Next-generation tissue-engineered heart valves with repair, remodelling and regeneration capacity

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Abstract | Valvular heart disease is a major cause of morbidity and mortality worldwide. Surgical valve repair or replacement has been the standard of care for patients with valvular heart disease for many decades, but transcatheter heart valve therapy has revolutionized the field in the past 15 years. However, despite the tremendous technical evolution of transcatheter heart valves, to date, the clinically available heart valve prostheses for surgical and transcatheter replacement have considerable limitations. The design of next-generation tissue-engineered heart valves (TEHVs) with repair, remodelling and regenerative capacity can address these limitations, and TEHVs could become a promising therapeutic alternative for patients with valvular disease. In this Review, we present a comprehensive overview of current clinically adopted heart valve replacement options, with a focus on transcatheter prostheses. We discuss the various concepts of heart valve tissue engineering underlying the design of next-generation TEHVs, focusing on off-the-shelf technologies. We also summarize the latest preclinical and clinical evidence for the use of these TEHVs and describe the current scientific, regulatory and clinical challenges associated with the safe and broad clinical translation of this technology.

Rheumatic fever
Inflammatory disease that mostly affects children aged 5–14 years and can cause permanent damage to the heart and heart valves.

Stenosis
Narrowing of the heart valve, which prevents proper opening, thereby reducing the blood flow through the valve.

Valve insufficiency
Also known as regurgitation or incompetence. Incomplete closure of the heart valve leaflets, which allows blood to flow backwards through the valve.

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Valvular heart disease affects a growing number of patients in both developed and developing countries[1,2]. The ageing of the population in both Europe and the USA has also led to an increase in the incidence of severe degenerative valve disease[3]. At the other end of the spectrum, up to 1% of newborn babies have congenital heart disease, which might require surgical or interventional treatment of the affected heart valves[4]. Rheumatic fever is the major cause of valvular pathologies in young individuals (aged 5–14 years) in many developing nations in Africa and Asia[5,6], increasing the demand for affordable valve replacement options. Valvular heart disease is characterized by a loss of valve functionality owing to stenosis and/or valve insufficiency. For some patient cohorts with degenerative valve insufficiency, such as high-risk patients with functional degenerative mitral valve regurgitation, new interventional techniques such as edge-to-edge repair offer good outcomes[7]. Numerous types of aortic insufficiency (including cusp perforation, annulus dilatation, cusp prolapse and cusp restriction) can be treated by annuloplasty, patch repair with autologous (or bovine) pericardium, shaving or resection[8]. Despite the advances in valvular repair techniques, as thoroughly reviewed previously for the different heart valves[9–12], the replacement of severely dysfunctional or stenotic valves can become unavoidable in many patients with severe valvular heart disease. The number of valve replacement procedures performed each year with a mechanical or bioprosthetic valve has been constantly increasing since its adoption and is expected to reach 850,000 implantations worldwide by 2050 (REF.[13]). The choice of the most appropriate replacement procedure is on the basis of valve-related factors that consider anatomy and pathology, and on other variables that determine the operative risk such as the presence of comorbidities, age, frailty and tolerance to anticoagulation therapy.

In this Review, we present a comprehensive overview of current clinically adopted valve replacement procedures. In addition, we discuss the design of next-generation tissue-engineered heart valves (TEHVs) with repair, remodelling and regenerative potential, with a focus on the most up-to-date evidence on in situ tissue engineering approaches. Finally, we detail the scientific, regulatory and clinical challenges that must be addressed before TEHVs can be safely translated into the clinic.

Key points

- Surgical heart valve replacement is the gold-standard treatment for aortic valve disease, but transcatheter valve implantation has revolutionized the field by providing a novel treatment option for patients of all risk profiles.
- Despite rapid advances in the field of heart valve therapy, an unmet clinical need remains for valve replacements with regenerative, remodelling and growth potential.
- In situ tissue engineering technologies can be used to produce a heart valve replacement that is readily available, manufactured using decellularized extracellular matrix or bioresorbable polymers, and transforms into a native-equivalent valve after implantation.
- Computational modelling is a powerful tool that can be used to improve and accelerate our understanding of tissue-engineered heart valve growth and remodelling and should be used in concert with in vitro and in vivo tissue engineering technologies.
- To ensure the good clinical safety, feasibility and efficacy of the tissue-engineered heart valve, researchers and clinicians should work according to Good Manufacturing Practices and Good Laboratory Practices as well as to International Organization for Standardization requirements.
- The field of heart valve tissue engineering still faces several challenges, such as issues related to immunocompatibility, haemocompatibility, remodelling and growth capacity, which need to be further investigated before broad clinical adoption is possible.

Annuloplasty
A procedure to tighten or reinforce the ring around a valve in the heart.

Bi-leaflet tilting disc
A valve made of a metal ring covered by polytetrafluoroethylene, whereby the metal ring holds a disc that opens and closes after one cardiac cycle.

Tetralogy of Fallot
A rare congenital condition caused by a combination of four heart defects: pulmonary valve stenosis, ventricular septal defect, overriding aorta and right ventricular hypertrophy.

SAVR and TAVR

Surgical aortic valve replacement (SAVR), first performed in the late 1960s, has until the past decade been the standard of care for the treatment of aortic stenosis, with excellent short-term and long-term results[1]. Although highly effective, SAVR requires invasive open-heart surgery with temporary cardiac arrest and the use of a cardiopulmonary bypass machine, which is associated with periprocedural risks[14] (FIG. 1). In the early 2000s, transcatheter aortic valve replacement (TAVR) revolutionized the field of heart valve therapy by providing a novel treatment option for patients who were considered inoperable or at high surgical risk such as elderly patients or those with multiple comorbidities[15–17]. Over the past 20 years, great progress in device technology, including improved stent designs and smaller and more efficient delivery systems in addition to reduced rates of paravalvular leaks and vascular complications, has substantially decreased the complication rate associated with TAVR[17]. Several access routes, including transapical, transfemoral and transaortic routes, have allowed safe implantation even in patients with vascular disease[18].

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At present, transcatheter techniques are routine for patients with aortic disease who were previously considered inoperable or at high surgical risk[18]. On the basis of findings from randomized, controlled trials conducted in the past 5 years that compared SAVR and TAVR in different cohorts of patients, the indication for TAVR has been extended to patients at intermediate[19,20] and low[21,22] surgical risk. TAVR is now the first-line therapy for patients with severe, symptomatic aortic stenosis.

Contemporary heart valve replacements

Mechanical valves. The first-ever heart valve surgery was performed in 1952 (REF.[23]). In 1957, the surgeon Albert Starr and the engineer Lowell Edwards created a mechanical valve with a ball-and-cage design, which became the first commercially available valve prosthesis[24]. In the next few decades, with the optimization of the surgical procedure and the evolution of mechanical valves with a bi-leaflet tilting disc (first developed in 1976), mechanical aortic valve replacement became the procedure of choice for younger patients with aortic stenosis with no contraindication to anticoagulation therapy[24,25]. However, the non-physiological valve geometry and the foreign surface of the mechanical valve contributed to an increase in thrombogenic risk for patients owing to the high shear stress around the hinge points and backflow jets that caused damage to red blood cells and increased platelet activation[25]. Valve thrombogenicity is still the most prevalent complication of using a mechanical valve, particularly during the first 6 months after the surgery[26]. To limit the risk of thrombosis, patients receive lifelong warfarin treatment. Importantly, the need for this lifelong anticoagulation therapy has a substantial effect on lifestyle and increases the lifetime risk of major bleeding. Therefore, mechanical valves are not recommended in athletes or in women intending to bear children[27].

Homograft heart valves. The first human homograft heart valve was implanted in 1956 (REF.[28]) and achieved wide clinical attention in the 1960s as a promising alternative to mechanical prostheses[29]. Human homograft heart valves represented an ideal valvular substitute given that the anatomical characteristics and tissue composition of these valves were associated with an improved haemodynamic profile, reduced thromboembolic risk and low immunogenicity compared with mechanical valves[30]. Excellent results in terms of valve durability and performance have been achieved with homograft valves implanted in the pulmonary position, as in patients with tetralogy of Fallot[31]. However, the use of homograft valves is also associated with major drawbacks such as limited availability given the shortage of human organ and tissue donors, and limited durability owing to residual immunogenicity and susceptibility to early calcifications that can induce structural valve degeneration[32,33]. Therefore, only 30–40% of homograft valves are still functional 20 years after implantation[33]. Furthermore, homograft valves are considered to be suboptimal for implantation in paediatric patients given their poorer long-term durability[34] and higher reoperation rates than mechanical valves[35].

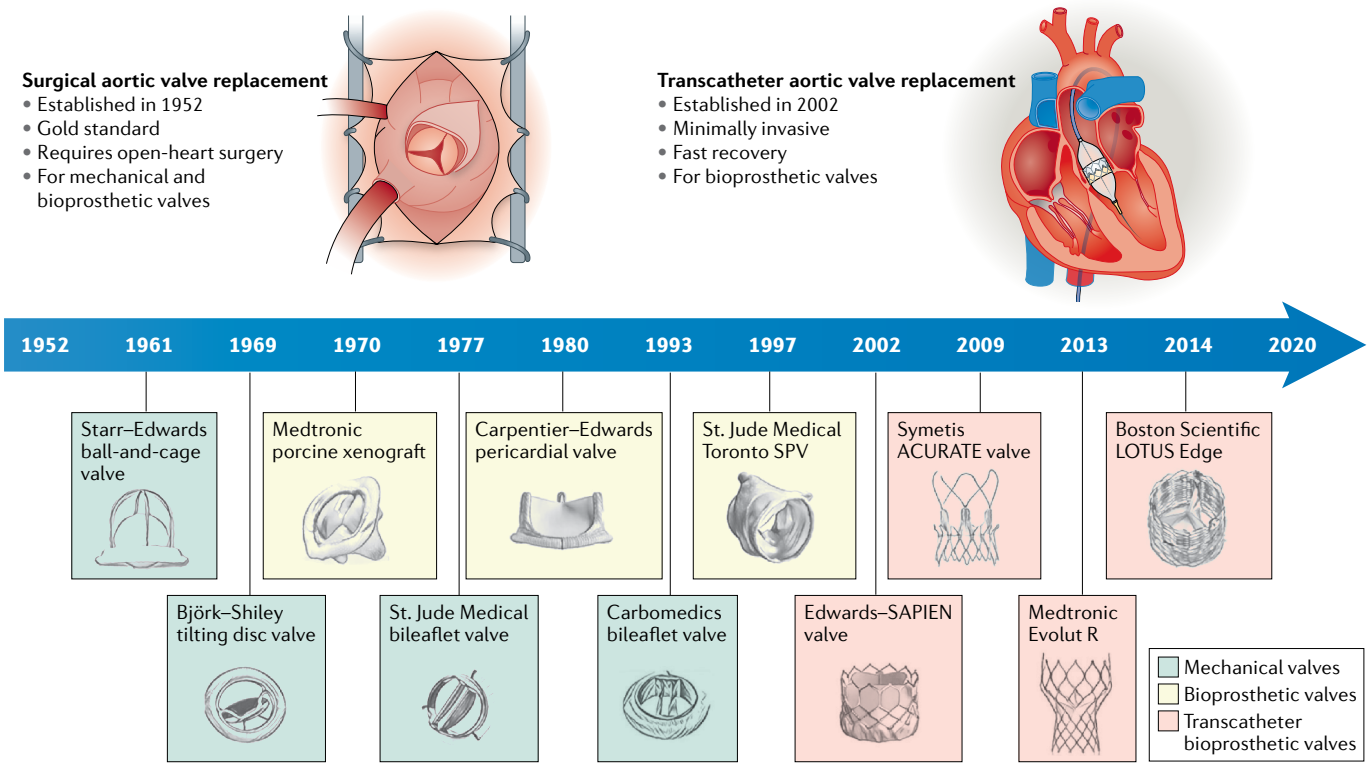

Fig. 1 | **Evolution of heart valve replacement options.** Timeline of the introduction of mechanical and (transcatheter) bioprosthetic aortic valves. SPV, stentless porcine valve. Adapted with permission from REF.[24], EMH Swiss Medical Publishers Ltd.

Since their introduction, the procedures for sterilization and storage of homograft valves have evolved from fresh aseptic harvesting with direct implantation to either cryopreservation in the vapour phase of liquid nitrogen or wet storage at 4°C after antibiotic sterilization[33]. Despite the reported improvements in the storage of homograft valves through the development of milder cryopreservation procedures (as reviewed elsewhere[30]), freezing and subsequent thawing of the homograft valves can lead to surface and structural damage[36,37]. As a consequence, valve insufficiency, leaflet shortening and thickening, vegetation, inflammation, and valve degeneration have been observed after implantation[38–40].

Bioprosthetic valves. In the 1970s, bioprosthetic valves were produced using widely accessible, glutaraldehyde-fixed xenogeneic materials, designed to overcome the problem of thrombogenicity observed with mechanical valves and to replicate the anatomical features of homograft valves by providing a valve with a native-like, tri-leaflet geometry and tissue composition[24]. When compared with mechanical valves, this improved valve geometry has an improved physiological-like haemodynamic profile and limited platelet adhesion and activation, thereby reducing the need for anticoagulation therapy[41]. The large range of surgical bioprosthetic valves available today includes valves that can be placed in the subannular or supra-annular position[42], valves with or without stent frames[43], sutureless valves[44] and valves with various anticalcification treatments[45].

