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

Next generation heart valve replacements with repair, remodelling and regeneration capacity

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Abstract

Valvular heart disease is a major cause of morbidity and mortality worldwide. While surgical valve repair or replacement has been the standard of care for many decades, transcatheter heart valve therapy has revolutionized the field in recent years. However, despite this tremendous technical evolution, to date, the clinically available heart valve prostheses for surgical and transcatheter replacement still have considerable limitations. The creation of next generation tissue engineered heart valves (TEHVs) with repair, remodelling, and regenerative capacity can address these unmet needs and may therefore represent a promising solution. In this review, we present a comprehensive overview of current clinically adopted heart valve replacements, with a particular focus on transcatheter prostheses. We then discuss the various concepts of heart valve tissue engineering to generate next-generation heart valve replacements with repair, remodelling, and regenerative potential focusing on off-the-shelf technologies. We summarize the latest preclinical and clinical evidence from the field. Finally, we tackle current scientific, regulatory, and clinical challenges that TEHVs are currently facing on its road towards safe and broad clinical translation.

1. Valvular heart disease and therapy option evolution

Valvular heart disease (VHD) affects a growing number of patients in both developed and developing countries [1,2], constituting a global disease burden. In Europe and the USA, the demographic change leads to an increase of elderly patients with severe degenerative valve disease [3]. At the other end of the spectrum, up to 1% of new-borns are affected by congenital heart disease which may at some point require surgical or interventional treatment of the affected heart valves. In developing countries, such as Africa and India, rheumatic fever is the major cause of valvular pathologies in young patients [4], and a considerable demand for affordable valve replacement options exists. Generally, VHD is characterized by loss of valve functionality due to stenosis and/or insufficiency. For selected patient cohorts affected by degenerative valve insufficiency, valve repair represents the preferred treatment option to restore competency of the valve. For the treatment of degenerative mitral valve regurgitation, (DMR) surgery represents the gold standard. In addition, for functional DMR new interventional techniques such as the edge-to-edge repair offer good results, especially in high risk population [5]. Different types of aortic insufficiency (i.e.: cusp perforation, annulus dilation, cusp prolapse, cusp restriction), can be treated by annuloplasty, patch repair with autologous (or bovine) pericardium, shaving, or resection [6]. Despite the advancements in valvular repair techniques, as thoroughly reviewed elsewhere for the different heart valves [7–10], replacement of severely dysfunctional or mainly stenotic valves is unavoidable for the majority of patients. The number of valve replacement procedures by either a mechanical or bioprosthetic valve performed each year is constantly increasing, and it is expected to reach 850,000 implants by 2050 [11]. The choice for the most appropriate replacement is based on valve related factors such as anatomy and pathology, and other factors such as comorbidities, age, frailty, that determine the operative risk, tolerance to anticoagulation, and life-style preferences.

In this review, we present a comprehensive overview of current clinically adopted valvular replacements, and of the implantation methods in use to substitute a diseased valve. In addition, we discuss how tissue engineering methods have been proposed to achieve next-generation valve replacements with repair, remodelling, and regenerative potential, with particular focus on the most up-to-date evidences of in-situ tissue engineering approaches. Finally, we tackle the scientific, regulatory, and clinical challenges that the field of heart valve tissue engineering has to face before being able to safely translate into clinics.

1.1 Surgical and transcatheter heart valve replacement

Surgical aortic valve replacement (SAVR), first performed in the late sixties, until now represents the standard of care for the treatment of aortic stenosis, with excellent short- and long-term results [1]. While highly effective, surgical valve replacement requires invasive open-heart surgery with temporary cardiac arrest and the use of a cardiopulmonary bypass which is associated with peri-procedural risks (Figure 1). Elderly patients or patients that were considered inoperable or at a high-risk for surgery were often underserved. In the early 2000s [12,13], transcatheter aortic valve replacement (TAVR) has revolutionized the field of heart valve therapy by providing a novel treatment option [14] (Figure 1). Over the past 20 years, great progress in device technology including improved stent designs, better and smaller sized delivery systems, reduced rate of paravalvular leaks and vascular complications have substantially decreased the complication rate [14]. Several access routes including transapical, transfemoral, and transaortic allow for a safe implantation even in patients with vascular limitations [15].

Today, transcatheter techniques are routine for previously considered inoperable or at high-risk patients [15]. Based on a number of randomized controlled trials comparing SAVR and TAVR, the indication for TAVR has been recently extended to intermediate[16,17] and low-risk [18,19] patient cohorts, becoming a first-line therapy for patients with severe, symptomatic tricuspid aortic stenosis as an alternative to SAVR.

1.2 Clinically-established heart valve replacements

As of today, the ideal valve substitute still does not exist. Four heart valve replacements types are being routinely used in clinics: mechanical valves, bioprostheses, and to a much lesser degree homografts and autografts.

1.2.1 Mechanical valves

Following the first-ever heart valve surgery performed in 1952, the first commercial valve prosthesis was a mechanical valve with ball-and-cage design, created by the surgeon Albert Starr and the engineer Lowell Edwards in 1957 [20]. As a consequence of the optimization of the surgical procedure, and the evolution of mechanical valves that are currently based on the bileaflet tilting disc design [21], first developed in 1976 [20], mechanical aortic valve replacement is the procedure of choice for younger patients with no contraindication to anticoagulation. However, the non-physiological valve geometry and the foreign surface lead to an increased thrombogenic risk due to the high shear stress around the hinge points and backflow jets that causes red blood cell damages and platelet activation [21]. Hence,

mechanical valve thrombogenicity is the most prevalent complication, particularly during the first six months after the surgery [22]. To limit the risk of thrombosis, patients are subjected to life-long warfarin treatment. The need for anti-coagulation therapy has a significant impact on the patient lifestyle and increases the life-long risk of major bleeding. Hence, mechanical valves are for instance not recommended in athletes as well as women with a desire for pregnancy [23].

1.2.2 Homografts

The first human homograft heart valve was implanted in 1956 [24] and achieved clinical attention in the 1960s [25], as a promising alternative for mechanical prostheses.

Human heart valve homografts represent an ideal valvular substitute, because of their physiological anatomy and tissue composition that leads to proper haemodynamic, reduced thromboembolic risk, and low immunogenicity [26]. Excellent results in terms of valve durability and performance have been achieved with homografts implanted in the pulmonary position as in tetralogy of Fallot [27]. However, homografts also display some major drawbacks such as the limited availability because of the shortage of human organ and tissue donors, and the limited durability, caused by residual immunogenicity and susceptibility to early calcifications that induce structural valve degeneration [28,29]. Hence, only a fraction of the homografts (30-40%) is still functional 20 years upon implantation [29] and they are considered suboptimal for implantation in paediatric patients because of poorer durability [30], and higher re-operation rates [31].

Along the years, the procedures for sterilization and storage of homografts have considerably evolved from the initial fresh aseptic harvesting with direct implantation. Currently, homografts can be either cryopreserved in the vapour phase of liquid nitrogen, or stored wet at 4°C after antibiotic sterilization [29] (cryopreserved homografts, cH). Nevertheless, despite the reported improvements in the development of milder cryopreservation procedures, as reviewed elsewhere [26], freezing and subsequent thawing of the homografts lead to surface and structural damages [32,33]. As a consequence, valve insufficiency, leaflet shortening and thickening, vegetation, inflammation, and degeneration have been observed upon implantation [34–36].

1.2.3 Bioprostheses

In the 1970, bioprosthesis, based on widely accessible glutaraldehyde-fixed xenogeneic materials were introduced to overcome the problem of thrombogenicity with mechanical valves and to replicate the

anatomical features of homografts by providing a native-like three-leaflet geometry and tissue composition [20].

When compared to mechanical valves, this improved valve geometry determines physiological-like haemodynamic that limit platelet adhesion and activation, therefore reducing the need for anti-coagulation therapy [37]. The large variety of surgical bioprosthesis available today includes valves that can be placed in the subannular or supraannular position [38], valves with or without stent-frames [39], sutureless valves [40], and valves with different anticalcification treatments [41].

With the event of TAVR, a multitude of innovative self-expandable and balloon-expandable valves have been developed, as reviewed elsewhere [42,43]. Although stents and delivery systems have different designs, transcatheter valve prostheses are manufactured using clinical-grade glutaraldehyde-fixed xenogeneic materials.

The current trend to preferentially use bioprostheses also in the younger population[37], comes at the cost of a structural valve failure, due to progressive calcification of the leaflets within 10-15 years after implantation [43]. On the other hand, valve durability up to 20 years has been reported for elderly patients [44]. Anti-mineralization treatments were developed to further improve bioprosthetic valve durability and to limit the onset of calcification [45]. While partially successful, this tissue processing is not sufficient to mitigate the degenerative phenomenon which is caused by the residual immunogenicity of the bioprosthetic material. Indeed, a multitude of studies, as reviewed elsewhere [46], showed that glutaraldehyde treatments are not sufficient to completely mask immunogenicity as standard fixation protocols may eliminate the immunogenicity of protein antigens, but immunogenic xenogeneic carbohydrate antigens persist, causing valve failure due to chronic inflammation and calcific nodule formation [47]. Bioprosthesis immunogenicity is even more profound in paediatric patients, causing early degenerative failure [48], making them an unsuitable choice for the treatment of congenital heart disease.

1.2.4 Autograft (Ross procedure)

The Ross procedure, firstly established by Donald Ross in 1967 [49], consists in the replacement of a diseased aortic root with the patient's autologous pulmonary valve, while a cH is implanted at pulmonary position to restore the right ventricular outflow track [50]. This technique was developed to overcome the lack of durable valve replacements for young patients affected by diseased aortic valves. Because of the greater life expectancy of younger patients, mechanical valves are the most frequently used option

to substitute the aortic valve. The Ross procedure, on the other hand, brings several advantages to the patients: no need for life-long anticoagulation therapy; replacement of the diseased valve with a living autologous substitute with regenerative and remodelling potential; physiological-like haemodynamic profile; and a long-term survival rate equivalent to age- and sex-matched healthy population [51]. In addition, recent reports have shown that the Ross procedure improves life expectancy [52] and freedom from cardiac- and valve-related mortality, as well as reduces the risk of stroke and bleeding when compared to mechanical valve replacement [51]. These results indicate that, when properly performed, the Ross procedure represents a valuable option to treat aortic valve disease in young patients and may even provide a more cost-effective approach compared to conventional aortic valve replacement using a mechanical valve [53]. However, the technical complexity still remains a major challenge of the Ross procedure and therefore limiting its broad clinical adoption [51].

1.3 Alternative heart valve replacement solutions

Polymeric valves, based on non-degradable polymers (i.e.: silicon [54], polytetrafluoroethylene [55], polyurethanes [56]) were initially designed to combine the immunocompatibility of the mechanical prostheses with the physiological haemodynamic profile provided by three-leaflet bioprostheses [57]. However, polymeric valves have shown limited durability and loss of functionality [58], variability between batches [59], thrombogenicity, pannus overgrowth [60], and calcification [58,61], thereby impeding their broad clinical translation. Over the past years, material scientists have been optimizing polymers in order to achieve durable biocompatible materials that have been safely used as a valvular component in ventricular assist devices [62]. More recently, siloxane poly(urethane-ureas)-based heart valves (Foldax Tria Aortic heart valve) demonstrated improved tear strength and creep resistance (i.e.: no permanent deformation) compared to conventional polyurethanes [63,64], in-vitro durability of 600 million cycles (equivalent to 15 years), and absence of calcification and thrombus formation in-vivo [57]. In light of these results, an early clinical feasibility study (ClinicalTrials.gov Identifier: NCT03851068) has been initiated. In the future, polymeric heart valves may be combined with minimally invasive transcatheter procedures, to provide a cost-efficient solution for the treatment of rheumatic valvular disease in emerging countries [57]. In this context, Strait Access Technologies developed a TAVR strategy for low resource countries that uses a supra-annular anchoring technique to provide correct valve positioning via tactile feedback and without the need for fluoroscopic imaging [57,65]. However, significant challenges still remain for polymeric valves: firstly, researchers need to balance durability and biocompatibility of

the material to ensure long-term in-vivo performance and improved valve longevity compared to bioprostheses; secondly, variability between different polymer batch synthesis and/or processing should be limited do not impact valve reproducibility; and, finally, polymer wear should be prevented at any time to limit the risk of tear and failure. Once these challenges will be solved, a novel prosthesis compatible with clinical standards could be achieved.

1.4 Clinical and societal problem

Despite tremendous technical evolution in the field of heart valve therapy, the currently available heart valve prostheses are not yet ideal. Prosthesis-associated problems occur within 10 years post-operatively [21]: progressive functional degeneration, limited durability, and the complications of lifelong anticoagulation therapy. In the paediatric population, the need for multiple surgeries to adjust to the somatic growth of young patients as well as redo-operations to replace failing valves pose additional problems [66]. The quality of life and the life expectancy of patients after heart valve replacement is substantially impacted compared to age-matched healthy individuals [67]. For the society, heart valve replacements are very costly (over €1 billion annually in Europe) [68] mainly because of post-surgery care costs and the need for repeated operations. Accordingly, there is a clear unmet clinical need for a heart valve prosthesis that does not degenerate and can adjust to functional and somatic changes and that can be implanted via minimally-invasive techniques.

