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JACC REVIEW TOPIC OF THE WEEK

Designing Biocompatible Tissue Engineered Heart Valves In Situ

JACC Review Topic of the Week

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ABSTRACT

Valvular heart disease is a globally prevalent cause of morbidity and mortality, with both congenital and acquired clinical presentations. Tissue engineered heart valves (TEHVs) have the potential to radically shift the treatment landscape for valvular disease by functioning as life-long valve replacements that overcome the current limitations of bioprosthetic and mechanical valves. TEHVs are envisioned to meet these goals by functioning as bioinstructive scaffolds that guide the in situ generation of autologous valves capable of growth, repair, and remodeling within the patient. Despite their promise, clinical translation of in situ TEHVs has proven challenging largely because of the unpredictable and patient-specific nature of the TEHV and host interaction following implantation. In light of this challenge, we propose a framework for the development and clinical translation of biocompatible TEHVs, wherein the native valvular environment actively informs the valve's design parameters and sets the benchmarks by which it is functionally evaluated. (J Am Coll Cardiol 2023;81:994–1003) © 2023 by the American College of Cardiology Foundation.

Valvular heart disease is a frequent cause of morbidity and mortality, with an estimated global prevalence of 49 million patients affected by rheumatic heart disease and calcific aortic valve disease combined.[1] Heart valve replacements are a component of at least 10% of adult cardiac surgeries in the United States, with a trend of further growth largely because of the aging population.[2,3] In the pediatric population, pulmonary valve replacements are regularly performed to treat congenital heart defects requiring reconstruction of the right ventricular outflow tract.[4]

Currently, the standard of care is use of mechanical or bioprosthetic valves, both of which have limitations. Mechanical valves are thrombogenic, and therefore subject patients to lifelong anticoagulation,[5] whereas bioprosthetic valves have limited durability, demonstrating structural degradation or calcification within 15 to 20 years of implantation.[6] In addition, both valves are unable to grow and remodel within the patient. Therefore, pediatric patients with valve replacements must undergo multiple open-heart surgeries to replace outgrown valves.[4]

In situ tissue engineering of heart valves has emerged as a means of overcoming these limitations by guiding the generation of an autologous valve replacement at the site of the original valve. Two types of in situ tissue engineered heart valves (TEHVs) will be the focus of this review: 1) *polymer-based TEHVs*,

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HIGHLIGHTS

- Biocompatible TEHVs represent potential alternatives to prostheses for replacement of diseased valves.
- Valve design and prototyping should be informed by the native valve environment.
- A predictable host interface will facilitate clinical application of engineered valves.

which are bioresorbable and acellular[7-9]; and 2) *decellularized TEHVs* derived from in vitro-grown tissues or explanted xenografts, in the form of an extracellular matrix (ECM) scaffold.[10-12]

Despite their immense promise, the clinical translation of TEHVs has been challenged by the complexities inherent to designing a valve capable of meeting several, at times conflicting, goals over multiple spatial and temporal scales. Complexities are further exacerbated by the unpredictability of in situ responses following implantation, as well as the unique challenges associated with regulatory approval and clinical implementation.

Here, we propose a framework for designing biocompatible TEHVs, composed of 3 interdependent phases: 1) defining design parameters informed by the native valve; 2) functional prototype testing; and 3) preclinical and clinical evaluations. Underlying each phase is the primary goal of designing a TEHV capable of guiding the in situ generation of a native-like autologous valve, which meets the logistic and regulatory requirements for clinical translation (**Central Illustration**).

DESIGN SPECIFICATIONS

The design specifications for a biocompatible TEHV are informed by the native valve physiology—specifically, how the form of the valve influences function, from the level of the matrix composition to the macrostructure. Simultaneously, valvular disease provides critical context for designing TEHVs capable of anticipating and responding to pathologic stimuli in situ.

