INVITED ARTICLE

TECHNOLOGY

Cell-free vascular grafts: Recent developments and clinical potential

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Recent advances in vascular tissue engineering have led to the development of cell-free grafts that are available off-the-shelf for on demand surgery. Challenges associated with cell-based technologies including cell sourcing, cell expansion and long-term bioreactor culture motivated the development of completely cell-free vascular grafts. These are based on decellularized arteries, decellularized cultured cell-based tissue engineered grafts or biomaterials functionalized with biological signals that promote *in situ* tissue regeneration. Clinical trials undertaken to demonstrate the applicability of these grafts are also discussed. This comprehensive review summarizes recent developments in vascular graft technologies, with potential applications in coronary artery bypass procedures, lower extremity bypass, vascular injury and trauma, congenital heart diseases and dialysis access shunts, to name a few.

***Keywords*: Vascular Grafts; Cardiovascular Disease; Decellularization; *In Situ* Tissue Regeneration; Functionalized Biomaterials; Tissue Engineering.**

INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States, claiming over 600,000 lives annually, according to the CDC-NCHS Report on Heart Disease (published February 2015). Coronary heart disease is the most prevalent among cardiovascular ailments, which is caused by blockages in major arteries around the heart, leading to heart attack or stroke[1]. The current effective remedy is an autologous transplant of saphenous vein (SV), radial artery (RA) or internal mammary artery (IMA) to the site of blockage, creating a bypass to restore normal blood cardiac flow. However, a number of complications and comorbidities such as hypertension, diabetes, or previous bypass or shunt procedures, especially in the elderly, can lead to unavailability of suitable autologous tissue options for grafting procedures[1]. This necessitates the development of alternative technologies such as tissue engineered blood vessels (TEBVs) that may be available in a cost-effective, timely and reproducible manner[2–4]. Many of the challenges facing the field have been overcome, particularly over the last decade, and successful TEBVs have been tested in animal models and a few human clinical trials are also underway[5,6]. However, the current state of tissue engineering requires extensive cell culture, bioreactor preconditioning and utilization of biomaterials that do not integrate within the host vasculature[3,7].

TEBVs in all studies imitate the architecture of native vessels, which consist of an inner endothelium in the lumen and circumferentially aligned contractile smooth muscle layers in an extracellular matrix (ECM) media, which provides mechanical strength. The inner endothelium not only prevents thrombosis, but is also responsible for maintaining and altering vascular tone to control blood flow. Smooth muscle is present in the medial layers of the vessels, along with collagen and elastin fibers which provide mechanical support. Smooth muscle cells (SMCs) possess contractile function and are required to expand and contract with blood flow, and produce ECM to replenish the degraded fibers. The smooth muscle in veins and arteries vary not only in thickness, but also in homogeneity and fiber alignment[8]. The more longitudinal fiber alignment in veins make them more distensible, and has been suggested that this makes them more prone to hyperplasia when used as replacement grafts[8,9]. In addition to function and structure, arteries and veins exhibit significantly different mechanical properties. In particular, the SV has a mean burst pressure of 1600 mm Hg while the IMA can withstand up to 3200 mm Hg[9]. TEBVs, in various studies reported here, are constructed to withstand pressures in similar ranges as the major arteries.

A TEBV is required to possess the following characteristics in order to function properly as a bypass graft: lack of thrombogenicity; ability to integrate into the host vasculature; and sufficient mechanical strength. The gold standard still remains to be the autologous SV graft, which possesses a pre-formed endothelium and does not induce an immunogenic response. That being said, the main challenge facing venous bypass grafts is occlusion[10]. Indeed, an endothelium may not be easily acclimated to high arterial shear stress and if removed, it may take months to reform a confluent healthy monolayer[11,12]. To overcome this problem, many studies have employed a pre-formed endothelial lining while utilizing synthetic graft materials, which provides a favorable blood-material interface[13,14]. Indeed, in a 9-year follow-up of implanted polytetrafluoroethylene grafts in human patients, 65% of endothelialized grafts remained patent, while 16% occluded[15]. This study utilized fibrin glue to coat the PTFE grafts

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Received 3 January 2017; accepted 27 February 2017; published online 31 March 2017; doi:10.1142/S2339547817400015

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to enable endothelial cell attachment, but these grafts do not promote cell attachment, spreading or growth. This revealed that the confluent endothelial layer is one of the largest contributor to patency. Nonetheless, many preclinical studies have reported rapid endothelialization especially in porcine and canine models. This is attributed to trans-anastomotic endothelial migration, a phenomenon that is known not to occur in humans. Trans-anastomotic endothelialization may be the biggest contributor to patency in pigs and dogs, and one must be careful in extrapolating the results obtained from these animal models to human physiology[16–18]. Human endothelialization occurs by intimal hyperplasia over suture sites that create a pannus, but pannus outgrowth is very slow — limited to about 3–5 mm in a year[11]. On the other hand, the ovine model is very similar to humans, exhibiting limited trans-anastomotic endothelial migration as well as similar primary hemostasis and coagulation patterns[19]. The main differences lie in secondary hemostasis, which for sheep is characterized by rapid initiation of contact activation, higher levels of protein C and FVIII, and higher clot firmness, thereby providing a stringent model for assessment of thrombogenesis[19].

Furthermore, TEBV need to be manufactured in a time and cost-effective fashion. The recent trends in tissue engineering are focused on the development of off-the-shelf options that may also be customizable. This mandates the advances in cell-free technologies, which utilize natural or synthetic matrices that are functionalized to be anti-thrombotic and self-healing promote *in situ* cellular infiltration upon implantation. The next generation grafts expected from tissue engineering will be capable of standard hospital storage, cause no immune response or complications in the host and most importantly, be readily available for on demand surgeries. This review focuses on the latest developments in such cell-free vascular grafts.

RECENT BREAKTHROUGHS IN TISSUE ENGINEERING

The first constructed TEBV by Weinberg and Bell in 1986 utilized a Dacron mesh with circumferentially aligned SMCs and fibroblasts forming the vascular wall and an endothelial lining to prevent clotting[20]. The burst pressure was proportional to the collagen content, as expected, and the graft was able to integrate into the host vasculature. The major challenges facing this graft included lack of elastin, a major contributor to the elasticity of the vascular wall, and limited host cell infiltration into the graft, which was restricted to the anastomoses sites[7,20]. Nevertheless, this study laid the foundation for the field of vascular tissue engineering.

Attempts with cell-based grafts

SMCs were originally obtained from host vasculature and cultured into scaffolds, or grown as cell sheets, which produced their own ECM[21–23]. The main challenge facing this approach was the limited cell growth, which slowed down the process of TEBV manufacture. Attempts were made to regain the replicative potential while maintaining the SMC properties, by overexpression of hTERT (human telomerase reverse transcriptase) to reverse the effects of cultural senescence[24,25]. This enabled isolation and culture of vascular SMCs even from aged patients[25] but the tumorigenic potential of hTERT expressing cells prevents clinical application of this approach. Recently, our group identified a transcription factor, Nanog, that could reverse senescence to restore SMC functionality in aged cells[26]. However, due to complications arising from genetic manipulations, SMCs were replaced by other sources, such as MSCs and fibroblasts, which were capable of producing smooth muscle proteins and ECM, and exerting contractile force[27,28]. Fibroblasts were very effective in production of ECM in bioreactors, yielding TEBVs with high burst pressures[29]. Co-cultures of SMCs and fibroblasts in fibrin scaffolds have been utilized in another study, where bioreactor preconditioning resulted in an aligned, differentiated, functional SMC layer within 30 days[30]. An interesting technique called "biotubes" also proved effective, where silicone tubes were inserted into subcutaneous pouches of rabbits, and allowed to be infiltrated with host fibroblasts which deposited ECM. These cellularized biotubes were then explanted from the subcutaneous patches and implanted as autologous vascular grafts[31,32]. Although these grafts were patent, two surgeries were required on the patient, which made mesenchymal stem cells (MSCs) the more attractive option.