With the introduction of TAVR, a multitude of innovative self-expandable and balloon-expandable valves have been developed[46,47]. Although stents and delivery systems have different designs, transcatheter valve prostheses are manufactured using clinical-grade, glutaraldehyde-fixed xenogeneic materials. However, the current trend to preferentially use bioprosthetic valves in patients aged <65 years[48] comes at the cost of potential structural valve failure owing to progressive calcification of the leaflets within 10–15 years after implantation[47]. By contrast, valve durability of up to 20 years has been reported in elderly patients[49]. Anti-mineralization treatments have been developed to improve bioprosthetic valve durability and to limit the onset of calcification[50]. Although partially successful, these treatments are not sufficient to mitigate valve degeneration caused by the residual immunogenicity of the bioprosthetic material. Furthermore, glutaraldehyde treatments are inadequate to completely mask immunogenicity because standard fixation protocols might eliminate the immunogenicity of protein antigens but immunogenic and xenogeneic carbohydrate antigens can persist, causing valve failure owing to chronic inflammation and calcific nodule formation[51,52]. Bioprosthesis immunogenicity is even more profound in paediatric patients and results in early degenerative failure, making bioprosthetic valves an unsuitable choice for the treatment of congenital heart disease[53].

Autograft valves (Ross procedure). The Ross procedure, first established by Donald Ross in 1967 (REF.[54]), involves the replacement of the diseased aortic root with the

Xenogeneic
Materials derived from tissues that originate from a different species to the recipient such as bovine pericardium or porcine valve leaflets.

patient's autologous pulmonary valve while a cryopreserved homograft is implanted in the pulmonary position to restore blood flow to the right ventricular outflow tract[55]. This technique was developed to overcome the lack of durable valve replacement options for young patients with diseased aortic valves, in whom mechanical valves were the most frequently used type of artificial valve. However, compared with those who receive mechanical valves, recipients of autograft valves using the Ross procedure do not need lifelong anticoagulation therapy[56]. Furthermore, autograft valves have regenerative and remodelling potential, a physiological-like haemodynamic profile and a long-term survival that is equivalent to the age-matched and sex-matched healthy population[56]. In addition, the Ross procedure has been reported to improve life expectancy and freedom from cardiac-related and valve-related death, and to reduce the risks of stroke and bleeding compared with mechanical valve replacement[56,57]. These findings suggest that, when properly performed, the Ross procedure is a valuable option to treat aortic valve disease in young patients and might even provide a more cost-effective approach than conventional aortic valve replacement using a mechanical valve[58]. However, the technical complexity of the procedure remains a major challenge, limiting its broad clinical adoption[56].

Alternative solutions. Polymeric valves, made using non-degradable polymers such as silicon[59], polytetrafluoroethylene[60] and polyurethanes[61], were initially designed to combine the immunocompatibility of the mechanical prostheses with the physiological-like haemodynamic profile provided by tri-leaflet bioprosthetic valves[62]. However, polymeric valves have since been linked with poor durability and a loss of functionality over time[63] as well as with variation between batches[64], thrombogenicity[64], pannus overgrowth[65] and calcification[63,66], impeding their broad clinical translation. Over the past 20 years, material scientists have attempted to optimize polymers in order to produce durable, biocompatible materials that can be safely used as a valvular component in ventricular assist devices[67]. Siloxane poly(urethane-urea)-based heart valves have demonstrated improved tear strength and creep resistance and no permanent deformation compared with conventional polyurethanes[68,69] as well as an in vitro durability of 600 million cycles (equivalent to 15 years) with the absence of calcification and in vivo thrombus formation[62]. In light of these results, Foldax have developed the Tria LifePolymer aortic heart valve and initiated an early clinical feasibility study, with 15 patients enrolled. Results on the early clinical experience using this novel polymeric valve are expected at the end of 2020[70,71]. In the future, polymeric heart valves might be combined with minimally invasive transcatheter procedures in developing countries to provide a cost-efficient solution for the treatment of rheumatic valvular disease[62]. In this context, Strait Access Technologies developed a TAVR strategy for developing countries that uses a supra-annular anchoring technique to provide accurate valve positioning via tactile feedback [62,72]. However, substantial challenges remain for polymeric valves. First, researchers need to balance the durability and biocompatibility of the material to ensure the long-term performance in vivo and improved valve longevity compared with bioprosthetic valves. Second, variability between the synthesis or processing of different polymer batches should be limited so that valve performance is consistent and reproducible. Finally, polymer wear should be prevented to limit the risk of tear and failure. When these challenges have been addressed, a novel prosthesis that is compatible with clinical standards can be achieved.

Creep resistance
Refers to a solid material's capacity to resist creep, that is, the tendency of a solid material to move slowly or deform over long-term exposure to high levels of stress.

Clinical and cost-effectiveness issues

Despite tremendous technical evolution in the field of heart valve therapy, an ideal heart valve prosthesis has not yet been developed. Prosthesis-associated issues, such as progressive functional degeneration, limited durability and the complications of lifelong anticoagulation therapy, occur within 10 years after implanatation[25]. In particular, paediatric patients require multiple surgeries to account for their somatic growth as well as repeat operations to replace failing valves[73]. The quality of life and the life expectancy of patients after heart valve replacement are lower than in age-matched healthy individuals[74]. Furthermore, heart valve replacements are very costly (costing >1 billion Euros annually in Europe), mainly owing to postoperative care costs and the need for repeat operations[75]. A clear unmet clinical need remains for a heart valve prosthesis that does not degenerate, can adjust to functional and somatic changes, and can be implanted via minimally invasive techniques.

Heart valve tissue engineering

In 1989, Dwight Emary Harken, a pioneer of heart valve surgery, summarized the characteristics and features of the ideal heart valve substitute in 'ten commandments', which included the capacity to self-repair, adaptively remodel, and grow and to be resistant to infections and thrombogenicity, all of which are fundamental characteristics of native heart valves[76]. No currently available heart valve prosthesis possesses all these properties. However, the design of TEHVs with a self-repair and remodelling capacity might address these unmet needs. A valve that continuously adjusts to the functional changes in the cardiovascular system, is not immunogenic and does not necessitate lifelong antithrombotic therapy could provide a lifelong, durable solution for patients with aortic stenosis, particularly paediatric and elderly patients.

From autologous to cell-free approaches

The fundamental paradigm of heart valve tissue engineering was initially established in 1993 by Langer and Vacanti and involved the use of autologous cells and tissue culture in a bioreactor to favour cell proliferation and extracellular matrix (ECM) deposition[77] (FIG. 2a). The repair and remodelling mechanisms of the TEHV were thought to improve the long-term durability of the valve[78]. Several clinical trials have been conducted to assess this autologous tissue engineering approach using either decellularized allogenic[79,80] or xenogeneic[81,82] valves. The paucity of homografts and the risk of

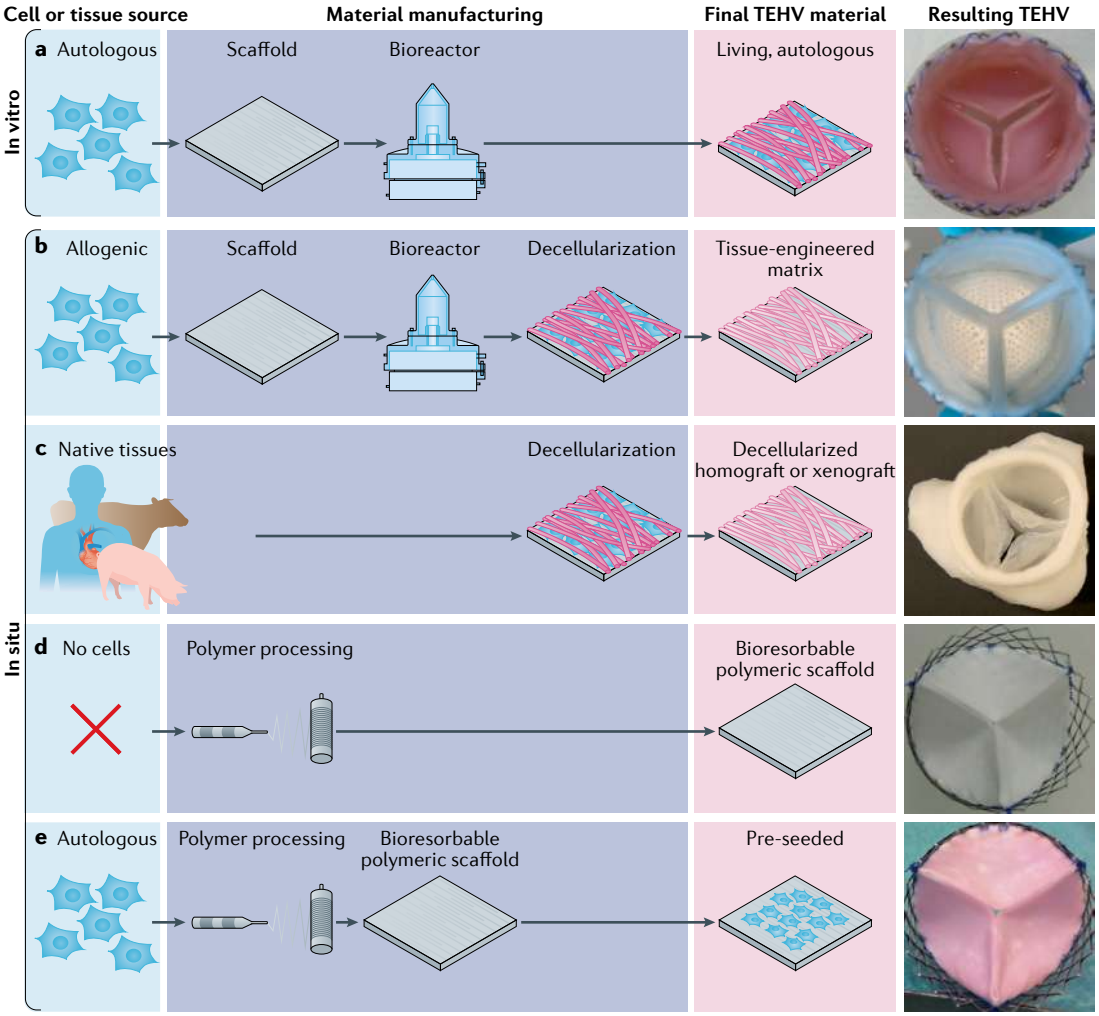

Fig. 2 | **Overview of various tissue-engineered approaches.** In vitro heart valve tissue engineering involves the isolation and seeding of autologous cells onto a bioresorbable scaffold that is then cultured into a bioreactor system[77]. The resulting living autologous tissue-engineered heart valve (TEHV) is then ready to be implanted into the patient (part **a**). In situ tissue-engineered approaches rely on the regenerative potential of the recipient to integrate and remodel an 'off-the-shelf' acellular implant that can be manufactured using various cell and tissue sources. Allogenic decellularized tissue-engineered matrices[144] (part **b**). Decellularized homograft or xenograft materials (part **c**). Bioresorbable polymeric scaffolds processed via electrospinning[98] or rotary jet spinning[157] that can be either cell-free (part **d**) or pre-seeded with autologous cells[164] (part **e**). TEHV image in part **a** adapted with permission from REF.[96], Elsevier. TEHV image in part **c** adapted with permission from REF.[155], Elsevier.

xenograft immunogenicity have motivated researchers to find alternative materials in natural (such as collagen and fibrin) and synthetic (poly(glycolic acid) and polycaprolactone) polymers, with promising results from in vitro[83–85] and preclinical in vivo[85–94] studies in terms of early functionality, implantation remodelling and endothelialization potential. However, to date, in vitro heart valve tissue engineering has not progressed into routine clinical use. The challenges of this tissue engineering approach are multifactorial. First, the process is technically and logistically complex, requiring the isolation and expansion of autologous cells before tissue culture. Furthermore, donor-to-donor variation can result in inconsistencies in the final product[95] and an uncontrolled thickening and shortening of the leaflets, with consequent valve insufficiency, has been repeatedly observed[87,88] even before implantation[96]. Finally, the long-term safety and efficacy of TEHVs have not yet been established.

To overcome the logistical hurdles of autologous TEHV use, such as the lack of scalability, high costs, donor-to-donor variation and logistical challenges in orchestrating valve manufacturing and implantation, in situ tissue engineering — a more straightforward and cost-effective approach — has gained much attention from the scientific community in the past decade. In situ valvular tissue engineering relies on the regenerative potential of the recipient's body to integrate and remodel an off-the-shelf implant that is designed to favour host cell adhesion and tissue formation and provide valve functionality immediately upon implantation[97]. The ideal substrate for in situ heart valve tissue engineering should have the capacity to remodel by selectively controlling host cell recruitment, adhesion

Decellularization
To deplete cells from biological tissues in order to remove DNA and immunological epitopes. Decellularization can be performed via chemical, enzymatic or physical methods to ensure a cell-free material that is available off the shelf.