2. Heart valve tissue engineering

D.E. Harken, a pioneer in heart valve surgery, summarized the characteristics and features of the ideal heart valve substitute in “ten commandments”, including the capacity of self-repair and adaptive remodelling, the resistance to infections, lack of thrombogenicity and the capability to grow. In fact, he stated the fundamental characteristics of a native heart valve [69]. To date, heart valve prostheses with the unique properties of a native heart valve do not exist. The creation of next-generation heart valve replacements via tissue engineering (TE) with self-repair and remodelling capacity may address these unmet needs. A valve that continuously adjusts to the functional changes in the cardiovascular system, which is not immunogenic and does not require any anti-thrombotic therapy could provide a life-long durable valvular solution, which will particularly benefit paediatric and young adult patients, but could be also attractive for the elderly.

2.1 From autologous to cell-free approaches

The fundamental paradigm of heart valve TE was initially established in 1993 [70] by using autologous cells and tissue culture in a bioreactor to favour cell proliferation and extracellular matrix (ECM) deposition (Figure 2A). Following the original protocols, the autologous tissue engineered heart valves (TEHVs), that comprised living cells, were then implanted in the patient. Repair and remodelling mechanisms were thought to grant the long-term durability [71]. Several clinical trials have been initiated using this autologous approach, also known as in-vitro TE, by using either decellularisation of allogenic [72,73] or xenogeneic[74,75] valves. The paucity of homografts, and the risk of xenograft immunogenicity have urged researchers to find alternative materials in natural (e.g.: collagen, fibrin) and synthetic (poly-glycolic acid (PGA) and poly-caprolactone (PCL)) polymers, with promising results in-vitro[76–78] and in preclinical [78–86] in-vivo studies in terms of early functionality, implant remodelling and endothelialisation potential. To date, however, in-vitro heart valve TE has not progressed into routine clinical use. The open challenges of this TE approach are multifactorial: 1) the process is technically and logistically complex, requiring the isolation and expansion of autologous cells prior tissue culture; 2) the final product may significant differ due to donor-to-donor variability; 3) uncontrolled thickening and shortening of the leaflets, with consequent valve insufficiency, which has been repeatedly observed [80,81], even prior to implantation [87]; and 4) long-term safety and efficacy have not been established yet.

To overcome the logistical hurdles, a more straight-forward and potentially cost-effective approach, the so called in-situ TE, is gaining popularity in the scientific community. In-situ TE relies on the regenerative potential of the recipient body to integrate and remodel an off-the-shelf available acellular implant, that is designed to favour host cell adhesion and tissue formation, while providing valve functionality immediately upon implantation [88]. The ideal substrate for in-situ heart valve TE should be able to remodel by selectively controlling host cell recruitment, adhesion, and differentiation and to support tissue formation while controlled degradation occurs, until a native-like functional tissue is achieved [88].

2.2 Strategies for in-situ heart valve TE

Multiple in-situ TE strategies have been established in the past years, focusing on different starting scaffolds to manufacture the heart valve replacement (Figure 2): homografts and xenografts, in-vitro grown tissue engineered matrices (TEM), and bioresorbable polymers with regenerative potential. To grant immunocompatibility and off-the-shelf availability to human-derived and animal-derived valvular

tissues, homografts and xenografts are processed via decellularisation [26] (Figure 2B). By depleting the cells and their DNA, the immunological epitopes are removed, leaving the integrity and functionality of the ECM mostly unchanged. The decellularisation technique has been also applied to TEM grown in-vitro, to ensure scalability, to grant off-the-shelf availability, and to reduce donor-to-donor variability [87] (Figure 2C). Finally, the last approach uses bioresorbable polymers with tuneable mechanical, chemical, and architectural properties, customized degradation rates, and tailored cell specific environment (i.e.: via functionalization), making them an interesting choice for the easy, rapid, and competitive manufacturing of scaffolds suitable for regenerative medicine purposes [89] (Figure 2D-E).

2.2.1 Decellularised pulmonary and aortic valve homografts

The in-vivo application of human-derived decellularised pulmonary and/or aortic valves (DPVH, DAVH) proved to be successful in both preclinical [90,91] and clinical studies (Table 1A). When compared to cH, (described in section 1.2.2), TEHV based on human-derived tissues can be achieved via decellularisation of freshly harvested (fDPVH, fDAVH) or cryopreserved pulmonary or aortic valve (cDPVH, cDAVH).

Fresh decellularised pulmonary and aortic valve homografts (fDPVH, fDAVH)

Since their introduction as pulmonary valve replacements for paediatric applications [72], fDPVH have demonstrated promising performance and spontaneous re-cellularisation potential [92]. As shown by the latest results of the ARISE and ESPOIR clinical trials (NCT02035540 and NCT02527629, respectively), fDPVH demonstrated excellent long-term performance with trivial regurgitation and better freedom from explantation compared to cH (Table 1A). fDAVH showed sustained performance up to 10 years with 100% freedom of reoperation and endocarditis (Table 1A).

Cryopreserved decellularised pulmonary and aortic valve homografts (cDPVH, cDAVH)

cDPVH and cDAVH have also demonstrated promising short term results in terms of valve immunocompatibility [93,94], performance and durability [95,96]. However, on the long run, these valves did not always show a significantly better freedom from reoperation or improved valve performance when compared to cH [95,97]. In particular, cDAVH demonstrated a comparable performance to cH [31], and similar mechanisms of degeneration [95] (i.e.: fibrosis, calcification, and lack of recellularisation[98,99]).

Remarkably, fDPVH and fDAVH appear to have a better performance when compared to decellularised cDPVH and cDAVH. These results suggest that the processing methodology matters and that the

cryopreservation and decellularisation techniques may have impacted the physical and mechanical properties of the original allogenic tissue, hindering in-situ regeneration [95]. Additionally, different sterilization methods are often used in these studies, and certain techniques, such as low-dose gamma radiation, have been reported to strongly influence the ECM integrity [100]. Hence, further studies should address the role of cryopreservation, decellularisation, and sterilization, on ECM preservation to ensure long-term homograft stability and remodelling. Finally, similarly to cH, the broad clinical application of these prostheses is limited by the donor shortage and by the need to implant this valvular replacement via open-heart surgery.

2.2.2 Decellularised xenografts

To overcome the limited availability of allogenic human tissue, decellularised xenografts based on porcine small intestinal submucosa (SIS, with the commercial name of CorMatrix, CorMatrix Cardiovascular, Inc, Roswell, GA) or porcine decellularised pulmonary valves (with the commercial name of Matrix P® or Matrix P plus®, AutoTissue GmbH, Berlin, Germany; or SynerGraft, CryoLife Inc, Kennesaw, GA) have been introduced in preclinical and clinical settings. As previously described for the homografts, the decellularisation process aims to provide an ECM-based scaffold with low immunogenicity and retained regenerative potential. However, substantial differences have been identified among the preclinical and clinical performance of decellularised xenogeneic valves.

<u>Small intestinal submucosa (CorMatrix)</u>

Preclinical trials testing SIS-based pulmonary [101] and tricuspid [102,103] valves indicated promising valve performance and host cell infiltration with endothelialisation [102], trilaminar tissue organization [103], and native-like valve growth of the annulus diameter [101] (Table 2). Inflammatory reactions were rarely observed in animal models, thereby further supporting SIS-ECM immunocompatibility.

In light of the promising preclinical validation, in 2010 CorMatrix moved forward to clinical translation. Since then, it has been used extensively in cardiovascular surgery as material for patch and valve repair [104], but also for valve/cusp replacement (Table 1B). However, independently of the indication, several studies reported that the use of CorMatrix resulted in thickening, fibrosis and calcification [105], material degeneration [106], and strong inflammatory response [107,108], requiring reoperation for valvular dysfunction in selected cases [105,108]. On the other hand, a series of clinical case reports demonstrated promising

results when using CorMatrix for repair and reconstruction of heart valves, heart valve leaflets [104], and as pulmonary valved conduit [109]. However, the short follow-up time point used in these case reports (<1 year [104]) cannot provide sufficient information on long-term material durability upon implantation and eventual degenerative phenomena. Importantly, as of 2019, a multi-center clinical trial on tricuspid valves using CorMatrix (NCT02397668) is recruiting patients from 1 to 70 years of age, and results are eagerly awaited.

Porcine decellularised valves (SynerGraft, Matrix P, Matrix P Plus)

Preclinical studies using decellularised xenografts based on porcine pulmonary or aortic valves (DPV and DAV, respectively, Table 2) showed promising results in terms of valve performance and host cell infiltration, but also endothelialisation [110,111], and collagen deposition [111]. Importantly, animal studies also demonstrated that endogenous retrovirus DNA, present in the porcine DPV, was not transmitted to the host animal (sheep) [112].

However, also for these xenografts, clinical trials led to contradictory results, with promising valvular performance, comparable to the native valve [113], and favourable outcome in the treatment of congenital diseases (for 3 / 7 patients) [98]. On the other hand, high incidence of failure [114], with stenosis and regurgitation [115], lack of remodelling or cellularisation [114], massive inflammation [116], and even sudden cardiac death in three paediatric patients [117] have been reported (Table 1B).

Independently on the approach and material used, the observed failure in clinical trials is most likely caused by the residual immunogenicity of the decellularised xenogeneic valves, that provoked a strong inflammatory response to the implanted tissue, calcification, and structural degeneration [117,118], that were however not observed in the preclinical sheep model. The striking differences between preclinical and clinical trials also indicate the need for improved in-vitro testing platforms to asses immunocompatibility (see also Section 3.1.1) and the use for animal models that are able to better mimic the human immune system (i.e.: non-human primates, such as the baboon [82,119,120]). Taken together, these results suggest that easily accessible, off-the-shelf available biomaterials with improved immunocompatibility are required to ensure a safe clinical translation.

2.2.3 Decellularised in-vitro grown TEM

Heart valve TE has been, so far, limited by the donor-to-donor variability in the in-vitro cultured ECM, as well as by the technical and logistical hurdles in timing valve manufacturing with the implantation

procedure. Starting in 2012, decellularisation of TEHVs was introduced to overcome these limitations, by developing off-the-shelf available non-immunogenic replacements with comparable mechanical and biological properties to the cellular counterpart [87]. As further advantage, decellularised in-vitro grown TEM for cardiovascular applications can be manufactured by using commonly used and easily accessible cell sources, such as myofibroblasts and dermal fibroblasts [87,121–128], instead of autologous, patient-derived cells. TEM-based heart valve replacements have been successfully investigated as pulmonary and aortic valve replacement in preclinical large animal models (Table 3). Remarkably, TEM-based valves have proved their compatibility with minimally invasive implantation techniques [81,119,122–124,129,130], even as an aortic valve replacement [125], showing good early functionality, host cell repopulation, endothelialisation, integration, and remodelling over time. In this context, a recent proof-of-concept study demonstrated that a computational modelling (CM) inspired TEHV design can guide tissue remodelling towards long-term functionality [122]. The ovine TEM-based TEHVs were implanted as TPVR in sheep, and followed up for one year, demonstrating a clinical-grade in-vivo performance, excellent durability, and native-like remodelling, with endothelialisation, collagen and elastin deposition, and the initial formation of sinuses of Valsalva [122]. Most importantly, in the past years several clinical trials using TEM-based vascular grafts have been initiated (Table 4A), with positive results in terms of host cell repopulation and graft patency when used as dialysis access conduits [131,132].

2.2.4 Bioresorbable polymers with regenerative potential

Synthetic bioresorbable polymers are gaining interest as potential starting materials for in-situ cardiovascular applications because of their tuneable mechanical, chemical, and architectural properties. TEHVs manufactured using bioresorbable polymers have the peculiarity of being naturally absorbed and metabolized by the human body as extensively reviewed elsewhere [133]. In addition, synthetic polymers are relatively easily tuneable, reproducible, off-the-shelf available, and scalable. In light of these advantages, researchers have investigated functionality and remodelling potential of bioresorbable polymeric valves in preclinical large animal models (Table 5A), even in combination to one-step pre-seeding procedures (Table 5B) to influence the early inflammatory response and the remodelling cascade. In addition, these materials are suitable for transcatheter applications either as pulmonary or aortic valve replacement (Table 5). In this context, bisurea-based (BU) and ureidopyrimidone (UPy)-based supramolecular polymers have been used to manufacture bioresorbable valves with regenerative potential, compatible with surgical [89,134] and transcatheter [135,136] implantation

techniques and demonstrating acceptable functionality for up to 12 months [89,134]. In addition, fast cellularisation, ECM deposition, and scaffold degradation were observed in the explanted valves, confirming the remodelling potential. In light of these promising results, international consortia (Intelligent materials for in-situ heart valve TE, ImaValve, FP7-NMP grant agreement ID: 604514; and One Valve for Life, 1-valve, CVON2012-01 1Valve) are further investigating the possibility to use these supramolecular polymers to develop aortic valve replacements compatible with minimally invasive implantation techniques. However, independent of the supramolecular polymer and the implantation technique used, intra-valve and inter-valve differences in tissue remodelling (i.e.: cellular infiltration, thickening, elastin deposition, scaffold resorption) have been reported [89,134,136], suggesting the need to further investigate polymer degradation and in-situ remodelling mechanisms to ensure a safe clinical translation.

