Review

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Elastin biology and tissue engineering with adult cells

Abstract: The inability of adult cells to produce well-organized, robust elastic fibers has long been a barrier to the successful engineering of certain tissues. In this review, we focus primarily on elastin with respect to tissue-engineered vascular substitutes. To understand elastin regulation during normal development, we describe the role of various elastic fiber accessory proteins. Biochemical pathways regulating expression of the elastin gene are addressed, with particular focus on tissue-engineering research using adult-derived cells.

Keywords: elastin; heparin; smooth muscle cell; vascular tissue engineering.

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Introduction

A major challenge in engineering artificial tissues is the difficulty in replicating normal extracellular matrix deposition within tissue constructs. This problem is pervasive; collagen fibers in cartilage must be properly organized in a hierarchical fashion to sufficiently support mechanical and cellular function (1), whereas biomimetic bone replacements need to organize macromolecular and hydroxyapatite components to resist compression and yet retain structural integrity (2). Similarly, tissue-engineered arteries must produce sufficient organized elastin and collagen to allow for repeated cycles of expansion and contraction in response to pulsatile flow (3). This review focuses primarily on blood vessel tissue engineering, particularly the challenge of inducing sufficient elastin expression from cells that colonize vascular constructs.

Developmental regulation of elastin dictates that synthesis is predominantly *in utero* and early childhood (4). New elastic fibers are not produced in appreciable amounts under normal physiological circumstances in adult cells. This deficit presents a significant challenge in tissue engineering of vascular constructs that seek to replicate the elastin content of natural vessels. This review considers factors involved in elastic fiber formation during normal development and addresses recent advances in the induction of elastin expression by cells from adult donors.

Vascular disease and elastin

Cardiovascular disease (CVD) remains the leading cause of death in the United States and a major cause of death worldwide (5). Common treatment options for CVD include coronary artery bypass grafting (CABG) and percutaneous transluminal coronary angioplasty (PTCA), predominantly in conjunction with stent implantation (6, 7). These surgical interventions have reduced mortality in recent years, but improvements are needed as thrombosis and restenosis are serious complications still associated with these procedures (8). CABG is typically performed using the autologous saphenous vein or internal mammary artery; however, these vessels are often unsuitable due to age, disease or repeat procedures (9). Commercial stents induce blood clotting, cause inflammation at the deployment site and interact poorly with vascular cells (10). Thus, there is a critical clinical need for a reliable vascular graft alternative that exhibits increased biocompatibility, utilizing normal biological responses to promote proper re-endothelialization

without triggering an inflammatory response or increasing clot formation.

One of the largest unmet challenges in the development of a sound tissue-engineered vascular graft (TEVG) is to promote a robust elastic fiber network upon recellularization of the graft. This is of critical importance, as elastic fibers are responsible for key mechanical and biochemical functions of the vessel (11). The primary challenge in promoting elastic fiber formation is that expression of the protein is developmentally restricted to the preadolescent stage, i.e., properly organized elastin is not expressed by adult cells.

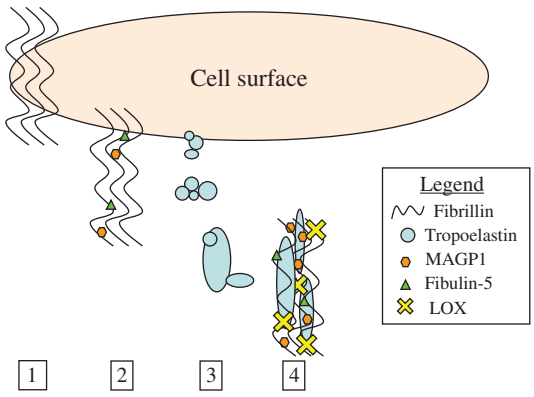

Figure 1 Elastin formation.
Initially, soluble fibrillin proteins are secreted from the elastogenic cell and deposited in the extracellular space, where they form microfibrils (Step 1). In Step 2 of assembly, microfibril-associated glycoprotein 1 (MAGP-1) and fibulin-5, along with additional fibulins and supplementary molecules, begin to interact with the fibrillin network. The ability of these molecules to facilitate the association of fibrillin and tropoelastin promotes assembly. Step 3 appears to occur concurrently with Step 2, as accumulating molecules of tropoelastin initiate association by coacervation, consisting of larger micron-sized assemblies that coalesce to form larger structures and continue to be deposited onto the microfibrillar scaffold in Step 4. Due to their oxidation by lysyl oxidase, processed tropoelastin molecules cross-link irreversibly to form the mature elastic fiber.