Functionalization
To include bioactive moieties, such as proteins, peptides and polysaccharides, into a scaffold by means of covalent or non-covalent binding to improve scaffold biocompatibility.

and differentiation and should support tissue formation while controlled degradation occurs, until a native-like functional tissue is achieved[97].

In situ tissue engineering strategies

Multiple in situ tissue engineering strategies have been established in the past decade that use various starting scaffolds to manufacture TEHVs (FIG. 2), including homografts and xenografts, in vitro-grown tissue-engineered matrices (TEMs) and bioresorbable polymers with regenerative potential. To confer immunocompatibility and off-the-shelf availability to human-derived and animal-derived valvular tissues, homografts and xenografts are processed via decellularization[30] (FIG. 2b). By depleting the cells and their DNA, the immunological epitopes are removed, leaving the integrity and functionality of the ECM mostly unchanged. This decellularization technique has also been applied to TEMs grown in vitro to ensure scalability and off-the-shelf availability and to reduce donor-to-donor variability[96] (FIG. 2c). Finally, bioresorbable polymers with tailored mechanical, chemical and architectural properties have also been used in the production of TEHVs. These polymers have customized degradation rates and are tailored to a cell-specific environment (via functionalization), making them a good choice for the easy, rapid and competitive manufacturing of scaffolds suitable for regenerative medicine purposes[98] (FIG. 2d,e).

Decellularized pulmonary valve and aortic valve homografts. The in vivo application of human-derived decellularized pulmonary valve homografts (DPVHs) and decellularized aortic valve homografts (DAVHs) has been met with success in both preclinical[99,100] and clinical studies[101–105] (TABLE 1). TEHVs produced using human-derived tissues can be obtained by the decellularization of freshly harvested or cryopreserved pulmonary or aortic valves. Freshly harvested DPVHs, first introduced as pulmonary valve replacements for paediatric applications[79], have demonstrated promising performance and spontaneous recellularization potential[102]. In the ARISE[101–103] and ESPOIR[104,105] clinical trials, freshly harvested DPVHs showed excellent long-term performance with trivial regurgitation and improved freedom from explantation compared with cryopreserved homografts[102,103] (TABLE 1). Freshly harvested DAVHs are also associated with sustained performance for up to 10 years, with no reported cases of reoperation or endocarditis[101,105,106] (TABLE 1).

Cryopreserved DPVHs and DAVHs have also demonstrated promising short-term results in terms of valve immunocompatibility, performance and durability[107,108]. However, at 8–10 years, these valves did not show significantly improved rates of freedom from reoperation or better valve performance compared with cryopreserved homografts[107,109]. In particular, cryopreserved DAVHs performed comparably to cryopreserved homografts[35] and had a similar degree of degeneration[107] in terms of fibrosis, calcification and lack of recellularization[110,111].

Remarkably, freshly harvested DPVHs and DAVHs seem to perform better than decellularized, cryopreserved DPVHs and DAVHs[107]. This finding suggests that the processing methodology is important and that the cryopreservation and decellularization techniques might affect the physical and mechanical properties of the original allogenic tissue, hindering in situ regeneration. Additionally, various sterilization methods are used in different studies, and certain techniques, such as low-dose gamma radiation, have been reported to have a strong influence on the integrity of the ECM[112]. Therefore, further studies should address the role of cryopreservation, decellularization and sterilization on ECM preservation to ensure long-term homograft stability and remodelling. Finally, similar to cryopreserved homografts, the broad clinical application of these prostheses is limited by donor shortage and the need to implant this valvular replacement via open heart surgery.

Decellularized xenografts. To overcome the limited availability of allogenic human tissue, decellularized xenografts generated using porcine small intestinal submucosa (CorMatrix, CorMatrix Cardiovascular) or porcine decellularized pulmonary valves (Matrix P or Matrix P plus, AutoTissue, SynerGraft, or CryoLife) have been used in preclinical and clinical settings. As previously described for the homografts, the decellularization process aims to provide an ECM-based scaffold with low immunogenicity and a retained regenerative potential. However, substantial differences have been identified between the preclinical and clinical performance of decellularized xenogeneic valves. In early preclinical trials, pulmonary[113] and tricuspid[114,115] valves produced using porcine small intestinal submucosa demonstrated promising valve performance and host cell infiltration, with endothelialization[114], trilaminar tissue organization[115] and native-like valve growth of the annulus diameter[113] (TABLE 2). However, in a study published in 2020, pulmonary valve conduits generated using porcine small intestine submucosa that were implanted into lambs and sheep showed unfavourable results[116]. Seven animals died during follow-up, and post-mortem analysis revealed a thickening of the explanted valve leaflets with signs of inflammation and endocarditis. Of the animals that completed follow-up, five showed severe stenosis and one had severe regurgitation. Only seven animals had well-functioning valves, albeit with limited signs of remodelling (TABLE 2). The study investigators identified several reasons for the poor performance of the valves, such as high mechanical stress and the host-specific inflammatory response to the implanted material, with decellularization repeatedly shown to be incomplete[116–118].

The CorMatrix porcine small intestinal submucosa was first assessed in the clinic in 2010 and has since been extensively used in cardiovascular surgery for patch and valve repair[119] and for valve and cusp replacement[120–123] (TABLE 3). However, independent of indication, and in line with preclinical findings[116], several studies reported that the use of CorMatrix resulted in thickening, fibrosis and calcification[124] as well as in material degeneration[120] and a strong pro-inflammatory response[121,125], all of which contributed to the need for reoperation for valvular

Table 1 | **Clinical applications of decellularized homograft-based valve replacements**

Trial (year)	Homograft (*n*)	Procedure (anticoagulation or anti-aggregation regimen)	Mean age (years)	Main findings	Ref.
ESPOIR (2019)	fDPVH (121)	SPVR (NR)	21.3 ± 14.4	Excellent performance with trivial regurgitation; better freedom from explantation than cH and BJV, indicating that fDPVHs are safe and effective	104
ESPOIR (2018)	fDPVH and fDAVH (5)	SAVR and SPVR (in children: aspirin for 3–6 months after implantation; in adults: warfarin for 2 months after implantation plus aspirin)	Range 2–38	Superior midterm SPVR results in children and young adults; good initial results for SAVR; fDPVH and fDAVH provide a new surgical option for young patients who previously required multiple valve procedures	105
ARISE (2016)	fDAVH (69)	SAVR (in children: aspirin for 3–6 months after implantation; in adults: warfarin for 2 months after implantation plus aspirin)	19.7 ± 14.6	fDAVHs can withstand the systemic circulation with trivial regurgitation and no evidence of dilatation or calcification; fDAVHs are suitable for young patients	101
Helder et al. (2016)	cDAVH (42) with cH (29) as controls	SAVR (NR)	49 ± 17	Although early outcomes of cDAVHs were promising, freedom from reoperation after 10 years is higher for cH than for cDAVH (80% vs 51%)	107
ARISE (2016)	fDPVH (93) with cH (93) and BJV (93) as controls	SPVR (aspirin for 3–6 months after implantation)	15.8 ± 10.2	At 10 years, fDPVHs were associated with 100% freedom from explantation and endocarditis, with sustained functionality over time	102
ESCORE (2011)	cDPVH pre-seeded with aECs (11)	SPVR (NR)	39.6 ± 10.3	Excellent haemodynamic performance over 10 years, with no evidence of calcification	194
Brown et al. (2011)	cDPVH (29) with cH (34) as controls	SPVR (aspirin for 3 months after implantation)	28.6 ± 16.0	Patients who received cDPVH did not need reoperation, and no evidence of deterioration was noted, with a good performance similar to that of cHs	108
ARISE (2011)	fDPVH (38) with cH (38) and BJV (38) as controls	SPVR (aspirin for 3 months after implantation)	12.7 ± 6.1	fDPVHs showed low pressure gradients and no evidence of dilatation or thickening, in addition to 100% freedom from explantation after 5 years; fDPVHs also showed evidence of adaptive growth	103
Burch et al. (2010)	cDPVH (47) with cH (47) as controls	SPVR (NR)	9.95 ± 7.96	cDPVHs were linked to non-significantly higher freedom from explantation after 8 years than cHs (79% versus 63%); no significant differences in valve performance were observed	109
Da Costa et al. (2010)	fDAVH or cDAVH (41)	SAVR (NR)	34 (0.1–71)	fDAVHs were associated with trivial to mild regurgitation, adequate haemodynamics and 98% freedom from reoperation; explanted fDAVHs showed low cellularization but stable structural integrity and a low rate of calcification	106
Dohmen et al. (2007)	cDPVH pre-seeded with aECs (11)	SPVR (NR)	44.0 ± 13.7	cDPVHs showed promising results in reconstructing the right ventricular outflow tract, with good performance and recellularization potential; however, the study was hampered by limited homograft availability	80
Cebotari et al. (2006)	fDPVH pre-cultured with aEPCs (2)	SPVR (aspirin for 1 month after implantation)	Ages 11 and 13	fDPVH were feasible and safe, with the potential to remodel and grow (increase in annulus diameter) and were associated with trivial valve regurgitation at 3.5 years and no signs of degeneration	79
Zehr et al. (2005)	cDAVH (22)	SAVR (no anticoagulation treatment)	53 ± 14	Panel-reactive antibody results were negative in 95% of patients after 1 year, and the valve showed good performance, with low transvalvular gradients	250
Hawkins et al. (2003)	cDPVH (14) with cH (20) as controls	SPVR (NR)	8.5 ± 7.9	The panel-reactive antibody levels for both class I and class II antibodies were significantly lower for cDPVHs than for cHs, but a similar functionality was observed after 1 year	251

aEC, autologous endothelial cell; aEPC, autologous endothelial progenitor cell; BJV, bovine jugular vein; cDAVH, cryopreserved decellularized aortic valve homograft; cDPVH, cryopreserved decellularized pulmonary valve homograft; cH, cryopreserved homograft; fDAVH, fresh decellularized aortic valve homograft; fDPVH, fresh decellularized pulmonary valve homograft; NR, not reported; SAVR, surgical aortic valve replacement; SPVR, surgical pulmonary valve replacement.

dysfunction in some patients[124,125]. By contrast, a series of clinical case reports demonstrated promising results when using CorMatrix for the repair and reconstruction of heart valves and heart valve leaflets and as a pulmonary valve conduit[119,126]. However, given the short follow-up time (<1 year) of these case reports, the long-term material durability and eventual degenerative phenomena of CorMatrix are not known[119]. A multicentre clinical trial on the performance of tricuspid valves using CorMatrix is ongoing and currently recruiting patients aged 1–70 years. Preliminary results presented in early 2020 demonstrated encouraging outcomes, with good

Table 2 | **Preclinical applications of decellularized homografts and xenograft-based TEHVs**

Homograft or xenograft (*n*)	Animal model (species)	Year	Procedure (anticoagulation or anti-aggregation regimen)	Study end points	Main findings	Ref.
Porcine SIS (20)	Lamb and sheep (Swifter)	2020	SPVR (lifelong treatment with anti-inflammatory analgesic or antithrombotic agent)	1, 3 and 6 months	Unreliable and unfavourable outcomes associated with the SIS valve; in total, seven animals died preterm owing to congestive heart failure and showed signs of leaflet thickening, bacterial endocarditis and strong inflammatory reaction with calcification, five animals had severe stenosis, and one had severe regurgitation; a well-functioning valve was observed in only seven animals, but leaflets showed limited signs of remodelling; overall, the unfavourable outcomes (that is, valve failure) might be related to the incomplete decellularization of the porcine SIS	116
Ovine DPVH-FD (3), ovine fDPVH (3) and porcine DPV-FD (3)	Sheep (NR)	2018	SPVR (dalteparin 2 weeks after implantation)	6 months	Freeze-drying is a promising method to extend the shelf-life of valvular grafts and does not affect early haemodynamics or cell infiltration; however, porcine DPV showed evidence of immunological reaction that did not impair early functionality	139
Porcine DAV (5)	Sheep (Polypay)	2017	SPVR (warfarin treatment 2 days after implantation)	5 months	Study showed feasibility of trans-species implantation with recellularization and sufficient haemodynamic function; thrombosis and bacterial or fungal contaminations were not observed; host cell infiltration was observed with the presence of collagen-producing myofibroblasts and endothelial cells	140
Porcine SIS as aortic cusp (4)	Pig (Landrace)	2017	SPVR (NR)	4 months	The implanted material remodelled and degraded over time, losing functionality owing to thickening and calcification	122
Porcine SIS as tricuspid valve (8), bioprostheses (2) and native valve (2)	Lamb and sheep (wethers and ewes)	2015	SPVR (heparin at surgery)	3 and 8 months	When implanted in lambs aged 3 months, the porcine SIS valve increased in annular diameter, with normal valvular performance; host cells migrated into the scaffold and tri-laminar ECM remodelling was observed	115
Porcine SIS as tricuspid valve (4)	Sheep (Western Range wether)	2014	SPVR (heparin at surgery)	3, 5, 8 and 12 months	Good valve performance with maintained coaptation was observed together with progressive tissue remodelling with collagen and elastin deposition and integration in the native annulus; no evidence of foreign body response was observed	114
Human DPV (5), baboon DPVH (3) and porcine cPV (6)	Baboon (NR)	2013	SPVR (NR)	10 and 26 weeks	Human and baboon DPV showed native-like haemodynamics; porcine cPV provoked the most intense antibody response	142
Porcine DAV (4)	Sheep (NR)	2012	SAVR (heparin at surgery; no anticoagulants after implantation)	1, 2 and 4 months	Good early performance, with no regurgitation under systemic pressure; evidence of smooth and pliable leaflets and full cellularization with host interstitial cells	81
Porcine DAV (8)	Dog (Mongrel)	2007	SPVR (NR)	1, 2 and 6 months	Efficient decellularization with minimal immune response and calcification; spontaneous endothelialization and host cell repopulation occurred within 2 months; stable valve performance was observed, with no regurgitation	129
Rabbit DAV (15)	Dog (NR)	2006	DAV was anastomosed in the transected abdominal aorta (no anticoagulants used during surgery or after implantation)	1, 3 and 6 months	Complete loss of valvular structure was seen, together with re-endothelialization and recellularization with a basic vascular cell component; no immunological response was observed	252
Porcine DPV (7)	Sheep (NR)	2005	SPVR (NR)	3 and 6 months	Valves showed smooth and pliable leaflets, with no evidence of thrombosis; endothelialization was observed, together with the presence of fibroblasts in the tissue; newly secreted collagen was detected in the absence of calcific deposits, suggesting remodelling potential of the implanted DPV	130

