Supramolecular polymer materials bring restorative heart valve therapy to patients

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Supramolecular polymer materials bring restorative heart valve therapy to patients

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The functional restoration of natural tissues in a variety of cardiovascular applications is the main objective of Endogenous Tissue Restoration (ETR). The recent progress in the development of biocompatible, biodegradable, and tunable biomaterials with unprecedented properties allow the next steps from laboratory studies to clinical studies. The independent control over mechanical properties and biodegradability provided by the combination of covalent and non-covalent bonds makes supramolecular polymers uniquely qualified for ETR. This paper will provide further details on the mechanism of ETR and will provide a perspective on the preclinical and clinical application of supramolecular polymers for ETR. In addition to various reports on chronic studies in large animal models, three world-first clinical studies are reported, demonstrating the potential of supramolecular technology in bringing ETR to patients.

Keywords: Supramolecular polymers; Heart valves; Tissue engineering; Clinical studies

Introduction

The development of new polymeric biomaterials made from both natural and synthetic building blocks has opened the creation of new biomedical devices with unprecedented properties [1]. While in the early days biomaterials have mainly been used in basic applications such as dialysis tubing or in dentistry implants, modern biomaterials are increasingly being found in demanding fields such as controlled drug delivery systems or regenerative applications [2]. In the latter, these biomaterials are typically used as scaffolds for (functional) tissue growth whereby the scaffold provides the necessary mechanical support and enables the new tissue to grow within the desired topologically constraints, such as for example the shape of a heart valve [3]. In this paper, we present a novel modular biomaterial platform based on supramolecular polymer chemistry, and its biomedical use for *in vivo* restorative heart valve therapy.

Every year more than 300,000 people worldwide get their heart valves replaced or repaired, because their current heart valve is not functioning properly. This number is anticipated to nearly triple to 850,000 by 2050 [4]. Most used today are bioprosthetic valves (BPV), which are constructed from animal-derived tissues [5,6]. An important drawback of this approach is the inherent limited durability of biological tissue. Since the human body does not recognize these materials as body-own, they eventually undergo a degenerative process associated with thrombosis and calcification, eventually resulting in loss of functionality [7]. Reintervention typically takes place between 10 and 15 years after surgery. Moreover, in younger patients this degenerative process goes faster. This is already apparent in comparing patients above and below 60 years of age [8], but it is even more apparent in pediatric applications, where children require several reoperations throughout childhood. Besides durability there are some other limitations to the use of biological tissues. Yield lim-

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itations due to inherent biological variations already put a burden on the availability of sufficient good quality tissue. Finally, none of valves based on biological tissue have the potential to grow with the patient, which may constitute the next hurdle for pediatric patients. An important trend to note is the rapid adoption of catheter-based therapies, which almost exclusively rely on biological tissue because of its pliability [9]. This is anticipated to boost a further increase in the use of biological tissue valves, while first signals are there that crimping the tissue into the catheter further reduces valve durability [10,11].

One approach to overcoming existing limitations with bioprosthetic materials is the use of biostable polymers for the valve material. Several clinical trials have been reported with enhanced poly(tetrafluoroethylene) (e-PTFE) based pulmonary valved conduits [12,13]. Early clinical investigation of a siloxane poly (urethane urea)-based surgical aortic valve is being pursued with Foldax' Tria valve [10,14], while biostable thermoplastic silicone-polycarbonate-urethane (TSPCU) transcatheter aortic valves reported promising in vivo performance in an ovine model [15-17]. None of these applications have found mainstream adoption yet. The success of a biostable approach is depending on the long-term inertness of the material inside the body, therefore long-term follow-up will be needed to establish the merit of this approach.

An alternative approach is to make use of the natural healing capacity of the human body by providing bioabsorbable materials that with time are replaced by new tissue built by the human body in a restorative approach [18,19]. The key to success for this approach is the development of bioabsorbable materials which are characterized by high strength, elasticity, durability, and controlled bio-absorption [20]. This has galvanized the development of a new modular biomaterial platform built on supramolecular polymers comprising ureido-pyrimidinone (UPy) hydrogen bonding units [21-23]. This unique hydrogen bonding supramolecular unit has been developed at Eindhoven University of Technology and is responsible for the strong non-covalent and reversible (supramolecular) interactions between the supramolecular polymers due to its unprecedented high self-association strength [24,25]. Because the supramolecular hydrogen bonding interactions can be modulated with polar organic solvents, these polymers can be processed relatively easily into porous implant structures using solvent processing techniques such as electrospinning. Owing to their biocompatibility, bio-absorption profile, and porous structure, these devices – once implanted – will be integrated by the patient's own tissue in a process called Endogenous Tissue Restoration (ETR). This constitutes an important paradigm shift compared to the current standards of care, in which foreign materials are designed and implanted to be biostable and as inert as possible, i.e. to minimize integration with the patient's own tissue. This paper will provide further details on the mechanism of ETR and will provide a perspective on the preclinical and clinical application of supramolecular polymers for ETR, largely based on work by the authors and their co-workers.

Endogenous tissue restoration

Current available heart valves suffer from many limitations. Importantly, the human body is unable to recognize the animal-derived tissues as its own, and therefore cannot adapt and remodel the valve in response to environmental changes, as it could do with a native heart valve. To overcome this limitation, several regenerative approaches have been pursued. In one attempt, animal-derived materials are processed with special techniques to wash away all xenocellular components, but without any glutaraldehyde-fixation [26-30], thus leaving a more natural scaffold compared to the glutaraldehyde-fixated valves that are used today. While this approach has shown some success with reendothelialization of the valve surface, reports on full cell penetration and natural tissue replacement are lacking. Furthermore, early clinical failure with incomplete removal of xenogeneic material warrants caution with this approach [28,31].

The tissue engineering approach, as postulated by Langer & Vacanti in their 1993 landmark paper [32], aims to overcome xenogeneic issues by recreating tissue using autologous cells and biomaterials. Using the *in vitro* tissue engineering method, cells are harvested from the patient and seeded onto a temporary biomaterial carrier. The combination is placed in a bioreactor in which it is typically exposed to physiological stresses and strains to stimulate production of autologous tissue. Shinoka et al. [33] were the first to report the replacement of a heart valve leaflet in a lamb model. Later, Hoerstrup et al. [34] demonstrated the replacement of full pulmonary valves, which exhibited remarkable native-like features after 20 weeks in the lamb. Building on this, the research group of Baaijens and Bouten [35,36] demonstrated tissue engineered human valves that were strong enough to withstand aortic pressures in an *in-vitro* test set-up.