The biology of elastin

Elastic fibers are predominantly found in the extensible tissues of the body, specifically skin, lungs and larger blood vessels (12). These tissues undergo repeated stretch and contraction throughout the lifetime of the host without significant damage, due to their robust mechanical properties. In addition, elastin and derived peptides regulate smooth muscle cell behavior biochemically, thus promoting maintenance of a healthy vessel (13, 14).

The elastic fiber assembly process has been thoroughly reviewed elsewhere (15, 16). Elastin fibers are composed mainly of cross-linked tropoelastin, supplemented by complex extra cellular matrix (ECM) molecular components. Fibrillins are the principal supplementary molecules, as they assist in directing the sites and spatial distribution of elastogenesis (16). Additional accessory proteins with important roles include microfibril-associated glycoprotein-1 (MAGP-1), the fibulin family (1, -2, -4 and -5), emilin-1 and the family of lysyl oxidases (LOX) that oxidize tropoelastin as a prelude to enzyme-free crosslinking (16).

An early step in elastic fiber assembly occurs when fibrillin-1 and -2 are secreted and assemble in the extracellular space (Figure 1). This fibrillin network provides a scaffold onto which tropoelastin is deposited. Tropoelastin deposition is initiated by the spontaneous assembly of monomers through a process called coacervation (17, 18). The unique biochemical properties of tropoelastin, in particular its multiple hydrophobic domains, facilitates self-assembly under physiological conditions. Upon secretion into the extracellular space, small tropoelastin coacervates form micron-sized spheres that are deposited onto microfibrils (12, 19). A feature of cross-linked tropoelastin, important for elastic fiber stability, is desmosine and isodesmosine linkages, which are unique to elastin and thus can be used in the laboratory for quantification (17). These accumulate with additional linear linkages allysine aldol and lysinonorleucine, all of which are derived from abundant lysines in tropoelastin following oxidation of the epsilon amino group by members of the LOX family (15).

The ELN gene

To determine the important factors in regulating tropoelastin secretion, an understanding of the gene encoding tropoelastin (ELN) and its regulation at the transcriptional level is needed. ELN is a single-copy gene found at chromosomal location 7q11.23 in humans (20). It is present in all vertebrates except for the cyclostomes and likely evolved due to increasing demands for extensible tissues due to higher pressure in a closed circulatory system (21). ELN is 45 kb, encompassing a total of 36 exons. The first exon encodes a proteolytically removed signal sequence that dictates secretion, whereas the remaining exons are subject to alternative splicing relative to events in different tissues and at different developmental stages (22, 23).

Regulation of expression is primarily controlled at the transcriptional level. Abundant evidence indicates that regulation of mRNA stability (as opposed to production) is the key point of regulation (24). mRNA is produced continually in both fetuses and adults, albeit at different levels,

but more rapidly degrades in adults (25). In analyzing the steps of ELN mRNA processing, it has been demonstrated that although mRNA levels correlate with protein levels, pre-mRNA levels are much higher, which indicates that a limiting factor in expression is the pre-mRNA to mRNA processing step (4). The promoter region of ELN extends at least 5 kb upstream of the start codon and contains response elements for Sp1, AP-1, AP-2 and TGFβ (26, 27).

Elastic fiber and human disease

As elastin is critical for elastic tissue development, it is not surprising that human disease has been linked to various mutations in ELN and elastin-related proteins. These disease states underscore the importance of a robust elastin network, as well as the consequences of imbalance in the regulation of ECM components. Specific examples are described below, with further information found in Table 1.

