

REVIEW

Bioengineered vascular scaffolds: the state of the art

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To date, there is increasing clinical need for vascular substitutes due to accidents, malformations, and ischemic diseases. Over the years, many approaches have been developed to solve this problem, starting from autologous native vessels to artificial vascular grafts; unfortunately, none of these have provided the perfect vascular substitute. All have been burdened by various complications, including infection, thrombogenicity, calcification, foreign body reaction, lack of growth potential, late stenosis and occlusion from intimal hyperplasia, and pseudoaneurysm formation. In the last few years, vascular tissue engineering has emerged as one of the most promising approaches for producing mechanically competent vascular substitutes. Nanotechnologies have contributed their part, allowing extraordinarily biostable and biocompatible materials to be developed. Specifically, the use of electrospinning to manufacture conduits able to guarantee a stable flow of biological fluids and guide the formation of a new vessel has revolutionized the concept of the vascular substitute. The electrospinning technique allows extracellular matrix (ECM) to be mimicked with high fidelity, reproducing its porosity and complexity, and providing an environment suitable for cell growth. In the future, a better knowledge of ECM and the manufacture of new materials will allow us to “create” functional biological vessels – the base required to develop organ substitutes and eventually solve the problem of organ failure.

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INTRODUCTION

Autologous vessels for repairing damaged or malformed arteries or veins have found an increasingly widespread use. Often, these are employed in vascular surgery to make coronary bypasses, lower limb bypasses, arteriovenous shunts, or repair congenital defects of the pulmonary outflow tract. However, their use may be extended to vascular injuries, mainly caused by penetrating trauma secondary to improvised explosive devices and high-velocity gunshot wounds, common in wartime and civilian trauma (1, 2). Polytrauma patients usually require a shorter operative time to facilitate resuscitation and normalization of hypothermia, coagulopathy, and metabolic acidosis; a rapid successful repair of traumatic arterial lesions is crucial to

stop ongoing hemorrhage as well as to facilitate distal blood flow to the extremities. Currently, among autologous native vessels, the saphenous vein is the most used for small-diameter arterial wall replacement (1-3). This “ready-to-use” patch is highly tolerated by the immune system, but the availability of harvesting sites can be limited in patients with diffuse vascular disease or trauma. Furthermore, in critically injured patients, the physiologic insult incurred during the harvesting of the vein must be weighed against the use of an alternative patching agent.

To reduce operative times, a biologic conduit may offer a wonderful substitute for a vascular graft. Biologic patch materials currently in use are limited to prosthetic materials and autologous, allogenic or xenogenic (glutaraldehyde-fixed) pericardium (4-10). Unfortunately, these patches

have well-known limitations, including infection, thrombogenicity, calcification, foreign body reaction, and lack of growth potential (4-10). Artificial vascular grafts manufactured from synthetic materials, for example, polyester and expanded polytetrafluoroethylene (ePTFE), have also been routinely used to reconstitute the blood flow in patients with various cardiovascular disorders. Conventional grafts have clinically shown satisfactory durability; however, they still have several disadvantages, such as thrombogenicity, late stenosis and occlusion from intimal hyperplasia (especially in small caliber grafts), susceptibility to infection, pseudoaneurysm formation, and lack of growth potential (3, 11-13).

Vascular tissue engineering has emerged as one of the most promising approaches to producing mechanically competent vascular substitutes. Clinical demands for a ready-to-use, reabsorbable vascular patch cover different fields of surgery: heart surgery (treatment of ischemic heart diseases), vascular surgery (distal revascularization of lower limbs), neurosurgery (repair of intracranial arteries), pediatric vascular surgery, and rare cases of microsurgical reconstruction after severe hand traumas.

Vascular tissue engineering: the biological bases

Completely bioresorbable vascular conduits capable of inducing regeneration and growth of a new vascular wall may overcome the limitations of contemporary artificial patches that are nonviable, artificial, or allogenic materials lacking the capacity of growth, repair, and remodeling. These intrinsic properties currently limit the long-term function of the artificial patch, creating a substantial burden of patch failure and related reoperations. The need for a prosthetic patch that performs as a vascular regeneration guide has led investigators to pursue many avenues in tissue engineering, starting from two axioms: (1) the native vascular function, particularly the mechanical function, depends on structure as much as it depends on composition, and (2) the tissue-engineered vessel should serve as a functional remodeling template, providing function during the remodeling, and, at the same time, a template for the alignment of the remodeled tissue. Therefore, from implantation to complete absorption, an ideal vascular scaffold should reproduce the typical mechanical and biological features of a natural vessel: it should have anti-inflammatory and anti-thrombotic properties, and minimize intimal hyperplasia; it should be compliant but, at the same time, resistant

to shear stress and blood pressure; it should avoid blood leakage or hemorrhage; and finally, it should be suturable, allowing the needle to pass, without lesions or lacerations. Apart from specific physical properties, tissue engineering strategies rely on the ability of cells (transplanted or host) to adhere to and migrate within the construct, and to remodel its composition and/or structure. Of course, the scaffold must be replaced by a functional cell-derived extracellular matrix (ECM) on the same time scale.

Remodeling also determines the ultimate mechanical and biological properties. Scientists in the field of tissue engineering are now applying the principles of cell biology, material science, and biomedical engineering to create biological substitutes that will restore and maintain normal function in diseased and injured tissues/organs (14-16). It has been demonstrated that tissue morphogenesis is heavily influenced by interactions between cells and ECM during normal tissue development. While simple polymeric scaffolds that have been used in the past provide architectural support for a neo-tissue development, they do not adequately mimic the complex interactions between tissue-specific cells and tissue-specific ECM that promote a functional tissue regeneration. Thus, future advances in tissue engineering will depend on the development of novel scaffolding systems that actively modulate cell behaviors to build healthy, vital tissue.

