Trends in Biotechnology

CellPress

Review

Bioengineering artificial blood vessels from natural materials

Matthew J. Moore [1,2,@], Richard P. Tan, [1,2] Nianji Yang, [1,2] Jelena Rnjak-Kovacina [3,@] and Steven G. Wise [1,2,4,*,@]

Bioengineering an effective, small diameter (<6 mm) artificial vascular graft for use in bypass surgery when autologous grafts are unavailable remains a persistent challenge. Commercially available grafts are typically made from plastics, which have high strength but lack elasticity and present a foreign surface that triggers undesirable biological responses. Tissue engineered grafts, leveraging decellularized animal vessels or derived *de novo* from long-term cell culture, have dominated recent research, but failed to meet clinical expectations. More effective constructs that are readily translatable are urgently needed. Recent advances in natural materials have made the production of robust acellular conduits feasible and their use increasingly attractive. Here, we identify a subset of natural materials with potential to generate durable, small diameter vascular grafts.

Highlights

Commercial synthetic vascular grafts are made from strong, stiff, chemically inert polymers. The properties of these materials are unsuited to the human vasculature, leading them to rapidly fail in low diameter (<6 mm) applications.

Natural biomaterials have inherent advantages that make them good candidates for incorporation into the next generation of grafts, including enhanced biological signaling and tunable mechanical properties.

Classical barriers to the wider adoption of natural materials include inadequate strength for vascular applications, rapid degradation, overly complex designs, and difficulty in translation out of a lab setting.

Advances in manufacturing, material source, and postprocessing have renewed the promise of natural materials, including silk, collagen, elastin, chitosan, and cellulose, to meet the challenges of developing effective artificial small diameter grafts.

The unmet need for small diameter artificial grafts

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, affecting approximately 523 million people [1]. The most common cause of CVD is atherosclerosis, the buildup of fatty plaques within arterial walls, which leads to blockages and reduced blood flow to downstream tissues [2]. An established treatment is surgery to insert a **vascular graft** (see Glossary) to bypass blood around the blockage. In the US alone, around 450 000 patients undergo a **vascular bypass** procedure each year [3,4]. Vascular grafts for this purpose are preferentially harvested from the patient (**autologous grafts**), commonly the saphenous vein from the leg or internal mammary artery from the chest wall. Arteries are highly effective in the long-term but are very limited in supply and length. In contrast, veins used for arterial bypass have mismatched mechanical properties, are predisposed to accelerated atherosclerosis, and ~50% fail within 10 years [5]. In around ~30% of cases, no autologous vessels are available due to disease or prior use, leaving surgeons reliant on artificial alternatives [6,7].

The two most common commercially available **artificial grafts** are made from common plastics, expanded polytetrafluoroethylene (ePTFE; known as Gore-Tex) or polyethylene terephthalate (PET; known as Dacron). Both are incredibly strong, chemically inert, can be manufactured at scale in a range of diameters and lengths, and stored for off-the-shelf use. However, these **synthetic materials** fail to replicate key elements of the native vasculature (Figure 1A) and neglect to regulate important biological mechanisms that determine graft fate. Commercial polymeric grafts are much stiffer than native vessels, have rough surfaces, and are highly hydrophobic, leading them to interact poorly with vascular cells and to trigger activation of the blood clotting cascade [8–10]. Accordingly, there are established failure modes that are essential to understand to facilitate the design of improved materials (Figure 1B).

[1]School of Medical Sciences, Faculty of Health and Medicine, University of Sydney, NSW 2006, Australia
[2]Charles Perkins Centre, University of Sydney, NSW 2006, Australia
[3]Graduate School of Biomedical Engineering, UNSW Australia, Sydney, NSW 2052, Australia
[4]The University of Sydney Nano Institute, University of Sydney, NSW 2006, Australia

*Correspondence: steven.wise@sydney.edu.au (S.G. Wise).
@Twitter: @bioeng_research (M.J. Moore), @JelenaRnjak (J. Rnjak-Kovacina), and @docswise (S.G. Wise).

The lack of an intact endothelium is central to the main failure modes of artificial grafts. A healthy endothelium is coated in a layer of glycoproteins and proteoglycans, collectively known as the glycocalyx, which resists thrombus formation by preventing platelets and red blood cells adhering to endothelial cells [11]. In contrast, synthetic materials that poorly recover the protective endothelial cell layer are prone to increased rates of blood clotting, the dominant mode of acute failure in artificial grafts. Their rough, hydrophobic surfaces generally cause higher blood cell interactions and protein adsorption, driving accelerated clot formation [12]. The lack of an endothelium also permits smooth muscle cell (SMC) migration to (and over-proliferation in) the intima, in a process called **neointimal hyperplasia**, the major cause of mid-to-late graft failure. Platelets and proinflammatory immune cells adhere to the graft surface and become activated, releasing growth factors and inflammatory cytokines that upregulate SMC proliferation [13]. In particular, macrophages are gaining recognition as master regulators of local inflammation [14] and their interactions with biomaterials and polarization state are increasingly important.

The ideal artificial vascular graft needs to satisfy several competing criteria (Box 1). Mechanical properties need to be tuned to combine high strength with elasticity that closely matches the native vasculature. The most promising materials will encourage endothelial cell attachment and growth, while also having high blood compatibility until **endothelialization** is complete. Materials that provide direct signaling to SMCs to favor a contractile phenotype are desirable, in addition to those that can regulate the local inflammatory response. Finally, capacity for scaled production, sterilization, and storage is preferred, making them compatible with existing clinical practice. **Natural materials** in general are more bioactive than synthetic polymers and a subset have favorable properties for vascular applications when manufactured appropriately [15]. Advances in the isolation, purification, and manufacturing processes of natural biomaterials have made the production of robust acellular conduits feasible and increasingly attractive. This review outlines the challenges for developing an effective artificial vascular graft, with a focus on recent advances that warrant a renewed focus on natural materials.

Revisiting natural materials for artificial blood vessels

The next generation of artificial vascular grafts aim to satisfy multiple design criteria, including finely tuned biological cues and mechanical properties that regulate blood compatibility and vascular cell growth (Figure 1C). A subset of natural materials have the potential to meet these requirements in light of improvements to scalable production of the source materials, compatible manufacturing techniques, and promising preclinical data. These include classical components of the mammalian **extracellular matrix (ECM)**, such as collagen and elastin, where recent advances offer renewed promise, and non-mammalian macromolecules, including silk, cellulose, and chitosan, which are emerging candidates (see Table 1 for a summary of properties).

However, the further development of naturally derived graft replacements has been limited by barriers traditionally linked to these sources, including lack of mechanical strength, rapid degradation, overly complex designs, and difficulty in translation. Recent innovations in these areas warrant a renewed examination of the potential of natural materials for clinical vascular applications (Figure 2, Key figure).

Appropriate mechanical properties for clinical vascular applications

Targeting **burst pressures** well in excess of normal human physiological conditions (>1000 mmHg) and high **tensile strength** (>1 MPa) combined with appropriate **compliance** (10–20%/100 mmHg) has meant natural materials have largely been considered to be too weak alone [16–19]. There has been a reliance on hybrid materials, combining with synthetic polymers of greater strength to achieve these benchmarks (discussed in detail later). However, enhancing

Glossary

Artificial graft: vascular graft made from either natural or synthetic materials.
Autologous graft: a vascular graft harvested from a patient's own blood vessels.
Burst pressure: internal pressure required to cause rupture of a vascular graft or blood vessel.
Compliance: the change in arterial volume for a given change in blood pressure.
Electrospinning: a manufacturing technique that applies high voltages to polymer solutions to produce fibers on nano- and micrometer scale.
Endothelialization: formation of an endothelial cell monolayer over the inner surface of an artificial or native blood vessel.
Extracellular matrix (ECM): a complex three-dimensional network made of macromolecules secreted by cells, such as collagen, elastin, and proteoglycans, that provides the structural support and biochemical cues essential for proper cell function.
Natural material: biomaterial derived from plant or animal sources, including proteins, polysaccharides, and proteoglycans.
Neointimal hyperplasia: the hyperproliferation of smooth muscle cells and fibroblasts within the intima of a blood vessel wall. Often occurs at anastomotic sites and progressively occludes the flow of blood.
Scaffold: a structural template that defines the three-dimensional geometry and architecture of the replacement tissue or organ.
Synthetic materials: biomaterials produced from polymers derived by chemical synthesis.
Tensile strength: ability of a material to withstand stretching forces.
Vascular bypass: surgical procedure that redirects blood flow around an occluded segment of blood vessel.
Vascular graft: conduit or tube that functions as a prosthetic blood vessel to redirect blood flow around a blockage or replace damaged blood vessels.