Supravalvular aortic stenosis (SVAS)

SVAS is typically due to functional loss of one copy of the elastin gene. Multiple mutations, including nonsense, translocations and within splice sites, are associated with this disease (28). SVAS can be either inherited as an autosomal dominant disorder or can arise through spontaneous mutation, such as the genome deletion events observed in Williams Syndrome (29, 30). Physiologically, SVAS presents as progressive narrowing of the supravalvular aortic and pulmonary vessels and may also affect other large vessels due to their higher elastin content. SVAS is rooted in elastin defects, consistent with the phenotypic observations from *Eln* $^{-/+}$ mice, which show many symptoms of the human disease (31). Each SVAS defect leads to truncated or aberrant tropoelastin monomers, leading to impaired elastin assembly and, ultimately, vascular insufficiency. In particular, smooth muscle cell (SMC) hyperproliferation associated with the disease is likely due to several factors, including a reduction in physical restriction of cell migration resulting from loss of highly organized elastic fibers and the presence of elastin fragments which are chemo-attractive to VSMCs (30, 32).

Cutis laxa

Cutis laxa is a collection of disorders characterized by defects in elastin-containing tissues, primarily skin and blood vessels (33). At the cellular level, defects appear in elastin fiber crosslinking. In cutis laxa patients, mutations in fibulin-5 and lysyl oxidase have been found, which provides a genetic link between specific elastin related proteins and the observed phenotypes (34, 35).

Marfan Syndrome

Marfan Syndrome (MFS) is characterized by a disruption of skeletal and lung phenotypes and weakening of arterial walls leading to aortic rupture (36). MFS arises from dominant-negative mutations in fibrillin-1, a key component of the elastic fiber (37). Mice with a mutation in fibrillin-1, recapitulating a common MFS mutation, give insight into the elastic fiber assembly process. Challenging the belief that deposition of a fibrillin scaffold was a necessary initial step in elastogenesis, mice expressing mutant fibrillin-1 deposit properly organized elastic fibers during development (38). This could be due to compensation by fibrillin-2 or some remaining ability of mutant fibrillin-1 to sufficiently polymerize to support fiber deposition. These fibers appear normal by microscopic analysis, yet they form vascular walls that are mechanically insufficient, leading to aortic rupture.

Defects in elastin homeostasis

Inflammatory disease typically leads to degradation of the ECM due to excessive cytokine and protease

Table 1 Elastin-related diseases.

Disease	Characteristics	Cause
Supravalvular aortic stenosis (SVAS)	Arterial wall weakness, aortic rupture	Functional loss of one copy of ELN
Cutis laxa	Loose skin, blood vessel deformities	Mutations in fibulin-5, ELN; defects in Cu^{2+} homeostasis
Marfan's syndrome	Thin vascular walls, aortic rupture	Mutations in fibrillin-1 gene
Emphysema	Degradation of lung elastin, excessive human leukocyte elastase (HLE)/MMP12 release	Multiple environmental causes
Scleroderma	Imbalance of ECM components, disorganized elastin	Autoimmune disease

secretion by inflammatory cells. Loss of elastin specifically is associated with emphysema, due to the release of macrophage elastase (MMP12) and human leukocyte elastase (HLE) in the lungs (39). In addition, aortic aneurysm (AA) is a consequence of a decrease in elastin in the large vessels (40). The release of TNFα and IL-1β induces changes in the SMC phenotype, which in turn leads to poor matrix remodeling and vascular calcification (41). Conversely, over-accumulation of poorly organized elastin due to aberrant deposition in adult tissues leads to pulmonary hypertension, scleroderma and fibrotic lung disease (Table 1) (42).

Elastin and smooth muscle cell phenotype

In the vasculature, elastin forms the highest percentage of ECM in the large vessels (approx. 50%), in contrast with 5% in the skin (17). Because of this abundance, most mutations affecting elastin cause significant cardiovascular defects. For example, homozygous elastin knockout mice ($Eln^{-/-}$) survive gestation, but die 4–5 days after birth due to vascular smooth muscle cell hyper-proliferation leading to arterial obliteration (32). In these mice, SMC proliferation and invasion of the vessel lumen is seen in the absence of inflammation, endothelial cell damage, disruption in collagen organization and even in the absence of blood flow. This indicates that an intact elastic lamina is required to maintain a quiescent phenotype in medial SMCs, which further emphasizes the tissue- and temporo-specific regulation by ELN, as the intestines contain both SMCs and elastin, though no intestinal disruption is indicated in this model (43).