NATIVE CELL BEHAVIOR. There are 2 main cell types in the valve. *Valvular interstitial cells* (VICs), the predominant cells of the valve leaflet, are responsible for ECM synthesis during development and regeneration.[13,14] Throughout growth and remodeling, VICs demonstrate an activated, myofibroblastic phenotype, characterized by proliferation, apoptosis, and expression of α-smooth muscle actin.[15] During injury, VICs temporarily differentiate back into the activated phenotype and deposit ECM, remodeling leaflet tissue.[13,14] *Valvular endothelial cells* (VECs) line the outer surfaces of the valve leaflet, where they communicate with VICs to facilitate remodeling and nutrient transport, while preventing inflammation and thrombosis.[16]

ABBREVIATIONS AND ACRONYMS

ECM = extracellular matrix

TEHV = tissue engineered heart valve

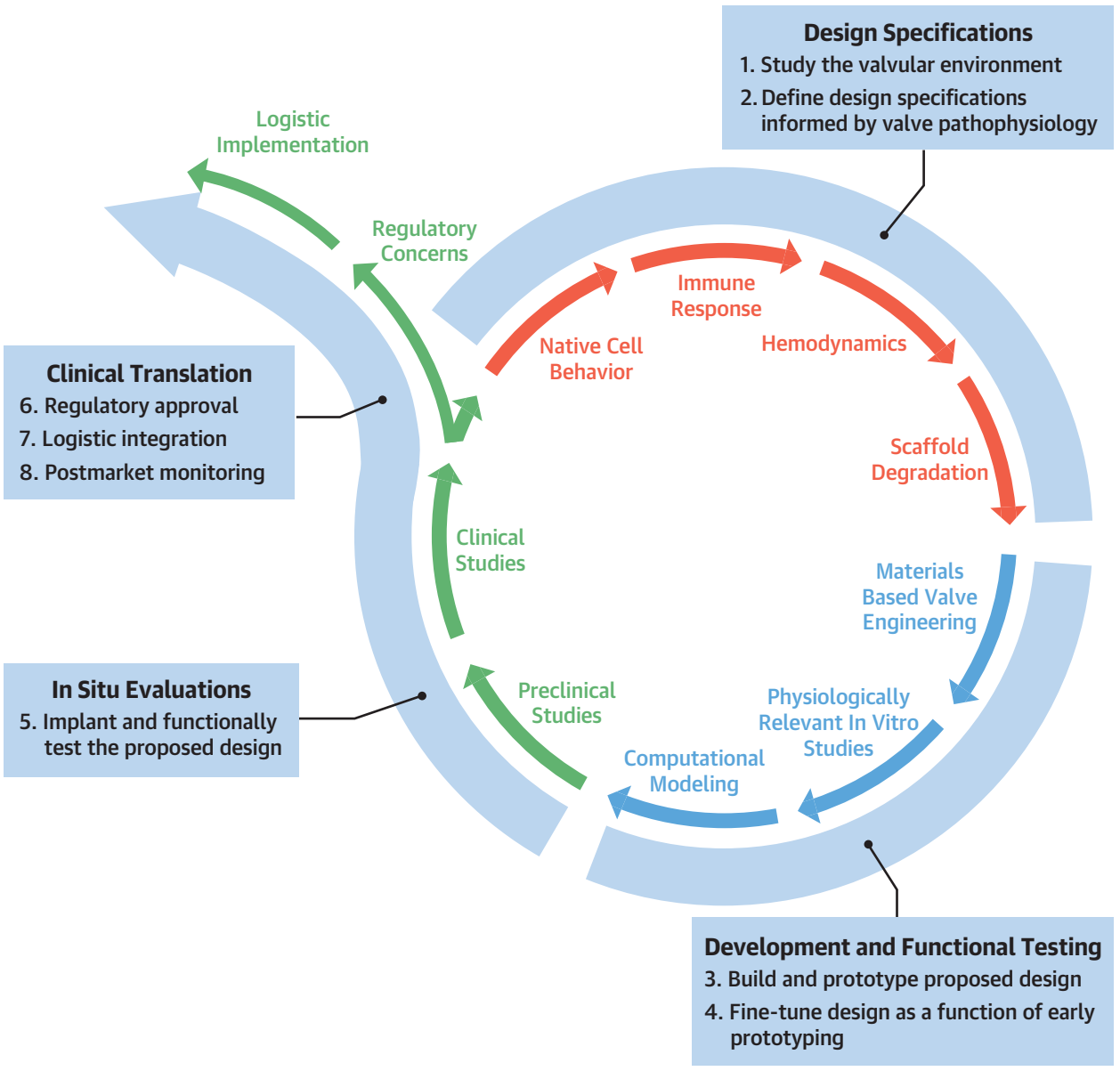
VEC = valvular endothelial cell

VIC = valvular interstitial cell

During remodeling, valve cells function as dynamic agents, processing their surroundings and generating appropriate responses: migration, proliferation, differentiation, ECM deposition, chemokine production, or release of remodeling enzymes. Depending on their environment, the cells may also output responses antagonistic to tissue homeostasis. For example, pathological VIC activation has consistently been associated with valve disease as it results in overexpression of catabolic enzymes, such as matrix metalloproteinases, and deposition of disorganized collagen fibers, creating fibrotic leaflet tissue.[17,18] Therefore, a central effort in valve tissue engineering has been to determine how environmental elements such as substrate stiffness,[19] loading patterns,[20] fiber architecture,[21] and the presence of ECM-like proteins[22,23] influence valve cell phenotypes.