MATERIALS AND METHODS IN VASCULAR TISSUE ENGINEERING

Decellularized tissue matrices

Due to a dearth of methods for *de novo* construction of a complex structure capable of imitating natural ECM, decellularized tissue matrices are currently considered an ideal scaffolding system due to their structural and mechanical similarity to native tissues and because they contain tissue-specific ECM proteins that remain after decellularization. Interestingly, Yoo and colleagues reviewed decellularization techniques and possible methods for using these decellularized matrices for whole organ engineering (17). Decellularized matrices have already been used for many tissue engineer applications. Kim et al developed a composite scaffold that was composed of a collagen matrix derived from decellularized porcine bladder submucosal matrix and synthetic poly(lactide-co-glycolide)

(PLGA) polymer (18). This composite scaffold provides a microenvironment that facilitates osteogenic differentiation of amniotic fluid-derived stem cells. Furthermore, Choi et al investigated the interactions between the ECM environment and human corneal endothelial cells to improve cell proliferation and function (19). Following this marked path, vascular tissue engineering has experimented with new decellularized constructs. Thanks to studies like those just mentioned, which have investigated the interactions between the ECM environment and mesenchyme-derived cells, it has been possible to develop tubular decellularized matrices supporting the growth of a neointima with endothelial and smooth muscle cells (20). Decellularized arteries, from allogenic and xenogenic sources, are attractive scaffolds for tissue-engineered vascular grafts (TEVGs) thanks to their mechanical and biological properties (20). However, these natural scaffolds are limited by the lack of precise manufacturing control of the physical and mechanical properties as well as by problems with inflammation and calcification (21). The search for a viable off-the-shelf, small-diameter vascular graft that can match autograft performance in terms of mechanical properties, cell compatibility, and vascular healing has been the focus of many research efforts, but, to date, has remained an elusive target.

Electrospinning

Another approach in the development of biomimetic structures has been suggested by the textile industry. To date, electrospinning is a chemical engineering technique widely used as a fabrication method to generate nanofibers with diameters ranging from 100 nm or less to several micrometers, for various textile applications. Although many other scaffold fabrication technologies are routinely used in tissue engineering, only a few provide constructs with critical similarities to natural ECM like those that electrospinning can provide. Electrospinning has become a popular alternative fabrication method during the last two decades, as it can be applied to many disciplines and it is relatively simple and inexpensive. Shin and colleagues reviewed the current approaches to the development of electrospun nanofibers as a scaffold for tissue engineering applications (22). These electrospun scaffolds can also be functionalized by adding biochemical and mechanical cues to enhance cellular interactions for tissue engineering applications.

Levorson et al described their work with fabrication and characterization of multi-scale electrospun scaffolds for cartilage regeneration (23). These scaffolds were able to maintain scaffold cellularity in serum-free conditions as well as aid the deposition of glycosaminoglycans. Xu et al presented a novel, controllable, dual protein delivery system through electrospun fibrous scaffolds with different hydrophilicities (24). Starting from these ideas, Zhang et al built up a double-layered electrospun membrane composed of chitosan hydrogel/poly(ethylene glycol)-*b*-poly(l-lactide-co-caprolactone) (PELCL) electrospun membrane loaded with VEGF (inner layer) and emulsion/PELCL loaded with PDGF (outer layer) to regulate proliferation of vascular endothelial cells and vascular smooth muscle cells (25). Only a rapid endothelialization along the lumen of grafts followed by proliferation of vascular smooth muscle cells around the exterior can prevent thrombosis and guarantee the right vascular compliance. Further applications of electrospinning technique are described in the following paragraphs.

Cell-seeded scaffolds

To improve biocompatibility features and promote remodeling and reabsorption, materials are usually seeded with human mesenchyme-derived cells. The first clinical application of a tissue-engineered vascular patch with pretreatment of human bone marrow cells, was reported by Shin'oka (26). Likewise, tissue-engineered biodegradable materials with autologous cell seeding before patch repair, where a bioreactor culture system was used, have been well documented as potential cardiovascular patches (27-33). Oh and Lee reviewed hydrophilization of synthetic biodegradable polymeric scaffolds for improving cell/tissue compatibility (34). This technique has been considered a simple and effective approach to achieve desirable *in vitro* cell culture and *in vivo* tissue regeneration within the synthetic polymeric scaffolds. In a very recent, cutting-edge study, polycaprolactone (PCL)/polylactide (PLA) and PCL/PLA/polyethylenglycol (PEG) electrospun small caliber vascular grafts and ePTFE grafts of the same size have been pre-coated with blood, gelatine or fibronectin and seeded with endothelial cells from human term placenta. The best results have been obtained with fibronectin-coated PCL/PLA/PEG grafts. Here, the number of attached viable cells was 78% to 81% higher than fibronectin pre-treated ePTFE grafts. Cells attached to PCL/PLA/PEG grafts appeared in physiological cobblestone morphology.

Viability analysis showed a high cell viability of more than 98% (35).

The feasibility of creating a completely biodegradable vascular regenerating guide directly *in vivo*, without chemical and cellular preconditioning *in vitro*, is of great interest. This approach may overcome the main problems related to present techniques. Tissue-engineered grafts currently being investigated require an extended period of preparation, and thus they cannot be used in emergency situations (36-38). Furthermore, the prolonged duration of culture increases the risk of infection and raises costs in terms of personnel, equipment, and materials needed. In TEVGs autologous cell seeding and culture or growth with bioreactors before the operation is usually necessary to improve their antithrombogenicity and performance. Many strategies have been attempted to facilitate regeneration of autologous tissue on tissue-engineered materials. A major method is preoperative cellular manipulation (26, 39, 40). Kaushal and coworkers (39) demonstrated functional small diameter neovessels produced with endothelial progenitor cells. Another effective method is the use of a bioreactor (39, 41, 42). Campbell and associates (43) suggested that the host's own peritoneal cavity is an effective bioreactor and showed that it is feasible to grow vascular prostheses within it. Also, Hoerstrup and colleagues (42) demonstrated the growth capacity of cell-seeded TEVGs grown *in vitro* for 21 days under biomimetic conditions.