Interestingly, we reported that as compared to vascular SMC, αSMA-expressing bone marrow MSC exhibited significantly higher elastogenic potential *in vitro* and *in vivo*[33,34]. Tissue engineered blood vessels that were prepared from these cells were implanted them into the jugular veins of lambs, where they remained patent and exhibited high amounts of elastin that was organized in a fibrillar network very similar to native vessels[35]. Niklason's group has also employed bone marrow MSCs in the preparation of TEVG (Tissue Engineered Vascular Graft) that were preconditioned in a bioreactor to yield robust implantable vascular grafts[27]. Another study demonstrated that EGFP-labeled MSCs, when seeded onto polyurethane scaffolds and implanted to Wistar rats, were able to proliferate and stained positive for SMC proteins after just 2 weeks *in vivo*[28]. Both SMC-like and EC (endothelial cell)-like cells were generated from bone-marrow derived MSCs and cultured onto decellularized native scaffolds, which performed very well as interpositional grafts in ovine carotid artery implantations[36].

The need for the presence of SMC in the vascular wall was challenged by a recent study from our group that compared vascular grafts with or without SMC. We concluded that the presence of SMCs in the medial layer was not necessary for successful implantation and remodeling of small intestinal submucosa (SIS)-fibrin composite vascular grafts in the arterial circulation of an ovine animal model[37]. Infiltration of highly proliferative host SMCs into the composite biomaterial and subsequent maturation into a smooth muscle phenotype rendered the initial donor SMCs redundant, especially after longer times *in vivo* (3 months), suggesting a simplified TEVG design with no cells in the vascular wall. Indeed, several implantation studies have been performed in large animal models where smooth muscle medial layer was not a contributor of patency or overall performance[38–40]. However, it was discovered that the presence of MSCs was an important contributing factor in the inflammatory response that may be required for vascular graft healing[41,42].

However, endothelial cells are still required to populate the lumen of SIS-based vascular grafts to prevent thrombus formation, as we demonstrated using an *ex vivo* arteriovenous shunt model[43]. ECs on several biomaterial grafts are known to prevent occlusion and enhance graft patency[14]. Endothelial lining enhances patency in Dacron grafts implanted in a canine model[16]. Using endothelial cells in "bio-ink", conduits or hydrogels (for microvasculature studies) in the lumen via thermal inkjet printing technique facilitated obtaining a confluent endothelium[44,45]. Bio-ink enabled use of printer cartridges filled with liquid cell suspensions, which could be programmed in patterns on surfaces. However, sources for endothelial cells are limited, and the yield from autologous skin biopsies are usually insufficient. The isolation often needs to be followed by FACS (Fluorescence assisted cell sorting), selection media and expansion up to several passages to obtain sufficient cell numbers[46].

Host circulating endothelial progenitor cells (EPCs) can be isolated, cultured and employed in TEBVs, which were shown to have lesser intimal hyperplasia as compared to autologous vein grafts[39]. Although EPCs are more readily expandable in culture than mature endothelial cells, they are rather scarce in circulation, around 50 cells in 1 mL of blood[47,48]. In order to overcome this issue, several attempts are being made to capture the EPC from the host directly using growth factor, VEGF (vascular endothelial growth factor), decorated onto the lumen of the grafts. One such study employed EDC chemistry on glutaraldehyde soaked PTFE grafts, with subsequent treatment with HAS bonds and covalent chemistry to bind VEGF onto the lumen to allow for endothelial cell migration and proliferation[12]. Another study used collagenous scaffolds which were treated

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with heparin benzalkonium chloride complex (HBAC) cross-linked via EDC chemistry to successfully prevent clotting of small diameter grafts in pigs[49]. In a recent study from our lab, heparin was employed to coat SIS by means of EDC-NHS chemistry, followed by immobilization of VEGF via binding to the heparin binding domain. These grafts exhibited high patency rates in the high flow environment of ovine carotid arteries and produced fully endothelialized, functional arteries as soon as one month post-implantation[50]. Unlike antibodies which have also been applied to capture EPCs from the host bloodstream[51,52], VEGF enhances the survival, proliferation and cell spreading of captured progenitors[53–56], making it a more attractive option for functionalizing TEBVs.

Design simplification and cell-free grafts

Cell-based technologies depend on patient cell harvest and expansion, bioreactor culture and preconditioning, which makes the manufacture of TEVGs a lengthy and expensive process[4,7]. Furthermore, primary adult cells or stem cells isolated from patients exhibit limited expansion potential, which further declines with patient age. To overcome these limitations, several groups including our own have developed completely acellular vascular grafts that may be available off-the-shelf and on demand and suitable as an arterial or venous replacement for treatment of cardiovascular disease.

An important advancement in engineering the next generation of TEBVs will be design simplification, which will make off-the-shelf grafts available for implantation[4]. We have recently shown in our laboratory that SMCs are not required in the medial layer of a vascular graft, with the correct choice of biomaterial[37]. In this context, biomaterial properties such as porosity become critical for graft patency and remodeling[20,57,58]. Another study used a biodegradable elastomer that was replaced by a neoartery in a rat animal model[59]. ECs have been replaced by growth factors and antibodies which are capable of capturing host circulating EPCs[50]. Zhou *et al*. have utilized heparin and VEGF to replace the endothelial lining, and have shown successful implantation in a canine arterial model[60].

In a recent case-study, L'Heureux and colleagues have used their "lifeline grafts" off-the-shelf by freeze-drying technique as dialysis access grafts[6]. Lifeline grafts are tubularized self-assembled cell sheets, which can be frozen and thawed a few days before the procedure and endothelialized with the patients' own cells[6]. In another human case study of a 4-year-old girl, poly caprolactone-polylactic acid (PCL-PLA) copolymer seeded with vascular SMCs isolated from the patients' own peripheral vein was used as a TEBV to replace a pulmonary artery[5]. Shinoka and colleagues have used grafts made in similar fashion in human clinical trials with success[61]. Niklason and colleagues at Humacyte have also developed cell-free grafts starting from traditional cell-based tissues. They employed banked human smooth muscle cells and pulsatile bioreactors to culture PLGA-based vascular constructs for 7–10 weeks in order to produce enough ECM to better mimic the *in vivo* environment. Then the grafts were decellularized prior to storage and surgery[22,62]. The cell-free grafts were employed in phase-2 single-arm clinical trials as upper arm brachial-to-axillary-straight grafts, where their secondary patency was reported to be 89% at 12 months post-implantation[63]. However, a major cause for concern remains that the primary patency was only at 63% at 6 months and dropped to 28% at 12 months[63]. This was mainly because of the propensity of cell-free grafts to occlude, which might be attributed to the lack of a healthy, confluent endothelium.