IMMUNE RESPONSE. In situ tissue generation is heavily influenced by the host immune response. Although some inflammation is necessary for neotissue formation, the nature of the host immune response determines the emergence of chronic inflammation or successful tissue regeneration.[24] Studies have indicated that the host immune response and scaffold design are interdependent, with elements such as valve geometry,[25] microarchitecture,[26] and composition[27] altering the nature of this response. For example, scaffolds that induce macrophage elongation or secretion of anti-inflammatory cytokines have shown promise in promoting regenerative macrophage phenotypes.[28] Studies of vascular tissue engineering suggest that localized scaffold release of a monocyte chemoattractant within hours of implantation is associated with early leukocyte infiltration as well as more rapid neo-tissue formation and increased collagen alignment over the course of 3 months.[29] This result is promising for the potential efficacy of functionalizing TEHVs with instructive bioactive molecules, even if they are released within relatively short timeframes. The role of the inflammatory response in influencing fibrosis has similarly been shown in cardiac remodeling, where postinfarct implantation of a cardiomyocyte-laden patch is associated with improved left ventricular function compared with

CENTRAL ILLUSTRATION **A Framework for Developing Biocompatible Tissue Engineered Heart Valves**



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The development of tissue engineered valves can be envisioned as 3 interdependent phases: 1) defining design parameters **(red arrows);** 2) experimental and computational prototyping **(blue arrows);** and 3) evaluating proposed designs in preclinical and clinical studies **(green arrows),** ultimately resulting in clinical translation.

implantation of patches without cardiomyocytes, a finding attributed to cardiomyocyte-induced paracrine effects.[30] Taken together, these findings indicate that scaffold design informed by inflammatory responses can influence the host environment toward demonstrating a proregenerative immune reaction.[24]

HEMODYNAMICS. Especially important to in situ valve regeneration are hemodynamics, which influence mechanobiology by contributing to leaflet deformation. Physiologically, changes in host hemodynamics—eg, because of development from birth to adulthood—induce growth and remodeling in the cardiac valve, with the preservation of mechanical tissue homeostasis.[31] Supraphysiological cyclic stretch of the valve leaflets can induce increased expression of matrix remodeling enzymes such as

matrix metalloproteinases and cathepsins.[32] In vitro, VECs can be stimulated to undergo endothelial-to-mesenchymal transformation through cyclic strain. However, the signaling pathway responsible for endothelial-to-mesenchymal transformation is dependent on the level of strain, where increasing strain to 20% is associated with a VEC response mimicking that which occurs in pathological valve loading.[20] Learning from these processes is important for understanding how evolving hemodynamics throughout scaffold remodeling may influence valve cell behavior and associated neo-tissue production.

SCAFFOLD DEGRADATION. To preserve valve function throughout remodeling, TEHV scaffolds should degrade at the rate of neo-tissue generation to provide an isomorphous tissue replacement with preserved integrity. If the scaffold degrades too quickly, valve function can be lost, and the infiltrating cells lose a guiding template for regeneration. On the other hand, delayed scaffold degradation may elicit a sustained inflammatory reaction in vivo.[24]

The choice of TEHV material is critical for defining scaffold degradation kinetics. Scaffolds can be composed entirely from decellularized valve tissue grown in vitro[33] or from bioresorbable polymers, either natural (ie, collagen and fibrin) or synthetic (ie, aliphatic polyesters and polyhydroxyalkanoates).[34] A central advantage of polymer-based scaffolds is their ability to influence scaffold degradation via the scaffold's chemical properties.[24] Understanding the mechanisms underlying scaffold degradation in situ is of great importance, as these determine the anticipated rate and pattern of degradation (ie, surface vs bulk erosion),[35] and thereby affect the valve's mechanical and structural properties throughout tissue remodeling.