Although the utility of these pretreatments is fully recognized, their short comings, including invasiveness, risk of contamination, and the need for preparation time, have led to hesitation in their clinical use. For these reasons, Torikai et colleagues (44) used *in situ* cellularization as an alternative method, more suitable for clinical application because of its ready availability (33, 45). The patch they designed was composed of three layers: the interior (luminal side) was composed of knitted PGA compounded with collagen microsphere, the middle layer was PCL, and the exterior was composed of woven PLA. This PGA/PLA graft showed evidence of good *in situ* cellularization, including relatively early endothelialization and population with functioning SMCs, after implantation into porcine aorta. The graft was durable enough to withstand high-pressure conditions for at least 12 postoperative months, despite the presence of a residual PLA layer, and also demonstrated the potential to acquire intrinsic physiologic vascular function. Considering the established use of artificial grafts for aortic surgery and the need for small-diameter prostheses, the complete and

early endothelialization demonstrated by their TEVG was meaningful, particularly in the setting of small diameter vascular structures, such as peripheral arteries. The limitations of study were the lack of regenerated elastin and the residual PLA layers.

Notwithstanding these encouraging results, the *ex vivo* cell-seeding procedure is complicated, invasive, expensive, and can cause contaminations. To overcome these problems, the last few years have marked a substantial paradigm shift in design criteria for modern synthetic biomaterials, and materials equipped with molecular cues mimicking certain aspects of the structure or function of natural extracellular microenvironments have quickly been developed (46-50).

Synthetic biodegradable polymeric scaffolds

Takahashi et al developed a novel tissue-engineered patch made of PGA/PLA that showed constructive *in situ* remodeling by site-specific host cells without prior *ex vivo* cell seeding (51). Unfortunately, PGA can induce a local inflammatory response following patch repair (52). More recently, Giammona et al have reported the *in vivo* application on a murine animal model of two electrospun biodegradable materials, specifically designed to create tubular structures. In one case a α , β -Poly [(N-hydroxyethyl)-dl-aspartamide] (PHEA)-PLA mixture was co-spun with silk fibroin (Fibro-PHEA-PLA) by a parallel electrospinning process to obtain a scaffold with two different polymeric fibers. In the other case, PHEA-PLA was mixed with PCL (PCL-PHEA-PLA) to obtain a hybrid fibers scaffold. The *in vitro* assay showed fibroblast colonization in both materials. Histopathological findings showed that after implantation a neutrophilic reaction associated to colliquative necrosis was predominant, particularly for PCL-PHEA-PLA. Fibro-PCL-PHEA caused a non-organized stromal reaction (53). Pitarresi et al have improved PHEA-PLA by producing a new copolymer named a,b-poly(N-2-hydroxyethyl) (2-aminoethylcarbamate)-D, L-aspartamide-graft-poly(lactic acid) (PHEA-EDA-g-PLA) as a starting material for producing a fibrillar scaffold for vascular regeneration (54). Thanks to the presence of free amino and hydroxyl groups in PHEA-EDA-g-PLA, the electrospun scaffold has been bound to heparin, a known natural anticoagulant molecule (55). Through electrostatic interaction, heparin is also able to retain growth factors like basic fibroblast growth factor (bFGF) (56-58). For this reason, the presence of heparin in avascular grafts could prevent

thrombosis phenomena and improve cell attachment and proliferation. In this case, the scaffolds obtained have been able to allow the formation of new endothelial tissue, making them of potential use for the production of bioengineered blood vessels (54). Due to their inflammatory potential many researchers have abandoned synthetic polymers and have tested vascular patches entirely made of a benzyl ester of hyaluronic acid (HA).

Hyaluronic acid scaffolds

HA can be processed to obtain several types of devices such as tubes, membranes, nonwoven fabrics, gauzes, and sponges. All these scaffolds are highly biocompatible. In the human body they do not elicit any adverse reactions and are reabsorbed by the host tissues (59). HA is the only nonsulphated glycosaminoglycan of ECM. Despite its simple structure, HA is involved in a great number of biological functions, such as cell proliferation and migration, morphogenesis, wound healing, inflammation, angiogenesis, and tumor growth (60-64). An important biological role is related to HA oligosaccharides that stimulate cytokine secretion and endothelial cell proliferation (60-64). Moreover, recent studies indicate that HA may inherently stimulate endothelialization, favoring the recruitment of endothelial cells and endothelial progenitor cells (EPCs) (43, 45). EPCs can migrate and adhere inside HA-based biomaterials, maintain their preendothelial phenotype, and express angiogenic factors, especially within the first week of growth (63, 65). These results indicate that HA-based biomaterials could be promising candidates as a vehicle for EPCs for regenerative medicine applications. They may also overcome problems related to current vascular implant materials that insufficiently recruit endothelial cells to form a normally functional and confluent endothelium, a key challenge to reinstating vascular homeostasis at the surgical site.