Importantly, supramolecular polymer-based cardiovascular replacements have advanced into clinical trials (Table 4B) [137]. Five paediatric patients (aged 4-12 years) received a supramolecular polymer-based graft as extracardiac cavopulmonary conduit for the treatment of univentricular cardiac malformation. The results show good recovery and improvement of the patients' general health condition 12 months after implantation. Over time, the graft performance was stable, with no significant differences at 12 months compared to early postoperative data. However, a longer follow-up is needed to further assess functionality, remodelling, and even growth potential of these bioresorbable vascular grafts. Finally, functionalization of synthetic polymers, either with antibodies, peptides, growth factors, or proteins, represent another advantage of using these materials for in-situ TE applications [138]. As an example, hybrid polymers, made from a combination of synthetic and biological polymers, can efficiently provide the necessary mechanical stability to withstand haemodynamic pressures as well as the biological interface needed by the cells to adhere and remodel the construct upon implantation. In this context, cell-free TEHVs for TPVR have been successfully implanted in an acute sheep model, showing good haemodynamic performance [139].

3. Road to clinical translation

Clinical translation of TEHV approaches has become a reality. TEHV based on decellularised homografts have shown good long-term performance with signs of adaptive growth [140] and cell repopulation [73,141,142]. On the other hand, stenosis and inflammation were repeatedly observed in TEHV

based on decellularised xenografts (Table 1). In addition, bioresorbable polymer-based TEHVs are currently being tested in clinical settings, after the same supramolecular polymer showed promising results in terms of patency and performance when used to treat congenital heart disease (Table 4). Considering the promising clinical results of TEM-based vascular grafts (Table 4), it is expected that heart valve replacements based on the same concept will soon reach the next phase of clinical translation, when clinical-grade functionality can be proven in a sheep model [122].

3.1 Scientific issues

Decellularised TEM or cell-free bioresorbable polymers, are available off-the-shelf but immunocompatibility, haemocompatibility, remodelling, and the capacity for in-situ growth need to be investigated further.

3.1.1 Immunocompatibility

Clinically-used xenograft bioprostheses rely on fixation treatment to block xenogeneic antigens, impeding, on the other hand, cell infiltration and tissue remodelling, fundamental pre-requisites for tissue engineered approaches. While TEHVs based on bioresorbable polymeric matrices are cell-free implants that do not contain any cell-derived components, decellularised regenerative TEHVs, based on in-vitro grown TEM, or on allogenic and xenogeneic grafts, will not be exposed to a fixation treatment to ensure cellular infiltration and matrix remodelling. Hence, decellularised tissues must be carefully tested for their potential of disease transmission and residual antigenicity.

In a pre-clinical study, Leyh et al. have demonstrated that, upon decellularisation, porcine pulmonary valves could not transmit residual retrovirus DNA to the host animal [112]. Clinical trials have demonstrated problems with the implantation of decellularised xenografts that were not completely decellularised [117] (see also section 2.1.2). Decellularised xenogeneic products may still contain epitopes, such as the alpha-galactose [143], that would elicit a strong inflammatory response, with subsequent calcification, and structural degeneration [117,118].

To solve this problem, researchers are now focusing on improving decellularisation protocols [144], and on developing antigen-reduction treatments (i.e.: the alpha-galactosidase treatment [145]) to limit residual antigenicity without affecting cell infiltration and matrix remodelling. To validate these new approaches, in-vivo studies are fundamental tools to demonstrate lack of immune cell adhesion and infiltration, as well as to confirm host cell repopulation and remodelling potential. However, it is sometimes difficult to

extrapolate the experimental results and to apply them to clinical settings, because of the sometimes drastic differences in the immune response between animals and humans [146]. This became apparent by the multiple failures reported in clinical trials when using decellularised xenografts (Table 1B) that had not been observed in pre-clinical studies (Table 2).

Hence, in-vitro testing of the immunocompatibility of a decellularised product should include the use of human cells. For this purpose, in-vitro screening platforms to apply physiological and pathological values of shear stress or cyclic strain [147,148] , have been established to characterize human blood-derived mononuclear cell recruitment into scaffolds. Potential chemotaxis signalling of the implanted decellularised matrix can be evaluated by using microfluidic systems to visualize blood-derived cell infiltration under flow conditions [149]. In addition, macrophage adhesion and polarization onto the implanted scaffold should be investigated to assess the presence of pro- or anti-inflammatory macrophages, respectively responsible for rapid matrix turnover or prolonged and detrimental inflammatory response and calcification [150].

3.1.2 Haemocompatibility

Biocompatibility is a critical feature of medical devices, and in the case of blood-contacting devices – such as cardiovascular implants – haemocompatibility is a fundamental criteria extensively regulated by ISO 10993-4 [151]. By direct contact with blood, the material surface of any heart valve replacement adsorbs plasma proteins that promote subsequent platelet and leukocyte adhesion and activation, thereby increasing the risk of thrombotic and thromboembolic complications [152].

A profound understanding of blood-implant interaction is needed to tailor anticoagulation treatment and prevent thrombotic and thromboembolic complications. This is even more important in transcatheter approaches, where the metal-based stent may further activate platelets and initiate coagulation cascade. For these reasons, preclinical and first clinical pilot investigation of TEHVs with in-situ regenerative potential is often subject to an early and temporary anti-coagulation or anti-aggregation therapy to reduce the risk of early thrombosis. Over time, the anti-thrombotic agents can then be reduced and ultimately completely removed as soon as the neo-endothelialisation process of the construct is completed to ensure a haemocompatible blood-scaffold interface. Unfortunately, different anti-thrombotic treatments (both for duration and type of drug used) have been used in association to the different TEHVs (Table 1, 2, 3, and 5), making it difficult to fully understand the thrombotic and

thromboembolic risks associated with these devices and to determine the effects anti-coagulants and anti-aggregation agents may have on the remodelling process.

In-vitro assessment of material haemocompatibility platforms to assess platelet adhesion [153] and fibrin clot structure [154] must be applied when developing novel materials that are in contact with blood. Briefly, blood is collected and analysed before being incubated in contact with the biomaterial in static, agitated, or dynamic set-ups, as extensively reviewed elsewhere [152]. These in-vitro systems have the advantage of having well established and controllable parameters (i.e.: blood flow, anticoagulant used) and can provide detailed and comparable information on blood cell adhesion and activation, protein adsorption, and markers for complement system and coagulation activation.

To reduce the thrombogenicity of a cardiovascular implant, different studies have evaluated the possibility to either functionalize the material to favour haemocompatibility, or to pre-seed and culture autologous endothelial cells (ECs) to ensure anti-thrombogenicity [155].

Material functionalization aims at facilitating endogenous EC adhesion and endothelium formation directly at the site of implantation. In-situ recruitment, adhesion, and differentiation of ECs and circulating endothelial progenitor cells (EPCs) may be possible to achieve by using CD34 [156,157] and CD133 [158] antibodies, fibronectin [159], fibrin [160], and laminin [161] derived peptides, and growth factors (i.e.: VEGF [157,162], SDF1α [163]). However, unspecific cell adhesion and differentiation in response to antibody-functionalized materials have been recorded [156], suggesting the need for a better understanding of the in-situ endothelialisation process.

Endothelial cell seeding aims at creating an anti-thrombogeneic surface by using autologous ECs to cover the implanted material. While in-vitro endothelialisation of small calibre vascular grafts have shown limited improvements in material haemocompatibility, as reviewed elsewhere [164], autologous EC pre-seeding onto pulmonary homografts ensured good haemodynamic performance pre-clinical [90,112,165] and in clinical [72,73,166] settings, where a confluent endothelium was observed. However, this approach still presents several limitations: EC isolation and expansion, as well as EPC isolation and differentiation, are time consuming and costly procedures; cell seeding may be inefficient due to limited and/or inhomogeneous cell adhesion on the material [167]; and cell retention and viability upon implantation via minimally-invasive transcatheter procedures is limited [168]. To overcome these limitations, a combination approach using SDF1α functionalization and EC pre-seeding has been

evaluated, demonstrating enhanced recellularisation, less inflammation, calcification, and platelet adhesion [163]. In addition, recently developed bioreactors for 3D cell seeding should be considered to ensure homogeneous and controlled EC distribution [169]. These results suggest that a more complex functionalization system, combining two or more bioactive components [161,170], as well as surface topography [171] and EC pre-seeding [163], should be further investigated to improve and control in-situ endothelialisation of regenerative materials.

3.1.3 Controlling the in-situ remodelling

Although vascular graft remodelling has been extensively evaluated even in clinical settings, it is still unknown to what extent these results are applicable to the field of heart valve TE, due to the more complex haemodynamic environment. The lack of understanding of the mechanisms driving cellular repopulation and neo-tissue formation upon TEHV implantation remains an unmet need to efficiently optimize TEHV design. Most of the studies investigating TEHV functionality and remodelling have reported loss of valve functionality within 12-24 weeks due to uncontrolled (adverse) tissue remodelling phenomena which translated into valvular leaflet thickening and/or shortening (retraction), resulting in valvular dysfunction, regurgitation, and ultimate failure [80,81,86,119,124,128,129,172]. Hence, understanding and guiding the remodelling processes, to avoid and prevent maladaptive remodelling phenomena, remain a major challenge for TEHV approaches to enable safe clinical translation. In this context, CM of valve mechanics and corresponding tissue remodelling has been suggested as a powerful strategy to predict the consequences of changes in valve design on the overall outcome. However, it has hardly been utilized to improve TEHV performance, nor has it been validated in clinically-relevant in-vivo models [173]. In this regard, a recent publication demonstrated the importance of combining in-silico models to TE application to optimize TEHV leaflet design to prevent leaflet retraction [122]. While CM is already used to optimize the performance of mechanical and bioprosthetic heart valve prostheses [174,175], it has hardly been utilized to improve performance TEHVs [173,176]. Emmert et al. [122] integrated a CM inspired heart valve design to guide tissue remodelling towards long-term functionality of TEHVs. Upon implantation in sheep as TPVR, the valves exhibited a preserved and good long-term in-vivo performance up to one year as predicted by the CM, and native-like remodelling characteristics.

Despite the advances in both in-vitro and in-silico model to assess functionality and remodelling potential of TEHVs, in-vivo animal experiments are still a mandatory step to move forward with clinical translation. Indeed, valve integration, cell repopulation, ECM formation and, eventually, growth, can

only be tested in-vivo. In this regard, TEHVs are usually tested in large animal models. Among the different mammals, the cardiovascular system of non-human primates closely resembles the one of human. However, due to costs and strict regulations, only few studies have evaluated TEHV in this animal model [82,120]. On the other hand, the sheep is the FDA-recommended animal model for the preclinical validation of heart valves. Briefly, the ovine model provides important information about valve functionality and durability by being able to induce progressive and accelerated degeneration and calcification. However, international guidelines do not provide requirements on the animal breed to be chosen for the preclinical studies. In this context, information about animal breed, gender, and/or age are often neglected in scientific publications, making extremely difficult, if not impossible, to evaluate and compare the remodelling outcomes between different studies.

Today, longitudinal studies assessing the mechanistic aspects and unravelling the biological and remodelling processes are still sparse. Kluin et al. [89] demonstrated proof-of-concept of in-situ heart valve TE using a slow degrading BU-modified supramolecular polymer-based TEHV. Upon implantation as surgical pulmonary valve replacement, the TEHV was populated by endogenous cells and, over the 12 months follow-up, 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. In particular, this specific aspect limits the predictability and reproducibility of the outcomes making the clinical translation of such replacements even harder. Hence, even longer follow-ups will be needed to validate this concept with particular regards to the risk of potential unpredictable (maladaptive) remodelling upon complete polymer degradation, which is not yet fully controllable.

In a recent study, Fioretta et al.[136] investigated the profound discrepancies observed in the remodelling of BU-modified supramolecular polymer-based TEHV. Briefly, these TEHV were implanted as TPVR in sheep and followed up for 6 months. In-depth evaluation of the explants demonstrated pronounced differences in cellular infiltration, scaffold degradation, and ECM deposition and highlighted the intra-valve and inter-valve variability arising after TEHVs implantation. Hence, future studies should further investigate the causes of the observed heterogeneous remodelling and the differences in regenerative mechanisms, to be able to predict valve functionality and remodelling, and to increase the safety profile of the replacement.

Finally, during the longitudinal evaluations over the course of the in-vivo follow-up, the scientific community needs to establish biological and mechanical markers which would enable the full characterization of the mechanistic events of valvular remodelling (Figure 3).

