Review

Cell-Free Versus Cell-Seeded Tissue-Engineered Heart Valves: Lessons From Preclinical and Clinical Studies

Tatsuya Watanabe[1,*], Kosuke Nakamae[1], Junya Matsuda[1], Annora Dasi[1], Chihiro Miyagi[1], Syed Faizullah Hussaini[1], Satoshi Yuhara[1], Hirotsugu Kurobe[2], Daisuke Onohara[1,3,†] and Toshiharu Shinoka[1,4,†]

[1]Center for Regenerative Medicine, Abigale Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH 43215, USA
[2]Department of Cardiovascular and Thoracic Surgery, Ehime University Graduate School of Medicine, 791-0295 Toon, Ehime, Japan
[3]Department of Pediatrics, The Ohio State University, Columbus, OH 43205, USA
[4]Department of Surgery, The Ohio State University, Columbus, OH 43210, USA
[†]These authors share senior authorship.

Abstract

Tissue-engineered heart valves (TEHVs) have been developed to overcome the limitations of current prosthetic valves, which lack growth, repair, and remodeling capacity. While early TEHV strategies relied on pre-seeded cells, recent approaches have shifted toward cell-free scaffolds designed to promote in situ tissue regeneration; however, the relative advantages and limitations of these strategies remain unclear. This review synthesizes evidence from preclinical and clinical studies of TEHVs, including fully synthetic biodegradable scaffolds, decellularized xenografts, decellularized allografts, engineered decellularized matrices, and cell-seeded constructs, with a focus on scaffold composition, recellularization, extracellular matrix (ECM) formation, and functional durability. Overall, cell-free TEHVs demonstrate the ability to recruit host cells and support tissue formation, but remodeling is frequently incomplete and heterogeneous, particularly in valve leaflets. Decellularized xenografts show limited durability due to persistent immunogenicity, whereas decellularized allografts provide the most consistent clinical performance but exhibit incomplete recellularization and variable long-term outcomes. Fully synthetic and engineered decellularized scaffolds enable in situ regeneration but are often associated with geometric instability and maladaptive remodeling. Cell-seeded TEHVs can enhance early endothelialization and matrix deposition; however, challenges remain in cell retention, survival, and regulation of remodeling, with no consistent long-term superiority. Taken together, neither purely cell-free nor cell-seeded strategies alone reliably achieve durable valve regeneration, and the primary challenge lies in controlling remodeling rather than the presence of seeded cells, supporting the development of integrated approaches that combine scaffold design with biological modulation to improve long-term valve function.

Keywords: Tissue-engineered heart valve, decellularization, cell seeding, in situ tissue engineering, heart valve regeneration, scaffold remodeling, cardiovascular biomaterials, regenerative medicine, preclinical study, clinical study.

***Address for correspondence**: Tatsuya Watanabe, Center for Regenerative Medicine, Abigale Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH 43215, USA. E-mail: tatsuya.watanabe@nationwidechildrens.org.

Introduction

Valvular heart disease remains a major global health burden, and valve replacement is often required when the disease progresses to advanced stages. Despite substantial advances in surgical and transcatheter techniques, currently available prosthetic valves are still fundamentally limited. Mechanical valves provide excellent durability but require lifelong anticoagulation and are associated with thromboembolic and bleeding complications [1,2]. In contrast, bioprosthetic valves avoid lifelong anticoagulation but are limited by structural valve deterioration and finite durability [3,4]. Importantly, none of these valve substitutes possesses the ability to grow, repair, or remodel, which represents a particularly important limitation in pediatric and congenital heart disease populations [2,5,6]. As a result, existing valve replacement options remain suboptimal, highlighting the need for a regenerative valve strategy that can achieve both durable function and biological adaptation [7,8].

To address these limitations, several alternative strategies have been explored, including homografts, xenografts, and autografts [6,9,10]. Although these options offer im-

portant biological and hemodynamic advantages, they still do not fully reproduce the adaptive and regenerative properties of native valves [5,6]. Even the Ross procedure, which provides growth potential and physiological valve function, still carries a substantial long-term risk of reintervention [11]. These limitations underscore the unmet clinical need for a truly regenerative valve substitute.

Tissue-engineered heart valves (TEHVs) have emerged as a promising regenerative strategy aimed at creating living valve substitutes capable of growth, repair, and remodeling [6,12]. In principle, TEHVs combine biomaterial scaffolds with biological processes to generate constructs that can integrate with the host and adapt over time [5,12]. More recently, the field has increasingly shifted toward cell-free and in situ regenerative approaches, particularly those based on decellularized or bioresorbable scaffolds, which seek to harness host-mediated recellularization and tissue remodeling while avoiding the complexity of *ex vivo* cell seeding and conditioning [12]. Current TEHV strategies can be broadly categorized into cell-seeded and cell-free approaches. Cell-seeded constructs aim to establish a biologically active tissue prior to implantation through in vitro cell expansion and conditioning [13–15], whereas cell-free strategies rely on host-driven recellularization following implantation [16,17]. In parallel, a range of scaffold platforms have been explored, including decellularized biological matrices and synthetic biodegradable materials, each with distinct advantages and limitations in terms of mechanical properties, remodeling capacity, and clinical feasibility.

However, despite encouraging early experimental and clinical results, the long-term outcomes of TEHVs, particularly those relying on in situ regeneration, have remained inconsistent across studies and platforms [9,10]. This heterogeneity likely reflects multiple interrelated factors, including variability in scaffold design and material properties, maladaptive remodeling such as fibrosis, degeneration, and calcification, incomplete or delayed recellularization, and strong host-specific biological and hemodynamic influences [9,10,12,18–20]. In particular, these findings suggest that the success of TEHVs depends not only on the initial scaffold design, but also on how effectively host-driven remodeling can be guided after implantation.

Several comprehensive reviews have summarized the development of TEHVs, with a primary focus on biomaterials, scaffold fabrication, and early-stage experimental studies [21–24]. These studies have provided important insights into material selection and biological responses under controlled conditions. However, comparatively less attention has been given to synthesizing evidence from a translational perspective, particularly in large-animal models and clinically relevant settings where physiological loading, host responses, and long-term remodeling can be more accurately assessed. In this context, a focused evaluation of translational outcomes is critical to understanding why promising early results often fail to translate into consistent long-term performance.

This review, therefore, examines the translational evidence for TEHVs, with a focus on whether cell-free strategies can achieve durable and biologically appropriate valve regeneration *in vivo*. By integrating findings from preclinical and clinical studies, we aim to clarify the major limitations of current TEHV strategies and the key requirements for achieving consistent long-term function and remodeling.

Materials and Methods

This study is a narrative review of the current literature on TEHVs, with a focus on translational and clinically relevant outcomes. Relevant studies were identified through comprehensive searches on PubMed. Eligible studies included preclinical investigations, particularly large-animal models, as well as clinical reports evaluating TEHV performance. Priority was given to studies reporting functional outcomes, remodeling characteristics, and long-term durability. Both cell-free and cell-seeded approaches were included to enable comparative analysis across different TEHV strategies, including fully synthetic biodegradable scaffolds, decellularized xenografts, decellularized allografts, and engineered decellularized matrices. Data extraction focused on scaffold composition, cell source, implantation model, duration of follow-up, and reported functional and histological outcomes.

Early Development of TEHVs

Early TEHV development was based on the concept of combining living cells with biodegradable polymer scaffolds to create living valve substitutes. This approach aimed to overcome the fundamental limitations of conventional prosthetic valves, including their inability to grow, repair, or remodel, as well as their risks of thrombogenicity and infection.

Shinoka *et al.* [13] developed one of the earliest tissue-engineered valve leaflet models by seeding a biodegradable polyglycolic acid (PGA) scaffold with autologous vascular-derived cells. Mixed cell populations isolated from ovine arteries were separated into fibroblasts and endothelial cells. Fibroblasts were first seeded onto the scaffold to form a tissue-like matrix, followed by endothelial cell seeding to establish a surface monolayer. The constructs were implanted to replace the right posterior leaflet of the pulmonary valve in a lamb model under cardiopulmonary bypass. In this initial study, both autologous and allogenic tissue-engineered leaflets were evaluated. Autologous constructs demonstrated favorable functional performance, with no stenosis and only trivial pulmonary regurgitation on postoperative echocardiography. In contrast, allogenic constructs showed inferior outcomes, including moderate regurgitation, leaflet deterioration, and inflammatory responses despite immunosuppression. Histological analy-

sis confirmed early extracellular matrix (ECM) formation within the engineered tissue, although matrix development remained immature at early time points.

Subsequent work further characterized the behavior of these constructs after implantation. In a follow-up study, Shinoka *et al*. [13–15] showed that autologous cell-seeded valve leaflets persisted after implantation and underwent progressive ECM development, including increased collagen deposition and the appearance of elastin fibers and endothelial markers. In contrast, non-seeded polymer controls degraded completely without forming organized replacement tissue, indicating that prior cell seeding was essential for tissue formation and durability. These findings suggested that transplanted autologous cells contributed directly to tissue development and remodeling after implantation [25] (Fig. 1).