While the *in-vitro* tissue engineering paradigm is conceptually and scientifically very appealing, there are several drawbacks which have limited its clinical application, especially for heart valves. Most importantly, the regulatory and manufacturing complexities associated with *in-vitro* production of autologous products significantly limit commercial application. Noteworthy is the work of Shinoka and Breuer, who reported already in 2001 on the first clinical application of a tissue engineered blood vessel in a so-called Fontan procedure, performed in Japan [37]. Now, 18 years later they are running a small clinical trial in the US [38]. Moreover, in their latest approach, the expensive bioreactor step is omitted by harvesting stem cells from the patient and seeding this onto the scaffold shortly before implantation. A slightly different approach is taken by Niklason et al, who have several clinical trials underway with *in-vitro* tissue engineered blood vessels, using a decellularization approach to remove all cellular components prior to implantation [39,40]. In this way, an allogeneic approach can be used, and long-term storage becomes feasible. The Hoerstrup and Baaijens groups report promising results out to 1 year in a sheep model using a similar approach with a computationally guided pulmonary valve design [41]. Most promising proof of concept has been provided by Tranquillo et al. who demonstrated 1 year performance of a pulmonary valve in a growing lamb model [42] and 6 months performance as a sheep aortic valve [43].

Despite the simplifications introduced, all these approaches are limited by the extensive *ex-vivo* manipulation of cells. To overcome this limitation, the *in-situ* tissue engineering paradigm was introduced. In this approach, scaffolds are seeded with cells which are harvested right before implantation (e.g. as in the

most recent work by Breuer and Shinoka [38]), or the cell seeding step is omitted and is replaced by the incorporation of bioactive factors into the scaffold in order to actively attract cells from the body and guide and stimulate formation of new tissue [44]. Although this approach is commercially and practically much more attractive, the use of bioactive factors still necessitates a pharmaceutical approach to the clinical development.

To overcome this final limitation, Endogenous Tissue Restoration (ETR) has been introduced. In this approach, fully synthetic polymeric scaffolds are implanted, without any additional cells or growth factors [45]. While these implants function as valves or vessels directly upon implantation, they rely on the innate capacity of the human body to gradually replace the implant by functional, body-own tissue. A key advantage of ETR over other regenerative approaches is the pure synthetic composition of the implant, which means that the well-known regulatory framework of medical devices applies. Moreover, there are numerous advantages compared to currently used bioprosthetic valves. First, manufacturing is predictable and scalable for synthetic scaffolds, as biological variation is no longer a factor. Further, biological tissues require storage in a solution, typically glutaraldehyde, while synthetic scaffolds can be sterilized and stored dry, thus omitting a time-consuming rinsing step during implant procedures. And most importantly, it is anticipated that ETR-based valves will avoid degenerative processes, such as thrombosis and calcification, associated with the animal-derived nature of bioprosthetic valves, thereby reducing the need for anticoagulation therapy and improving valve durability. A recent study demonstrated that lower calcification potential of electrospun supramolecular polymer scaffolds in comparison to glutaraldehyde-treated pericardium may be associated with a pro-healing cellular response to the supramolecular polymer versus a pro-inflammatory cellular response to the pericardium [46].

A schematic overview of the ETR process is provided (Fig. 1). Firstly, the device is made using bio-restorative polymer materials and implanted at the intended position in the patient. At this point in time, the functionality is fully carried by the device. Secondly, the device is pervaded with cells that start forming neo-tissue, which gradually takes over functionality. In the final phase, the neo-tissue is strong enough to completely take over functionality, and the remnants of the polymeric devices are gradually absorbed. Clearly, an appropriate balance between tissue restoration and device absorption is essential for ensuring adequate functionality throughout the ETR process.

At the microscopic level, ETR is defined as “the natural process by which patient’s own native cells infiltrate an absorbable implant and trigger a cascade of physiologic events with gradual replacement by functional native tissue” [47]. Upon implantation, the device is infiltrated with inflammatory cells, predominantly macrophages, which release growth factors that attract endothelial cells, (myo)fibroblasts and smooth muscle cells, taking care of neotissue formation. The macrophages also govern absorption of the implanted device, by secreting enzymes and oxygen radicals. Once the implant is absorbed, the inflammatory trigger is resolved and the inflammatory cells disappear, thus leaving a quiescent, natural tissue that has taken over functionality from the original polymeric implant.

An important element in ETR is the creation of a three-dimensional matrix that is sufficiently porous to allow cell infiltration and subsequent ETR, while also providing adequate shape and function to allow the ETR process to resulting in a functional body part. A promising technique for this purpose is electrospinning (Fig. 1). Electrospinning is used to produce medical products such as wound dressings and implant coatings since 1977. From 2000 onwards research has focused on tissue regeneration owing to the ability of electrospun meshes to mimic the microstructure of the extracellular matrix [48]. In the electrospinning technique, a polymer dissolved in an organic solvent or mixture of solvents is dispensed from a needle into an electric field that is applied between the needle and a rotating collector target. The electric charge pulls threads of polymer solution towards the rotating target upon evaporation of the solvent. A non-woven microporous mesh is formed that depending on the geometry of the target can be electrospun into a three-dimensional heart valve shape. Due to its versatility, electrospinning allows tuning of the scaffold microstructure made up by the mesh to enable ETR. Note that other applications such as 3D printing typically lack the resolution for mimicking the extracellular matrix. However, hybrid approaches such as melt electrospinning are in development that may in future allow sufficient resolution in combination with more precise control over the deposited architecture [49].

The most crucial condition for ETR is found in the polymer material requirements. To start with, the material needs to be easily processable to reach the desired porous structure to enable cell integration. The material also needs to be biocompatible to make sure that no local or systemic adverse event will occur during the integration of the implant. And most importantly, the polymeric material needs to ensure an adequate balance between tissue formation and implant absorption. As this balance is different for every application, and even different in different parts of the same device [50-52], it is evident that these properties cannot be obtained with one single material. Therefore, the versatility of supramolecular polymer chemistry has been instrumental in creating a material platform that enables ETR.

Supramolecular polymers

In 1988, Lehn described supramolecular chemistry as “chemistry beyond the molecule” in which well-defined non-covalent interactions determine the (dis)-assembly, conformation, and function of the molecular system [53]. Initially, supramolecular chemistry was focused on the development of well-defined aggregates or supramolecular assemblies in solution as pioneered by Cram, Lehn, and Pedersen, which was honored with a Nobel Prize in 1987. Today, because of the development of directional and strong non-covalent, supramolecular binding units that are synthetically accessible, supramolecular chemistry has grown beyond small aggregates in solution towards real supramolecular materials that are made of one-dimensional supramolecular polymers held together by these reversible, yet strong, non-covalent interactions [54,55]. These supramolecular polymers display not only the material properties that have made polymeric materials so successful, but also unique properties, such as responsiveness and self-healing, properties that most classic covalent