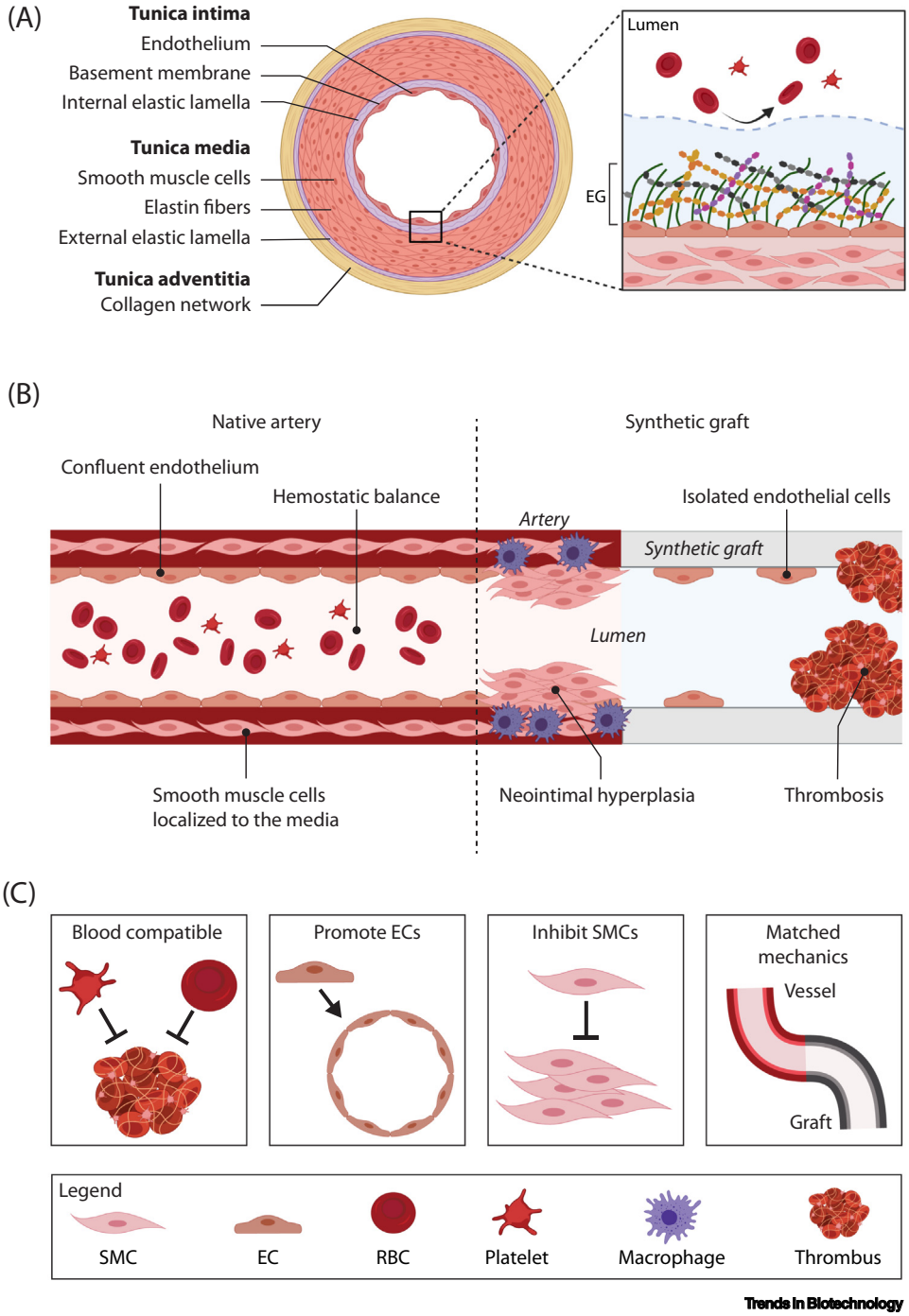


Figure 1. Vascular graft failure modes and design criteria. (A) General structure of an artery. (B) Factors contributing to the failure of synthetic vascular grafts. The delayed formation of an endothelium leaves the hydrophobic graft surface exposed. Blood plasma proteins rapidly adsorb to the exposed graft, followed by platelet adhesion and activation, which results in the formation of a platelet-rich thrombus. The biologically incompatible graft material provokes an immune response, resulting in macrophage infiltration and expression of inflammatory cytokines that drive smooth muscle cells to over-proliferate, developing neointimal hyperplasia. (C) Criteria for the rational design of artificial blood vessels. Image not to scale. Abbreviations: EC, endothelial cell; EG, endothelial glycocalyx; RBC, red blood cell; SMC, smooth muscle cell.

Box 1. Vascular graft design criteria and constraints

Following many attempts to manufacture an artificial blood vessel, a standardized set of performance criteria have been developed. Many of these criteria are outlined in ISO 7198:2017 'Cardiovascular implants and extracorporeal systems – tubular vascular grafts and vascular patches'. These criteria are strongly aligned to counter the dominant failure modes of artificial blood vessels.

The challenge in developing the next generation of grafts is that it is a complex, multifaceted problem combining elements of mechanical engineering, vascular biology, and immunoregulation. Beginning with mechanical considerations, it is generally accepted that close mimicry of the human internal mammary artery (the best performing bypass conduit) is the goal (Table I). The core paradox is achieving high strength while retaining compliance, a problematic challenge for traditional polymer systems. While the systolic blood pressure of a severely hypertensive patient could be as high as ~180 mmHg, native vessels have burst pressures up to 3000 mmHg. The bioengineering consensus is that grafts with a burst pressure >1000 mmHg are desirable, in combination with compliance that is as close a match to the native tissue as possible.

Further, developing grafts with appropriate vascular biology also has multiple considerations. Corresponding to the known failure modes (Figure 1), implanted materials will optimally have high blood compatibility, minimizing immediate failure from blood clotting. In the mid-term the most blood-compatible surface is in intact endothelial cell layer; implants that rapidly recover a functional endothelium perform better. Lastly, control of SMC over-proliferation needs to be considered, either via regulation of the immune response or direct signaling to these cells.

Overall, these competing elements need to be brought together in a manner considerate of regulatory requirements and compatible with scaled manufacture and sterilization in order to be appropriately translated. To date, this goal remains elusive.

Table I. Mechanical properties of human and artificial blood vessels

Mechanical property	Human artery[a]	Artificial blood vessel benchmarking	Refs
Burst pressure (mmHg)	3196 ± 1264 4225 ± 1368 2031 ± 872[b]	>1000	[17,18]
Compliance (%/100 mmHg)	11.5 ± 3.9 16.3 ± 12.3[c]	10–20	[17,73]
Strength (MPa)	4.3 ± 1.8	>1	[19]
Suture retention (N)	1.35 ± 0.49 1.96 ± 1.17	>1	[17,18]

[a]Left internal mammary artery harvested from unused segments during coronary artery bypass surgery.
[b]Vessel defects present.
[c]Calculated from values in [73] using the compliance equation in [17].

the physical properties of natural polymers so that they can be used alone is increasingly feasible due both to sustained improvements in manufacturing and processing and the identification of new material sources. At the forefront has been an increase in the use of **electrospinning** as a technique to generate naturally derived grafts. Electrospinning uses high voltage applied to materials dissolved in volatile solvents to generate dry fibers that can be collected to form conduits. Originally adapted from the textile industry using rudimentary equipment, widespread use has seen significant improvements in hardware, reproducibility, and control of architectural features. It facilitates fine control of fiber size and deposition and allows for co-blending of materials. New approaches for simultaneous spinning of multiple components [20], formation of hollow shell fibers [21], and derivative techniques such as gel spinning [22] have also been developed to add further value to this approach.