These early investigations demonstrated several key principles. First, biodegradable polymer scaffolds served as temporary templates that supported cell attachment, proliferation, and matrix formation, while gradually degrading as new tissue developed [13,25]. Second, autologous cell use appeared critical for functional performance and biocompatibility, avoiding immune-mediated deterioration observed in allogenic constructs. Third, implanted cell-seeded constructs were capable of generating ECM components *in vivo*, suggesting the potential for creating living valve substitutes [25].

However, these studies were preliminary feasibility investigations and also revealed several limitations. The engineered leaflets were thicker and less flexible than native valve tissue, and ECM maturation remained incomplete at the early time points examined [13,14]. In addition, the constructs were evaluated only in the low-pressure pulmonary circulation, and their ability to withstand systemic hemodynamic stress remained uncertain. These limitations left important unresolved questions regarding the optimal cell source and the long-term durability of engineered tissues.

Overall, these foundational studies demonstrated that living, cell-seeded valve leaflets could be generated and function *in vivo*, establishing the initial biological basis for TEHV development and later efforts to create more durable and clinically applicable valve substitutes.

Current Approaches to Cell-Free TEHVs

Emergence of the Cell-Free TEHV Paradigm

Early preclinical efforts in TEHVs relied on *in vitro* cell seeding of scaffolds with autologous cells, aiming to create living constructs capable of growth and remodeling after implantation [26]. Although this approach demonstrated feasibility, including early clinical application using autologous progenitor cell-seeded decellularized allografts, it required complex processes such as cell isolation, expansion, and bioreactor conditioning [26,27].

In parallel, the limitations of conventional biological substitutes, including homografts and xenografts, became increasingly evident. These included immune-mediated degeneration, calcification, and limited long-term durability, particularly in pediatric patients [27]. Early decellularization strategies were therefore developed to reduce graft immunogenicity by removing donor cellular components while preserving the ECM. However, initial clinical experiences with decellularized xenogeneic valves, such as SynerGraft, demonstrated that incomplete decellularization and residual matrix immunogenicity could lead to severe inflammatory responses and early structural failure [28].

These findings shifted the field toward cell-free TEHV strategies based on synthetic, xenogeneic, allogeneic, or fully engineered decellularized scaffolds. Although these platforms differ in material origin and biological behavior, they all seek to achieve the same objective: durable valve function through host-driven recellularization and remodeling after implantation. The following sections examine the translational performance of these platforms and the major barriers that continue to limit successful in situ valve regeneration.

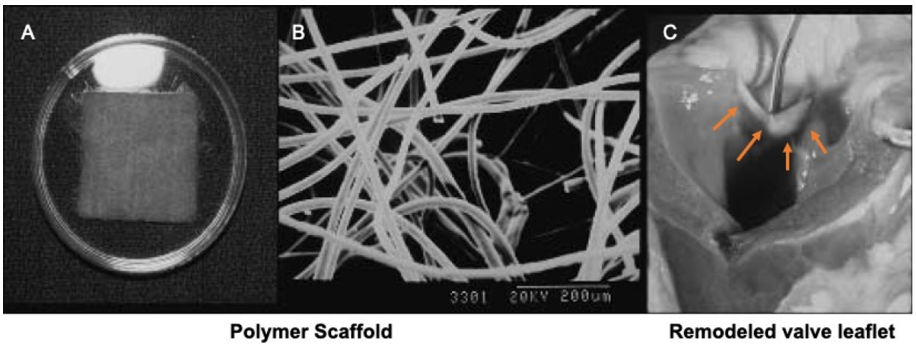

Fig. 1. Polymer scaffold and remodeled valve leaflet. (**A**) Macroscopic view of tissue-engineered fabric scaffold composed of polyglycolic acid (PGA) prior to implantation. (**B**) Scanning electron microscopy image demonstrating the fibrous architecture of the scaffold, with a porous network of randomly oriented polymer fibers. Scale bar: 200 μm. (**C**) Macroscopic view of the explanted valve leaflet after *in vivo* remodeling, showing tissue formation and leaflet morphology. Arrows indicate regions of neotissue development. (Reproduced with permission from reference 25, copyright 2002 Wiley) [25].

Fully Synthetic Biodegradable TEHVs

Fully synthetic biodegradable scaffolds represent an emerging platform for cell-free TEHVs, in which a resorbable polymeric implant provides initial mechanical function while serving as a temporary template for endogenous cell infiltration and in situ tissue formation. In this concept, the scaffold is not intended to persist long term, but rather to guide a host-driven regenerative process through progressive replacement of the material by newly formed ECM under physiological loading conditions.

Preclinical studies have demonstrated the feasibility of this platform. Kluin *et al*. [29] showed that a fully synthetic supramolecular elastomeric heart valve, based on bisurea-modified polycarbonate (PC-BU), implanted in the pulmonary position in sheep maintained functional performance for up to 12 months, while gradual endogenous cell infiltration and matrix deposition led to the formation of collagen- and elastin-containing neotissue. Similarly, Serruys *et al*. [30] evaluated a bioresorbable supramolecular elastomeric pulmonary valved conduit (Xeltis XPV) and demonstrated favorable hemodynamic performance up to 24 months, with low pressure gradients and preserved valve competence in most cases, accompanied by progressive cellular infiltration and ECM formation. Mechanistic analysis by De Kort *et al*. [31] further revealed that scaffold degradation, inflammation, and tissue formation occur in a highly heterogeneous and spatiotemporally variable manner, with collagen maturation occurring primarily between 6 and 12 months and marked variability across animals and within individual leaflets (Fig. 2).

Early clinical translation has also been reported. Prodan *et al*. [32] described the first-in-human implantation of the Xeltis bioabsorbable pulmonary valved conduit, made of poly-carbonate-based 2-ureido-4[1H]-pyrimidone (UPy), demonstrating procedural feasibility and acceptable early outcomes with two-year follow-up, although a subset of patients developed pulmonary insufficiency due to leaflet prolapse. As an additional proof-of-concept for minimally invasive delivery, Zakko *et al*. [33] demonstrated the feasibility of a fully synthetic transcatheter TEHV using electrospun polycaprolactone leaflets mounted on a bioabsorbable stent, with preserved valve function following percutaneous implantation in a fetal ovine model.

Other recent large-animal studies using microfibrous bioresorbable elastomeric scaffolds implanted as valve prostheses in the aortic position in sheep and followed for 12 months have demonstrated preserved valve integrity in some cases, but also leaflet thickening, retraction, and

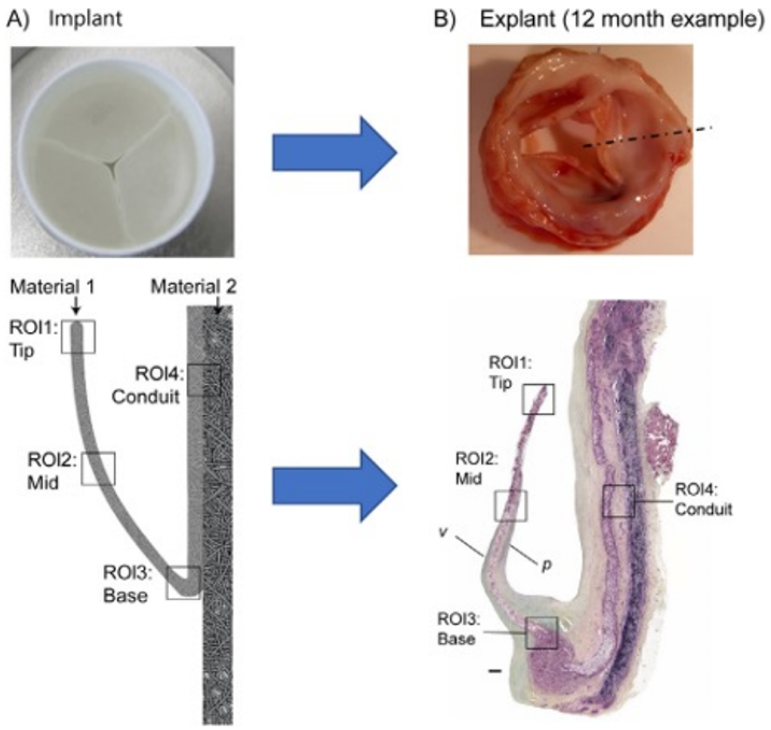

Fig. 2. Remodeling of a bioresorbable synthetic polymer valve following implantation. (**A**) Macroscopic view of the electrospun valved conduit at implantation, illustrating the composite scaffold design and regions of interest (ROIs) along the leaflet (tip, mid, base) and conduit. (**B**) Representative explanted valve at 12 months, demonstrating *in vivo* remodeling. Gross and histological images show progressive host cell infiltration originating from the conduit/base region, with migration toward the leaflet. Notable structural changes include leaflet shortening and thickening, as well as degradation of the polymer scaffold, particularly at the base region. Regions of interest corresponding to the tip, mid, base, and conduit are indicated. (Reproduced with permission from reference 31, copyright CC BY 4.0) [31].

structural deterioration in others [34]. Similarly, electrospun biodegradable polyester urethane urea (PECUU) scaffolds implanted as pulmonary leaflet replacements in sheep and evaluated over 1–12 weeks showed early host cell infiltration and matrix formation, but also progressive leaflet thickening and reduced leaflet motion over time [35]. Ascione *et al*. [36] evaluated styrene-block-ethylene/butylene-block-styrene (SEBS) polymer valve prototypes implanted in the mitral position in juvenile sheep under cardiopulmonary bypass, with 6-month follow-up demonstrating acceptable short-term safety and function, although long-term remodeling remains to be defined.

