integrate into the host, has provided unique solutions to treating neonatal congenital heart disease (CHD). While treatment has improved over-time, neonatal CHD still remains a pressing clinical concern with 1–2% of newborns suffering from CHD and with it being the primary cause of birth defect-related deaths in newborns [1–3]. Surgical based interventions remain a pillar of care with ~25% of CHD patients requiring surgery in the first year of life [4,5]. Prenatal diagnosis of CHD newborns that will require surgery continues to improve as well. From 2006 to 2012, the prenatal diagnosis rate of CHD patients that would require surgery within the first 6 months of life increased from 26% to 42% representing improved surgical planning capabilities [6]. With the improved surgical intervention and the survival of neonates/infants with CHD into adult hood reaching 90%, the rate of reoperation continues to rise as well [7–9]. Reoperation in CHD patients brings additional challenges such as increased surgical complications from pericardial adhesions and increased associated mortality [10–14]. These downstream associated risks necessitate careful surgical planning and management of CHD early in a neonate's life. In this capacity, tissue engineering provides novel approaches to CHD surgical interventions addressing the limitations of current implantable materials such as restricted growth, thrombosis, infection, and limited endothelialization [15–18]. Notably, tissue engineering allows for the replacement of synthetic and grafted materials with biodegradable scaffolds that can grow and remodel in the neonate [19–22]. In this review, we address the use of cardiovascular tissue engineering to create vascular grafts, heart patches, and heart valves for use in surgical management of neonatal CHD.

2. Fetal cardiac embryology stages overview and description

The fetal circulatory system is one of the earliest organ systems to form beginning in week 3 of fetal development. Formation of this system is marked by the initial movement of the mesodermal cells that will act as pre-cursor cells to form the heart. The early heart forms as a straight tube with an ordered structure for the atria, ventricles, sinus venosus, and common outflow tract. In addition to this tube, a second heart field

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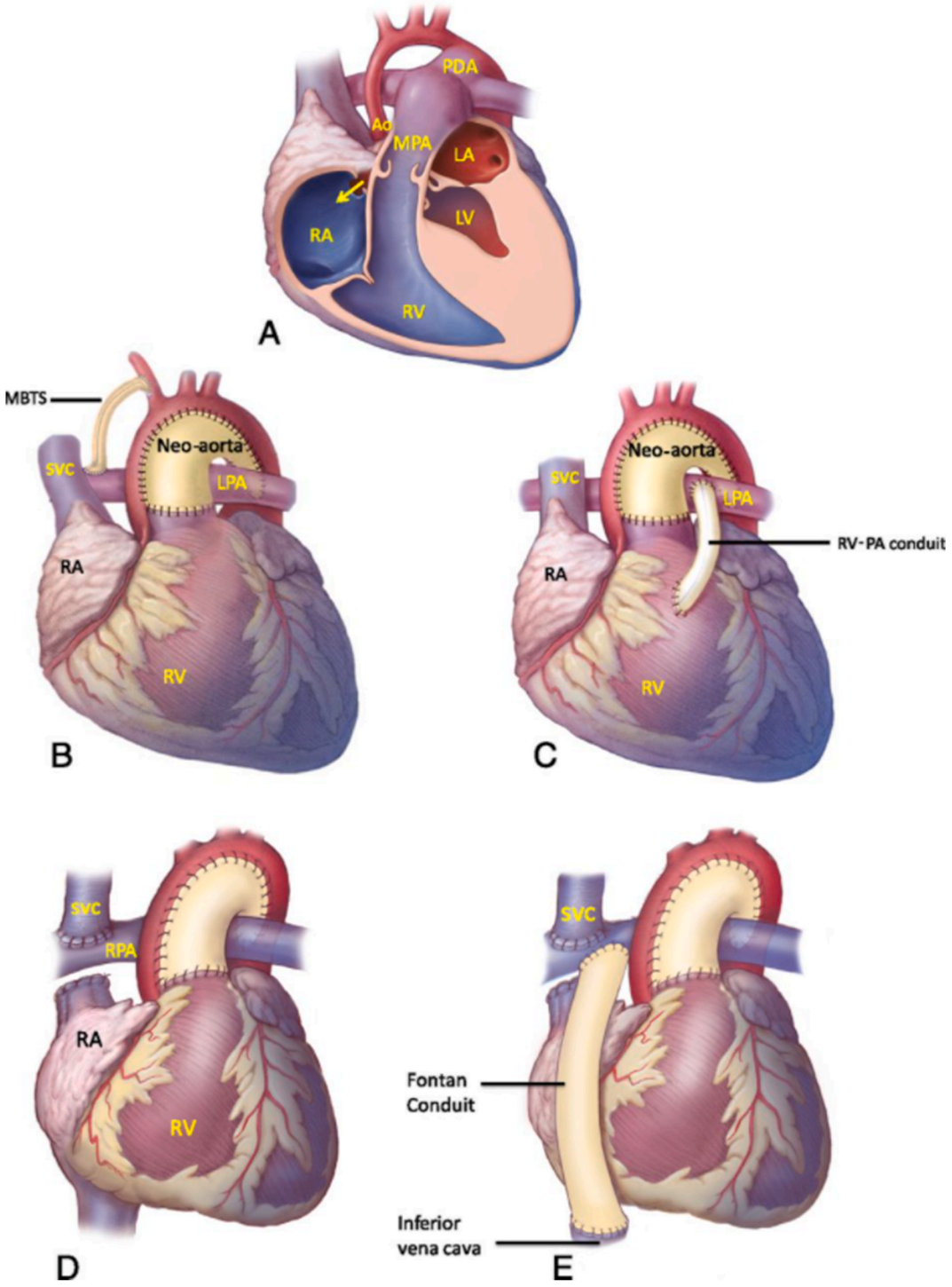

Fig. 1. The anatomy of hypoplastic left heart syndrome with underdeveloped left ventricle (LV). (A) The arrow indicates the atrial septum defect. **(B)** Stage 1 palliation using a modified Blalock-Taussig shunt (MBTS). **(C)** Stage 1 palliation using a right ventricle (RV)–to–pulmonary artery (PA) conduit (Sano modification). **(D)** Stage 2 palliation using the bidirectional Glenn shunt. It is a direct anastomosis of the superior vena cava (SVC) to the right PA (RPA). **(E)** Stage 3 palliation. The extra-cardiac Fontan uses a tube graft to connect the inferior vena cava to the central PA. Ao = aorta, LA = left atrium, LPA = left pulmonary artery, MPA = main pulmonary artery, PDA = patent ductus arteriosus, RA = right atrium. Reproduced from Ref. [144].

forms that is marked by its contribution to proper folding and the predominant cellular source for the right ventricle and the outflow tract. Over the course of several weeks, this tube undergoes a well-defined series of folds and changes to create the 4 separate chambers [23]. The precise embryonic origins of the various CHD malformations that can arise from errors in these early steps is an evolving, complicated topic well covered in other reviews [24,25]. Ultimately, many CHD malformations likely arise from a combination of genetic and environmental mechanisms dynamically interacting that results in a malformed heart [26].

3. Surgical management of congenital heart malformations

Surgical management of various CHD malformations can be

categorized as either palliative or curative with the need for new tissue dependent upon the specific surgical approach. The most prevalent, serious congenital heart malformations that necessitate surgery include hypoplastic left heart syndrome (HLHS), tetralogy of Fallot (TOF), tricuspid valve atresia, and transposition of the great arteries. HLHS is a sequela of left-sided defects including aortic valve and mitral valve stenosis or atresia, an underdeveloped or non-existent left ventricle, and underdevelopment of the aortic arch/aorta (Fig. 1) [27]. The failed development of the left side of the heart necessitates dependence on the right side to supply circulation through the ductus arteriosus [28]. Without surgical intervention, prognosis is almost inevitability fatal [29,30]. The inheritance and genetic abnormalities that predispose fetuses to developing HLHS remains complicated and not fully understood. In mice, lack of Tbx5, as well as mutations in Sap130 & Pcdha9, has been linked to development of HLHS. In humans, mutations in GJA1 gene and NOTCH1 have been linked to HLHS [31,32]. HLHS has additionally been linked to a pattern of familial inheritance as well as associated with Turner syndrome and other additional genetic defects [33,34]. The standard surgical management of HLHS is a three-stage palliative surgical approach beginning with the Norwood procedure and ending in a Fontan procedure allowing blood to bypass the ventricles and travel directly to the lungs (Fig. 1) [35]. The Fontan procedure can be further separated into either a lateral tunnel Fontan or an extra-cardiac Fontan approach with both ultimately connecting the inferior vena cava (IVC) to the pulmonary artery [36]. The extra-cardiac Fontan relies on non-autologous materials for creating the conduit that utilize either synthetic expanded polytetrafluoroethylene (ePTFE or Gore-Tex) or donor tissue such as aortic homograft [36,37]. The use of synthetic materials poses additional challenges to short-term and long-term surgical success. In the first-stage palliative care for HLHS, aortic arch reconstruction with synthetic or donor tissue suffers from rapid outgrowing by the patient [38].