3.1.4 Functional growth

Current clinically available valvular replacement are non-living and non-regenerative prostheses which lack growth potential, hence requiring substitution in children to accommodate for somatic growth. For this reason, growth represents a milestone in TE as well as a fundamental prerequisite for paediatric applications. By remodelling into a native-like tissue upon implantation, TEHVs have been hypothesized to be able to grow with the host. However, only few studies have, so far, investigated the growth capacity of TEHVs. Preclinical investigations of decellularised xenogeneic valves in the lamb model demonstrated an increase in TEHV annular diameter compatible with animal growth [75,103]. Additionally, TEM-based vascular grafts have shown signs of somatic growth (i.e.: increased inner diameter, increased conduit length) when implanted as pulmonary artery replacement in lamb [172]. TEM-based TEHVs implanted in the juvenile sheep were shown to increase in annulus diameter with animal growth and to match the neighbouring pulmonary artery well. However, this event was associated with impaired valve functionality to incremental valvular insufficiency. This result was compatible with the observed leaflet shortening and annular growth [172]. Clinical trials using decellularised pulmonary homografts have shown also promising signs of growth, measured as an increase in inner valve diameter [140]. However, annulus size and valve diameter are not sufficient measurements to discriminate between functional growth and tissue dilation. First of all, valve performance parameters (such as: pressure gradient, peak velocity and gradient, and effective orifice area, flow patterns, etc) should always be within the physiological range in case of growth. On the other hand, maladaptive remodelling phenomena such as dilation of the valve annulus may result in central regurgitation, pathological flow phenomena, and jets, that need to be clearly discriminated from somatic growth. In addition, it is to be recognized that tissue dilation is usually associated to morphological changes such as thinning of the valvular walls which may result in a reduction of mechanical properties. To assess that, and state of the art imaging techniques such as echocardiography computed tomography (CT) and/magnetic resonance tomography scans represent very valuable tools.

Taken together, these results demonstrate how little is known about growth capacity of TEHVs and emphasize the need for better methodologies to efficiently discriminate growth from tissue dilation [177].

Hence, a combination of important parameters should be considered in order to systematically demonstrate the growth potential of a cardiovascular TE implant: increase in body weight during animal growth, increase in diameter and length of the implant, good functionality over time (i.e.: no regurgitation), absence of thrombi, calcifications, stenosis, and aneurysm, and balanced tissue formation, with gradual substitution of the implant by functional ECM (i.e.: non-fibrotic tissue, with a limited number of contractile cells). These parameters underline the capacity of a cardiovascular TE construct to adapt to their environment while growing with the patient and retaining their functionality. Additionally, matrix and polymer turnover should be balanced in order to prevent loss of implant integrity, thereby increasing the risk of developing dilation.

3.2 Regulatory and logistical challenges

To ensure a safe bench-to-bedside translation of cell-free TEHVs with in-situ regenerative potential, regulatory, logistical, and infrastructural challenges need to be overcome.

Cell-free TEHVs can be obtained by using different materials, even in combination, with their distinct biological (i.e.: ECM proteins, peptides, bioactive moieties), physical (i.e.: porosity, fiber diameter, thickness), and mechanical (i.e. elastic modulus, tensile strength, elongation) properties. Despite technical heterogeneity, depending on the national/international regulatory legislation, cell-free TEHVs can be either classified as medical devices or biological products. On the other end, functionalized TEHVs may be classified as biological or combination products, if they incorporate bioactive molecules that provides a distinct pharmacological or immunological function upon implantation. Additionally, if such bioactive molecules are considered as medicinal products (drugs), their quality, safety and efficacy shall be proven as well.

A strict requirement for any biomedical device is standardization. By controlling the manufacturing procedures used to obtain a cell-free TEHV, it would be possible to ensure reproducible and consistent batches of product according to pre-defined quality criteria, posing the foundation for commercialization and safe clinical translation. To address this challenge, Good Manufacturing Practices (GMP) processes, Good Laboratory Practices (GLP) testing, and, in USA, Good Tissue Practices should be implemented for the manufacturing and validation of TEHVs. [178]

Quality control procedures are therefore essential to ensure that every product is compliant to the pre-defined desired characteristics. Besides the general standards/regulations covering quality

management for medical devices (e.g. ISO 13485, FDA Quality System Regulation, GMP), several technical standards guide in the development of cardiovascular devices, such as ISO 7198 (Cardiovascular implants and extracorporeal systems - Vascular prostheses - Tubular vascular grafts and vascular patches), ISO 5840 standards (Cardiovascular implants: cardiac valve prostheses), ISO 25539 standards (Cardiovascular implants: endovascular devices) [179]. However, further investigations may be required for TEHVs, specifically those based on in-vitro derived TEM, to ensure biocompatibility (e.g. ASTM F2027-16, ASTM F2383-11, ASTM F2150-13), immunocompatibility, and homogeneous tissue development [180,181].

Finally, to ensure commercialization and broad clinical application, many other aspects should be fulfilled: sterilization, packaging, and storage life. By being cell-free, terminal sterilization of TEHVs for in-situ applications has been achieved with a variety of methods. As an example, human TEM-based TEHVs have been, so far, sterilized in ethanol and antibiotic solution [123,125]. On the other hand, bioresorbable polymeric valves can be sterilized by using gamma-radiation, plasma treatment, or ethylene oxide [89]. However, selection of the optimal sterilization protocol [182] should be evaluated and implemented early in the TEHV development process, due to the effect of sterilization on the TEHV material, as well as its impact on the biocompatibility and safety profile of the TEHV.

3.3 Clinical requirements

The safe translation of TEHVs strongly depends on the observance of strict clinical requirements and the development of clinical guidelines. In fact, beside the scientific challenges (see section 3.1), additional features such as the definition of clinical indications, patient selection based on specific inclusion and exclusion criteria (i.e. comorbidities, regenerative potential), monitoring strategies (i.e. echocardiography, CT-Scan, MRI), and the implementation of back-up plans in case of failure (i.e. bail out strategies), and eventual emergency treatments need to be established in order to ensure safe clinical translation. There are multiple challenges required for clinical translation of TEHVs. First, the valve prototype should be tested in a first-in-man clinical trial, where the safety and performance/effectiveness are monitored. After the early feasibility is assessed, the valve design can be further adjusted and optimized according to the first-in-man outcome. To proceed with further evaluation (i.e.: further trials), a frozen-design that cannot be further modified, should be implemented and tested in clinical trials in accordance to the Good Clinical Practices (GCP) guidelines.

Additionally, the selection of suitable candidates needs to follow a risk stratification procedure and assess their regenerative potential prior the initiation of any treatment in order to exclude comorbidities (e.g. diabetes or immunosuppression), hence preventing potentially fatal failures. The strong natural variability existing into the human innate and adaptive immune response has to be considered and correlated to differences in age or gender. Finally, in order to reduce the risk of human errors during valve replacement procedures, usability of valve substitutes and delivery devices need to be easy to handle and including detailed user instructions and standard operating procedures (SOPs). In this regard, TEHVs should be delivered to the operational room packaged, sterile, and with a summary of the product characteristics. In this context, the reduction of the associated logistical hurdles and the development of a standardized manufacturing process and quality control system is of extreme importance.

3.4 Economical considerations

Degeneration of the aortic valve is the most prevalent indication for valve replacement in elderly patients and has a major impact on society. Importantly, incidence of severe aortic stenosis has been predicted to increase at a rate of 4.4‰/year in patients ≥ 65 years, with an increasing number of patients, up to 115'000 per year, eligible for TAVR procedure in EU and North America [183]. Hence, (aortic) valve diseases have a clear impact on health care resource planning and a repercussion on health care costs, reaching more than 1 Billion Euro in Europe only [68]. Recent cost-effectiveness analyses showed that TAVR procedures appear to be less cost-effective (range 18,421 – 120,779 Euro) than traditional surgeries (range 14,108 – 40,944 Euro) [184,185]. Recent market analysis revealed that TAVR procedures will be more advantageous than surgical approaches only in intermediate-risk patients, due to several influencing factors such as prostheses costs, age, fewer comorbidities, lower complication rates, and length of hospital stay [186]. However, following the new FDA indication to expand TAVR to low-risk and younger patients, TAVR procedure rates will increase in the near future and they might prove to be more cost-effective than the past.

The potential cost-effectiveness of TEHVs was recently assessed in a study of Huygens et al., in which they compared costs and effects of SAVR and TAVR in elderly patients. Results showed that, assuming equal costs of TEHVs compared to clinical heart valve replacements, long-term durability of TEHV had the highest impact on cost-effectiveness. National saving in the first decade after implantation, varied from 2.8-11.2 Mio Euro for SAVR and 3.2-12.8 Mio Euro for TAVR [187].

Unfortunately, due to the large discrepancies in TEHVs manufacturing procedures, an exact estimate of production costs and laboratory performance tests of TEHV are still largely unknown, mainly because TEHVs are not yet commercially available [188].

4. Future research directions

4.1 Computational modelling

CM, particularly when integrated with experimental studies, can significantly improve and accelerate our comprehension of TEHV growth and remodelling [122,189]. CM has been extensively used to increase our understanding of valve haemodynamic, to improve valve prosthesis designs, and even create patient-specific designs [190,191]. As mechanical cues (e.g. stresses and strains) induced by the haemodynamic loading conditions are well known to drive cardiovascular tissue adaptation (Figure 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 understanding in-vivo experimental observations, computational simulations have greatly contributed to understanding the appearance of inter-animal differences in TE vascular grafts [192] and TEHV [122] adaptation, differential remodelling and functionality [193], and the interplay between immuno-driven and mechano-mediated regenerative processes [194,195]. CM is also very valuable to support the safe clinical translation of TEHVs, due to its ability to predict long-term valve adaptation and corresponding functionality as a function of patient-specific in-vivo environment and the initial conditions provided by the scaffold [122,189]. Particularly, CM predictions of the evolution of TEHV properties and function after implantation can elucidate to what extent certain (patient-specific) factors affect the anticipated remodelling profile [122], and thereby give important information regarding the robustness of TEHV performance. Moreover, the identification of factors that determine successful regeneration and functional adaptation of TEHVs on the one hand, or maladaptation and failure on the other hand, can aid in the establishment of risk stratification procedures. Finally, given the myriad of possible combinations of scaffold properties, it is not realistic to optimize TEHV designs with respect to long-term function using experimental studies alone. The ability of computational models to predict the in-vivo evolution of TEHV properties should therefore be leveraged to efficiently optimize scaffold design in order to guide TEHV adaptation and ensure long-term functionality. In a recent proof-of-concept

734 study, Emmert et al. used an integrated computational-experimental approach to demonstrate that CM
735 inspired changes in TEHV design [176,196] can indeed radically improve the long-term in-vivo outcome [122].
736 To build on these promising results, the development and use of CM optimization techniques, for
737 example similar to the recently developed framework by Szafron et al. [197], are important for identifying
738 the most promising combinations of scaffold properties. Consequently, only small selections of valve
739 designs need to be evaluated experimentally, which is expected to tremendously accelerate the
740 improvement and clinical translation of TEHVs.

741 The increased adoption and improvement of computational models in the area of cardiovascular TE
742 also comes with a number of challenges. For example, the increasing incorporation of biological growth
743 and remodelling phenomena in computational models usually correlates with an increase in model
744 parameters, which may lead to difficulties in identifying parameter values (uniquely). Integrating model
745 development with systematic experimental studies can help to (partly) overcome this problem.
746 Additionally, increases in model complexity can lead to problems related to numerical tractability. The
747 development of numerical methods to simplify model descriptions [198–200] is essential for efficiently
748 predicting TEHV adaptation and optimizing scaffold properties.

749 Finally, the proof that computational models can indeed accurately predict valve remodelling in humans
750 on a patient-specific basis is still pending. The expectation that this will be possible is supported by
751 promising pre-clinical studies that have demonstrated the principal feasibility of predicting differences
752 in cardiovascular tissue engineering outcomes due to variations in animal-specific conditions [122,192] or
753 haemodynamics [193]. Additionally, a recent proof-of-concept clinical study [201], where computational
754 models of the patient-specific electrophysiology of atrial fibrillation patients have been successful in
755 predicting effective personalized ablation strategies, further indicates that computational models have
756 the capacity to accurately simulate complex patient-specific cardiovascular phenomena. To
757 successfully predict the evolution of TEHVs in human, patient-specific conditions, it is important that
758 computational models adequately incorporate the relevant physical/biological/chemical phenomena
759 that affect valve adaptation and function. Furthermore, available data from clinical studies need to be
760 appropriately analysed to enable the development and analysis of patient-specific models. Regarding
761 the first point, mechanistic in-vitro and pre-clinical studies should form the foundation of the different
762 mechanisms incorporated in computational models of valve remodelling, and can provide relevant
763 information on the general impact of differences in patient-specific conditions on valve adaptation and

function. To address the second part, the integration of computational models with machine learning algorithms represents a promising strategy for dealing with large data sets, exploring massive design spaces, and quantifying uncertainty [202].

