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REVIEW

Off-the-shelf tissue engineered heart valves for *in situ* regeneration: current state, challenges and future directions

Sarah E. Motta[a*], Valentina Lintas[a*], Emanuela S. Fioretta[a], Simon P. Hoerstrup[a,b] and Maximilian Y. Emmert[a,b,c]

[a]Institute for Regenerative Medicine (IREM), University of Zurich, Zurich, Switzerland; [b]Wyss Translational Center Zurich, University and ETH Zurich, Zurich, Switzerland; [c]Heart Center Zurich, University Hospital Zurich, Zurich, Switzerland

ABSTRACT

Introduction: Transcatheter aortic valve replacement (TAVR) is continuously evolving and is expected to surpass surgical valve implantation in the near future. Combining durable valve substitutes with minimally invasive implantation techniques might increase the clinical relevance of this therapeutic option for younger patient populations. Tissue engineering offers the possibility to create tissue engineered heart valves (TEHVs) with regenerative and self-repair capacities which may overcome the pitfalls of current TAVR prostheses.

Areas covered: This review focuses on off-the-shelf TEHVs which rely on a clinically-relevant *in situ* tissue engineering approach and which have already advanced into preclinical or first-in-human investigation.

Expert commentary: Among the off-the-shelf *in situ* TEHVs reported in literature, the vast majority covers pulmonary valve substitutes, and only few are combined with transcatheter implantation technologies. Hence, further innovations should include the development of transcatheter tissue engineered aortic valve substitutes, which would considerably increase the clinical relevance of such prostheses.

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1. Introduction

Congenital or acquired heart valve diseases and defects, such as valvular stenosis and valvular insufficiency, are nowadays treated with advanced procedures like valve repair or valve replacement. The treatment option depends on the type and severity of the valve disorder, and in case of severe aortic valve diseases, heart valve replacement represents the standard of care [1]. Current valve implantation techniques comprise open-heart surgery and transcatheter aortic valve replacement (TAVR). While the former is considered the preferred choice, the latter has tremendously advanced in the past years to treat patients with severe aortic valve stenosis ineligible or considered at high risk for surgical valve replacement [2,3]. TAVR options are less invasive for the patients and offer quicker recovery requiring shorter hospitalization than conventional open-heart surgery [4]. Recently, these techniques were shown to provide comparable or even superior results to surgical valve replacement, with regard to survival rate, cardiovascular mortality, complications, functionality, and hemodynamics [4–7]. In light of these advantages, TAVR has also been used to treat aortic valve regurgitation [7].

Today's therapeutic options for open-heart valve substitution include mechanical and bioprosthetic replacements. Mechanical prostheses have longer durability but impose lifelong anticoagulation medication to prevent the thromboembolic effects associated to their relatively nonphysiological hemodynamics [8]. Bioprostheses are manufactured from glutaraldehyde-fixed xenografts (i.e. porcine heart valves/cusps and porcine or bovine pericardium) and can better reproduce the physiological hemodynamics. However, despite the fixation treatment performed to reduce their immunogenicity, bioprostheses are intrinsically associated to calcific degeneration and structural failure within 10–15 years after implantation. The structural valve failure and onset of micro-calcific nodules have been shown to be linked with chronic inflammation induced by residual xenogeneic epitopes within the bioprostheses [9]. This leads to cell differentiation toward osteoblastic-like phenotypes and expression of pro-inflammatory molecules, driving long-term tissue degeneration [9]. Hence, multiple re-interventions may be required during patients' lifetime [10], and therefore bioprostheses are contraindicated in younger patient populations.

Valve substitutes currently used for TAVR consist of crimpable stented valvular grafts, manufactured with glutaraldehyde-fixed bioprostheses, similar to the ones used for surgical valve replacement. Therefore, even if the currently available data for TAVR are short- to mid-term follow-ups (<10 years), and little is known about the durability of TAVR prostheses [5], it is reasonable to assume that the long-term failure mode of TAVR will be prevalently calcification and structural degeneration [11], as these complications are peculiarly inherent to the used bioprostheses. Due to their limited durability, bioprostheses, including also all TAVR devices, are

CONTACT Maximilian Y. Emmert maximilian.emmert@usz.ch
*These authors contributed equally to this article.