In addition to HLHS, the Fontan procedure is utilized as palliative management of tricuspid atresia with initial treatment for these patients focusing on management until a Fontan can be performed [39–41]. Tricuspid atresia represents 1% of all congenital heart defects and is marked by failure of the tricuspid valve to form, presenting as a single ventricle anomaly. If left untreated, the defect is fatal in infancy [40,41]. There is limited understanding of the potential underlying genetic defects behind this disease, with one study reporting a connection with mutations in NFTAC1 and a few reports linking it with a familial inheritance pattern [42–44]. TOF is another serious CHD presentation that ultimately requires either palliative or curative surgical treatment [45]. It is characterized by a combination of 4 defects: ventricle septal defect, pulmonary stenosis, right ventricle hypertrophy, and an overriding aorta [46]. Without surgical intervention, prognosis for long-term survival is limited, with less than 50% survival by age 3 [46]. Clinical presentation include cyanosis with hypercyanotic spells and presence of a systolic murmur [45,47]. From a molecular genetic standpoint, TOF has been linked with deletion of chromosome 22q11.2, NKX2-5 gene mutation, and JAGGED1 gene mutation among several others [48–50]. Additionally, there are studies demonstrating a familial inheritance pattern in TOF [51–54]. It is further associated with Trisomy 21, 18, and 13 as well as 1p36 deletion syndrome [34]. Palliative TOF management commonly involves utilizing a modified Blalock-Taussig (BT) shunt composed of ePTFE, but this does not resolve the underlying malformation and faces serious complications such as graft thrombosis [45,55]. Curative TOF management in neonates necessitates the use of cardiopulmonary bypass to repair the ventricular septal defect with an ePTFE patch, and rectify the pulmonary artery if needed [45,56–58]. An evolving and exciting avenue of treatment for TOF involves pulmonary valve replacement with tissue engineered constructs. While ePTFE, pulmonary homografts, aortic homografts, xenograft valves, and mechanical valves all remain options for post-corrective TOF surgical patients, no single option has prevailed as the gold standard in clinical use [59–61].

While advancement in the surgical techniques and clinical management of neonatal CHD has allowed more neonates to reach adulthood, the long-term success remains unclear. A recent large-scale report of post-Fontan patient follow-up found 90% survival at 30 years of age and 80% survival at 40 years of age. However, the majority (59%) had serious complications with 21% requiring re-operation [62]. Another report found the incidence of heart failure in post-Fontan for single ventricle repair to be 40% [63]. In the Fontan procedure, the extra-cardiac Fontan continues to grow in popularity as the preferred approach due to advantages such as its applicability to a broad spectrum of anatomic presentations and amenability in a heart transplant procedure [64,65]. This technique's rise in use brings with it a greater necessity for donor tissue and/or material to form the conduit; however, concerns remain over the increased risk of thrombosis and stenosis with using a conduit. Non-synthetic donor tissue includes autologous pericardium, bovine xenografts, and homografts [66–72]. While these options poise some advantages, such as the potential for luminal endothelialization seen in pedicled pericardium, they ultimately face disadvantages due to limited donor tissue availability and the risk of venous thrombosis as seen in the bovine xenografts [68,73–75]. Dacron and Gore-Tex (ePTFE) synthetic conduits pose a viable alternative due to their ease of availability, but still face challenges. Dacron grafts used in the extra-cardiac Fontan result in a high rate of stenosis [76]. Gore-Tex conduits face their own challenge of patient somatic overgrowth leading to surgical delay in the newborn until the IVC has approached a diameter closer to that of its final adult size [77–79]. Tissue engineered vascular grafts (TEVGs) address the limitations faced by other implantable materials in the extra-cardiac Fontan by being readily available, patient specific, thrombosis resistant, and capable of growth [80].

While the long-term survival of TOF patients has been shown to reach 92% at 20 years, the percent of these survivors requiring reoperation is around 53% [81]. Notably, the majority of post corrective TOF patients will experience pulmonary regurgitation at 5–10 years post-surgical management. Pulmonary valve replacement thus represents a common follow-up intervention with 36%–40% of surviving TOF patients requiring it [82,83]. Concern over pulmonary valve replacement occurring too late in TOF patients to be beneficial necessitates re-evaluation of intervention earlier after corrective TOF surgery [84]. The obvious consequence of this is questions over the longevity of pulmonary valve replacements and complications that can arise over a patient's life. Surgical based pulmonary valve replacement in TOF patients has shown modern success. Mechanical and homograft replacement valves have been shown to have 10-year re-operation free rate among survivors of 87% (mechanical) and 74%–89% (homograft) [85–87]. Longer term follow-up to determine the longevity of these valve replacements in aging TOF patients remains to be determined.

4. Tissue engineering topical overview

As described above, outcomes of current surgical procedures used in CHDs are limited by the use of prosthetic materials used to create replacement heart valves, vascular grafts, and cardiopulmonary patches. Use of these materials is often complicated by limited durability and the risks of infection, host immune response, and thrombotic complications. The lack of growth and remodeling potential is also a particularly hazardous limitation in the case of pediatric patients. The field of tissue engineering holds promise for surgical solutions for these patients that can rise above these issues.

Tissue engineering, first described as a field by Langer and Vacanti in 1993, promotes the ideal of using the body's natural growth and regeneration processes to repair and replace damaged and nonfunctioning organs with healthy, native tissue [88]. Many approaches exist within tissue engineering, including the use of biodegradable polymeric scaffolds, decellularized extracellular matrix, stem cells, and harvested patient cells [89]. Each of these techniques has had its own successes, and each is characterized by its own set of limitations.

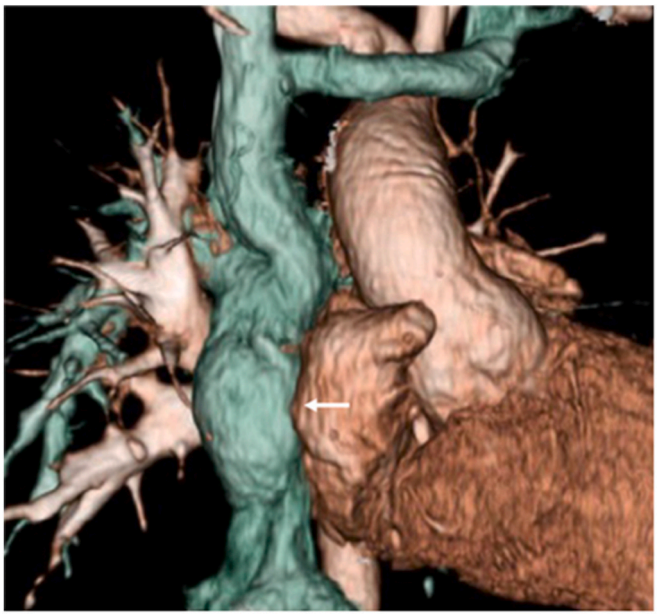

Fig. 2. Tissue Engineered Vascular Graft. Three-dimensional computed tomography of the tissue engineered vascular graft (arrow) used as a Fontan conduit in a clinical patient. Reproduced from Ref. [100].

Tissue engineering devices can also be designated by their preparation method. Products can be prepared *in vitro* prior to implantation by culturing cells on a scaffold, or *in situ* by allowing all remodeling to occur within the body [90]. *In situ* tissue engineering benefits from not requiring culturing time, allowing for a more off-the-shelf solution ready at the time of diagnosis [91]. A crucial problem still to be overcome in the pediatric population is the lack of growth and remodeling potential of the grafts currently used. Multiple cell sources and seeding and culturing mechanisms have been attempted, but optimal solutions remain to be elucidated [92].

An early attempt to use the body's regenerative potential to improve outcomes for congenital heart disease patients came as a modification to the traditional non-degradable Dacron grafts used in the Fontan circuit. Endothelial cell seeding of the Dacron grafts demonstrated improved performance over non-seeded grafts in preventing clotting, and the seeded grafts were better able to resist the calcification seen in Dacron grafts after longer implant times [93]. However, these seeded Dacron grafts remain lacking in growth potential, and their non-resorbing nature make them prone to long-standing inflammation and calcification the longer they are implanted.

Tissue engineering has also shown promising results for repair of congenital valve defects. Mechanical and biologic heart valve replacements both have their own drawbacks. Mechanical valves require lifelong anticoagulation therapy, and biological valves have a known shortened lifetime within the body before failure [94–96]. In addition, neither of these solutions holds growth potential for pediatric patients. Tissue engineering has potential to aid in these developments, aiming to create autologous heart valve substitutes using degradable substrates and patient cells [97].

5. Tissue engineered vascular grafts

The field of congenital cardiovascular tissue engineering that has advanced furthest to date is the TEVG. These conduits are used as cavopulmonary conduits during the final stage of the Fontan procedure (Fig. 2). The goal of these conduits is the formation of a vessel that is made of native tissue that can heal, remodel, and grow as the child ages; in contrast to the currently-used conduits with no growth potential.

The first human TEVG implantations were performed in 1999 in Tokyo. The grafts used in this study was made of a woven layer of either poly-glycolic acid or poly-lactic acid with a porous inner and outer layer of poly-caprolactone/lactide. Scaffolds were seeded with patient-derived cells; the first patient receiving a graft seeded with saphenous vein cells, while the next three patients' grafts were seeded with bone marrow mononuclear cells [98]. The early success of this technique provided much hope for the field of tissue engineering and led to larger clinical studies in Japan as well as the United States of America (USA).