Despite these encouraging findings, important limitations have emerged. A consistent finding across preclinical and early clinical studies is the marked variability in how synthetic scaffolds degrade, recruit host cells, and remodel into functional valve tissue. Even when overall valve function is maintained, substantial heterogeneity persists in cellular infiltration, ECM organization, and leaflet structure [31]. Snyder *et al*. [37] recently reported persistent activation of valvular interstitial cell-like populations and sustained myofibroblast phenotypes after implantation of an electrospun polymeric scaffold in a sheep right ventricular outflow tract (RVOT) model. These findings suggested a role of non-immune factors in maladaptive remodeling.

In clinical settings, this variability can translate into functional complications such as leaflet prolapse and regurgitation, indicating that maintaining stable valve geometry during scaffold degradation remains a major challenge [32]. In addition, the remodeling process is strongly influenced by the host inflammatory response, which, while necessary for regeneration, can also lead to maladaptive outcomes if not properly regulated.

This platform highlights several key requirements for successful cell-free TEHVs. Scaffold design must provide not only initial mechanical competence but also geometric stability throughout degradation, particularly at the leaflet level. In addition, the host inflammatory response must be appropriately modulated, and tissue formation must be spatially and temporally controlled to achieve consistent ECM organization and durable valve function. These findings suggest that, although fully synthetic scaffolds demonstrate the feasibility of in situ valve regeneration, successful translation depends on tighter control over valve scaffold degradation, host inflammatory responses, tissue remodeling, and the preservation of valve geometry.

Decellularized Xenograft Valves

Decellularized xenogeneic valves represent one of the earliest cell-free TEHV strategies [38], but immune-mediated degeneration has remained a major limitation of this platform. In this approach, valves derived from animal tissues, most commonly porcine, are decellularized to remove immunogenic cellular components while preserving the native ECM architecture. These scaffolds were initially expected to overcome key limitations of conventional biological valve substitutes, particularly the lack of growth potential and regenerative capacity, and were therefore considered especially attractive for pediatric applications [39].

The SynerGraft® valve (CryoLife, Inc., Kennesaw, GA, USA) represents one of the earliest clinical applications of this concept. These grafts, composed of decellularized aortic composite grafts or pulmonary roots, were designed to reduce antigenicity while preserving a biologically instructive ECM capable of supporting host-cell repopulation after implantation. Early experimental and clinical experience initially suggested that decellularized xenografts could provide functional valve replacement and support *in vivo* repopulation.

However, early clinical failure soon raised major concerns in 2003. Simon *et al*. [28] reported rapid degeneration following implantation in four pediatric patients. Three patients died, including two with severe graft degeneration at 6 weeks and 1 year, and one due to graft rupture on postoperative day 7. The remaining patient underwent prophylactic explantation of the graft 2 days after implantation. Macroscopic and histological analyses revealed severe inflammation, structural deterioration, leaflet degeneration, and calcification, with a prominent foreign body reaction characterized by neutrophil and macrophage infiltration [28].

These findings suggested that decellularization alone was insufficient to prevent early inflammatory destruction of xenogeneic valve scaffolds. Because heart valve prostheses are exposed to substantial biomechanical stress immediately after implantation, such inflammatory injury may critically weaken the scaffold and accelerate structural failure [40]. Consistent with this interpretation, Rieder *et al*. [41] showed that decellularized porcine valve scaffolds retained the capacity to attract monocytes and inflammatory cells despite removal of donor cellular material, indicating persistent immunogenicity after implantation. Although Seebacher *et al*. [42] reported that decellularized porcine pulmonary valve conduits preserved favorable biomechanical strength and extensibility compared with cryopreserved homografts, these mechanical advantages alone did not resolve the biological limitations of xenograft scaffolds.

Subsequent iterations of decellularized xenograft valves sought to improve these outcomes. The Matrix P® bioprosthesis (AutoTissue GmbH, Berlin, Germany) was among the first decellularized porcine pulmonary heart valves to receive regulatory approval in Europe and become commercially available [43]. It is processed to remove xenogeneic cellular components from the ECM, thereby preserving an intact yet cell-free connective tissue scaffold [43]. In 2011, Konertz *et al*. [44] reported intermediate results with Matrix P in patients undergoing RVOT reconstruction, including 8.2% early mortality and 87% freedom from valve dysfunction at 3 years postoperatively. Additionally, computed tomography and magnetic resonance

imaging studies demonstrated normal structural features, with no evidence of calcification. However, long-term follow-up was less favorable. Christ *et al*. [43] reported high rates of reoperation and reintervention over 10 years following the Ross procedure using Matrix P prostheses, with frequent valve dysfunction, elevated pressure gradients, and a 10-year reoperation rate exceeding 40%. Similarly, Sabateen *et al*. [45] reported that freedom from catheter-based interventions at 9 years was limited to 64%. They attributed the limitations of this valve, at least in part, to the decellularization process itself, particularly the use of non-toxic methods intended to preserve ECM integrity. Notably, even successful recellularization did not prevent progressive structural deterioration, highlighting a fundamental limitation of this regenerative strategy.

Porcine small intestinal submucosa-extracellular matrix (pSIS-ECM; CorMatrix Cardiovascular, Inc., Roswell, GA, USA) was also investigated as a regenerative xenogeneic scaffold for valve repair and replacement [46]. By eliminating xenogeneic cellular components while preserving matrix architecture, this material was intended to promote host-cell infiltration, tissue remodeling, and eventual formation of a living valve substitute [46]. Early large animal and human clinical studies demonstrated favorable valve function [47–49], along with progressive endothelialization and constructive remodeling in a short-term porcine pulmonary artery (PA) implantation model [50].

However, these findings did not translate into durable performance. Miller *et al*. [50] evaluated pSIS-ECM pulmonary valve replacement (PVR) in a porcine model and reported that all animals developed at least moderate pulmonary regurgitation within 6 months despite ongoing remodeling. Additionally, Van Rijswijk *et al*. [46] demonstrated early failure of these valves used as custom-made valved conduits constructed from pSIS-ECM for pulmonary valve replacement in 10 lambs and 10 sheep with up to 6 months of follow-up (Fig. 3).

Within 2 months after surgery, five sheep and two lambs died of heart failure, and the explanted valve leaflets showed marked thickening with inflammatory changes. Among the animals that survived to the planned follow-up, one of five sheep developed severe regurgitation, whereas five of eight lambs exhibited significant pulmonary stenosis [46]. In clinical settings, the largest studies of CorMatrix used for valve repair have shown functional failure at short- and mid-term follow-up, with high reoperation rates and histologic evidence of inflammation, fibrosis, and calcification [51–53]. These findings indicate that avoidance of glutaraldehyde alone is insufficient to confer durable regenerative performance if the scaffold fails to support constructive remodeling and appropriate host-cell infiltration.

Another recent approach has focused on enhancing durability through chemical stabilization, such as pentagalloyl glucose (PGG)-treated decellularized bovine jugular vein (BJV) valved conduits implanted in the pulmonary position in sheep, which demonstrated preserved valve function and host cell repopulation *in vivo* [54].

To date, only a limited number of decellularized

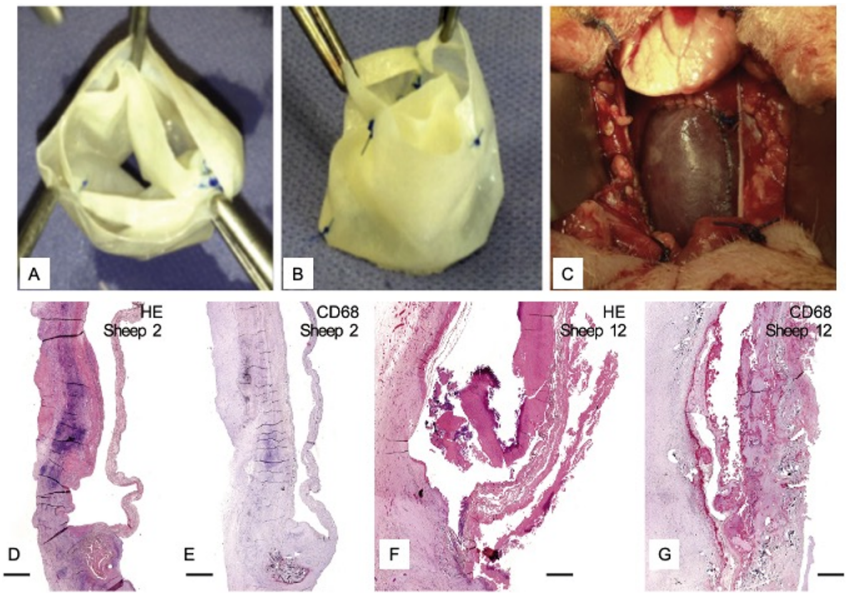

Fig. 3. *In vivo* performance and inflammatory response of a pSIS-ECM valve conduit. (**A**,**B**) pSIS-ECM valve conduit before implantation. (**C**) Implanted valve conduit. (**D**,**E**) Histology of explanted valves from sheep that survived to the planned follow-up. (**F**,**G**) Histology of valves from sheep that died before the planned follow-up (1 month post-implantation). (**D**,**F**) Hematoxylin and eosin (H&E) staining. (**E**,**G**) Scale bar = 1 mm. CD68 staining. Marked inflammatory infiltration is observed in the pSIS-ECM and surrounding tissues, predominantly consisting of neutrophils and $CD68^{+}$ macrophages. pSIS-ECM, porcine small intestinal submucosa-extracellular matrix. (Reproduced with permission from reference 46, copyright 2020 Elsevier) [46].