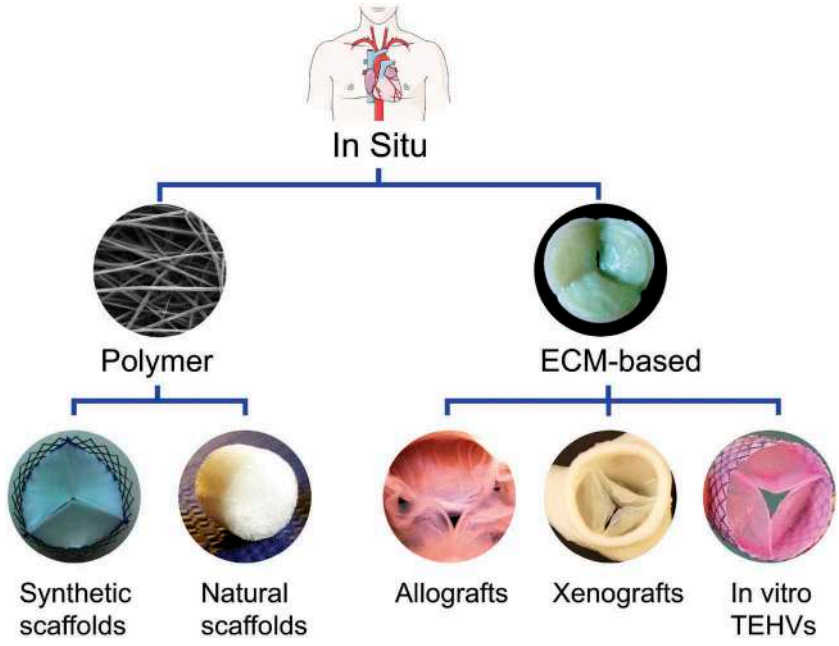

Figure1. Overview of the different approaches for *in situ* TEHVs. Polymeric (image adapted from www.rci.rutgers.edu) and ECM-derived TEHVs (image adapted from Schmidt et al. [32]) represent the two main families available for *in situ* TEHV regeneration. From these two classes different materials and approaches give rise to the several prostheses subtypes. Biodegradable polymeric TEHVs can be made with either synthetic or natural (image adapted from www.cellecbiotek.com) scaffolds. ECM-derived substitutes can be divided into decellularized allografts (image adapted from Jana et al. [33]), decellularized xenografts (image adapted from Hopkins [34]), and *in vitro*-grown TEHVs.

currently employed preferably in old patients. Therefore, in order to extend TAVR to lower-risk and younger patient populations (<60 years old), the need for next-generation prostheses with regenerative and self-repair properties, which can last for the patient's lifetime, has become of pivotal importance [12].

Alternatively to bioprosthetic or mechanical valves, synthetic nonbiodegradable heart valves for surgical or catheter-based techniques have been explored since the 1950s, but have not been included in clinical routine [13]. Among the various approaches reported in literature, recent studies concerning the *in vivo* performance of poly(carbonate urea) [14] or poly(tetrafluoroethylene)-based [15] valves showed controversial outcomes, mainly because of their tendency to calcify, stiffen, or undergo mechanical failure. Poly(urethane) surgical valves, when implanted *in vivo* in the ovine or porcine model, experienced thrombus formation, mild calcification, cusp thickening, and regurgitation [16–18]. Conversely, poly(styrene-b-isobutylene-b-styrene) valves, initially reported to undergo calcification and thrombus formation in the sheep [19], have been recently improved and showed reduced thrombogenicity and promising hemodynamic profiles [20]. However, in order to optimize the material resistance to *in vivo* deterioration and thus reduce the risk for structural failure, future studies should investigate novel strategies to improve the material stability and avoid excessive material degradation *in vivo*.

To resume, each class of the clinically available heart valve prostheses has peculiar advantages and drawbacks. However, all current valve substitutes share a common disadvantage, as they lack the capacity to repair, remodel, and grow, therefore requiring multiple reoperations or interventions throughout the patient's lifetime. Indeed, apart from acute functionality upon implantation and resistance to acute thromboembolic events, an ideal valve replacement should offer such important characteristics lifelong. To achieve unlimited durability, the implant should preserve the delicate hemodynamic and mechanical environment of the native valve [21], as any deviation from the physiological loading yields to a plethora of complications, including thrombi, pannus formation, infections, calcification, and structural failure [22]. As reviewed elsewhere, a growing body of evidence suggests that together with molecular mechanisms, physical cues modulate immune and inflammatory pathways, and that pathophysiological mechanical stimuli yield negative tissue remodeling and trigger inflammation [23].

Tissue engineering (TE) offers the possibility of creating biocompatible living valve substitutes with regenerative and self-repair potential that may overcome the shortcomings of the current clinically used prostheses [24]. The classical TE paradigm combines three cardinal players, namely autologous cells, biodegradable scaffolds, and bioreactors, for the production of *in vitro*-grown, patient-specific organ substitutes [25]. Besides this traditional dogma, various alternative methods have been developed to reduce the production costs/time and permit on-the-fly available prostheses. Among the many approaches for producing tissue engineered heart valves (TEHVs) reported in literature and reviewed elsewhere [24], acellular off-the-shelf TEHVs are of particular interest, due to their abundant availability and scalability when compared to classical *in vitro* TE concepts [24,26]. Such approaches rely on the *in situ* TE notion, which has emerged as a potential technique to regenerate TEHVs *in vivo* directly at the site of implantation, exploiting the natural regenerative potential of the human body [27,28]. In

Table 1. Tissue engineered heart valve studies under clinical or preclinical investigation.