Purified *Bombyx mori* (silkworm) silk fibroin is a stand-out as a natural material that can be engineered to have appropriate mechanical properties as single-component graft. Silk fibroin can be electrospun from either an aqueous solution or dissolved in an organic solvent [23]. Electrospun silk fibroin grafts spun from aqueous solution and crosslinked with water vapor

Table 1. Advantages and disadvantages of natural materials

Material	Source	Type	Advantages	Disadvantages	Refs
Collagen	Extracted from bovine or porcine tissue, or *Sarcophyton* corals	Fibrous protein	Abundant supply available Compatible with established prefabrication techniques, such as electrospinning Well characterized biomaterial	Isolation from complex tissue disorganizes the structure responsible for *in vivo* strength. Binds platelets which trigger a prothrombotic cascade.	[27,54]
Elastin	Extracted from bovine or porcine tissue, or recombinant production	Fibrous protein	Potentially reduces neointimal hyperplasia by limiting SMC proliferation and migration Non-thrombogenic	Insufficient strength alone for vascular applications, requires blending. Hard to source in large quantities.	[29,37,52,53]
Silk fibroin	Various species of spider (e.g., *Nephila edulis*) and silkworms (e.g., *Bombyx mori*)	Fibrous protein	Tunable mechanical properties Well tolerated *in vivo* Low thrombogenicity Controlled degradation	Heterogenous supply from animal sources. Limited bioactivity, no direct signaling to SMCs.	[8,22,26,39]
Bacterial cellulose	Produced by bacteria (e.g., *Komagataeibacter xylinus*)	Polysaccharide	High water content enhances tissue integration and blood compatibility	Incompatible with traditional prefabrication techniques.	[40,74,75]
Chitosan	Derived from chitin, a component of arthropod exoskeletons	Polysaccharide	Antimicrobial activity Antithrombotic Structurally similar to glycosaminoglycans in the endothelial glycocalyx	Brittle in pure form. Usually requires blending with additional polymer.	[33,34,76]

achieved an ultimate tensile strength of 1 MPa and burst pressure of ~850 mmHg, approaching values for native rat aorta (2.4 MPa and ~1900 mmHg, respectively). These grafts were also more than seven times more elastic than ePTFE, with a Young's modulus of 4.2 MPa, demonstrating a combination of strength with a degree of elasticity [8]. Further, spinning the same amount of silk from an organic solvent led to conduits with increased porosity that were significantly stiffer (5.44 MPa) and stronger (1.22 MPa), leading to a burst pressure that more closely matched native rat tissue (1441 mmHg) (Figure 3A) [23]. Bi-layered silk conduits generated using a combination approach of freeze-drying molded silk, before augmenting with gel-spun fibers, showed burst pressure results similar to aqueous electrospun silk (827 mmHg), but with greater elasticity (0.31 MPa), demonstrating that manufacturing approaches can drive modifications to the final mechanical properties using the same material [24]. Another study of note used gelatin alone to derive a series of robust electrospun conduits using a modified technique to deposit fibers orientated at 45° to the graft wall. After crosslinking, these grafts were relatively elastic (0.1–1.2 MPa) though weaker than silk (0.1–0.8 MPa), suggesting that continued evolution of novel production methods could see natural materials produced as stand-alone grafts [25].

A further area of development for natural materials that could impact mechanical properties is the identification of new material sources. In the case of silk, the widespread use of *B. mori* silk is being augmented by a growing enthusiasm for spider-sourced material. Woven spider silk grafts exhibited similar compliance compared with the internal mammary artery (12.1% versus 11.5%, respectively), albeit with a lower burst pressure (675 mmHg versus 3196 mmHg) [26]. Similarly, as an alternative to mammalian sources, collagen derived from soft *Sarcophyton* corals is hyper-elastic and viscoelastic [27]. A vascular graft produced by wrapping these fibers achieved compliance (4.8 ± 0.99%/100 mmHg) comparable with coronary arteries (Figure 3B) [28]. Finally, elastin-like recombinamers (ELRs), artificial polymers based on the VPGVG polypeptide sequence that is highly prevalent in natural elastin, offer the opportunity for improved control of mechanical properties. Grafts cast from ELRs demonstrated a burst pressure of 436 mmHg and a compliance comparable with native sheep carotid artery (9.6% versus 12–14%/100 mmHg)

Key figure

Bioengineering artificial blood vessels from natural materials

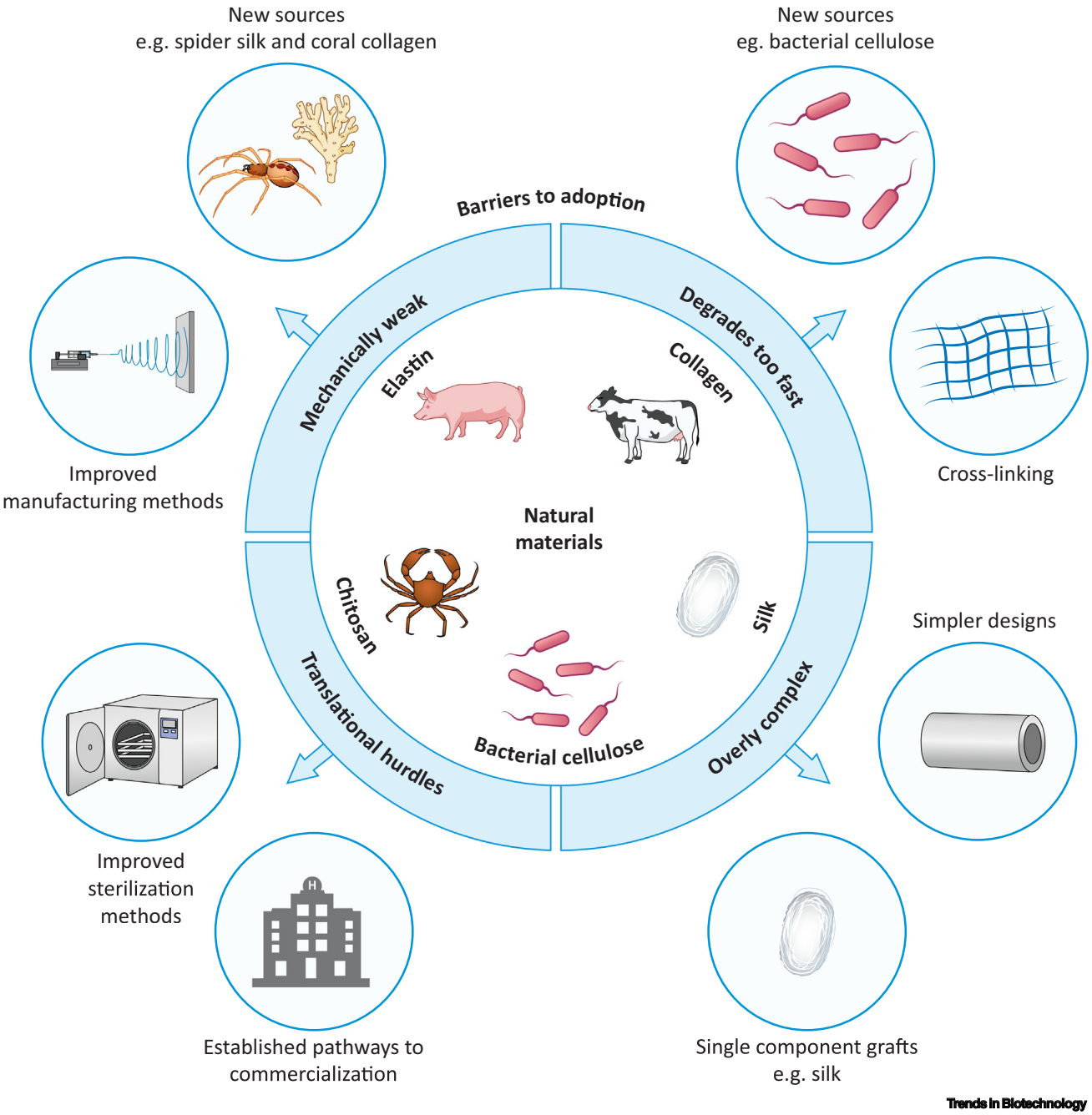
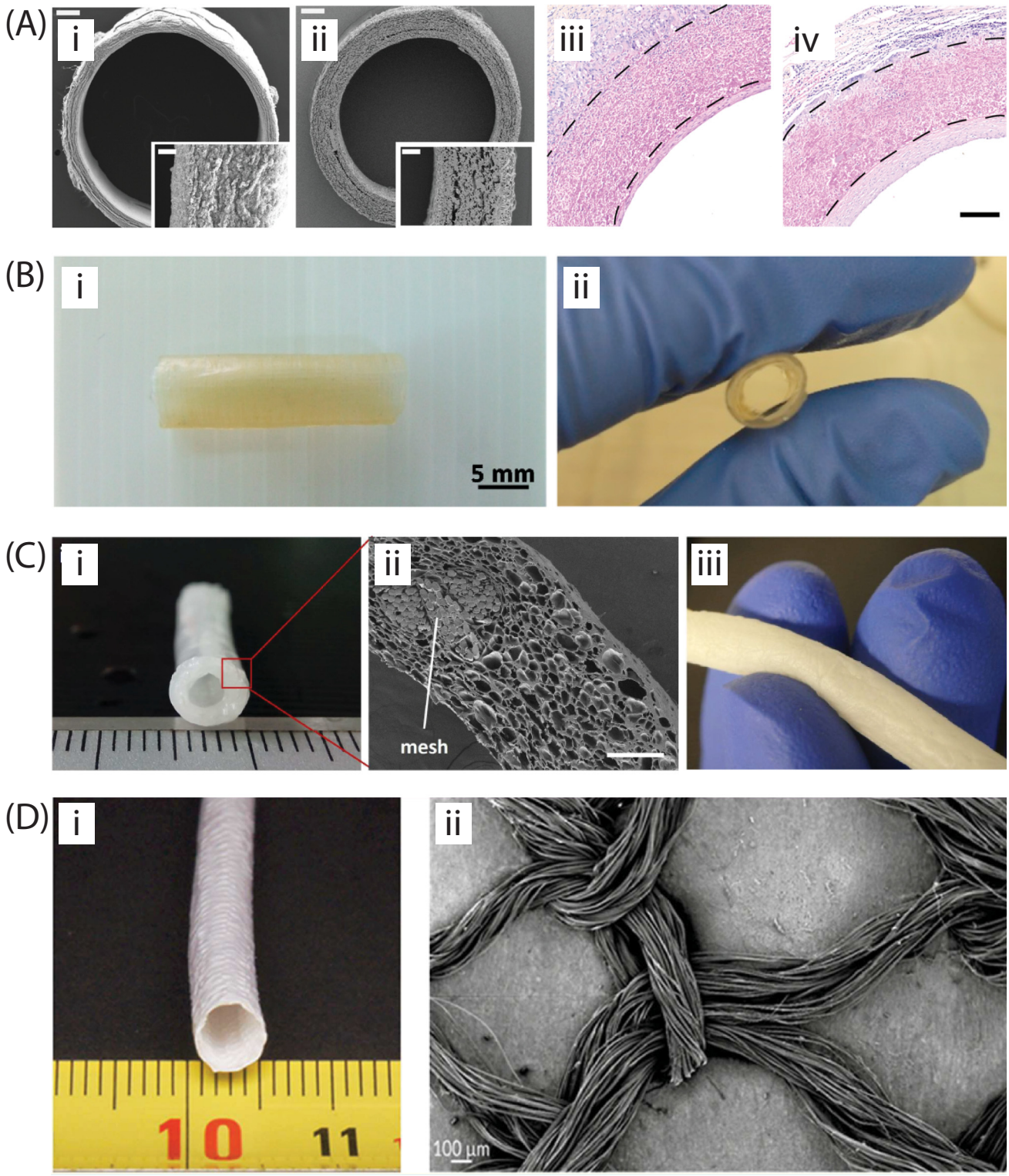