A difficulty intrinsic to in situ valve remodeling is reliably predicting the spatial and temporal kinetics of scaffold degradation. Computational modeling has contributed to understanding how cell-mediated remodeling affects valve geometry and mechanics.[36] Nevertheless, patient-specific hemodynamics, immune response to the scaffold, as well as cell recruitment and behavior all play roles in determining the scaffold degradation kinetics. And, vice versa, scaffold degradation may concomitantly affect valve cell phenotype and ECM deposition.[37] Therefore, methods of serially evaluating the balance between scaffold degradation and neo-tissue formation, and correlating this with valve function, are needed to understand and predict how this balance functionally influences TEHV performance.[38] This would help answer the critical questions: What patterns of scaffold remodeling lend themselves to preserved TEHV function? And, what are acceptable benchmarks for heterogeneity throughout remodeling?

DEVELOPMENT AND FUNCTIONAL TESTING

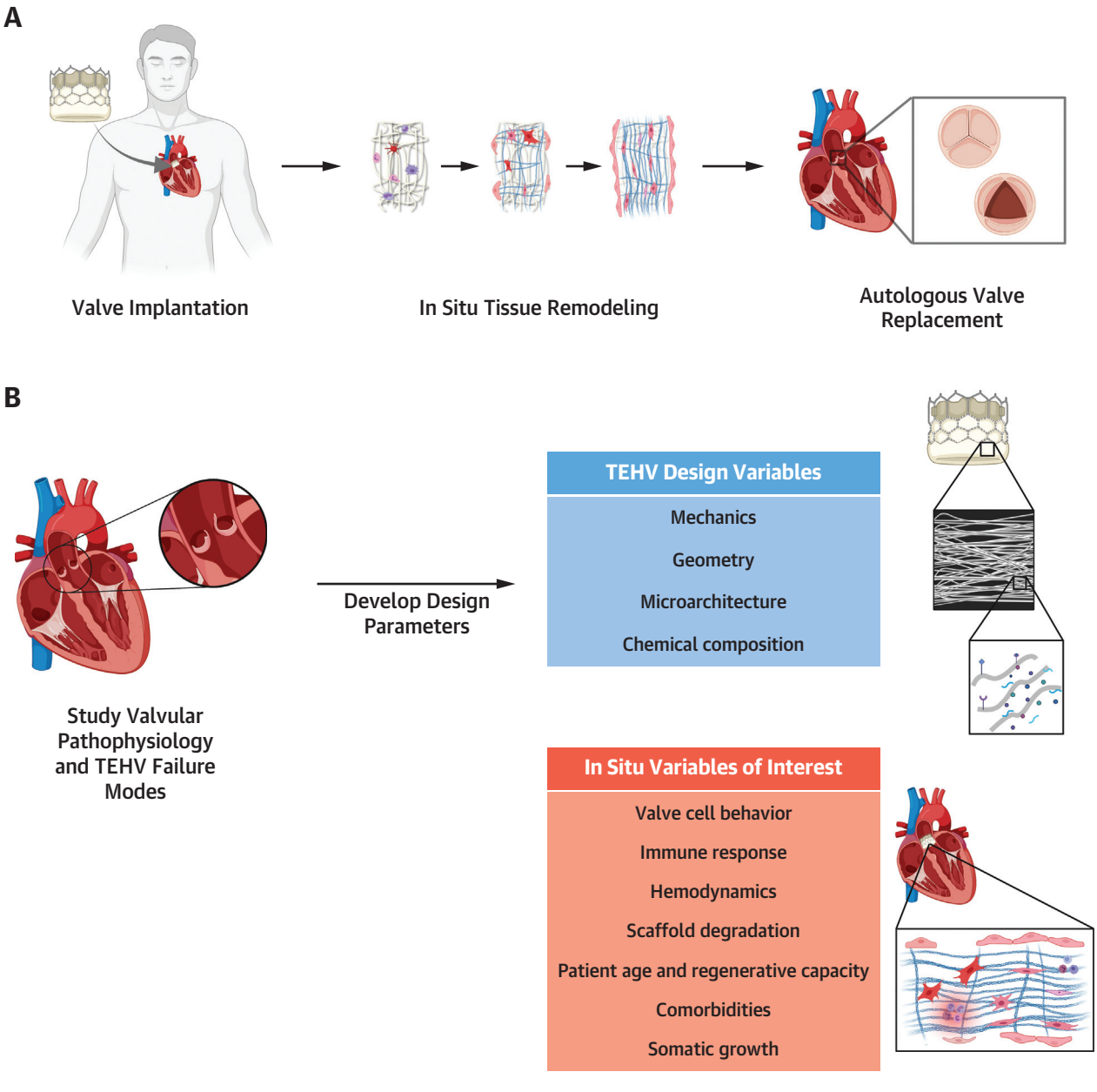
One of the greatest complexities of heart valve tissue engineering is the interplay between several parameters over multiple spatial and temporal scales. Interdependence of the valve's design specifications raises questions about their interactive effects. For example, how do changes to the valve microarchitecture influence the mechanical properties? How do shifts in the mechanical properties affect load-bearing behavior and subsequent valve cell phenotype? And, how do the valve phenotype and the subsequent remodeling influence, in turn, the microarchitecture? Such a circle can be either virtuous or vicious, depending on the molecular, mechanical, and architectural features of the scaffold and their constant interaction.

