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VASCULAR ELASTIC LAMINAE: ANTI-INFLAMMATORY PROPERTIES AND POTENTIAL APPLICATIONS TO ARTERIAL RECONSTRUCTION

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1. ABSTRACT

Biomaterials, including non-biodegradable and biodegradable polymers, and collagen and fibrin matrices, have been used in experimental and clinical arterial reconstruction. While these biomaterials exhibit various characteristics suitable for arterial reconstruction, the patency of biomaterial-based arterial substitutes remains problematic because of inflammation and thrombogenesis. Endothelial cell seeding of biomaterials has been proposed and used for reducing the thrombogenicity of biomaterials. However, difficulties in cell retention hamper the application of such an approach. Although autogenous vein grafts offer satisfactory results, not all patients possess veins available for arterial replacements. Thus, a critical issue in arterial reconstruction is developing arterial substitutes that are inflammation/thrombosis-resistant while possessing the characteristics of natural arteries. Here we show that allogenic vascular elastic laminae exhibit anti-inflammatory properties and may be considered a potential material for arterial reconstruction. In this article, we briefly review the composition, structure, and function of vascular elastic laminae, summarize recent discoveries on the role of elastic laminae in regulating leukocyte adhesion and vascular smooth muscle cell proliferation and migration, and discuss potential applications of allogenic elastic laminae to arterial reconstruction.

2. INTRODUCTION

Arterial reconstruction is an effective approach for the treatment of atherosclerosis. A number of materials, including biodegradable (1-4) and non-biodegradable (5-8) polymers, and collagen (8-12) and fibrin (13) matrix scaffolds, have been used in clinical and experimental arterial reconstruction. However, these materials induce various degrees of inflammation and thrombosis, hampering the performance of arterial substitutes (14-16). To overcome this problem, endothelial cell seeding on polymer and bio-matrix scaffolds has been proposed and tested in experimental models (5-7,17,18). However, difficulties in cell retention during and after arterial reconstruction hinder the application of such an approach (19). Although autogenous vein grafts offer satisfactory results, the availability of vein grafts is problematic in a considerable fraction of patients (19). Thus, a critical issue in vascular reconstruction is developing arterial substitutes that are inflammation/thrombosis-resistant while possessing natural characteristics, such as structural stability, mechanical strength and compliance, and vascular cell compatibility. Here we show that allogenic arterial elastic laminae exhibit such characteristics and may be considered a potential surface material for arterial reconstruction.

3. BIOCHEMISTRY, STRUCTURE, AND FUNCTION OF ARTERIAL ELASTIC LAMINAE

3.1 Composition and formation of elastic laminae

Arterial elastic laminae, the prevalent structure of the arterial media, are comprised of concentric sheets of tightly organized elastic fibers. These elastic fibers, which are arranged predominantly in the circumferential direction, are composed of microfibrils and amorphous elastin (20,21). Elastin is the most abundant protein found in large arteries and composes approximately half the dry mass of the vessel (20,21). Mature elastin, a highly insoluble and hydrophobic protein, is formed by the cross-linking of its 72-kDa precursor, tropoelastin (20,22). In higher vertebrates, including humans, approximately 75% of tropoelastin is composed of four amino acids, glycine,

valine, alanine, and proline (21). Tropoelastin is produced by several cell types, including smooth muscle cells (SMCs) and endothelial cells (21,23), and is released into the extracellular space where cross-linking and elastin formation take place (20). Mature elastin contains two major types of domains. The first type is hydrophobic and rich in the non-polar amino acids, including glycine, valine, proline, and alanine, which are often arranged in repeats of three to six amino acid peptides, such as GVGVP, GGVP, and GVGVAP (21,24). The second type includes cross-linking domains and is rich in alanine and lysine, the latter of which is subject to enzymatic cross-linking by lysyl oxidase (24). For tropoelastin, the lysine-containing domains, as well as the C-terminus, appear to be widely conserved, while the hydrophobic domains display considerable variability (21,22). This structural conservation renders elastin a highly inert and non-immunogenic protein.

Tropoelastin deposition, alignment, and cross-linking are dependent on various proteins and molecular interactions. Trafficking tropoelastin from the cytoplasm to the elastin assembly site in the extracellular space is made possible by a 67-kDa protein known as elastin binding protein (EBP), which protects tropoelastin from intracellular aggregation and proteinase attack (25). EBP, which is an inactive, alternatively-spliced form of beta-galactosidase (26), binds the hydrophobic sequences VGVAPG of elastin and LGTIPG of laminin (23). EBP also has a galactosugar-binding site, which, when occupied, causes a conformational change that results in the release of tropoelastin (27). This characteristic provides a mechanism for galactosugar-containing microfibrillar associated glycoproteins (MAGPs) to disengage tropoelastin from its chaperone EBP at the site of elastic fiber assembly (28). Subsequently, EBP is recycled by the cell in order to chaperone additional tropoelastin molecules back to the site of elastin polymerization (25).