Our group has recently reported the development of cell-free grafts that were implanted successfully in a large, preclinical animal model. These grafts were based on SIS that was functionalized sequentially with heparin and VEGF, in order to prevent thrombosis and attract endothelial progenitor cells. When implanted as interpositional grafts in the carotid arteries of an ovine model, they exhibited 92% patency rate[50]. By employing VEGF, which captures progenitor cells from the bloodstream, these grafts were fully endothelialized within a month, as evidenced by immunostaining for mature endothelial markers such as vWF, CD144 and eNOS. The vascular wall also showed significant infiltration of host cells expressing smooth muscle proteins and exhibiting vascular contractility in response to vasoconstrictors and dilators. After three months, the endothelium aligned in the direction of flow and the medial layer comprised of uniformly distributed and circumferentially aligned smooth muscle cells closely resembling native carotids. This study demonstrated the feasibility of engineering cell-free vascular conduits that can serve as "off-the-shelf" vascular grafts to treat cardiovascular disease.

DECELLULARIZATION STRATEGIES

The goal of decellularization is to effectively isolate the ECM of a biological tissue from its immunogenic materials such as cellular and nuclear contents. While any xenogeneic or allogeneic cellular antigens are very likely to trigger an adverse immune response by the host, leading to graft rejection, the composition of the ECM is similar between species. The ECM is a protein-abundant microstructure comprised of glycoproteins, proteoglycans, glycosaminoglycans and structural proteins such as collagens, elastin, fibronectin and laminin providing a scaffold that enables cell adhesion, proliferation and differentiation[64]. The microstructure and morphology of the ECM vary between organs suggesting that decellularization protocols that have been developed for one organ/tissue may not be as effective for others[65]. For instance, while Triton X-100 has been an effective detergent for removing superficial cellular and nuclear material from heart valves, it was not as effective in decellularizing tendons, ligaments and blood vessels[66]. For our purpose, we will focus our discussion on strategies employed to decellularize tissues for use in vascular tissue engineering.

Small diameter grafts (< 6 mm) derived from synthetic materials have shown poor clinical performance as they continue to exhibit low patency and high rates of thrombus formation. Additionally, studies have shown that they are highly susceptible to intimal hyperplasia at the sites of anastomosis[67,68]. The current challenges facing synthetic grafts may be attributed to the mismatches in mechanical properties — specifically compliance — between the native and synthetic vessels and the surface chemistry that governs important cellular functions such as infiltration and proliferation[69,70]. Alternatively, decellularization of biological tissues yields scaffolds that closely resemble native ECM in composition and micro-anatomical structure, and are therefore more suitable for vascular grafting. As the removal of cellular material is typically accomplished by physical or chemical treatments, enzymatic agents or a combination thereof, the process involves steps that may affect the structure and/or composition of the ECM. Despite such drawbacks, decellularization protocols are more effective than synthetic biomaterial strategies in producing scaffolds mimicking the composition and structure of native ECM. Consequently, many research groups have been investing time and effort in developing decellularization strategies that maintain the biochemical composition and structural integrity of native ECM, thereby preserving its function and suitability as vascular implant.

A standard decellularization approach, in general, involves the extraction of tissues through dissection, which is followed by with a suitable buffer and treatment with antibiotic and antimicrobial agents (penicillin, streptomycin and amphotericin B) in order to keep the tissues hydrated and resistant to bacterial growth. The tissues may then be subjected to physical treatments, detergents and/or enzymatic agents to remove cellular content and chromosomal DNA. Subsequently, the decellularized scaffolds may be seeded with cells, or functionalized with biological factors and implanted *in vivo*. In an attempt to optimize decellularization and ensure consistency of the final product, various groups have opted to automate the process by developing relatively simple devices that integrate all the desired chemical and/or physical processes and may involve several cycles and the use of different solvents[70–72]. These steps are described in more detail below.

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Physical treatments

Physical treatments are intended to lyse cells by releasing cytoplasmic material, however, they tend to be insufficient at removing all the immunogenic content, which is why they are oftentimes coupled with chemical detergents and/or enzymatic agents to remove remaining cellular debris. Common physical treatments involve sonication, pressure, shear forces, freezing and thawing[73]. Hypotonic and hypertonic solutions have also been found to be effective at mediating the cell lysing process[71]. Azhim and coworkers have performed studies on decellularization of porcine aorta via sonication at the luminal side while immersed in 2% SDS (sodium dodecyl sulfate) solution[74]. Their findings showed that sonication at 15 W removed their DNA content with insignificant change in mechanical properties[75]. Despite the predominant tendency toward chemical washes and enzymatic digestion processes, some groups claim to have developed novel physical decellularization approaches that do not require the action of harsh chemicals or enzymes[76], thereby preserving the integrity of the tissues. The experimental setup involves suspending aortic porcine blood vessels in saline solutions followed by exposure to high pressure, under temperature control to avoid freezing that may cause rupture of collagen fibers. Good temperature-control resulted in material with significantly less DNA content, better preserved ECM structure, and superior mechanical properties. No inflammatory response was observed upon implantation and the lumen was repopulated by the host's endothelial cells, rendering the grafts patent for up to 24 weeks *in vivo*.

Chemical treatments

A wide variety of chemical treatments have been reported in the literature ranging from alkaline and acidic solutions to ionic and non-ionic detergents[73]. Each of these detergents has their own unique way of altering the structure and components of the cells as well as the three-dimensional structure of the ECM. Acid and alkaline-based treatments are used to dissociate cell membranes and intracellular organelles. Acid solutions, in particular, have the potential to denature and solubilize nucleic acids. Nevertheless, drastic changes in pH affect the morphology and functionality of biological tissues by disrupting the surface chemistry and solubilizing free amine residues and activity of growth factors and glycosaminoglycans. The same effect is observed when using ionic detergents, such as sodium deoxycholate (DCA) and SDS. Therefore, these detergents are used in moderate concentrations.

On the other hand, non-ionic detergents like Triton X-100, are superior in preserving the architecture of the ECM since they mostly target lipid-lipid interactions, but they are not as efficient in removing DNA. Zwitterionic detergents, which exhibit the properties of both ionic and non-ionic detergents are harsher to the ECM structure relative to Triton X-100, but they do not lead to significant loss of mechanical properties. The most popular zwitterionic detergent that has been employed to decellularize arteries is 3-[(3 cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Both Triton X-100 and CHAPS elute the glycosaminoglycans present in biological tissues[71]. Finally, EDTA and EGTA have also been included in decellularization protocols to facilitate cell removal by chelating calcium and magnesium and disrupt cell-cell and cell-matrix adhesion.

Enzymatic treatment

Enzymes are, without a doubt, a powerful decellularization tool due to their high specificity and can be used to digest nucleic acids (DNA and RNA) or to remove unwanted ECM residue. Trypsin and nucleases are consistently employed in decellularization protocols. Fetal bovine serum (FBS) is a good source of nucleases. In fact, incubation of decellularized tissues in FBS-containing media results in low levels of β-actin, major histocompatibility complex (MHC) and DNA content[77].