An Italian team obtained stimulating results in testing vascular prostheses entirely made of HA in rat and pig experimental models (65-68). In this case, human vascular cells, such as endothelial cells (62) and smooth muscle cells (SMCs) (63), were grown *in vitro* on benzylic esters of hyaluronic acid (namely HYAFF-11 biomaterials) constructs to develop new tissue-engineered vascular substitutes. Previous *in vivo* studies (65-68) confirmed that HYAFF tubes could sequentially orchestrate the vascular regeneration events needed for very small artery reconstruction. HYAFF-11 was shown

to be very well tolerated and to elicit no adverse reactions in clinical practice (62, 63, 69). More recently, Du et al have fabricated well-aligned nanofibrous scaffolds with PCL using a high-speed rotating collector. They then modified those surfaces with HA and studied the synergistic effect of the scaffolds on the *in vitro* behavior of the endothelial cells. The results of adhesion and the morphology of human umbilical vein endothelial cells (HUVECs) showed that the HA-coating aligned PCL (HA-aPCL) nanofibrous scaffolds could highly promote attachment and guide the HUVECs bipolar spread with the parallel, aligned nanofibers. Furthermore, HUVECs on the HA-aPCL formed a confluent monoendothelial cell layer and exhibited superior protein expression levels of von Willebrand factor. This study suggested that the combination of aligned nanostructure and HA modification was more capable of promoting the regeneration of functional endothelium for vascular tissue engineering than the use of either individually (70).

HA is also often mixed with other ECM polymers to better reproduce the complexity and biological role of the natural ECM. In some studies, HA has been combined with collagen fibers, showing advantages such as the enhancement of cell migration and division compared to either material on its own (71, 72). Another potential material applicable to TEVG field is HA-EDA-g- α -elastin copolymer. In this case HA was functionalized with EDA and the resulting derivative was reacted with α -elastin, a water-soluble protein that maintains the main biological performance of native elastin. The presence of unreacted amino groups in HA-EDA-g- α -elastin allow chemical crosslinking with ethylene glycol diglycidyl ether (EGDGE) and the production of porous scaffolds. The authors evaluated attachment, viability, and proliferation of primary rat dermal fibroblasts and umbilical artery smooth muscle cells, obtaining good results. Furthermore, the prepared scaffold showed good water affinity, a resistance in simulated physiological fluid, and an appropriate susceptibility to hydrolysis by hyaluronidase. Undoubtedly, thanks to the presence of HA and α -elastin, a potential application of the investigated scaffolds could be dermal or vascular regeneration, since both components are abundant in these tissues where they play important biological functions (73).

Elastin into scaffolds: the keystone of TEVGs

A major structural element of arterial walls is elastic fiber, which endows vessels with the critical property of

elastic recoil (74). In arteries, elastin dictates tissue mechanics at low strains before stiffer collagen fibers are engaged. It confers elasticity, preventing dynamic tissue creep by stretching under load and recoiling to its original configuration after the load is released. In addition to mechanical responsiveness, elastin is a potent autocrine regulator to vascular SMC activity, and this regulation is important for preventing fibrocellular pathology. Finally, elastin degradation associated with matrix metalloproteinase (MMP) activity is a cell-mediated process, observed in almost all types of vascular calcification. Elastin peptides associated with transforming growth factor- β could induce osteogenic gene expression in SMCs, possibly via elastin laminin receptor (ELR) signaling (13, 74). Their presence in a vascular graft would greatly enhance design and patency; furthermore, elastic fibers also influence vascular cell behavior through direct interaction and by regulation of growth factor activation. Elastin knockout studies and clinical observations have revealed the essential regulatory function of elastin during artery development. In the absence of extracellular elastin accumulation, smooth muscle proliferation leads to arterial stenosis (75-79). Thus, to ensure appropriate mechanical function and prevent serious complications, successful artery replacements must incorporate an elastic component.

Approaches to date have included building tissue replacements on an elastin scaffold isolated from cadaveric tissues, supplying soluble tropoelastin to a cell culture, or designing biocompatible synthetic elastic polymers (13). One of the most promising approaches was described by L'Heureux et al (80-82), who developed a completely autologous technique called sheet-based tissue engineering. Dermal fibroblasts are obtained from a small skin biopsy and grown in conditions that promote an engineered complete vessel, ready to be implanted in six months. This approach is time consuming, with total production time at ~24 weeks. It is conceivable that in previous techniques (26, 83-86), the change from two-dimensional to three-dimensional culture conditions utilizing porous scaffolds may have contributed to a loss in elastin biosynthesis by SMCs. It is now known that the scaffold must provide the appropriate chemistry for SMCs to secrete elastin. Few scaffolds are able to promote elastin biosynthesis.

One of the most promising ECM-derived polymers is hyaluronan, as discussed earlier. *In vitro* studies with gel preparations of hyaluronan (87, 88) have confirmed elastogenesis in neonatal SMCs. In 2012, McKenna et al (89) electrospun

small-diameter vascular grafts containing recombinant human tropoelastin (rTE), the monomer unit of elastin, that, when cross-linked, mimics native elastin fibers. The material provided both the compliance and support of endothelial cell adhesion for a component of a vascular graft biomaterial, but it lacked the tensile strength to support *in vivo* arterial pressures. The authors concluded that, for vascular graft applications, the tensile strengths of the prTE scaffolds should be further increased by reinforcement or co-spinning with either collagen or synthetic materials. Interestingly, Bashur et al (90) have reviewed many important issues related to elastin-based TEVGs. Starting from a description of the current challenges that exist in the field of elastic matrix engineering, specifically for vascular applications, they continue by introducing the specific targets and processes that impact elastogenesis, and conclude with a review of current progress toward achieving robust elastogenesis in TEVGs aimed at clinical use.