Elastin assembly occurs at microfibrils, which are filaments 8 – 16 nm in diameter (21). Microfibrils comprise the non-elastin component of elastic fibers (29), and are composed of 350-kDa glycoproteins known as fibrillins and several MAGPs, including MAGP-1 and MAGP-2 (21,30). It is thought that microfibrils are established prior to elastin assembly, thus providing a scaffold for the deposition, alignment, and cross-linking of tropoelastin (20,31). MAGPs have been proposed to mediate the interaction between microfibrils and tropoelastin. One possible role of MAGPs includes their association with the C-terminal of tropoelastin during deposition (32). In this case, disulfide bonds formed between the C-terminal and the MAGPs may further stabilize tropoelastin prior to enzymatic cross-linking (21,30,33). The importance of the intact C-terminal is reinforced by the observation that tropoelastin lacking this terminal fails to assemble into proper elastic laminae (34,35). These regulatory processes may determine the alignment and organization of elastic fibers in arteries. However, it remains poorly understood what controls the directionality of the microfibrils during development.

After the organized deposition of tropoelastin at the microfibril, the final enzymatic cross-linking process ensues. Nearly all of the lysine residues of tropoelastin participate in cross-linking (36). The copper-dependent enzyme lysyl oxidase subjects epsilon-amino groups of targeted lysine residues to oxidative deamination, thus producing the alpha-amino adipic delta-semi-aldehyde, allysine (21,31,33,37). Condensation of lysine and allysine residues spontaneously follows, resulting in the formation of various bi-functional cross-links known as lysinonorleucines, and tetra-functional, elastin-specific cross-links known as desmosines and isodesmosines (21,22,33). It has been proposed that lysinonorleucine cross-links form a bridge between one elastin chain and two additional anti-parallel chains (37). The end result is an extensively cross-linked and considerably hydrophobic, insoluble material that precludes monomeric disassembly (37) and allows only non-specific digestion and degradation.

In addition to EBP, microfibril proteins, and lysyl oxidase, other proteins may play critical roles in the development of arterial elastic laminae. For instance, negatively-charged extracellular glycosaminoglycans may interact with the positively-charged lysine residues of tropoelastin to promote mature elastin assembly (38). Conversely, glycosaminoglycans containing galactose derivatives, such as dermatan and chondroitin sulfate, have been linked to impaired elastogenesis (34,39). The study of Merrilees and colleagues has demonstrated that cells over-expressing chondroitin sulfate-deficient versican variant V3 increased tropoelastin expression and elastic fiber formation in arterial SMC cultures, and resulted in elastic lamina formation in balloon injured carotid arteries (40). Another protein, latent transforming growth factor beta binding protein 2 (LTBP-2), is coexpressed with tropoelastin (41). Because LTBP-2 exists in numerous elastin-containing structures, and comprises an integral component of some microfibrils, it may contribute to elastic fiber formation (41). These examples demonstrate that a range of interactions and consequences may be possible due to the participation of various extracellular components, though the precise influences of these factors on tropoelastin assembly are still unclear.

In mammals, arteries contain concentric elastic laminae with circumferentially aligned elastic fibers, whereas veins are comprised of a network of elastic fiber bundles predominantly aligned in the axial direction of the vessel. When observed by optical and electron microscopy, elastic laminae or fibers appear amorphous under physiological conditions. Historically, it has been thought that elastic laminae and fibers are stable structures that undergo little turn-over and remodeling (22,42,43). However, recent studies have demonstrated that mechanical stretch in hypoxia-induced pulmonary hypertension can induce expansion of elastic laminae and exposure of the microfibrils within several hours (44,45). These observations suggest that elastic laminae and fibers may undergo dynamic remodeling in response to environmental stimulations.

3.2 Regulation of elastin gene expression

The human elastin gene is found in chromosome 7q11.1-21.1 (46), containing approximately 45 kbs (47,48), with the major hydrophobic and cross-linking domains encoded by separate exons (21,24). The elastin gene is highly expressed in conjunction with elastin synthesis during the early stage of embryonic development, though the level of mRNA is undetectable in normal adult blood vessels (42). A number of regulatory *cis* – elements are found in the untranslated, intronic, and promoter regions of the elastin gene. For instance, negative regulatory elements exist in the intronic region, while both positive and negative regulatory elements exist in the promoter region (21).

A variety of factors may play roles in the regulation of elastin gene expression. Growth factors, such as insulin-like growth factor (49) and transforming growth factor beta-1 (50), as well as other agents, including cGMP (51) and nitric oxide (52), have been shown to up-regulate elastin gene expression. Conversely, numerous factors, such as basic fibroblast growth factor (53), epidermal growth factor-like growth factor (54), interleukin-1-beta (55), and angiotensin II (56), have been demonstrated to down-regulate elastin synthesis. Conditions such as hypoxia (57) and age (58) have also been shown to contribute to elastin mRNA down-regulation and mRNA destabilization, respectively. In addition, the accumulation of tropoelastin in the extracellular space may influence the translation of elastin mRNA (21). Thus, a range of growth factors, agents, and environmental conditions can affect tropoelastin synthesis and, ultimately, elastin production.

3.3 Structural function of elastic laminae

Large arteries are comprised of multiple layers of elastic laminae. These laminae have long been known to contribute to the structural stability and mechanical strength of the arterial wall (44,45). Arteries are subject to extensive mechanical stress induced by arterial blood pressure. Without the support of the elastic laminae, vascular cells may be over-stretched under arterial blood pressure. For instance, elastic laminae degradation has long been known as a major factor in reducing arterial wall strength and inducing arterial aneurysms (59-62). The importance of the elastic laminae can also be demonstrated in experimental arterial reconstruction with vein grafts. Veins and arteries both possess a strong collagen-containing adventitia, though veins have only loosely organized elastic fibers instead of elastic laminae. When a vein is used as a graft and exposed to arterial blood pressure, ~60% of endothelial cells and SMCs die within 12 hours of implantation due to mechanical stretch (63). Thus, the elastic laminae are a critical structure for the stability and mechanical performance of a blood vessel.