xenograft valves have reached clinical application, and their long-term durability has remained suboptimal, increasingly implicating immune-mediated mechanisms. The host immune response to xenogeneic valve scaffolds appears to involve both innate and adaptive immune pathways [55]. Human and Zilla *et al*. [56] demonstrated that graft-specific antibody responses may directly contribute to bioprosthetic calcification and subsequent valve degeneration. In particular, the cell-surface epitope galactose-α1,3-galactose (α-Gal), which is present on porcine valves [55,57], has been strongly implicated in xenograft valve degeneration [57]. At the same time, Helder *et al*. [55] proposed that although decellularization may remove much of the abundant α-Gal antigen, non-α-Gal antigens may persist and continue to trigger immune activation. These observations support the need for rigorous antigen testing and biological characterization of xenogeneic scaffolds prior to clinical application.

Overall, these findings suggest that preservation of native ECM alone is insufficient in decellularized xenograft valves if xenogeneic antigens persist and continue to drive immune-mediated degeneration. Although xenogeneic scaffolds can initially provide structural support and, in some cases, evidence of host-cell infiltration, their long-term performance remains fundamentally constrained by incomplete elimination of immunogenicity and the resulting failure to achieve stable, constructive remodeling.

Decellularized Allograft Valves

Decellularized allograft valves (Table 1) represent one of the most clinically advanced platforms for cell-free TEHVs. In this approach, donor-derived human valves are processed to remove cellular components while preserving the native ECM architecture, mechanical anisotropy, and layered leaflet structure. The fundamental concept is that this biologically derived scaffold can provide immediate physiological valve function while enabling host-mediated recellularization and matrix remodeling after implantation, thereby functioning as a living valve substitute without the need for pre-seeded cells.

Preclinical studies have demonstrated both the functional performance and regenerative potential of this platform. Quinn *et al*. [66] investigated detergent/enzyme–decellularized pulmonary allografts implanted in the right ventricular outflow tract of juvenile sheep and showed that, at 20 weeks, decellularized valves exhibited hemodynamic performance comparable to cryopreserved grafts, with minimal regurgitation and similar pressure gradients. Importantly, calcification was observed in cryopreserved and bioprosthetic grafts but not in decellularized valves, and histologic analysis demonstrated autologous recellularization of the arterial wall. Similarly, Van Hoof *et al*. [59] evaluated decellularized pulmonary valve allografts with subsequent cryopreservation in an ovine model and reported comparable transvalvular gradients and valve competence between groups at six months, but with reduced inflammatory infiltration, pannus formation, and calcification in decellularized grafts.

In a growing sheep model, Tudorache *et al*. [61] provided further evidence of long-term performance and regenerative capacity. Decellularized aortic allografts implanted in the systemic circulation maintained competent valve function for up to 20 months, with preservation of native three-layer leaflet architecture and repopulation by endothelial and interstitial cells. Notably, enlargement of the valve annulus during somatic growth suggested the potential for physiological adaptation of the implanted scaffold. Additional studies have demonstrated that host-mediated recellularization is not limited to juvenile environments. Theodoridis *et al*. [67] showed that decellularized pulmonary valves implanted in elderly sheep maintained adequate function with cellular repopulation of both the valve wall and cusps within 3–6 months. Complementary *in vitro* validation studies further confirmed that advanced decellularization protocols can achieve greater than 99% removal of donor DNA while preserving ECM components such as collagen, elastin, and glycosaminoglycans, without compromising biomechanical strength [69].

These preclinical findings have translated into substantial clinical experience, particularly in PVR. Sarikouch *et al*. [62] reported a decade-long experience with 131 decellularized pulmonary homografts (DPHs), demonstrating superior durability compared with cryopreserved homografts and BJV conduits, with 100% freedom from explantation at 10 years. The prospective ESPOIR Trial further supported these results, reporting low mortality (2.5%), stable hemodynamic performance, and 97.5% freedom from explantation or reintervention at 5 years in 121 patients [58]. Comparative analyses have similarly demonstrated improved long-term outcomes, including higher freedom from explantation and lower incidence of infective endocarditis compared with BJV conduits [65]. Favorable outcomes have also been reported in pediatric RVOT reconstruction, with high freedom from reintervention and minimal calcification [60], as well as in aortic valve replacement and more complex congenital procedures, where stable hemodynamics and preserved structural integrity were observed during follow-up [64,68]. However, not all studies have shown clear superiority, as meta-analyses have reported no significant differences in mortality, graft dysfunction, or reintervention rates compared with cryopreserved homografts at mid-term follow-up, indicating that long-term advantages remain uncertain [63].

Histologic and explant analyses provide important insight into the biological behavior of this platform after implantation. Sarikouch *et al*. [70] demonstrated substantial repopulation of decellularized valves by recipient-derived endothelial and mesenchymal cells, along with evidence of active ECM production, such as procollagen synthesis.

Table 1. Preclinical and clinical outcomes of decellularized allografts and homografts.

Reference	Model	Surgery type	Material	N	Follow-up duration	Key results
Bobylev *et al*. [58]	human	PVR	DPH	121 (trial), 319 (registry)	median 5.9 years (up to 15 years)	excellent outcomes; freedom from explantation 97.5%, superior to cryopreserved homografts
Van Hoof *et al*. [59]	sheep	PVR (RVOT)	decellularized vs cryopreserved allograft	19	6 months	similar hemodynamics; less inflammation, pannus, and calcification
da Costa *et al*. [60]	human	RVOT reconstruction	decellularized allograft	59	mean 5.4 years (up to 8 years)	low reintervention (90.9% freedom); minimal calcification
Tudorache *et al*. [61]	sheep	aortic valve replacement (Ross model)	decellularized aortic allograft	10	20 months	good function; repopulation and growth potential
Sarikouch *et al*. [62]	human	PVR	DPH	93	4.6 years (up to 10 years)	100% freedom from explantation at 10 years; superior durability
Ahmed *et al*. [63]	human meta-analysis	RVOT (Ross)	decellularized vs cryopreserved	1687	5–6 years	no significant difference in major outcomes
Bobylev *et al*. [64]	human	double semilunar valve replacement	decellularized homograft	5	median 31 months	no mortality; excellent function
Bobylev *et al*. [65]	human	PVR	decellularized vs BJV conduit	319 vs 319	10 years	higher freedom from explantation (95.5%)
Quinn *et al*. [66]	sheep	RVOT reconstruction	decellularized pulmonary allograft	18	20 weeks	no calcification; evidence of recellularization
Theodoridis *et al*. [67]	sheep (elderly)	PVR	decellularized pulmonary allograft	18	3–12 months	matrix-guided regeneration even in the elderly
da Costa *et al*. [68]	human	aortic valve replacement	decellularized aortic allograft	41	mean 19 months	low gradients; minimal calcification

PVR, pulmonary valve replacement; DPH, decellularized pulmonary homograft; RVOT, right ventricular outflow tract; BJV, bovine jugular vein.

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Early explant studies, such as that by Sayk *et al*. [71], showed initial infiltration of inflammatory cells, predominantly macrophages, representing an early phase of host-mediated remodeling without acute rejection. More detailed analyses have revealed that recellularization is spatially heterogeneous. Kugo *et al*. [72] reported that cellular repopulation was more prominent in the ventricularis layer and distal arterial wall, whereas the proximal root region remained relatively sparsely populated, suggesting that local hemodynamic conditions and matrix structure strongly influence host-cell infiltration patterns.

Despite these strengths, several important limitations remain. Although recellularization consistently occurs, it is often incomplete and uneven, particularly within the leaflet interstitium, which is essential for maintaining long-term structural integrity and function [70,72]. In addition, while decellularization reduces immunogenicity, it does not eliminate immune responses entirely, and low-grade inflammation may persist and contribute to progressive remodeling or degeneration over time [59,71]. Furthermore, the variability in long-term clinical outcomes and the lack of consistent superiority over conventional homografts suggest that decellularization alone may be insufficient to fully prevent structural valve deterioration [63].