Combination treatments

The combination of physical, chemical and enzymatic treatments may be required for optimal decellularization and may also reduce the need of concentrated chemicals or severe agitation. In one study, Gui and coworkers successfully developed functional, small-diameter, tissue engineered vessels derived from human umbilical cords, using a novel decellularization approach based on a combination of zwitterionic and ionic detergents, and enzymatic treatment[78]. The two-step decellularization process involved treatment with CHAPS buffer, followed by treatment with SDS. The samples were further incubated in endothelial growth media-2 (EGM-2) containing 12% fetal bovine serum with the required enzymatic cocktail to digest unwanted cellular debris. This strategy produced promising results as the decellularized grafts maintained their ECM content and promoted adhesion of human umbilical endothelial cells (HUVECs) *in vitro*. Upon implantation into nude rats, these cell-free grafts exhibited less intimal hyperplasia as compared to non-decellularized control vessels and promoted smooth muscle cell adhesion to the vascular wall and robust collagen matrix deposition. However, patency was poor (only 30% at 4 weeks) as these decellularized implants were thrombosed by 8 weeks postoperatively, most likely due to lack of an endothelial monolayer.

Another strategy for decellularizing ovine arteries employed treatment with 0.05% Trypsin, 0.02% EDTA and 1% antibiotic/antimycotic (AA) in PBS, followed by treatment with 1% Triton X-100, 1% AA and enzymatic treatment with DNase and RNase[79]. The treated arteries contained negligible amount of DNA and were completely devoid of laminin. On the other hand, the ECM structure and collagen and fibronectin content were preserved as shown by scanning electron microscopy and immunostaining. Interestingly, the decellularized arteries supported adhesion of human MSCs and exhibited mechanical properties e.g. tensile strength and compliance that were statistically similar to those of untreated control arteries. In contrast, while effective as a decellularization agent, 1% SDS had a negative effect on the mechanical properties of decellularized tissues, as shown in the same study[79].

An unconventional, yet quite successful, decellularization protocol for SIS was proposed by Cook Biotech (US patent #6,206,931). Porcine small intestines were isolated from fully grown adult pigs and subjected to disinfection with 0.5% (v/v) perpropionic acid, 0.3% (v/v) peracetic acid or 5% (w/v) chlorhexidine digluconate in ethanol. Further washes were carried out in hypotonic solution of hydrogen peroxide, which enhanced the removal of pathogens, pyrogens and blood constituents, while maintaining the porosity and structural integrity of the material. The submucosal layer was then isolated with the aid of a casing machine and used to prepare SIS-based vascular grafts, which showed slightly lower compliance than the average native carotid artery and significantly higher burst pressure than the average systolic pressure[80]. Such mechanical properties made them highly suitable for use as arterial replacement grafts.

Devices for decellularization

Decellularization devices have been developed to automate and accelerate the process while providing consistent results. One group developed a device to decellularize swine abdominal aorta and carotid arteries using DCA and DNase I treatments[69,81]. The device was composed of one reservoir and pump for each step of fluid exchange, a decellularization chamber and a pump to draw fluids into a waste container. Process parameters such as fluid flow rates, temperature and shaking rate were controlled by the control unit and the whole process was repeated for a total of 4 cycles. The resulting decellularized arteries exhibited well preserved ECM structure and similar Young's modulus and UTS (ultimate tensile strength) as native arteries. However, some residual DNA was still present in the tissues. A subsequent study optimized the device further to provide longitudinal and radial strain under sterile conditions using two pumps, a heat exchanger

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and a microcontroller-based unit[70]. Vascular grafts produced with this device were seeded with human iliac artery endothelial cells (hIAEC), and implanted in the iliac arteries of pigs where they remained patent for up to 70 days post-implantation. Interestingly, hIAEC were replaced by host endothelial cells and smooth muscle cells were observed to migrate from the adventitia to the medial layer of the vessel wall.

Another approach utilized a perfusion-driven process that could successfully decellularize tissues using low concentrations of chemicals and short exposure times[71]. Vessels harvested from the placenta were attached to a perfusion capillary and assembled to a recirculation system with a peristaltic pump, which allowed for application of reverse osmotic pressure for cell lysis. The process started with cell lysis via osmotic pressure using hypotonic (0.4% NaCl) and hypertonic (1.2% NaCl) saline solutions, followed by washes with a solution containing 1% Triton X-100 and 0.02% w/w EDTA in PBS and treatment with DNase I and 0.1% w/v of peracetic acid (PAA) prior to being stored at 4°C in PBS. The process preserved the collagen and glycosaminoglycan (GAG) content, thereby promoting endothelial cell attachment and preserving the mechanical properties (elastic modulus and UTS) of the decellularized grafts. Finally, application of pulsatile force during decellularization of human umbilical cords significantly reduced the time required for complete removal of cellular material (only 3 hours), but *in vivo* performance was not determined[72].

Decellularization of cell-based tissue engineered vascular grafts

In addition to native tissues, some groups developed processes to decellularize tissue engineered vascular grafts. Similar to prior work with skin tissue substitutes (Dermagraft, Advanced Tissue Sciences[82,83]), Humacyte Inc. decellularized tissue engineered vascular grafts (Lifeline) that have been used recently in clinical trials[63] (IND#15263, FDA). To this end, they engineered vascular tissues using polyglycolic acid scaffolds that were populated with banked human smooth muscle cells and cultured in bioreactors for 7–10 weeks, long enough time to produce adequate amounts of ECM and remodel the polymeric scaffold to a composition that closely resembles the native vessel wall. The grafts were then decellularized and were either implanted directly to replace large diameter grafts[62]; or following endothelialization of their lumen to replace small diameter grafts, which are known to occlude more readily[39,84]. These grafts were also implanted as arteriovenous fistulas for use in dialysis patients. In the interim report[63], the primary patency rate at 12 and 24 months post-implantation was only 28% and 15%, respectively, requiring surgical intervention to restore patency. Subsequently, the secondary patency rate remained high at 80% for 24 months, enabling long-term clinical use of these grafts.

This technique has also been employed by another group that employed fibrin hydrogels as scaffolds[29,85]. These scaffolds were seeded with allogeneic ovine dermal fibroblasts, which were integrated into the fibrin gels during fibrinogen polymerization with thrombin. After static culture for 2 weeks, these were transferred to a custom-designed pulsatile bioreactor for 5 weeks to enable graft maturation. The resultant tubes were then decellularized with SDS, and Triton X-100 and subjected to nuclease treatments. These decellularized fibrin-based grafts were implanted into lambs as pulmonary artery substitutes and the animals were allowed to grow to adulthood (1 year). The results showed that the volume and diameter of the implanted vessel increased with the size of the host, indicating somatic growth potential of these grafts[86].

This strategy obviates the need for autologous cells and can provide off-the-shelf grafts for clinical use. However, the costs and processing time associated with cell isolation and banking, bioreactor culture and decellularization is significant, necessitating development of strategies that eliminate the need for cells and bioreactors. Such a strategy may entail building "neo-vessels" *in situ* starting with cell-free grafts. This alternative approach may require functionalization of the lumen in order to prevent occlusion and attract host endothelial cells to ensure long-term patency. In addition, functionalization may attract the patients' own cells to form the smooth muscle medial layers required for contractile function.