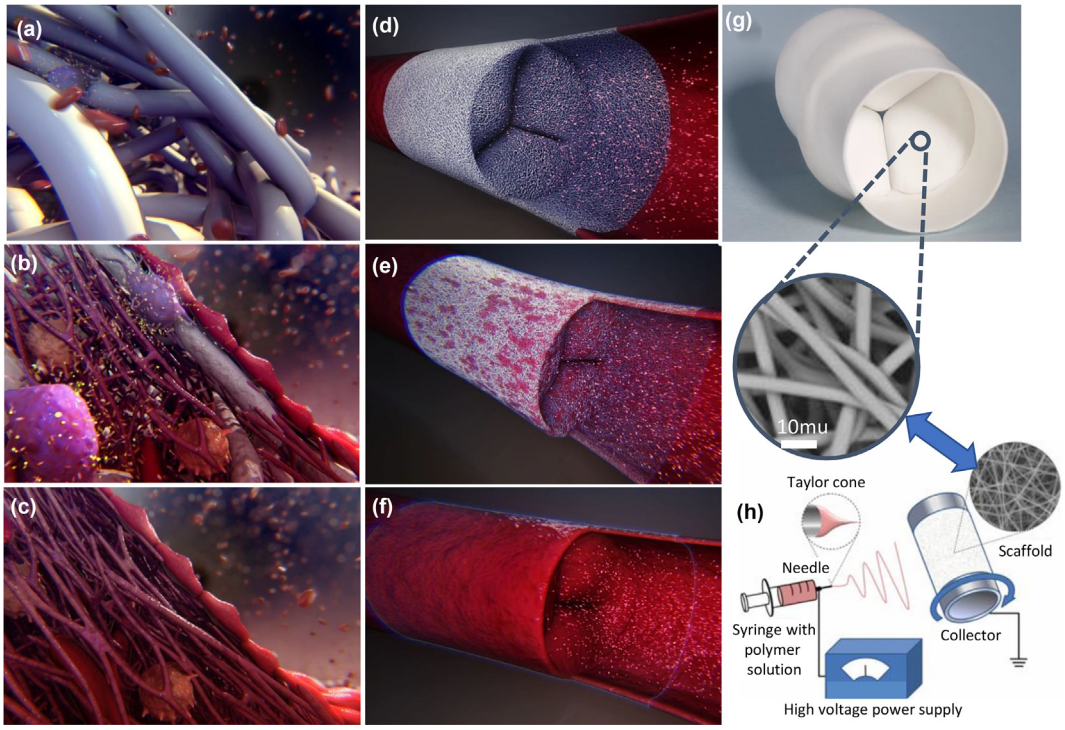


FIGURE 1

Schematic view of endogenous tissue restoration (ETR) process in a valved conduit before (a, d), during (b, e) and after ETR (c, f) at microscopic (a–c) and macroscopic level (d–f). Pulmonary Valved Conduit macroscopic photo (g) with high magnification Scanning Electron Microscopy inset showing microstructure created by electrospinning process (h).

polymers lack [56]. The development of supramolecular biomaterials started with the seminal work of Stupp et al. who designed peptide amphiphiles as supramolecular building blocks to make bioactive, supramolecular hydrogels [57-61]. The amphiphiles are composed of a hydrophobic alkyl tail, a beta-sheet forming region, a charged region and if applicable a bioactive epitope. It has been shown that these peptide amphiphiles form fibrous structures that can be formulated into supramolecular hydrogels. Importantly, co-assembly of so-called filler, *i.e.* non-active, amphiphiles with bioactive amphiphiles resulted in bioactive hydrogels that could be used for an impressive list of regenerative medicine and drug delivery applications.

While directionality and strength of the non-covalent interactions are important when developing applications for supramolecular polymers, also additional requirements must be met. The most challenging of these requirements are synthetic availability, costs, and chemical robustness of the supramolecular unit. The unit that has succeeded in coping with all these challenges, and thereby bringing this field to everyday reality, is the self-complementary quadruple hydrogen bond unit as developed by Sijbesma and Meijer in 1997 [23]. The key to its success was the use of hydrogen-bonding arrays in which cooperative hydrogen-bonding interactions are present. Consequently, their self-complementary quadruple hydrogen bonding units based on 2-ureido-4[1*H*]-pyrimidinones (UPy) display a strong self-association and resulted for the first time in a supramolecular H-bonding unit that was strong, stable and synthetically easily accessible (Fig. 2) [62]. Moreover, this strong self-association allowed for the first time the formation of supramolecular polymers with high degrees of polymerization.

A multitude of low molecular-weight polymers have been functionalized with UPy units in order to benefit from the resulting strong supramolecular interactions between the polymer chains, thereby increasing their apparent molecular weight and consequently improving their material properties while keeping their beneficial processing stays at high temperatures or in polar solvents [63]. Since the sensitivity of hydrogen bonding to temperature, solvent polarity, and concentration, heating or dilution will lead to a drop in binding strength. As a result, the reversible (cross-linking) interactions between the polymers weaken and the apparent molecular weight of the polymers is much lower resulting in a low viscous, tractable materials, that can be processed in solution (*e.g.* electrospinning) or by various thermal processing methods (e.g. extrusion, injection molding and 3D-printing). The status of these supramolecular polymers around 2004 was published in Materials Today [64].

Due to their synthetic flexibility, enhanced processing, material performance, and compatibility with existing biocompatible, and optionally bioabsorbable, polymer technologies, UPy-based supramolecular materials have become a very attractive materials platform to be used for biomedical applications. Especially, their biocompatibility, (tunable) bioabsorption, and strength are of great importance. In the past years supramolecular UPy-based polymers have therefore evolved into a modular material platform in which all necessities have been met to become a very successful material in cardio-vascular devices designed to enable ETR.

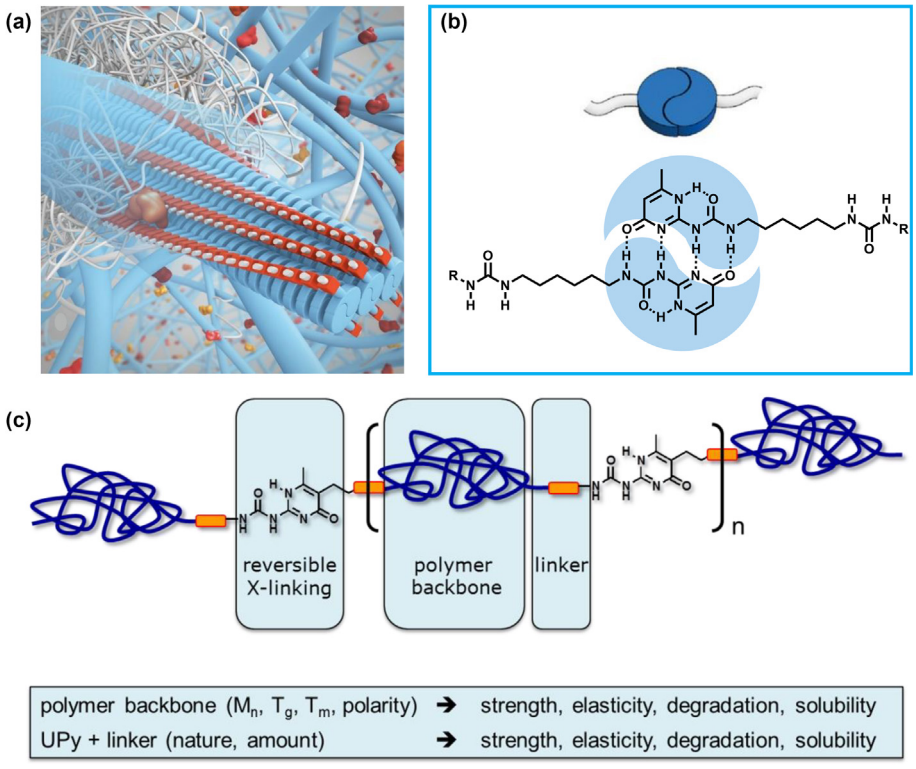


FIGURE 2

Schematic design of UPy polymers for ETR, highlighting their modular nature. Adapted with permission from [57]. A. UPy-units can dimerize and form fibrous structures of 3–4 stacks. These stacks can be biofunctionalized with functional UPy-additives resulting in bioactivated materials. B. The dimerization of two UPy-units. C. The UPy-unit is used as hydrogen bonded reinforcement of the hard phase in a thermoplastic elastomeric polymer structure.