Subsequent cell culture experiments further emphasize the important effect that elastin has on the smooth muscle cell phenotype. Elastin was originally thought to play primarily a mechanical role in vessels, but the increased proliferative capacity of SMCs from $Eln^{-/-}$ points to a broader role in cell signaling (13). SMCs from $Eln^{-/-}$ mice show disorganized actin cytoskeletons and focal adhesions, further indications of their proliferative phenotype. Importantly, exogenous recombinant elastin can rescue this phenotype, showing that endogenously organized elastin fibers are not required for this biological effect. Consistent with the hypothesis that cell surface receptor activation could initiate this phenotypic switch, the G_i type of G-protein coupled receptor, signaling through RhoA, is necessary for elastin-initiated quiescence in SMCs (13).

From a tissue-engineering perspective, a porcine carotid stent model using an elastin-coated stent was shown to reduce SMC hyper-proliferation as compared to control, thus demonstrating the biochemical regulation of the SMC phenotype *in vivo* (13).

Studying elastin: *in vitro* models of elastin formation

Elastin formation *in vitro* has predominantly been studied using primary fetal/neonatal cultures of smooth muscle cells or fibroblasts (44). As primary cells quickly demonstrate decreased elastin expression as the passage number increases, mouse or rat cells are frequently used because they are more easily obtained (45). Variables such as serum concentration, growth factor additives, cell density and time affect elastin formation and complicate some studies, so models have been developed to address the technical limitations imposed by the preference for primary neonatal cells (15, 46).

One informative model uses immortalized pigmented epithelial (PE) cells derived from human eyes (47). These cells express microfibrillar components, as they are found in close proximity to the retinal basement in the Bruch's membrane. Though PE cells do not naturally express ELN, transfection with a bovine ELN construct is sufficient for extensive cross-linked elastin deposition on microfibrils, though the three dimensional organization seen in normal tissue is lacking (48).

In another model, recombinant tritiated tropoelastin is added to elastogenic cultured neonatal rat aortic SMCs (47). The amount of added tropoelastin incorporated into existing elastin fibers is measured by the quantity of tritiated desmosine and isodesmosine linkages. When a non-elastogenic cell type is used as a source of lysyl oxidase, recombinant tropoelastin is incorporated. This incorporation, combined with data showing the process is inhibited by β-aminoproprionitrile (BAPN), a lysyl oxidase inhibitor, confirms: 1) the importance of lysyl oxidase in elastin formation and 2) the ability of exogenous tropoelastin to become a substantial component of elastic fibers. This work has implications for therapeutic treatment of elastolytic diseases like cutis laxa and emphysema, as it raises the possibility of a combination therapy using recombinant tropoelastin and LOX (48).

Live imaging studies have been instrumental for demonstrating the temporal appearance of each component of the elastic fiber during assembly. Wagenseil et al. showed that the first component to appear is fibronectin, then fibrillin, MAGP-1, fibulins and, finally, the dominant component elastin. In these experiments, primary and fluorescently labeled secondary antibodies were applied to living cells, imaged and then removed after 1 h (15).

The three-dimensional tissue environment where cells naturally grow is difficult to mimic *in vitro*, but no doubt plays an important role in cell behavior and ECM organization. In a series of studies, Faris et al. used long-term (up to 43 weeks) cultures of neonatal rat SMCs to examine elastin formation between layers of SMCs in the vasculature (46). These studies take advantage of a somewhat unique ability of SMCs to grow in multiple layers in culture, where other cell types generally become quiescent due to contact inhibition. It was shown that elastin forms between layers of SMCs in a pattern very similar to that of the native vessel, which emphasizes the importance of the 3D environment in elastogenesis.

Elastin-interacting molecules

The following provides an overview of the most characterized of the elastin-interacting proteins, though these have been reviewed in more depth previously (15–17). These molecules are summarized in Table 2.

Fibrillins

The fibrillins (1, 2 and 3) are large extracellular matrix glycoproteins that polymerize as major components of microfibrils to give a beaded periodicity (16). Dogma states that fibrillin-1 is required for elastic fiber deposition, where it forms a scaffold onto which tropoelastin is deposited and cross-linked (49, 50). Fibrillins interact directly and indirectly with molecules on the cell surface through a variety of interaction domains, including RGD sequences (interacting with integrins), and heparin-binding domains (51). Fibrillin-1 and -2 bind directly to tropoelastin in biochemical experiments and are closely localized *in vivo* (49). Defects in fibrillin-1 lead to Marfan's syndrome in humans, characterized by early mortality due to aortic aneurysm in most patients (38). Transgenic mice that contain a deletion of five exons in the middle region of fibrillin-1 recapitulate many aspects of Marfan's syndrome, including death due to hemorrhage. However, contrary to the expected results, the disrupted fibrillin does not interfere with normal elastin deposition, but only with remodeling in this system (38).