Three-dimensional (3D) printed vessels

Several rapid prototyping techniques and materials, from designs as simple as a single channel (91), to designs that recreate the complex geometries of vascular pathways (92), and even scaffolds based on complex collagen forms (93), have been used to achieve a vasculature reconstruction that is as natural as possible. Currently, solid freeform fabrication (SFF) as well as inverted colloidal crystal (ICC) fabrication or the newly emerging multi-photon absorption polymerization (MAP), provide precise controllability over 3D matrix architecture at a micro-scale level. Limited material selection and lack of submicro-scale structural resolution are the major shortcomings of these techniques. Bioprinting (94), in which cells and matrix are deposited dropwise, has been developed over the past decade but also is a slow, serial process with limitations on print resolution, materials, and cells.

In contrast to these methods, 3D sacrificial molding provides an intriguing alternative (95-97). Proof-of-concept studies have shown that a network of channels can be fabricated by creating a rigid 3D lattice of filaments, casting the lattice into a rubber or plastic material, and then sacrificing the lattice to reveal a microfluidic architecture in the bulk material. However, 3D sacrificial molding of perfusable channels has so far required the use of cytotoxic organic solvents or processing conditions for either removing the sacrificial filaments or casting the surrounding material, and thus could not be accomplished with aqueous-based ECMs

or in the presence of living cells. In their work, Miller et al (98) describe a biocompatible sacrificial material – a simple glass made from mixtures of inexpensive and readily available carbohydrates – and a means to 3D print the material to facilitate the rapid casting of patterned vascular networks in engineered tissues. In this case, they used the material as a cytocompatible sacrificial template in engineered tissues containing primary rat hepatocytes to generate cylindrical networks that could be lined with endothelial cells and perfused with blood under high-pressure pulsatile flow. Unfortunately, it was difficult to achieve the cellular densities of native tissues (approximately 10–500 million cells per mL), but it demonstrated an avenue for building and studying such tissue mimics, in which the vasculature appears not to constrain the design space for the tissue itself, allowing for arbitrary cell types, matrices, and their patterning.

CONCLUSIONS

The way to create a perfectly absorbable structure capable of guiding the regeneration of a native human vessel is neither easy nor close to being achieved, but it is not too far off. Materials and methods used by the scientific community are amazingly complex and many efforts have been made to reach the perfection of animal structures,

like ECM. Actually, ECM is an admirable intricate network of filamentous proteins connected by countless joints, immersed in a hydrogel rich in nutrients and growth factors, where the cellular component can perform its action of continuous control and remodeling. It is a very difficult challenge to try to reproduce and imitate the complexity of the ECM, but, probably, as often happens in scientific discoveries, the solution to the problem may be discovered through some chance, simple intuitions. Certainly, the application of bioengineered materials in humans to repair damaged vascular structures will revolutionize current surgical approaches to vascular pathology and will anticipate the transplantation of the whole organ into human being, giving an important boost to research in the field of regenerative medicine.

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REFERENCES

1. Fox CJ, Gillespie DL, O'Donnell SD, et al. Contemporary management of wartime vascular trauma. *J Vasc Surg.* 2005;41(4):638-644.
2. Tsai JW, Ayubi FS, Rice RD, Zhang Z, Armstrong PJ. Permacol (porcine dermal collagen) and Alloderm (acellular cadaveric dermis) as a vascular patch repair for common carotid arteriotomy in a rabbit model. *Ann Vasc Surg.* 2009; 23(3):374-381.
3. Muto A, Nishibe T, Dardik H, Dardik A. Patches for carotid artery endarterectomy: current materials and prospects. *J Vasc Surg.* 2009;50(1):206-213.
4. Tremblay D, Zigras T, Cartier R, et al. A comparison of mechanical properties of materials used in aortic arch reconstruction. *Ann Thorac Surg.* 2009;88(5):1484-1491.
5. Smaill BH, McGiffin DC, Legrice IJ, Young AA, Hunter PJ, Galbraith AJ. The effect of synthetic patch repair of coarctation on regional deformation of the aortic wall. *J Thorac Cardiovasc Surg.* 2000;120(6):1053-1063.
6. Hertzner NR, Mascha EJ. A personal experience with coronary artery bypass grafting, carotid patching, and other factors influencing the outcome of carotid endarterectomy. *J Vasc Surg.* 2006;43(5):959-968.
7. Jacobowitz GR, Kalish JA, Lee AM, Adelman MA, Riles TS, Landis R. Long-term follow-up of saphenous vein, internal jugular vein, and knitted Dacron patches for carotid artery endarterectomy. *Ann Vasc Surg.* 2001;15(3):281-287.
8. AbuRahma AF. Patch closure improves results with carotid endarterectomy. *Semin Vasc Surg.* 2004;17(3):243-252.
9. Wong P, Hopkins S, Vincente D, Williams K, Macri N, Berguer R. Differences in neointima formation between impervious and porous polytetrafluoroethylene vascular patch material. *Ann Vasc Surg.* 2002;16(4):407-412.
10. Bond R, Rerkasem K, Naylor R, Rothwell PM. Patches of different types for carotid patch angioplasty. *Cochrane Database Syst Rev.* 2004;2(2):CD000071.