UPy-based polymer platform and ETR

Today, most available biomaterials for biomedical applications are based on aliphatic polyesters such as polycaprolactone (PCL) or derivatives thereof [65]. The mechanical properties of these bioabsorbable materials are mainly determined by their high molecular weights (M_w > 100 kDa), the presence of chemical cross-links or of crystalline domains. The need for high molecular weight polymers to get the desired material properties usually hampers their solution-processing due to the resulting high viscosities. Furthermore, although the crystalline domains are beneficial for the initial high strength of the material, they also have a strong impact on the bioabsorption process. Crystalline domains in aliphatic polyesters absorb very slowly in general and may cause an immunological response [66]. Additionally, the crystalline domains may have a negative impact on the long-term elastic behavior of the material due to their tendency to induce fatigue, which is especially concerning in cardiovascular applications. Another class of available biomaterials is based on polyurethane copolymers [67]. Although, these polyurethanes show the desired elastic behavior, they are often hard to process from solution with solvents that are toxic and teratogenic, like DMF and DMAc, and they are predominantly based on aromatic hard-block units comprising methylene diphenyl diisocyanate (MDI). The aromatic MDI in these polyurethane materials hampers their possible use as bioabsorbable biomedical materials, since MDI is known to result in degradation products that can comprise highly toxic aniline and derivatives thereof [68].

The clear need for absorbable materials for heart valve restoration, which are characterized by high strength, elasticity, durability, and controlled absorption, has led to the development of a new modular platform built on UPy-materials. Besides these critical characteristics, they also need to be easily processable into the porous 3D-structures that constitute the biomedical implants for ETR, such as conduits or heart valves. The first biocompatible and bioabsorbable UPy-based materials addressing these needs have been prepared by Dankers and Meijer in 2005 [69]. Here, low molecular weight telechelic PCL was end-capped with two UPy units which resulted in reasonable material performance. However, they still lack the elasticity and durability needed for cardio-vascular implants. Consequently, in the next generation of UPy-materials, telechelic polymers were chain-extended with UPy-moieties, resulting in copolymers with multiple UPy-units in the polymer chain [21], as depicted in Fig. 2c. The synthetic freedom created by combining telechelic polymers with these bifunctional UPy moieties makes this approach highly modular as the material properties can be largely tuned by the selection of a specific telechelic polymeric building block (see Fig. 2c). The multiple UPy units in the polymer chain form physical cross-links with neighboring UPy-polymers thereby strongly improving the mechanical properties of the resulting materials. This ultimately resulted in a library of flexible and bioabsorbable UPy-materials with tunable strength, durability and bioabsorption profiles [21,70].

These second generation UPy-polymers comprise multiple components (low molecular weight telechelic polymer back-

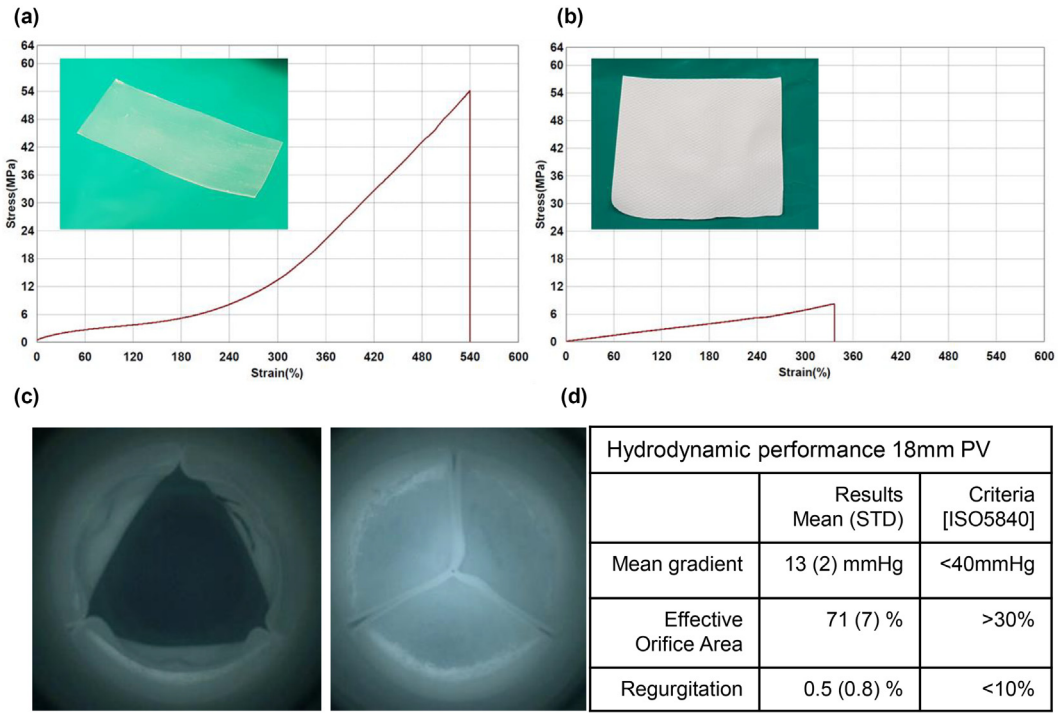
bones, linker molecules, and chain-extending UPy-units) that contribute each to the chemical, physical and biological properties of the resulting materials. The polymer backbone is typically a biocompatible and biodegradable aliphatic polyester or aliphatic polycarbonate with a relatively low molecular weight (<5 kDa). In the resulting supramolecular polymer materials, the hydrogen-bonded UPy-dimers are phase-separate as hard block from the (soft) polymer backbone matrix, which results in a three-dimensional physical network comprising dynamic cross-links [71]. The presence of these reversible cross-links delivers strength and elasticity to the supramolecular materials. Consequently, by changing the composition of the polymer ingredients, a wide range of mechanical properties can be obtained from soft to elastic, and even stiff, while keeping materials that are easily processable, biodegradable and biocompatible [21].