Fibulins

The fibulin family consists of seven characterized, related extracellular matrix proteins (34, 55). They comprise repeats of Ca^{2+}-binding, EGF-like domains (cbEGF-like domains). Fibulins also share a similar C-terminal domain structure with the fibrillins. Fibulin-1 and -2 are significantly larger than their three to five counterparts and all undergo alternative splicing. Fibulin-5, the only fibulin to contain a RGD sequence, can bind integrins and elastin and is proposed to facilitate tropoelastin deposition (52).

Fibulin-1 is detectable in serum and neuronal cell cultures, which indicates a possible nerve-specific ECM function, particularly as fibulin-1 also binds to the β amyloid protein (53, 54). Though associated with basement membranes, fibulin-1, -2 and -3 are involved directly with elastin fibers. The fibulin-4 knockout mouse shows defects in vascular function, with aortic narrowing and tortuosity, leading to perinatal death. In these mice, elastin is aggregated and disorganized, with greatly reduced cross-linking, despite normal levels of both elastin and LOX. These features, combined with the observation that fibulin-4 binds both elastin and fibrillin, infer that fibulin-4 is likely to play an important role in fiber assembly (55).

Table 2 Elastin-interacting molecules.

Molecule	Description	Function
Fibrillins	Large polymeric glycoprotein	Forms microfibrils
Fibulins 1–5	Ca^{2+}-binding	Binds both elastin and fibrillin, likely bridges them during and after assembly
MAGP-1 and -2 (microfibril associated glycoprotein-1 and-2)	Small glycoprotein	Binds elastin and fibrillin
EMILINs (elastin microfibril interface located protein)	Glycosylated, with collagen type-VIII homology domain	Interacts with elastin and fibulin-5
Lysyl oxidase (LOX) family	Cu^{2+}-dependent amine oxidase	One or more members required for elastin crosslinking
Versican	Large glycoprotein	V3 variant promotes elastic fiber formation
Elastin-binding protein (EBP)	Surface localized splice variant of β-galactosidase	Elastin receptor, may escort tropoelastin monomers to nascent fibers in extracellular space

The fibulin-5 knockout mouse mimics some of the effects of fibrillin disruption, including loose skin, malformation in the major vessels and inadequate alveolarization of the lungs, a phenotype that is similar to the human disease cutis laxa. *In vitro*, fibulin-5 co-localizes and binds to elastin, and overexpression of fibulin-5 can induce elastic fiber formation (56). Commensurate with other animal models that display disruptions in elastin fibers, the fibulin-5 knockout mouse exhibits increased SMC proliferation and migration, both *in vivo* and *in vitro* (57). In a mouse carotid ligation model, both fibulin-5 and elastin are up-regulated in response to injury (57). Fibulin-5 is a ligand of superoxide dismutase (SOD), an enzyme responsible for the modulation of reactive oxygen species in the vessel and thus may also aid in vessel responses to free radicals (58).

Microfibril-associated glycoproteins (MAGP)

MAGP-1 and -2 (*mfap-2*, and *-5*, respectively in the mouse) are 20-kDa glycoproteins that co-localize with fibrillin/elastin junctions *in vivo*. Though the *mfap2*$^{-/-}$ mouse has no observed defects in elastin formation, MAGP-1 binds both fibrillin-1 and -2 and tropoelastin and may therefore perform a molecular bridging function (15). Interestingly, recently reported data describe a role for MAGP-1 in bone remodeling, where MAGP-1 may increase osteoclast differentiation by TGFβ-mediated up-regulation of RANKL (15).