FUNCTIONALIZATION STRATEGIES

The major cause of failure in synthetic and biomaterial-based engineered vessels is occlusion by formation of thrombus[3]. It has long been known that the presence of certain peptide sequences e.g. REDV (Arg-Glu-Asp-Val) promote a non-thrombogenic surface and at the same time favor endothelial cell attachment, spreading and proliferation[87]. Prevention of thrombus formation has since then been attempted mostly by heparin coating or bonding that activates anti-thrombin II (ATII), thereby inhibiting the thrombin clotting cascade[60,88]. Previous studies used heparin that was ionically or electrostatically bound to the polymer and was designed to be released over time in order to maintain patency. In one study, PCL and Chitosan (positively charged) were co-electrospun, in order to make a scaffold with net positive charge so that it binds negatively charged heparin[89]. Once implanted into the rat abdominal aorta, these grafts remained patent for 1 month, most likely due to the elution of heparin from the

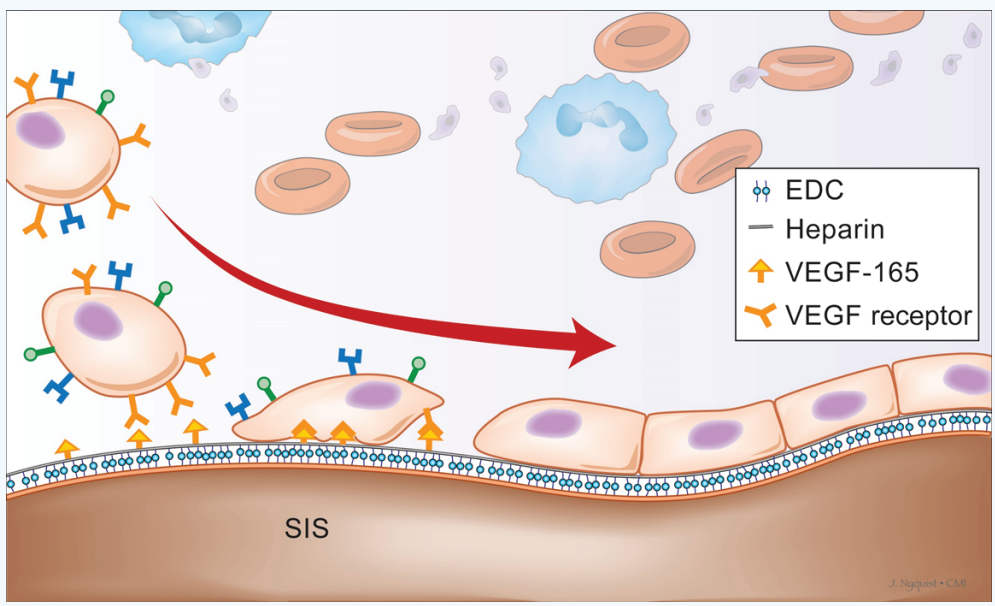

Figure 1 Schematic of cell capture on the lumen of vascular grafts decorated with heparin and heparin-bound VEGF. This figure is used with permission from *Biomaterials*[46].

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Table 1 Recent tissue engineering strategies employed for development of acellular vascular grafts and their application in animal models/clinical trials.

Study	Model	Site of implantation	Comments	Reference
Implantation of PCL-PLA polymer	Human	Pulmonary artery	Successful	5
Self-assembled and devitalized Lifeline graft	Human	AV shunt-brachial to axillary	Endothelial cells needed prior to implantation	6
Endothelialized versus unseeded PTFE	Human	Infrainguinal	Endothelialized grafts perform better	15
Endothelialized versus unseeded dacron	Canine	Thoraco-abdominal bypass	Endothelialized grafts performed better	16
Fibrin-based grafts, decellularized prior to implantation	Ovine	Femoral artery	No need for cell seeding	85
Biotube generated by subcutaneous implantation of silicone rods	Rabbit	Small caliber grafts	Biotubes mostly consisted of autologous fibroblasts	31
TEVGs are grown with SMC in bioreactor and decellularized	Porcine	Carotid	EC or EPC seeding still required	39
Collagen-based acellular grafts	Rabbit	Arterial bypass	Clotting appears in smaller caliber grafts	49
Fast degrading elastomer	Rat	Abdominal aorta	Neoartery formed in 3 months	59
Decellularized vessel with heparin and VEGF	Canine	Carotid artery	Not patent with heparin alone	60
Growing lamb model, decellularized cultured fibrin	Ovine	Pulmonary artery	Graft grows along with patient	98
Humacyte grafts	Human	Dialysis shunt	Primary patency was not high, but secondary patency was achieved	63
VEGF-heparin functionalized SIS	Ovine	Carotid artery	High primary patency, with complete integration into host	50
Knitted polyester with SDF1α and Fibronectin	Ovine	Carotid artery	Stem cell mobilization into graft maintained patency	94

graft[89]. A similar approach was used with a different composite material made of PCL, polyvinyl alcohol (PVA) and gelatin, in which the fibers degraded to release heparin *in vivo* in a controlled manner[90]. Another biodegradable and positively charged material, polymerized allylamine (PAA) has also been used to bind heparin electrostatically, promoting attachment and physiological function of HUVECs as evidenced by release of NO and prostaglandin[89].

While these studies have shown short-term patency, others have proposed that covalent bonding of heparin on the lumen may be preferred due to concerns with heparin-induced thrombocytopenia (HIT), caused by high levels of heparin in the blood and prompting regulatory concerns with heparin-eluting grafts and stents[91]. Indeed, covalent bonding of heparin to grafts by cross-linking or amide linkages has been proven to be effective in maintaining patency in several models. Immobilization of heparin can be easily achieved by EDC chemistry or glutaraldehyde crosslinking[49,92]. Heparin with benzalkonium chloride complex could be bound onto the a decellularized intestinal collagen layer by EDC chemistry, which performed adequately as an interpositional graft in a rabbit artery model, but required an additional collagen layer in the lumen in order to attract endothelial cells for longer term patency[49]. Comparable studies utilized fibronectin in addition to covalent bonding of heparin[93], addition of gelatin or chitosan into the scaffold[92], addition of specific peptides and growth factors[94–96], and antibodies for capture of circulating progenitor cells[52]. However, binding of cells to antibodies blocked the function of the targeted receptor, thereby reducing the function of the newly-formed endothelium[52]. Recently, poly(carboxybetaine methacrylate) or PCMBA was functionalized with a peptide that is known to capture and support the growth and spread of EPC but not mature endothelial cells such as HUVECs[95].

Similarly, growth factors such as stromal derived factor-1α (SDF-1α) and VEGF have been used to capture EPCs. SDF-1α has been used in two recent studies to capture EPCs, after implantation in the rat aorta[96] or in the sheep carotid artery[94]. VEGF has also been shown to capture EC even under flow[30,50,55,60], suggesting that it may be efficient in endothelializing cell-free grafts. Interestingly, two *in vivo* studies, reported that immobilized heparin was insufficient in maintaining long term patency (>1 month) and required the presence of VEGF, which was bound via the heparin binding domain[50,60]. Our laboratory recently demonstrated the ability of heparin-bound, surface immobilized VEGF to capture endothelial cells (EC) under flow *in vitro*[56]. Subsequently, we employed this strategy to immobilize VEGF on the surface of a natural biomaterial, namely Small Intestinal Submucosa (SIS) to engineer a cell free vascular graft. Our A-TEV (A-cellular Tissue Engineered Vessel) was made with tubular laminated SIS, with heparin conjugated via EDC-NHS chemistry and subsequent coating of VEGF, which is covalently bound to heparin via its HBD (heparin binding domain). See **Fig. 1**.