For medical applications it is a prerequisite that the polymers can easily be prepared and processed into porous structures in a robust and repeatable process without introducing elements or residuals that would elicit an adverse response *in vivo*. The method of choice is electrospinning, a solution-based processing technique. In solution, the reversible UPy cross-links can be temporarily removed by using a polar, hydrogen-bonding competitive, co-solvent which will result in a formulation with sufficient low viscosity as needed for electrospinning. Note that these polar solvents are typically not biocompatible. Therefore, standards have been established on how to demonstrate sufficiently low residual solvent concentrations to allow safe use in medical applications [72]. Upon evaporation of the polar solvent, hydrogen bonds are reformed, and the mechanical strength of the polymer is restored. Fig. 3 displays the tensile properties of a typical example of a UPy-polymer as a film (Fig. 3a) and as a porous mesh after electro-spinning (Fig. 3b). Bench data has demonstrated that UPy-based electrospun pulmonary and aortic valves are able to withstand the hemodynamic conditions required per regulatory standards [73].

UPy-based hydrogen bonding polymers have unique properties that can completely fulfill the high demands of a biomedical material to be used in ETR based heart valve repair, due to their ease of preparation, the tunable mechanical and biodegradable properties, the durability, and ease of processing. Feasibility has been demonstrated by the Dankers group, who implanted electrospun PCL-based UPy-polymers in an aortic interposition rat model [74]. Interestingly, the supramolecular scaffold appeared strong enough for leak-free connections to the adjacent aortic ends, no clinical signs of distant thromboembolic effects were observed, and cellularization of the scaffold was already observed four hours after implantation. Moreover, in this study Dankers showed that these scaffolds could be rendered non cell-adhesive by mixing in only 10 w% of a non-fouling UPy modified PEG (polyethylene glycol) supramolecular polymer, thereby illustrating the modularity and power of supramolecular mixing to obtain functional materials [69]. Even more sophisticated examples of supramolecular functionalized polymers can be found in recent work of Dankers, in which was shown that UPy-PCL fibers functionalized with heparin-IL-4 (interleukin) modulate macrophage phenotype and protein secretion [75], whereas, a supramolecular composite of UPy-modified poly(L-lactic acid caprolactone) (PLLCL) and SDF1α derived peptides was shown to attract and stimulate specific leukocyte populations [76]. In a different approach, the supramolecular mix of poly(hexyl carbonate)-UPy polymers with UPy-modified cyclic RGD peptide moieties showed promising results as a bioactive, yet mechanically robust, biomaterial for the treatment of pelvic organ prolapse (POP) [77]. Electrospun meshes comprising this supramolecular mix showed promoted tissue integration and accelerated tissue ingrowth, and reduced scar formation in an incisional hernia model. Moreover, muscle atrophy was prevented, and the inflammatory response was modulated resulting in a delayed degradation process. This example clearly shows the possibilities when an elastic, mechanically tuned, biodegradable polymer is combined with selective biological cues. Fig. 4 provides a timeline overview of the progression of the UPy-based platform and a selection of its applications.

The modular design of the UPy-material platform gives not only flexibility in its mechanical performance, but also allows one to mix and match all components to tune its biodegradation properties. For example, the biodegradation can be tuned by changing the polymeric backbone which indeed can be chosen from a wide range of available low molecular weight biocompatible polymers, all with their own unique biodegradation profiles. *In-vitro* degradation studies using lipase, esterase, or oxidative conditions (H_2O_2/Co(II)Cl_2), have confirmed the biodegradability of chain-extended UPy-polymers comprising polycaprolactone backbones [78]. Most interestingly, this study revealed that, in contrast to regular polycaprolactone, the degradation was not governed by (enzymatic) hydrolysis, but rather by an oxidative pathway, since esterase and lipase had almost no effect on mass loss or MW-reduction, whereas the peroxide-medium displayed a pronounced mass loss in time. It was hypothesized that the bioresorption of these UPy-polymers was resulting from a chemical degradation of the UPy-moieties rather than ester-hydrolysis in the PCL-backbone. More specifically, oxidative degradation is thought to predominantly degrade the urethane and urea linkages by a-proton extraction [62,79]. Clearly, this implies that *in vivo* absorption only takes place when surrounding, active oxygen producing, cells are present. This might safeguard the scaffold for only being absorbed when newly grown tissue has formed. In a recent paper, the biocompatibility of possible low molecular weight oxidative degradation products of the UPy-moiety were investigated in vitro [80]. This study showed that the investigated UPy and isocytosine-derivatives have no effect on cell viability, they do not interfere with several endothelial functions, and are not mutagenic nor immunogenic, even at high concentrations. Thereby strongly indicating that these degradation products are biocompatible.

Moreover, on a more macroscopic level, these supramolecular polymers are known to display self-healing properties, i.e. mechanical damage can be repaired by the material itself [56]. Therefore, initial degradation of polymer chains can be corrected for by the supramolecular polymers themselves thanks to their reversible and dynamic nature and prevent failure due to micro-fractioning. Hence, using the chemical toolbox available to these UPy-polymers, an extensive library of biocompatible supramolecular polymers has been created by SupraPolix with controlled degradation profiles defined by molecular, supramolecular, and biological interactions.



Hydrodynamic performance 18mm PV		
	Results Mean (STD)	Criteria [ISO5840]
Mean gradient	13 (2) mmHg	<40mmHg
Effective Orifice Area	71 (7) %	>30%
Regurgitation	0.5 (0.8) %	<10%

FIGURE 3

(a, b) Tensile properties of a UPy-polymer as a film (a) and as porous mesh after electro-spinning (b). (c) Opening and closing behavior of an 18 mm UPy-polymer Pulmonary Valve (PV) conduit during hydrodynamic testing according to ISO5840. (d) Hydrodynamic test results at normotensive conditions compliant with ISO5840.