In addition to mechanical integrity, the elastic laminae contribute to the elasticity of arteries. The recoil of the arterial wall is a critical mechanism for the continuation of blood flow during diastole when cardiac ejection is ceased. The unique amino acid organization and cross-linking patterns of elastin are commonly regarded as important determinants for the elasticity of the elastic fibers and laminae (64). Investigations by nuclear magnetic resonance have demonstrated that the backbones of elastin amino acid chains are highly mobile and individual amino acid residues are also able to move freely (65). The cross-links help organize the tropoelastin peptide chains into a filamentous network, an efficient structure for the storage of recoiling energy under mechanical stretch. Observations by electron microscopy suggest the presence of ordered filamentous structures in elastic fibers under extensive mechanical stretch (150 – 200%), while amorphous appearance is observed without mechanical stretch (20). The structure and organization of elastin provide a basis for the elastic properties of elastic fibers. Though, elastic laminae have long been considered a structure that provides arteries with shape, strength, elasticity, and stability, recent investigations have demonstrated that elastin may also serve as an extracellular signaling molecule (66-68).

4. ROLE OF ELASTIC LAMINAE IN VASCULAR PATHOGENESIS

4.1 Regulation of SMC proliferation and migration *in vitro*

SMC proliferation and migration are processes induced by a number of factors, including growth factors, inflammatory stimuli, and endothelial and vessel wall injury. SMC proliferation is often associated with migration, and both processes are regulated via similar mechanisms. SMC migration from the arterial media to the intima, together with SMC proliferation, are critical processes that contribute to neointimal formation and atherogenesis (69-71). Extracellular matrix components, including collagen and elastin, have been shown to mediate SMC proliferation and migration (72,73). While collagen serves as a stimulating factor for these processes (68,73,74), the role of elastin has been controversial.

A number of cell types commonly exhibit migration and proliferation *in vitro* in response to tropoelastin (68,75), elastin degradation products (67,74-81), and elastin peptides (78,82-87). Unlike other types of extracellular matrix proteins such as collagen, fibronectin, and laminin, elastin is not known to interact with integrins (67,68,88-90). However, blood and vascular cells, including SMCs, express a non-integrin receptor known as the elastin/laminin receptor, which has been shown to directly interact with elastin and mediate elastin-induced cellular activities (91,92). This elastin/laminin receptor is comprised of three subunits, including the 67-kDa EBP, a 61-kDa neuraminidase, and a 55-kDa protective protein (67). In addition to chaperoning tropoelastin to the extracellular space during elastic fiber assembly, EBP also acts as the elastin binding unit of the elastin/laminin receptor. Galactosugar-binding elicits a conformational change in EBP in both its chaperone and receptor modes. While in its chaperone mode, the conformational change causes EBP to release tropoelastin for elastic fiber assembly. However, while in its receptor mode, the conformational change not only induces EBP to release extracellular elastin, but also causes EBP to disengage from the elastin/laminin receptor (27). This attribute has provided investigators with a useful mechanism to remove EBP from cells in order to discern cell-elastin interactions.

EBP has been shown to mediate elastin-induced mitogenic activities (66,91). This role has been supported by the observation that the removal of EBP with lactose significantly reduces these mitogenic activities (81,88). It has also been suggested that EBP signals through a G-protein-related mechanism since elastin peptide-induced cell migration is inhibited by pertussis toxin (67,68). Additionally, *in vitro* studies have demonstrated that tropoelastin, elastin degradation products, and elastin peptides may act through EBP to increase intracellular Ca^{2+} (93) and NO levels (94), O_2^- production (89), tyrosine phosphorylation (67), and cause vasorelaxation (93,95). Lactose (67,93), pertussis toxin (67,89), and EBP antibody (67) inhibited these effects. Additionally, VGVAPG upregulates MMP-2, MT1-MMP, and TIMP-2 in a lactose- and pertussis toxin-sensitive manner (96). These observations reinforce the notion that a G-protein pathway possibly mediates EBP-related signaling events.

However, multiple mechanisms may exist by which elastin-cell interactions take place. For instance, chemotactic activity induced by elastin peptides prepared with elastase was not abolished completely by competitive VGVAPG binding (82). This observation suggests roles for non-VGVAPG peptides interacting, perhaps, with unidentified receptors. In support of the function of non-VGVAPG peptides, the elastin peptide VGVGVA has been reported to stimulate O_2^- production, elastase release, and intracellular Ca^{2+} level in a manner less sensitive to pertussis toxin than VGVAPG (89). Another peptide, GVAPG, was shown to induce monocyte migration that was insensitive to lactose, suggesting a role for a non-elastin/laminin receptor (86). It has also been demonstrated that elastin fiber binding to the elastin/laminin receptor is enhanced and not competitively diminished by the addition of elastin degradation products (97). This observation suggests a non-competitive relationship of multiple elastin-associating proteins (98). Perhaps one explanation for the spectrum of results elicited by various forms of elastin is that multiple types of elastin-cell interactions may exist, including that of insoluble elastin components with cells, and that of soluble peptides with cells (99). Since there is evidence that elastin-associating proteins other than those comprising the elastin/laminin receptor exist (99-101), elastin-cell interactions may involve various types of receptors (82,86,98).