4.2 3D bioprinting

3D bioprinting is gaining importance and is expanding its reach also to direct printing of living cells and ECM proteins into implantable scaffolds [203,204]. Recently, a group at Carnegie Mellon University demonstrated the ability to manufacture 3D-bioprint collagen to engineer the human heart by using an improved second-generation freeform reversible embedding of suspended hydrogels (also termed FRESH) [205]. In this context, tri-leaflet collagen heart valves were successfully 3D-printed and demonstrated sustained functionality in a flow loop system (≤15% regurgitation). Another group based in Switzerland, recently presented the fabrication of a 3D-printed silicone-based heart valve featuring reinforced leaflets and fully customizable to patient's anatomy using a versatile multi-material additive technology [206]. Computer simulations demonstrated that bioinspired fiber design and customized leaflet shape reduced the maximum stress during valve diastole thereby improving valve durability. Additionally, in-vitro haemodynamic tests carried out at aortic conditions showed promising regurgitation fractions of ≤6%. However, despite the enormous strides towards the clinical translation of such technology, numerous challenges have still to be faced such as the need of preclinical evaluations and large-scale clinical trials before 3D printed heart valves can be used as therapeutic devices in standard clinical practice [207]. Limitations for the clinical translation of 3D bioprinting reside in the in-vivo solubility, structural stability, and cell toxicity of bioinks, in the sterility of using a 3D printer in a surgical setting, and finally the establishment of regulatory standards for clinical use [203].

4.3 Bioresorbable stent technologies

Particularly ambitious is the combination of heart valve TE with minimally-invasive transcatheter techniques to avoid open heart surgery. Transcatheter valves are mounted onto a metal stent that provides a supporting frame to guide the crimping procedure necessary for minimally-invasive valve delivery. In addition, the stent enables precise valve positioning above the native valve, limit paravalvular regurgitation, and prevent coronary artery occlusion in the setting of aortic valve replacement. For TE applications, the stent should also promote integration of the engineered tissue with the native host tissue. While current stents are clinically well-known and established for heart valve

prostheses, they do not have the potential to remodel over time, becoming a potential site for complications (e.g.: infection, thrombosis, or intimal hyperplasia). For this reason, novel biodegradable stents have been proposed. Similarly to clinically used stents, bioresorbable stents should allow for minimally-invasive transcatheter implantation, support valve anchoring, and favour initial integration in the annulus. Over time, the stent should be reabsorbed to obtain a fully biological valve prosthesis with the potential to grow. In case of success, this will open up the possibility of translating TAVR to young/paediatric patients. Few studies have, so far, achieved a bioresorbable stent for heart valve replacement. 3D-printed bioresorbable polymeric stents have been designed and tested in-vitro with the aim to treat congenital heart disease in paediatric patients via minimally-invasive implantation techniques [208]. Despite the promising preliminary results in terms of crimping and degradation rate of the developed polymeric stent, in-vivo proof-of-concept of this approach is still lacking. On the other hand, dynamic degradation and haemocompatibility of AZ31 magnesium alloy-based stents have been extensively evaluated [209,210]. In addition, a recent study confirmed the possibility to combine such innovative stent material with a bioresorbable polymeric valve for surgical pulmonary valve replacement, thereby obtaining a fully regenerative implant. Acute functionality was assessed in pig up to 12 hours, demonstrating feasibility of the implantation procedure [211]. However, further improvements should be made to translate this design to a transcatheter-compatible approach with long-term in-vivo good performance.

4.4 Cardiovascular TE in the foetus

Early reports of foetal cardiac interventions date back to 1975 addressing intrapartum surgery of foetal ventricular tachycardia and heart block [212]. Lately, the interest in foetal cardiac interventions has increased, especially for conditions in which the foetus is at high risk for pre- or neonatal death [213]. In regard to heart valve surgery, the most common closed foetal intervention is aortic valvuloplasty, firstly performed in 1989 [214]. Despite unsuccessful preliminary reports, which showed foetal death after intervention, improvements in the technical feasibility was achieved for 75-80% of the procedures [215]. Despite the progress in the field, foetal surgery is controversial, as poses high risks for the developing foetus and the mother associated to the large uterine incisions used for the approach [216], and several and frequent post-natal surgical interventions are still required after pre-natal treatment, hence impairing the quality of life of newborn [213].

More recently, the possibility to couple minimally-invasive implantation techniques to TEHVs has determined potential future therapies. As an example, percutaneous implantation of stents [217] and TEHVs [218,219] in foetal sheep has been investigated. The regenerative capacity of the foetal environment is remarkably high and has the ability to heal ECM architecture, strength, and function, making the foetus a prime target as a model for tissue regeneration [219]. In this context, a preliminary study show the feasibility of implanting TEHVs as pulmonary replacement via transventricular approach in two 110 days gestational foetal lambs, that then showed normal postnatal valve function [218]. Albeit promising for otherwise fatal congenital heart diseases, further studies need to be performed aiming at the optimization of the design and implantation procedure of (TE) valve replacements to limit the risks associated with this technique.

5. Outlook & Conclusions

While surgical valve repair and replacement has been the standard of care for decades, transcatheter approaches have revolutionized the field of heart valve therapy and are expected to become a first line therapy for many patients in the near future. However, despite this tremendous technical evolution, the currently clinically adopted heart valve prostheses still come with significant limitations, and, to date, a heart valve prosthesis with the unique properties of a native heart valve does not exist.

The recent extension of TAVR towards eligible lower risk and younger patients may be particularly relevant for the transcatheter valve market, which is expected to grow considerably in the near future [183]. The currently available bioprostheses utilized for TAVR approaches are made from glutaraldehyde-fixed xenogeneic material. Hence, they are associated with significant limitations such as the permanent risk for infection, thrombotic issues (i.e. leaflet thrombosis) and, most importantly, the fact that they are prone to continuous degeneration. This is known to be accelerated in younger patients and may require multiple re-interventions which come with an increased morbidity and mortality. This clearly underscores the urgent clinical need for next-generation heart valve replacements which remain functional throughout the patients` life, and which do not require any anticoagulation therapy.

Tissue engineered heart valves (TEHVs) with repair, remodelling, and regenerative capacity can address these unmet needs and have been repeatedly suggested as a potential solution. Notably, systematic data from preclinical studies have advanced such technologies into first human trials and have made such next generation valves a clinical reality. However, despite this substantial progress

the overall clinical translation is still hampered with numerous challenges (i.e. scientific, logistical and regulatory issues) that need to be systematically addressed in order to increase the clinical relevance of such next generation heart valves. To date, the largest clinical experience exists for TEHVs based on decellularised homografts which demonstrated promising long-term results in terms of performance and freedom from re-intervention when implanted as pulmonary valve replacement. Bioresorbable polymer-based TEHVs have been recently advanced into a first-in-men clinical trial. Next, based on the encouraging results of the clinical trials in the setting of vascular grafts, the initiation of clinical pilot studies using cell-free ECM based valves is awaited in the near future. Results from such ongoing and future early phase clinical trials will be instrumental in many ways as they will help i) to provide important safety and performance data of such next generation heart valve concepts which will ultimately set the basis for their broad clinical translation and implementation; ii) to define potential indications, to enable proper patient selection and to determine monitoring as well as bail out strategies in case of malfunction or failure; and most importantly, iii) to reveal important insights into the valve remodelling process in humans which is key to achieve long-term performance and native-like tissue configurations. In addition, future studies should also take into consideration to extend TEHVs towards the atrioventricular valves, and, in particular, the mitral valve. International guidelines strongly suggest, whenever possible, to use mitral repair techniques over mitral replacement [220]. However, with the recent advent of transcatheter techniques, a slow but steady increase in transcatheter mitral replacement procedures is foreseen in the next future [221], with the inherent risks of bioprosthetic valve degeneration leading to reoperation in patients younger than 60 years old [220]. Hence, a regenerative mitral valve replacement will be desirable. Finally, technologies such as 3D bioprinting, next generation bioresorbable stent concepts, and in particular, computational modelling for the prediction and guidance of the complex mechanobiological processes during valve remodelling, represent powerful tools to further increase the potential of such next generation heart valves and enhance their clinical relevance.

6. Key learning objectives

1. Surgical heart valve replacement represents the gold-standard treatment, however, transcatheter valve implantation has revolutionized the field by providing a novel treatment option to high, intermediate, and low-risk patients.

2. Despite the rapid evolution in the field of heart valve therapy, there is still an unmet clinical need for valve replacements with regenerative, remodelling, and growth potential.

3. In-situ TE aims at providing an off-the-shelf available heart valve replacement, based on decellularised ECM or bioresorbable polymers, that transforms into a native-analogous valve upon implantation.

4. To improve and accelerate our understanding of TEHVs growth and remodelling, computational modelling represents a powerful tool and should be combined with in-vitro and in-vivo TE strategies.

5. Clinical translation of TEHV approaches is a reality. To ensure safety, feasibility, and efficacy of the TEHV, researchers and clinicians should work according to GMP/GLP and ISO-standards.

6. Heart valve TE still faces several challenges (immunocompatibility, haemocompatibility, remodelling, and growth capacity) that need to be further investigated before broad clinical adoption will be possible.

7. Glossary items

Bileaflet tilting disc: Valve made of a metal ring covered by ePTFE. The metal ring holds a disc that opens and closes following the cardiac cycle.

Frozen design: A design freeze is a binding decision defining the while product, its parts, or parameters and allows the development of the design based on that criteria.

Rheumatic fever: Inflammatory disease affecting children between 5-15 years of age. Rheumatic fever can cause permanent damage to the heart and heart valves.

Stenosis: Narrowing of the heart valve, which does not allow its proper opening, thereby reducing the blood flow through the valve. Rheumatic fever is the main cause of mitral valve stenosis.

Insufficiency: Valve insufficiency (or regurgitation or incompetence), is a disease in which the heart valve does not close tightly, allowing the blood to flow backward.

Annuloplasty: Procedure to tighten or reinforce the ring around a valve in the heart.

Valvuloplasty: Balloon valvuloplasty or balloon valvotomy, is a procedure to repair a stenotic heart valve by expanding a balloon catheter inside the valve to increase the valve opening area.

Comorbidity: Presence of one or more additional conditions occurring concomitantly with the primary disease. Typical valvular disease comorbidities are hypertension, respiratory insufficiency, peripheral arterial stenosis, and chronic renal failure.

Transcatheter: Minimally-invasive implantation techniques based on the use of a catheter to deliver the valve substitute,

Ball-and-cage valve: A valve replacement consisting of an occluder (ball) in a silicone-coated stainless-steel cage. During diastole, the occluder would sit into the sewing ring, preventing retrograde flow.

Warfarin: Name of an anticoagulant compound that inhibits the vitamin-K dependent synthesis of biologically active forms of the clotting factors

Homograft: Also known as allograft, is a tissue/organ from a donor, of the same species as the recipient, but not genetically identical (e.g. human-human).

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.

Xenogeneic: Materials derived from tissues derived from another species than the recipient, such as bovine pericardium or porcine valve leaflets.

Self-expandable: Prostheses used in combination with a shape memory nitinol stent, which achieves valve deployment through stent self-expansion at 37 °C during transcatheter valve delivery

Balloon expandable: Prostheses used in combination with a stent which is not self-expandable, and which are deployed by inflating a balloon that plastically deforms the stent.

Crimping: Procedure used to reduce the diameter of the valve prosthesis of more than three folds to fit it into the catheter used for minimally-invasive implantation.

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

Synthetic biodegradable polymers: Polymers based on biocompatible and bioresorbable starting materials such as Polycaprolactone (PCL), Polylactic-acid (PLA), Polyglycolic acid (PGA), and Poly-4-hydroxy butyrate (P4HB).

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

Storage life: Length of time a product can be stored under specified conditions, without its functionality or performance being affected.

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

8. Tables

Table 1: Overview of the clinical studies evaluating performance and remodelling potential of decellularised (A) homograft-based and (B) xenografts-based TEHVs for in-situ regeneration.

