Review

Elastin biosynthesis: The missing link in tissue-engineered blood vessels

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Abstract

Nearly 20 years have passed since Weinberg and Bell attempted to make the first tissue-engineered blood vessels. Following this early attempt, vascular tissue engineering has emerged as one of the most promising approaches to fabricate orderly and mechanically competent vascular substitutes. In elastic and muscular arteries, elastin is a critical structural and regulatory matrix protein and plays an important and dominant role by conferring elasticity to the vessel wall. Elastin also regulates vascular smooth muscle cells activity and phenotype. Despite the great promise that tissue-engineered blood vessels have to offer, little research in the last two decades has addressed the importance of elastin incorporation into these vessels. Although cardiovascular tissue engineering has been reviewed in the past, very little attention has been given to elastin. Thus, this review focuses on the recent advances made towards elastogenesis and the challenges we face in the quest for appropriate functional vascular substitutes.

Keywords: Extracellular matrix; Smooth muscle; Tissue engineering; Arteries

1. Introduction

Elastin is the dominant extracellular matrix protein deposited in the arterial wall and can contribute up to 50% of its dry weight [1]. The protein product of the elastin gene is synthesized by vascular smooth muscle cells and secreted as a tropoelastin monomer, that is soluble, non-glycosylated and highly hydrophobic. After posttranslational modifications, tropoelastin is crosslinked and organized into elastin polymers that form concentric rings of elastic lamellae around the medial layer of arteries. In arteries, elastin dictates tissue mechanics at low strains before stiffer collagen fibers are engaged [2]. Elastin also confers elasticity, preventing dynamic tissue creep by stretching under load and recoiling to their original configurations after the load is released. In addition to the mechanical responsiveness, elastin is a potent autocrine regulator of vascular smooth muscle cells activity and this regulation is important for preventing fibrocellular pathology. Elastin signals via a non-integrin heterotrimeric G-protein-coupled pathway, induces actin stress fiber organization, inhibits smooth muscle cell proliferation, and regulates migration [3]. Indeed, elastin knockout studies and clinical observations have revealed an essential regulatory function since, in the absence of extracellular elastin, smooth muscle cells proliferation stenoses arteries [4]. Thus to ensure appropriate mechanical function of the vessel and to prevent vessel stenosis, successful tissue-engi-
2. The structure of blood vessels as basis for vascular tissue engineering

The schematic view of elastic and muscular arteries is shown in Fig. 1. For the detailed anatomy, the reader is referred to that described elsewhere [5].

Blood vessels are composed of three concentric tunics. The tunica intima forms the innermost lining composed of non-thrombogenic monolayer endothelial cells. By secreting specific molecules like nitric oxide, endothelial cells inhibit platelet activation and prevent thrombus formation [6,7]. The tunica adventitia forms the external layer and contains a collagenous extracellular matrix and fibroblast cells. In this review, we will provide a detailed description of the tunica media (middle layer) where most of the elastin is found.

The tunica media is generally composed of a dense population of concentrically organized smooth muscle cells and is separated from the tunica intima by an internal elastic lamina. In elastic arteries, such as the aorta and its largest branches including the brachiocephalic, common carotid, subclavian and iliac arteries, smooth muscle cells synthesize the elastin molecules that are incorporated into elastic fibers, which are then arranged into concentric rings of elastic lamellae around the arterial media. The elastic lamellae allow the artery to maintain sufficient blood pressure with variations in hemodynamic stress of the cardiac systole and diastole [8]. In muscular arteries, however, elastin is assembled as fibers [9]. Under physiological condition, smooth muscle cells possess a quiescent contractile phenotype and control the dilation and constriction of blood vessels thus regulating blood flow. Under pathological