More recent studies have demonstrated that elastin can also elicit inhibitory effects on the proliferation of vascular SMCs in culture models (68,73,102). In several *in vitro* investigations, SMC proliferation and migration were observed in collagen gels in the presence of elastin degradation peptides (103,104) and on surfaces coated with elastin degradation peptides (74). However, it was found that SMC proliferation and migration were significantly reduced in collagen gels with elastin degradation peptides in a dose-dependent manner (102). Similarly, these SMC activities were significantly suppressed on elastin peptide-coated surfaces compared with surfaces coated with collagen and fibronectin (74). Such effects are possibly mediated by trimeric G-protein signaling pathways since a treatment with pertussis toxin reduces or abolishes the inhibitory effect of elastin (68). Furthermore, Rho kinase is possibly involved in the regulation of elastin-induced, G-protein-mediated inhibition of SMC proliferation (68). As a result, actin polymerization increases and SMC proliferation decreases in response to a treatment with elastin degradation peptides (68). These observations suggest that elastin not only inhibits SMC proliferation, but also facilitates the transformation of SMCs from the proliferative type to the contractile type (105).

The variability in results may be attributed to differences in cell origins and experimental conditions. In addition, the selection of various control states may lead to different conclusions. The role of elastin peptides in regulating SMC activities may be observed in relevance to the quiescent state of SMCs or by comparing with the role of other biological and polymeric materials, such as collagen and polyglycolic acid. While elastin peptides may exhibit stimulatory effects on SMC proliferation and migration relative to the physiological quiescent state of SMCs, these peptides may induce lower SMC proliferation and migration compared with collagen and polymeric materials, demonstrating relative inhibitory effects on SMC activities. To fully understand the role of elastin peptides, a wide spectrum of experimental conditions should be taken into account. Finally, the action of elastin may depend on the interaction of elastin with multiple cell membrane receptors. The activation of the elastin/laminin receptor may promote mitogenic activities, whereas the activation of an inhibitory receptor, which has yet been identified, may suppress these activities. Further investigations are necessary to clarify these issues.

4.2 Regulation of vascular morphogenesis and pathogenesis *in vivo*

Proper deposition and organization of elastin are critical for vascular development. Defects of elastic laminae due to elastin gene mutations are implicated in numerous diseases (48,106-109), and are associated with excessive cell accumulation and abnormal arterial development (106,110-112). Examples include Williams Syndrome and supravalvular aortic stenosis, in which elastic fibers and laminae are composed of lower elastin levels (111,113). In the latter case, stenoses develop from vascular SMC proliferation and intimal hyperplasia (106,110). Surprisingly, affected vessels produce abnormally high numbers of elastic laminae, compensating for the elastin shortage and permitting vasculature of nearly normal compliance (111). Mice lacking the elastin gene die within days of birth from vascular occlusion due to subendothelial cell accumulation (110).

Given the alternating arrangement of elastic laminae and SMC layers in the arterial wall, elastic laminae and SMCs may mutually influence each other during vascular development. SMCs produce tropoelastin and likely play a role in the formation, organization, and directionality of elastic fibers and laminae. Conversely, elastic laminae may act through various cell membrane receptors to regulate SMC mitogenic activities in coordination with the stage and state of development. Galactosugars present in the extracellular space have been

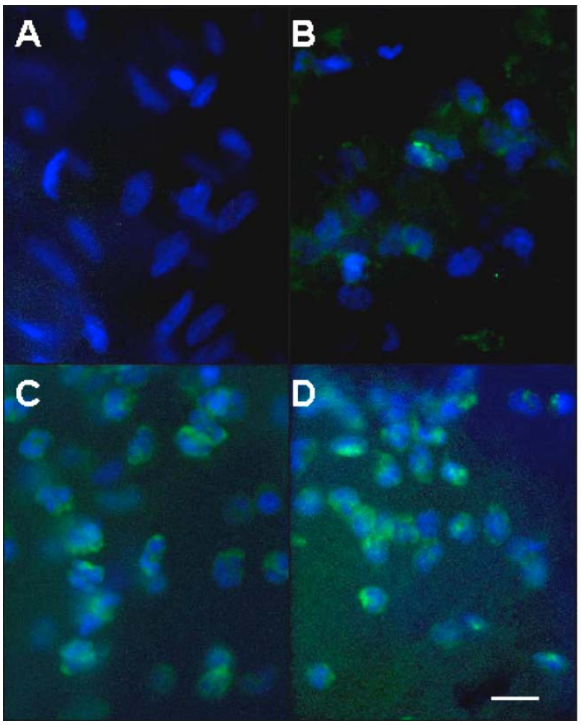

Figure 1. Fluorescent micrographs showing the influence of various surfaces, including the endothelium of a control aorta (A), elastic lamina (B), basal lamina (C), and adventitial collagen (D) on the adhesion of leukocytes labeled with an anti-CD 11 b/c antibody (green in color) at 30 min after scaffold implantation. The blue color represents cell nuclei. Scale bar: 10 micrometers. Reprinted with permission (116).