(A) Clinical application of decellularised homograft-based valve replacements with in-situ regenerative potential					
Homograft	**Trial Number Name Year**	**Procedure (Anti-coagulation /anti-aggregation regimen)**	**Age [years]**	**Main Findings**	**Year/Ref.**
fDPVH (n=121)	NCT02035540 ESPOIR (2014-2016)	SPVR (N/A)	21.3±14.4	Excellent performance with trivial regurgitation. Better freedom from explantation compared to cH and BJV. fDPVH are safe and efficient.	2019 [222]
fDPVH and fDAVH (n=5)	NCT02035540 ESPOIR (2011-2017)	SAVR and SPVR (children: Aspirin for 3-6 months PI; adults: warfarin for 2 months PI and aspirin)	2-38	Superior mid-term results in children and young adults for SPVR. Good initial results for SAVR. fDPVH and fDAVH provide a new surgical option for young patients previously subjected to multiple valve procedures.	2018 [223]
fDAVH (n=69)	NCT02527629 ARISE (2008-2015)	SAVR (children: Aspirin for 3-6 months PI; adults: warfarin for 2 months PI and aspirin)	19.7±14.6	fDAVH withstand systemic circulation, with trivial regurgitation, no dilation, no calcification. fDAVH are suitable for young patients	2016 [224]
cDAVH (n=42); cH as control (n=29)	N/A (2002-2004)	SAVR (N/A)	49±17	While early outcomes of cDAVH have been promising, freedom from reoperation after 10 years is greater for cH than cDAVH (80% vs 51%).	2016 [95]
fDPVH (n=93); cH (n=93) and BJV (n=93) as controls	NCT02527629 ARISE (2005-2015)	SPVR (Aspirin for 3-6 months PI)	15.8±10.2	At 10 years, 100% freedom from explantation and endocarditis for fDPVH. Sustained functionality over time.	2016 [92]
cDPV pre-seeded with aECs (n=11)	N/A ESCORE (2000-2002)	SPVR (N/A)	39.6±10.3	Excellent haemodynamic performance over 10 years, with no signs of calcifications	2011 [166]
cDPVH (n=29) (SynerGraft); cH as control	N/A (1993-2009)	SPVR (Aspirin for 3 months PI)	28.6±16.0	No patient required reoperation; no deterioration of the valve; good performance, comparable to cH.	2011 [96]
fDPVH (n=38); cH (n=38) and BJV (n=38) as control	NCT02527629 ARISE (2005-2010)	SPVR (Aspirin for 3 months PI)	12.7±6.1	fDPVH showed low pressure gradients, no dilation, no thickening. Freedom from explantation was 100% after 5 years. fDPVH also exhibited adaptive growth.	2011 [140]
cDPV (n=47); cH (n=47) as control	N/A (2000-2005)	SPVR (N/A)	9.95±7.96	cDPVH showed improved but non-significant freedom from explantation after 8 years compared to cH (79% vs 63%). No significant improvements in valve performance were detected.	2010 [97]
fDAVH or cDAVH (n=41)	N/A (2005-2010)	SAVR (N/A)	34 (0.1-71)	fDAVH showed trivial to mild regurgitation, adequate haemodynamics and 98% freedom from reoperation. Explanted fDAVH showed low cellularisation, but stable structural integrity and low rate of calcification.	2010 [141]
cDPVH pre-seeded with aECs (n=11)	N/A 2000-2003	SPVR (N/A)	44.0±13.7	cDPVH showed promising results to reconstruct the right ventricular outflow tract, with good performance and recellularisation potential. However, the study was hampered by the limited homograft availability.	2007 [73]

fDPVH pre-cultured with aEPCs (n=2)	N/A (2002)	SPVR (Aspirin for 1 month PI)	11, 13	Feasible and safe; potential to remodel and grow (increase in annulus diameter), trivial valve regurgitation at 3.5 years; no signs of degeneration	2006 [72]
cDAVH (SynerGraft) (n=22)	N/A (2002-2003)	SAVR (No anticoagulation treatment)	53±14	Panel reactive antibody results were negative in 95% of patients after 1 year. Good performance with low transvalvular gradients.	2005 [93]
cDPVH (CryoValve SG) (n=14); cH as control	N/A	SPVR (N/A)	8.5 ± 7.9	The panel-reactive antibody level for both class I and class II antibodies are significantly lower for cDPVH compared to cH. Similar functionality after 1 year.	2003 [94]
(B) Clinical application of decellularised xenograft-based valve replacements with in-situ regenerative potential					
Xenograft	**Study design/ Year**	**Procedure (Anti-coagulation/anti-aggregation regimen)**	**Age [years]**	**Main Findings**	**Year/Ref.**
Porcine SIS (CorMatrix) as aortic cusp (n=6)	April-July 2013	SAV repair (N/A)	2m – 14y	Significant insufficiency in 5 patients in the post-operative (119-441 days). Inflammatory cells and chronic inflammation of the implanted tissue were observed.	2017 [107]
Porcine SIS (CorMatrix) for aortic valve repair (n=1)	Case report	SAVR (N/A)	12	Stable valvular performance for 2 years. After 4 years, the valve required a substitution because of calcification, fibrosis, and retraction.	2017 [105]
Porcine SIS (CorMatrix) for pulmonary and aortic valve repair (n=22)	Single-center, prospective, non-randomized	SAV repair (n=4) SPV repair (n=18) (N/A)	2m –14y	The implanted material did not show significant advantages compared to polytetrafluoroethylene, in particular when used for pulmonary valve reconstruction, where functional deterioration was observed	2016 [106]
Porcine DPV (Matrix P) (n=1)	Case report	SPVR (N/A)	6	Severe conduit stenosis, moderate regurgitation, hypertrophic right ventricle. Several aneurysms in the conduit, characterized by lack of cellularisation with no evidence of inflammatory or foreign body response.	2014 [115]
Porcine DPV (Matrix P Plus) (n=21)	2007-2008	SPVR (N/A)	49	Massive inflammatory reaction and necrosis, graft stenosis, not recommended for reconstruction in adults.	2014 [116]
Porcine DPV (Matrix P and Matrix P Plus) (n=26)	2006-2008	SPVR and TPVR (N/A)	12.4 (0.8-38.7)	52% of the patients needed reoperation due to stenosis with moderate to severe insufficiency. Histology showed wall thickening with severe foreign body reaction and inflammation. Endothelialisation was not detected.	2013 [225]
Porcine DPV (Matrix P and Matrix P Plus) (n=93)	2006-2010	SPVR (N/A)	20 (0.16 - 290) months	DPV failure was caused by stenosis, pseudo aneurysm, or conduit dilatation. Poor host cell infiltration with presence of inflammatory giant cells. DPV failure rate was 35.5%, and dysfunction rate was 29%.	2012 [114]
Porcine DPV (Matrix P and Matrix P Plus) (n=61)	2006-2008	SPVR (N/A)	7 (9d – 50y)	Valve failure in 4 patients, with need for re-operation. 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.	2011 [98]
Porcine DPV (Matrix P Plus) (n=16)	2007-2008	SPVR (Heparin at surgery: Aspirin PI)	14 ± 11	Graft obstruction in 38% of the cases after 10 months. Histological examination revealed stenosis, due to inflammation and calcification.	2010 [226]

Porcine DPV pre-seeded with aECs (n=12)	2000-2003	SPVR (N/A)	44.0±13.7	Porcine DPV showed promising performance and recellularisation potential, similarly to decellularised homografts.	2007 [73]
Porcine DPV (Matrix P) (n=50)	2002-2004	SPVR (N/A)	46	Physiological like performance of the valve, with unremarkable haemodynamic and low-pressure gradients.	2005 [113]
Porcine DPV (SynerGraft 500 and 700) (n=4)	2001	SPVR (N/A)	2.5-11	DPV showed good performance post-operatively. Sudden death for structural failure, degeneration, and rupture of the valve occurred in 3 patients. Severe inflammation with granulocytes and macrophages, as well as calcific deposits were observed in the explants. Samples from the pre-implant DPV demonstrated incomplete decellularisation.	2003 [117]

fDPVH: fresh decellularised pulmonary valve homograft; fDAVH: fresh decellularised aortic valve homograft; cDPVH; cryopreserved decellularised pulmonary valve homograft; cDAVH: cryopreserved decellularised aortic valve homograft; DPV: decellularised pulmonary valve; DAV: decellularised aortic valve; SAVR: surgical aortic valve replacement; SPVR: surgical pulmonary valve replacement; aEPCs: autologous endothelial progenitor cells; aECs: autologous endothelial cells; aEPCs: autologous endothelial progenitor cells; cH: cryopreserved homograft; BJV: bovine jugular vein; SIS: small interstitial submucosa; N/A: not available

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Table 2: Overview of the pre-clinical studies evaluating performance and remodelling potential of decellularised homografts and xenografts-based TEHVs for in-situ regeneration.

Pre-clinical application of decellularised homografts and xenograft-based TEHV with in-situ regenerative potential						
Homograft / Xenograft	**Animal model (species)**	**Number of animals**	**Procedure (Anti-coagulation/anti-aggregation regimen)**	**End-points**	**Main findings**	**Year/Ref.**
Porcine DAV	Sheep (Polypay)	5	SPVR (Warfarin, 2 days PI)	5 months	Feasibility of the trans-species implantation with recellularisation and sufficient haemodynamic function. No thrombosis, bacterial or fungal contaminations. Host cell infiltration with presence of collagen-producing myofibroblasts and endothelial cells.	2017 [227]
Porcine SIS (CorMatrix) as aortic cusp	Pig (Landrace)	4	SPVR (N/A)	4 months	The implanted material remodelled and degraded over time, losing functionality due to thickening and calcification	2017 [228]
Ovine DPVH-FD (n=3) *Ovine fDPVH (n=3)* *Porcine DPV-FD (n=3)*	Sheep (N/A)	9	SPVR (Dalteparin, 2 weeks PI)	6 months	Freeze-drying is a promising method to extend the shelf-life of valvular grafts and does not affect early haemodynamics and cell infiltration. Porcine DPV, on the other hand, showed evidence of immunological reaction that did not impair early functionality.	2017 [229]
Porcine SIS (CorMatrix) as tricuspid valve (n=8) *Bioprostheses (n=2)* *Native valve (n=2)*	Sheep (6 wethers and 6 ewes)	12	SPVR (Heparin at surgery)	3 months (n=6) 8 months (n=6)	When implanted in a 3 month old lamb model, the CorMatrix valve showed an increased of the annular diameter with normal valvular performance. Host cells migrated into the scaffold and trilaminar ECM remodeling was observed.	2015 [103]
Porcine SIS (CorMatrix) as tricuspid valve (n=4)	Sheep (Western Range wether))	4	SPVR (Heparin at surgery)	3, 5, 8, 12 months (n=1 for time point)	Good valve performance with maintained coaptation. Progressive tissue remodeling with collagen and elastin deposition, and integration in the native annulus. No evidence of foreign body response.	2014 [102]
Human DPV (n=5) *Baboon DPVH (n=3)* *Porcine cPV (n=6)*	Baboon (N/A)	14	SPVR (N/A)	10 weeks 26 weeks	Human and baboon DPV showed native-like haemodynamics. Porcine cPV provoked the most intense antibody response.	2013 [120]
Porcine DAV	Sheep (N/A)	4	SAVR (Heparin at surgery; no anticoagulants PI)	1 month (n=1) 2 months (n=1) 4 months (n=2)	Good early performance, with no regurgitation under systemic pressure. Smooth and pliable leaflets, full cellularisation with host interstitial cells.	2012 [74]

Porcine DAV	Dog (Mongrel)	8	SPVR (N/A)	1 month (n=3) 2 months (n=3) 6 months (n=2)	Efficient decellularisation with minimal immune response and calcification. Spontaneous endothelialisation and host cell repopulation occurred within 2 months. Stable valve performance with no regurgitation.	2007 [110]
Rabbit DAV	Dog (N/A)	15	Abdominal aorta (no anticoagulants during surgery or PI)	1 month (n=5) 3 months (n=5) 6 months (n=5)	Complete loss of valvular structure. Reendothelialisation and recellularisation with basic vascular cell component. No immunological response was observed.	2006 [230]
Porcine DPV	Sheep (N/A)	7	SPVR (N/A)	3 months (n=4) 6 months (n=3)	Valves showed smooth and pliable leaflets. No evidence of thrombosis. Endothelialisation was observed, with presence of fibroblasts in the tissue. Newly secreted collagen was detected in the absence of calcific deposits, suggesting remodelling potential of the implanted DPV.	2005 [111]
Porcine DAV (n=12) *Porcine DAV-Fn-HGF (n=15)* *Porcine DAV-HGF (n=12)*	Dog (Beagle)	39	SPVR (Heparin at surgery; no anticoagulants PI)	1 week (n=17) 1 month (n=17) pre-term death (n=5)	At 1 week, Fn-HGF functionalized DAV showed partial endothelial coverage, that became complete endothelialisation at 1 month. A greater number of cells was observed in the Fn-HGF group, compared to the controls.	2005 [231]
Porcine SIS (CorMatrix) as pulmonary valve (n=12)	Pig (farm pig)	12	TPVR (Heparin at surgery)	1 day (n=1) 1 month (n=1) 3 months (n=3) 6 months (n=3) 12 months (n=3) 1 animal died before valve replacement	Good performance up to 12 months, with no stenosis and trivial regurgitation. Intensive remodeling of the SIS was observed, with endothelialization and host fibroblast infiltration.	2005 [101]
Porcine DPV (n=3), *Porcine DPV pre-seeded with aECs and aMFBs (n=5)*	Sheep (N/A)	8	SPVR (N/A)	6 months	Porcine endogenous retrovirus DNA was initially observed in porcine DPV samples. However, it 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.	2003 [112]
Porcine DPV pre-seeded with aECs (n=8)	Sheep (N/A)	8	SPVR (Heparin at surgery; no anticoagulants PI)	7 days (n=1) 3 months (n=4)	The explanted DPV showed a confluent endothelial cell monolayer at any time points, with no signs of	2003 [165]

				6 months (n=3)	calcification and low calcium content. An increasing number of fibroblasts was observed in the valvular tissue over time.	
Porcine DAV (n=5) *Ovine cAV (n=2)*	Sheep (Suffolk)	7	SPVR (N/A)	5 months	Good valve performance over time with no macroscopic damages to the leaflets. Cellular repopulation by fibroblasts was observed and no signs of calcification were detected, suggesting that decellularisation can stabilize xenogeneic valves.	1999 [232]

DPVH: decellularised pulmonary valve homograft; fDPVH: fresch decellularised pulmonary valve homograft; DPV: decellularised pulmonary valve; DAV: decellularised aortic valve; cPV: cryopreserved pulmonary valve; cAV: cryopreserved aortic valve; SAVR: surgical aortic valve replacement; SPVR: surgical pulmonary valve replacement; FD: freeze-dried; aECs: autologous endothelial cells; aMFBs: autologous myofibroblasts; Fn-HGF: fibronectin-hepatocyte growth factor; HGF: hepatocyte growth factor; ECM: extracellular matrix; PI: post implantation; SIS: small interstitial submucosa; N/A: not available.