In summary, decellularized allograft valves provide strong clinical proof-of-concept that cell-free TEHVs can achieve both functional performance and partial biological integration in humans. At the same time, this platform highlights several critical requirements for successful in situ valve regeneration. Preservation of native ECM architecture is essential for immediate function and for guiding host cell infiltration. However, passive host-driven recellularization is not sufficient to achieve uniform and functionally appropriate tissue regeneration, particularly within the leaflet interstitium. In addition, even reduced immune responses must be actively controlled to prevent maladaptive remodeling. These findings indicate that, although decellularized allografts represent the most clinically advanced realization of cell-free TEHVs, further refinement in scaffold design and biological control of recellularization is required to achieve consistent long-term durability.

Decellularized Fully Engineered TEHVs

Engineered decellularized TEHVs (Table 2) represent a hybrid platform that combines features of both synthetic scaffolds and biologically derived matrices. In this approach, tissue constructs are first generated *in vitro* by seeding cells onto biodegradable scaffolds to produce a structured ECM, followed by decellularization prior to implantation. The resulting "off-the-shelf" valve retains a biologically formed matrix with preserved architecture while eliminating the need for patient-specific cell sourcing and *in vitro* conditioning at the time of implantation [73–76]. The central concept is that this pre-formed matrix provides a more instructive microenvironment for host cell infiltration and remodeling compared with purely synthetic scaffolds, thereby enabling in situ regeneration while maintaining clinical practicality.

Extensive preclinical work has demonstrated both the feasibility and dynamic remodeling behavior of this platform. Early studies of engineered extracellular matrix–based valves, particularly those developed from fibrin- and fibroblast-derived matrices, demonstrated not only functional performance but also the capacity for somatic growth using a juvenile sheep PA implantation model with up to 52 weeks of follow-up, as evidenced by increases in valve diameter during follow-up along with extensive recellularization and minimal calcification [81].

Efforts have also been made to integrate engineered ECM valves with minimally invasive delivery systems. Stented decellularized tissue-engineered valves produced from vascular-derived cells cultured on biodegradable scaffolds and subsequently decellularized have also been implanted percutaneously in the pulmonary position of sheep, demonstrating preserved early hemodynamic performance together with rapid host-cell repopulation and active extracellular matrix remodeling [77]. Complementary studies using homologous "off-the-shelf" engineered valves have similarly demonstrated sustained valve function together with rapid recellularization and matrix remodeling, further supporting the concept that biologically pre-formed scaffolds can facilitate in situ regeneration.

This concept has also been extended to transcatheter valve platforms. Human cell–derived ECM TEHVs mounted on transcatheter stent systems have demonstrated feasible acute implantation in the aortic position, with preserved leaflet motion, no paravalvular leakage, and early host-cell infiltration into the scaffold [78]. More recently, incorporation of anatomically relevant sinus geometry into engineered tissue valves has been shown to improve physiologic leaflet motion and flow patterns, highlighting the importance of scaffold geometry in modulating biomechanical loading and valve durability [79] (Fig. 4).

Histological analyses across these studies provide important insight into the remodeling behavior of these constructs after implantation. Following implantation, host-cell infiltration occurs rapidly, with progressive recellularization of both leaflet and wall regions and increasing extracellular matrix deposition, including collagen and glycosaminoglycans, that in some studies approaches native tissue composition over time [77,80,81]. Endothelialization of the valve surface is also observed, indicating restoration of a functional blood-contacting interface. However, remodeling is not uniform, and regional differences in cellular infiltration and matrix organization are frequently observed, particularly between the wall and leaflet regions [77,80].

Table 2. Fully engineered decellularized TEHVs with preclinical and translational evaluation.

Reference	Valve concept	Cell source / Strategy	Scaffold / Material	Model	Follow-up	Key findings
Reimer *et al*. [76]	tubular TEHV	fibroblast-derived engineered ECM (decellularized)	fibrin-based engineered tissue tubes	growing lamb (pulmonary)	8 weeks	early functional performance; host cell infiltration and ECM deposition; failure due to leaflet shortening and commissural instability
Schmitt *et al*. [77]	percutaneous TEHV (stented)	*in vitro* engineered tissue → decellularized	PGA/P4HB-derived ECM	sheep (transcatheter pulmonary)	up to 6 months	successful percutaneous implantation; rapid recellularization and remodeling; progressive regurgitation due to suboptimal design
Lintas *et al*. [78]	human cell-derived TEHV (TAVR platform)	human cell-derived ECM (decellularized)	engineered ECM scaffold	sheep (transcatheter)	short-term	demonstrated feasibility of off-the-shelf human-derived TEHV for transcatheter delivery
Motta *et al*. [79]	TEHV with integrated Valsalva sinuses	human cell-derived ECM (decellularized)	engineered ECM with sinus geometry	sheep (transcatheter pulmonary)	Mid-term	Improved leaflet kinematics and more physiological valve geometry compared to earlier designs
Emmert *et al*. [80]	geometry-optimized TEHV	engineered homologous ECM (decellularized)	*in vitro* grown matrix (design-optimized)	sheep (aortic position)	1 year	sustained valve function with minimal leaflet contraction; demonstrates the importance of geometry in preventing remodeling failure
Syedain *et al*. [81]	tri-tube valved conduit	fibroblast-derived ECM (decellularized)	collagen-rich engineered matrix	growing lamb (pulmonary)	up to 52 weeks	demonstrated somatic growth, endothelialization, and sustained function; reduced calcification and improved durability

TEHV, tissue-engineered heart valve; PGA, polyglycolic acid; ECM, extracellular matrix; TAVR, transcatheter aortic valve replacement.

European Cells and Materials Vol.58 2026 (pages 136–157) DOI: 10.22203/eCM.v058a11

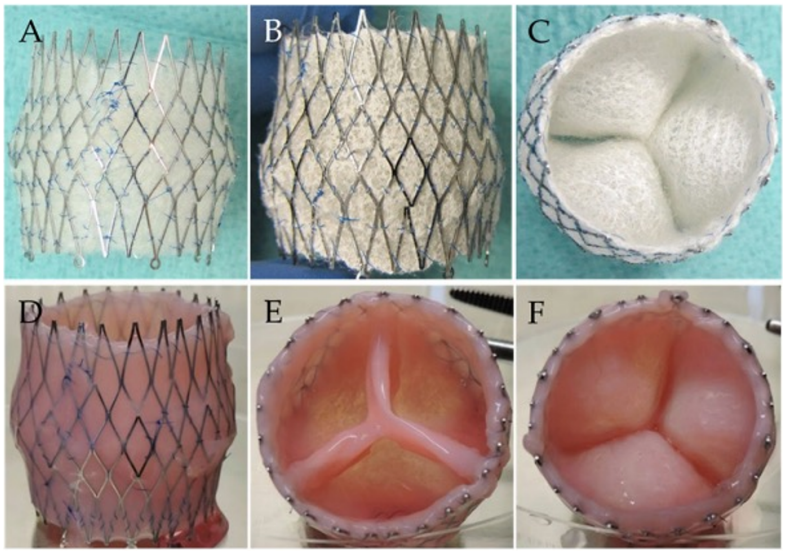

Fig. 4. Manufacturing process of a cell-derived decellularized tissue-engineered heart valve (TEHV) with integrated stent. (**A**–**C**) Polymer-based TEHV prior to tissue formation, showing a polyglycolic acid (PGA) scaffold integrated within a nitinol stent and coated with biodegradable polymer. (**D**,**E**) Cell-seeded and matured TEHV following *in vitro* conditioning, demonstrating dense and homogeneous tissue formation and development of valve leaflet structure. (**F**) Final decellularized TEHV, showing a smooth, tissue-derived leaflet architecture after removal of cellular components while preserving ECM structure. (Reproduced with permission from reference 79, copyright CC BY 4.0) [79].

Despite these favorable regenerative features, important functional limitations have emerged. Progressive deterioration in leaflet coaptation and increasing regurgitation over time have been consistently reported, often associated with structural remodeling such as leaflet shortening, hinge displacement, and tissue compaction [77,80]. These findings indicate that biologically active remodeling processes are not inherently coupled with preservation of valve geometry. Even in constructs demonstrating robust recellularization and matrix formation, uncontrolled or spatially heterogeneous remodeling can lead to functional valve failure.

Engineered decellularized TEHVs provide important insights into the requirements for successful cell-free valve regeneration. First, biologically derived ECM scaffolds can promote rapid host-cell repopulation and active tissue remodeling, supporting the importance of biologically instructive cues [77,80,81]. However, these studies demonstrate that recellularization and matrix formation alone are insufficient to ensure durable valve function [77,80]. Second, preservation of leaflet geometry, particularly at the hinge and coaptation regions, is essential and must be tightly coupled to the remodeling process. Third, spatial and temporal control of remodeling remains a major challenge, as heterogeneous tissue formation can lead to asymmetric deformation and loss of valve competence. These findings highlight that successful cell-free TEHVs require not only biologically active scaffolds but also precise control of geometry and remodeling dynamics to maintain long-term functional integrity.