Figure 2. A subset of natural materials have favorable mechanical or biological properties that make their incorporation into artificial vascular grafts desirable. Traditional barriers, including poor strength, rapid degradation, overly complex design, and lack of translation have limited the wider adoption of natural materials. Innovations in manufacturing, material sources, postprocessing strategies, simplified designs, and breakthroughs in commercialization warrant revisiting these materials.



Trends in Biotechnology

(See figure legend at the bottom of the next page.)

(Figure 3C) [29]. With further development, these sources could enhance the capacity for strong, elastic conduits made solely of natural materials.

Controlled degradation and remodeling

A key characteristic of artificial vascular grafts is that their structural integrity is adequate to support arterial pressures without rupturing or forming aneurysms. This requires that any degradation is balanced by new tissue formation, replacing lost material with native proteins. This is especially challenging as degradation and remodeling vary significantly amongst patient cohorts, with older and sicker patients having reduced capacity compared with younger groups [30]. Natural materials are subject to more accelerated and uncontrolled degradation rates than synthetic polymers, which has hindered their adoption as vascular grafts. Recent advances using natural materials have focused on slowing graft degradation through the tuning of physical properties or selection of materials resistant to degradation in humans.

As a benchmark, the most widely used synthetic polymer in vascular graft research, poly (caprolactone) (PCL) grafts, typically degrade by ~70–80% of their original weight after 18 months implantation in an abdominal aorta rat model. In these grafts, calcification and stenosis are the two major remodeling events in the graft lumen, occurring as early as 1.5 months [31,32]. The addition of chitosan in PCL/chitosan grafts, evaluated in sheep carotid models, showed improved remodeling resulting in a lumen fully covered with endothelium, organized contractile SMC, and deposition of collagen and elastin structured similarly to native sheep arteries. However, after the 6-month evaluation period, only 9.1 ± 5.4% of original PCL/chitosan grafts remained, indicating the chitosan addition greatly accelerated degradation [33]. Further studies show that chitosan degradation is highly tunable through the degree of acetylation or crosslinking of its glucosamine subunits with increased acetylation or crosslinking yielding more lasting grafts [34,35]. Similar approaches utilizing blending and structuring of only natural materials have shown that simply mimicking native vessel architecture helps to extend graft longevity. Tri-layered grafts made of elastin–collagen–collagen show no aneurysm formation and no degradation up to 4 weeks [36]. Similarly, collagen/elastin degradation resistance can be tailored and improved through alterations in the crosslinking process with chemical crosslinkers 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC) and glutaraldehyde [37].

In the absence of blends, stand-alone natural materials such as silk can be manipulated through various processing parameters to improve degradation. Silk degradation can be tailored through its fabrication methods, ranging from hours to years. Silk grafts prepared from the organic solvent hexafluoroisopropanol (HFIP) showed no degradation up to 1 year, while water-dissolved silk grafts fully degraded within 6 months [38]. Silk degradation can be further delayed by higher degrees of crystalline β-sheet. Varying the molecular weight of silk alters its final pore architecture, with higher molecular weights corresponding to higher porosity. Higher porosity grafts were associated with faster cell colonization and infiltration, leading to more extensive ECM deposition [22]. Addressing the mechanical and controlled remodeling requirements, a tri-layered graft constructed from inner and outer layers of electrospun silk, sandwiching an intermediate layer

Figure 3. Vascular grafts manufactured from natural biomaterials. (A) Scanning electron micrographs (SEM) of silk grafts electrospun using (i) water, and (ii) hexafluoroisopropanol (HFIP) solvents (scale bar = 200 μm, inset scale bar = 50 μm). Hematoxylin and eosin staining of HFIP graft cross-sections in a rat abdominal aorta model show minimal neointimal hyperplasia at (iii) 3 weeks, and (iv) 24 weeks (dotted black lines indicate the graft wall, scale bar = 200 μm). (B) (i, ii) Graft constructed from collagen derived from *Sarcophyton* coral and alginate. (C) (i) Macro view of ElastinGraft device. Scale in mm. (ii) SEM of (i) showing the inner polyvinylidene fluoride (PVDF) reinforcing textile mesh surrounded by macroporous click-elastin like recombinamer (ELR) graft. (iii) Graft from (i) coated in electrospun polycaprolactone (PCL) sheath. (D) (i) SilkGraft device with 5 mm inner diameter. Scale in mm. (ii) SEM showing the braided textile layer (foreground) coupled to electrospun layer (background). Images adapted, with permission, from [23] (A), [28] (B), [29] (C), and [39] (D).

of braided silk fibers, was developed (Figure 3D) [39]. The electrospun layers were designed to enhance cellular infiltration, whilst the braided layer provided mechanical strength. The proliferation and metabolism of endothelial, smooth muscle, and fibroblast cells seeded onto the graft exceeded those on tissue culture plate. Further, the graft showed no relevant hemolytic activity or complement activation. After 1 month in a sheep carotid model, the graft remained free from obstruction and showed signs of positive tissue remodeling.