observed to instigate EBP shedding which impacts both elastin assembly (28) and cell adhesion (114). For example, in the normal fetal ductus arteriosus, an elevated level of chondroitin sulfate, a galactose derivative-containing glycosaminoglycan, is suspected to induce EBP shedding, thus impairing elastic fiber assembly, and promoting SMC invasion and ductus arteriosus closure (28,115). *In vitro*, ductus arteriosus SMCs with impaired EBP migrate through elastin scaffolds, while aortic SMCs with normal EBP presentation attach to the elastin surface, but fail to migrate through the scaffolds (114). Removal of EBP by exposure to chondroitin sulfate, however, allows aortic SMCs to migrate into elastin scaffolds, similar to the EBP-impaired ductus arteriosus SMCs (114). Additionally, in Hurler Disease, a high level of dermatan sulfate, an epimerized derivative of chondroitin sulfate, is thought to act similarly on reducing EBP efficacy, impairing elastic fiber construction, increasing fibroblast proliferation, and ultimately contributing to coronary artery stenosis (39). These observations suggest that elastin inhibits SMC mitogenic activities via the mediation of EBP.

Recent investigations have provided further evidence that elastic fibers and laminae elicit a predominantly inhibitory effect on SMC proliferation, thus preventing the development of intimal hyperplasia (109,110). In transgenic mice lacking the elastin gene, intimal hyperplasia develops rapidly, resulting in arterial stenosis and animal death shortly after birth (110). Furthermore, exogenous insoluble elastin inhibits Hurler fibroblast proliferation (39). These observations support the role of elastic fibers and laminae in the negative control of vascular mitogenic activities.

In vivo experiments have not yielded the range of results demonstrated by *in vitro* studies. While elastin fibers and peptides have both instigated and inhibited cell proliferation and migration *in vitro*, the effect of elastin *in vivo* has consistently been inhibitory. This disparity may be a byproduct of the controlled environment of *in vitro* studies where naturally-occurring physiological components are absent. This environment, therefore, may provide a non-physiological control state with which experimental observations are compared. However, the challenges associated with *in vivo* experimentation have impeded the dissection of elastin-mediated pathways. It is apparent that further investigation is warranted in order to reconcile the different observations between *in vitro* and *in vivo* studies.

4.3 Leukocyte adhesion to vascular elastic laminae

Leukocytes can be activated via cell membrane receptors interacting with inflammation initiators, chemotactic factors released by blood and vascular cells, and extracellular matrices, including collagen and fibronectin. Activated leukocytes, capable of adhering to injured endothelial cells or exposed matrix surfaces, release growth factors and chemotactic factors that attract additional leukocytes and stimulate SMC proliferation and migration. These processes contribute significantly to thrombogenesis and neointimal formation. Thus a critical issue in preventing thrombogenesis and neointimal formation in arterial substitutes is reducing leukocyte activation and adhesion.

Recent studies have shown that arterial elastic laminae, once implanted into a host artery and exposed to leukocytes, exhibit anti-inflammatory characteristics (116). Compared with the adventitial collagen matrix and basal lamina, elastic laminae were associated with significantly lower leukocyte adhesion (figure 1). Furthermore, the density of platelets on the elastic laminae was apparently lower than that on adventitial collagen and the basal lamina (figure 2). However, the density of leukocytes and platelets on the elastic laminae was higher than that on the endothelium of control aorta (figure 1, figure 2, figure 3). These observations suggest that, although elastic laminae are not completely inert, the elastic laminae are relatively inflammation/thrombosis-resistant compared with collagen-containing matrices.

4.4 Neointimal formation on vascular elastic laminae

Neointimal formation in arterial substitutes is a process initiated by leukocyte and platelet adhesion to the substitute surface, followed by thrombus formation. Subsequently, SMCs migrate from the host artery into the thrombus, resulting in an atheroma-like structure. Such a process contributes significantly to the failure of arterial substitutes. The thickness of the neointima has been

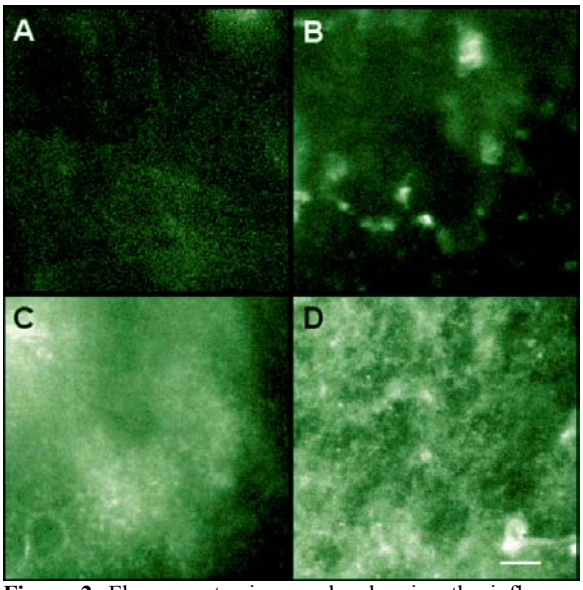

Figure 2. Fluorescent micrographs showing the influence of various surfaces, including the endothelium of a control aorta (A), elastic lamina (B), basal lamina (C), and adventitial collagen (D) on the adhesion of platelets labeled with an anti-GPIIb/IIIa antibody. Because of the overwhelming aggregation of platelets on the basal lamina and adventitial collagen, it was difficult to measure the density of platelets. Scale bar: 10 micrometers. Reprinted with permission (116).