At one year after implantation in the Japanese trial, the grafts demonstrated a 10–20% increase in cross sectional area change from baseline, demonstrating potential growth of the neovessel within patients [99]. Intermediate follow-up demonstrated no graft related mortality; however, 28% of patients developed a stenosis. All stenotic patients successfully underwent angioplasty [100]. Notably, early imaging was not done on these patients unless they became symptomatic [22]. In a clinical trial in the United States, all patients received early imaging, and asymptomatic stenosis was diagnosed and treated in three of four patients, leading to a halting of the clinical trial and a return to animal-based research for more analysis [101]. Follow-up research has since suggested that the asymptomatic stenosis seen in the United States trial was likely also present in the Japanese trial but went unrecognized as imaging was not routinely performed on asymptomatic patients. A graft explanted from a patient following a non-graft related death 13 years after implantation demonstrated a neovessel indistinguishable from the adjacent pulmonary artery or superior vena cava [77].

Follow-up studies on the clinical trial patients as well as animal work demonstrated many important factors in the development of neovessels from the grafts. Seeded cells acted to decrease the development of neointimal hyperplasia, but were notably lost quite rapidly after seeding, suggesting a paracrine mechanism of action rather than a direct functional effect as a stem cell [102]. Macrophages were found to be critical to neotissue formation, with a lack of macrophages leading to a loss of neotissue formation, and an overabundance of macrophages leading to higher levels of stenosis [103,104]. These results suggest that a careful balance of the immune system is vital for optimal neovessel formation.

Targeted positron emission tomography-computed tomography (PET-CT) imaging of matrix metalloproteinase (MMP) activity in TEVGs in sheep demonstrated changes in the inflammatory state of the neovessel over time as the scaffold degrades [105]. During the early phase after implantation, the graft exhibits an enhanced inflammatory response, as an increased number of inflammatory cells, and eventually smooth muscle cells, endothelial cells, and fibroblasts inhabit the graft. As the graft degrades, the inflammatory stimulus subsides, leaving primarily the non-inflammatory, more native vascular cell populations. In addition, this study found that bone marrow mononuclear cell seeding, often used in tissue engineering applications, had a beneficial effect at reducing early inflammation and associated neotissue overgrowth in the early phase of neotissue development.

Mathematical modeling of small animal experiments with similar implant materials demonstrated that the remodeling process is driven by two main processes: the inflammation-driven response to the implanted foreign material, and the mechano-mediated remodeling of the resulting neovessel [106]. The balance of these two factors changes dynamically over time as the stiff inflammatory graft material degrades, shifting mechanical stress and strain onto the newly infiltrating smooth muscle cells and newly deposited matrix.

In fact, mathematical modeling, as well as large animal studies, demonstrated that the early stenosis that occurred in the USA TEVG clinical trial was mathematically predicted, and interestingly was predicted to self-resolve over time as the scaffold degraded, and native mechano-mediated vessel remodeling processes could take over from the inflammation-driven reaction to the implant (Fig. 3) [101]. Sheep studies confirmed these findings, demonstrating the development of asymptomatic stenosis that spontaneously resolved as the scaffold degraded. Over time, the TEVG developed histological make-up nearly

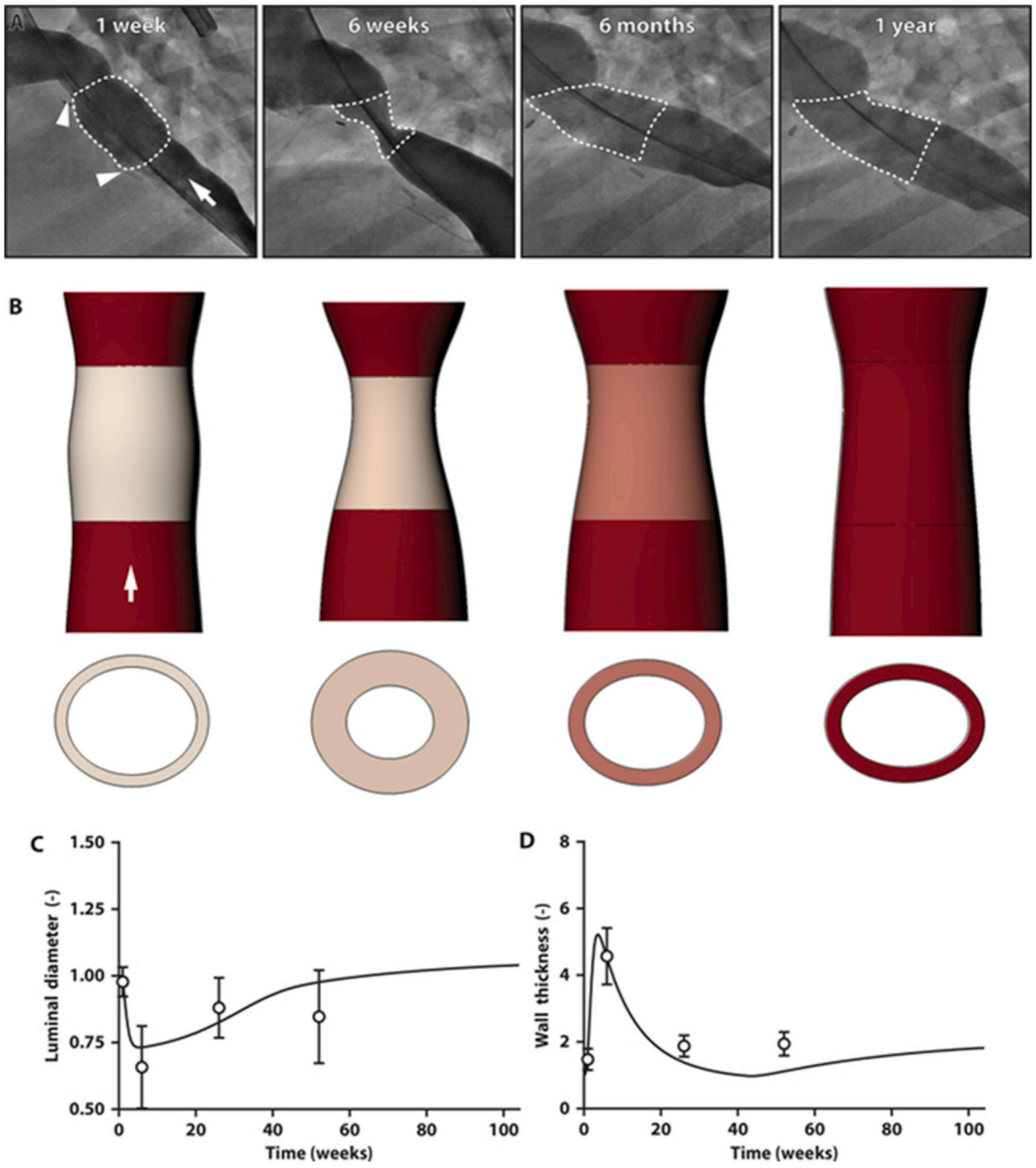

Fig. 3. Tissue engineered vascular graft (TEVG) stenosis spontaneously reverses in an ovine model. (A) Serial angiographic images of a representative ovine TEVG 1 week, 6 weeks, 6 months, and 1 year after implantation. Anastomoses are identified with white arrowheads, and the TEVG is identified by a white dotted outline. White arrow indicates direction of blood flow. **(B)** Image reconstructions of serial intra-vascular ultrasound (IVUS) and angiographic measurements of luminal area, wall thickness, and length of the TEVG, confirming the development of stenosis at 6 weeks but spontaneous resolution by 6 months after implantation. White arrow indicates direction of blood flow. When based on lamb data, the model simulations (solid lines) for the midgraft **(C)** luminal diameter and **(D)** wall thickness fit well the hydraulic diameters calculated from the IVUS measurements (symbols) over the 1-year study. Ovine IVUS measurements are represented as means ± SD (n = 22 at 1 week, n = 22 at 6 weeks, n = 20 at 6 months, n = 15 at 1 year). Reproduced from Ref. [101].

identical to that seen in the native IVC.

Separate groups have also had success in developing tissue engineered vascular grafts for the Fontan circulation, while focusing on different graft production methods. Work by the Tranquillo group has focused on decellularized tubes as vascular grafts. These tubes, when implanted as pulmonary arteries in sheep and followed for 1 year, showed an increase in both diameter and volume with the growth of the lambs [107]. Clinical work performed by the Xeltis Corporation focused on using electrospun polyester scaffolds for Fontan. These grafts appeared to perform well over the first year following implantation [108].

Overall, tissue engineered vascular grafts have been the source of a great amount of research both in the laboratory as well as the clinic. The seemingly relatively simple functionality of a vascular conduit compared to more active tissues such as cardiac muscle or heart valves made this tissue a prime target for early investigation. However, as the above studies show, formation of neovessels from these scaffolds is far from a passive process, with a multitude of biological and biomechanical factors playing roles in how grafts will perform in both the short and long term.