Category	Material	Model	Position	Time point	Major results	Limitations	Reference
Polymeric	Fibrin-based	*In vitro*	Pulmonary	4 weeks	Reduced tissue shrinkage	Limited extracellular matrix (ECM) synthesis; No elastin fibers	[41]
	Bis-urea-modified poly(carbonate)	Sheep	Pulmonary	1–12 months	Sustained functionality; Remodeling with layered collagen and elastic fibers	Incomplete scaffold reabsorption	[31]
	Polyester-urethanes with ureidopyrimidinone supramolecular binding motif	Sheep	Pulmonary and aortic	1–24 months	Good hemodynamics	Incomplete scaffold reabsorption	[44,46]
	60/40 P4HB/Gelatin solutions with PGA	Sheep	Pulmonary	15 hours	Compatible with transcatheter techniques; Good functionality	Incomplete cellular infiltration	[48]
ECM-Derived	Decellularized Allografts	Human	Pulmonary and aortic	1–10 years	Good short-term functionality	Unknown long-term functionality; controversial recellularization	[58–61]
	Non-fixed cell-free Xenografts	Human	Pulmonary	6 weeks to 1 year	Death	Severe immune response	[66]
	Non-fixed cell-free Xenografts	Sheep	Pulmonary and aortic	1–4 months	Good hemodynamics; Complete recellularization	Risk for immune reaction	[65]
	In vitro derived ECM-based TEHVs	Sheep	Pulmonary and aortic	4–24 weeks	Good functionality; Good hemodynamics; Good cellular infiltration	Leaflet shortening; Unknown long-term functionality	[68,69,72–74,76,77]

particular, *in situ* TE aims at restoring the native tissue function and structure by providing the optimal microenvironment necessary to recruit host cells, then maintain their physiological phenotype, migration, and remodeling properties [29], and ensure long-term tissue homeostasis [26].

In this context, the scaffold is not a trivial component, since it has to sustain biomechanical conditions and biologically promote host cell repopulation and subsequent *in situ* tissue remodeling [26]. Ideally, the scaffold material for *in situ* TEHVs should not only be bioresorbable and biocompatible but also antithrombogenic and able to withstand the hemodynamic loading [24]. To date, synthetic polymers, biological sources, or a combination of both (the so-called hybrid scaffolds), and decellularized extracellular matrix (ECM)-derived scaffolds are currently under investigation in order to determine the most favorable material for the development of next-generation off-the-shelf available valve substitutes [30]. In this review, we summarize and critically compare the different off-the-shelf TEHV concepts for *in situ* regeneration with regard to their preclinical performance, their potential and challenges for clinical translation, and, where available, first experiences in the clinical setting (Figure 1, Table 1) [31–34].

2. Biodegradable polymeric scaffolds

Scaffolds based on biodegradable natural, synthetic (artificial), or hybrid polymers, are an interesting choice for the development of valves for *in situ* TE. The main advantage of using biodegradable polymers is the possibility to easily tune mechanical properties, degradation rate, and scaffold architecture to match the specific tissue of interest and to provide a tailored environmental niche to endogenous cells [24]. Moreover, the easy and rapid manufacture procedure, unlimited supply, and scalability properties make biodegradable polymers convenient materials to be investigated [24].

2.1. Natural polymers

Natural polymers (mainly in the form of hydrogels) exploit components of the native ECM as starting materials (e.g. collagen [35], fibrin [36], hyaluronic acid [37], chitosan [38], and gelatin [39]) and are, therefore, attractive for TE applications. As reviewed elsewhere, properties like mechanical stability, controlled porosity, degradation rate, and mass transport are currently under *in vitro* evaluation by several research groups [38]. So far, collagen- and fibrin-based acellular polymers seem to be the most promising substitutes for TEHV application. Indeed, several *in vitro* studies demonstrate controlled degradation, excellent cell retention upon seeding, optimal cell infiltration, and good tissue development [38,40,41]. However, so far cell-free TEHVs based on these materials are neither in preclinical nor clinical studies, due to their low mechanical strength, high thrombogenicity, and cell-mediated shrinkage [38].