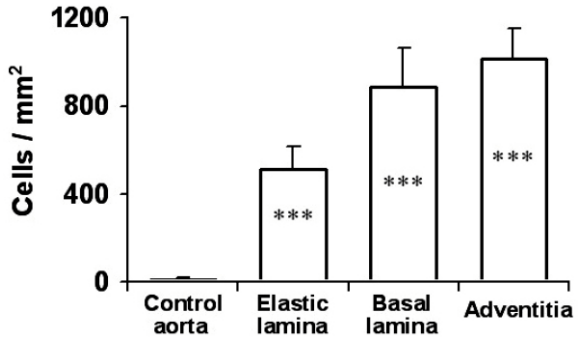

Figure 3. Variations in the density of leukocytes on various surfaces at 30 min. ***: p less than 0.001 for comparison between matrix surface and the control aorta. Reprinted with permission (116).

considered an index for assessing the performance of arterial substitutes. To date, almost all biomaterials used in arterial reconstruction, including polymers, collagen matrix, as well as the standard autogenous vein graft, cause neointimal formation. Thus the inhibition or reduction of neointimal formation is one of the most important issues in arterial reconstruction.

Recent studies have demonstrated that, in a rat model with implanted adventitial, basal lamina, and elastic laminae patches, the elastic laminae patch was associated with significantly less neointimal formation than the collagen and basal lamina patches (figure 4, figure 5) (116). Furthermore, the thickness of the SMC layer and the rate of SMC proliferation, detected by using a BrdU incorporation method, were significantly less on the elastic lamina patch compared with those on collagen-containing patches (figure 6, figure 7) (116). Similar results were observed when rat aortic matrix scaffolds were used as aortic substitutes with adventitial collagen and elastic lamina surfaces (Liu *et al*., unpublished data). The thickness of SMC-containing neointima on the elastic lamina surface of the aortic substitute was even less than that of the autogenous vein graft in the same animal. These observations suggest that the vascular elastic laminae not only possess anti-inflammatory characteristics, but also induce anti-proliferative effects on SMCs, thus resulting in reduced neointimal formation.

Elastic laminae have been shown consistently to inhibit cell mitogenic activities *in vivo*. Perhaps the first *in vivo* demonstration of the anti-thrombotic nature of elastic laminae is provided by Kabemba and colleagues, who demonstrated that proteolytically-exposed elastic laminae of dog femoral arteries resist clot formation even in the absence of their intimal layer (117). In a more recent *in vivo* study, stents coated with elastin sheaths, prepared from carotid artery digestion, elicit inflammatory or thrombotic responses significantly lower than that in uncoated stents (68). Taken together, these previous investigations support the inhibitory role of vascular elastin and elastic laminae, rendering elastic laminae a suitable material for the construction of arterial substitutes.

5. POTENTIAL APPLICATIONS TO ARTERIAL RECONSTRUCTION

The anti-inflammatory properties of elastin have been captured in elastin-derived peptides and used to combat cell and protein adhesion (118), as well as postoperative abdominal adhesions (119). In the role of vascular reconstruction, the anti-inflammatory and anti-SMC proliferative features of vascular elastic laminae render these laminae a potential material for arterial replacement. Allogenic arterial elastic laminae scaffolds have been recently produced by NaOH treatment of fresh arterial specimens and used for experimental arterial reconstruction (Liu et al., unpublished data). Preliminary investigations have provided promising results for elastic laminae-based arterial reconstruction.

While the elastin matrix is proven a relatively anti-thrombogenic structure, the collagen matrix remains a necessary component for the construction of vascular substitutes. First, a collagen matrix provides the structural framework and mechanical strength of a vascular scaffold. A pure elastin scaffold may not be able to withstand tensile stretch due to internal blood pressure. Second, collagen fibers have been shown to play a role in matrix-cell signal transduction, a critical process for cell survival, proliferation, and migration (120,121). As shown in a recent study, a collagen matrix attracts more SMCs than the elastic laminae, which may be beneficial to the process of scaffold vascularization (116). However, due to its thrombogenicity, collagen may not be a suitable surface material for vascular reconstruction.