6. Tissue engineered heart patches

The holy grail of tissue engineering for patients with congenital heart disease may be the creation of an autologous whole heart for replacement surgery. The ability to create an entirely autologous, healthy heart to replace a damaged or congenitally malformed heart would be an ideal solution for patients, particularly in pediatric patients where donor hearts are scarce [109,110]. While the field is far from completing this lofty goal, many laboratory successes have occurred in the creation of heart patches. Tissue engineering of heart tissue is made more difficult by the limited regeneration potential of heart tissue after birth [111]. Due to the complex nature and function of cardiac muscle, development of functional cardiac patches requires careful understanding of the biology at play, with functional heart patches utilizing mixtures of chemical, mechanical, and electrical stimulation in order to develop patches with *in vivo* functionality [112].

In vitro culturing of cardiomyocytes onto specially designed scaffolds to drive appropriate bundle orientations led to cardiac patches with good structure similar to that of native cardiac muscle [113]. Physiological function of transplanted heart patches has been shown to be improved by prevascualrization, which allows for increased neotissue survival through integration into the host's vascular network [114].

In the research laboratory, much success has occurred in the decellularization and recellularization of heart tissue, up to and including an entire adult heart being decellularized and recellularized [115,116]. However, even after recellularization with appropriate cell types, formation of clinically functional grafts and organs remains a challenge.

In a study to evaluate the utility of injected stem cells on heart remodeling, human embryonic stem cell-derived cardiomyocytes were injected into the hearts of non-human primates after creation of large myocardial infarctions. One month post-implantation saw a 10% improvement in left ventricular ejection fractions, which improved by another 12% by 3 months [117]. It is important to note that injected and seeded stem cells, previously believed to transdifferentiate into functional cells within tissue engineered constructs, actually play an immunomodulatory role, releasing a number of important factors affecting the neotissue formation process [118,119].

These techniques of creating heart patches and regenerating heart tissue are not without their own drawbacks, as the well-performing products are prone to the development of arrhythmias [111,117].

7. Tissue engineered heart valves

There have been many attempts at creating tissue engineered heart valves (TEHVs), both in the research lab and the clinic. However, an optimal design remains to be determined. The overall goal for tissue engineered heart valves is a valve capable of full *in vivo* function at the time of implantation and regenerative capacity to create a functional native valve over time. *In vitro* and preclinical studies have been promising on many fronts, but clinical translation requires an outperformance of current prosthetic options, which has yet to occur [120]. Despite many benchtop-based successes, TEHVs have had a difficult history in the clinic, being used in patients after only limited animal models, and suffered from several complications in early studies. These difficulties led to a return to laboratory research to improve the designs, but improvements are still often done on an empiric approach, rather than rational design. Mechanistic studies of tissue formation in TEHVs are required for the improved design and eventual translation of these technologies to the clinic [121].

The first use of tissue engineering in the cardiovascular system was in heart valves in 1995, when Shinoka et al. implanted 7 adult lambs with a polyglycolic acid (PGA) heart valve leaflet seeded with animal-specific cells. Following implantation, the valves developed no stenosis and only trivial regurgitation, and the leaflets developed extra cellular matrix (ECM) and cellular architecture approaching that of the native valve [122].

An early attempt to bring TEHVs to the clinic came in the form of the Matrix P decellularized porcine valve [123]. These valves were used as right ventricle outflow tract (RVOT) replacements in children. Unfortunately, these valves were subject to high rates of stenosis, aneurysm, and dissection formation, including higher than expected levels of inflammation. These high rates of early and late failure, leading to patient death, lead to the removal of this valve from the clinic, and a clinical and regulatory wariness of new tissue engineering developments. Further evaluation suggested that the cause of the high inflammation rates may have been due to incomplete decellularization of the valves used in the studies.

Many studies have been done in large animal models to further evaluate TEHVs designs. Valves made from seeding mesenchymal stem cells onto a scaffold and culturing for one month were implanted into an adult sheep and followed for 20 weeks. The valves were well cellularized prior to implantation, and had a development of more valve-like cellular makeup over time while experiencing only trivial to mild regurgitation [124]. While the valve diameter remained unchanged, the cusps became attenuated and regurgitant over time [125].

Mixed positive and negative outcomes such as these are commonplace for many TEHVs designs. For example, we will take the case of valves made from sheep fibroblast-created tissue which was then decellularized and formed into a valve [126]. These valves, primarily made up of collagen, were used in adult sheep as aortic valve replacements for 24 weeks. No stenosis or thickening of the leaflets was observed over 24 weeks, but mild to moderate regurgitation was developed for 3 of the 4 valves implanted. No calcification was observed; however, it was noted that grafts were cellularized primarily at the base, with some cells found towards the free edge. Elastin and proteoglycans were found at explant in recellularized regions [126].

Similar decellularized tubes formed into heart valves were implanted as a pulmonary valve in a young lamb. As the valve remodeled, the valve demonstrated leaflet shortening, with worsening valvular insufficiency over 8 weeks. However, the leaflets showed endothelialization and ECM deposition over the implantation time [127]. This discrepancy in outcomes between valve performance in an adult versus young lamb highlights the challenge in designing a functional tissue engineering product for a young and growing patient.

The Xeltis pulmonary valve conduit is an electrospun polymeric heart valve designed to degrade slowly while promoting endogenous tissue regeneration to create native heart valves [128]. In a 12-month sheep model, the valve was shown to develop mild to moderate regurgitation as the scaffold was degraded and infiltrated with macrophages and smooth muscle cells as well as protein-rich deposits. The conduits were covered by a neointima by 2 months, and only one animal of the six in the study developed severe calcification by 12 months. The Xeltis valve is currently undergoing clinical trials.

An orthotopic pulmonary valve replacement was performed in a non-human primate model by *in vitro* TEHV preparation. Cells were seeded onto a scaffold, allowed to deposit matrix, and then the scaffolds were decellularized prior to implantation leaving behind their deposited extracellular matrix. The primates were followed for 8 weeks after a minithoracotomy and transapical implantation. The valves showed rapid recellularization with mild-moderate insufficiency and leaflet shortening. The valves were notably non-immunogenic, in comparison with polymeric and biologic valve substitutes [129].

As with other tissue engineering areas, many developments up until recently have been done in an empiric manner, with investigative hunches being used to improve designs and experiment to find more successful outcomes. However, more recent studies have shown great success in taking a more rational design approach to tissue engineering. Combining in-depth mathematical modeling with carefully designed experiments, the unknown mechanisms guiding tissue formation can be better elucidated. This advanced understanding can then be used to develop grafts with more ideal properties to lead to better outcomes for neotissue formation and performance over the short and long term. By

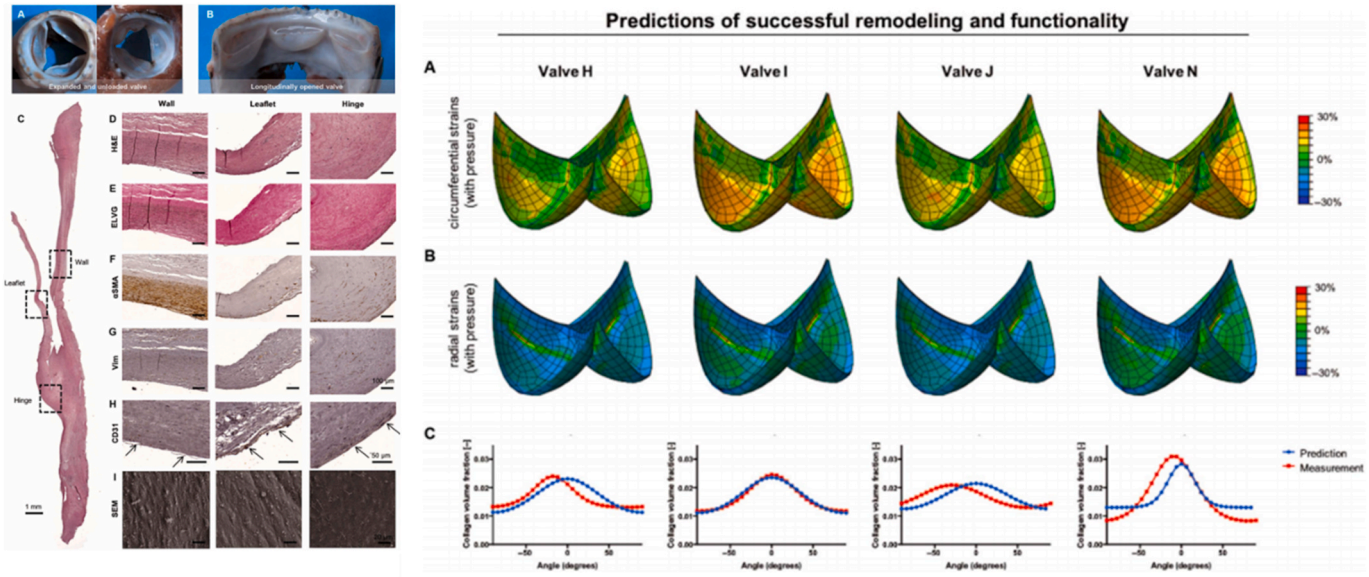

Fig. 4. Computational modeling of tissue engineered heart valve (TEHV) in sheep. (**Left panel**) Postmortem histological analyses of an exemplary TEHV explant after 1 year *in vivo* (valve N). **(A)** Gross images of the valve after harvest with distal and proximal views of the fully expanded unloaded valve and **(B)** of the three leaflets after the valve was cut open longitudinally through one of the commissural points. **(C)** Hematoxylin and eosin (H&E) staining of the longitudinal transection of entire valve and **(D)** of the higher magnification of the insets (black boxes) from the wall, leaflet, and hinge areas (scale bars, 100 μm). **(E to H)** Stainings for Elastica van Gieson (ELVG), alpha smooth muscle actin (αSMA), vimentin, and CD31 (arrows indicate endothelial cells) in the wall, leaflet, and hinge areas (scale bars, 100 μm). **(I)** Scanning electron microscopy (SEM) analysis of the wall, leaflet, and hinge surfaces of the explanted TEHV (scale bars, 20 μm). **(Right panel)** Computational predictions of valve remodeling based on valve-specific tissue properties at explantation. **(A)** Circumferential and **(B)** radial strains predicted for the different valves during hemodynamic loading. **(C)** Measured (red) and predicted (blue) collagen architecture for each valve. Reproduced from Ref. [130].

combining computational modeling with a sheep model of transcatheter valve replacement, a sheep tissue engineered pulmonary valve was delivered on a stent and followed for 1 year [130]. Explanted tissue showed strong agreement with predicted remodeling (Fig. 4).