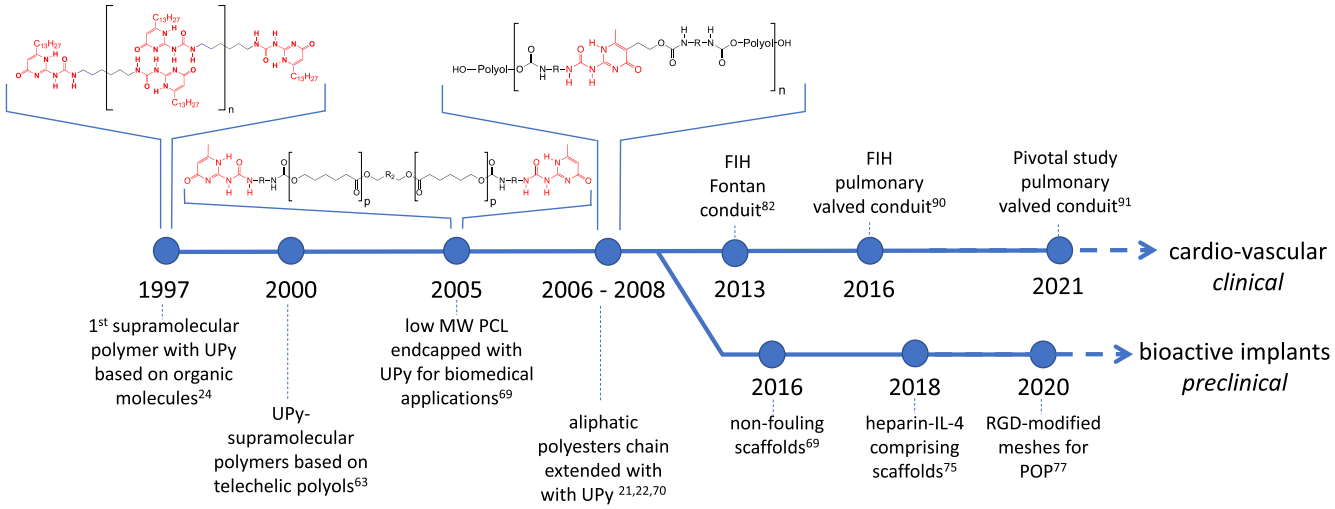


FIGURE 4

Development of UPy-materials towards biomedical applications, starting from supramolecular molecular assemblies in 1997 towards supramolecular polymers comprising biodegradable polyester or polycarbonate polyols in the decennia afterwards, resulting in supramolecular materials that can be electro-spun into scaffolds that combine flexibility with high ultimate tensile strengths at high elongations and the possibility load these biodegradable implants with bioactive cues.

Examples & applications

Supramolecular polymers have been applied with success in several applications in a preclinical setting. Moreover, first clinical applications have been reported. This section summarizes the key results thus far, focusing on large animal models and clinical study results. The technology was used in different application from simple tubes to more complex valve geometries. Examples from preclinical and clinical applications will be used to illustrate how the technology proved to be successful.

Pre-clinical: pulmonary artery

A first demonstration of ETR in a chronic large animal model was reported by Schoen et al [81], in a pulmonary artery interposition model in 9 adult sheep using an electrospun conduit, composed

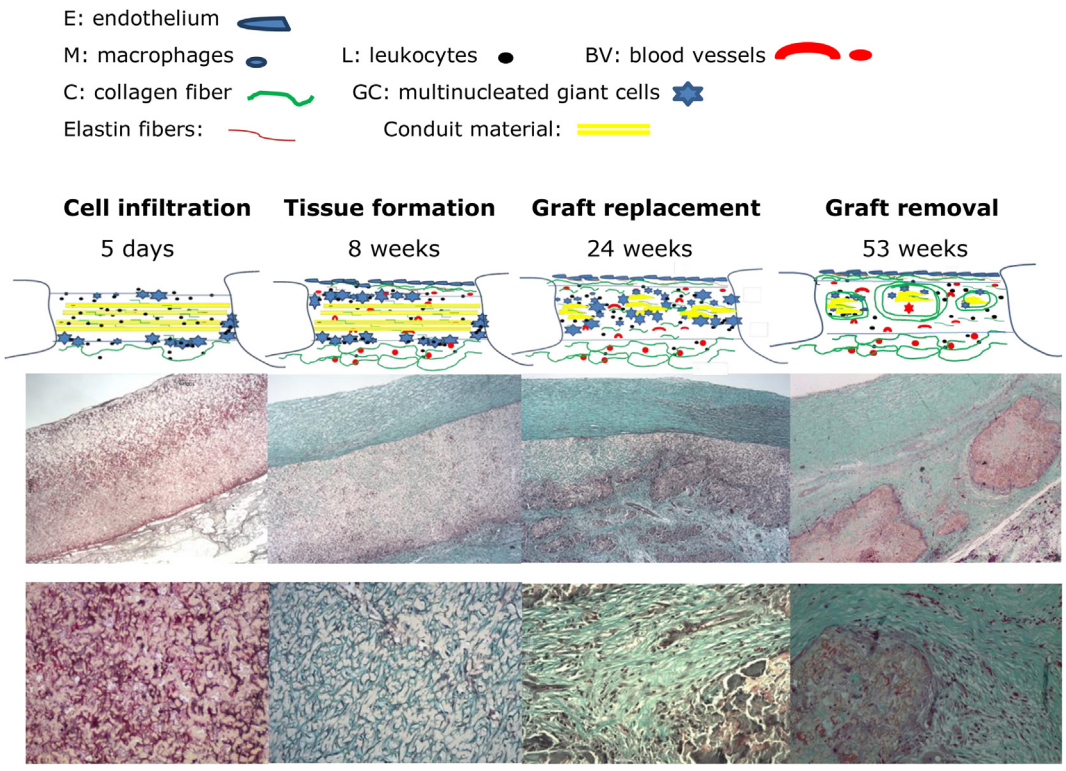


FIGURE 5

Histopathological progression of ETR with time in an ovine pulmonary artery interposition model. Left cell infiltration (red dots) is shown within 5 days after implantation. At 8 weeks, new tissue (blue-green) has formed at the luminal side and within the implant. At 24 weeks the implant starts fragmenting and is progressively replaced with tissue, which continues at 53 weeks. Adapted with permission from Bockeria et al. [82]

of a polycaprolactone-based UPy polymer. Follow-up ranged from 5 days to 1 year. Mechanical testing performed on the explanted grafts showed that no mechanical contribution could be measured from the implanted polymer beyond 11 weeks, and the burst pressure of the newly formed tissue was well above safety requirements for commercial conduits. Histologically, progress

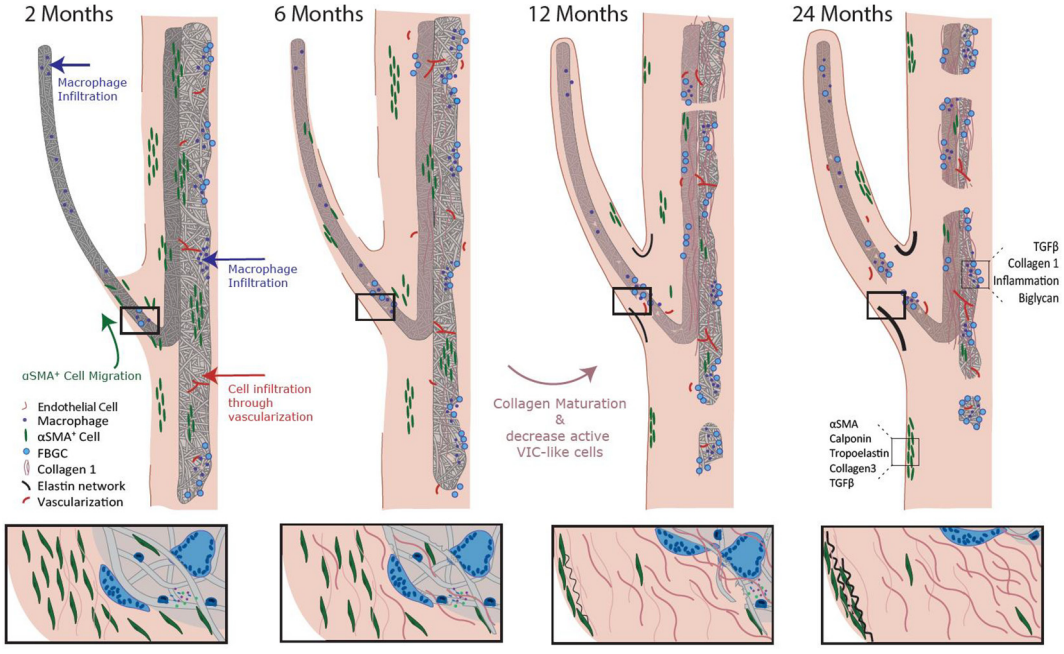


FIGURE 6

Schematic overview of essential processes involved in in-situ heart valve regeneration. (Adapted with permission from De Kort et al. [84]).

absorption of the graft material was accompanied by simultaneous collagen formation, as intended by the ETR concept (Fig. 5).