2.2. Synthetic polymers

Synthetic bioresorbable heart valves are mainly made of biodegradable (i.e. naturally dissolving) polymers (e.g. poly(glycolic acid) (PGA)/poly(lactic acid) (PLA) [42], poly (hydroxyalkanoate) [43], and bis-urea-modified poly(carbonate) (PC-BU) [31]), which are able to be naturally absorbed

by the human body and finally metabolized [44]. Such scaffolds are attractive for their reproducibility, fast production, controlled and tailored properties, and inherent off-the-shelf availability [26]. Moreover, combining synthetic scaffolds with the *in situ* TE approach can lead to patient-specific functionalized immunomodulatory valve substitutes, inducing endogenous tissue regeneration by controlling the inflammatory response upon implantation [26]. Bioresorbable valves based on a supramolecular elastomeric polymer (PC-BU), compatible with both surgical and transcatheter implantation techniques, maintained functionality up to 12 months as pulmonary valve replacements in an ovine model [31]. In this proof-of-concept study, cell-driven remodeling gradually replaced the scaffold with newly synthetized layered ECM, containing elastic fibers, glycosaminoglycans, and collagen. Considering the complexity of the valve anatomy and hemodynamics, it is not surprising that only few of these polymeric valves reached preclinical investigation.

However, and remarkably, vascular conduits based on a similar technology using poly-caprolactone with 2-ureido-4 [1H]-pyrimidinone as scaffold material have already advanced into clinical application. In this clinical pilot trial, such conduits were used as cavo-pulmonary shunts in five human patients with severe cardiac congenital malformations and displayed a good safety profile without the occurrence of adverse events at 12-month follow-up [45]. Moreover, just recently, additional data using the same technology became available demonstrating further evidence of ongoing tissue remodeling at 2-year follow-up in the pulmonary position in sheep [44], and good acute hemodynamic performance as aortic valve replacement in an ovine model [46].

Notwithstanding these encouraging results, it is to mention that due to the limited experience on long-term performance of such synthetic materials *in vivo* (as TEHVs or conduits), there is still poor understanding of the effects of polymer degradation, remodeling mechanisms, and infiltrating cell phenotypes [30]. Longer-term follow-up studies will also help to investigate their ability to grow with the recipient.

2.3. Hybrid polymeric scaffolds

Hybrid scaffolds are made of synthetic polymers (e.g. poly (glycerol sebacate):poly(caprolactone) [47], PGA/poly(4-hydroxybutyrate) (P4HB) [48], poly(ethylene glycol)-diacrylate [49]) combined with natural compounds (e.g. methacrylated hyaluronic acid [47], gelatin [47,48], alginate [49]), mainly manufactured via electrospinning or 3D bioprinting [30]. Ideally, their tissue organization mimics the tri-layered architecture and mechanical properties of native heart valves. Theoretically, contrarily to purely synthetic biodegradable or natural polymeric scaffolds alone, the hybrid materials efficiently preserve the needed mechanical support while improving the distribution of cells, by combining the positive properties of both approaches [50]. Recently, a cell-free manufacturing technique for the production of biomimetic pulmonary valve replacement has been developed [48]. The valves were produced starting from synthetic polymer/protein composites of 60/40 P4HB/Gelatin solutions with the addition of PGA, and finally jet spinned over a customized mandrel using the so-called jet spinning technology. The tunable composition, together with tailored mechanical properties and scaffold architecture, allowed the rapid production of reproducible and scalable valves, which have the potential to mimic their native counterparts [48]. Moreover, this feasibility study demonstrated that jet spinned valves are suitable for minimally invasive pulmonary valve replacement [48]. However, due to the packed fibers organization, the cell infiltration of these constructs has demonstrated debatable outcomes [30]. Therefore, long-term studies should clarify the cell repopulation and remodeling potential of such technologies.

Another advantage of these hybrid starter matrices is the possibility to include functionalization and loading of bioactive components to improve polymeric scaffolds performance by favoring host cell repopulation and tissue formation [26]. These treatments might promote the modulation of the evoked inflammatory response toward a positive cellular and tissue fate [28]. A proof-of-principle *in vitro* study investigated poly(ethylene glycol) dimethacrylate-poly(lactic acid) for hybrid off-the-shelf TEHVs with promising results in terms of mechanical properties and cells viability under static conditions [51]. Upon seeding with valvular endothelial and interstitial cells on the polymer surface, good proliferative and metabolic activity was achieved. In the same study, the proof-of-concept functionalization of these TEHVs with collagen type-1 gel and versican was reported. However, long-term *in vivo* evaluation is required to assess the regenerative potential of these off-the-shelf TEHVs when exposed to the complex physiological hemodynamic environment.

3. ECM-derived scaffolds

ECM-derived scaffolds are based on matrices containing native mature ECM [52] or *in vitro* synthetized matrices which mimic native tissue features [53]. The advantages of such scaffolds comprise biocompatibility, physiological-like mechanical properties, and native-like structure. Within this review, we will describe three different types of cell-free ECM-derived scaffolds: decellularized allografts (human-derived), not-fixed cell-free xenografts (animal-derived), and decellularized *in vitro*-derived ECM-based TEHVs.