An innovative solution to the problem of rapid degradation may come from new natural materials that are not readily broken down in mammalian systems. Bacterial cellulose is a high molecular weight polysaccharide produced by microorganisms, principally *Komagataeibacter xylinus*. It is produced extracellularly in the form of nanofibers that intertwine and coalesce into a highly hydrated three-dimensional fibrillar network of pure cellulose. The high water retention and abundant hydrophilic hydroxyl groups provide low thrombogenicity and immunogenicity, whilst high crystallinity and strength produce flexible, strong materials. Multiple studies have demonstrated the potential of bacterial cellulose as a stand-alone material for vascular grafts, including a layer-by-layer fabrication approach that demonstrated high burst pressure (792 ± 22.8 mmHg) and suture retention strength (4.58 ± 0.28 N) [40]. Long-term (9 month) preclinical studies in sheep confirmed that bacterial cellulose materials are resistant to degradation and, depending on the degree of cellular infiltration and ECM deposition, artificial blood vessels made from bacterial cellulose may increase in strength over time [40].

This aspect of graft performance has a number of issues that remain to be addressed. There is currently no consensus on the optimal degradation rate of vascular grafts, with initial enthusiasm for very rapidly degrading constructs replaced with a more conservative trend toward slower breakdown. Clinical adoption will certainly be easier for materials that are long-lasting and robust, consistent with the current commercial options. *In vitro* simulations and short-term small animal studies remain dominant, making it difficult to forecast long-term degradation rates and appropriateness for clinical translation. Longitudinal, large animal model evaluation of the best candidates is needed but has a high barrier to entry and is resource and cost intensive.

Balancing complexity and efficacy

In practice, surgical bypass is performed with either autologous grafts freshly harvested from the patient, or prepackaged, sterilized polymer grafts that can be stored on the shelf, ready at the time of use. To date, strategies for engineering artificial vascular grafts have centered on three common approaches: cell-assembled **scaffolds**, decellularized tissue, and prefabricated scaffolds (Box 2). Each strategy presents a trade-off between the degree of biomimicry, manufacturing time, and costs. Cell-assembled grafts have dominated research efforts for the past decade, following transformational work demonstrating that robust conduits could be derived from human cell culture [18]. Achieving a high degree of biomimicry alone does not lead to clinical translation, with economic and logistical factors, including off-the-shelf availability, remaining as primary barriers.

Cell-assembly strategies have both the highest complexity and degree of biomimicry, with variations developed using an array of human cell types (SMC, fibroblasts, and induced pluripotent stem cells) cultured for long periods to produce *de novo* conduits [41]. A leading candidate employs human SMCs grown on a degradable polymer scaffold in a bioreactor for 7–10 weeks, to grow grafts comprising a collagen-rich matrix [42]. These grafts showed significant promise in preclinical evaluation, justifying human trials. However, in Phase II studies, only 28% of these grafts remained open at 12 months, a result akin to commercial ePTFE, despite the time and resource-

intensive production approach [43]. Stepping down in complexity, decellularized tissue-based grafts aim to exploit the architecture and mechanical properties of vessels derived from animal and human cadaveric tissue, removing cells before use to reduce harmful immune reactions. These grafts are available for clinical use, though have not been widely adopted [44]. Clinical performance has been limited by high rates of thrombosis, infection, and rapid degeneration leading to aneurysm formation [45,46]. Accordingly, decellularized grafts are again only comparable with commercial ePTFE in long-term efficacy [47,48]. Further, variations in source material continue to present challenges for manufacturing reproducibility and the complete removal of tissue antigens remains a major issue.

Prefabrication strategies, the least complex approach, are best suited for the application of natural materials. As described earlier, there are few natural materials that have sufficient strength to be employed as stand-alone materials. This is evident in the reliance on synthetic polymers, dominantly PCL, in recent graft research [49–51]. Many of these constructs are accordingly multicomponent systems, generated either by co-blending synthetic and natural polymers during electrospinning, or by adding natural surface coatings to synthetic base materials. It is also increasingly common for grafts to have three or more components (e.g., heparin/silk/PLCL) to mimic the multilayered architecture of native vessels more closely. Similarly, surface coatings of increasing complexity have been developed to provide the necessary biological signal to simultaneously promote endothelialization while having low thrombogenicity. From this work there is consensus that the presence of natural materials improves biological function. Elastin coatings reduce platelet adhesion, with a corresponding increase in endothelial cell attachment compared with equivalent uncoated grafts [52], while grafts comprising elastin increased the proportion of SMCs in a contractile phenotype [53], suggesting a beneficial effect in limiting neointimal hyperplasia. Collagen improved endothelial cell viability, attachment, and migration when incorporated in poly(lactic acid) (PLA) knitted [54], PCL bilayered [55], and poly(vinyl alcohol) (PVA) [56] vascular scaffold *in vitro*. Hybrid PCL collagen grafts also reduced accumulation of oxidized lipid species and increased contractile SMCs when implanted *in vivo*. Similarly, coatings of gelatin (derived from collagen) on PCL grafts promote endothelialization, while inhibiting SMC growth, in further support of the favorable vascular cell signaling provided by collagen sequences [57]. Addition of chitosan to PCL grafts [co-electrospun, CS/PCL] also increases endothelialization but

Box 2. Strategies for bioengineering artificial blood vessels

There are three common strategies for bioengineering artificial blood vessels that have dominated research efforts to date (Figure I): cell-assembled, decellularized tissue, and fully prefabricated grafts.

Cell-assembled grafts are composed of *de novo* extracellular matrix (ECM) deposited by cells during extended culturing. Cells are extracted from a patient or donor and the desired cell type is isolated and expanded *in vitro*. Cells are then seeded into a degradable porous scaffold that provides a three-dimensional template of the final graft. The cell/scaffold construct is then cultured in a bioreactor system for several weeks, during which time the cells simultaneously degrade the scaffold whilst depositing new proteins that form the final graft structure. Pulsatile blood flow may be simulated during cell culture to mechanically condition the construct. Finally, the construct is decellularized prior to implantation to remove cellular antigens that could provoke an immune response. This approach is resource and time intensive, making production expensive, with a high regulatory burden.

Decellularized tissue grafts are derived from the ECM of native tissue harvested from animal or human cadaveric source. Tissues previously used for harvest include blood vessels, small intestinal submucosa, the ureter, and amnionic membrane. Cells and cellular material are removed from the tissue to reduce immune reactivity. Tissue decellularization strategies may be chemical (detergents and solutions of different pH and tonicity), biological (DNase and RNase enzymes), and/or physical (pressure treatments, sonication, and freeze–thaw cycles). Decellularization benefits from leveraging some elements of the native tissue structure, but grafts generated using this approach have poor blood interaction due to the high collagen content and are subject to ongoing regulatory issues.

Prefabricated grafts are constructed from synthetic or naturally derived polymers, or a hybrid. Common manufacturing techniques include electrospinning, mold casting, knitting or braiding, gel-spinning, and freeze drying. This mode of production has the lowest level of biomimicry; the only materials to reach the clinic are polymers without any biological cues. However, they also have the lowest regulatory burden and can be compatible with scaled production, sterilization, and off-the-shelf use.