Preclinical: pulmonary valved (PV) conduit

Pulmonary valved conduits provide or restore pulmonary valve function, thus enabling blood to flow effectively from the right ventricle to the lungs. Children born with a malformation of their Right Ventricular Outflow Tract (RVOT) often require replacement of part of their native pulmonary artery and valve with a PV conduit. A restorative PV conduit may provide significant clinical benefits if the number of reoperations could be reduced. Several studies reported promising performance out to 1 year. Kluin et al. implanted bis-urea-modified polycarbonate (PC-BU) pulmonary in adult sheep (n = 10) up to 1 year with generally good functionality through-out follow-up [51] and Uiterwijk et al. showed similar performance with isotropic (n = 10) and anisotropic (n = 10) electrospun polycarbonate UPy-based PV in adult sheep, also out to 1 year [83].

Longest follow-up thus far has been reported with a supramolecular pulmonary valved conduit developed by Xeltis, demonstrating good functionality out to 2 years in an adult sheep model [47,50,84]. Pulmonary valved conduits were manufactured by electrospinning and were composed of two polymers: the conduit was based on a PCL-UPy while the leaflets were constructed from a polycarbonate based UPy-polymer and evaluated in an ovine model (n = 20). Pressure gradients were favorable through-out follow-up, and no severe regurgitation was observed. Systolic lumen diameter increased with time, while pressure gradient decreased, indicating an increase in compliance. There were no signs of stenosis or aneurysms, while the control group (Hancock, n = 3) showed significant neo-intimal thickening at 6 months follow-up. Detailed histopathological assessment demonstrated that the ETR process is similar throughout the valve, although it happens first in the base of the leaflet, eventually followed by the tip of the leaflet (Fig. 6).

Preclinical: aortic valve

While pulmonary valved conduits mainly address an unmet clinical need in congenital heart valve disease, the incidence of aortic valve diseases in adults is much greater. Ongoing efforts on developing a transcatheter UPy-based restorative aortic valve report good acute performance but also emphasize the importance of iterative design and material optimization [85,86]. One study reports chronic follow-up demonstrating that 1 of 5 tested UPy-based material configurations was superior in showing good functionality out to 1 year as a transapical aortic valve in an adult sheep (Fig. 7) [87].

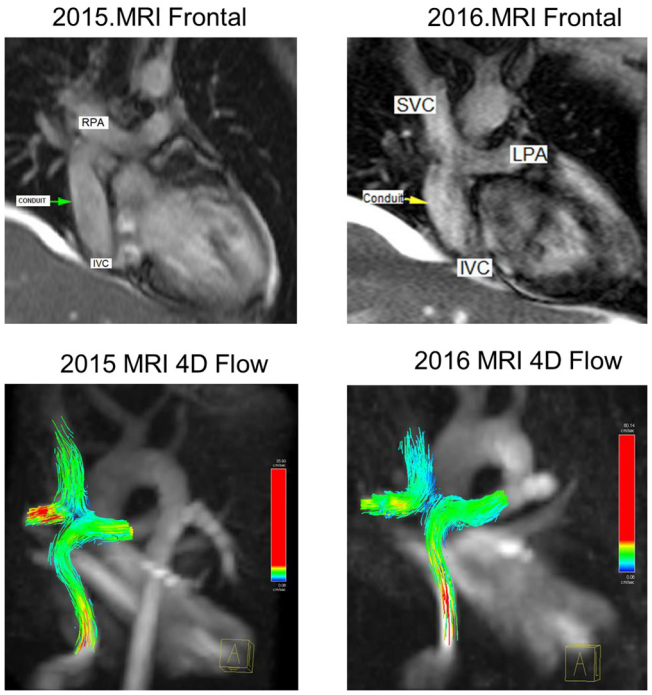


FIGURE 8

One and two-year MRI controls on frontal views showing the EC-TCPC connecting Inferior Vena Cava (IVC) to the left (LPA) and right pulmonary artery (RPA). 4D flow evaluation shows good laminar flow in the vascular graft with no turbulence. SVC: Superior Vena Cava. Adapted with permission from Bockeria et al. [88].

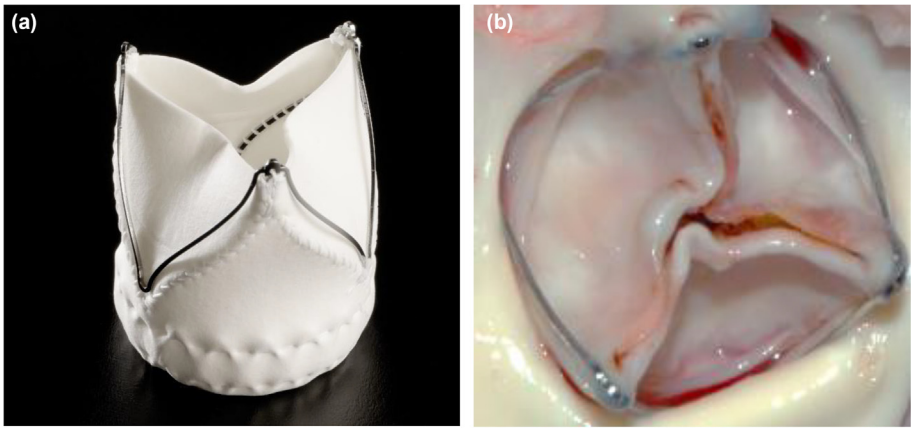


FIGURE 7

Photograph (a) of supramolecular transapical aortic valve and explant picture (b) showing fully intact valve 12 months after implantation in the aortic position in a sheep model. Note that the supramolecular polymer is mounted on a non-degradable nitinol frame to enable transcatheter valve delivery [87].