11. Edelman ER. Vascular tissue engineering : designer arteries. *Circ Res.* 1999;85(12):1115-1117.
12. Schmidt CE, Baier JM. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials.* 2000;21(22):2215-2231.
13. L'Heureux N, Dusserre N, Marini A, Garrido S, de la Fuente L, McAllister T. Technology insight: the evolution of tissue-engineered vascular grafts—from research to clinical practice. *Nat Clin Pract Cardiovasc Med.* 2007;4(7):389-395.
14. Atala A. Recent developments in tissue engineering and regenerative medicine. *Curr Opin Pediatr.* 2006;18(2):167-171.
15. Atala A. Engineering organs. *Curr Opin Biotechnol.* 2009;20(5):575-592.
16. Orlando G, Wood KJ, Stratta RJ, Yoo JJ, Atala A, Soker S. Regenerative medicine and organ transplantation: past, present, and future. *Transplantation.* 2011;91(12):1310-1317.
17. Arenas-Herrera JE, Ko IK, Atala A, Yoo JJ. Decellularization for whole organ bioengineering. *Biomed Mater.* 2013;8(1):014106.
18. Kim J, Jeong SY, Ju YM, et al. *In vitro* osteogenic differentiation of human amniotic fluid-derived stem cells on a poly(lactide-co-glycolide) (PLGA)-bladder submucosa matrix (BSM) composite scaffold for bone tissue engineering. *Biomed Mater.* 2013;8(1):014107.
19. Choi JS, Kim EY, Kim MJ, et al. *In vitro* evaluation of the interactions between human corneal endothelial cells and extracellular matrix proteins. *Biomed Mater.* 2013;8(1):014108.
20. Quint C, Arief M, Muto A, Dardik A, Niklason LE. Allogeneic human tissue-engineered blood vessel. *J Vasc Surg.* 2012;55(3):790-798.
21. Steinhoff G, Stock U, Karim N, et al. Tissue engineering of pulmonary heart valves on allogenic acellular matrix conduits: *in vivo* restoration of valve tissue. *Circulation.* 2000;102(19)(Suppl 3):III50-III55.
22. Rim NG, Shin CS, Shin H. Current approaches to electrospun nanofibers for tissue engineering. *Biomed Mater.* 2013;8(1):014102.
23. Levorson EJ, Raman Sreerexha P, Chennazhi KP, Kasper FK, Nair SV, Mikos AG. Fabrication and characterization of multiscale electrospun scaffolds for cartilage regeneration. *Biomed Mater.* 2013;8(1):014103.
24. Xu W, Atala A, Yoo JJ, Lee SJ. Controllable dual protein delivery through electrospun fibrous scaffolds with different hydrophilicities. *Biomed Mater.* 2013;8(1):014104.
25. Zhang H, Jia X, Han F, et al. Dual-delivery of VEGF and PDGF by double-layered electrospun membranes for blood vessel regeneration. *Biomaterials.* 2013;34(9):2202-2212.
26. Shin'oka T, Matsumura G, Hibino N, et al. Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. *J Thorac Cardiovasc Surg.* 2005;129(6):1330-1338.
27. Cho SW, Park HJ, Ryu JH, et al. Vascular patches tissue-engineered with autologous bone marrow-derived cells and decellularized tissue matrices. *Biomaterials.* 2005;26(14):1915-1924.
28. Schmidt D, Mol A, Neuenschwander S, et al. Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. *Eur J Cardiothorac Surg.* 2005;27(5):795-800.
29. Stock UA, Sakamoto T, Hatsuoka S, et al. Patch augmentation of the pulmonary artery with bioabsorbable polymers and autologous cell seeding. *J Thorac Cardiovasc Surg.* 2000;120(6):1158-1167, discussion 1168.
30. Yang C, Sodian R, Fu P, et al. *In vitro* fabrication of a tissue engineered human cardiovascular patch for future use in cardiovascular surgery. *Ann Thorac Surg.* 2006;81(1):57-63.
31. Mettler BA, Sales VL, Stucken CL, et al. Stem cell-derived, tissue-engineered pulmonary artery augmentation patches *in vivo*. *Ann Thorac Surg.* 2008;86(1):132-140, discussion 140-141.
32. Sales VL, Mettler BA, Lopez-Illasaca M, Johnson JA Jr, Mayer JE Jr. Endothelial progenitor and mesenchymal stem cell-derived cells persist in tissue-engineered patch *in vivo*: application of green and red fluorescent protein-expressing retroviral vector. *Tissue Eng.* 2007;13(3):525-535.
33. Mendelson K, Aikawa E, Mettler BA, et al. Healing and remodeling of bioengineered pulmonary artery patches implanted in sheep. *Cardiovasc Pathol.* 2007;16(5):277-282.
34. Oh SH, Lee JH. Hydrophilization of synthetic biodegradable polymer scaffolds for improved cell/tissue compatibility. *Biomed Mater.* 2013;8(1):014101.
35. Pfeiffer D, Stefanitsch C, Wankhammer K, et al. Endothelialization of electrospun polycaprolactone (PCL) small caliber vascular grafts spun from different polymer blends. *J Biomed Mater Res A.* 2014. epub ahead of print.
36. Ye Q, Zünd G, Jockenhoevel S, et al. Tissue engineering in cardiovascular surgery: new approach to develop completely human autologous tissue. *Eur J Cardiothorac Surg.* 2000;17(4):449-454.
37. Zhang WJ, Liu W, Cui L, Cao Y. Tissue engineering of blood vessel. *J Cell Mol Med.* 2007;11(5):945-957.
38. Kerdjoudj H, Moby V, Berthelemy N, et al. The ideal small arterial substitute: Role of cell seeding and tissue engineering. *Clin Hemorheol Microcirc.* 2007;37(1-2):89-98.
39. Kaushal S, Amiel GE, Guleserian KJ, et al. Functional small-diameter neovessels created using endothelial progenitor cells expanded *ex vivo*. *Nat Med.* 2001;7(9):1035-1040.
40. He H, Shirota T, Yasui H, Matsuda T. Canine endothelial progenitor cell-lined hybrid vascular graft with nonthrombogenic potential. *J Thorac Cardiovasc Surg.* 2003;126(2):455-464.
41. Niklason LE, Gao J, Abbott WM, et al. Functional arteries grown *in vitro*. *Science.* 1999;284(5413):489-493.
42. Hoerstrup SP, Cummings MrCS I, Lachat M, et al. Functional growth in tissue-engineered living, vascular grafts: follow-up at 100 weeks in a large animal model. *Circulation.* 2006;114(1)(Suppl):I159-I166.