These A-TEVs have been tested in a preclinical ovine carotid model for up to 3 months[50]. To date we have tested n = 25 sheep with 92% patency. A functional confluent endothelium was formed in the lumen of these grafts as early as 1 month post-implantation, as evidenced by scanning electron microscopy and immunohistochemistry. Remodeling also occurred within the host, through an inflammation-mediated macrophage invasion that was shown to be necessary for host-cell infiltration, remodeling and ultimately, development of biological function[3,40–42]. Although SIS is porcine in its origin, the nucleic acid content remaining after processing is negligible (< 0.2 ng/mg; see Patent #6,206,931, COOK Biotech). Therefore, macrophage invasion is most likely part of the normal wound healing response that may be facilitated by the structure and porosity of this natural scaffold[97]. In addition to the capability for self-endothelialization, the vascular wall was populated by host smooth muscle cells, which were functional and responded to vaso-active agonists. After 3 months, the grafts not only resembled the native carotid in structure and function, but also in mechanical properties such as mechanical strength and compliance.

CONCLUSIONS

Overall, development of strategies to engineer functional and implantable, cell-free vascular grafts may overcome many of the issues associated with the presence of cells, including immune rejection of allogeneic cells,

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challenges associated with senescence and expansion of autologous cells (especially from older patients), long-culture times in bioreactors, and the high cost of cellular therapies. Since the feasibility of these grafts has already been demonstrated in large animal models, the field will gain significant momentum by successful testing of these grafts in human clinical trials.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health grants, R01 HL086582 (S.T.A., D.D.S) and R43 HL134439 (D.D.S., S.T.A).

DISCLOSURE

All authors declare financial interest in Angiograft LLC.

REFERENCES

1. Mozaffarian, D. *et al*. Heart disease and stroke statistics — 2015 update: A report from the American Heart Association. *Circulation* **131**, e29–322 (2015).
2. Huang, A.H. & Niklason, L.E. Engineering of arteries *in vitro*. *Cell. Mol. Life Sci*. **71**, 2103–2118 (2014).
3. Naito, Y. *et al*. Vascular tissue engineering: Towards the next generation vascular grafts. *Adv. Drug Deliv. Rev*. **63**, 312–323 (2011).
4. Peck, M., Gebhart, D., Dusserre, N., McAllister, T.N. & L'Heureux, N. The evolution of vascular tissue engineering and current state of the art. *Cells Tissues Organs* **195**, 144–158 (2012).
5. Shin'oka, T., Imai, Y. & Ikada, Y. Transplantation of a tissue-engineered pulmonary artery. *N. Engl. J. Med*. **344**, 532–533 (2001).
6. Wystrychowski, W. *et al*. Case study: First implantation of a frozen, devitalized tissue-engineered vascular graft for urgent hemodialysis access. *J. Vasc. Access* **12**, 67–70 (2011).
7. Zilla, P., Bezuidenhout, D., & Human, P. Prosthetic vascular grafts: Wrong models, wrong questions and no healing. *Biomaterials* **28**, 5009–5027 (2007).
8. Canham, P.B., Finlay, H.M. & Boughner, D.R. Contrasting structure of the saphenous vein and internal mammary artery used as coronary bypass vessels. *Cardiovasc. Res*. **34**, 557–567 (1997).
9. Konig, G. *et al*. Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. *Biomaterials* **30**, 1542–1550 (2009).
10. Conte, M.S. The ideal small arterial substitute: A search for the Holy Grail? *FASEB J*. **12**, 43–45 (1998).
11. Berger, K., Sauvage, L.R., Rao, A.M. & Wood, S.J. Healing of arterial prostheses in man: Its incompleteness. *Ann. Surg*. **175**, 118–127 (1972).
12. Crombez, M. *et al*. Improving arterial prosthesis neo-endothelialization: Application of a proactive VEGF construct onto PTFE surfaces. *Biomaterials* **26**, 7402–7409 (2005).
13. Mansfield, P.B., Wechezak, A.R. & Sauvage, L.R. Preventing thrombus on artificial vascular surfaces: True endothelial cell linings. *Trans. Am. Soc. Artif. Intern. Organs* **21**, 264–272 (1975).
14. Schneider, P.A., Hanson, S.R., Price, T.M. & Harker, L.A. Preformed confluent endothelial cell monolayers prevent early platelet deposition on vascular prostheses in baboons. *J. Vasc. Surg*. **8**, 229–235 (1988).
15. Deutsch, M., Meinhart, J., Fischlein, T., Preiss, P. & Zilla, P. Clinical autologous *in vitro* endothelialization of infrainguinal ePTFE grafts in 100 patients: A 9-year experience. *Surgery* **126**, 847–855 (1999).
16. Graham, L.M. *et al*. Immediate seeding of enzymatically derived endothelium in Dacron vascular grafts. Early experimental studies with autologous canine cells. *Arch. Surg*. **115**, 1289–1294 (1980).
17. Herring, M. *et al*. Patency in canine inferior vena cava grafting: Effects of graft material, size, and endothelial seeding. *J. Vasc. Surg*. **1**, 877–887 (1984).
18. Ombrellaro, M.P., Stevens, S.L., Kerstetter, K., Freeman, M.B. & Goldman, M.H. Healing characteristics of intraarterial stented grafts: Effect of intraluminal position on prosthetic graft healing. *Surgery* **120**, 60–70 (1996).
19. Foley, S.R. *et al*. A comprehensive study of ovine haemostasis to assess suitability to model human coagulation. *Thromb. Res*. **134**, 468–473 (2014).
20. Weinberg, C.B. & Bell, E. A blood vessel model constructed from collagen and cultured vascular cells. *Science* **231**, 397–400 (1986).
21. L'Heureux, N., McAllister, T.N. & de la Fuente, L.M. Tissue-engineered blood vessel for adult arterial revascularization. *N. Engl. J. Med*. **357**, 1451–1453 (2007).