EMILINs

EMILIN-1 is a matrix glycoprotein associated with elastic fibers (59). It is found throughout elastic tissues, partially co-localizing with elastin, though it appears earlier in development. Biochemically, the 115-kDa protein shares sequence homology with collagen type VIII and complement C1q protein and is highly glycosylated (60). Another family member, EMILIN-2, shows a similar protein structure but a slightly different tissue distribution (61). The EMILINs are of particular interest because, in addition to their close association at the interface between elastin and microfibrils, the proteins interact directly with elastin and fibulin-5. Additionally, the EMILIN-1 knockout mouse exhibits disrupted elastin in the aorta and skin, and derived cells are deficient in elastin assembly *in vitro* (62). Thus, although the precise role of EMILIN in elastin development and remodeling is undefined, there is sufficient evidence of its involvement to support consideration in future studies.

Lysyl oxidase (LOX)

LOX is a copper-containing amine oxidase that oxidizes lysine residues, which facilitates cross-linking between lysine resides on adjacent tropoelastin monomers. This includes the desmosine and isodesmosine linkages that impart stability and durability to the elastic fiber (63). LOX also oxidizes collagen fibers and is required for the stable formation of collagen structures (12). Inhibition of LOX using BAPN disrupts elastin integrity and decreases cross-linking (48). Mice lacking the LOX gene die perinatally with a phenotype typical of animals lacking proper elastic fibers, i.e., fragmented elastic fibers, aortic aneurysms and narrowed vessels. Less commonly, obstructed airways and skin defects are also observed and collagen cross-linking is disrupted (64). Curiously, an overexpression of LOX is associated with the onset of Alzheimer's disease and is suspected to be involved in increased senile plaque formation in the neuronal ECM (65).

There are four other members of the LOX family, which are known as lysyl oxidase-like protein-1 through -4 (LOXL1 through LOXL4). The specific role of each in elastin cross-linking is not yet known, however some information has come from knockout mouse studies. Liu et al. demonstrated widespread disruption of elastin in the LOXL1 knockout mouse, but collagen fibers were found to be intact and normal, which indicates that LOXL1 may be an elastin-specific cross-linking enzyme (66). Given the general lack of elastin expression in the adult, it is interesting to note that the elastic fibers in LOXL1 knockout mice appear normal initially, but when challenged by injury, fail to remodel appropriately. Specifically, they display normal uterine physiology in virgin mice, but these mice cannot remodel elastic fibers appropriately postpartum. As elastin is normally regenerated in the uterus and cervical tissue after labor, the lack of this function in the knockout mouse may indicate an adult-specific role for LOXL1 (66).

Versican

Versican is an extracellular chondroitin-sulfate containing glycoprotein, which undergoes differential splicing to produce the V0, V1, V2 and V3 variants. Of these, the V3 variant, which lacks chondroitin sulfate domains, promotes elastic fiber formation when overexpressed in rat SMCs (67). Not surprisingly, the V3 versican promotes changes in SMC morphology, with decreases in proliferation and protein secretion (68).

Elastin-binding protein (EBP)

The putative elastin receptor complex is made up of three subunits. The largest, EBP, is a 67-kDa cell-surface-associated protein that is a splice variant of β-galactosidase (69). The other two subunits are membrane-bound, neuraminidase (Neu-1) and cathepsin A. Elastin-derived peptides bind the elastin receptor complex and induce up-regulation of genes, including pro-MMP1, in a Neu-1-dependent manner, providing evidence for a feedback loop in elastin maintenance (69, 70).

Regulation of elastin expression throughout development

During development, elastin expression is tightly regulated in ways that likely differ between tissues, as the organization of fibers also varies. Elastin in the blood vessel wall forms concentric lamellar layers in between layers of SMCs, whereas in the lungs, elastin forms more fenestrated sheets, with wispy fibers and less dense packing (71). The architecture in the skin is also different, characterized by branched fibers and less compacted lamella. Additionally, elastin constitutes only 3–4% of the dry weight of skin, though it imparts important mechanical properties to the dermis, e.g., degradation of elastin contributes to wrinkles during aging (72).

As elastin production substantially drops in early childhood, yet more elastin is required to sustain the adult as the diameter of the aorta and other tissues increases during growth. To address this paradox, during growth, elastin is deposited initially at the vessel's luminal side, then along the inside edge of lamellar fenestrations, presumably in response to lamellar stretching (50). On this basis, in a transgenic mouse construct with a CAT-reporter driven by the 5′ promoter end of the elastin gene, high expression levels were seen in the aorta and lungs, i.e., tissues composed of a significant amount of elastin, and a lower level in skin, commensurate with its levels of elastin (73).