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947 **Table 3:** Overview of the pre-clinical studies evaluating performance and remodelling potential of
948 decellularised TEM-based TEHVs for in-situ regeneration.

Pre-clinical application of decellularised TEM-based TEHV with in-situ regenerative potential						
Tissue engineered matrix (TEM)	**Animal model (species)**	**Number of animals**	**Procedure (Anti-coagulation/anti-aggregation regimen)**	**End-points**	**Main findings**	**Year/Ref.**
PGA-P4HB-based human TEM	Sheep (White alpine)	3	TPVR (Heparin at surgery)	Acute	Principal feasibility of human cell-derived TEM to manufacture TEHV comprising Valsalva sinuses. Acute valve functionality was excellent. Explanted valve showed the presence of blood-derived cells in the tissue.	2019 [123]
PGA-P4HB-based human TEM	Sheep (White alpine)	5	TAVR (Heparin at surgery)	Acute	Principal feasibility of TAVR using clinically-relevant TEHV based on human cell-derived TEM. Good haemodynamic performance, with free coronary flow, no stenosis, no paravalvular leak. Explanted valve showed the presence of blood-derived cells in the tissue.	2018 [125]
PGA-P4HB-based ovine TEM	Sheep (Grey horned heathes)	11	TPVR (Heparin at surgery; Aspirin and Dalteparin for 5 days PI)	12 months	Computational modelling-inspired valve design optimized to control the deformation of the leaflets. Excellent long-term in-vivo performance, with no regurgitation nor stenosis. Native-like remodelling without leaflet shortening. Remodelling results were predicted by computational modelling.	2018 [122]
PGA-P4HB-based ovine TEM	Sheep	3	TPVR (Heparin at surgery: N/A for PI)	Acute (n=1) 4 months (n=2)	First prototype of TEHV with integrated Valsalva sinuses. 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.	2018 [124]
Fibrin-based ovine TEM	Sheep (Dorset)	8	SPVR (Heparin for the duration of the study)	5 months	Good performance up to 8 weeks, followed by increased regurgitation due to evident leaflet shortening.	2017 [172]

					Remodelling was evident with host cell infiltration, collagen and elastin deposition.	
Fibrin-based ovine TEM	Sheep (Dorset)	4	SAVR (Heparin for the duration of the study)	3 months (n=1) 6 months (n=3)	First chronic evaluation of a TEHV as aortic valve replacement in a preclinical model. Good haemodynamic performance, with no stenosis. Evident matrix remodelling, collagen and elastin synthesis, and host endothelial and interstitial cells.	2015 [128]
PGA-P4HB-based ovine TEM	Sheep	12	TPVR (N/A)	1 day (n=2) 2 months (n=2) 4 months (n=4) 6 months (n=4)	Good early performance, with mild regurgitation starting at 8 weeks and progressing to moderate at 24 weeks due to a compromised leaflet coaptation. Significant host cell repopulation and matrix remodelling without calcification were observed.	2014 [129]
PGA-P4HB-based human TEM	Baboon (Chacma)	6	TPVR (Aspirin and Warfarin for the duration of the study)	1 month (n=3) 2 months (n=3)	After 2 months, valve functionality showed signs of regurgitation (mild to moderate). Leaflets were thin and mobile, but shortened overtime. Remarkably rapid cellular repopulation was observed, compared to the human native heart valve used as a control, confirming the substantial remodelling potential of TEM.	2013 [119]

SAVR: surgical aortic valve replacement; SPVR: surgical pulmonary valve replacement; TAVR: transcatheter aortic valve replacement; TPVR: transcatheter pulmonary valve replacement; TEM: tissue engineered matrix; TEHV: tissue-engineered heart valve; PGA: polyglycolic acid; P4HB: poly(4-hydroxybutyrate); ECM: extracellular matrix; PI: post implantation; N/A: not available.

Table 4: Overview of the clinical studies evaluating performance and remodelling potential of (A) human TEM-based and (B) bioresorbable polymer-based TEHV and/or grafts for in-situ regeneration.

(A) Clinical application of decellularised hTEM-based vascular grafts with in-situ regenerative potential						
hTEM	**Trial Number Study Year Design**	**Procedure**	**Number of patients**	**Age [years]**	**Main Findings**	**Year/Ref**
hTEM	NCT017444418 and NCT01840956 2012-2026 Two single-arm phase 2 trial	Surgical	60	18-80	Host myogenic, endothelial, and progenitor cell repopulation of acellular human grafts. Functional multi-layered living tissues. Self-healing after cannulation injury.	2019 [131]
hTEM	NCT017444418 and NCT01840956 2012-2026 Two single-arm phase 2 trial	Surgical	60	18-80	No dilatation and rarely post-cannulation bleeding. At 6 months, 63% of patients had primary patency, 73% had primary assisted patency, and 97% had secondary patency. Most loss of primary patency because of thrombosis. At 12 months, 28% had primary patency, 38% had primary assisted patency, and 89% had secondary patency.	2016 [132]
hTEM	NCT03183245 Phase 3 trial	Surgical	240	≤18	Ongoing	-
hTEM	NCT02644941 Randomized	Surgical	355	≤18	Ongoing	-
hTEM	NCT03631056	Surgical	N/A	Child, Adult, Older adult	N/A	-
hTEM	NCT03005418 Non-randomized, Phase 2 trial	Surgical	40	18-85	Ongoing	-
hTEM	NCT02887859 Single group, Phase 2 trial	Surgical	25	18-85	Ongoing	-
hTEM	NCT01872208 Single group Pilot study	Surgical	20	18-80	Ongoing	-
(B) Clinical application of bioresorbable polymer-based TEHV and grafts with in-situ regenerative potential						
Polymer	**Trial Number Study Year Design**	**Procedure**	**Number of patients**	**Age [years]**	**Main Findings**	**Year/Ref**
Upy-polyester-urethanes (EC-TCPC)	NCT02377674 2013-2019 Single group assignment	EC-TCPC	5	4-12	Good patient recovery, with no complications; no device-related adverse events; anatomical and functional stability of the implanted graft in all patients; significant improvement in the patients' general condition	2017 [137]
Upy-polyester-urethanes (Xplore 1)	NCT02700100 2016-2022 Non-randomized, Single group assignment	SPVR	12	2-22	N/A	N/A
Upy-polyester-urethanes (Xplore 2)	NCT03022708 2017-2023 Non-randomized, Single group assignment	SPVR	10	2-22	N/A	N/A

Upy-polyester-urethanes	NCT03405636 2018-2024 Non-randomized, Single group assignment	SPVR	55	≤22	N/A	N/A

EC-TCPC: extracardiac total cavopulmonary connection; SPVR: surgical pulmonary valve replacement; hTEM: decellularised human tissue engineered matrix: N/A: not available

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Table 5: Overview of the pre-clinical studies evaluating performance and remodelling potential of (A) bioresorbable polymer-based TEHV and (B) their combination with a one-step pre-seeding procedure using autologous bone marrow mononuclear cells.

(A) Preclinical application of bioresorbable polymer-based TEHV with in-situ regenerative potential						
Polymer	**Animal model (species)**	**Number of animals**	**Procedure (Anti-coagulation / anti-aggregation regimen)**	**End-points**	**Main findings**	**Year/Ref.**
Polycarbonate urethane urea + AZ31 magnesium alloy stent	Pig (Yorkshire)	5	SPVR (Heparin at surgery)	Acute	Successful implantation, normal leaflet function, no thrombosis, no regurgitation, no degradation	2019 [211]
Upy-polyester-urethanes	Sheep	18	SPVR (N/A)	2 months (n=6) 6 months (n=6) 12 months (n=6)	Ongoing remodelling process with neointima formation. Neointimal thickness showed a peak at 6 months. Inflammation is maximum at 6 months; degradation peak is at 12 months.	2018 [134]
UPy-polyester-urethanes (XPV)	Sheep (Ile de France)	33	TAVR (N/A)	Acute	Good haemodynamic performance, comparable to commercially available valves, with acceptable degree of regurgitation	2017 [135]
UPy-polyester-urethanes (XPV)	Sheep (Swifter)	20	SPVR (N/A)	Acute (n=11) 3 months (n=1) 6 months (n=1) 12 months (n=5) 24 months (n=2)	Favourable and durable haemodynamic performance, no stenosis, no obstruction and no severe regurgitation was observed	2017 [233]
Bisurea-polycarbonate	Sheep (Swifter)	10	SPVR (Heparin at surgery; N/A PI)	2 months (n=1) 6 months (n=5) 12 months (n=4)	Sustained functionality up to 12 months, remodelling with de-novo collagen and elastin synthesis, incomplete scaffold reabsorption	2017 [89]
P4HB-gelatin	Sheep	4	TPVR (N/A)	Acute	Good haemodynamic performance and competence upon implantation	2017 [139]
(B) Preclinical application of bioresorbable polymer-based TEHV pre-seeded with autologous bone marrow mononuclear cells						
Polymer	**Animal model (species)**	**Number of animals**	**Procedure**	**End-points**	**Main findings**	**Year/Ref.**
Bisurea-polycarbonate (n=6) *Bisurea-polycarbonate pre-seeded with aBMMNCs (n=7)*	Sheep	13	TPVR (Heparin at surgery, Calciparin 30 days PI)	Acute (n=3) 1 month (n=4) 6 months (n=6)	BMMNC pre-seeding was feasible but it causes severe regurgitation and calcification. Independently on pre-seeding, differential leaflet remodelling was observed.	2019 [136]
PGA-P4HB pre-seeded with aBMMNCs	Sheep	4	TAVR (N/A)	Acute	Sufficient positioning, no obstruction of the coronaries, no structural damages, no stent migration. Paravalvular leakage and central aortic regurgitation was observed.	2014 [234]

PGA-P4HB pre-seeded with aBMMNCs	Sheep	12	TAVR (Aspirin daily)	Acute (n=4) 2 days (n=5) 1-2 weeks (n=3)	Adequate leaflet mobility and functionality. Intact leaflet structure, no signs of thrombus or structural damage. Early cellular remodelling after 2 weeks.	2012 [235]
PGA-P4HB pre-seeded with aBMMNCs	Sheep	1	TAVR (N/A)	Acute	Technical feasibility of minimally invasive aortic replacement with TEHV; adequate leaflet mobility and coaptation, without thrombus formation or structural damages	2011 [236]
PGA-P4HB pre-seeded with aBMMNCs	Chacma baboon	6	TPVR (Aspirin and Warfarin for 4 weeks)	Acute (n=1) 1 month (n=5)	Preserved valvular structures and adequate functionality. Substantial cellular remodelling, layered and endothelialised tissues	2011 [82]

TAVR: transcatheter aortic valve replacement; TPVR: transcatheter pulmonary valve replacement; PGA: polyglycolic acid; P4HB: poly(4-hydroxybutyrate; UPy: ureido-pyrimidinone; aBMMNC: autologous bone marrow mononuclear cells; PI: post implantation; N/A: not available.

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Figure legends

Figure 1: Evolution of heart valve replacement options. Schematic representation of mechanical and (transcatheter) bioprosthetic aortic valve prostheses evolution over time. Valve prostheses images have been adapted from Russo et al. [20].

Figure 2: Overview of different TE approaches. A) In-vitro heart valve TE based on the isolation and seeding of autologous cells onto a bioresorbable scaffold that is then cultured into a bioreactor system [70]. The resulting living autologous TEHV is ready to be implanted into the patient. Image adapted from Dijkman et al.[87] with permission. B-E) In-situ TE approaches relay on the regenerative potential of the recipient body to integrate and remodel an off-the-shelf available acellular implant that can be manufactured using different cell and tissue sources: B) allogenic decellularised TEM-based matrices (as reported in Emmert et al. [122]); C) decellularised homografts or xenografts materials (image adapted from Jana et al. [133]); D-E) bioresorbable polymeric scaffolds processed via i.e.: electrospinning[89] or rotary jet spinning [139], that can be either D) cell-free or E) pre-seeded (as reported by Fioretta et al. [136]).