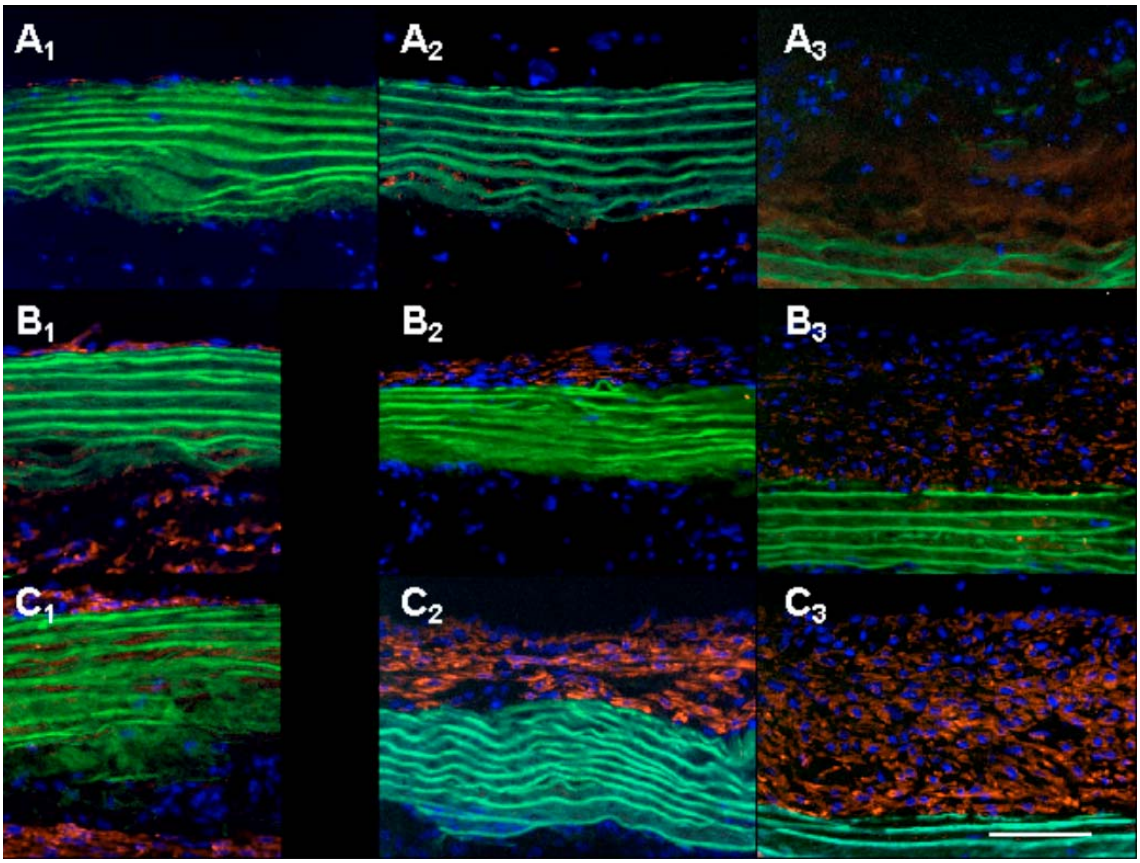

Figure 4. Fluorescent micrographs showing the influence of various matrix surfaces on neointima formation. Row A, B, and C demonstrate the anti-alpha-actin antibody-labeled SMCs (red in color) in matrix scaffolds collected at day 5, 10, and 20, respectively. Subscripts 1, 2, and 3 indicate various matrix surfaces, including the elastic lamina, basal lamina, and adventitial collagen, respectively. The blue color represents cell nuclei, and the green represent elastic laminae. Scale bar: 100 micrometers. Reprinted with permission (116).

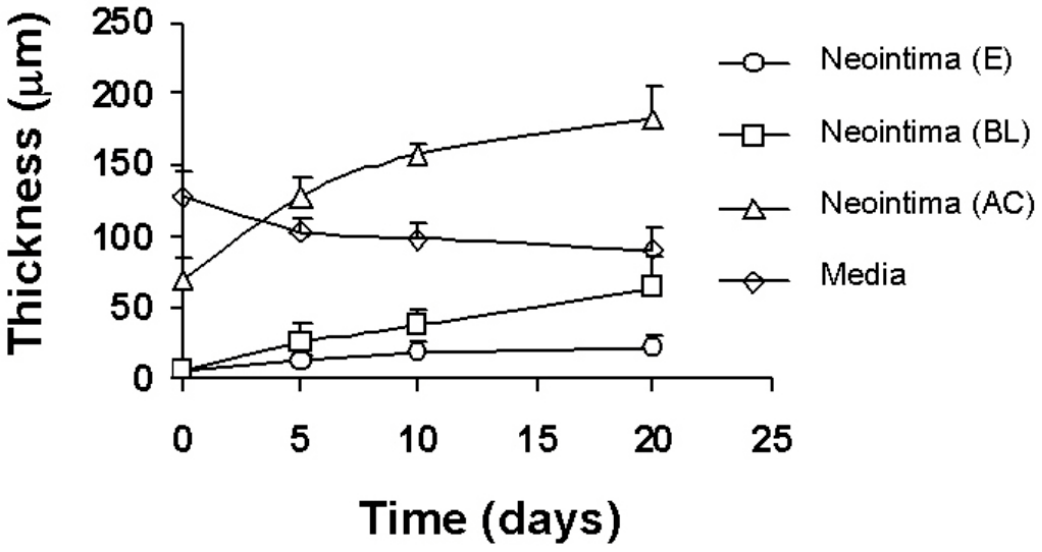

Figure 5. Changes in the thickness of neointima on various matrix surfaces, including elastic lamina (E), basal lamina (BL), and adventitial collagen (AC). All changes were statistically significant from time 0 (control) to day 20 (ANOVA p less than 0.01). The thickness of the media of the matrix scaffold is also presented, which decreased from day 5 to 20. Reprinted with permission (116).