Table 2 (cont.) | **Preclinical applications of decellularized homografts and xenograft-based TEHVs**

Homograft or xenograft (*n*)	Animal model (species)	Year	Procedure (anticoagulation or anti-aggregation regimen)	Study end points	Main findings	Ref.
Porcine DAV (12), porcine DAV-Fn-HGF (15) and porcine DAV-HGF (12)	Dog (Beagle)	2005	SPVR (heparin at surgery, no anticoagulants after implantation)	1 week, 1 month and preterm death	At 1 week, the Fn-HGF-functionalized DAV showed partial endothelial coverage that resulted in complete endothelialization at 1 month; a greater number of cells was observed in the Fn-HGF group compared with the DAV and DAV-HGF groups	253
Porcine SIS as pulmonary valve (12)	Pig (farm pig)	2005	TPVR (heparin at surgery)	1 day and 1, 3, 6 and 12 months; one animal died before valve replacement	Good performance up to 12 months was observed, with no stenosis and trivial regurgitation; intensive remodelling of the SIS was observed, with endothelialization and host fibroblast infiltration	113
Porcine DPV (3) and porcine DPV pre-seeded with aECs and aMFBs (5)	Sheep (NR)	2003	SPVR (NR)	6 months	Porcine endogenous retroviral DNA was initially observed in porcine DPV samples but was not detected in the host animal blood samples, suggesting that porcine DPV did not transmit the retroviral DNA; after 6 months, cellular infiltration with endothelial and interstitial cells was observed	131
Porcine DPV pre-seeded with aECs (8)	Sheep (NR)	2003	SPVR (heparin at surgery, no anticoagulants after implantation)	7 days and 3 and 6 months	The explanted DPV showed a confluent endothelial cell monolayer at all time points, with no signs of calcification and low calcium content; an increasing number of fibroblasts were observed in the valvular tissue over time	193
Porcine DAV (5) and ovine cAV (2)	Sheep (Suffolk)	1999	SPVR (NR)	5 months	Good valve performance over time was observed, with no macroscopic damage to the leaflets; cellular repopulation by fibroblasts was also observed, with no signs of calcification, suggesting that decellularization can stabilize xenogeneic valves	254

aEC, autologous endothelial cell; aMFB, autologous myofibroblast; cAV, cryopreserved aortic valve; cPV, cryopreserved pulmonary valve; DAV, decellularized aortic valve; DPV, decellularized pulmonary valve; DPVH, decellularized pulmonary valve homograft; ECM, extracellular matrix; fDPVH, fresh decellularized pulmonary valve homograft; FD, freeze-dried; Fn, fibronectin; HGF, hepatocyte growth factor; NR, not reported; SAVR, surgical aortic valve replacement; SIS, small interstitial submucosa; SPVR, surgical pulmonary valve replacement; TEHV, tissue-engineered heart valve; TPVR, transcatheter pulmonary valve replacement.

valve performance and only mild regurgitation at early follow-up (range of 6–12 months) in eight out of ten patients[127] (TABLE 3). The long-term results of this trial are eagerly awaited[128].

Preclinical studies of decellularized xenografts generated using porcine pulmonary or aortic valves (TABLE 2) showed promising results in terms of valve performance, host cell infiltration, endothelialization and collagen deposition[129,130]. Importantly, a sheep study also demonstrated that endogenous retrovirus DNA, present in the porcine pulmonary valve, was not transmitted to the host animal[131]. However, clinical trials assessing these xenografts have generated mixed findings. In one clinical study, xenografts showed promising valvular performance, similar to that of the native valve[132], and favourable outcomes in three out of a total of seven patients with congenital heart disease[110]. By contrast, xenografts have been associated with a high incidence of structural valve failure[133], stenosis and regurgitation[134,135] as well as massive inflammation[136], a lack of remodelling or cellularization[134], and even sudden cardiac death[137] (TABLE 3). On the basis of these findings, the clinical use of decellularized xenografts should proceed with caution.

Regardless of the approach and material used, the reasons underlying the poor performance of xenografts in clinical trials are multifactorial, with host-specific factors (such as age, presence of comorbidities and individual immune response), tissue-related factors (including animal origin, processing and degree of decellularization) and implantation-specific factors (such as annulus anatomy, haemodynamic forces and stress distribution, and valve geometry) all contributing to early valve deterioration and dysfunction[116]. The majority of commercially available decellularized tissues still contain traces of DNA that are insufficient to elicit an immune response in the host. However, various studies have shown that xenogeneic tissues, such as decellularized porcine small intestine submucosa[116,118], can contain up to 6 μg/mg of nucleic acid debris, thereby exceeding the recommended threshold of 50 ng/mg that is commonly accepted to be non-immunogenic[138]. The presence of residual DNA can provoke a strong inflammatory response in the implanted decellularized xenogeneic tissue, causing calcification and structural degeneration[117,137]. However, these adverse effects were often not observed in preclinical studies in sheep, again highlighting the translational limitations of animal models[81,129–131,139,140] (TABLE 2). The striking differences in outcomes of xenograft implantation between preclinical and clinical trials also indicate the need for improved in vitro testing platforms to assess xenograft immunocompatibility and to identify animal models with an immune system that more closely resembles the human immune system (such as baboons and other non-human primates[89,141,142]). Taken together, these results suggest that the use of completely

Table 3 | **Clinical applications of decellularized xenograft-based valve replacements**

Xenograft (*n*)	Study design (year)	Procedure (anticoagulation or anti-aggregation regimen)	Age (years)	Main findings	Ref.
Porcine SIS as tricuspid valve (10)	FDA clinical feasibility trial (2020)	Surgical tricuspid replacement (NR)	NR	After an average follow-up of 8 months (range 6–12 months), good valve performance was reported; trace–moderate regurgitation was reported at implantation and only mild regurgitation was noted at all subsequent follow-up appointments in eight patients; one patient showed papillary dehiscence at 10 weeks and another patient progressed from mild to moderate regurgitation after an accident	127
Porcine DPV (492)	Single-centre, prospective (2019)	SPVR (NR)	57 ± 11 (range 21–73)	A high incidence of porcine DPV dysfunction and resulting right ventricular failure was observed, which necessitated reoperation or reintervention in 30.5% of patients; freedom from porcine DPV reoperation at 5.0 years, 10.0 years and 12.5 years was 76.2 ± 2.1%, 58.6 ± 2.9% and 53.4 ± 3.4%, respectively, and lower than with autografts (91.8 ± 1.4%, 86.1 ± 2.0% and 86.1 ± 2.0%, respectively)	133
Porcine SIS as aortic cusp (6)	Case report (2017)	SAV repair (NR)	Range 0.2–14.0	Clinically significant insufficiency was observed in five patients in the postoperative follow-up period (range 119–441 days); a migration of inflammatory cells, which induced structural and functional changes in the implanted tissue, was observed	121
Porcine SIS for aortic valve repair (1)	Case report (2017)	SAVR (NR)	12	Stable valvular performance was observed for 2 years; after 4 years, a replacement valve was required owing to calcification, fibrosis and retraction	124
Porcine SIS for pulmonary and aortic valve repair (22)	Single-centre, prospective, non-randomized (2016)	SAV and SPV repair (NR)	Range 0.2–14.0	The implanted material was not advantageous compared with polytetrafluoroethylene, particularly when used for pulmonary valve reconstruction, for which functional deterioration was observed	120
Porcine DPV (1)	Case report (2014)	SPVR (NR)	6	Severe conduit stenosis, moderate regurgitation and hypertrophy of the right ventricle were observed; several aneurysms were present in the conduit, characterized by a lack of cellularization with no evidence of an inflammatory or foreign body response	135
Porcine DPV (21)	Case report (2014)	SPVR (NR)	49 (range 33–56)	A massive inflammatory reaction, necrosis and graft stenosis were observed; not recommended for reconstruction in adults	136
Porcine DPV (26)	Case report (2013)	SPVR and TPVR (NR)	12.4 (range 0.8–38.7)	A total of 52% of patients needed reoperation owing to stenosis with moderate-to-severe insufficiency; histology results showed wall thickening with severe foreign body reaction and inflammation; endothelialization was not observed	255
Porcine DPV (93)	Case report (2012)	SPVR (NR)	20 months (range 5 days to 290 months)	DPV failure was caused by stenosis, pseudoaneurysms or conduit dilatation; poor host cell infiltration was observed, with the presence of inflammatory giant cells; the DPV failure rate was 35.5% and the dysfunction rate was 29.0%	134
Porcine DPV (61)	Case report (2011)	SPVR (NR)	Range 9 days to 50 years	Valve failure occurred in four patients, necessitating re-operation; valve showed unremarkable functionality and normal structural features, with no evidence of calcification; the intermediate-term performance of the DPV was favourable in patients with congenital heart disease	110
Porcine DPV (16)	Case report (2010)	SPVR (heparin at surgery, aspirin after implantation)	14 ± 11	Graft obstruction was observed in 38% of patients after 10 months; histological examination revealed stenosis owing to inflammation and calcification	256
Porcine DPV pre-seeded with aECs (12)	Case report (2007)	SPVR (NR)	44.0 ± 13.7	Porcine DPV showed promising performance and recellularization potential, similar to decellularized homografts	80

Table 3 (cont.) | **Clinical applications of decellularized xenograft-based valve replacements**

Xenograft (*n*)	Study design (year)	Procedure (anticoagulation or anti-aggregation regimen)	Age (years)	Main findings	Ref.
Porcine DPV (50)	Case report (2005)	SPVR (NR)	46	Physiological-like performance of the valve, with unremarkable haemodynamics and low pressure gradients	132
Porcine DPV (4)	Case report (2003)	SPVR (NR)	Range 2.5–11.0	DPV showed good postoperative performance; sudden death owing to valve failure, valve degeneration and rupture of the valve occurred in three patients; severe inflammation with granulocytes and macrophages as well as calcific deposits were observed in the explants; samples from the pre-implant DPV showed incomplete decellularization	137

aEC, autologous endothelial cell; DPV, decellularized pulmonary valve; NR, not reported; SAV, surgical aortic valve; SAVR, surgical aortic valve replacement; SIS, small interstitial submucosa; SPV, surgical pulmonary valve; SPVR, surgical pulmonary valve replacement; TPVR, transcatheter pulmonary valve replacement.

decellularized xenografts manufactured with easily accessible, off-the-shelf biomaterials with improved immunocompatibility must undergo rigorous preclinical and clinical testing to ensure safe clinical translation.

Decellularized TEMs grown in vitro. To date, TEHVs have been limited by donor-to-donor variability in cultured ECM as well as by the technical and logistical hurdles in the timing of valve production with the implantation procedure. The process of decellularization of TEHVs was introduced in 2012 to overcome these limitations through the development of non-immunogenic substitutes that are available off-the-shelf with similar mechanical and biological properties to those of their cellular counterparts[96]. Furthermore, decellularized TEMs grown in vitro for cardiovascular applications can be manufactured using common and readily accessible cell sources, such as myofibroblasts and dermal fibroblasts, as opposed to patient-derived cells, which are required for autologous valve production[96,143–150]. TEM-based heart valve replacements have been used as substitutes for pulmonary and aortic valves in large-animal models[144–147,150–152] (TABLE 4). Importantly, TEM-based valves can be implanted via minimally invasive transcatheter techniques, even as an aortic valve replacement, and have shown favourable early functionality, host cell repopulation, endothelialization, integration and remodelling over time[82,121,124–127,131,132]. In this context, a proof-of-concept study published in 2018 demonstrated that a TEHV designed using computational modelling can guide tissue remodelling towards improved long-term performance[144]. Ovine TEM-based TEHVs were used to replace the pulmonary valve in sheep and, after 1 year, demonstrated clinical grade in vivo performance, excellent durability and native-like remodelling, with evidence of endothelialization, collagen and elastin deposition, and the initial formation of sinuses of Valsalva[144]. In the past 4 years, several clinical trials using TEM-based vascular grafts have been initiated (TABLE 5), with favourable results in terms of host cell repopulation and graft patency when used as dialysis access conduits[153,154].