While many TEHVs have been developed, and several have seen clinical investigation, widespread adoption of the devices has yet to occur. This is likely due to the fact that, although current heart valve replacement outcomes are suboptimal, particularly in the pediatric population, tissue engineered heart valves have yet to outperform these artificial replacements [120]. As research progress continues and tissue engineered heart valve technology improves, a design capable of outperforming current prostheses may arise leading to more widespread clinical application.

8. Tissue engineering in the fetal environment

Many congenital cardiac anomalies can be discovered and diagnosed *in utero* during routine physician appointments [6]. In fact, many CHDs that require emergent surgery after birth can have their causes traced back to dysfunctional valves within the fetal stages. If these valves could be repaired *in utero*, there is the potential to provide curative treatment for CHD before birth, preventing the need for any surgeries. This idea has been implemented clinically by balloon valvuloplasty in the fetus to open stenotic valves [131]. While often successful in opening the valve, this technique typically damages the native valve, leading to hindered valve performance after birth [131]. The fetal millieu also provides a unique environment for tissue engineering due to its high regenerative capacity. Fetal tissue has been shown to exhibit scar-free wound healing [132]. Combination of this idea of fetal intervention with tissue engineering holds potential in the future to create curative procedures for CHDs.

Human fetal amniotic fluid holds promise as a source to create TEHV *in vitro*, and has the additional benefit of being easy to access with limited risk to mother or fetus [133]. By taking amniotic cells from a fetal sheep and seeding them onto a heart valve scaffold, a TEHV was developed. The fetus was then exposed and implanted with the valve which was followed for 1 week before analysis [134].

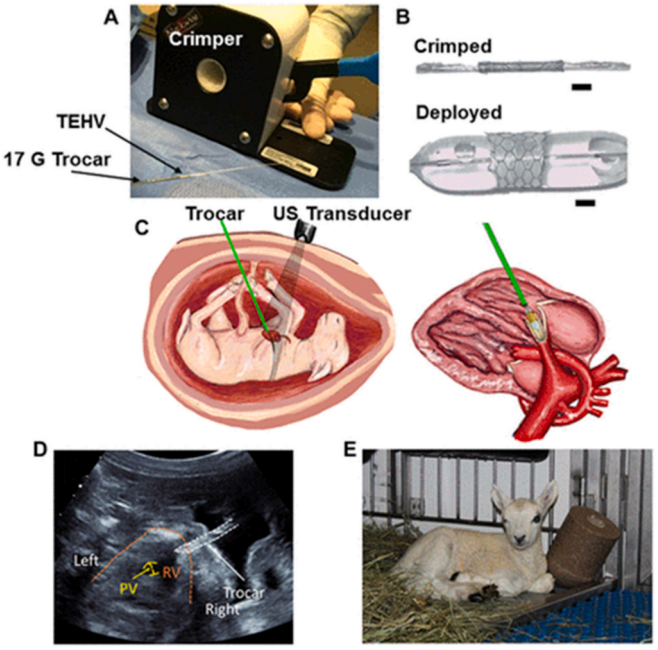

Fig. 5. Implantation of Fetal tissue engineered heart valve (TEHV) in Sheep. (A) The TEHV was crimped with a manual crimper onto **(B)** a balloon catheter and was deployed by inflation of the balloon. Scale bar = 2 mm. Implantation of the TEHV was performed through percutaneous transapical approach via **(C)** a 17-gauge (G) trocar guided by **(D)** ultrasound (US) into the pulmonary annulus. **(E)** The fetal lamb was born at term. PV = pulmonary valve; RV = right ventricle. Reproduced from Ref. [135].

Recently, our group in conjunction with many other teams was able

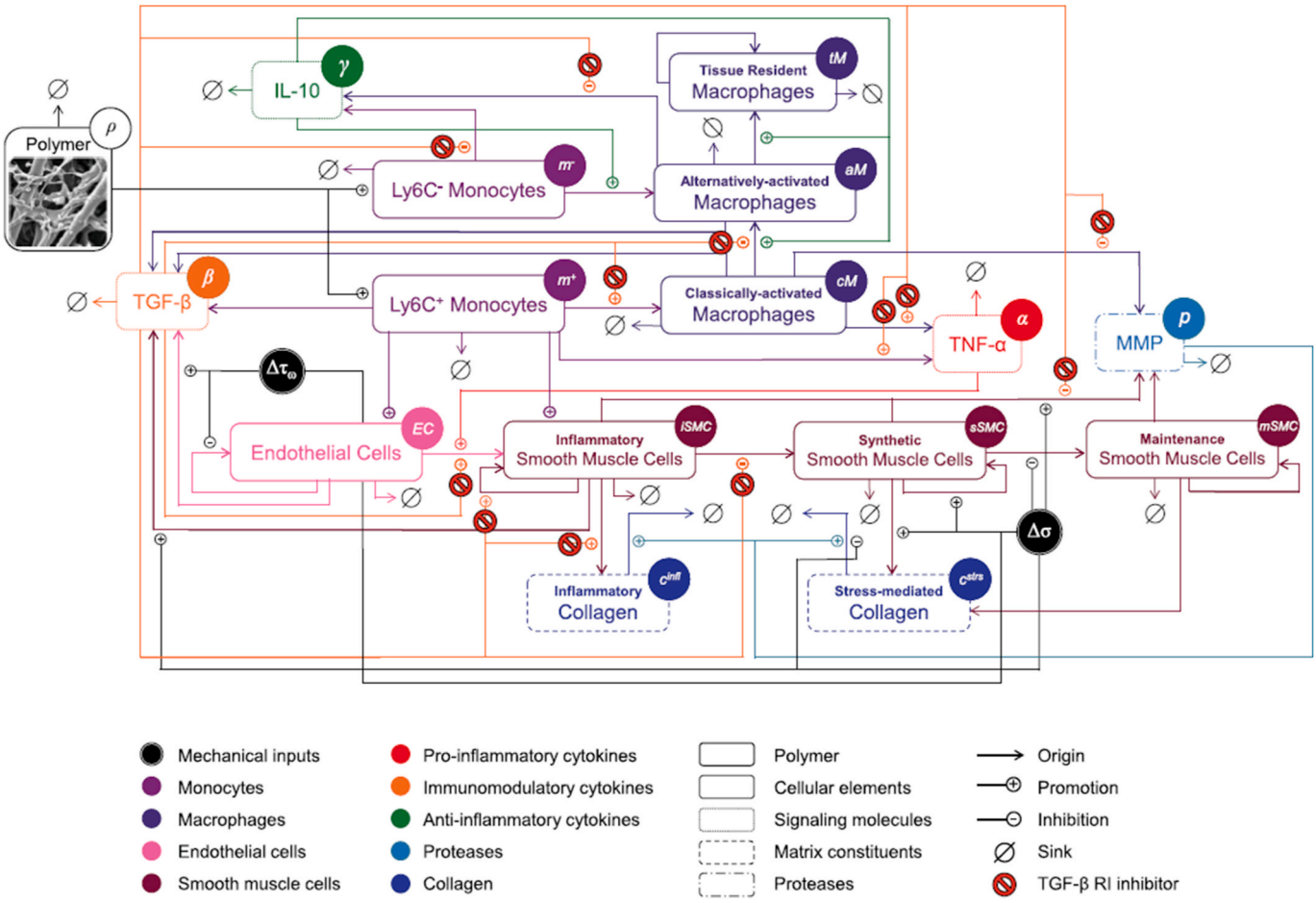

Fig. 6. The Bio-Chemo-Mechanical model of tissue engineered vascular graft (TEVG) development. Represented graphically using a schematic similar to that originally developed by Ref. [145]. The model is shown following pharmacological intervention with a transforming growth factor-beta receptor 1 (TGF-β R1) inhibitor. Legend and color scheme for the mechanistic pathways are below the figure, and variables used in the governing equations can be found within circles next to the name of each element in the diagram. Reproduced from Ref. [139]. IL: interleukin; TNF-α: tumor necrosis factor-alpha; MMP: matrix metallaoproteinase.

to develop a completely resorbable TEHV within a resorbable stent capable of being implanted percutaneously through a catheter into a fetal heart (Fig. 5) [135]. This TEHV was created using a specially designed zinc alloy stent and electrospun polycaprolactone (PCL) valves designed to fit through a 17G Trocar. This technique was developed and performed on fetal lambs, replacing the native pulmonary valve. After implantation, the valve showed good function, and the fetal sheep was allowed to develop and be born at term [135].