3.1. Decellularized allografts

Human heart valve allografts still represent a valid valve substitute [54]. Allogeneic heart valve transplantation started over 50 years ago and revealed optimal properties like hemodynamics, high resistance to infections (e.g. endocarditis), reduced thromboembolic risks, and limited immunogenic reactions when compared to mechanical or biological prostheses [54,55]. In order to reduce the immune response, allografts in preclinical and clinical trials are decellularized in a first step and then cryopreserved until further use [55]. A study of Lichtenberg et al. using a sheep model demonstrated a significant decrease in the immunological response after removal of cells and cellular debris from the implanted valves [56]. Moreover, repopulation by endogenous cells resulted in positive *in vivo* remodeling and no signs of degeneration, suggesting that an

eventual pre-seeding with autologous cells may be redundant [55]. Another study [57] showed that decellularization might have a beneficial effect for *in vivo* regeneration by avoiding an excessive immune response. Recently, decellularized allografts have been implanted using surgical procedures in several human cohort studies [58–60]. Mid-term results of decellularized allografts in children and young adults demonstrated good functionality as pulmonary and aortic valve replacements, and reduced reoperation rates compared to standard cryopreserved allografts and conventional xenogeneic prostheses suggesting that such decellularized allografts represent a promising solution for the future [58–60]. However, longer follow-up studies are required to confirm the encouraging mid-term outcomes. In fact, a recent study of 10-year follow-up of decellularized allografts for aortic valve replacement reported results similar to standard cryopreserved allografts [61]. In this report, explanted decellularized allografts revealed extensive fibrosis, edematous degeneration, calcification, and minimal recellularization. Indeed, the decellularization technique employed in this specific study may have altered the matrix integrity, therefore impairing the allograft durability in the long-term (>5 years) [60,61]. Therefore, standardization of decellularization technique and longer-term trials are needed before the broad use of decellularized allografts in clinical practice may be warranted.

Beside this, allografts present several disadvantages, especially for pediatric patients. Indeed, there is a general limited availability for human allogenic substitutes, and they present with further drawbacks as their inability to grow [24,27]. Moreover, heart valve replacement using an allograft requires open-heart procedures and may also need multiple reoperations during the course of the patient's life [55]. An important question mark regarding these substitutes is for how long they will represent the best option as pulmonary or aortic valve replacement. Indeed, as the world population is aging, also degenerative cardiovascular diseases are increasing in prevalence, therefore limiting even more the availability of such allografts.

3.2. Non-fixed cell-free xenografts

Alternatively to the glutaraldehyde-fixed bioprostheses currently employed in clinical settings, surgical non-fixed decellularized xenogeneic heart valves [62] have raised attention in the past years, showing potential for cell infiltration in preclinical models [63,64]. Particularly, recent experiences revealed complete recellularization of porcine decellularized valves in juvenile sheep after 4 months in the aortic position, with excellent hemodynamic performances and absence of regurgitation and calcification [65]. However, the clinical translation of decellularized xenografts in young patients resulted in early prosthesis fatal failure, mainly due to severe immune reaction [66]. The risk of immune rejection and early calcification, together with insufficient cell repopulation in humans, remain the main pitfalls of this approach, raising concerns on its safety and limiting its clinical potential [67].

3.3. *In vitro*-derived ECM-based TEHVs

As previously mentioned, classical *in vitro* heart valve TE focuses on the development of TEHVs by combining the use of natural or synthetic biodegradable scaffold materials (e.g. fibrin, PGA, P4HB), homologous cells, *in vitro* culture, and, more recently, decellularization techniques to largely provide off-the-shelf prostheses. Indeed, these technologies require longer production time in comparison to the previously discussed categories of TEHVs, in order to replace the initial polymer with neo-tissue. In fact, the final goal is to permit cellular infiltration and the formation of a dense collagen-rich ECM while the scaffold degrades [24,27]. Vascular-derived myofibroblasts are a relevant cell source for the cardiovascular field, and previous studies have demonstrated their potential to produce ECM [68,69]. However, also other cell sources such as dermal fibroblasts are gaining new insight in the production of *in vitro*-grown ECM scaffolds [70,71]. In fact, dermal fibroblasts have demonstrated their potential in the production of dense and organized collagenous ECM comparable to native valve leaflets and applicable to fibrin-derived TEHVs [70]. A recent study of Syedain et al. demonstrated good valve functionality and *in vivo* recellularization of off-the-shelf TEHVs after 6 months as aortic replacement in the sheep model [72]. In parallel to these surgically implanted TEHVs, a pilot study of 2010 proved the feasibility of combining heart valve TE with transcatheter valve replacement [73]. TEHVs demonstrated *in vivo* functionality for up to 8 weeks with mobile cusps, which, however, thickened over time [73]. Further follow-up studies compared surgically implanted and transcatheter TEHVs and introduced the concept of decellularized TEHVs [74,75], which showed good functionality *in vivo* for up to 24 weeks in the ovine model [76,77]. The progressive regurgitation observed in these valves can be ascribed to cell-mediated leaflet retraction *in vivo*. In this regard, the valve design and the hemodynamic load experienced upon implantation are crucial in influencing the cell behavior (migration, differentiation, remodeling) [21,22], as also proved by predictive computational models of the TEHV remodeling process under dynamic pulmonary and aortic pressure conditions [78,79].