Bioresorbable polymers with regenerative potential. Synthetic bioresorbable polymers are gaining interest as potential starting materials for in situ cardiovascular applications owing to their adaptable mechanical, chemical and architectural properties. TEHVs manufactured using bioresorbable polymers have the advantage of being naturally absorbed and metabolized by the body, as extensively reviewed previously[155]. In addition, synthetic polymers are reproducible, available off-the-shelf and scalable. In light of these advantages, researchers have investigated the functionality and remodelling potential of bioresorbable polymeric valves in large-animal models[98,156–160] (TABLE 6). Furthermore, bioresorbable polymer-based TEHVs can also adopt a one-step preseeding procedure using autologous bone marrow mononuclear cells to modulate the early inflammatory response and the remodelling cascade[89,161–164] (TABLE 7). Synthetic bioresorbable polymers are suitable for transcatheter applications either as a pulmonary or as an aortic valve replacement. In this context, bisurea-based and ureidopyrimidone-based supramolecular polymers are compatible with surgical[98,159] and transcatheter[158,164] implantation techniques and have been used to manufacture bioresorbable valves that have regenerative potential, demonstrating acceptable functionality for up to 12 months[98,159]. In addition, rapid cellularization, ECM deposition and scaffold degradation were observed in the explanted valves, confirming their remodelling potential. In light of these promising results, international consortia are currently investigating the potential of these supramolecular polymers in the development of next-generation TAVR[165]. However, independent of the supramolecular polymer and the implantation technique used, intra-valve and inter-valve differences in tissue remodelling (such as cellular infiltration, thickening, elastin deposition and scaffold resorption) have been reported[98,159,164], suggesting the need to further investigate the mechanisms involved in polymer degradation and in situ remodelling to ensure safe clinical translation.

Importantly, supramolecular polymer-based cardiovascular replacements have been explored in early clinical trials (TABLE 8). Five patients aged 4–12 years received a supramolecular polymer-based graft as an extracardiac cavopulmonary conduit for the treatment of univentricular cardiac malformation[166]. No device-related adverse events were observed after implantation, and graft performance was stable. An improvement in

Table 4 | **Preclinical applications of decellularized TEM-based TEHVs with in situ regenerative potential**

TEM (*n*)	Animal model (species)	Year	Procedure (anticoagulation or anti-aggregation regimen)	Study end points	Main findings	Ref.
PGA–P4HB-based human TEM (3)	Sheep (White Alpine)	2019	TPVR (heparin at surgery)	Acute	The study showed the feasibility of using human cell-derived TEM to manufacture TEHVs comprising Valsalva sinuses; acute valve functionality was excellent; explanted valves showed the presence of blood-derived cells in the tissue	145
PGA–P4HB-based human TEM (5)	Sheep (White Alpine)	2018	TAVR (heparin at surgery)	Acute	The study showed the feasibility of TAVR using clinically relevant TEHVs designed on the basis of human cell-derived TEM; good haemodynamic performance was observed, with free coronary flow, no stenosis and no paravalvular leak; explanted valves showed the presence of blood-derived cells in the tissue	147
PGA–P4HB-based ovine TEM (11)	Sheep (Grey Horned Heath)	2018	TPVR (heparin at surgery, aspirin and dalteparin for 5 days after implantation)	12 months	Computational modelling-based valve design was optimized to control the deformation of the leaflets; excellent long-term in vivo performance was observed, with no regurgitation or stenosis, together with native-like remodelling; remodelling outcomes were predicted by computational modelling	144
PGA–P4HB-based ovine TEM (3)	Sheep (NR)	2018	TPVR (heparin at surgery)	Acute and 4 months	This study assessed the first prototype of a TEHV with integrated Valsalva sinuses; findings showed encouraging acute and short-term valve performance, but leaflet shortening and regurgitation were observed after 4 months; host endothelial and interstitial cells with ECM synthesis and remodelling were observed	146
Fibrin-based ovine TEM (8)	Sheep (Dorset)	2017	SPVR (heparin for the duration of the study)	5 months	Good performance for up to 8 weeks, followed by increased regurgitation owing to evident leaflet shortening; remodelling was evident with host cell infiltration and collagen and elastin deposition	152
Fibrin-based ovine TEM (4)	Sheep (Dorset)	2015	SAVR (heparin for the duration of the study)	3 and 6 months	The study was the first long-term evaluation of a TEHV as an aortic valve replacement in a preclinical model; good haemodynamic performance was observed, with no evidence of stenosis; ECM remodelling, collagen and elastin synthesis, and the presence of host endothelial and interstitial cells were observed	150
PGA–P4HB-based ovine TEM (12)	Sheep	2014	TPVR (NR)	1 day and 2, 4 and 6 months	Good early performance, with mild regurgitation starting at 8 weeks and progressing to moderate at 24 weeks owing to compromised leaflet coaptation; substantial host cell repopulation and ECM remodelling without calcification were observed	151
PGA–P4HB-based human TEM (6)	Baboon (Chacma)	2013	TPVR (aspirin and warfarin for the duration of the study)	1 and 2 months	After 2 months, valve functionality showed signs of mild-to-moderate regurgitation; leaflets were thin and mobile but shortened over time; rapid cellular repopulation was observed in the tissue-engineered valve compared with the human native heart valve used as a control, confirming the substantial remodelling potential of TEM	141

ECM, extracellular matrix; NR, not reported; P4HB, poly(4-hydroxybutyrate); PGA, poly(glycolic acid); SAVR, surgical aortic valve replacement; SPVR, surgical pulmonary valve replacement; TAVR, transcatheter aortic valve replacement; TEHV, tissue-engineered heart valve; TEM, tissue-engineered matrix; TPVR, transcatheter pulmonary valve replacement.

the patients' general health condition was reported at 12 months. However, longer follow-up is needed to further assess the graft performance, remodelling potential and even growth capacity of these bioresorbable vascular grafts. In the Xplore-1 study[167,168], pulmonary valve conduits designed using the same supramolecular materials were evaluated in 12 patients aged 2–12 years (TABLE 8). Preliminary reports at 24 months demonstrated unfavourable findings. Although no major clinical events (death or need for re-operation or intervention) were reported, echocardiography assessment showed the presence of moderate-to-severe pulmonary insufficiency in 11 out of 12 patients starting at 6 months after implantation. In addition, a protruding leaflet was observed in six of the implanted valves. However, in the Xplore-2 follow-up trial[169], a second-generation device, which was produced through a more controlled manufacturing process than the device in the Xplore-1 trial, was implanted into six children aged 2–9 years. Preliminary reports from the 12-month follow-up showed good

valve performance with no insufficiency, but one case of valve stenosis was observed and one patient required a re-operation[167]. Given the mixed outcomes of these two trials, further clinical data with a longer follow-up period are needed to determine the overall safety and efficacy of supramolecular polymer-based pulmonary valve conduits.

Clinical translation of TEHVs

The clinical translation of TEHV approaches has become a reality. Decellularized homologous TEHVs have shown good long-term performance with evidence of adaptive growth[103] and cell repopulation[80,106,170]. By contrast, TEHVs designed on the basis of decellularized xenografts have been associated with stenosis and increased inflammation (TABLE 3). Bioresorbable polymer-based TEHVs are currently being tested in clinical settings after a supramolecular polymer showed promising results in terms of patency and performance when used to treat congenital heart disease[167] (TABLE 8). Considering the promising clinical results of TEM-based vascular grafts, heart valve replacements designed using a similar concept are expected to reach the next phase of clinical translation after clinical-grade functionality has been proven in a sheep model[144].

Challenges associated with TEHV use

Immunocompatibility. Xenograft bioprostheses used in the clinical setting rely on fixation treatment to block xenogeneic antigens. However, this fixation treatment can also impede cell infiltration and tissue remodelling, which are fundamental prerequisites for all TEHVs[96]. Although TEHVs manufactured with bioresorbable polymeric matrices are cell-free implants that do not contain any cell-derived components, decellularized and regenerative TEHVs that are made with in vitro-grown TEMs or use allogenic and xenogeneic grafts do not require fixation treatment to ensure cellular infiltration and matrix remodelling[96]. Therefore, decellularized tissues must be carefully tested for residual antigenicity or for the potential to transmit disease.

A preclinical study has demonstrated that, after decellularization, porcine pulmonary valves cannot transmit residual retrovirus DNA to the host animal[131]. However, issues associated with the implantation of decellularized xenografts that had not been completely decellularized have also been highlighted by a clinical study[137]. Incompletely decellularized xenogeneic products might still contain epitopes, such as α-galactose[171], that can elicit a strong inflammatory response, which can in turn result in calcification and structural degeneration[117,137]. To address this issue, researchers have sought to improve decellularization protocols[172] and to develop antigen-reduction treatments (such as α-galactosidase[173]) to limit residual antigenicity without affecting cell infiltration and matrix remodelling. In vivo studies are needed to validate these new approaches and to ensure the absence of immune cell adhesion and infiltration as well as to confirm the host cell repopulation and remodelling potential. However, experimental data are often difficult to extrapolate into the clinical setting given the vast differences in the immune

Table 5 | **Clinical applications of decellularized hTEM-based vascular grafts**

Trial design (year)	Procedure	*n*	Age range (years)	Main findings	Ref.
Single-group, phase II trials (2019)	Surgical	60	18–80	Findings showed host myogenic, endothelial and progenitor cell repopulation of acellular human grafts; functional multi-layered living tissues were identified, which self-healed after cannulation injury	153
Request for expanded access (compassionate use) of human acellular vessels (2018)	Surgical	NR	NR	Ongoing	257
Phase III trial (2017)	Surgical	240	≤18	Ongoing	258
Single-group, phase II trials (2016)	Surgical	60	18–80	No dilatation was observed and post-cannulation bleeding was rare; at 6 months, 63% of patients had primary patency, 73% had primary assisted patency and 97% had secondary patency; the loss of primary patency occurred predominantly owing to thrombosis; at 12 months, 28% had primary patency, 38% had primary assisted patency and 89% had secondary patency	154
Interventional randomized, parallel assignment trial (2016)	Surgical	355	≤18	Ongoing	259
Non-randomized, phase II trial (2016)	Surgical	40	18–85	Ongoing	260
Single-group, phase II trial (2016)	Surgical	25	18–85	Ongoing	261
Single-group pilot study (2013)	Surgical	20	18–80	Ongoing	262

hTEM, human tissue-engineered matrix; NR, not reported.

Table 6 | **Preclinical applications of bioresorbable or biodegradable polymer-based TEHVs**

Polymer	Animal model (species)	Year	*n*	Procedure (anticoagulation or anti-aggregation regimen)	End points	Main findings	Ref.
Polycarbonate urethane urea and AZ31 magnesium alloy stent	Pig (Yorkshire)	2019	5	SPVR (heparin at surgery)	Acute	Successful implantation, with normal leaflet function; no evidence of thrombosis, regurgitation or degradation was observed	160
UPy-polyester-urethanes	Sheep (NR)	2018	18	SPVR (NR)	2, 6 and 12 months	Ongoing remodelling process with neointima formation was observed; neointimal thickness and inflammation peaked at 6 months, whereas degradation peaked at 12 months	159
UPy-polyester-urethanes	Sheep (Ile de France)	2017	33	TAVR (NR)	Acute	Good haemodynamic performance was observed, similar to that of commercially available valves, with an acceptable degree of regurgitation	158
UPy-polyester-urethanes	Sheep (Swifter)	2017	20	SPVR (NR)	Acute and 3, 6, 12 and 24 months	Favourable and durable haemodynamic performance was observed, with no evidence of stenosis, obstruction or severe regurgitation	156
Bisurea-polycarbonate	Sheep (Swifter)	2017	10	SPVR (heparin at surgery, lifelong treatment with anti-inflammatory analgesic)	2, 6 and 12 months	Sustained functionality up to 12 months, with evidence of remodelling with de novo collagen and elastin synthesis and incomplete scaffold reabsorption	98
P4HB-gelatin	Sheep (NR)	2017	4	TPVR (NR)	Acute	Good haemodynamic performance and competence after implantation	157

NR, not reported; P4HB, poly(4-hydroxybutyrate); SPVR, surgical pulmonary valve replacement; TAVR, transcatheter aortic valve replacement; TEHV, tissue-engineered heart valve; TPVR, transcatheter pulmonary valve replacement; UPy, ureido-pyrimidinone.

response between animals and humans[174]. This discrepancy became evident through the numerous valve failures associated with decellularized xenografts reported in clinical trials (TABLE 3) that were not observed in preclinical studies (TABLE 2).