Fig. 1. (A) The structure of blood vessels showing the three tunics. (B) The distribution of elastin within the vessel wall is shown for a muscular artery (left) and elastic artery (right); taken from [5] with permission.
conditions, smooth muscle cells convert to a synthetic and non-contractile phenotype. This synthetic phenotype results in the proliferation and increased matrix production in the tunica media. Smooth muscle cells respond to a variety of signals in the dynamic environment of the arterial wall. These signals can be biochemical and/or biomechanical in nature and may lead to changes in cell function and phenotype, both under physiological and pathological conditions [10,11]. Biochemical signals must pass through the matrix surrounding the smooth muscle cells and, in some cases, they are sequestered and released by the matrix. Biomechanical signals are also transmitted by the smooth muscle cells to the matrix via cell receptors that link the external environment to the cytoskeleton [12]. In the native blood vessel, the smooth muscle cells are surrounded by the elastic lamellae or fibers. The transition between phenotypes and the production of extracellular matrix components in the synthetic state are dependent on the signals passed through elastin. Thus, elastin stabilizes the arterial structure by regulating proliferation, phenotypic modulation and organization of vascular smooth muscle cells [1,13].

The contents of the extracellular matrix surrounding the vascular cells consist primarily of collagen (types I and III), elastin, some proteoglycans and glycoproteins. The major mechanical properties related to blood vessel function are tensile stiffness, elasticity and compressibility. Collagen provides the tensile stiffness for the resistance against rupture, elastin dictates the elastic properties, proteoglycans contribute to the compressibility; and combined with collagen, elastin prevents irreversible deformation of the vessel against pulsatile blood flow [14,15].

3. Tissue engineering of blood vessels

Tissue engineering has emerged as a promising technology in the design of an ideal, responsive, living conduit with properties similar to that of the native tissue. To date, it has focused mainly on generating tissues to repair bone, cartilage, tendon, ligament, skin, blood vessels and heart valves [16–20].

Tissue-engineered blood vessels have specific advantages over traditional synthetic grafts since they are designed to be non-thrombogenic and to be responsive both mechanically and biologically with respect to the load that changes with the hemodynamic environment. Tissue-engineered blood vessels are specifically more attractive for pediatric patients since they have the capacity to grow, repair, and remodel as required over time. It may be argued that the first successful clinical application of tissue-engineered blood vessel in pediatric surgery was due to this fact [21–23]. The challenges faced by the approach of tissue engineering for replacing blood vessels are also substantial. Those include sufficient strength and elasticity to withstand the hemodynamic cyclic loading, matched compliance with the adjacent native vessel, and a lining of the lumen with non-thrombogenic properties. While engineering approaches for other tissue substitutes can rely on in vivo remodeling to approach functionality with time, tissue-engineered blood vessels must function immediately on implantation [14].

Following decades of research to improve the properties of clinically used Dacron® (polyethylene terephthalate; PET) and Teflon® (expanded polytetrafluoroethylene; ePTFE) grafts in cardiovascular surgery, a different approach for vascular tissue substitutes was taken in the late 1980s. Weinberg and Bell were the first to report their attempts to design in vitro tissue-engineered blood vessel using collagen gel scaffold and bovine aortic cells [24]. In spite of this novel approach, the vessel could not withstand the load of arterial hemodynamic environment and failed to show adequate burst strength even after reinforced with a Dacron® mesh. L’Heureux et al. [25] also used a similar approach using collagen gel scaffold and human umbilical vein cells but encountered the same mechanical limitations. Hirai et al. [26,27] fabricated a vascular replacement from collagen gel and cells harvested from canine jugular vein which was then implanted into the vena cava position of canine model. Their attempt, however, failed since the graft ruptured within few days of implantation. Although there have been numerous attempts and failures, tissue engineering of blood vessels continues to be an active area of research [28–34]. However, despite the great promise tissue-engineered blood vessels have to offer, elastin, a critical structural component, was notably absent from most of the approaches utilized to engineer vessels. Although progress has been made towards understanding the underlying principles of elastin biosynthesis and incorporation into fibers in vivo, many of the conditions and mechanisms required to form viable elastin-containing vessels are still elusive. In the following sections, we will highlight attempts made towards fabricating mechanically and biologically responsive blood vessels incorporating elastin.