As emerged from these studies, hemodynamics, scaffold design, and cellular behavior are parameters which strongly influence the final outcome of implanted TEHVs. Future research studies should focus on the interplay existing between these factors and establish how they contribute to a positive remodeling. Moreover, the clinical relevance of such therapeutic option would be improved when preferring human cell sources over xenogeneic cells for TEHV production.

4. Not only a scientific challenge

Despite the promising outcomes of the preclinical and in-human trials, the clinical translation of TEHVs is limited to date. From a scientific standpoint, the clinical translation of TEHVs cannot disregard the proof of short-term safety, long-term durability, stable functionality, regeneration, self-repair capacity, and potential growth. These aspects are of utmost importance especially in younger patients. Current data on

long-term *in vivo* performance of TEHVs do not exceed the 2-year follow-up [44], and the holy-grail lifelong durable TEHVs, which is ideally able to restore and maintain valve functionality, has not been fully demonstrated yet. The multiple reasons for the hindered application of TEHVs in clinical practice, however, go beyond the scientific challenges described above and include regulatory, clinical, technical, and logistical requirements (as reviewed elsewhere [80]), often disregarded and neglected in the current studies. The exact classification of TEHVs might be impaired by the heterogeneity of the approaches reported in literature and the discrepancies in the official classification guidelines of the different regulatory authorities (e.g. tissue engineered medical products are classified differently by European regulatory agencies and US FDA, as medicinal products or as medical products, respectively) [80]. Concerning additional technical requirements for the clinical translation of TEHVs, compliance to the guidelines of the International Organization for Standardization (ISO) is needed. Furthermore, the translational potential of TEHVs, which today is a must, would be only effective when production processes and performance of the devices follow the Good Manufacturing and Good Laboratory Practices (GMP, GLP) [80]. Despite the rapid advancements in the scientific and clinical application of tissue engineered products, their commercialization is still limited by the inconsistency of the regulatory guidelines among different countries. Furthermore, irrespectively of the country or authority, the current regulatory classifications apply only to non-regenerative products, such as the current valve replacements (i.e. bioprostheses and mechanical valves). In order to facilitate the bench-to-bed translation, the current norms should include novel classes of devices with regenerative capacities [81], as well as guidelines for the implementation of such prostheses in the clinical practice (e.g. clinical indication, target patients, monitoring and surveillance strategies, risk analysis), to guarantee patient safety and outcome predictability.

5. Conclusion

In summary, TEHVs have the potential to become the needed next-generation prostheses superior to the currently used substitutes. However, in order to achieve that, long-term proof of good performance along with favorable remodeling capacities in the absence of degeneration phenomena is needed. While much progress has been made in recent years, further developments and optimization with regard to biomaterials, TE technologies, rapid manufacturing processes, as well as regulatory pathways will further support clinical translation.

6. Expert commentary

Transcatheter TEHVs might represent the next frontier of clinical heart valve therapy and have gained importance during the recent years [73,74,76,82–86]. The underlying principle would be to provide alternative lifelong off-the-shelf heart valves, able to adaptively self-regenerate and self-repair, in combination with the fast patient recovery and short hospitalization offered by a catheter-based valve replacement [4].

Surgical open-heart valve replacement has been the gold standard for decades. Therefore, this invasive implantation technique has been used for the majority of TEHVs approaches reported in literature [31,70,72]. However, minimally invasive approaches have progressed rapidly, hence currently becoming attractive also for new-generation TEHVs. Preclinical studies highlighted the promising remodeling potential of such emerging transcatheter TEHVs, based on synthetic polymeric or ECM-derived scaffolds [31,44,76,77,83–88].

Polymeric TEHVs represent a very attractive solution for future heart valve concepts due to their advantages such as off-the-shelf availability, scalability, and rapid manufacturing. From a translational standpoint, these scaffolds offer many advantages over the classical TE strategies, such as cost-effectiveness, and off-the-shelf availability. However, their long-term remodeling capacities are still unknown. This concept completely relies on the self-regenerative potential of recipient which may vary depending on patient's age and general medical history. This aspect becomes particularly important when such polymeric technologies are applied to patients with a limited regenerative potential (i.e. elderly or multimorbid patients). Therefore, the safety and durability of purely polymeric TEHVs still needs to be fully explored, especially at the critical time point when complete scaffold degradation has occurred and the newly formed tissue has to solely ensure mechanical integrity of the valve.