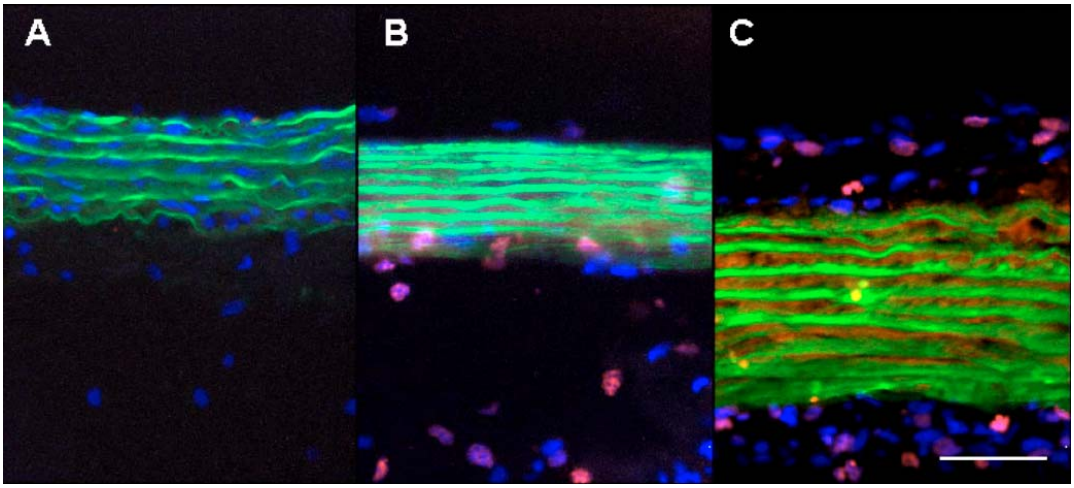

Figure 6. Fluorescent micrographs showing the influence of various surfaces, including the endothelium of a control aorta (A), elastic lamina (B), and basal lamina (C), on BrdU incorporation at day 10. The lower side of each scaffold is the collagen-containing adventitia. The red color represent anti-BrdU antibody-labeled cell nuclei, the blue represents total cell nuclei, and the green represents elastic laminae. Scale bar: 100 micrometers. Reprinted with permission (116).

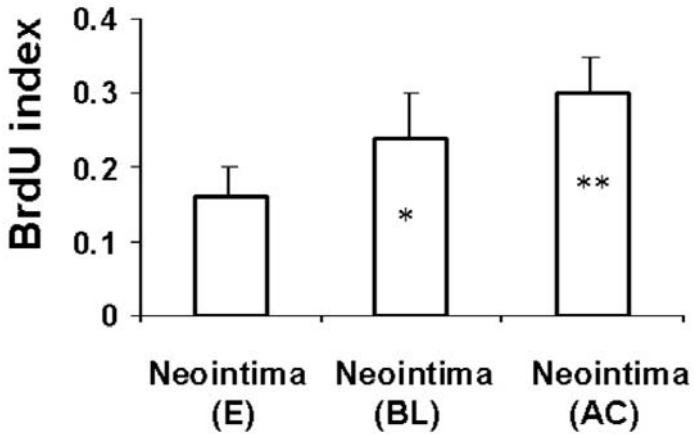

Figure 7. Comparison in the BrdU-labeling index, defined as the ratio of the density of BrdU-labeled cells to that of total cells, between various matrix surfaces at day 10. * and **: p less than 0.05 and 0.01, respectively, for comparisons between the elastic lamina and another surface. E: elastic laminae, BL: basal lamina, and AC: adventitial collagen. Reprinted with permission (116).

The immune response of a host to an allogenic tissue, which induces acute rejection, has been a major concern in tissue and organ transplantation. Fortunately, it is the cellular components that cause acute host immune reactions, a process requiring the participation of living cells and signaling molecules (116). Extracellular matrix proteins, including elastin and collagen, exhibit little immunogenicity and do not cause acute vascular rejection (116). These investigations support the use of allogenic vascular matrix for vascular reconstruction.

However, we have noticed a gradual decrease in the thickness of the elastin matrix after implantation (figure 5), suggesting a process of elastin degradation (116). Although chronic host immune reactions are among potential factors, the lack of immune cells within the elastin matrix at all observation times suggests a negative role for immune reactions. Previous studies have shown that elastin degradation is mediated by matrix metalloproteinases (116). Whether and how these proteinases influence the integrity of elastin matrix in this model remain to be assessed.

Another concern with elastic laminae-based arterial reconstruction is the lack of cell migration into the elastin matrix. While a high density of leukocytes and SMCs was observed within the adventitial collagen matrix, few cells were present in the spaces between elastic laminae. To date, how elastin matrix inhibits leukocyte infiltration and SMC migration remains to be assessed. Although an acellular elastin matrix may not significantly influence the mechanical performance and strength of a vascular scaffold within a short term, the lack of SMCs possibly influences the vascularization and contractility of the scaffold. These issues should be addressed before an elastin matrix is used for vascular reconstruction.

6. PERSPECTIVE

Elastin has demonstrated both structural and signaling functions. Although *in vitro* experiments have yielded both stimulatory and inhibitory results, *in vivo* investigations have consistently shown an inhibitory role for elastin in mediating SMC migration and proliferation. Similarly, allogenic elastic laminae grafts have exhibited anti-inflammatory characteristics *in vivo*. Although further investigations are needed to clarify the mechanisms by which elastin and the elastic laminae exert their effects, recent evidence indicates that allogenic arterial elastic laminae may be considered a potential blood-contacting material for arterial reconstruction.

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