3.1. The effect of the type of scaffold on elastin biosynthesis

Scaffolds play an important role in tissue engineering. Scaffolds are basically three-dimensional structural templates which support cell adhesion, migration, differentiation, proliferation and provide guidance for neo tissue formation [35]. Ideally, after fulfilling their function as a template, they should degrade into nontoxic byproducts. The coordinated rates of scaffold degradation and neo tissue generation are essential elements to be considered [36]. The chosen scaffold material should be biocompatible and reproducible without any batch-property variation with high porosity and inter-connected microstructure. Thus far, it has not been possible to meet the above criteria using a particular scaffold since biological and synthetic scaffolds have both advantages and disadvantages (Table 1).

Interestingly when smooth muscle cells are cultured in conventional tissue culture dishes, they are known to secrete
both soluble (precursor) and insoluble elastin [37]. In addition, it has been reported that static stretching of vascular smooth muscle cells in culture dishes stimulated elastin biosynthesis as well as crosslinking [38]. It is conceivable that in previous approaches [24–26,28,30] the change from two-dimensional culture to three-dimensional culture conditions utilizing porous scaffolds may have contributed to a loss in elastin biosynthesis by vascular smooth muscle cells. Therefore, the scaffold should provide the right chemistry for the smooth muscle cells to secrete elastin.

3.1.1. Biological scaffolds

Biological scaffolds are made of either single or a few extracellular matrix components like elastin, collagen, glycosaminoglycans and fibrin. Collagen and fibrin are clinically approved natural scaffold materials studied for their potential promotion of elastin biosynthesis in three-dimensional arrangement. Scaffolds made from collagen were utilized by a number of groups for the development of tissue-engineered blood vessels [24,25,32]. When vascular smooth muscle cells were seeded and allowed to mature in vitro, however, no elastin was detected. Long and Tranquillo [2] investigated elastin biosynthesis by vascular smooth muscle cells harvested from neonatal rats using both collagen and fibrin gel scaffolds. Elastogenesis was significantly higher (by a factor of 8) on the fibrin gel compared with the collagen gel scaffold. The fact that more elastin was secreted on fibrin gels than on collagen gels may explain why earlier investigators who used collagen gels did not succeed in obtaining good elastic properties [24,25,27]. The amount of elastin secreted was also strongly a function of the cell source since aortic smooth muscle cells from adult rats secreted less elastin than cells from neonatal rats [2].

Ramamurthi and Vesely [39] studied hyaluronan gel crosslinked with divinyl sulfone as scaffolds for culturing neonatal rat aortic smooth muscle cells and for their ability to promote elastogenesis. Under similar sets of experimental conditions, cells cultured on standard polystyrene tissue culture plates were compared with cells grown on the hyaluronan gel. Their data showed an increased amount of elastin (both soluble and insoluble) on the hyaluronan gel. Microscopical analysis of the elastin structure isolated by alkali digestion revealed that elastin was organized into smooth, highly fenestrated sheets composed of fibers, visible at the sheet edges. Interestingly such sheets and fibers were not observed on the elastin obtained from cells grown on tissue culture plates.

3.1.2. Synthetic scaffolds

Synthetic scaffold materials allow precise control over properties such as molecular weight, porosity, microstructure, degradation time and mechanical properties [40]. However, they often show reduced extracellular matrix remodeling [41]. The most widely investigated synthetic scaffold materials are degradable polyesters composed of lactide and glycolide and their copolymers.