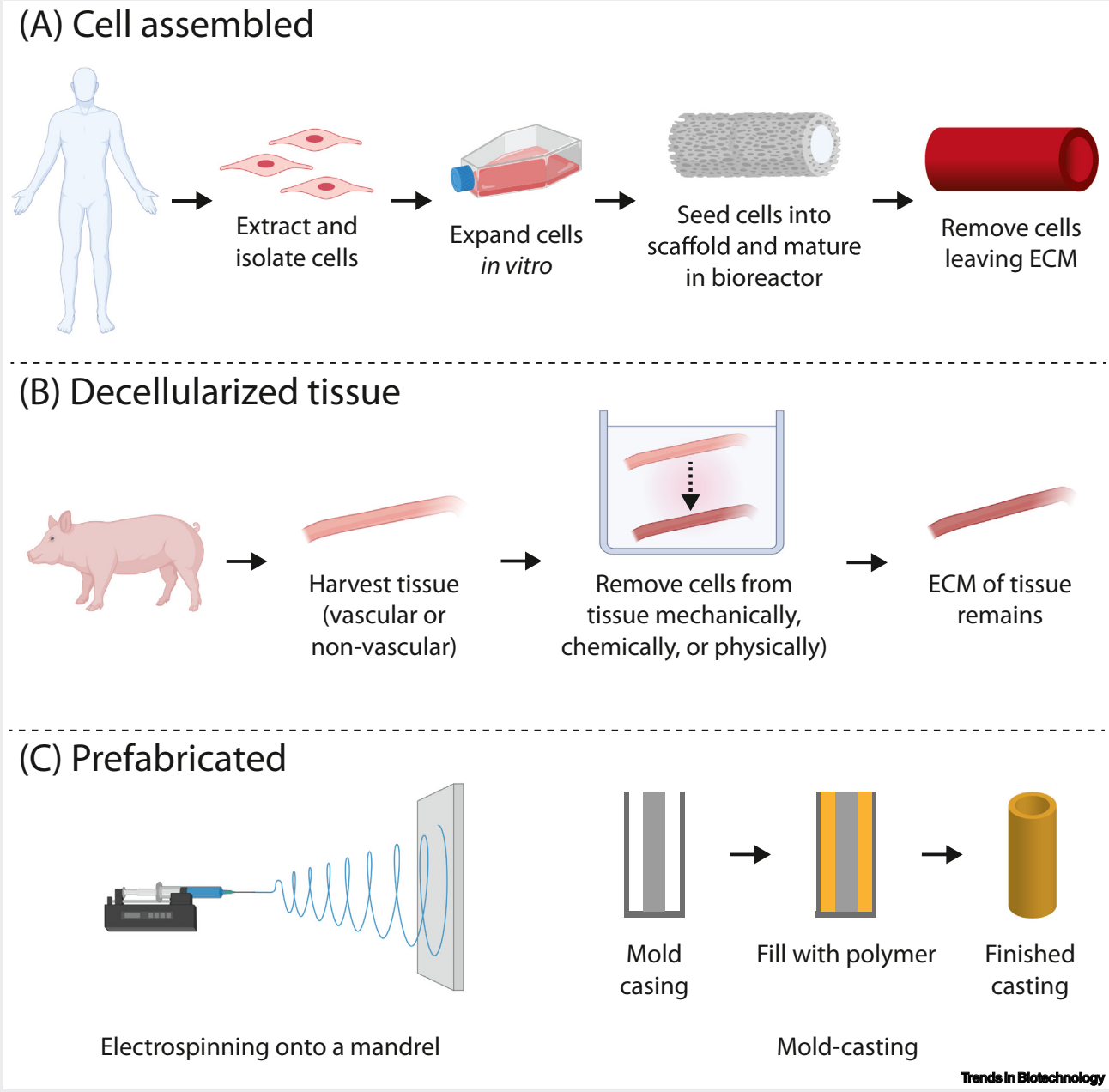


Figure I. Strategies for bioengineering artificial blood vessels. (A) Cell-assembled grafts are composed of *de novo* extracellular matrix (ECM) deposited into a degradable polymer scaffold by cells during extended culturing. (B) Decellularized tissue grafts are derived from the ECM of native tissue harvested from animal or human cadaveric source. (C) Prefabricated grafts are constructed from synthetic or naturally derived polymers using techniques such as electrospinning, mold-casting, or freeze drying.

additionally adds resistance to thrombosis [33]. To date, evaluations in small animal models for these increasingly complex multicomponent conduits have shown some promise, but further development into larger preclinical models is rare.

Recent findings suggest that a return to simpler, single component systems has merit. As seen earlier, single component systems with sufficient mechanical strength are limited, with silk being a stand-out exemplar. Silk-only conduits support endothelial cell growth and resist platelet adhesion and blood clot formation *in vitro* and have had detailed longitudinal analysis in a rat abdominal aorta grafting model for up to 6 months [8]. These studies show that silk conduits rapidly form a complete endothelium by 12 weeks, while ePTFE grafts in the same study remain uncovered even at 24 weeks. Interestingly, equivalent silk grafts with thicker fibers, leading to higher porosity, endothelialize even faster and are completely covered by 6 weeks, suggesting that the architecture of these prefabricated electrospun grafts plays an important role [23]. Building on this example, further design approaches that allow for finer control of porosity, such as ice templating, are increasingly prominent and could be applied to the next generation of conduits [58]. Overall, the emerging opportunity is to take advantage of improvements in manufacturing capability to design single component natural polymer systems with embedded architecture to provide biological cues, leading to an optimal combination of simplicity and efficacy.

Feasibility of translation

Despite a large number of studies showing the promise of natural materials in small animal models, few have been evaluated in larger animals and fewer still progressed further along the translational pathway. Again, there are several classical barriers preventing further development, including wide availability of the source material (scalability), compatibility with clinical sterilization/packaging protocols, and regulatory approval.

Prominent natural materials are typically sourced from animals. Collagen is abundant and readily extracted from cows, pigs, sheep, and fish. Gelatin, derived from collagen following hydrolysis, has the same sources and can be obtained cheaply in large quantities. Similarly, elastin is a major component of the vascular extracellular matrix and can be obtained from large animals, though in smaller quantities [59]. The limitation for these natural polymers is that their high level of *in vivo* crosslinking means that isolated materials have either limited solubility or need to be exposed to harsh chemical regimes to make them suitable for biomaterial applications. Other natural polymers such as chitosan are derived from the partial deacetylation of chitin, an abundant polysaccharide in arthropod exoskeletons and the cell wall of fungi. As mentioned earlier, silk is dominantly sourced from *B. mori* silkworms, where farming presents some issues with batch variability, and increasingly from spiders, where the small amounts produced make large-scale production unrealistic.

Advances in recombinant protein technology and transgenic animals offer increasingly good solutions to supply problems. Recombinant human collagen has a long history in biomaterials applications, including recent application in high-strength spun fibers [60], injectable hydrogel for application in myocardial repair [61], and modified for compatibility with 3D printing [62]. This approach also allows for the production of blended constructs that combines favorable properties from each, such as collagen–chitosan, for example [63]. Similarly, tropoelastin, the soluble precursor of elastin, has been optimized for recombinant production from an *Escherichia coli* expression system and applied to a wide range of applications, including for vascular graft coating [64] and wound healing [65]. The recombinant production of tropoelastin for human use continues to be developed commercially by Allergan Inc., initially as an injectable biomaterial. Elastin-like recombinamers, based on major sequence motifs in the full-length protein, continue to show promise, as described earlier. Finally, for silk, transgenic host species for producing recombinant spider silk proteins means industrial scale quantities can be produced [66] and manufacturing processes have advanced to the point where spinning recombinant silk proteins into native-like silk is achievable [67].

The US Food and Drug Administration (FDA) guidelines require all vascular prosthesis must be sterilized with techniques that meet a sterility assurance limit of 10^{-6}, which is the probability that not more than one viable microorganism is detected per 1 million sterilized final products (www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/vascular-prostheses-510k-submissions-guidance-industry-and-fda-staff). While the favorable biological properties of natural materials make them well suited for use as vascular grafts, this also leads to an increased susceptibility to infections when used *in vivo*. The translation of natural materials for use as commercial grafts will require increased scrutiny over appropriate sterilization techniques that can be precisely controlled to avoid compromising their structural and/or biochemical integrity. In the absence of a universal technique applicable to a wide range of polymers, sterilization of grafts made from natural materials will most likely be evaluated case by case. The differences in structural and biochemical features of natural materials vary more significantly than synthetic polymers. Therefore, different sterilization techniques will need to be further investigated before being declared safe and effective for natural material grafts. This may involve specific changes to and/or combinations of common sterilization approaches, including heat, chemical, and irradiation techniques. Leading candidate materials, including tropoelastin, collagen, and silk have published detailed studies showing compatibility with at least one of these methods [68–70].

In addition to the commercial development of tropoelastin-based materials by Allergan Inc. described earlier, other natural materials are moving along the translational pathway. For collagen, CollaMedix Inc. was founded in 2018 to produce medical devices made from their proprietary load-bearing pure collagen filaments, CollaFabric® (www.collamedixinc.com). In the case of silk, Silk Biomaterials is an Italian-based medical device start-up that is developing silk fibroin for several applications, including peripheral vascular disease, drug delivery, and nerve regeneration (www.silkbiomaterials.com). Similarly, Sofregen Medical Inc. from the US is an early stage commercial biotechnology company developing products for medical aesthetics and reconstructive surgery. In 2019, Sofregen announced that their Silk Voice® implant had received FDA approval for augmenting vocal fold tissue for phonation improvement. This was a significant hallmark in silk commercialization as the first and only FDA-approved product made from solubilized silk protein. Taken together, these examples demonstrate a feasible pathway for scalable delivery of regulatory approved natural materials for human use.