22. Niklason, L.E. *et al*. Functional arteries grown *in vitro*. *Science* **284**, 489–493 (1999).
23. Zhang, Y. *et al*. [Primary research of reconstruction of complete biological tissue engineered blood vessel]. Sheng wu yi xue gong cheng xue za zhi [*J. Biomed. Eng*.] **22**, 448–451 (2005) (in Chinese).
24. McKee, J.A. *et al*. Human arteries engineered *in vitro*. *EMBO Rep*. **4**, 633–638 (2003).
25. Poh, M. *et al*. Blood vessels engineered from human cells. *Lancet* **365**, 2122–2124 (2005).
26. Han, J. *et al*. Nanog reverses the effects of organismal aging on mesenchymal stem cell proliferation and myogenic differentiation potential. *Stem Cells* **30**, 2746–2759 (2012).
27. Gong, Z. & Niklason, L.E. Small-diameter human vessel wall engineered from bone marrow-derived mesenchymal stem cells (hMSCs). *FASEB J*. **22**, 1635–1648 (2008).
28. Mirza, A. *et al*. Undifferentiated mesenchymal stem cells seeded on a vascular prosthesis contribute to the restoration of a physiologic vascular wall. *J. Vasc. Surg*. **47**, 1313–1321 (2008).
29. Syedain, Z.H., Meier, L.A., Bjork, J.W., Lee, A. & Tranquillo, R.T. Implantable arterial grafts from human fibroblasts and fibrin using a multi-graft pulsed flow-stretch bioreactor with noninvasive strength monitoring. *Biomaterials* **32**, 714–722 (2011).
30. Gui, L. *et al*. Construction of tissue-engineered small-diameter vascular grafts in fibrin scaffolds in 30 days. *Tissue Eng. Part A* **20**, 1499–1507 (2014).
31. Ma, N. *et al*. Development of the novel biotube inserting technique for acceleration of thick-walled autologous tissue-engineered vascular grafts fabrication. *J. Mater. Sci. Mater. Med*. **22**, 1037–1043 (2011).
32. Watanabe, T., Kanda, K., Ishibashi-Ueda, H., Yaku, H. & Nakayama, Y. Autologous small-caliber "biotube" vascular grafts with argatroban loading: A histomorphological examination after implantation to rabbits. *J. Biomed. Mater. Res. B Appl. Biomater*. **92**, 236–242 (2010).
33. Han, J., Liu, J.Y., Swartz, D.D. & Andreadis, S.T. Molecular and functional effects of organismal ageing on smooth muscle cells derived from bone marrow mesenchymal stem cells. *Cardiovasc. Res*. **87**, 147–155 (2010).
34. Liu, J.Y., Peng, H.F. & Andreadis, S.T. Contractile smooth muscle cells derived from hair-follicle stem cells. *Cardiovasc. Res*. **79**, 24–33 (2008).
35. Liu, J.Y. *et al*. Functional tissue-engineered blood vessels from bone marrow progenitor cells. *Cardiovasc. Res*. **75**, 618–628 (2007).
36. Zhao, Y. *et al*. The development of a tissue-engineered artery using decellularized scaffold and autologous ovine mesenchymal stem cells. *Biomaterials* **31**, 296–307 (2010).
37. Row, S. *et al*. Arterial grafts exhibiting unprecedented cellular infiltration and remodeling *in vivo*: The role of cells in the vascular wall. *Biomaterials* **50**, 115–126 (2015).
38. Neff, L.P. *et al*. Vascular smooth muscle enhances functionality of tissue-engineered blood vessels *in vivo*. *J. Vasc. Surg*. **53**, 426–434 (2011).
39. Quint, C. *et al*. Decellularized tissue-engineered blood vessel as an arterial conduit. *Proc. Natl. Acad. Sci. U.S.A*. **108**, 9214–9219 (2011).
40. Roh, J.D. *et al*. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *Proc. Natl. Acad. Sci. U.S.A*. **107**, 4669–4674 (2010).
41. Hibino, N. *et al*. A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. *FASEB J*. **25**, 4253–4263 (2011).
42. Naito, Y. *et al*. Characterization of the natural history of extracellular matrix production in tissue-engineered vascular grafts during neovessel formation. *Cells Tissues Organs* **195**, 60–72 (2012).
43. Peng, H., Schlaich, E.M., Row, S., Andreadis, S.T. & Swartz, D.D. A novel ovine *ex vivo* arteriovenous shunt model to test vascular implantability. *Cells Tissues Organs* **195**, 108–121 (2012).
44. Cui, X. & Boland, T. Human microvasculature fabrication using thermal inkjet printing technology. *Biomaterials* **30**, 6221–6227 (2009).
45. Zhao, L., Lee, V.K., Yoo, S.S., Dai, G. & Intes, X. The integration of 3-D cell printing and mesoscopic fluorescence molecular tomography of vascular constructs within thick hydrogel scaffolds. *Biomaterials* **33**, 5325–5332 (2012).
46. Hewett, P.W. & Murray, J.C. Human microvessel endothelial cells: Isolation, culture and characterization. *In Vitro Cell Dev. Biol. Anim*. **29A**, 823–830 (1993).
47. Foresta, C. *et al*., Blood levels, apoptosis, and homing of the endothelial progenitor cells after skin burns and escharectomy. *J. Trauma* **70**, 459–465 (2011).
48. Guven, H., Shepherd, R.M., Bach, R.G., Capoccia, B.J. & Link, D.C. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J. Am. Coll. Cardiol*. **48**, 1579–1587 (2006).
49. Huynh, T. *et al*. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. *Nat. Biotechnol*. **17**, 1083–1086 (1999).
50. Koobatian, M.T. *et al*. Successful endothelialization and remodeling of a cell-free small-diameter arterial graft in a large animal model. *Biomaterials* **76**, 344–358 (2016).
51. Custodio, C.A. *et al*. Photopatterned antibodies for selective cell attachment. *Langmuir* **30**, 10066–10071 (2014).
52. Markway, B.D. *et al*. Capture of flowing endothelial cells using surface-immobilized anti-kinase insert domain receptor antibody. *Tissue Eng. Part C Methods* **14**, 97–105 (2008).
53. Anderson, S.M., Siegman, S.N. & Segura, T. The effect of vascular endothelial growth factor (VEGF) presentation within fibrin matrices on endothelial cell branching. *Biomaterials* **32**, 7432–7443 (2011).
54. Gui, L., Muto, A., Chan, S.A., Breuer, C.K. & Niklason, L.E. Development of decellularized human umbilical arteries as small-diameter vascular grafts. *Tissue Eng. Part A* **15**, 2665–2676 (2009).