While these results are quite promising and represent the newest developments in the combination of engineering, science, and medicine, there is much to learn and accomplish before these techniques are ready for safe clinical use.

9. Future perspectives

In order to design and translate tissue engineering products to the clinic, more detailed understanding of the mechanisms involved in neotissue formation and remodeling are required. There is a large benefit of utilizing translational research in tissue engineering, comparing results and designing appropriate studies between humans, large animals, small animals, and computational models [136,137]. The rational design of new tissue engineering iterations relies on the development and careful understanding of both large and small animal models. A recently developed model for heart valve and heart valve leaflet transplantation in a mouse allows for mechanistic studies of how TEHVs develop, and the importance of different graft and host characteristics [138].

Improvements in computational modeling coupled with carefully designed animal studies may allow for a more detailed, mechanistic understanding of the pathway from implanted biomaterial to functional neotissue (Fig. 6). Computational modeling of the bio-chemo-mechanics of the environment including interactions between cell types, implanted graft parameters, and cytokines allows for the prediction *in silico* of the effect of pharmacologic modifications and interventions on neotissue development [139]. Separately, a model evaluating the effect of changing graft parameters allowed for the prediction of short and long-term outcomes of graft design changes, and predicted design parameters based on the preferred long-term outcomes [140].

Three-dimensional imaging and analysis have become commonplace in the clinic to evaluate congenital malformations and plan corrective surgeries. Combination of 3D imaging with the rapidly improving field of 3D printing has provided many clinicians with additional tools for diagnosis, management, and education of CHDs. As these technologies continue to improve and combine with the field of tissue engineering, we are beginning to see the development of patient-specific stents, grafts, and patches [141].

Combination of tissue engineering products with genetic engineering approaches may allow for improving tissue engineering approaches; however, much study still remains in both of these areas, as well as their effects on each other within a growing patient [142]. In conjunction with this area of research, there is concern that cells from diseased patients may not be suitable for tissue engineering since they are

Practice points

- Treatment of CHD remains a pressing clinical concern with 1–2% of all live births suffering from CHD.
- Tissue engineering has shown promise in the creation of surgical solutions for CHD capable of creating new autologous tissues that can integrate with the host, including tissue engineered vascular grafts, heart patches, and heart valves.
- Further work involving rational design of tissue engineered products is paramount to the creation of products capable of successful clinical use.

inherently damaged as well [97].

Previous clinical missteps such as those with early TEHVs demonstrates the need for regulatory oversight of tissue engineered products to prevent patient morbidity and mortality. However, regulatory strategies become more complicated with tissue engineering devices, as traditional definitions of implanted materials and the testing strategies typically applied to determine suitability do not appropriately predict tissue engineering device effectiveness [143].

10. Conclusions

Overall, tissue engineering has come far since its inception in the early 1990s, and has seen advancements with direct application to treatment of CHDs. TEVGs are currently seeing use in the clinic, with TEHVs beginning to return to clinic as well after initial pitfalls. Bringing tissue engineered heart valves into the fetal milieu shows great promise on the horizon for advanced treatments for CHDs.

Declaration of competing interest

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References

[1] Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002;39(12):1890–900.
[2] Roger VL, et al. Heart disease and stroke statistics–2011 update: a report from the American Heart Association. Circulation 2011;123(4):e18–209.
[3] Matthews TJ, MacDorman MF, Thoma ME. Infant mortality statistics from the 2013 period linked birth/infant death data set. Natl Vital Stat Rep 2015;64(9):1–30.
[4] Triedman JK, Newburger JW. Trends in congenital heart disease: the next decade. Circulation 2016;133(25):2716–33.
[5] Simeone RM, et al. Pediatric inpatient hospital resource use for congenital heart defects. Birth Defects Res A Clin Mol Teratol 2014;100(12):934–43.
[6] Quartermain MD, et al. Variation in prenatal diagnosis of congenital heart disease in infants. Pediatrics 2015;136(2):e378–85.
[7] Holst KA, et al. Current interventional and surgical management of congenital heart disease: specific focus on valvular disease and cardiac arrhythmias. Circ Res 2017;120(6):1027–44.
[8] Diller GP, Breithardt G, Baumgartner H. Congenital heart defects in adulthood. Dtsch Arztebl Int 2011;108(26):452–9.
[9] Fuller SM, et al. Estimating mortality risk for adult congenital heart surgery: an analysis of the society of thoracic surgeons congenital heart surgery database. Ann Thorac Surg 2015;100(5):1728–35. discussion 1735-6.
[10] Holst KA, et al. Risk factors and early outcomes of multiple reoperations in adults with congenital heart disease. Ann Thorac Surg 2011;92(1):122–8. discussion 129-30.
[11] Londe S, Sugg WL. The challenge of reoperation in cardiac surgery. Ann Thorac Surg 1974;17(2):157–62.
[12] D'Agostino RS, et al. The society of thoracic surgeons adult cardiac surgery database: 2018 update on outcomes and quality. Ann Thorac Surg 2018;105(1):15–23.
[13] Roselli EE, et al. Adverse events during reoperative cardiac surgery: frequency, characterization, and rescue. J Thorac Cardiovasc Surg 2008;135(2):316–23. 323. e1-6.
[14] Jacobs JP, et al. Reoperations for pediatric and congenital heart disease: an analysis of the Society of Thoracic Surgeons (STS) congenital heart surgery database. Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu 2014;17(1):2–8.
[15] Guidoin R, et al. Expanded polytetrafluoroethylene arterial prostheses in humans: histopathological study of 298 surgically excised grafts. Biomaterials 1993;14(9):678–93.
[16] Dorigo W, et al. A multicenter comparison between autologous saphenous vein and heparin-bonded expanded polytetrafluoroethylene (ePTFE) graft in the treatment of critical limb ischemia in diabetics. J Vasc Surg 2011;54(5):1332–8.
[17] Nassar GM, Ayus JC. Clotted arteriovenous grafts: a silent source of infection. Semin Dial 2000;13(1):1–3.
[18] Bachleda P, et al. Infected prosthetic dialysis arteriovenous grafts: a single dialysis center study. Surg Infect 2012;13(6):366–70.
[19] Brennan MP, et al. Tissue-engineered vascular grafts demonstrate evidence of growth and development when implanted in a juvenile animal model. Ann Surg 2008;248(3):370–7.
[20] Roh JD, et al. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. Proc Natl Acad Sci U S A 2010;107(10):4669–74.
[21] Cheung DY, Duan B, Butcher JT. Current progress in tissue engineering of heart valves: multiscale problems, multiscale solutions. Expet Opin Biol Ther 2015;15(8):1155–72.
[22] Hibino N, et al. Late-term results of tissue-engineered vascular grafts in humans. 436 J Thorac Cardiovasc Surg 2010;139(2):431–6. e1-2.
[23] Carachi R, Doss S. Clinical embryology: an atlas of congenital malformations. Springer Nature; 2019.
[24] Gittenberger-de Groot AC, et al. Embryology of the heart and its impact on understanding fetal and neonatal heart disease. Semin Fetal Neonatal Med 2013;18(5):237–44.
[25] Cook AC, Yates RW, Anderson RH. Normal and abnormal fetal cardiac anatomy. Prenat Diagn 2004;24(13):1032–48.
[26] Bajolle F, Zaffran S, Bonnet D. Genetics and embryological mechanisms of congenital heart diseases. Arch Cardiovasc Dis 2009;102(1):59–63.
[27] Norwood WI, Lang P, Hansen DD. Physiologic repair of aortic atresia-hypoplastic left heart syndrome. N Engl J Med 1983;308(1):23–6.
[28] Gobergs R, Salputra E, Lubaua I. Hypoplastic left heart syndrome: a review. Acta Med Litu 2016;23(2):86–98.
[29] Sinha SN, et al. Hypoplastic left ventricle syndrome. Analysis of thirty autopsy cases in infants with surgical considerations. Am J Cardiol 1968;21(2):166–73.
[30] Ishino K, et al. The modified Norwood procedure for hypoplastic left heart syndrome: early to intermediate results of 120 patients with particular reference to aortic arch repair. J Thorac Cardiovasc Surg 1999;117(5):920–30.
[31] Dasgupta C, et al. Identification of connexin43 (alpha1) gap junction gene mutations in patients with hypoplastic left heart syndrome by denaturing gradient gel electrophoresis (DGGE). Mutat Res 2001;479(1–2):173–86.
[32] Durbin MD, et al. Hypoplastic left heart syndrome sequencing reveals a novel NOTCH1 mutation in a family with single ventricle defects. Pediatr Cardiol 2017;38(6):1232–40.
[33] McBride KL, et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: segregation, multiplex relative risk, and heritability. Am J Med Genet 2005;134A(2):180–6.
[34] Muntean I, Togănel R, Benedek T. Genetics of congenital heart disease: past and present. Biochem Genet 2017;55(2):105–23.
[35] Ohye RG, Schranz D, D'Udekem Y. Current therapy for hypoplastic left heart syndrome and related single ventricle lesions. Circulation 2016;134(17):1265–79.
[36] Fredenburg TB, Johnson TR, Cohen MD. The Fontan procedure: anatomy, complications, and manifestations of failure. Radiographics 2011;31(2):453–63.
[37] d'Udekem Y, et al. The Fontan procedure: contemporary techniques have improved long-term outcomes. Circulation 2007;116(11 Suppl):I157–64.
[38] Jonas RA. Intermediate procedures after first-stage Norwood operation facilitate subsequent repair. Ann Thorac Surg 1991;52(3):696–700.
[39] Gale AW, et al. Fontan procedure for tricuspid atresia. Circulation 1980;62(1):91–6.
[40] Sumal AS, Kyriacou H, Mostafa AMHA. Tricuspid atresia: where are we now? J Card Surg 2020;35(7):1609–17.
[41] Franklin RC, et al. Tricuspid atresia presenting in infancy. Survival and suitability for the Fontan operation. Circulation 1993;87(2):427–39.
[42] Abdul-Sater Z, et al. Two heterozygous mutations in NFATC1 in a patient with Tricuspid Atresia. PloS One 2012;7(11):e49532.
[43] Lin AE, Rosti L. Tricuspid atresia in sibs. J Med Genet 1998;35(12):1055–6.
[44] Bonnet D, et al. Tricuspid atresia and conotruncal malformations in five families. J Med Genet 1999;36(4):349–50.