43. Campbell JH, Efendy JL, Campbell GR. Novel vascular graft grown within recipient's own peritoneal cavity. *Circ Res*. 1999; 85(12):1173-1178.
44. Torikai K, Ichikawa H, Hirakawa K, et al. A self-renewing, tissue-engineered vascular graft for arterial reconstruction. *J Thorac Cardiovasc Surg*. 2008;136(1):37-45,e1.
45. Iwai S, Sawa Y, Ichikawa H, et al. Biodegradable polymer with collagen microsphere serves as a new bioengineered cardiovascular prosthesis. *J Thorac Cardiovasc Surg*. 2004; 128(3):472-479.
46. Iwai S, Sawa Y, Taketani S, Torikai K, Hirakawa K, Matsu-da H. Novel tissue-engineered biodegradable material for reconstruction of vascular wall. *Ann Thorac Surg*. 2005; 80(5):1821-1827.
47. Avci-Adali M, Paul A, Ziemer G, Wendel HP. New strategies for *in vivo* tissue engineering by mimicry of homing factors for self-endothelialisation of blood contacting materials. *Biomaterials*. 2008;29(29):3936-3945.
48. Avci-Adali M, Ziemer G, Wendel HP. Induction of EPC homing on biofunctionalized vascular grafts for rapid *in vivo* self-endothelialization—a review of current strategies. *Bio-technol Adv*. 2010;28(1):119-129.
49. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials*. 2009;30(30):5910-5917.
50. Monchaux E, Vermette P. Effects of surface properties and bioactivation of biomaterials on endothelial cells. *Front Biosci (Schol Ed)*. 2010;S2:239-255.
51. Takahashi H, Yokota T, Uchimura E, et al. Newly developed tissue-engineered material for reconstruction of vascular wall without cell seeding. *Ann Thorac Surg*. 2009;88(4): 1269-1276.
52. Ceonzo K, Gaynor A, Shaffer L, Kojima K, Vacanti CA, Stahl GL. Polyglycolic acid-induced inflammation: role of hydrolysis and resulting complement activation. *Tissue Eng*. 2006;12(2):301-308.
53. Lo Monte AI, Licciardi M, Bellavia M, et al. Biocompatibility and biodegradability of electrospun PHEA-PLA scaffolds: our preliminary experience in a murine animal model. *Digest J Nanomater Biostruct*. 2012;7:841-851.
54. Pitarresi G, Fiorica C, Palumbo FS, Rigogliuso S, Ghersi G, Giammona G. Heparin functionalized polyaspartamide/ polyester scaffold for potential blood vessel regeneration. *J Biomed Mater Res A*. 2014;102(5):1334-1341.
55. Linhardt RJ, Turnbull JE, Wang HM, Loganathan D, Gallagher JT. Examination of the substrate specificity of heparin and heparan sulfate lyases. *Biochemistry*. 1990;29(10):2611-2617.
56. Jayson GC, Gallagher JT. Heparin oligosaccharides: inhibitors of the biological activity of bFGF on Caco-2 cells. *Br J Cancer*. 1997;75(1):9-16.
57. Soker S, Goldstaub D, Svahn CM, Vlodavsky I, Levi BZ, Neufeld G. Variations in the size and sulfation of heparin modulate the effect of heparin on the binding of VEGF165 to its receptors. *Biochem Biophys Res Commun*. 1994; 203(2):1339-1347.
58. Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. *Science*. 1984; 223(4642):1296-1299.
59. Vindigni V, Cortivo R, Iacobellis L, Abatangelo G, Zavan B. Hyaluronan benzyl ester as a scaffold for tissue engineering. *Int J Mol Sci*. 2009;10(7):2972-2985.
60. Genasetti A, Vigetti D, Viola M, et al. Hyaluronan and human endothelial cell behavior. *Connect Tissue Res*. 2008;49(3-4): 120-123.
61. Ibrahim S, Ramamurthi A. Hyaluronic acid cues for functional endothelialization of vascular constructs. *J Tissue Eng Regen Med*. 2008;2(1):22-32.
62. Turner NJ, Kieley CM, Walker MG, Canfield AE. A novel hyaluronan-based biomaterial (Hyaff-11) as a scaffold for endothelial cells in tissue engineered vascular grafts. *Biomaterials*. 2004;25(28):5955-5964.
63. Remuzzi A, Mantero S, Colombo M, et al. Vascular smooth muscle cells on hyaluronic acid: culture and mechanical characterization of an engineered vascular construct. *Tissue Eng*. 2004;10(5-6):699-710.
64. Pasquinelli G, Vinci MC, Gamberini C, et al. Architectural organization and functional features of early endothelial progenitor cells cultured in a hyaluronan-based polymer scaffold. *Tissue Eng Part A*. 2009;15(9):2751-2762.
65. Lepidi S, Grego F, Vindigni V, et al. Hyaluronan biodegradable scaffold for small-caliber artery grafting: preliminary results in an animal model. *Eur J Vasc Endovasc Surg*. 2006; 32(4):411-417.
66. Pandis L, Zavan B, Abatangelo G, Lepidi S, Cortivo R, Vindigni V. Hyaluronan-based scaffold for *in vivo* regeneration of the rat vena cava: Preliminary results in an animal model. *J Biomed Mater Res A*. 2010;93(4):1289-1296.
67. Zavan B, Vindigni V, Lepidi S, et al. Neoarteries grown *in vivo* using a tissue-engineered hyaluronan-based scaffold. *FASEB J*. 2008;22(8):2853-2861.
68. Lepidi S, Abatangelo G, Vindigni V, et al. *In vivo* regeneration of small-diameter (2 mm) arteries using a polymer scaffold. *FASEB J*. 2006;20(1):103-105.
69. Tonello C, Zavan B, Cortivo R, Brun P, Panfilo S, Abatangelo G. *In vitro* reconstruction of human dermal equivalent enriched with endothelial cells. *Biomaterials*. 2003;24(7): 1205-1211.
70. Du F, Zhao W, Zhang M, Mao H, Kong D, Yang J. The synergistic effect of aligned nanofibers and hyaluronic acid modification on endothelial cell behavior for vascular tissue engineering. *J Nanosci Nanotechnol*. 2011;11(8):6718-6725.
71. Zhu C, Fan D, Wang Y. Human-like collagen/hyaluronic acid 3D scaffolds for vascular tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2014;34:393-401.
72. Kim HJ, Kim KK, Park IK, Choi BS, Kim JH, Kim MS. Hybrid scaffolds composed of hyaluronic acid and collagen