MATERIALS-BASED VALVE ENGINEERING. In creating a fibrous scaffold suitable for in situ valve regeneration, the ideal polymer would be elastomeric, with anisotropic stress-strain behavior similar to that of the native leaflet. It would also be fatigue-resistant, bioresorbable, and nonthrombogenic.[34,39] Further developments in polymer science would facilitate increased control over scaffold degradation mechanisms and immune response, allowing for the fabrication of scaffolds designed to meet patient-specific regenerative capacity.[40]

It is important to consider how synthetic and natural polymers can be bioinstructive.[41,42] Scaffold architecture, composition, and functionalization with bioactive molecules can all contribute toward cell recruitment and behavior.[24] The fibrous scaffold microarchitecture is of great importance here. First, the valve leaflet's trilayered architecture contributes to its unique combination of tensile strength and flexibility, while providing instructive cues during development, healing, and homeostasis.[43] Second, the scaffold microarchitecture directs neo-tissue architecture by contact-guiding infiltrating cells toward the desired configuration.[44]

Decellularized TEHVs are bioinstructive in their use of extracellular matrix as a biomimetic substrate, which is intended to promote cell infiltration and homogeneous remodeling. Two prominent fabrication approaches are as follows: 1) in vitro engineering of valves in mechanically conditioning bioreactors (Hoerstrup and Emmert team); and 2) the development of valves constructed from tissue tubes

FIGURE 1 Heart Valve Pathophysiology Informs the Design of Tissue Engineered Valves



(A) In situ heart valve tissue engineering begins with the implantation of the valvular scaffold, followed by remodeling, resulting in the generation of an autologous valve replacement **(arrows). (B)** Tissue engineered heart valve (TEHV) design involves variables at the valve, leaflet, and matrix levels, which together support heart valve generation. Although design variables influence TEHV outcome, they can be altered by in situ factors, such as age, tissue viability, comorbidities, and somatic growth.

(Tranquillo group), both of which have demonstrated preserved performance up to 1 year in vivo.[10,12]

PHYSIOLOGICALLY RELEVANT IN VITRO STUDIES. Directing tissue formation in vivo via scaffold design cues is challenging because of competing environmental stimuli—rendering postimplant valvular remodeling the most unpredictable aspect of TEHVs. Environmental elements can be patient-specific, can be heterogeneous, and can override guiding cues integrated into the scaffold, therefore highlighting the need for physiologically relevant TEHV prototyping (Figure 1). It is currently possible to evaluate TEHV function (before implantation) through the use of "pulse-duplicating" bioreactors recapitulating native

hemodynamics. In developing the next generation of TEHV prototyping methods, the remodeling process should be accounted for. Methods for integrating hemodynamic valve function testing with "live" tissue generation would facilitate real-time studies of valve remodeling, illuminating the interactions among valve cell phenotype, tissue formation, and valvular function over spatial and temporal scales. Circulating biological factors responsible for remodeling, such as macrophages, or well-recognized inflammatory cytokines like transforming growth factor-β, can also be incorporated.

Importantly, a patient's biological response to the TEHV may be influenced by elements such as age or comorbidities. Subcutaneous scaffold implantation may be a proxy for host immune response, recellularization, and scaffold degradation, thereby informing TEHV design.[45] However, such an invasive test would be impractical to administer in humans as a surrogate for individual patients' biological responses. Moving forward, tissues-on-a-chip may present a noninvasive means of predicting the rate and quality of neo-tissue formation in a patient-specific fashion by harnessing stem cell-derived VICs and VECs toward generating individualized models.