Cell-Seeded TEHVs

Cell-seeded TEHVs (Table 3) represent the earliest and most intuitive strategy for creating living valve substitutes, based on the concept that pre-seeding scaffolds with cells prior to implantation can accelerate tissue formation and guide remodeling. In this approach, autologous or progenitor cells, such as endothelial cells, fibroblasts, or stem cells, are seeded onto biodegradable or decellularized scaffolds and often conditioned in bioreactors before implantation. The underlying rationale is that these cells will actively produce ECM, promote rapid endothelialization, and establish a functional, living tissue capable of growth and repair after implantation.

Extensive preclinical studies have demonstrated the feasibility of this strategy and provided insight into its biological behavior. Flanagan *et al*. [87] developed autologous fibrin-based TEHVs seeded with vascular-derived cells and conditioned under pulsatile flow prior to implantation in the pulmonary position of sheep. After three months, the valves remained structurally intact and exhibited extensive tissue remodeling, including endothelial coverage, mature collagen formation, and ingrowth of microvasculature within the tissue. The original fibrin scaffold was largely resorbed and replaced by newly synthesized ECM, indicating active tissue regeneration. However, leaflet contraction led to valve insufficiency, suggesting that cell-mediated remodeling can also drive adverse structural changes.

Table 3. Cell-seeded TEHVs: preclinical and clinical *in vivo* studies.

Reference	Valve concept	Cell source / Strategy	Scaffold	Model	Follow-up	Key findings
Tudorache *et al.* [82]	orthotopic aortic TEHV	autologous endothelial cells (bioreactor-conditioned)	decellularized allograft	sheep (aorta)	3 months	maintained valve function without degeneration; absence of calcification and inflammation compared to controls
Metzner *et al.* [83]	percutaneous TE valved stent	autologous ECs + myofibroblasts	xenograft + SIS scaffold	sheep (pulmonary, transcatheter)	4 weeks	functional valve performance with preserved leaflet motion; successful percutaneous deployment
Movileanu *et al.* [84]	living TEHV (layer-specific seeding)	ADSC-derived fibroblasts + endothelial cells	decellularized pulmonary valve	sheep (orthotopic pulmonary)	6 months	good hemodynamics and structural preservation; maintained leaflet integrity and endothelialization
Al Hussein *et al.* [85]	ADSC-seeded pulmonary TEHV	ADSC-seeded valves	decellularized scaffold	sheep (pulmonary)	6 months	no significant improvement vs decellularized valves in survival or complications; highlights limits of current seeding strategies
Dohmen *et al.* [86]	clinical TE pulmonary valve (Ross procedure)	autologous endothelial cells	decellularized allograft/xenograft	Human (clinical)	up to 5 years	excellent hemodynamics; no calcification; good functional status in most patients
Cebotari *et al.* [26]	pediatric TE pulmonary valve	autologous cell-seeded homograft	decellularized homograft	human (clinical)	mid-term	demonstrated growth potential and durability in pediatric patients

TEHV, tissue-engineered heart valve; SIS, small intestinal submucosa; ADSC, adipose-derived stem cell; ECs, endothelial cells.

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Other studies have evaluated reseeding of decellularized biological scaffolds. Tudorache *et al*. [82] demonstrated that decellularized ovine aortic valves reseeded with autologous endothelial cells functioned effectively in the systemic circulation, with preserved cusp mobility, minimal transvalvular gradients, and absence of calcification after implantation in sheep with 3-month follow-up. Similarly, Vincentelli *et al*. [88] investigated bone marrow–derived cell seeding in decellularized porcine pulmonary valves using a sheep PA replacement model with 4-month follow-up, and found that both mononuclear and mesenchymal stem cell (MSC)–seeded valves exhibited recellularization and endothelialization. Notably, MSC–seeded valves showed more organized ECM structure and lower pressure gradients, whereas mononuclear cell–seeded valves developed leaflet thickening and increased inflammatory infiltration, highlighting the importance of cell type selection in determining remodeling outcomes (Fig. 5).

Additional studies further illustrate both the potential and limitations of pre-seeding strategies. Kajbafzadeh *et al*. [89] demonstrated that decellularized aortic valve conduits pre-seeded with bone marrow–derived MSCs remained patent and showed extensive recellularization in a sheep model followed for up to 19 months. Although these findings support the capacity of pre-seeded constructs to undergo biological integration, the model involved conduit implantation in the descending thoracic aorta rather than orthotopic valve replacement, which limits direct interpretation of valve durability and remodeling under physiological leaflet loading.

From a scaffold design perspective, *in vitro* studies using layered collagen constructs highlight the importance of reproducing native valve microarchitecture. Tedder *et al*. [90] developed stem cell–seeded tri-layered collagen constructs mimicking fibrosa, spongiosa, and ventricularis layers, and showed high cell viability, elongation, and differentiation toward valvular interstitial cell–like phenotypes under bioreactor conditioning. These findings suggest that appropriate structural organization and mechanical cues may help direct seeded cell behavior toward physiologic remodeling, although *in vivo* validation remains necessary.

The feasibility of combining cell seeding with minimally invasive delivery has also been explored. Metzner *et al*. [83] developed autologous tissue-engineered valved stents by seeding endothelial cells and myofibroblasts onto scaffold-covered stents, followed by dynamic conditioning. These constructs were successfully implanted percutaneously into the pulmonary valve position in juvenile sheep. At four weeks of follow-up, most valves demonstrated normal leaflet motion and preserved valve function. Immunohistochemistry confirmed the presence of endothelial and interstitial cell-like phenotypes within the remodeled tissue, supporting the feasibility of combining tissue engineering with minimally invasive valve delivery.

Related strategies have also attempted to enhance endogenous cell recruitment rather than relying solely on con-

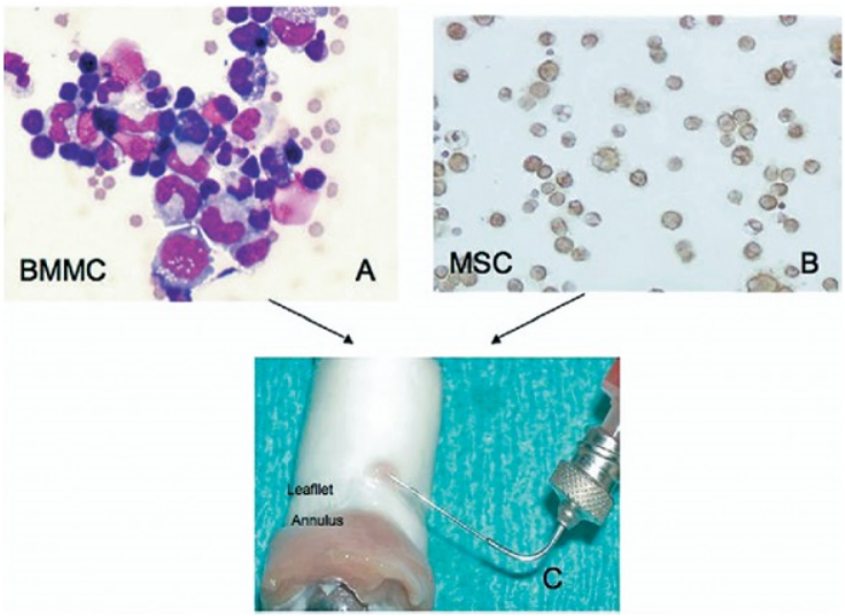

Fig. 5. Cell seeding strategy for TEHVs using bone marrow–derived cells. (**A**) Representative bone marrow mononuclear cells (BMMCs) following May–Giemsa staining. (**B**) Mesenchymal stem cells (MSCs) characterized by immunocytochemical staining. (**C**) Decellularized porcine pulmonary valve mounted on a dilator, demonstrating the cell seeding procedure. Cells are injected into the inner surface of the pulmonary arterial wall using a customized right-angled needle to create channels within the scaffold, facilitating cell distribution and infiltration. TEHVs, tissue-engineered heart valves. (Reproduced with permission from reference 88, copyright 2007 Elsevier) [88].

ventional pre-seeding. Jordan *et al*. [91] developed "self-seeding" heart valves by modifying decellularized scaffolds to capture circulating endothelial progenitor cells, and Chen *et al*. [92] reported that aptamer-based approaches can enhance endothelial progenitor cell adhesion and re-endothelialization of decellularized scaffolds. Although these studies are relevant to the broader goal of biologically guided valve remodeling, they are conceptually distinct from direct pre-seeding strategies. These approaches highlight a shift toward harnessing endogenous circulating cells to overcome limitations of direct seeding. In parallel, biohybrid strategies combining decellularized scaffolds with targeted reseeding have been explored in transcatheter settings. Koenig *et al*. [93] evaluated a decellularized and reseeded biohybrid valve for transcatheter aortic valve implantation in an *in vitro* model. Their study showed that the construct could be generated and conditioned to display physiologic behavior before crimping, with confluent endothelial and fibroblast-like cell layers and substantial ECM formation. However, crimping caused marked cell injury, and subsequent perfusion revealed loss of seeded cells and damaged cell layers, together with increased intercellular adhesion molecule (ICAM) expression suggestive of an inflammatory response. These findings support the promise of biohybrid, cell-seeded valves for transcatheter applications, while also demonstrating that currently used crimping procedures can substantially compromise seeded-cell viability and may limit translation of living TEHVs to TAVI settings.