- for cartilage regeneration. *Tissue Eng Regen Med*. 2012; 9:57-62.
73. Palumbo FS, Pitarresi G, Fiorica C, Rigogliuso S, Ghersi G, Giammona G. Chemical hydrogels based on a hyaluronic acid-graft- α -elastin derivative as potential scaffolds for tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2013;33(5):2541-2549.
74. Patel A, Fine B, Sandig M, Mequanint K. Elastin biosynthesis: The missing link in tissue-engineered blood vessels. *Cardiovasc Res*. 2006;71(1):40-49.
75. Karnik SK, Brooke BS, Bayes-Genis A, et al. A critical role for elastin signaling in vascular morphogenesis and disease. *Development*. 2003;130(2):411-423.
76. Long JL, Tranquillo RT. Elastic fiber production in cardiovascular tissue-equivalents. *Matrix Biol*. 2003;22(4):339-350.
77. Li DY, Brooke B, Davis EC, et al. Elastin is an essential determinant of arterial morphogenesis. *Nature*. 1998;393(6682):276-280.
78. Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci*. 2002;115(Pt 14):2817-2828.
79. Ratcliffe A. Tissue engineering of vascular grafts. *Matrix Biol*. 2000;19(4):353-357.
80. L'Heureux N, Germain L, Labbé R, Auger FA. *In vitro* construction of a human blood vessel from cultured vascular cells: a morphologic study. *J Vasc Surg*. 1993;17(3):499-509.
81. L'Heureux N, McAllister TN, de la Fuente LM. Tissue-engineered blood vessel for adult arterial revascularization. *N Engl J Med*. 2007;357(14):1451-1453.
82. L'Heureux N, Dusserre N, König G, et al. Human tissue-engineered blood vessels for adult arterial revascularization. *Nat Med*. 2006;12(3):361-365.
83. Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. *Proc Natl Acad Sci U S A*. 2002;99(19):12025-12030.
84. Mann BK, West JL. Tissue engineering in the cardiovascular system: progress toward a tissue engineered heart. *Anat Rec*. 2001;263(4):367-371.
85. Matsumura G, Hibino N, Ikada Y, Kurosawa H, Shin'oka T. Successful application of tissue engineered vascular auto-grafts: clinical experience. *Biomaterials*. 2003;24(13):2303-2308.
86. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science*. 1986; 231(4736):397-400.
87. Joddar B, Ibrahim S, Ramamurthi A. Impact of delivery mode of hyaluronan oligomers on elastogenic responses of adult vascular smooth muscle cells. *Biomaterials*. 2007;28(27):3918-3927.
88. Joddar B, Ramamurthi A. Elastogenic effects of exogenous hyaluronan oligosaccharides on vascular smooth muscle cells. *Biomaterials*. 2006;27(33):5698-5707.
89. McKenna KA, Hinds MT, Sarao RC, et al. Mechanical property characterization of electrospun recombinant human tropoelastin for vascular graft biomaterials. *Acta Biomater*. 2012;8(1):225-233.
90. Bashur CA, Venkataraman L, Ramamurthi A. Tissue engineering and regenerative strategies to replicate biocomplexity of vascular elastic matrix assembly. *Tissue Eng Part B*. 2012;18(3):203-217.
91. Zhao L, Lee VK, Yoo SS, Dai G, Intes X. The integration of 3-D cell printing and mesoscopic fluorescence molecular tomography of vascular constructs within thick hydrogel scaffolds. *Biomaterials*. 2012;33(21):5325-5332.
92. Wu W, DeConinck A, Lewis JA. Omnidirectional printing of 3D microvascular networks. *Adv Mater*. 2011;23(24):H178-H183.
93. Liu CZ, Xia ZD, Han ZW, Hulley PA, Triffitt JT, Czernuszka JT. Novel 3D collagen scaffolds fabricated by indirect printing technique for tissue engineering. *J Biomed Mater Res B Appl Biomater*. 2008;85B(2):519-528.
94. Visconti RP, Kasyanov V, Gentile C, Zhang J, Markwald RR, Mironov V. Towards organ printing: engineering an intra-organ branched vascular tree. *Expert Opin Biol Ther*. 2010;10(3):409-420.
95. Theriault D, White SR, Lewis JA. Chaotic mixing in three-dimensional microvascular networks fabricated by direct-write assembly. *Nat Mater*. 2003;2(4):265-271.
96. Bellan LM, Singh SP, Henderson PW, Porri TJ, Craighead HG, Spector JA. Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. *Soft Matter*. 2009;5:1354-1357.
97. Wu W, Hansen CJ, Aragón AM, Geubelle PH, White SR, Lewis JA. Direct-write assembly of biomimetic microvascular networks for efficient fluid transport. *Soft Matter*. 2010;6:739-742.
98. Miller JS, Stevens KR, Yang MT, et al. Rapid casting of patterned vascular networks for perfusable engineered 3D tissues. *Nat Mater*. 2012;11(9):768-774.