[45] Bailliard F, Anderson RH. Tetralogy of fallot. Orphanet J Rare Dis 2009;4:2.
[46] Bertranou EG, et al. Life expectancy without surgery in tetralogy of Fallot. Am J Cardiol 1978;42(3):458–66.
[47] Apitz C, Webb GD, Redington AN. Tetralogy of fallot. Lancet 2009;374:1462–71. 9699.
[48] Momma K, et al. Tetralogy of Fallot associated with chromosome 22q11.2 deletion in adolescents and young adults. Genet Med 2001;3(1):56–60.
[49] Goldmuntz E, Geiger E, Benson DW. NKX2.5 mutations in patients with tetralogy of fallot. Circulation 2001;104(21):2565–8.
[50] Eldadah ZA, et al. Familial Tetralogy of Fallot caused by mutation in the jagged1 gene. Hum Mol Genet 2001;10(2):163–9.
[51] Pankau R, Siekmeyer W, Stoffregen R. Tetralogy of Fallot in three sibs. Am J Med Genet 1990;37(4):532–3.
[52] Jones MC, Waldman JD. An autosomal dominant syndrome of characteristic facial appearance, preauricular pits, fifth finger clinodactyly, and tetralogy of Fallot. Am J Med Genet 1985;22(1):135–41.
[53] Pacileo G, Musewe NN, Calabrò R. Tetralogy of Fallot in three siblings: a familial study and review of the literature. Eur J Pediatr 1992;151(10):726–7.
[54] Digilio MC, et al. Recurrence risk figures for isolated tetralogy of Fallot after screening for 22q11 microdeletion. J Med Genet 1997;34(3):188–90.
[55] Kiran U, et al. The blalock and taussig shunt revisited. Ann Card Anaesth 2017;20 (3):323–30.
[56] Devendran V, et al. Tetralogy of Fallot with subarterial ventricular septal defect: surgical outcome in the current era. Ann Pediatr Cardiol 2015;8(1):4–9.
[57] Jeon B, et al. Surgical treatment of tetralogy of Fallot in symptomatic neonates and young infants. J Thorac Cardiovasc Surg 2020;159(4):1466–1476.e2.
[58] Hirsch JC, Mosca RS, Bove EL. Complete repair of tetralogy of Fallot in the neonate: results in the modern era. Ann Surg 2000;232(4):508–14.
[59] Harrild DM, et al. Pulmonary valve replacement in tetralogy of Fallot: impact on survival and ventricular tachycardia. Circulation 2009;119(3):445–51.
[60] Starr JP. Tetralogy of fallot: yesterday and today. World J Surg 2010;34(4): 658–68.
[61] Mosca RS. Pulmonary valve replacement after repair of tetralogy of Fallot: evolving strategies. J Thorac Cardiovasc Surg 2016;151(3):623–5.
[62] Dennis M, et al. Clinical outcomes in adolescents and adults after the fontan procedure. J Am Coll Cardiol 2018;71(9):1009–17.
[63] Piran S, et al. Heart failure and ventricular dysfunction in patients with single or systemic right ventricles. Circulation 2002;105(10):1189–94.
[64] Kogon B. Is the extracardiac conduit the preferred Fontan approach for patients with univentricular hearts? The extracardiac conduit is the preferred Fontan approach for patients with univentricular hearts. Circulation 2012;126(21): 2511–5. discussion 2515.
[65] Haas GS, et al. Extracardiac conduit fontan procedure: early and intermediate results. Eur J Cardio Thorac Surg 2000;17(6):648–54.
[66] Kavarana MN, et al. Seven-year clinical experience with the extracardiac pedicled pericardial Fontan operation. Ann Thorac Surg 2005;80(1):37–43. discussion 43.
[67] Adachi I, et al. Fontan operation with a viable and growing conduit using pedicled autologous pericardial roll: serial changes in conduit geometry. J Thorac Cardiovasc Surg 2005;130(6):1517–22.
[68] Adachi I, et al. Immunohistological findings for an extracardiac conduit in Fontan pathway constructed with pedicled autologous pericardium. Eur J Cardio Thorac Surg 2006;29(6):1059–60.
[69] Adachi I, et al. Immunohistologic examination of pedicled autologous pericardium 9 years after implantation for an extracardiac conduit in Fontan pathway: comparison with in situ pericardium and pulmonary arterial tissue from the same patient. J Thorac Cardiovasc Surg 2007;133(4):1101–3.
[70] Baslaim G, et al. Bovine valved xenograft conduits in the extracardiac Fontan procedure. J Thorac Cardiovasc Surg 2003;126(2):586–8.
[71] Baslaim G. Bovine valved xenograft (Contegra) conduit in the extracardiac Fontan procedure: the preliminary experience. J Card Surg 2008;23(2):146–9.
[72] Metin SK, et al. Fresh homografts obtained through a national organ-sharing programme for repair of congenital heart disease. J Int Med Res 2005;33(1): 123–31.
[73] Yuan SM. The Contegra valved bovine conduit: a biomaterial for the surgical treatment of congenital heart defects. Arq Bras Cardiol 2012;99(6):1159–66.
[74] Schoof PH, et al. Bovine jugular vein thrombosis in the Fontan circulation. J Thorac Cardiovasc Surg 2002;124(5):1038–40.
[75] Schoof P, Waterbolk T. Contegra and extracardiac fontan. J Thorac Cardiovasc Surg 2009;138(1):258–9.
[76] van Brakel TJ, et al. High incidence of Dacron conduit stenosis for extracardiac Fontan procedure. J Thorac Cardiovasc Surg 2014;147(5):1568–72.
[77] Shinoka T. What is the best material for extracardiac Fontan operation? J Thorac Cardiovasc Surg 2017;153(6):1551–2.
[78] Lee C, et al. Midterm follow-up of the status of Gore-Tex graft after extracardiac conduit Fontan procedure. Eur J Cardio Thorac Surg 2007;31(6):1008–12.
[79] Alexi-Meskishvili V, et al. Optimal conduit size for extracardiac Fontan operation. Eur J Cardio Thorac Surg 2000;18(6):690–5.
[80] Patterson JT, et al. Tissue-engineered vascular grafts for use in the treatment of congenital heart disease: from the bench to the clinic and back again. Regen Med 2012;7(3):409–19.
[81] Park CS, et al. The long-term result of total repair for tetralogy of Fallot. Eur J Cardio Thorac Surg 2010;38(3):311–7.
[82] Frigiola A, et al. Physiological and phenotypic characteristics of late survivors of tetralogy of fallot repair who are free from pulmonary valve replacement. Circulation 2013;128(17):1861–8.
[83] Cuypers JA, et al. Unnatural history of tetralogy of Fallot: prospective follow-up of 40 years after surgical correction. Circulation 2014;130(22):1944–53.
[84] Therrien J, et al. Pulmonary valve replacement in adults late after repair of tetralogy of fallot: are we operating too late? J Am Coll Cardiol 2000;36(5): 1670–5.
[85] Egbe AC, et al. Use of mechanical valve prostheses in adults with tetralogy of Fallot. Int J Cardiol 2019;291:45–9.
[86] van de Woestijne PC, et al. Right ventricular outflow tract reconstruction with an allograft conduit in patients after tetralogy of Fallot correction: long-term follow-up. Ann Thorac Surg 2011;92(1):161–6.
[87] Meijer FMM, et al. Excellent durability of homografts in pulmonary position analysed in a predefined adult group with tetralogy of Fallot. Interact Cardiovasc Thorac Surg 2019;28(2):279–83.
[88] Langer R, Vacanti JP. Tissue engineering. Science 1993;260(5110):920–6.
[89] Naito Y, et al. Beyond burst pressure: initial evaluation of the natural history of the biaxial mechanical properties of tissue-engineered vascular grafts in the venous circulation using a murine model. Tissue Eng 2014;20(1–2):346–55.
[90] Melchiorri AJ, et al. Vitro endothelialization of biodegradable vascular grafts via endothelial progenitor cell seeding and maturation in a tubular perfusion system bioreactor. Tissue Eng C Methods 2016;22(7):663–70.
[91] Bouten CVC, Smits AIPM, Baaijens FPT. Can we grow valves inside the heart? Perspective on material-based in situ heart valve tissue engineering. Front Cardiovasc Med 2018;5:54.
[92] Avolio E, Caputo M, Madeddu P. Stem cell therapy and tissue engineering for correction of congenital heart disease. Front Cell Dev Biol 2015;3:39.
[93] Nugent HM, Edelman ER. Tissue engineering therapy for cardiovascular disease. Circ Res 2003;92(10):1068–78.
[94] Durães AR, et al. Usefulness and safety of rivaroxaban in patients following isolated mitral valve replacement with a mechanical prosthesis. Am J Cardiol 2018;122(6):1047–50.
[95] Salem DN, et al. Antithrombotic therapy in valvular heart disease–native and prosthetic: the seventh ACCP conference on antithrombotic and thrombolytic therapy. Chest 2004;126(3 Suppl):457S–82S.
[96] Head SJ, Çelik M, Kappetein AP. Mechanical versus bioprosthetic aortic valve replacement. Eur Heart J 2017;38(28):2183–91.
[97] Neuenschwander S, Hoerstrup SP. Heart valve tissue engineering. Transpl Immunol 2004;12(3–4):359–65.
[98] Matsumura G, et al. Successful application of tissue engineered vascular autografts: clinical experience. Biomaterials 2003;24(13):2303–8.
[99] Shin'oka T, et al. Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. J Thorac Cardiovasc Surg 2005;129 (6):1330–8.
[100] Sugiura T, et al. Tissue-engineered vascular grafts in children with congenital heart disease: intermediate term follow-up. Semin Thorac Cardiovasc Surg 2018; 30(2):175–9.
[101] Drews JD, et al. Spontaneous reversal of stenosis in tissue-engineered vascular grafts. Sci Transl Med 2020;12(537).
[102] Hibino N, et al. A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. Faseb J 2011;25 (12):4253–63.
[103] Kurobe H, et al. Concise review: tissue-engineered vascular grafts for cardiac surgery: past, present, and future. Stem Cells Transl Med 2012;1(7):566–71.
[104] Hibino N, et al. Tissue-engineered vascular grafts form neovessels that arise from regeneration of the adjacent blood vessel. Faseb J 2011;25(8):2731–9.
[105] Stacy MR, et al. Targeted imaging of matrix metalloproteinase activity in the evaluation of remodeling tissue-engineered vascular grafts implanted in a growing lamb model. J Thorac Cardiovasc Surg 2014;148(5):2227–33.
[106] Szafron JM, et al. Immuno-driven and mechano-mediated neotissue formation in tissue engineered vascular grafts. Ann Biomed Eng 2018;46(11):1938–50.
[107] Syedain Z, et al. Tissue engineering of acellular vascular grafts capable of somatic growth in young lambs. Nat Commun 2016;7:12951.
[108] Bockeria LA, et al. Total cavopulmonary connection with a new bioabsorbable vascular graft: first clinical experience. J Thorac Cardiovasc Surg 2017;153(6): 1542–50.
[109] Thrush PT, Hoffman TM. Pediatric heart transplantation-indications and outcomes in the current era. J Thorac Dis 2014;6(8):1080–96.
[110] Doroshow RW, Ashwal S, Saukel GW. Availability and selection of donors for pediatric heart transplantation. J Heart Lung Transplant 1995;14(1 Pt 1):52–8.
[111] Laflamme MA, Murry CE. Heart regeneration. Nature 2011;473(7347):326–35.
[112] Birla RK. A methodological nine-step process to bioengineer heart muscle tissue. Tissue Cell 2020;67:101425.
[113] Madden LR, et al. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. Proc Natl Acad Sci U S A 2010;107(34):15211–6.
[114] Stevens KR, et al. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc Natl Acad Sci U S A 2009;106 (39):16568–73.
[115] Tang-Quan KR, et al. Whole cardiac tissue bioscaffolds. Adv Exp Med Biol 2018; 1098:85–114.
[116] Guyette JP, et al. Bioengineering human myocardium on native extracellular matrix. Circ Res 2016;118(1):56–72.
[117] Liu YW, et al. Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. Nat Biotechnol 2018;36(7): 597–605.
[118] Harrington JK, et al. Determining the fate of seeded cells in venous tissue-engineered vascular grafts using serial MRI. Faseb J 2011;25(12):4150–61.