Hybrid materials represent a valuable alternative, as they own advantages derived from both natural and synthetic materials. However, these hybrid scaffolds still lack to fully prove their capacity to attract endogenous cells, which is the basis for a positive and functional remodeling in the long term. In this context, functionalized hybrid scaffolds have been the focus of some recent researches, where bioactive compounds have been integrated into electrospun meshes to favor host cell repopulation and tissue formation [51]. In this sense, such scaffolds might be suitable for antithrombogenic or drug-delivery purposes. Nevertheless, despite the promising performances of polymer-based prostheses, future studies are needed to demonstrate the safety and effectiveness of such visionary conceit and explore their potential as durable, regenerative valve substitutes.

Among ECM-derived TEHVs, cell-free allografts and xenografts represent the most advanced ones with a substantial clinical experience so far. Indeed, the concept of decellularization treatments (to obtain acellular non-fixed xeno-, or allogeneic materials) was introduced to achieve better performance in preclinical and clinical trials [55], when compared to the commercially available glutaraldehyde-fixed biomaterials. Maintenance of the native geometry and tissue structure is probably the major advantage of allografts and xenografts. However, besides further long-term proof, additional specific research is needed to further improve and optimize the decellularization protocols, in order to preserve the structural ECM properties and completely eradicate the risk of adverse host response [60,61,66,89]. Concerning the allografts, the decellularization might reduce the leukocytes infiltration and therefore the severity of the immune reaction at 3 months postimplantation, when compared to analogous cryopreserved substitutes [90]. Moreover, the

controversial results reported for cell-mediated response upon implantation of allogeneic substitutes may be ascribed to the role of memory T cells [91], as suggested by Benichou and co-workers [91].

On the other hand, *in vitro*-derived ECM-based TEHVs show encouraging remodeling prospects *in vivo*, with host cell repopulation and *de novo* ECM formation [72,73,76,77,84,92], yet with progressive valve insufficiency.

As a common denominator for all TEHV approaches, valve geometry appears to play a key role in determining the hemodynamics and cusp kinetics, which finally influence and determine cell migration, cell phenotype and ECM remodeling, as well as potential negative remodeling phenomena. Hence, if the valve design is not fully representative of the native features, it may yield to nonphysiological hemodynamic loading, affecting the deformation profile of the cusps, inducing a negative remodeling response (i.e. leaflet shortening or retraction) and ultimately leading to valve dysfunction due to progressive regurgitation [93]. Hence, future studies should focus on the development and implementation of a more physiological-like valve design, possibly including the Valsalva sinuses [94], which accommodate proper hemodynamic loading sensed by the resident cells in order to address deleterious or maladaptive remodeling.

Additionally, the growth capacity of TEHVs still needs further validation. However, some of the described off-the-shelf approaches seem promising in this sense. In fact, *in vitro*-derived ECM-based vascular grafts have shown capacity to grow when implanted in the young lamb model, with both acellular and autologous ECM-derived TE grafts [95,96], hence it is reasonable to expect similar outcomes for analogous TEHVs, while other technologies still need validation for growth. Finally, yet importantly, all today's off-the-shelf TEHVs still need to demonstrate long-term durability, reproducibility, and antithrombogenic properties via large trials with long-term follow-up.

To date, only few studies combined off-the-shelf TEHVs with transcatheter implantation techniques [31,48,76,77,87]. Transcatheter prostheses undergo significant dimensional change during the crimping step performed before valve delivery. The resistance to crimping and the absence of detectable matrix damages are paramount for the long-term outlook of transcatheter prostheses. As TAVR is expected to be extended to lower-risk patients, it is evident that competitive next-generation TEHVs shall prove not only tissue remodeling and growth but also resistance to crimping-induced adverse effects and safety of catheter-based delivery [12].

Furthermore, the majority of preclinical and clinical reports of TEHVs primarily concern their application as pulmonary valve substitutes. Few exceptions were reported recently, mostly implicating open-heart surgery with ECM-based TEHVs in the aortic position [59,72,83–86,90], showing good functionality. This said, further innovations should include the development of transcatheter TE aortic valve substitutes [86], which would increase the clinical relevance of such prostheses.