Therefore, in vitro testing of the immunocompatibility of a decellularized product should include the use of human cells. For this purpose, in vitro screening platforms that incorporate physiological and pathological values of shear stress or cyclic strain have been established to characterize human blood-derived mononuclear cell recruitment into scaffolds[175,176]. Potential chemotaxis signalling of the implanted decellularized matrix can be evaluated by using microfluidic systems to visualize blood-derived cell infiltration under flow conditions[177]. In addition, potential macrophage adhesion and polarization onto the implanted scaffold should be investigated to respectively assess the presence of pro-inflammatory or anti-inflammatory macrophages, which are responsible for rapid matrix turnover or for a prolonged and detrimental inflammatory response and calcification[178].

Haemocompatibility. Biocompatibility is a crucial feature of all medical devices. The International Organization for Standardization (ISO) requires an assessment of haemocompatibility for any medical device that comes into contact with circulating blood, including heart valve implants (ISO 10993-4)[179]. Through direct contact with blood, the material surface of any heart valve replacement absorbs plasma proteins that promote platelet and leukocyte adhesion and activation, thereby increasing thrombotic and thromboembolic risk[180]. A thorough understanding of the blood–implant interaction is needed to tailor anticoagulation treatment and to prevent thrombotic and thromboembolic complications, particularly for transcatheter approaches, given that the metal-based stent might further activate platelets to initiate the coagulation cascade. For these reasons, animal models and clinical trial participants involved in studies of TEHVs with in situ regenerative potential are often subject to early and temporary anticoagulation or anti-aggregation therapy to reduce the risk of early thrombosis (TABLES 2,4,6,7). Over time, the antithrombotic agents can be reduced in dosage and eventually completely stopped as soon as the neo-endothelialization process of the construct has been accomplished to ensure a haemocompatible blood–scaffold interface. Unfortunately, given that investigators have used different types of antithrombotic treatment at various durations for different TEHVs, we do not yet understand the thrombotic and thromboembolic risks associated with these devices and the effects that anticoagulants and anti-aggregation agents might have on the remodelling process.

When developing novel materials that will come into contact with blood, material haemocompatibility platforms must be used to identify potential platelet adhesion[181] and assess the fibrin clot structure[182]. During this process, blood is collected and analysed before being incubated together with the biomaterial in static, agitated or dynamic set-ups, as previously reviewed[180]. These in vitro systems have the advantage of having well-established and controllable parameters (such as rate of blood flow and anticoagulant used) and can provide detailed information on blood cell adhesion and activation, protein adsorption, and the presence of markers of the complement system and coagulation activation.

Crimping
A procedure used to reduce the diameter of the valve prosthesis by more than threefold to fit the prosthesis into the catheter used for minimally invasive implantation.

In an attempt to reduce the thrombogenicity of a cardiovascular implant, numerous studies have evaluated the strategies of either functionalizing the material to favour haemocompatibility or pre-seeding and culturing autologous endothelial cells to increase their anti-thrombogenic properties[183]. Material functionalization is aimed at facilitating endogenous endothelial cell adhesion and endothelium formation directly at the site of implantation. In situ recruitment, adhesion and differentiation of endothelial cells and circulating endothelial progenitor cells might be achieved using CD34 (REFS[184,185]) and CD133 (REF.[186]) antibodies, fibronectin[187], fibrin[188], laminin-derived peptides[189] and growth factors such as vascular endothelial growth factor[185,190] and stromal cell-derived factor 1α (SDF1α)[191]. However, non-specific cell adhesion and differentiation in response to antibody-functionalized materials have been reported[184], suggesting the need for improved understanding of the in situ endothelialization process.

Endothelial cell seeding is performed to generate an anti-thrombogenic surface by using autologous endothelial cells to cover the implanted material. Although in vitro endothelialization of small-calibre vascular grafts has shown limited benefits for material haemocompatibility[192], autologous endothelial cell pre-seeding onto pulmonary homografts is associated with favourable haemodynamic performance in both the preclinical[99,131,193] and clinical[79,80,194] settings, in which a confluent endothelium was observed. However, several disadvantages have limited the use of this approach. Endothelial cell isolation and expansion as well as endothelial progenitor cell isolation and differentiation are time consuming and costly procedures. Furthermore, cell seeding might be inefficient owing to limited or inhomogeneous cell adhesion on the material[195], and the rates of cell retention and viability after the crimping procedure required for transcatheter implantation are low[196]. To overcome these limitations, a combination approach using SDF1α functionalization and endothelial cell pre-seeding was evaluated[191]. This approach resulted in increased recellularization as well as less inflammatory cell infiltration, calcification and platelet adhesion compared with xenogeneic heart valves treated with basic fibroblast growth factor. In addition, bioreactors for 3D cell seeding should be considered to ensure a homogeneous and controlled endothelial cell distribution[197]. Together, these results suggest that a more complex functionalization strategy, combining two or more bioactive components[189,198] as well as surface topography[199] and endothelial cell pre-seeding[191], should be further investigated to improve the in situ endothelialization of regenerative materials.

Regulation of in situ valve remodelling. Although vascular graft remodelling has been extensively evaluated in the clinical setting, to what extent these results can be applied to the field of heart valve tissue engineering remains unknown owing to the complex haemodynamic environment. The lack of understanding of the mechanisms driving cellular repopulation and neo-tissue formation after TEHV implantation remains a barrier to the efficient optimization of TEHV design. The majority of the studies investigating TEHV functionality and remodelling have reported a loss of valve functionality within 12–24 weeks after implantation owing to uncontrolled, adverse tissue remodelling, resulting in valvular leaflet thickening or shortening (retraction) and subsequent valvular dysfunction, regurgitation and

Table 7 | **Preclinical application of bioresorbable or biodegradable polymer-based TEHVs pre-seeded with aBMMNCs**

Polymer	Animal model (species)	Year	*n*	Procedure	End points	Main findings	Ref.
Bisurea-polycarbonate and bisurea-polycarbonate pre-seeded with aBMMNCs	Sheep (White Alpine)	2019	13	TPVR (heparin at surgery, calciparin 30 days after implantation)	Acute and 1 and 6 months	aBMMNC pre-seeding was feasible but resulted in severe regurgitation and calcification; differential leaflet remodelling was observed, independently of pre-seeding	164
PGA–P4HB pre-seeded with aBMMNCs	Sheep (White Alpine)	2014	4	TAVR (NR)	Acute	Sufficient positioning of valve, with no obstruction of the coronary arteries, and no evidence of structural damage or stent migration; paravalvular leakage and central aortic regurgitation were also noted	163
PGA–P4HB pre-seeded with aBMMNCs	Sheep (White Alpine)	2012	12	TAVR (aspirin daily)	Acute, 2 days and 1–2 weeks	Adequate leaflet mobility and functionality was observed with intact leaflet structure and no signs of thrombus or structural damage; early cellular remodelling was seen after 2 weeks	162
PGA–P4HB pre-seeded with aBMMNCs	Sheep (White Alpine)	2011	1	TAVR (NR)	Acute	The study showed technical feasibility of minimally invasive aortic replacement with TEHVs and adequate leaflet mobility and coaptation, with no evidence of thrombus formation or structural damage	161
PGA–P4HB pre-seeded with aBMMNCs	Baboon (Chacma)	2011	6	TPVR (aspirin and warfarin for 4 weeks after implantation)	Acute and 1 month	Preserved valvular structures and adequate functionality were noted as well as evidence of substantial cellular remodelling and layered and endothelialized tissues	89

aBMMNC, autologous bone marrow mononuclear cell; NR, not reported; P4HB, poly(4-hydroxybutyrate); PGA, poly(glycolic acid); TAVR, transcatheter aortic valve replacement; TEHV, tissue-engineered heart valve; TPVR, transcatheter pulmonary valve replacement.

Table 8 | **Clinical applications of bioresorbable polymer-based TEHVs and grafts**

Polymer (trial)	Study design (year)	Procedure	*n*	Age range (years)	Main findings	Ref.
UPy-polyester-urethanes (Xplore-2)	Non-randomized, single-group assignment (2020)	SPVR	6	2–9	At 1 year, echocardiography performed in six patients did not reveal severe regurgitation or protruding leaflets; however, one valve stenosis was reported and one patient required a re-operation	167
UPy-polyester-urethanes (Xplore-1)	Non-randomized, single-group assignment (2020)	SPVR	12	2–12	At 2 years, no major clinical events (death or re-operation) and no signs of aneurysm or stenosis were seen; however, echocardiography detected moderate or severe regurgitation in 11 out of 12 patients; 6 valves showed a protruding leaflet	167
UPy-polyester-urethanes	Non-randomized, single-group assignment (2018)	SPVR	55	≤22	NR	263
UPy-polyester-urethanes	Single-group assignment (2017)	EC-TCPC	5	4–12	Good patient recovery with no complications; no device-related adverse events were observed; the implanted graft was anatomically and functionally stable in all patients, and all patients showed an improvement in their general condition	166
UPy-polyester-urethanes (Xplore-2)	Non-randomized, single-group assignment (2017)	SPVR	10	2–22	NR	169
UPy-polyester-urethanes (Xplore-1)	Non-randomized, single-group assignment (2016)	SPVR	12	2–22	NR	168

EC-TCPC, extracardiac total cavopulmonary connection; NR, not reported; SPVR, surgical pulmonary valve replacement; TEHV, tissue-engineered heart valve; UPy, ureido-pyrimidinone.

failure[87,88,93,141,146,150–152]. Therefore, a thorough understanding of the remodelling process is needed to prevent maladaptive remodelling, which remains a major challenge for the clinical adoption of TEHV approaches. In this context, computational modelling of valve mechanics with corresponding tissue remodelling has been suggested as a powerful strategy to predict the consequences of changes in valve design on the overall outcome[200]. Although computational models have already been used to optimize the performance of mechanical and bioprosthetic heart valves[201,202], reports of their use in predicting and improving the performance of TEHVs are limited[200,203]. Our group integrated a computational modelling-inspired heart valve design to guide tissue remodelling towards the long-term functionality of TEHVs[144]. After implantation in sheep as a transcatheter replacement for the pulmonary valve, the TEHV showed favourable long-term in vivo performance up to 1 year, as predicted by the computational model, and retained native-like remodelling characteristics.

Despite the advances in both in vitro and in silico models to assess the functionality and remodelling potential of TEHVs, positive in vivo findings in animal models are required before clinical testing. Indeed, valve integration, cell repopulation, ECM formation and valve growth can be tested only in vivo. In this regard, TEHVs are usually assessed in large-animal models. Among all mammals, the cardiovascular system of non-human primates most closely resembles that of humans. However, owing to high costs and strict ethical regulations, very few studies have evaluated TEHVs in non-human primates[89,142]. Conversely, the ovine model is FDA-recommended for the preclinical validation of heart valves and can provide important information regarding valve functionality and durability as well as potential progressive and accelerated degeneration and calcification. However, international guidelines do not provide requirements on the specific animal breed to be chosen for the preclinical studies. Given that information about animal breed, sex and age are often not included in scientific publications, the evaluation and comparison of the remodelling outcomes between different studies have proven difficult, if not impossible.

At present, longitudinal studies that have assessed the mechanistic aspects of the biological and remodelling processes are still sparse. Kluin et al. reported a proof-of-concept study that assessed an in situ TEHV designed using a slow-degrading, bisurea-modified supramolecular polymer[98]. After surgical implantation to replace the pulmonary valve, the TEHV was populated by endogenous cells and, over the 12-month follow-up period, the implant was gradually replaced by de novo ECM. Despite these promising results, only partial cell-driven polymer resorption occurred after 12 months and, importantly, the explanted valves had a very heterogeneous appearance. This heterogeneity limits the predictability and reproducibility of the outcomes, making the clinical translation of these tissue-engineered replacements even more difficult. Therefore, an even longer follow-up period will be needed to validate this concept, particularly with regard to the risk of potential maladaptive remodelling after complete polymer degradation, which cannot yet be controlled.

In 2019, Fioretta et al. investigated the heterogeneity in remodelling of bisurea-modified, supramolecular polymer-based TEHVs[164]. Briefly, these TEHVs were implanted as a transcatheter pulmonary valve replacement in sheep. At 6 months, marked differences in

cellular infiltration, scaffold degradation and ECM deposition were observed within the same valve explant and between different explants, highlighting the intra-valve and inter-valve variation associated with TEHV implantation. Future studies are needed to investigate the reasons underlying the observed heterogeneous remodelling and the differences in regenerative mechanisms to better predict valve functionality and remodelling and to increase the safety profile of TEHVs (FIG. 3).