Concluding remarks

A durably effective, small diameter artificial vascular graft has been described as the holy grail of bioengineering [71,72]. With the increasing incidence of cardiovascular risk factors, including diabetes and obesity, the need for artificial blood vessels will continue to grow [1]. Following significant research into complex tissue-engineered constructs, the most promising have reached human trials but have failed to meet expectations. The future of graft engineering should leverage the innate beneficial properties of natural materials, including tunable mechanical properties and improved interactions with blood and vascular cells. Traditional barriers to entry are gradually being overcome, with improvements in manufacturing, the identification of new sources, and the growing number of companies moving past regulatory hurdles. To maximize the likelihood of translation to the clinic, the simplest designs with the fewest components should be considered. A realization of this approach, extrapolating from current trends, could be a vascular graft comprised of a single natural material, with finely tuned mechanical properties, slow degradation, and in-built architectural cues to direct local cell interactions (see Outstanding questions). Chosen from a scalable source, compatible with clinical sterilization techniques, as is the case for several of the natural materials identified here, such a graft would be compatible with off-the-shelf use and meet a pressing unmet need in vascular medicine. The immediate challenge is to identify

Outstanding questions

What is the ideal degradation rate for grafts made from natural materials and should this be tailored for patient age and disease?

How can the field make large animal models more accessible/commonplace to enable better evaluation of the best new graft candidates?

Is compliance to the surrounding native vasculature matching alone sufficient to drive meaningful improvements in graft performance?

Can a single component graft combine the necessary mechanical and biological cues to be an effective artificial graft?

Will the commercial use of natural materials for other indications (e.g., wound healing) translate to clinical vascular applications?

the most promising candidates and evaluate them in the large preclinical animal models (e.g., sheep and pig) that are a necessary step along the path to clinical translation. High-cost barriers and the requirements for significant infrastructure and expertise has meant that these studies are rarely completed and highly promising materials fail to advance beyond small animal model (mouse and rat) evaluation. More frequent graduation to this higher level of preclinical testing is critical to demonstrating that natural materials have developed sufficiently to overcome the traditional barriers to translation (Figure 2). The near-term focus should be on developing and increasing the frequency of the missing elements of the preclinical translational pathway, giving confidence that simpler, naturally derived grafts are a viable alternative for small diameter applications.

Acknowledgments

This work was supported by the National Health and Medical Research Council (APP1162969; S.G.W., and J.R-K.), Australian Research Council (LP180100540; S.G.W., and J.R-K.), and Sydney Local Health District (S.G.W). M.J.M. was supported by an Australian Government Research Training Program Scholarship. The authors also acknowledge the financial support of E. Brackenreg. Figures 1, 2, and I (Box 2) were created with the assistance of BioRender.

Declaration of interests

No interests are declared.

References

1. Roth, G.A. *et al.* (2020) Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 study. *J. Am. Coll. Cardiol.* 76, 2982–3021
2. Aboyans, V. *et al.* (2017) 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society For Vascular Surgery (ESVS): document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries endorsed by: the European Stroke Organization (ESO) the task force for the diagnosis and treatment of peripheral arterial diseases of the European Society Of Cardiology (ESC) and of the European Society For Vascular Surgery (ESVS). *Eur. Heart J.* 39, 763–816
3. Kim, L.K. *et al.* (2015) Trends in hospital treatments for peripheral arterial disease in the United States and association between payer status and quality of care/outcomes, 2007–2011. *Catheter. Cardiovasc. Interv.* 86, 864–872
4. Benjamin, E.J. *et al.* (2017) Heart disease and stroke statistics-2017 update: a report from the American Heart Association. *Circulation* 135, e146–e603
5. Caliskan, E. *et al.* (2020) Saphenous vein grafts in contemporary coronary artery bypass graft surgery. *Nat. Rev. Cardiol.* 17, 155–169
6. Poletti, L.F. *et al.* (1998) Should vein be saved for future operations? A 15-year review of infrainguinal bypasses and the subsequent need for autogenous vein. *Ann. Vasc. Surg.* 12, 143–147
7. Biancari, F. *et al.* (2001) Redo bypass surgery to the infrapopliteal arteries for critical leg ischaemia. *Eur. J. Vasc. Endovasc. Surg.* 21, 137–142
8. Filipe, E.C. *et al.* (2018) Rapid endothelialization of off-the-shelf small diameter silk vascular grafts. *JACC Basic Transl. Sci.* 3, 38–53
9. Bao, L. *et al.* (2020) Physicochemical properties and in vitro biocompatibility of three bacterial nanocellulose conduits for blood vessel applications. *Carbohydr. Polym.* 239, 116246
10. Dekker, A. *et al.* (1991) Adhesion of endothelial cells and adsorption of serum proteins on gas plasma-treated polytetrafluoroethylene. *Biomaterials* 12, 130–138
11. Moore, K.H. *et al.* (2021) The glycocalyx: a central regulator of vascular function. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 320, R508–R518
12. Jaffer, I.H. *et al.* (2015) Medical device-induced thrombosis: what causes it and how can we prevent it? *J. Thromb. Haemost.* 13, S72–S81
13. Collins, M.J. *et al.* (2012) Therapeutic strategies to combat neointimal hyperplasia in vascular grafts. *Expert. Rev. Cardiovasc. Ther.* 10, 635–647
14. Tan, R.P. *et al.* (2021) Macrophage polarization as a novel therapeutic target for endovascular intervention in peripheral artery disease. *JACC Basic Transl. Sci.* 6, 693–704
15. Joyce, K. *et al.* (2021) Bioactive potential of natural biomaterials: identification, retention and assessment of biological properties. *Signal Transduct. Target. Ther.* 6, 122
16. Seifu, D.G. *et al.* (2013) Small-diameter vascular tissue engineering. *Nat. Rev. Cardiol.* 10, 410–421
17. Konig, G. *et al.* (2009) Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. *Biomaterials* 30, 1542–1550
18. L'Heureux, N. *et al.* (2006) Human tissue-engineered blood vessels for adult arterial revascularization. *Nat. Med.* 12, 361–365
19. Stekelenburg, M. *et al.* (2009) Dynamic straining combined with fibrin gel cell seeding improves strength of tissue-engineered small-diameter vascular grafts. *Tissue Eng. Part A* 15, 1081–1089
20. Yin, A. *et al.* (2017) Coaxial electrospinning multicomponent functional controlled-release vascular graft: optimization of graft properties. *Colloids Surf. B* 152, 432–439
21. Ranjan, V.D. *et al.* (2020) In vitro cell culture in hollow microfibers with porous structures. *Biomater. Sci.* 8, 2175–2188
22. Rodriguez, M. *et al.* (2020) Fabricating mechanically improved silk-based vascular grafts by solution control of the gel-spinning process. *Biomaterials* 230, 119567
23. Chan, A.H.P. *et al.* (2019) Altered processing enhances the efficacy of small-diameter silk fibroin vascular grafts. *Sci. Rep.* 9, 17461
24. Gupta, P. *et al.* (2020) Bioresorbable silk grafts for small diameter vascular tissue engineering applications: in vitro and in vivo functional analysis. *Acta Biomater.* 105, 146–158
25. Elsayed, Y. *et al.* (2016) Fabrication and characterisation of biomimetic, electrospun gelatin fibre scaffolds for tunica media-equivalent, tissue engineered vascular grafts. *Mater. Sci. Eng. C* 61, 473–483
26. Dastagir, K. *et al.* (2020) In vitro construction of artificial blood vessels using spider silk as a supporting matrix. *J. Mech. Behav. Biomed. Mater.* 101, 103436
27. Shelah, O. *et al.* (2021) Coral-derived collagen fibers for engineering aligned tissues. *Tissue Eng. Part A* 27, 187–200