COMPUTATIONAL MODELING. Computational models are particularly promising in advancing TEHV design, enabling quantitative evaluations of how design parameters (such as valve geometry and fiber architecture[46]), and environmental elements (such as cell-mediated remodeling[36]) influence neo-tissue formation. In achieving clinical translation of TEHVs, computational models will become increasingly valuable in predicting how environmental stimuli interface with scaffold design, especially with regard to directing tissue regeneration at the cellular level (as reviewed by Loerakker et al[47]). Design testing should consider the effects of ECM synthesis—importantly, the spatial and temporal dynamics of collagen deposition,[36] which have implications for tissue anisotropy, compaction, and load-bearing. Taken together, integrating computational techniques into the TEHV development pipeline represents a unique means of studying the host/scaffold interface, while facilitating high-throughput isolation of design parameters most suited towards valve regeneration (**Figure 2**).[10]

IN SITU EVALUATIONS

Although in vitro and computational evaluations provide a platform for designing and prototyping TEHVs, the host environment plays a critical role in directing the outcome of scaffold remodeling.[48] Achieving long-term physiological valve tissue generation thus requires rigorously evaluating TEHVs in situ and applying outcomes toward further refining scaffold design.

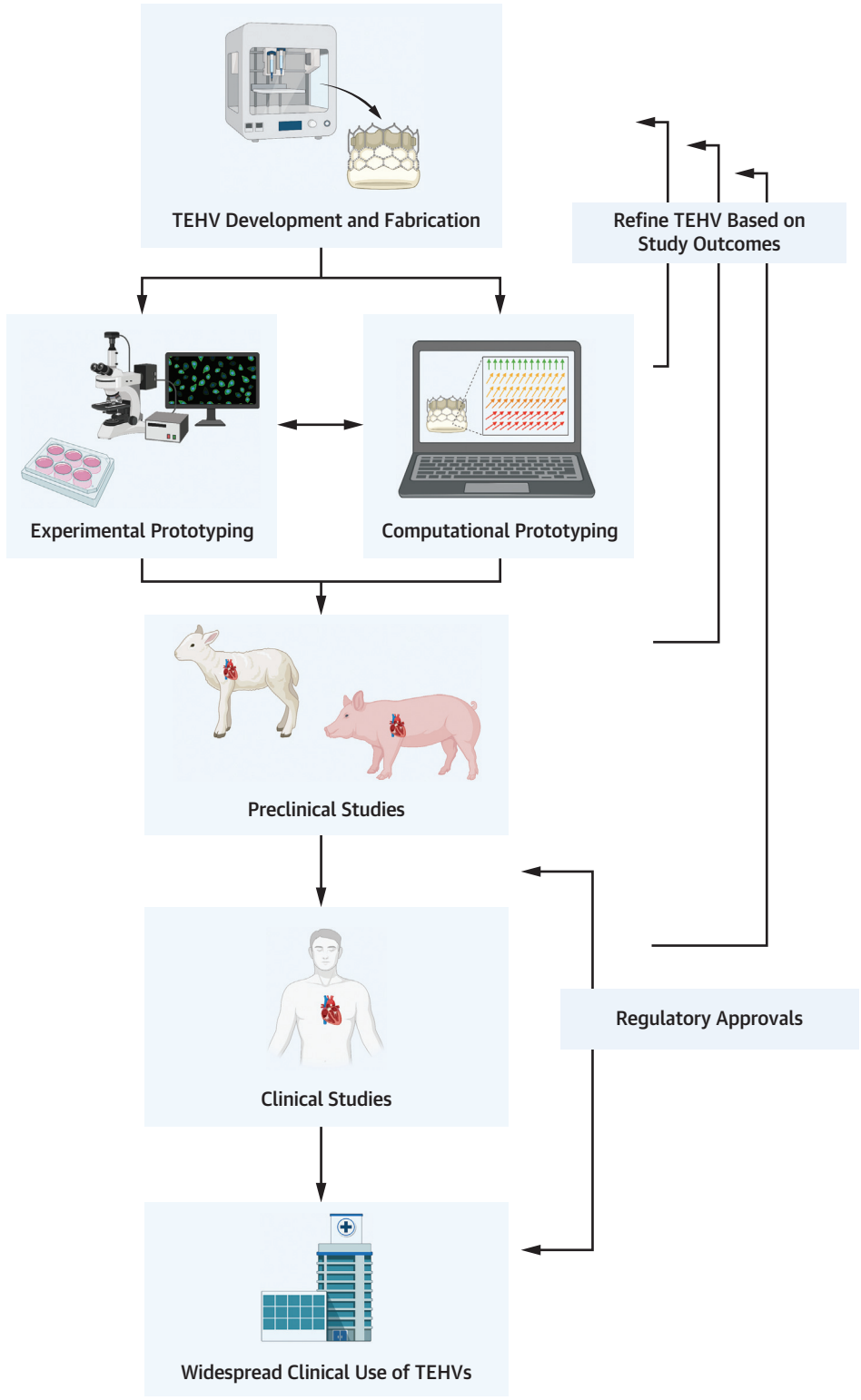
PRECLINICAL STUDIES. To date, the majority of preclinical TEHV evaluations have been carried out in porcine and ovine models,[7,8,10,12] which have proven pivotal in assessing valve validity (safety and efficacy). A prime example has been the Emmert and Hoerstrup team's preclinical evaluations of a "second-generation" TEHV, featuring a physiological leaflet geometry with increased belly curvature and a coaptation area intended to anticipate in situ leaflet shortening. In ovine models, the second-generation designs demonstrated reductions in leaflet thickening and preserved valve function for 1 year, as well as reduced α-smooth muscle actin expression and increased endothelization of the hinge region.[10,25] These results were indicative of a relatively proregenerative biological response, and were observed as early as 8-weeks follow-up, possibly indicating that early host responses strongly influence long-term outcomes.[25]