A multidisciplinary approach to TE would shed light on the mechanisms underlying *in vivo* remodeling, at different scale levels (organ, tissue, cell) [23]. Particularly, the integration of computational modeling (e.g. finite element models, computational fluid dynamics and fluid-structure interaction) with bench experiments would allow selecting the optimal methods for TEHV production, informing on the functional performance and deformation profile. In this sense, technologies such as cardiac biosimulator platforms [97,98] combined with advanced imaging technologies (e.g. 4D-magnetic resonance imaging) might help in evaluating TEHVs early functionality [99]. Moreover, the inclusion of patient-specific characteristics in preoperative simulations of prosthesis replacement may lead to improved success rates and reduced risk of complications, thereby increasing safety and outcome predictability [100]. Therefore, hybrid approaches and interdisciplinary analysis for the evaluation of TEHVs will increase the relevance and clinical translatability of these valve replacement options. Since the perfect remodeling and growing substitute is not determined yet, we can hypothesize that a combination of tissue and bioengineering, fluid dynamics, and material science knowledge might lead toward the optimal (pre)clinical solution.

7. Five-year view

As to recent developments and achievements in the field, we expect further rapid advances in both TAVR and TEHV technologies within the coming years. Long-term outcomes of the ongoing randomized clinical trials on high- and intermediate-risk patients will provide important data and insight on current TAVR prostheses with regard to their durability. In parallel, clinical trials on TAVR in low-risk and younger patients are currently underway and short-term preliminary results in these patient populations should become available shortly. Moreover, latest-generation TAVR devices including strategies for preventing paravalvular leakage, that represented the Achille's heel of TAVR in the first years, are currently under evaluation.

Concerning TEHV technologies, a bioresorbable polymeric-based technology platform for *in situ* tissue restoration, has been recently advanced into a pilot clinical trial (Xplore, clinical trial NCT03022708) showing a good initial safety profile. In addition, recently published data of valves using the same technology have demonstrated encouraging results as pulmonary valve replacements in the ovine model, showing first evidence of endogenous tissue restoration [31,44]. Hence, the initiation of clinical pilot trials is awaited in the near future.

In parallel, decellularized allografts showed good results as pulmonary valve replacement, with superior freedom from any re-intervention or re-operation after 10 years, compared to conventional cryopreserved homografts and xenografts (ESPOIR program, clinical trial NCT02035540). Building on these encouraging outcomes, a sister project received approval for the clinical evaluation of these prostheses in the aortic setting (ARISE program; clinical trial NCT02527629).

Meanwhile, *in vitro*-derived, ECM-based TEHVs are under continuous development toward clinical translation of another potential class of next-generation grafts and valve substitutes with substantial regeneration and self-repair capacity. Although not yet clinically applied in the

setting of heart valves, this approach has already been advanced into clinical translation as small caliber conduits for dialysis shunts (clinical trials NCT01744418 and NCT01840956) providing safe and functional hemodialysis access [101].

Remarkably, both pure polymeric and acellular ECM-based TEHVs share the advantage of off-the-shelf availability, reducing the logistic burden compared to autologous, patient-specific approaches, and probably facilitating the regulatory pathways toward clinical application and commercialization.

In conclusion, based on the tremendous progress in recent years, clinical translation of heart valve TE is within reach and may become a clinical reality in the near future. Besides that, and importantly, when combined with transcatheter implantation techniques, TEHVs may serve as a basis for future regenerative TAVR devices with lifelong durability, hence overcoming the pitfalls of current valve prostheses in the rapidly growing transcatheter market.

Key issues

- In the last years, transcatheter aortic valve replacement is evolving and expanding constantly. Minimally invasive valve implantation procedures coupled with lifelong valve prostheses would further extend this therapeutic option to younger patient populations. In this prospect, tissue engineered heart valves with regenerative capabilities represent a suitable candidate and may overcome the drawbacks of current TAVR prostheses.
- *In situ* tissue engineering, based on the body's regenerative potential, enables to produce off-the-shelf available valve substitutes. *In situ* TEHVs reported in clinical or preclinical trials include synthetic biodegradable polymeric valves, decellularized allografts and xenografts, and acellular *in vitro* grown ECM-based valves.
- Synthetic bioresorbable polymers offer the advantages of reproducible, controllable and tunable mechanical and structural properties, unlimited supply, and can be functionalized for modulating the recipient's immune response. When combined with biological materials, synthetic polymers provide the basis for hybrid scaffolds. Long-term follow-up studies are needed to additionally investigate the polymer degradation and remodeling *in vivo*.
- Decellularized allografts and xenografts preserve the native tissue organization and mechanical profile. The limited availability of allografts and the potential risk for transspecies disease transmission associated with the xenografts are the major limitations of these approaches. The reported *in vivo* cell repopulation of such valves is still controversial.
- Decellularized *in vitro* produced ECM-based TEHVs potentially represent regenerative implants with the capacity to grow. Current valves experienced progressive regurgitation *in vivo*, probably due to suboptimal valve design.
- Only few TEHVs have been implanted with transcatheter techniques. Moreover, the majority of the studies have been performed on the low-pressure circulation. Therefore, the experience of minimally invasive TEHVs that can sustain the aortic valve loading is still limited.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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