The effect of poly-L-lactic acid (PLLA) bonding over polyglycolic acid (PGA) scaffold surface on the properties of tissue-engineered vascular constructs was investigated by Kim and Mooney [42]. Aortic smooth muscle cells from adult rats were harvested and seeded on the scaffold and allowed to grow and secrete matrix proteins. Over a period of 7 weeks in culture, matrix protein quantification revealed a large amount of elastin deposition (30% of the dry mass of the vascular tissue construct excluding the polymer content) on the bonded scaffolds. This finding is interesting since the cell attachment property of the bonded scaffold was comparatively poor, yet elastogenesis was favored. In contrast, the elastin content was only 17% on unbonded PGA scaffold after 4 weeks in culture, and did not increase with time. These results strongly suggest that elastin biosynthesis is affected by scaffold shrinkage or degradation rate. Indeed for the PGA scaffold, both shrinkage and degradation were significant during the culture time. PGA hydrolysis releases glycolic acid that could result in lower values of pH. The localized acidic microenvironment could inhibit cell proliferation and/or function including matrix protein deposition. Recent investigations by Williams and Wick [43] and Higgins et al. [44] demonstrated that the high degradability of PGA scaffold altered cellular function as a result of pH changes. In their experiments, these authors observed very low elastin biosynthesis after prolonged periods of tissue cultivation in a bioreactor.

Shum-Tim et al. [45] used an in vivo strategy for elastin biosynthesis utilizing composite scaffolds made from
polyglycolic acid and polyhydroxyalkanoate. Vascular cells from lamb carotid arteries were harvested, expanded in vitro, and seeded onto 7-mm diameter tubular scaffolds as mixed cell populations. The construct was matured for 7 days and implanted into the abdominal aortic segments of lambs from which the cells were originally harvested. Despite the random cell seeding in vitro, histological analysis of retrieved engineered vessels showed uniformly organized elastic and collagen fibers aligned according to the direction of blood flow. The investigators did not report whether elastin was secreted during the 7-day culture before implantation. However, the in vivo remodeled vascular tissue showed mechanical strength approaching that of the native vessel although some permanent deformation was still apparent after 6 months of implantation. This indicated that the elastin observed by histochemical analysis was either insufficient or non-crosslinked. If an appropriate amount of crosslinked elastin network in the tissue-engineered vascular construct was formed, it would have prevented the observed permanent deformation by dynamic recoiling after stretch. Since crosslinked elastin network is essential for the compliance of the vessel, such permanent deformation would likely cause the construct to fail [46].

In a separate study by the same group of researchers [47], Stock et al. attempted to promote elastogenesis using similar composite scaffolds with some differences: (i) the scaffolds were coated with laminin to enhance initial cell attachment and; (ii) cells were seeded over a period of 4 days. A tissue-engineered blood vessel matured exclusively in vitro was used as a control while others were implanted into lambs. Interestingly, no elastin was detected on the control, while 4% elastin was found in the implanted engineered vascular tissue. This finding clearly indicates that the signals controlling the expression of elastin mRNA are not mimicked during in vitro vascular tissue engineering and demonstrates the challenges we face in promoting in vitro elastogenesis utilizing three-dimensional scaffolds.

More recently, Opitz et al. [48] seeded smooth muscle cells harvested from lambs onto poly 4-hydroxybutyrate scaffolds and subjected the constructs to a pulsatile flow bioreactor for 24 days. Following endothelial cell seeding, the construct was externally wrapped with ovine small intestinal submucosa and implanted in the descending aortas of juvenile sheep up to 24 weeks. Analyses of retrieved tissues indicated the presence of elastin in tissue-engineered vessels but the fibers were poorly organized. It also showed a marked decrease in desmosine content compared with the native aorta. Reduced desmosine content indicates that either less elastin was biosynthesized or the level of elastin crosslinking was low. One may argue that the migration of smooth muscle cells from the adjacent native aorta into the tissue-engineered vessel could result in the deposition of elastin. The uniform distribution of elastin over the entire length of the vessel after a short in vivo period, however, strongly supports the assumption that the elastin was indeed derived from the seeded cells. During the first few weeks following implantation, the lack of mature elastic components was compensated by the elasticity of the scaffold. However, after degradation of the scaffold material, the newly formed vascular tissue was too weak to withstand the aortic blood pressure resulting in a dilatation. The observed lack of mature elastin structures thus constitutes a serious limitation for tissue engineering of functional blood vessels designed for the high-pressure circulation. Biosynthesis and subsequent crosslinking of elastin appears to be one of the most complex and tightly regulated processes during the maturation of blood vessels [49].