Preclinical evaluations also inform scaffold design (**Figure 2**). For example, the Tranquillo group's preclinical evaluations of a tubular heart valve in a growing lamb model led to a revised design capable of overcoming the original valve's primary failure modes. This updated design improved commissure stability by shifting load to a tube surrounding the valve, and successfully functioned as a pulmonary valve in a growing lamb model for up to 52 weeks, outperforming clinically used bioprosthetic valves.[12]

Taken together, these examples demonstrate preclinical studies' essential role in providing functional readouts characterizing the TEHV-host interaction over time. Moving forward, preclinical studies should function as a testing-ground for the development of surrogate markers that enable live evaluation of host response to the scaffold (ie, imaging or biomarkers), which, in combination with current clinical readouts, would be capable of assessing and predicting the quality of valve tissue generation.[49] Some examples may include serum inflammatory markers, cytokine and antibody generation, and advanced imaging (ie, strain echocardiography, 4-dimensional cardiac magnetic resonance, positron emission tomography for visualization of cell metabolism, or integration of specialized contrast agents).

CLINICAL STUDIES. To date, the Xeltis valve is the only in situ TEHV to undergo in-human trials. The first trial illuminated a primary failure mode of

FIGURE 2 Development and Prototyping of TEHVs



Achieving clinical translation of in situ tissue engineered heart valves (TEHVs) involves continuous scaffold design development and prototyping. Although stages of development are sequential **(downward arrows),** each provides functional readouts that are applied toward fine-tuning the original design **(upward arrows).**

pulmonary valve regurgitation in the setting of leaflet prolapse, prompting a re-evaluation of leaflet design. Informed by computational techniques, the valve's fatigue resistance was enhanced by introducing a homogenous leaflet thickness. The new design was validated in vitro, leading to the second clinical trial, which demonstrated largely retained valvular function over the course of 1 year.[9] Significant improvements in outcomes between the first and second clinical studies highlights how dynamic valve development can work synergistically with ongoing functional tests to improve clinical outcomes.[9]

CLINICAL TRANSLATION

To translate research findings to the clinical setting, it is essential to consider the quality control objectives, regulatory hurdles, and logistic challenges inherent to TEHV commercialization.

REGULATORY CONCERNS. A central objective is establishing rigorous quality control metrics for TEHV fabrication. Of particular importance is creating quality control standards for reducing interbatch variability, especially for in situ decellularized TEHVs, where cell behavior and culture conditions influence the final product. Good manufacturing practices[50,51] provide a foundation for these requirements, which should include standardized fabrication protocols and equipment (ie, regulated tissue culture, bioreactors, and polymer synthesis). Following fabrication, functional metrics should be assessed (response to fatigue testing, hemodynamic testing, and biocompatibility), in tandem with characterization of the scaffold's architecture (anisotropy, porosity, fiber diameter) and composition.