Functional growth. Current clinically available valvular replacements are non-living and non-regenerative prostheses that lack growth potential and therefore need to be replaced in paediatric patients to accommodate somatic growth. Therefore, good growth potential is an important goal in tissue engineering as well as a fundamental prerequisite for paediatric applications. Given that TEHVs have the potential to remodel into a native-like tissue after implantation, they have been hypothesized to be able to grow with the host. However, to date, very few studies have investigated the growth capacity of TEHVs. Preclinical investigations of decellularized xenogeneic valves in a lamb model demonstrated an increase in TEHV annular diameter that is compatible with animal growth[82,115]. Additionally, TEM-based vascular grafts have shown signs of somatic growth (that is, increased inner diameter and conduit length) when implanted as a pulmonary artery replacement in lambs[152]. TEM-based TEHVs implanted in lambs have also been shown to increase in annulus diameter with animal growth and matched well with the growth of the neighbouring pulmonary artery[152]. However, this growth was associated with impaired valve functionality and incremental valvular insufficiency, which was consistent with the observed leaflet shortening and annular growth[152]. A clinical trial using decellularized pulmonary homografts has also shown promising evidence of growth, measured as an increase in inner valve diameter[103]. However, measurements of annulus size and valve diameter cannot be used to discriminate between functional growth and tissue dilatation. First, valve performance parameters, including pressure gradient, peak velocity, effective orifice area and flow pattern, should always be within the physiological range in the context of somatic growth. By contrast, maladaptive remodelling processes, such as dilatation of the valve annulus, might result in central regurgitation, pathological flow, and jets and therefore need to be clearly distinguished from physiological growth[204]. In addition, tissue dilatation is known to be associated with morphological changes, such as thinning of the valvular walls, which might result in an impairment of mechanical properties. In this context, imaging techniques such as echocardiography, CT and cardiac MRI are very useful tools.

Taken together, these results demonstrate how little is known about the growth capacity of TEHVs and emphasize the need for better approaches to differentiate between growth and tissue dilatation[204]. Numerous parameters should be considered in order to systematically demonstrate the growth potential of a cardiovascular tissue-engineered implant, including the increase in body mass during animal growth, an increase in diameter and length of the implant, good functionality over time (that is, no regurgitation), an absence of thrombi, calcifications, stenoses and aneurysms, and balanced tissue formation with gradual substitution of the implant by functional ECM (that is, non-fibrotic tissue with a limited number of contractile cells). These parameters highlight the capacity of a tissue-engineered cardiovascular construct to adapt to its environment while growing with the patient and retaining functionality. Additionally, matrix and polymer turnover should be balanced to prevent the loss of implant integrity, thereby decreasing the risk of tissue dilatation.

Regulatory and logistical challenges

Numerous regulatory, logistical and infrastructural challenges need to be overcome to ensure the safe bench-to-bedside translation of cell-free TEHVs with in situ regenerative potential. Cell-free TEHVs can be obtained by using different materials with distinct biological properties (such as ECM proteins, peptides or bioactive moieties), physical traits (including porosity, fibre diameter or thickness) and mechanical features (such as elastic modulus, tensile strength or elongation). Given the differences in the technical protocols, cell-free TEHVs can be classified as either medical devices or biological products, depending on national or international regulatory legislation. Conversely, functionalized TEHVs can be classified as biological or combination products if they contain bioactive molecules that provide a distinct pharmacological or immunological function after implantation. Additionally, if these bioactive molecules are considered to be medicinal products (drugs), their quality, safety and efficacy need to be proven.

A strict requirement for any biomedical device is standardization[205]. By controlling the manufacturing procedures used to obtain a cell-free TEHV, the consistency and reproducibility of the batches of product made according to predefined quality criteria can be ensured, setting the foundation for commercialization and safe clinical translation. To address this challenge, a Good Manufacturing Practices system (to provide guidance for the manufacturing, testing and quality assurance for the sale of food, pharmaceutical products, medical devices and others), a Good Laboratory Practices system (a quality control system to ensure the consistency, quality and reliability of chemical non-clinical safety tests) and Good Tissue Practices system (which governs the methods and facilities used for the manufacturing of human cells, tissues, and cellular-based and tissue-based products, and is enforced by the FDA) should be implemented for the manufacturing and validation of TEHVs[205].

Quality control procedures are therefore essential to ensure that every product is compliant with the predefined desired characteristics. Aside from the general standards and regulations covering quality management for medical devices (ISO 13485, FDA quality system regulation and Good Manufacturing Practice), several technical standards provide guidance for the development of cardiovascular devices such as ISO 7198 (which specifies the requirements for cardiovascular implants and extracorporeal systems, vascular prostheses, and tubular vascular grafts and vascular patches), ISO 5840 (which

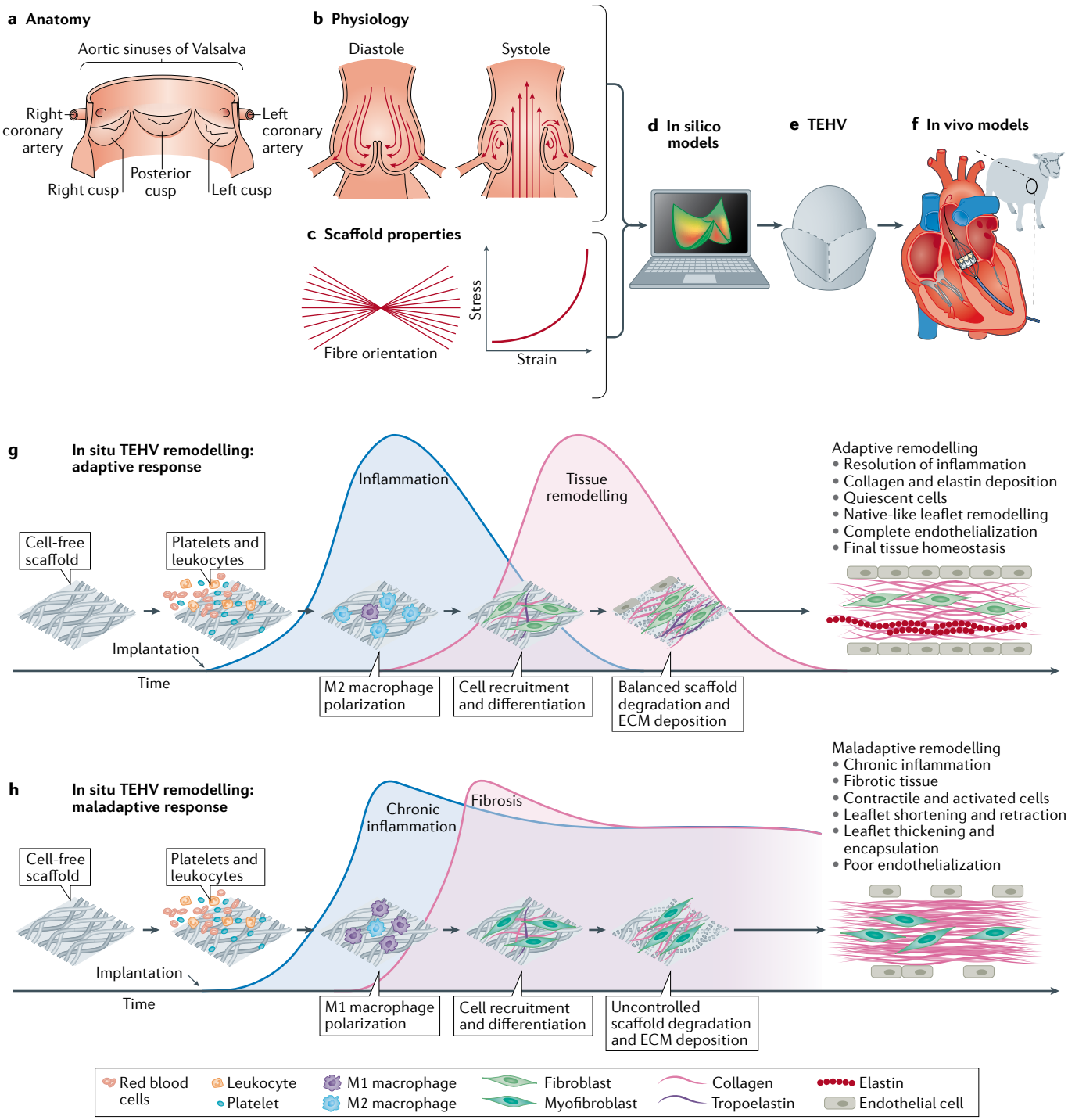

Fig. 3 | **Challenges and future technologies for the successful generation of TEHVs. a–c** | Tissue-engineered heart valve (TEHV) design should take into account the anatomy of the native heart valve as well as the physiological haemodynamic environment, whereby different mechanical forces act on the valve leaflet during systole and diastole[264]. **d–f** | These characteristics, in combination with the material properties of the TEHV, should be used to develop in silico modelling tools to better understand and predict the mechanical forces acting on the valve leaflets and to determine the most appropriate valve design to ensure long-term functionality. After in vitro characterization, TEHV performance and remodelling potential should be evaluated in a preclinical animal model by using, for example, minimally invasive transcatheter procedures to implant the valve. Longitudinal preclinical studies, comprising multiple time points, should be performed to investigate the inflammation-mediated remodelling potential of the TEHVs. **g** | In situ adaptive remodelling of a TEHV towards a native-like leaflet starts with the recruitment of inflammatory cells (such as monocytes, macrophages and lymphocytes), followed by the recruitment of progenitor cells, extracellular matrix (ECM)-producing cells (such as myofibroblasts) and endothelial cells in the scaffold. Over time, the implanted scaffold is completely reabsorbed, and new ECM is secreted. Inflammation eventually resolves, leading to maturation of the tissues towards a native-like structure. **h** | In situ maladaptive remodelling is characterized by the constant presence of inflammatory and ECM-producing contractile cells, which leads to an exacerbation of the immune reaction, chronic inflammation and fibrosis, resulting in an abundance of contractile cells, implant encapsulation and/or TEHV leaflet shortening.

provides general requirements for cardiac valve prostheses) and ISO 25539 (which specifies the requirements for the assessment of endovascular systems)[206]. However, further investigation might be needed for TEHVs, specifically those using in vitro-derived TEMs, to ensure biocompatibility (such as American Society for Testing and Materials (ASTM) standards F2027-16, F2150-13 and F2383-11), immunocompatibility and homogeneous tissue development[207,208].

Finally, to ensure commercialization and broad clinical application, many other aspects of the TEHV should be tested and validated, including sterilization protocols, packaging and storage life. By being cell-free, terminal sterilization of TEHVs for in situ applications has been achieved using a variety of methods. For example, human TEM-based TEHVs have been sterilized in ethanol and antibiotic solution[145,147], whereas bioresorbable polymeric valves can be sterilized by using gamma radiation, plasma treatment or ethylene oxide[98]. However, an optimal sterilization protocol should be identified and implemented early in the TEHV development process owing to the effect of sterilization on the TEHV material as well as its influence on the biocompatibility and safety profile of the TEHV[209].

Clinical requirements

The safe translation of TEHVs strongly depends on the observance of strict clinical requirements and the development of clinical guidelines. Indeed, aside from the scientific challenges listed above, numerous clinical requirements need to be fulfilled before TEHVs can be assessed and used in the clinical setting. These requirements include the establishment of clinical indications, specific inclusion and exclusion criteria for patient selection (such as the presence of comorbidities or potential for valve regeneration), monitoring strategies (including echocardiography, CT or MRI), and bail-out strategies in the event of failure and eventual emergency treatments. First, the valve prototype should be tested in a first-in-human clinical trial, in which valve safety and performance or effectiveness are monitored. After early feasibility is assessed, the valve design can be further adjusted and optimized according to the outcomes of the trial. To proceed with further trials, a frozen design that cannot be further modified should be implemented and tested in clinical trials in accordance with the good clinical practice guidelines.

Additionally, the selection of suitable candidates needs to follow a risk-stratification procedure, whereby the regenerative potential of the valve in the patient should be assessed before the initiation of any treatment in order to exclude comorbidities (such as diabetes mellitus or immunosuppression), thereby preventing potentially fatal valve failures. The natural variation in the innate and adaptive immune response between patients must be considered and correlated with differences in age or sex. Finally, to reduce the risk of human errors during valve replacement procedures, valve substitutes and delivery devices need to be easy to handle and must have detailed user instructions and standard operating procedures. TEHVs should be delivered to the operating room packaged and sterile and contain information on the product characteristics. In this context, a reduction in the associated logistical hurdles and the development of a standardized manufacturing process and quality control system are of extreme importance.

Economic considerations

The degeneration of the aortic valve is the most prevalent indication for valve replacement in elderly patients. Importantly, the incidence of severe aortic stenosis has been predicted to increase at a rate of 4.4% per year in patients aged ≥65 years, with an increasing number of patients (up to 115,000 per year) who are eligible for a TAVR procedure in Europe and North America[210]. Consequently, aortic valve diseases have a large influence on health-care resource planning and costs associated with its management and treatment have reached >1 billion Euros in Europe[75]. Cost-effectiveness analyses from the past 2 years showed that TAVR procedures seem to be less cost-effective (range 18,421–120,779 Euros) than traditional surgeries (14,108–40,944 Euros)[211,212]. Market analyses revealed that TAVR procedures are more advantageous than surgical approaches only in intermediate-risk patients owing to several factors such as prostheses costs, age, comorbidities, lower complication rates and lower length of hospital stay compared with high-risk patients[213]. However, after the new FDA indication to expand TAVR to low-risk and younger patients, TAVR procedure rates will increase in the near future, which might improve their cost-effectiveness.