### 3.2. The effect of mechanical stimuli on elastin biosynthesis

Kim et al. [53–55] studied the correlation between biomechanical signals and elastin biosynthesis under dynamic stimulation. They seeded rat aortic smooth muscle cells on collagen sponge scaffold and polyglycolic acid (PGA) scaffold. The PGA scaffold was bonded with poly(L-lactic acid) and treated by fibronectin coating. Compared with static culture conditions, elastin biosynthesis on bonded PGA scaffold was upregulated by dynamic stimulation. Elastin biosynthesis on collagen sponge scaffold, however, did not respond to the stimulation. In this case, it can be concluded that biomechanical stimulation alone was not sufficient for elastin biosynthesis but the nature of the scaffold also played a role. This finding is in agreement with the observation by Seliktar et al. who reported elastin mRNA expression to be independent of the dynamic stimulation on collagen gel scaffold [56]. The fact that collagen-based scaffolds appeared to be unfavorable for elastin biosynthesis is discouraging since decellularized collagen is frequently used as vascular substitutes. It is important to note, however, that recently, Isenberg and Tranquillo applied sustained dynamic stimulation to vascular cell-seeded collagen gel scaffold in an attempt to promote elastogenesis [57]. Their data revealed, for the first time, significant elastin biosynthesis on collagen scaffolds. Thus, we should reconsider the contradictory results obtained with collagen scaffolds and carefully develop new tests in future research.

In an interesting study on the effect of dynamic stimulation on elastin biosynthesis, Opitz et al. seeded vascular smooth muscle cells on poly-4-hydroxybutyrate scaffold and dynamically pulsed the construct [58]. For the time under investigation, the elastin content reached 9% relative to the native aorta whereas no elastin was detected in statically matured blood vessels. Although the level of
elastin was still significantly lower than that found in the native tissue and, additional biomechanical or biochemical signals are required for physiologically acceptable levels of elastin biosynthesis, their finding is encouraging.

In an effort to design an ideal tissue-engineered blood vessel, many investigators examined different types of scaffolds and mechanical stimulation techniques using bioreactors. Although most of them succeeded in improving the general mechanical properties, such as burst strength, as a result of collagen biosynthesis, most failed to induce elastin biosynthesis [28,30,31,59–61]. To study the effect of dynamic stimulation on cell behavior and the final extracellular composition by providing important biochemical and biomechanical signals similar to that observed in physiological condition, development in bioreactor technology for tissue engineering of blood vessels has also been recognized and progressed rapidly [16,43,62–66].

3.3. The effect of growth factors on elastin biosynthesis

In the extracellular matrix, the amount and arrangements of elastin are important for inducing cellular responses since specific cell-surface receptors like G-protein-coupled receptors and integrin α₅β₃, are able to bind cells with tropoelastin. Thus, elastogenesis can be mediated by different receptors and by various growth factors and specific culture conditions [3,13]. For instance, lysyl oxidase helps to crosslink the tropoelastin to give elastic fibers. In the absence of lysyl oxidase, tropoelastin tends to associate with glycosaminoglycan due to the presence of α-amino groups in the elastin lysine residues. These residues offer positive charges for binding with negative charges of glycosaminoglycan. In the absence of lysyl oxidase such electrostatic interaction could be important and prevent newly synthesized tropoelastin molecules from spontaneous random aggregation far from the cell surface [37,67]. Long and Tranquillo [2] studied the effect of TGF-β1 and insulin on elastogenesis in rat aortic smooth muscle cells. In the presence of these growth factors, the cells synthesized nonpolar amino acids which comprise about two-thirds of the elastin residues. This composition is similar to that found in human aortic elastin. Other investigators showed calcitriol and retinoic acid as factors that promote elastogenesis [68–70].