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55. Sharon, J.L. & Puleo, D.A. Immobilization of glycoproteins, such as VEGF, on biodegradable substrates. *Acta Biomater.* **4**, 1016–1023 (2008).
56. Smith Jr., R.J., Koobatian, M.T., Shahini, A., Swartz, D.D. & Andreadis, S.T. Capture of endothelial cells under flow using immobilized vascular endothelial growth factor. *Biomaterials* **51**, 303–312 (2015).
57. Kusaba, A., Fischer 3rd, C.R., Matulewski, T.J. & Matsumoto, T. Experimental study of the influence of porosity on development of neointima in Gore-Tex grafts: A method to increase long-term patency rate. *Am. Surg.* **47**, 347–354 (1981).
58. Sterpetti, A.V., Hunter, W.J., Schultz, R.D. & Farina, C. Healing of high-porosity polytetrafluoroethylene arterial grafts is influenced by the nature of the surrounding tissue. *Surgery* **111**, 677–682 (1992).
59. Wu, W., Allen, R.A. & Wang, Y. Fast-degrading elastomer enables rapid remodeling of a cell-free synthetic graft into a neoartery. *Nat. Med.* **18**, 1148–1153 (2012).
60. Zhou, M. *et al.* Development and validation of small-diameter vascular tissue from a decellularized scaffold coated with heparin and vascular endothelial growth factor. *Artif. Organs* **33**, 230–239 (2009).
61. Hibino, N. *et al.* Late-term results of tissue-engineered vascular grafts in humans. *J. Thorac. Cardiovasc. Surg.* **139**, 431–436, 436 e431–432 (2010).
62. Dahl, S.L. *et al.* Readily available tissue-engineered vascular grafts. *Sci. Transl. Med.* **3**, 68ra69 (2011).
63. Lawson, J.H. *et al.* Bioengineered human acellular vessels for dialysis access in patients with end-stage renal disease: Two phase 2 single-arm trials. *Lancet* **387**, 2026–2034 (2016).
64. Kim, S.-H., Turnbull, J. & Guimond, S. Extracellular matrix and cell signalling: The dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J. Endocrinol.* **209**, 139–151 (2011).
65. Yu, Y., Alkhawaji, A., Ding, Y. & Mei, J. Decellularized scaffolds in regenerative medicine. *Octotarget* **7**, 58671–58683 (2016).
66. Gilbert, T.W., Sellaro, T.L. & Badylak, S.F. Decellularization of tissues and organs. *Biomaterials* **27**, 3675–3683 (2006).
67. Teebken, O.E. & Haverich, A. Tissue engineering of small diameter vascular grafts. *Eur. J. Vasc. Endovasc. Surg.* **23**, 475–485 (2002).
68. Ravi, S. & Chaikof, E.L. Biomaterials for vascular tissue engineering. *Regen. Med.* **5**, 107 (2010).
69. Pellegata, A.F. *et al.* Detergent-enzymatic decellularization of swine blood vessels: Insight on mechanical properties for vascular tissue engineering. *BioMed. Res. Int.* **2013**, 918753 (2013).
70. Pellegata, A.F. *et al.* Arterial decellularized scaffolds produced using an innovative automatic system. *Cells Tissues Organs* **200**, 363–373 (2014).
71. Schneider, K.H. *et al.* Decellularized human placenta chorion matrix as a favorable source of small-diameter vascular grafts. *Acta Biomater.* **29**, 125–134 (2016).
72. Chen, S., Li, J. & Dong, P. Utilization of pulsatile flow to decellularize the human umbilical arteries to make small-caliber blood vessel scaffolds. *Acta Cardiol. Sin.* **29**, 451–456 (2013).
73. Boccafoschi, F. *et al.* Decellularized biological matrices: An interesting approach for cardiovascular tissue repair and regeneration. *J. Tissue Eng. Regen. Med.*, doi: 10.1002/term.2103 (2015).
74. Azhim, A., Yamagami, K., Muramatsu, K., Morimoto, Y. & Tanaka, M. The use of sonication treatment to completely decellularize blood arteries: A pilot study. In: *2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. (30 August — 3 September 2011), pp. 2468–2471.
75. Syazwani, N., Azhim, A., Morimoto, Y., Furukawa, K.S. & Ushida, T. Decellularization of aorta tissue using sonication treatment as potential scaffold for vascular tissue engineering. *J. Med. Biol. Eng.* **35**, 258–269 (2015).
76. Funamoto, S. *et al.* The use of high-hydrostatic pressure treatment to decellularize blood vessels. *Biomaterials* **31**, 3590–3595 (2010).
77. Gui, L., Chan, S.A., Breuer, C.K. & Niklason, L.E. Novel utilization of serum in tissue decellularization. *Tissue Eng. Part C Methods* **16**, 173–184 (2010).
78. Gui, L., Muto, A., Chan, S.A., Breuer, C.K. & Niklason, L.E. Development of decellularized human umbilical arteries as small-diameter vascular grafts. *Tissue Eng. Part A* **15**, 2665–2676 (2009).
79. Mancuso, L., Gualerzi, A., Boschetti, F., Loy, F. & Cao, G. Decellularized ovine arteries as small-diameter vascular grafts. *Biomed. Mater.* **9**, 045011 (2014).
80. Roeder, R. *et al.* Compliance, elastic modulus, and burst pressure of small-intestine submucosa (SIS), small-diameter vascular grafts. *J. Biomed. Mater. Res.* **47**, 65–70 (1999).
81. Pellegata, A.F., Asnaghi, M.A., Zonta, S., Zerbini, G. & Mantero, S. A novel device for the automatic decellularization of biological tissues. *Int. J. Artif. Organs* **35**(3), 191–198 (2012).
82. Edmonds, M.E., Foster, A.V. & McColgan, M. 'Dermagraft': A new treatment for diabetic foot ulcers. *Diabet. Med.* **14**, 1010–1011 (1997).
83. Herndon, D.N. Multiple piece use of Dermagraft-TC. *J. Burn Care Rehabil.* **18**, S14-15 (1997).
84. Quint, C., Arief, M., Muto, A., Dardik, A. & Niklason, L.E. Allogeneic human tissue-engineered blood vessel. *J. Vasc. Surg.* **55**, 790–798 (2012).
85. Syedain, Z.H., Meier, L.A., Lahti, M.T., Johnson, S.L. & Tranquillo, R.T. Implantation of completely biological engineered grafts following decellularization into the sheep femoral artery. *Tissue Eng. Part A* **20**, 1726–1734 (2014).
86. Syedain, Z. *et al.* Tissue engineering of acellular vascular grafts capable of somatic growth in young lambs. *Nat. Commun.* **7**, 12951 (2016).
87. Hubbell, J.A., Massia, S.P., Desai, N.P. & Drumheller, P.D. Endothelial cell-selective materials for tissue engineering in the vascular graft via a new receptor. *Biotechnology (N. Y.)* **9**, 568–572 (1991).
88. Kwon, I.K. & Matsuda, T. Co-electrospun nanofiber fabrics of poly(L-lactide-co-epsilon-caprolactone) with type I collagen or heparin. *Biomacromolecules* **6**, 2096–2105 (2005).
89. Yang, Z., Tu, Q., Wang, J. & Huang, N. The role of heparin binding surfaces in the direction of endothelial and smooth muscle cell fate and re-endothelialization. *Biomaterials* **33**, 6615–6625 (2012).
90. Tan, Z., Wang, H., Gao, X., Liu, T. & Tan, Y. Composite vascular grafts with high cell infiltration by co-electrospinning. *Mater. Sci. Eng. C Mater. Biol. Appl.* **67**, 369–377 (2016).
91. Warkentin, T.E. Heparin-induced thrombocytopenia. *Curr. Opin. Crit. Care* **21**, 576–585 (2015).
92. Zhu, Y., Gao, C., Liu, X. & Shen, J. Surface modification of polycaprolactone membrane via aminolysis and biomacromolecule immobilization for promoting cytocompatibility of human endothelial cells. *Biomacromolecules* **3**, 1312–1319 (2002).
93. Sun, M., Deng, J. & Gao, C. The correlation between fibronectin adsorption and attachment of vascular cells on heparinized polycaprolactone membrane. *J. Colloid Interface Sci.* **448**, 231–237 (2015).
94. De Visscher, G., Mesure, L., Meuris, B., Ivanova, A. & Flameng, W. Improved endothelialization and reduced thrombosis by coating a synthetic vascular graft with fibronectin and stem cell homing factor SDF-1alpha. *Acta Biomater.* **8**, 1330–1338 (2012).
95. Li, Q. *et al.* Functionalization of the surface of electrospun poly(epsilon-caprolactone) mats using zwitterionic poly(carboxybetaine methacrylate) and cell-specific peptide for endothelial progenitor cells capture. *Mater. Sci. Eng. C Mater. Biol. Appl.* **33**, 1646–1653 (2013).
96. Yu, J. *et al.* The effect of stromal cell-derived factor-1alpha/heparin coating of biodegradable vascular grafts on the recruitment of both endothelial and smooth muscle progenitor cells for accelerated regeneration. *Biomaterials* **33**, 8062–8074 (2012).
97. Hiles, M.C., Badylak, S.F., Geddes, L.A., Kokini, K. & Morff, R.J. Porosity of porcine small-intestinal submucosa for use as a vascular graft. *J. Biomed. Mater. Res.* **27**, 139–144 (1993).

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