At present, the lack of centralized regulatory requirements for the clinical approval of TEHVs remains a primary challenge to commercialization.[50] Exacerbating this challenge, the approval pipeline for TEHVs differs between countries.[50] International Organization for Standardization guidelines provide a foundation for synchronizing regulations, but must be adapted to account for complexities inherent to tissue engineering (most prominently, dynamic remodeling within a patient).[50] Before valve implantation, these adaptations would likely involve evaluating the patient's regenerative capacity, projected immune response, and candidacy for alternative interventions. Following implantation, requirements may take the form of standardized follow-up protocols, with clinically informed functional benchmarks and predetermined interventions if a valve fails to meet these.[38,50] Clinical studies are essential in continuously refining these requirements. Trial design should be tailored to specific TEHVs, both in terms of population selection (ie, faster-degrading scaffolds for the pediatric population) and readouts (ie, focus on antigenicity for decellularized scaffolds).

LOGISTIC IMPLEMENTATION. Well-defined strategies for the logistic integration of TEHVs will greatly improve efforts toward clinical translation. Here, clinical use of valvular xenografts and homografts provides a model for the preparation, storage, and distribution of TEHVs. This would entail dedicated fabrication and tissue culture centers, with standard operating procedures for TEHV processing, sterilization, and storage. To enhance clinical availability, valves should be stored "off-the-shelf" and accessible on-site for selection at the time of intervention.

Polymer-based valves (such as Xeltis) are exceptionally well-poised for clinical translation given their relatively rapid manufacturing, off-the-shelf availability, and ease of storage. Polymeric scaffolds lend themselves to mass customization; valves can be designed in a wide range of sizes and fabricated from polymers with degradation profiles intended to meet patient-specific regenerative capacity. Unlike bioprosthetic grafts or decellularized TEHVs, their acellular nature renders them free from concerns of antigenicity.

Although polymeric TEHVs have demonstrated functionality in ovine models up to 24 months,[52] considerable questions remain regarding heterogeneity in neo-tissue formation within and between patients as the original scaffold degrades, especially given differences in age, genetics, and comorbidities.[38,49,53] To meet this challenge, the spatial and temporal kinetics of scaffold degradation can be visually monitored in situ by integrating specialized contrast agents[54] or nanoparticles[55] into the TEHV polymer. This information would contribute toward developing clinical predictors of remodeling and regenerative capacity, a possible prerequisite to the widespread clinical translation of polymeric TEHVs.

Decellularized TEHVs have been anticipated to decrease remodeling heterogeneity.[56] These scaffolds have the advantage of acting as a homogeneous "starter-matrix" for remodeling, thereby reducing the risk of a prolonged inflammatory response to residual polymer.[56] However, there are logistical challenges to their clinical translation. Tissues are cultured for 1 to 2 months, highlighting the significant time, resources, and labor required to engineer decellularized

valves. This limits scalability, especially when bioreactors are necessary for tissue maturation. Fabrication of decellularized TEHVs requires rigorously standardizing tissue-culture protocols—including cell sources and culture reagents. This reduces opportunities to customize scaffold mechanical properties or degradation kinetics to accommodate specific patients. Nevertheless, unintended variability remains a possibility caused by unpredictable cellular behavior during in vitro tissue engineering. Moreover, immunogenicity (from in vitro- or xenograft-derived tissue) may unexpectedly hamper healthy neo-tissue generation.

Taken together, in evaluating approaches for TEHV development, primary success criteria can be envisioned as the following: 1) development of a scaffold capable of instructing predictable and reproducible tissue generation; 2) minimization of requirements for patient-specific scaffold customization; and 3) practicality of clinical implementation (elements such as cost, storage, ease-of-use, and scalability).

Following clinical translation, TEHVs would have widespread utility—especially for patients with congenital heart disease, where freedom from valve replacement failure can be only months.[57] Critical risk factors for valve graft failure are younger age at implantation and smaller graft size, highlighting the urgent need to develop a replacement capable of remodeling and growing. Preclinically, TEHVs have proven capable of this, improving freedom from valve dysfunction.[12] Although it remains to be seen whether these results are recapitulated long-term in humans, the Xeltis trials underscore TEHVs' feasibility and safety and, importantly, their capacity for integration into the clinical sphere.

CONCLUSIONS

TEHVs have the potential to dramatically improve treatments for valve disease, acting as life-long valve replacements that can grow and remodel. Designing a TEHV capable of achieving long-term functionality in situ requires a design process actively guided by valve pathophysiology, coupled with rigorous prototyping. The form and function of the native valve inform TEHV design and establish benchmarks for assessing valvular function. Unifying each phase of this design framework is the primary goal of creating TEHVs that respond to in situ stimuli to ultimately instruct the formation of an autologous valve replacement.

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