Early clinical translation of cell-seeded TEHVs has also been reported. Dohmen *et al*. [86] evaluated decellularized pulmonary valves seeded with autologous endothelial cells for RVOT reconstruction. In 23 patients followed for up to five years, the valves demonstrated stable hemodynamic performance and absence of calcification on imaging in most cases, providing early clinical proof-of-concept for the feasibility of cell-seeded TEHVs.

Despite these promising results, important limitations have emerged. A major challenge is the difficulty in controlling cell behavior after implantation. Seeded cells exhibit variable survival, differentiation, and phenotypic stability, leading to unpredictable remodeling outcomes. In several studies, cell-mediated matrix remodeling resulted in leaflet contraction, thickening, or retraction, ultimately compromising valve function [87,88]. Importantly, even advanced preclinical models have demonstrated that the presence of seeded cells can contribute to fibrosis, cusp retraction, and valve insufficiency, reflecting maladaptive remodeling responses [84].

Another critical limitation is the uncertain added value of pre-seeding itself. Comparative studies have shown that acellular scaffolds can undergo rapid host-driven recellularization after implantation, raising questions about whether pre-seeded cells provide a consistent advantage in long-term remodeling. For example, Al Hussein *et al*. [85] reported no significant differences in operative parameters, postoperative complications, or six-month survival between decellularized valves with or without ADSC seeding in a sheep model. Similarly, recent preclinical work suggests that cell-seeded valves may perform comparably to acellular constructs *in vivo*, further supporting the importance of host-driven regeneration [84].

Furthermore, technical challenges related to cell delivery remain substantial. The role of bioreactor conditioning in overcoming these limitations remains debated; while dynamic conditioning can enhance ECM production and endothelialization, inappropriate or excessive mechanical loading may damage seeded cells and impair valve performance [94]. Conventional seeding methods often result in poor cell retention, with a significant proportion of cells lost immediately after implantation due to shear stress and blood flow [83,85]. In addition, maintaining cell viability within the scaffold is difficult due to limited nutrient diffusion and mechanical loading, further reducing the effectiveness of pre-seeding strategies [83,87]. These issues complicate the interpretation of *in vivo* outcomes, as it is often unclear whether observed tissue formation is mediated by the implanted cells or by host-derived repopulation [10,85].

Taken together, these findings indicate that cell-seeded TEHVs can influence early tissue formation, endothelialization, and matrix organization, but their long-term benefit remains difficult to define because remodeling outcomes remain variable and direct comparisons with acellular approaches have not shown consistent superiority. While host-driven recellularization can occur in acellular scaffolds, pre-seeding may offer a way to influence the initial cellular environment, particularly with respect to endothelialization and early matrix organization. However, whether this translates into consistent long-term functional benefit remains unresolved [10,12,18,85]. Importantly, these findings suggest that the key issue is not simply whether cells are seeded, but how early remodeling is controlled after implantation.

Overall, these insights refine the possible role of cell seeding: rather than simply adding cells to scaffolds, the more relevant question is whether early biological conditions can be engineered in a way that improves long-term valve remodeling and function. This perspective supports a more integrated framework in which cell seeding, scaffold design, and host response are considered together when evaluating how durable and physiologic tissue regeneration might be achieved [10,12,18,27].

Discussion

A central question in TEHV development is whether decellularization alone can generate a scaffold that not only preserves initial mechanical integrity but also supports durable biological remodeling after implantation. Decellularization is an attractive strategy because it creates an off-the-shelf scaffold while preserving key ECM architecture

and avoiding several practical limitations of cell-seeded TEHVs, including manufacturing complexity, storage difficulty, and regulatory burden [73,74,95]. However, the evidence reviewed here indicates that decellularized TEHVs do not yet achieve sufficiently consistent biological regeneration.

Across multiple studies, host-cell repopulation of decellularized valves is consistently observed, confirming that these scaffolds are not biologically inert [96,97]. However, recellularization is typically partial and regionally heterogeneous. Cellular infiltration is usually greatest in the wall, hinge, and blood-contacting surfaces, whereas the leaflet interstitium, particularly toward the distal free edge, remains relatively sparsely populated [96,98]. This is a critical limitation because long-term durability depends not only on endothelial coverage, but also on viable interstitial cells capable of maintaining matrix homeostasis and adapting the ECM to physiological loading conditions [96,97].

These findings suggest that the key limitation of decellularization is not the absence of host response, but the lack of sufficiently rapid, spatially organized, and biologically appropriate recellularization within the leaflet itself. *In vitro* studies reinforce this point by showing that even under controlled conditions, infiltration into dense leaflet matrices remains limited and is often confined to superficial or subsurface regions [99]. Even cell populations with relatively greater repopulation potential, such as mesenchymal stem cells compared with mononuclear cells or valvular interstitial cells (VICs), still require active seeding and environmental support for meaningful integration [98,100]. Thus, recellularization is not an automatic consequence of decellularization, but a biologically constrained process shaped by matrix architecture and local mechanical and biochemical conditions. These observations suggest that intrinsic properties of current scaffold designs may actively limit cell infiltration, particularly within the leaflet interstitium.

A consistent finding across these studies is the limited cellular infiltration into the leaflet interstitium of decellularized and synthetic TEHV scaffolds. This reflects not a lack of host response, but the presence of physicochemical and mechanobiological barriers that constrain interstitial cell migration. From a structural perspective, native valve leaflets consist of a dense and highly organized ECM with limited intrinsic porosity. Decellularization and subsequent processing may further reduce effective pore size and matrix permeability through collagen compaction and loss of glycosaminoglycans, creating an environment that restricts cell infiltration beyond superficial layers. Studies of three-dimensional cell migration demonstrate that pore size and matrix density are fundamental physical constraints, with reduced porosity significantly limiting cell invasion [101,102]. Mechanical properties also play a critical role. Effective cell migration requires a balance between matrix stiffness and degradability, whereas highly stiff or crosslinked matrices can resist cellular traction and limit remodeling. Matrix stiffness is a well-established regulator of cell behavior, influencing migration, spreading, and differentiation [103,104]. In valve leaflets, the requirement for mechanical durability under cyclic loading may therefore create a mechanically restrictive environment for interstitial repopulation. In addition, local hemodynamic conditions further limit infiltration. Valve leaflets are exposed to high shear stress and rapid cyclic deformation, which favor surface endothelialization but are less conducive to stable cell adhesion and subsequent migration into the matrix. Shear stress regulates endothelial behavior while also limiting attachment of circulating cells under flow [105]. Finally, diffusion limitations contribute to spatially heterogeneous recellularization. While endothelial coverage can be rapidly established at the surface, deeper regions of the leaflet lack vascular supply and are constrained by limited oxygen and nutrient diffusion, typically within 100–200 μm from the surface [106]. Taken together, these factors help explain why passive recellularization remains incomplete in current TEHV platforms and highlight the importance of scaffold designs that enable spatially appropriate cell infiltration under physiological conditions.

In this context, scaffold origin and design play a central role in determining how effectively these physicochemical and mechanobiological barriers can be addressed. Decellularized engineered tissues may permit deeper cellular infiltration and more active matrix remodeling than decellularized native valves, suggesting that the matrix composition and architecture strongly affect how effectively host cells colonize the scaffold [73,74]. In parallel, biofunctionalized and immunomodulatory scaffold strategies indicate that host responses can be steered toward more constructive remodeling without direct cell delivery [107]. Together, these observations suggest that successful cell-free TEHVs will depend not simply on decellularization, but on scaffold designs that actively promote appropriate host-cell recruitment, endothelialization, and interstitial remodeling.

Immune-driven remodeling represents a critical determinant of TEHV outcomes, where the trajectory of the host response, ranging from constructive remodeling to chronic inflammation, is largely dictated by macrophage polarization [10,21]. Although an initial M1-like inflammatory phase is essential for debris clearance and the initiation of tissue repair, successful and durable regeneration necessitates a timely transition toward an M2-like reparative phenotype [108,109]. Failure to complete this transition may result in persistent macrophage activation, predisposing the graft to foreign body reactions, fibrosis, calcification, and subsequent structural valve deterioration [108,110]. This immunological response is highly material-dependent: while ECM-derived scaffolds, such as pSIS-ECM, are designed to foster M2-like remodeling through bioactive degradation products [109,111], they may undergo uncontrolled protein infiltration and macrophage accumulation in

the valvular environment, leading to inflammatory thickening [112]. Conversely, fully synthetic biodegradable scaffolds offer the potential to modulate macrophage behavior through the optimization of fiber architecture, porosity, stiffness, and degradation kinetics [10,108]. Consequently, scaffold failure should be redefined not merely as insufficient recellularization, but as a failure to orchestrate macrophage-mediated remodeling toward an organized and functional tissue response [10,21].

The findings from "Cell-Seeded TEHVs" also make clear that the limitations of decellularized valves should not be interpreted as evidence that cell seeding is unnecessary. Rather, the comparison between cell-free and cell-seeded approaches suggests that the central challenge is not seeded versus unseeded valves, but how to achieve durable and biologically appropriate remodeling. Decellularized scaffolds reveal the regenerative capacity of the host, but also the limits of passive recellularization. Cell-seeded approaches show that the early biological environment can be shaped, but that this process is difficult to control [82–88,94]. Thus, decellularization alone is not sufficient if the goal is to reproducibly generate a living valve with appropriate leaflet cellularity, matrix homeostasis, and long-term adaptability.