Figure 3: Schematic representation of the challenges and future technologies for the successful generation of TEHVs. Successful TEHV design should take into account A) the native heart valve anatomy, comprising, among other, the Valsalva sinuses (marked with *, image adapted from Miyazaki et al. [237]) and B) the physiological haemodynamic environment, with different mechanical forces acting on the valve leaflet during systole and diastole [238]. These data, in combination with C) TEHV material properties, such as fibre orientation and mechanical properties, should be used to develop D) in-silico modelling tools to better understand and predict the mechanical forces acting on the valve leaflets (images adapted from Sanders et al. [176]) and E) determine the most indicated valve design to ensure long-term TEHV functionality. After in-vitro characterization, TEHV performance and remodelling should be evaluated E) in-vivo 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. F) Predicted in-vivo adaptive remodelling of a TEHV towards a native-like leaflet starts with the initial recruitment of inflammatory cells (e.g.: monocytes, macrophages, lymphocytes) followed by the recruitment of progenitor cells, ECM-producing cells (e.g.: myo-fibroblasts) and endothelial cells in the scaffold. Over time, the implanted scaffold will be completely reabsorbed, while new ECM is secreted and, finally, the inflammation will resolve leading to maturation of the tissues towards a native-like structure. F) Predicted in-vivo maladaptive remodelling characterized by a constant presence of inflammatory and ECM-producing contractile cells which lead to exacerbation of the immune reaction, chronic inflammation and fibrosis, resulting in abundance of contractile cells, implant encapsulation and/or TEHV leaflet shortening.

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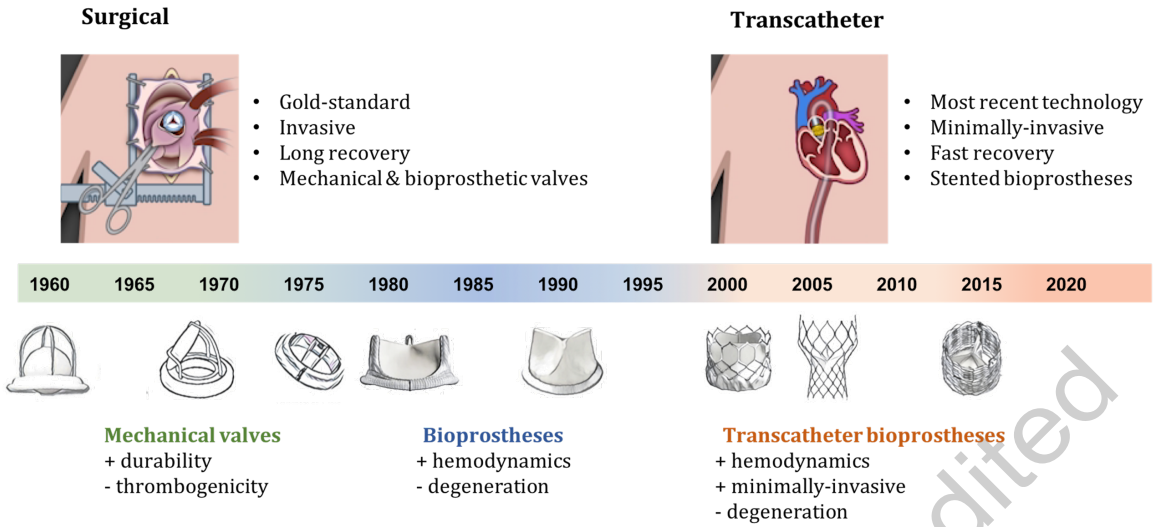

1554

1555 **Figure 1: Evolution of heart valve replacement options.** Schematic representation of mechanical
1556 and (transcatheter) bioprosthetic aortic valve prostheses evolution over time. Valve prostheses images
1557 have been adapted from Russo et al. [20].

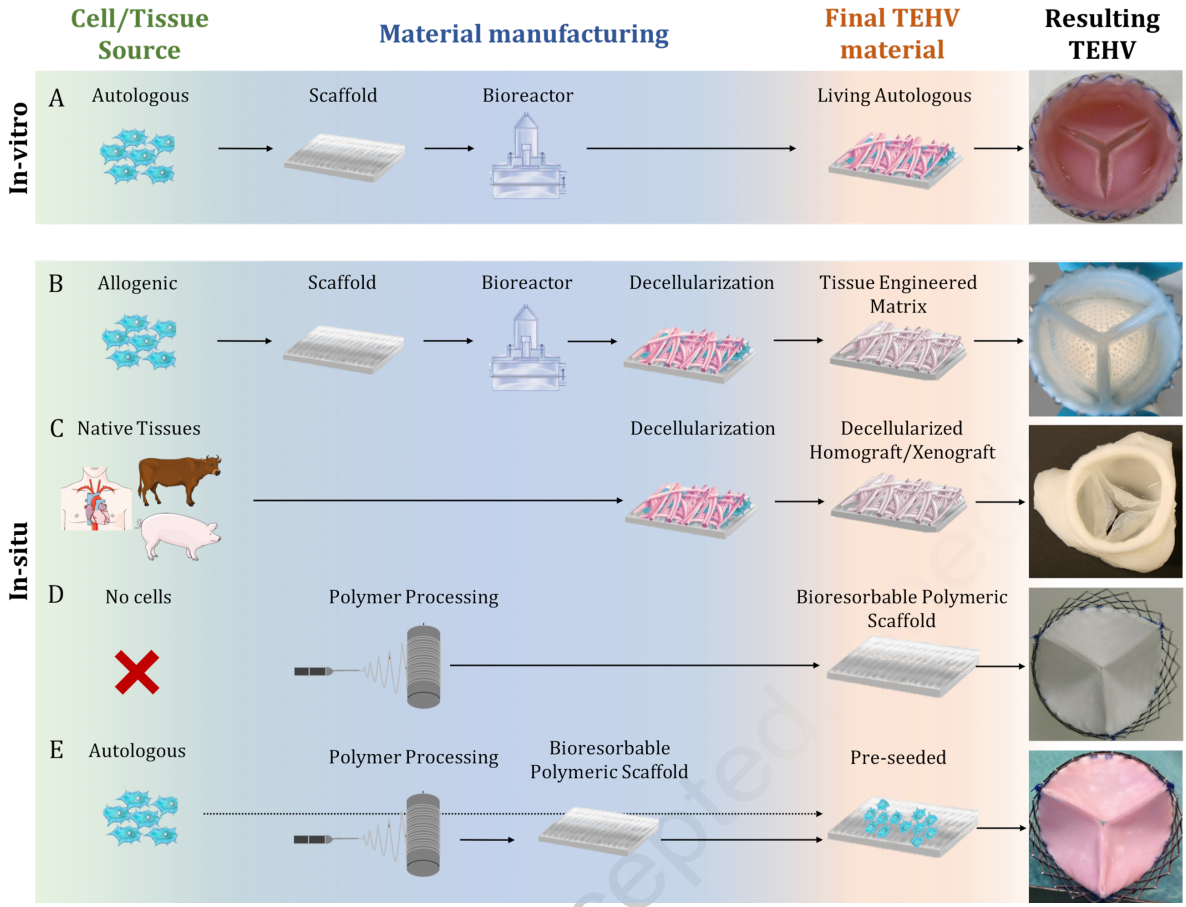

Figure 2: Overview of different TE approaches. A) In-vitro heart valve TE based on the isolation and seeding of autologous cells onto a bioresorbable scaffold that is then cultured into a bioreactor system [70]. The resulting living autologous TEHV is ready to be implanted into the patient. Image adapted from Dijkman et al.[87] with permission. B-E) In-situ TE approaches relay on the regenerative potential of the recipient body to integrate and remodel an off-the-shelf available acellular implant that can be manufactured using different cell and tissue sources: B) allogenic decellularised TEM-based matrices (as reported in Emmert et al. [122]); C) decellularised homografts or xenografts materials (image adapted from Jana et al. [133]); D-E) bioresorbable polymeric scaffolds processed via i.e.: electrospinning[89] or rotary jet spinning [139], that can be either D) cell-free or E) pre-seeded (as reported by Fioretta et al. [136]).

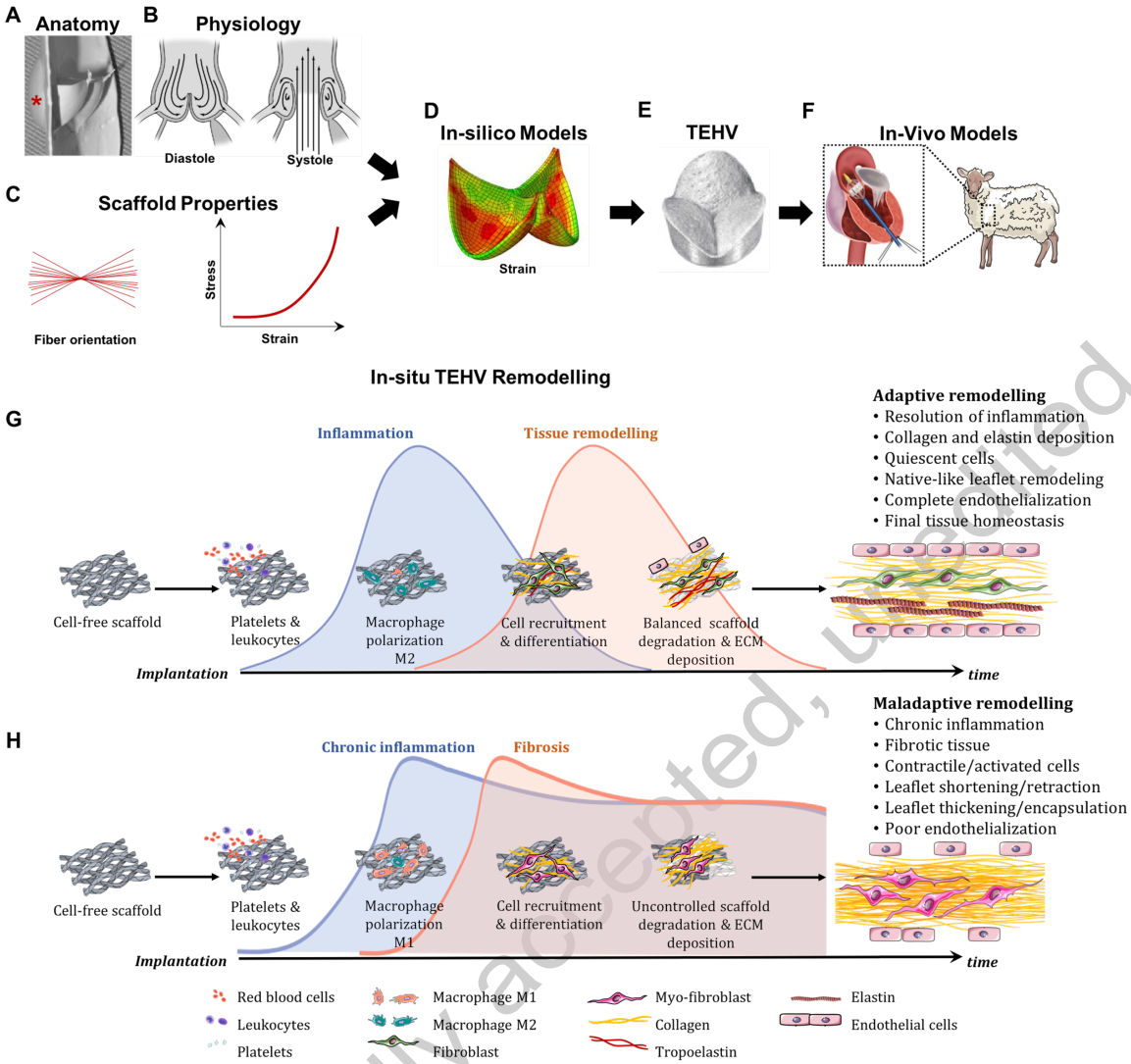

Figure 3: Schematic representation of the challenges and future technologies for the successful generation of TEHVs. Successful TEHV design should take into account A) the native heart valve anatomy, comprising, among other, the Valsalva sinuses (marked with *, image adapted from Miyazaki et al. [237]) and B) the physiological haemodynamic environment, with different mechanical forces acting on the valve leaflet during systole and diastole [238]. These data, in combination with C) TEHV material properties, such as fibre orientation and mechanical properties, should be used to develop D) in-silico modelling tools to better understand and predict the mechanical forces acting on the valve leaflets (images adapted from Sanders et al. [176]) and E) determine the most indicated valve design to ensure long-term TEHV functionality. After in-vitro characterization, TEHV performance and remodelling should be evaluated E) in-vivo 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. F) Predicted in-vivo adaptive remodelling of a TEHV towards a native-like leaflet starts with the initial recruitment of inflammatory cells (e.g.: monocytes, macrophages, lymphocytes) followed by the recruitment of progenitor cells, ECM-producing cells (e.g.: myo-fibroblasts) and endothelial cells in the scaffold. Over time, the implanted scaffold will be completely reabsorbed, while new ECM is secreted and, finally, the inflammation will resolve leading to maturation of the tissues towards a native-like structure. F) Predicted in-vivo maladaptive remodelling characterized by a constant presence of inflammatory and ECM-producing contractile cells which lead to exacerbation of the immune reaction, chronic inflammation and fibrosis, resulting in abundance of contractile cells, implant encapsulation and/or TEHV leaflet shortening.