3.4. Other approaches on elastin biosynthesis

3.4.1. The self-assembly approach

An innovative approach was introduced by L’Heureux et al. [71] to design tissue-engineered blood vessels without the use of a scaffold. These vessels were exclusively made of cultured human vascular cells by a sequential rolling technique using a 3-mm diameter polytetrafluoroethylene mandrel. In step 1, a sheet of cultured smooth muscle cells was rolled using a mandrel to form the tubular medial layer. After 1 week of media maturation, a sheet of fibroblast cells was rolled around the cylindrical sheet of smooth muscle cells to form an adventitial layer. The cell composite was allowed to grow for 7 weeks in a luminal flow bioreactor, after which the mandrel was removed. Finally, endothelial cells were seeded into the lumen and the entire construct was further matured for a week. The resulting engineered blood vessel had high burst strength similar to that of native vessels, presumably due to collagen production. Immunohistochemical analysis showed the presence of elastin in the internal interfaces of the fibroblastic adventitial layer rather than within the muscular medial layer. However, the authors did not quantify the relative amounts of elastin. Although the entire procedure took more than 3 months of culturing and maturation, it would be considered as the most successful engineered blood vessel of its kind. Vasoactivity and other pharmacological investigation suggested this approach to be an excellent three-dimensional in vitro tissue model [72]. The authors implanted unendothelialized tissue-engineered human blood vessel (to avoid immediate thrombosis associated with the acute rejection of endothelialized vessel) to the femoral artery of dogs. In spite of the high burst strength, the lack of viscoelastic response under physiological condition due to the compliance mismatch occluded the vessel in just 7 days of implantation. Inadequate elastin biosynthesis into the medial layer is likely the main reason for compliance mismatch. This method of vascular tissue fabrication was the subject of a patent [73] and recently announced its first clinical use [74,75].

3.4.2. The peritoneal cavity “bioreactor” approach

Campbell et al. [76–78] reported a “designer” artery grown in the peritoneal cavity. Silastic® tubing of variable dimensions were inserted in the peritoneal cavity of rats and rabbits. In that position, macrophage-derived granulation tissue was formed on the surface of the Silastic® tubing as a result of inflammation. After the Silastic® tubing had been removed, the tissue was everted. This living vessel derived from inflammatory cells resembled native blood vessels, with an inner lining of mesothelium (endothelium-like cells), middle layer of myofibroblast cells and an outer layer comprising collagenous connective tissue. However, only 9% elastin relative to the native aorta content was secreted after 2 weeks in the peritoneal cavity. In terms of elastogenesis, this approach does not seem to be beneficial since similar amount of elastin has been reported using in vitro bioreactor [58]. The everted tissues were then grafted, by end-to-end anastomoses, into rat abdominal aortas or rabbit common carotid arteries of the same animals in which the grafts had grown. After 4 months of implantation in these animal models, the authors reported 50% patency for the peritoneal derived autologous vessels. These vessels were also found to respond partially to vasoactive agents. Although the presence of structures resembling elastic lamellae after 1 month of implantation in the rat aortic segments is reported, it was not quantified. Thus, this study is not conclusive for additional elastogenesis after the
vessels were grafted into arterial positions. The viability of the peritoneal cavity as a “bioreactor” has also been studied [79]. When the tubes were removed from the peritoneal cavity of dogs after 3 weeks, there was no significant elastin present. This was consistent with the rabbit and rat models [76]. The high burst strength reported (2500 mm Hg) is likely due to the high collagen content of the granulation tissue [80], suggesting the lack of elastin. After the grafts were interposed into the femoral artery of the same animals to which they were grown, smooth muscle-like cells were observed indicating transdifferentiation of the myofibroblast cells. Since inflammatory cells transdifferentiated to myofibroblasts and then to smooth muscle-like cells, the end point of this continued transdifferentiation is not apparent. Other than qualitative immunostaining, the authors also did not quantify the elastin content. In addition, unlike the rabbit and rat models, the retrieved grafts from dogs did not respond to vasoactive agents. This raises a question to the long-term application of the vessels and needs to be addressed before it is considered as a success story. The most striking observation in these grafts, however, was the presence of vasa vasorum that was not reported in any tissue-engineered vessels to date.