Comparisons have been made between the costs and benefits of SAVR and TAVR among elderly patients with aortic valve disease. Assuming equal costs of TEHVs compared with clinical heart valve replacements, the long-term durability of TEHVs had the greatest influence on cost-effectiveness[214]. National savings in the first decade after implantation were in the range of 2.8–11.2 million Euros for SAVR and 3.2–12.8 million Euros for TAVR. Unfortunately, given the large discrepancies in TEHV manufacturing procedures, exact estimates of production costs and laboratory performance tests for TEHVs are still largely unknown, mainly because TEHVs are not yet commercially available[215].

Future directions

Computational modelling

Computational modelling, particularly when integrated with experimental studies, can substantially improve and accelerate our comprehension of TEHV growth and remodelling[144,216]. Computational modelling has been used extensively to increase our understanding of valve haemodynamics, to improve valve prosthesis design and even to create patient-specific designs[217,218]. Given that mechanical cues, such as stresses and strains induced by the haemodynamic loading conditions, are known to drive cardiovascular tissue adaptation (FIG. 3), many developments in this area have focused on capturing the biomechanical behaviour and mechanobiological growth and remodelling processes of engineered cardiovascular tissues in mathematical and computational models.

In the context of in vivo experimental observations, computational simulations have greatly contributed to our understanding of inter-animal differences in tissue-engineered vascular grafts[219] and TEHV

First-in-human
An early feasibility clinical study used to evaluate the initial clinical safety and performance in patients.

adaptation[144], differential remodelling and functionality[220], and the interaction between immune system-driven and mechanical stress-mediated regenerative processes[221,222]. Computational modelling can also support the safe clinical translation of TEHVs given its capacity to predict long-term valve adaptation and the corresponding functionality as a function of the patient-specific in vivo environment and the initial conditions provided by the scaffold[144,216]. In particular, computational modelling predictions of the changes in TEHV properties and function after implantation can help to elucidate to what extent certain (patient-specific) factors can affect the anticipated remodelling profile[144] and thereby provide important information on the robustness of TEHV performance. Moreover, the identification of factors that determine the successful regeneration and functional adaptation of TEHVs or, conversely, factors that determine maladaptation and failure can aid in the establishment of risk-stratification procedures. Finally, given the myriad of possible combinations of scaffold properties, TEHV designs cannot realistically be optimized for long-term function using experimental studies alone. The capacity of computational models to predict the in vivo change in TEHV properties over time should therefore be leveraged to optimize scaffold design in order to guide TEHV adaptation and ensure long-term functionality. In a proof-of-concept study, our group used an integrated computational experimental approach to demonstrate that computational modelling-inspired changes in TEHV design[203,223] can indeed markedly improve long-term in vivo outcomes[144]. To build on these promising results, the development and use of computational modelling optimization techniques, such as those used in a framework designed by Szafron et al.[224], are important for identifying the most favourable combinations of scaffold properties. Consequently, only a small selection of valve designs will need to be evaluated experimentally, which is expected to accelerate the clinical translation of TEHVs.

The increased adoption of, and advances in, computational models of cardiovascular tissue engineering applications comes with a number of challenges. For example, the increased incorporation of features of biological growth and remodelling in computational models usually correlates with an increase in model parameters, which might lead to difficulties in parameter discrimination and identification. The integration of computational model development with systematic experimental studies can help to partially overcome this problem. Additionally, increases in model complexity can lead to problems related to numerical tractability. The development of numerical methods to simplify model descriptions is essential for the prediction of TEHV adaptation and for the optimization of scaffold properties[225–227].

Finally, proof that computational models can accurately predict valve remodelling in humans on a patient-specific basis is still pending. Promising preclinical studies have demonstrated the feasibility of predicting differences in cardiovascular tissue engineering outcomes owing to variations in animal-specific conditions[144,219] or haemodynamics[220]. Additionally, a proof-of-concept clinical study involving the use of computational models of the patient-specific electrophysiology of individuals with atrial fibrillation has been successful in predicting effective personalized ablation strategies[228], further indicating that computational models have the capacity to accurately simulate complex, patient-specific cardiovascular phenomena. To successfully predict the evolution of TEHVs in patient-specific conditions, computational models must adequately incorporate the relevant physical, biological and chemical phenomena that affect valve adaptation and function. Furthermore, data from clinical studies need to be appropriately evaluated to facilitate the development and analysis of patient-specific models. The integration of computational models with machine learning algorithms is a promising strategy for the analysis of large datasets and for quantifying uncertainty[229].

3D bioprinting

Cardiovascular 3D bioprinting is emerging as an important tool that can facilitate the printing of living cells and ECM proteins onto implantable scaffolds[230,231]. Researchers used 3D bioprinted collagen to engineer a human heart through an improved second-generation freeform, reversible embedding approach to suspend hydrogels[232]. In this context, tri-leaflet collagen heart valves were successfully printed and showed sustained functionality in a flow loop system (≤15% regurgitation). Another group described a 3D-bioprinted, silicone-based heart valve featuring reinforced leaflets that was fully customizable to the patient's anatomy using a versatile multi-material additive technology[233]. Computer simulations demonstrated that a bioinspired fibre design and customized leaflet shape reduced maximum stress during valve diastole, thereby improving valve durability. Additionally, in vitro haemodynamic testing showed a promisingly low regurgitation fraction of ≤6%. Despite the enormous advances towards the clinical translation of this technology, numerous challenges still remain, such as the need for preclinical evaluation and large-scale clinical trials, before 3D printed heart valves can be used as therapeutic devices in standard clinical practice[234]. Limitations for the clinical translation of 3D bioprinting include uncertainties about in vivo solubility and structural stability as well as the potential cell toxicity of bioinks, the questionable sterility of using a 3D printer in a surgical setting, and the need to establish regulatory standards for clinical use[230].

Bioresorbable stent technologies

Heart valve tissue engineering combined with minimally invasive transcatheter techniques has been suggested as a potential strategy to avoid the open-heart surgery required for valve repair. Transcatheter valves are mounted onto a metal stent that provides a supporting frame to guide the crimping procedure that is necessary for minimally invasive valve delivery[235,236]. In addition, the stent allows for precise valve positioning above the native valve, limits paravalvular regurgitation and prevents coronary artery occlusion in the setting of aortic valve replacement. For tissue engineering applications, the stent should also promote the integration of the engineered tissue with native host tissue.

Although currently available stents have been clinically established for use as heart valve prostheses, they do not have the potential to remodel over time and are associated with complications such as infections, thrombosis and intimal hyperplasia[235,237]. Therefore, novel biodegradable stents have been proposed as an advantageous alternative. As with other clinically approved stents, bioresorbable stents should be implanted using a minimally invasive transcatheter approach, support valve anchoring and favour initial integration in the annulus. Over time, the stent should be reabsorbed and leave a fully biological valve prosthesis with the potential to grow. If successful, this technology could enable TAVR in paediatric patients. However, to date, very few studies have identified an effective bioresorbable stent for heart valve replacement. 3D-printed bioresorbable polymeric stents have been designed and tested in vitro with the aim of treating congenital heart disease in paediatric patients using minimally invasive implantation techniques[238]. Despite the promising preliminary results in terms of crimping and the degradation rate of the developed polymeric stent[238], an in vivo proof-of-concept study that can validate this approach is still lacking. Conversely, the dynamic degradation and haemocompatibility of AZ31 magnesium alloy-based stents have been extensively evaluated[239,240]. In addition, the combination of innovative stent materials with a bioresorbable polymeric valve to obtain a fully regenerative implant for surgical pulmonary valve replacement has been proposed. Acute functionality was assessed in a pig model for up to 12 h and demonstrated the feasibility of the implantation procedure[160]. However, further improvements should be made to translate this design to a transcatheter-compatible approach with good long-term performance.

Tissue engineering for fetal applications

The first report of fetal cardiac interventions dates back to 1975, when intrapartum surgery was performed to treat ventricular tachycardia and heart block in the fetus[241]. The past 20 years has seen an increase in the interest in fetal cardiac interventions, especially for conditions associated with a high risk of prenatal or neonatal death[242]. In terms of heart valve surgery, the most common closed fetal intervention is aortic valvuloplasty, which was first performed in 1989 (REF.[243]). Although preliminary attempts were unsuccessful, resulting in fetal death after intervention, improvements in technical feasibility were achieved for 75–80% of the procedures[244]. This field has advanced substantially in the past decade, but fetal surgery remains controversial given the high risks to the developing fetus and the mother as a result of the large uterine incisions used for this approach[245]. Furthermore, postnatal surgical interventions are still frequently required after prenatal treatment[242].

The combination of minimally invasive implantation techniques with TEHVs has been evaluated using fetal animal models. Specifically, the percutaneous implantation of stents[246] and TEHVs in sheep fetuses has been described[236,247]. Fetuses have a high regenerative capacity as well as a large capacity for repair of the ECM architecture and function, making them a prime target as a model of tissue regeneration[247]. A preliminary study has demonstrated the feasibility of implanting TEHVs as a pulmonary replacement via the transventricular approach in two gestational fetal lambs (aged 110 days), which showed normal postnatal valve function[236]. Although this strategy is promising for the treatment of otherwise-fatal congenital heart diseases, further studies need to be performed to optimize the design and implantation of tissue-engineered valve replacements to limit the risks associated with this technique.

Valvuloplasty
Balloon valvuloplasty or balloon valvotomy is a procedure that repairs stenotic heart valves by expanding a balloon catheter inside the valve to increase the valve opening area.

Conclusions

Although surgical repair and replacement have been the standard of care for patients with valvular heart disease for many decades, transcatheter approaches have revolutionized the field of heart valve therapy and are expected to become a first-line therapy for many patients with valvular heart disease in the near future. However, despite this tremendous technical evolution, clinically adopted heart valve prostheses are still associated with substantial limitations, and a heart valve prosthesis that encompasses all the unique properties of the native heart valve still does not exist.

Given the expanded indications of TAVR for low-risk and younger patients with aortic stenosis, the transcatheter valve market is expected to grow considerably in the near future[210]. However, the currently available bioprostheses for TAVR approaches are made from glutaraldehyde-fixed xenogeneic material and are associated with substantial limitations such as the permanent risk of infections and thrombosis. Most importantly, bioprostheses are also prone to continuous degeneration. This degenerative process is known to be accelerated in younger patients who often require multiple reinterventions, which further increase the risk of morbidity and mortality. These limitations clearly underscore the urgent clinical need for next-generation heart valve replacements that remain functional throughout the life of a patient and that do not require the patient to undergo lifelong anticoagulation therapy.

TEHVs with repair, remodelling and regenerative capacity can address these unmet needs. Of note, promising data from preclinical studies have led to early trials in humans, making these next-generation valves a clinical reality. However, despite this substantial progress, translation of this technology for patient use is still hampered by numerous scientific, logistical and regulatory challenges that need to be systematically addressed in order to increase the clinical relevance of next-generation heart valves. To date, TEHVs designed using decellularized homografts have been the most widely studied in patients and have shown promising long-term results in terms of performance and freedom from reintervention when implanted as a pulmonary valve replacement. Bioresorbable polymer-based TEHVs have also been evaluated in first-in-human clinical trials. Furthermore, given the encouraging results of the clinical trials that evaluated the safety and efficacy of vascular grafts, clinical pilot studies using cell-free, ECM-based valves are eagerly awaited.

These early-phase clinical trials will be instrumental in providing important safety and performance data on next-generation heart valves, which will ultimately

set the basis for their broad clinical translation and implementation. Furthermore, these data will help to define the potential indications to enable appropriate patient selection and to establish monitoring as well as bail-out strategies in the event of valve malfunction or failure. Most importantly, these data will also reveal important insights into the valve remodelling process in humans, which are essential for the development of valves with long-term performance and native-like tissue configurations.

Future studies should also evaluate the use of TEHVs to replace the mitral and atrioventricular valves. International guidelines strongly suggest, whenever possible, to use mitral repair techniques over mitral replacement[248]. However, with the advent of transcatheter techniques in the past 15 years, a slow but steady increase in transcatheter mitral replacement procedures is foreseeable[249], with the inherent risks of bioprosthetic valve degeneration leading to reoperation in patients aged <60 years[248]. Therefore, a regenerative mitral valve replacement is desirable. Finally, technologies such as 3D bioprinting, next-generation bioresorbable stents and, in particular, computational modelling are powerful tools for the prediction and guidance of the complex mechanobiological processes during valve remodelling and will further increase the clinical potential of next-generation heart valves.

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E.S.F., S.E.M., V.L., S.L. and M.Y.E. wrote the manuscript and contributed substantially to discussion of its content. M.Y.E. developed the overall design and concept of the article. All the authors contributed to reviewing and editing the manuscript before submission.

Competing interests

F.P.T.B. and S.P.H. are shareholders at LifeMatrix and Xeltis. V.F. declares financial activities with Boston Scientific, Edwards Lifesciences and Medtronic in relation to educational grants (including travel support), fees for lectures and speeches, fees for professional consultation, and research and study funds. M.Y.E. is a shareholder at LifeMatrix. The other authors declare no competing interests.

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