Taken together, current evidence supports the view that decellularization is necessary but not sufficient for optimal TEHV regeneration. The future of decellularized TEHVs depends on moving beyond passive acellular scaffolds toward matrices that actively promote rapid, uniform, and functionally appropriate recellularization *in vivo*. The most promising direction is therefore not a purely cell-free paradigm, but an integrated regenerative strategy in which scaffold design, host response, and, where appropriate, targeted cellular support are coordinated to achieve durable valve function.

If decellularized TEHVs reveal the limitations of passive host-driven remodeling, cell-seeded TEHVs reveal the opportunities and the difficulties of actively shaping the early biological environment. Early studies established that implanted cells can contribute to ECM formation, endothelialization, and tissue organization, supporting the concept that living valve substitutes can be created through engineered cellular participation [13,25,82,83,86–90]. More recent work suggests that seeded cells may be most relevant during the early post-implantation phase, when the biological trajectory of the construct is being established. In this context, cell seeding should be viewed less as a way to "fill" a scaffold with cells and more as a strategy to initiate and regulate early remodeling.

However, the literature also makes clear that cell-seeded TEHVs face substantial biological and translational limitations. The major challenge is not simply cell survival, but control of cell behavior after implantation. Seeded cells may support constructive remodeling, but they may also promote fibrosis, cusp thickening, contraction, or leaflet retraction, leading to structural and functional deterioration [84,87,88,113]. These findings indicate that the problem is not the presence of cells themselves, but the lack of precise control over cell phenotype, persistence, and mechanobiological behavior within the scaffold.

This limitation is closely related to the unresolved question of cell source. Many TEHV studies have relied on fibroblasts, bone marrow mononuclear cells, MSCs, or endothelial cells because they are accessible and technically feasible [88–90,113]. Yet none fully reproduces the specialized biological functions of native VICs and valvular endothelial cells (VECs), which regulate ECM turnover, maintain leaflet structure, and respond dynamically to mechanical stimuli. Recent advances in stem cell biology, including protocols for deriving VIC- and VEC-like cells from induced pluripotent stem cells, offer a promising route toward more physiologically relevant and potentially patient-specific TEHVs [114]. Nevertheless, major challenges remain in achieving mature, stable, and reproducible phenotypes that behave appropriately after implantation.

Technical barriers further complicate the translation of cell-seeded approaches. Manufacturing typically requires cell harvesting, expansion, scaffold seeding, and prolonged conditioning, increasing variability, cost, and regulatory complexity [115]. Cell retention is often poor, and substantial cell loss may occur during implantation because of blood flow and shear stress [83,85,116,117]. Cell viability may also be compromised by limited nutrient diffusion and mechanical loading within the scaffold [83,87,117]. These challenges are even greater in transcatheter settings, where crimping and deployment can directly damage seeded cells and disrupt the engineered cellular surface [93]. Although bioreactor conditioning may improve tissue maturation and endothelialization, inappropriate conditioning can also damage cells or alter their phenotype unfavorably [94].

Another major unresolved issue is how to evaluate the true contribution of seeded cells *in vivo*. In large-animal models, implanted cells may be lost rapidly because of species mismatch, immune clearance, or inflammatory responses, making it difficult to determine whether later remodeling reflects persistence of the seeded population or replacement by host-derived cells [118–120]. Small-animal and immunodeficient models can clarify cell fate and host–graft interactions at higher mechanistic resolution [121–124], but they do not fully recapitulate the geometry, loading, and durability requirements of clinical valve replacement. As a result, the field still lacks an ideal framework for determining when, where, and how seeded cells meaningfully improve long-term valve remodeling.

There is also an important consideration that preclinical durability should be interpreted in the context of the animal model used. Young and adolescent sheep models are prone to rapid calcification and remodeling [125], which may lead to valve failure at a faster rate than in the human clinical setting. In contrast, porcine models offer

anatomical and physiological similarities to humans and may be useful for longer-term functional and structural assessment [126]. Goat models provide a practical platform for orthotopic valve implantation and for evaluating degeneration mechanisms such as macrophage accumulation and circulating protein infiltration [112]. However, none of these models fully reproduces human valve biology, immune response, growth pattern, calcification tendency, or long-term clinical durability. Thus, the results of TEHV studies should be interpreted with careful consideration of species-specific characteristics, age, growth status, hemodynamic position, remodeling pattern, and follow-up duration.

Despite these limitations, the current literature does not support dismissing cell seeding as unnecessary. Rather, it suggests that the role of cell seeding should be refined. Seeded cells may not need to remain the dominant long-term cellular population of the final valve, but they may still be important for establishing early endothelialization, guiding initial matrix organization, or modulating the host response in a favorable direction [82–88,91–94]. In this framework, cell-seeded and cell-free approaches should not be viewed as opposing paradigms, but as complementary strategies for solving the same core problem: how to generate a valve that not only functions mechanically at implantation, but also remodels into a biologically appropriate and durable living tissue. Efforts to improve preclinical relevance include the development of genetically engineered large-animal models, such as Gal-knockout sheep, designed to replicate human anti-Gal immune responses and provide a more clinically relevant platform for evaluating biological valve performance [127].

Overall, the most important lesson from the translational literature is that the field should move beyond the simplistic question of whether seeded or unseeded valves are superior. The central issue is how to control remodeling so that it becomes durable, spatially organized, and physiologically appropriate. Cell-free strategies reveal both the promise and the limits of passive host repopulation, whereas cell-seeded strategies reveal both the promise and the limits of actively shaping the early biological environment. Future progress will likely depend on integrating the strengths of both, including scaffolds with appropriate geometry, mechanics, and immunoregulatory properties, biologically relevant cells or cell-guiding signals, and experimental systems capable of defining how early remodeling events determine long-term valve performance. Such an integrated framework may offer the most realistic path toward truly regenerative and clinically durable heart valve replacements.

In this context, and in light of the limitations of current preclinical evaluation models discussed above, it remains possible that the regenerative potential of cell-seeded TEHVs has been incompletely characterized. Achieving a functional valve requires the simultaneous coordination of mechanical integrity, continuous dynamic loading, and spatially organized cellular infiltration conditions that are difficult to reproduce in existing experimental systems. Future efforts should therefore focus not only on scaffold optimization, but also on the development of experimental platforms that more accurately capture these coupled biological and mechanical processes. At the same time, the next generation of TEHVs will likely require instructive scaffold designs that actively guide host-driven regeneration while also enabling effective integration of biologically relevant cell populations. An ideal scaffold should combine controlled porosity and matrix architecture to permit interstitial cell infiltration, mechanical properties matched to the native valve to support physiological loading, and bioactive or immunomodulatory cues that promote constructive remodeling. Importantly, rather than replacing cell-based strategies, such approaches may provide a framework in which appropriately selected and delivered cells can function more effectively. With recent advances in cell sourcing, including induced pluripotent stem cell–derived valve-specific cell populations, re-evaluating cell-seeded TEHVs within optimized experimental frameworks may help clarify their true regenerative potential.

Conclusions

Current evidence indicates that neither cell-free nor cell-seeded TEHV strategies alone consistently achieve durable and functional valve regeneration. While cell-free approaches simplify translation and enable host-driven remodeling, they often result in incomplete and heterogeneous tissue formation. Conversely, cell-seeded strategies can enhance early tissue development but face challenges in cell retention, survival, and long-term control of remodeling. The central limitation across all approaches is the inability to reliably regulate the remodeling process. Future progress in TEHV development will depend on integrated strategies that combine optimized scaffold design with targeted biological modulation to achieve consistent, long-term valve function.

List of Abbreviations

TEHV, tissue-engineered heart valve; ECM, extracellular matrix; PGA, polyglycolic acid; SIS, small intestinal submucosa; pSIS, porcine small intestinal submucosa; VIC, valve interstitial cell; VEC, valve endothelial cell; MSC, mesenchymal stem cell; H&E, hematoxylin and eosin; RVOT, right ventricular outflow tract; PVR, pulmonary valve replacement; DPH, decellularized pulmonary homograft; BJV, bovine jugular vein; PA, pulmonary artery; ADSC, adipose-derived stem cell; TAVR, transcatheter aortic valve replacement; PC-BU, bis-urea-modified polycarbonate; PECUU, electrospun biodegradable polyester urethane urea; SEBS, styrene-block-ethylene/butylene-block-styrene; UPy, poly-carbonate-based 2-ureido-4[1H]-pyrimidone; α-Gal, galactose-α1,3-galactose; ECs, en-

dothelial cells; ICAM, intercellular adhesion molecule.

Availability of Data and Materials

Not applicable.

Author Contributions

TW and DO conceived and designed the study. TW and KN drafted the manuscript. DO supervised the study and critically revised the manuscript for important intellectual content. JM, AD, CM, SFH, SY, HK and TS reviewed and edited the manuscript. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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