Clinical: Fontan conduit

Besides various preclinical applications as mentioned above, the first clinical applications of supramolecular polymers have been reported as well. Bockeria et al. performed a world-first clinical feasibility trial, in which five children (aged 4–12 years) received an electrospun PCL-based UPy polymer conduit as part of a Fontan procedure [82,88]. This procedure is performed on children with a congenital heart defect, possessing only a single functional ventricle. All patients received an 18 or 20 mm diameter conduit to create the extracardiac total cavopulmonary connection (EC-TCPC) during the final stage of the Fontan procedure. All procedures were successful and a significant improvement in patients' general conditions were reported at 1 and 2 years, with no (adverse) changes to the grafts compared to early postoperative data [88,89]. Good haemodynamics, and anatomical and functional stability of the graft was demonstrated using MRI (Fig. 8). While the number of patients is still small, this first clinical study provides important data on the initial clinical safety and feasibility of restoring cardiovascular function using a supramolecular polymer-based approach.

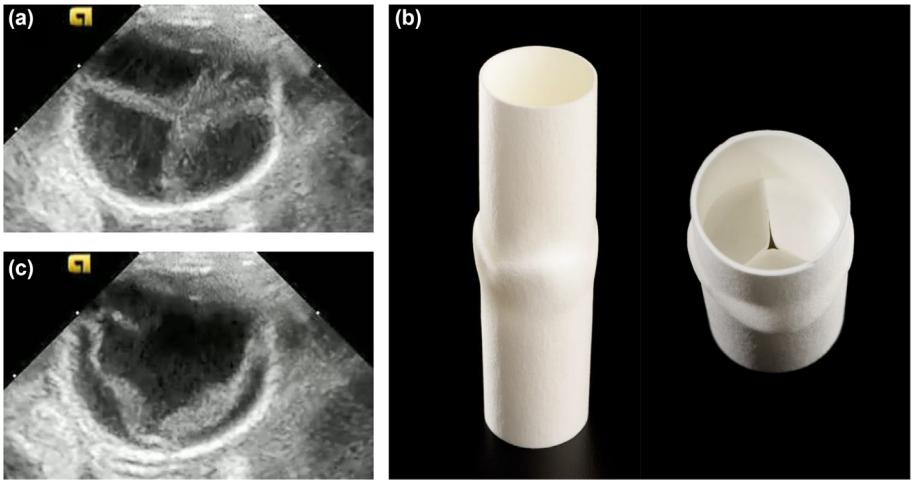


FIGURE 9

Epicardial echocardiogram of the PV conduit after implantation in opened (a) and closed position (b). See supplementary movie 1 for an illustration of the dynamic opening and closing behavior of the PV conduit. (c) Macroscopic view of the PV conduit. Echocardiogram courtesy of Dr. Jaquiss, Children's Medical Center Dallas, Texas.

TABLE 1

Summary of Xeltis Pulmonary Valved Conduit clinical trial patient demographics and 12-month outcomes [94].

Patient demographics	**Xplore-1**	**Xplore-2**
Number of patients	12	6
Median age & (range)	5 years (range 2–12)	5 years (3–9)
Median weight & (range)	17 kg (10–43)	21 kg (14–29)
Individual diagnoses	Tetralogy of Fallot (4) Pulmonary Atresia with VSD (4) Common Arterial Trunk (3) Transposition of Great Arteries with VSD and pulmonary stenosis (1)	Tetralogy of Fallot (1) Pulmonary Atresia with VSD (1) Common Arterial Trunk (1) Transposition of Great Arteries with VSD and pulmonary stenosis (1) Ross procedure (2)
Xeltis PV implant diameter	16 mm (5) 18 mm (7)	18 mm (6)
12-month outcomes	**Xplore-1**	**Xplore-2**
No death	100%	100%
No reoperation	100%	83%[1]
No reintervention	100%	100%
No endocarditis	100%	100%
No aneurysm	100%	100%
No severe valve leakage	58%	100%

[1] One patient was diagnosed with underlying tuberous sclerosis with rabdhomyoma which resulted in valve replacement.

Clinical: pulmonary valved conduit

In addition to the extracardiac conduit, a pulmonary valved conduit has been progressed into clinical evaluation. The company Xeltis currently has 2 clinical feasibility trials ongoing with their pulmonary valved conduit [90,91]. In the first trial twelve patients, with an age ranging from 2 to 12 years, were enrolled in a prospective, non-randomized, open-label study to assess the safety of a supramolecular pulmonary valved conduit, in subjects undergoing RVOT reconstruction. Conduit diameters were either 16 or 18 mm, these electrospun implants were composed of two polymers: the conduit was based on a PCL-UPy while the leaflets were constructed from a polycarbonate based UPy-polymer. The investigators reported 100% technical success without mortality, reoperation, or reintervention until two years after surgery for all twelve patients (Fig. 9) [92,93].

Based on learnings from this study a further improved version of the PV was introduced in a second feasibility trial enrolling 6 more patients in 4 centers in a US-based Early Feasibility Study (EFS). At 1-year follow-up the second study showed similarly positive safety data with superior performance on valve regurgitation [94]. Key study parameters and 12-months outcomes are summarized in Table 1. Based on results in these 2 trials, Xeltis has been granted FDA approval to expand the EFS study into a pivotal clinical trial aimed at obtaining regulatory approval for their supramolecular pulmonary valved conduit.

Conclusions

Almost 30 years after the landmark paper of Langer & Vacanti on the concept of tissue engineering to overcome xenogeneic issues by recreating tissue using autologous cells, the first clinical applications to functionally restore pulmonary arteries and heart valves are successfully performed. The endogenous approach makes use of a new biomaterials platform in which designed supramolecular interactions are taking care of the unique combination of properties required to be successful. These supramolecular polymer materials offer a versatile toolset for enabling ETR (Endogenous Tissue Restoration), resulting in functional restoration of natural tissues in a variety of (cardiovascular) applications. In addition to various reports on chronic studies in large animal models, three world-first clinical studies were reported as well, demonstrating the potential of supramolecular technology in bringing ETR to patients.

Future perspective

With first human implants of supramolecular conduits performed in 2013 [82], we are nearing a decade of clinical application of supramolecular cardiovascular implants, suggesting that these materials are safe and will be gradually replaced by functional natural tissue. While further clinical studies with more patients and longer follow-up times are still needed to fully assess the potential, we believe that this is only the beginning in using supramolecular materials as biomaterials in ETR as the synthetic conduits and grafts have some of the dynamic molecular properties of natural tissue and therefore are coming closer to the life-like material so desperately required to combine synthetic and natural materials. As current clinical experience is still limited to low pressure applications, an important next step is to make the clinical translation for high pressure heart valves and blood vessels. With worldwide 17.8 million deaths every year from cardiovascular disease – 31% of all global deaths – there is huge upside potential if supramolecular materials and ETR continue to deliver on their promise [95].

Declaration of Competing Interest

The manuscript is written by people of the Eindhoven University of Technology, Xeltis and Suprapolix. Both Xeltis and Suprapolix have an interest to bring the devices presented to the clinic and have a financial interest. Hence also the people involved as they are either members of staff (Mes, Serrro and Bauer) or c-founders (Bosman and Cox) for Suprapolix and Xeltis, respectively. Dankers started another company making use of the materials of Suprapolix and has an indirect interest. Meijer is co-founder of Suprapolix and a consultant at Xeltis and hence has a financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mattod.2021.12.003.

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