28. Wertheimer, S. *et al.* (2021) Bio-composites reinforced with unique coral collagen fibers: towards biomimetic-based small diameter vascular grafts. *J. Mech. Behav. Biomed. Mater.* 119, 104526
29. Fernández-Colino, A. *et al.* (2019) Small caliber compliant vascular grafts based on elastin-like recombinamers for in situ tissue engineering. *Front. Bioeng. Biotechnol.* 7, 340
30. Brown, B.N. *et al.* (2017) Effects of age-related shifts in cellular function and local microenvironment upon the innate immune response to implants. *Semin. Immunol.* 29, 24–32
31. Li, W. *et al.* (2018) Long-term evaluation of vascular grafts with circumferentially aligned microfibers in a rat abdominal aorta replacement model. *J Biomed Mater Res B Appl Biomater* 106, 2596–2604
32. de Valence, S. *et al.* (2012) Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model. *Biomaterials* 33, 38–47
33. Fukunishi, T. *et al.* (2016) Tissue-engineered small diameter arterial vascular grafts from cell-free nanofiber PCL/chitosan scaffolds in a sheep model. *PLoS One* 11, e0158555
34. Kong, X. and Xu, W. (2015) Biodegradation and biocompatibility of a degradable chitosan vascular prosthesis. *Int. J. Clin. Exp. Med.* 8, 3498–3505
35. Vroman, I. and Tighzert, L. (2009) Biodegradable polymers. *Materials* 2, 307–344
36. Koens, M.J.W. *et al.* (2015) Vascular replacement using a layered elastin-collagen vascular graft in a porcine model: one week patency versus one month occlusion. *Organogenesis* 11, 105–121
37. Ryan, A.J. *et al.* (2020) Hierarchical biofabrication of biomimetic collagen-elastin vascular grafts with controllable properties via lyophilisation. *Acta Biomater.* 112, 52–61
38. Wang, Y. *et al.* (2008) In vivo degradation of three-dimensional silk fibroin scaffolds. *Biomaterials* 29, 3415–3428
39. Alessandrino, A. *et al.* (2019) Three-layered silk fibroin tubular scaffold for the repair and regeneration of small caliber blood vessels: from design to in vivo pilot tests. *Front. Bioeng. Biotechnol.* 7, 356
40. Weber, C. *et al.* (2018) Patency and in vivo compatibility of bacterial nanocellulose grafts as small-diameter vascular substitute. *J. Vasc. Surg.* 68, 177S–187S
41. Kimicata, M. *et al.* (2020) Extracellular matrix for small-diameter vascular grafts. *Tissue Eng. Part A* 26, 1388–1401
42. Dahl, S.L.M. *et al.* (2011) Readily available tissue-engineered vascular grafts. *Sci. Transl. Med.* 3, 68ra69
43. Lawson, J.H. *et al.* (2016) Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: two phase 2 single-arm trials. *Lancet* 387, 2026–2034
44. Lin, C.-H. *et al.* (2018) In vivo performance of decellularized vascular grafts: a review article. *Int. J. Mol. Sci.* 19, 2101
45. Madden, R.L. *et al.* (2004) Experience with cryopreserved cadaveric femoral vein allografts used for hemodialysis access. *Ann. Vasc. Surg.* 18, 453–458
46. Sharp, M.A. *et al.* (2004) A cautionary case: the synergraft vascular prosthesis. *Eur. J. Vasc. Endovasc. Surg.* 27, 42–44
47. Jadlowiec, C.C. *et al.* (2015) An outcomes comparison of native arteriovenous fistulae, polytetrafluorethylene grafts, and cryopreserved vein allografts. *Ann. Vasc. Surg.* 29, 1642–1647
48. Chemla, E.S. and Morsy, M. (2008) Randomized clinical trial comparing decellularized bovine ureter with expanded polytetrafluoroethylene for vascular access. *Br. J. Surg.* 96, 34–39
49. Wu, P. *et al.* (2020) Construction of vascular graft with circumferentially oriented microchannels for improving artery regeneration. *Biomaterials* 242, 119922
50. Qin, K. *et al.* (2020) Hyaluronan promotes the regeneration of vascular smooth muscle with potent contractile function in rapidly biodegradable vascular grafts. *Biomaterials* 257, 120226
51. Kuang, H. *et al.* (2020) Construction and performance evaluation of Hep/silk-PLCL composite nanofiber small-caliber artificial blood vessel graft. *Biomaterials* 259, 120288
52. Tanaka, T. *et al.* (2021) Development of small-diameter elastin-silk fibroin vascular grafts. *Front. Bioeng. Biotechnol.* 8, 622220
53. Nguyen, T.-U. *et al.* (2019) Electrochemical fabrication of a biomimetic elastin-containing bi-layered scaffold for vascular tissue engineering. *Biofabrication* 11, 015007
54. Zhang, F. *et al.* (2021) A hybrid vascular graft harnessing the superior mechanical properties of synthetic fibers and the biological performance of collagen filaments. *Mater. Sci. Eng. C* 118, 111418
55. Park, S. *et al.* (2019) Fabrication of strong, bioactive vascular grafts with PCL/collagen and PCL/silica bilayers for small-diameter vascular applications. *Mater. Des.* 181, 108079
56. Bates, N.M. *et al.* (2020) Bioconjugation of a collagen-mimicking peptide onto poly(vinyl alcohol) encourages endothelialization while minimizing thrombosis. *Front. Bioeng. Biotechnol.* 8, 621768
57. Xing, Y. *et al.* (2021) Gelatin coating promotes in situ endothelialization of electrospun polycaprolactone vascular grafts. *J. Biomater. Sci. Polym. Ed.* 32, 1161–1181
58. Joukhdar, H. *et al.* (2021) Ice templating soft matter: fundamental principles and fabrication approaches to tailor pore structure and morphology and their biomedical applications. *Adv. Mater.* 33, 2100091
59. Daamen, W.F. *et al.* (2005) Isolation of intact elastin fibers devoid of microfibrils. *Tissue Eng.* 11, 1168–1176
60. Yaari, A. *et al.* (2016) Wet spinning and drawing of human recombinant collagen. *ACS Biomater. Sci. Eng.* 2, 349–360
61. McLaughlin, S. *et al.* (2019) Injectable human recombinant collagen matrices limit adverse remodeling and improve cardiac function after myocardial infarction. *Nat. Commun.* 10, 4866
62. Tytgat, L. *et al.* (2020) High-resolution 3D bioprinting of photo-cross-linkable recombinant collagen to serve tissue engineering applications. *Biomacromolecules* 21, 3997–4007
63. Deng, A. *et al.* (2021) Preparation of a recombinant collagen-peptide (RHC)-conjugated chitosan thermosensitive hydrogel for wound healing. *Mater. Sci. Eng. C* 119, 111555
64. Oliveira, S. *et al.* (2020) Tubular fibrous scaffolds functionalized with tropoelastin as a small-diameter vascular graft. *Biomacromolecules* 21, 3582–3595
65. Mithieux, S.M. *et al.* (2018) Tropoelastin implants that accelerate wound repair. *Adv. Healthc. Mater.* 7, 1701206
66. Whittall, D.R. *et al.* (2021) Host systems for the production of recombinant spider silk. *Trends Biotechnol.* 39, 560–573
67. Rising, A. and Johansson, J. (2015) Toward spinning artificial spider silk. *Nat. Chem. Biol.* 11, 309–315
68. Yeo, G.C. *et al.* (2017) A sterilizable, biocompatible, tropoelastin surface coating immobilized by energetic ion activation. *J. R. Soc. Interface* 14, 20160837
69. Delgado, L.M. *et al.* (2014) Influence of sterilisation methods on collagen-based devices stability and properties. *Expert Rev. Med. Devices* 11, 305–314
70. Rnjak-Kovacina, J. *et al.* (2015) The effect of sterilization on silk fibroin biomaterial properties. *Macromol. Biosci.* 15, 861–874
71. Kakisis, J.D. *et al.* (2005) Artificial blood vessel: the holy grail of peripheral vascular surgery. *J. Vasc. Surg.* 41, 349–354
72. Slaughter, M.S. (2010) The ideal conduit for surgical revascularization. *Circulation* 122, 857–858
73. Girerd, X.J. *et al.* (1992) Incompressibility of the human arterial wall: an in vitro ultrasound study. *J. Hypertens.* 10, S111–S114
74. Bao, L. *et al.* (2021) Implantation of air-dried bacterial nanocellulose conduits in a small-caliber vascular prosthesis rabbit model. *Mater. Sci. Eng. C* 122, 111922
75. Wacker, M. *et al.* (2020) In vitro hemo- and cytocompatibility of bacterial nanocelluose small diameter vascular grafts: impact of fabrication and surface characteristics. *PLoS One* 15, e0235168
76. Wang, Y. *et al.* (2020) A chitosan modified asymmetric small-diameter vascular graft with anti-thrombotic and anti-bacterial functions for vascular tissue engineering. *J. Mater. Chem. B* 8, 568–577