Encouraged by the work of Campbell et al. [76], Cebotari et al. [81] conducted a similar experiment using decellularized allogenic tissue as a tube. In contrast to Campbell’s group, they observed extracellular matrix degeneration and calcification after 21 days of implantation and concluded this method of blood vessel fabrication to be nonviable. Indeed, similar tissue degeneration was reported as early as 1973 [82] for vessels derived from inflammatory cells. The issue of in vivo tissue degeneration is an important aspect that should be addressed to overrule any possibility of graft failure in long-term application.

Instead of relying on elastin biosynthesis, many investigators turned their attention towards elastin-based scaffolds [15, 83–90]. However, these elastin-based scaffolds are not immune from enzymatic degradation. In fact, in vitro experiments demonstrated that elastin is continually degraded by elastase-type endopeptidases and new elastic fibers are continuously deposited [13]. If biochemical and biomechanical signals favoring elastin biosynthesis by vascular smooth muscle cells are not promoted, elastin-based scaffolds will ultimately fail to function due to enzymatic attack.

4. The use of stem cells for vascular tissue engineering to promote elastogenesis

Obviously, one of the critical elements required for successful tissue engineering of blood vessels is the appropriate selection of cells and a reliable cell source. Most efforts in the past decade have been focused on differentiated vascular cells. Recent advances in stem cells have been rapid, and their use as a cell source represent the next era of vascular tissue engineering research. Endothelial progenitor cells seeded on biodegradable scaffolds have been investigated as microvessels [91], and as a lining for decellularized porcine iliac vessels to promote patency [92]. We refer the reader to excellent reviews on the potential use of stem cell in vascular tissue engineering [93–96]. However, none of these reviewed works has yet generated any significant advances towards elastogenesis [97].

5. Conclusions

The ideal tissue-engineered blood vessel should possess sufficient mechanical strength, compliance, suture retention and contractile smooth muscle phenotype at the time of implantation. In all these aspects elastin plays an important role. Extracellular elastin provides mechanical elasticity to tissues and contributes towards the process of tissue remodeling by affecting cell–cell signaling. The formation of elastic fibers requires the assembly and crosslinking of tropoelastin monomers, and organization of the resulting insoluble elastin matrix into functional fibers. The molecules and mechanisms involved in this process are poorly understood. In current tissue-engineered blood vessels, the presence of this important structural and regulatory matrix is scanty. If we plan to succeed in our endeavors to replace the current prosthetic vascular grafts with tissue-engineered living tissues, the question why this important matrix is not laid down by the cells must be addressed. Approaches to incorporate elastin in tissue-engineered products by utilizing exogenous elastin scaffolds isolated from cadaveric tissues, supplying soluble tropoelastin to a cell culture, and designing biocompatible synthetic elastic polymers are, arguably, insufficient.

6. The way forward

Coronary artery and peripheral vascular diseases are the causes for high mortality rates in the Western society. Limited autologous veins or arteries with appropriate dimensions are available as ideal substitutes for vascular interventions but their clinical outcome is not satisfactory. From Voorhees et al.’s landmark paper on porous grafts in the 1950s [98] and that of Herring et al. [99] who pioneered endothelial cell seeding in the late 1970s, to the current approaches of tissue engineering, the quest for the ideal vascular substitute is still in its infancy. Tissue engineering of the ideal blood vessel is dependent on many variables, and the greatest challenge is to meet all the criteria simultaneously. In order to improve elastin biosynthesis in tissue-engineered blood vessels, several factors have to be studied. Scaffold material chemistry, appropriate cell source, culture conditions, growth factors, nutrient flow rates and frequency in dynamic stimulation are some of the variables that need to be considered for in vitro studies. As
we have seen, both scaffold chemistry and the mechanical stimuli play a significant role in elastin biosynthesis. Not only the qualitative presence of elastin but also its amount, its crosslinked structure and three-dimensional arrangement in tissue-engineered vessels are equally important. Successful tissue-engineered blood vessels incorporating elastin are, therefore, the Holy Grail of future vascular interventions [100].

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References