[119] Chien KR, et al. Regenerating the field of cardiovascular cell therapy. Nat Biotechnol 2019;37(3):232–7.
[120] Dijkman PE, et al. Heart valve replacements with regenerative capacity. Transfus Med Hemotherapy 2016;43(4):282–90.
[121] Blum K, Drews J, Breuer CK. Tissue engineered heart valves: a call for mechanistic studies. Tissue Eng Part B Rev; 2018.
[122] Shinoka T, et al. Tissue engineering heart valves: valve leaflet replacement study in a lamb model. Ann Thorac Surg 1995;60(6 Suppl):S513–6.
[123] Perri G, et al. Early and late failure of tissue-engineered pulmonary valve conduits used for right ventricular outflow tract reconstruction in patients with congenital heart disease. Eur J Cardio Thorac Surg 2012;41(6):1320–5.
[124] Sutherland FW, et al. From stem cells to viable autologous semilunar heart valve. Circulation 2005;111(21):2783–91.
[125] Gottlieb D, et al. In vivo monitoring of function of autologous engineered pulmonary valve. J Thorac Cardiovasc Surg 2010;139(3):723–31.
[126] Syedain Z, et al. 6-month aortic valve implantation of an off-the-shelf tissue-engineered valve in sheep. Biomaterials 2015;73:175–84.
[127] Reimer J, et al. Implantation of a tissue-engineered tubular heart valve in growing lambs. Ann Biomed Eng 2016;45(2):439–51.
[128] Bennink G, et al. A novel restorative pulmonary valved conduit in a chronic sheep model: mid-term hemodynamic function and histologic assessment. J Thorac Cardiovasc Surg 2018;155(6):2591–601. e3.
[129] Weber B, et al. Off-the-shelf human decellularized tissue-engineered heart valves in a non-human primate model. Biomaterials 2013;34(30):7269–80.
[130] Emmert MY, et al. Computational modeling guides tissue-engineered heart valve design for long-term in vivo performance in a translational sheep model. Sci Transl Med 2018;10(440).
[131] Tulzer A, et al. Immediate effects and outcome of in-utero pulmonary valvuloplasty in fetuses with pulmonary atresia with intact ventricular septum or critical pulmonary stenosis. Ultrasound Obstet Gynecol 2018;52(2):230–7.
[132] Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. Plast Reconstr Surg 2010;126(4):1172–80.
[133] Weber B, Zeisberger SM, Hoerstrup SP. Prenatally harvested cells for cardiovascular tissue engineering: fabrication of autologous implants prior to birth. Placenta 2011;32(Suppl 4):S316–9.
[134] Weber B, et al. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation. Biomaterials 2012;33(16):4031–43.
[135] Zakko J, et al. Development of tissue engineered heart valves for percutaneous transcatheter delivery in a fetal ovine model. JACC Basic Transl Sci 2020;5(8):815–28.
[136] Breuer CK. The development and translation of the tissue-engineered vascular graft. J Pediatr Surg 2011;46(1):8–17.
[137] Loerakker S, et al. Controlling the adaption behaviour of next-generation tissue-engineered cardiovascular implants via computational modelling. Eur Heart J 2020;41(10):1069–73.
[138] Lee YU, et al. Transplantation of pulmonary valve using a mouse model of heterotopic heart transplantation. J Vis Exp 2014;89.
[139] Khosravi R, et al. A computational bio-chemo-mechanical model of in vivo tissue-engineered vascular graft development. Integr Biol (Camb) 2020;12(3):47–63.
[140] Szafron JM, et al. Optimization of tissue-engineered vascular graft design using computational modeling. Tissue Eng C Methods 2019;25(10):561–70.
[141] Yoo SJ, et al. 3D printing in medicine of congenital heart diseases. 3D Print Med 2015;2(1):3.
[142] Nappi F, Singh SSA. Gene therapy and regenerative tissue engineering in congenital heart disease. Transl Pediatr 2019;8(5):356–9.
[143] Emmert MY, Fioretta ES, Hoerstrup SP. Translational challenges in cardiovascular tissue engineering. J Cardiovasc Transl Res 2017;10(2):139–49.
[144] Javed R, et al. Hypoplastic left heart syndrome: an overview for primary care providers. Pediatr Rev 2019;40(7):344–53.
[145] Aparício P, Thompson MS, Watton PN. A novel chemo-mechano-biological model of arterial tissue growth and remodelling. J Biomech 2016;49(12):2321–30.