Elastin is regulated temporally as well as spatially in humans, rats, mice, chickens and other species. Elastin is first expressed early in development (between weeks 17–19 in human prenatal tissue) with prominent elastin fibers becoming visible late in gestation and continuing to increase through postnatal growth. Production drops to nearly undetectable levels in childhood for humans, or at approximately 3 months of age in mice (74). Elastin has a sufficiently long half-life that substantial expression in the adult is not required, except in cases of injury or disease (15, 42, 75).

Elastin and tissue engineering

Elastin biochemistry

Coacervation, the spontaneous association of tropoelastin monomers, is affected by temperature, pH, salt content and protein concentration (76). It is a key intermediate process in the assembly of native elastin fibers and is also a useful property from a bioengineering standpoint because it allows the development of elastomeric constructs in the laboratory (17, 77). Interestingly for its potential in vascular applications, heparin, a commonly used anticoagulant, promotes the coacervation of recombinant tropoelastin monomers *in vitro* (25, 78). We found that heparin promotes elastic fiber formation in cultures of adult human SMCs (79).

Molecular regulation of elastin expression

Many growth factors, cytokines and ECM components alter elastin levels as reviewed in detail previously (80).

Insulin-like growth factor-1 (IGF-1)

IGF-1 up-regulates elastin in neonatal SMCs, but not in lung fibroblasts (80, 81). In these studies, tropoelastin mRNA increased only from 2 h after IGF-1 treatment. It may be that there are longer-term effects from IGF-1 treatment, either as a result of a short initial delivery or over continuous exposure, as elastogenesis in culture requires a minimum of several days of confluent culture (82).

The IGF-1 responsive element is between -195 bp and -136 bp in the promoter. IGF-1 induces up-regulation at the transcriptional level (83). IGF-1 most likely activates transcription by competing with a negative regulator from the promoter region. Taken with later data for several Sp1 binding sites in this area of the promoter, it seems likely Sp1 contributes to this process (81).

Heparin

Heparin treatment of neonatal cells has been shown by our group to enhance elastogenesis (79). It has also been

demonstrated that neonatal rat lung fibroblasts treated with heparin accumulate elastin within the cell layer with a concomitant reduction in soluble tropoelastin (84). Thus, heparin may help to promote deposition of tropoelastin onto existing fibers, possibly through direct binding.

Using embryonic chick SMCs, it was also demonstrated that heparin induces elastin expression in this system, with a heparin effect dependent upon the SMC growth state (85). In proliferating cells, heparin addition induces elastin expression in a PKC-dependent manner. However, in cells that are already growth-arrested, heparin decreases elastin expression. It is unclear if the induction of elastin is due to heparin activation of a signaling cascade, or simply a result of heparin-induced growth arrest (85).

Cytokines TNFα, IFN-γ, bFGF and IL-1β

Using both human foreskin fibroblasts (HFFs) and rat SMCs, Kahari et al. found that TNFα treatment drastically down-regulates elastin expression after only 12 h of treatment (86). The repressing effect of TNFα is markedly less in SMCs than HFFs, indicating some tissue specificity of the TNFα response (86). Interestingly, the inhibition is seen on native mRNA and a transfected CAT-reporter construct, which indicates that this inhibition occurs at the initiation of transcription, not during subsequent processing steps. In contrast, treatment with IFNγ shows only a repression of endogenous mRNA, and no effect on the CAT reporter construct. This indicates IFNγ represses elastin expression by regulating mRNA levels post-translationally. IL-1β, another macrophage-released cytokine inhibits elastin expression in rat lung fibroblasts via NFκB induction, with a simultaneous transition towards a proliferative SMC phenotype (85).

Basic fibroblast growth factor (bFGF)

In rat lung fibroblasts, bFGF inhibits elastin transcription via the MAPK pathway, which initiates binding of c-Jun at the AP1 inhibitory site in the elastin promoter (87). Heparin is also involved in bFGF signaling and thus may warrant further study in this system (88).

Transforming growth factor β (TGFβ)

TGFβ predominantly exists in the latent pro-TGFβ form, which is ECM associated and generally associated with latent TGFβ binding proteins (LTBPs) to stabilize the inactivated form. Upon cleavage, active TGFβ is released and activates cell surface receptors, where it mediates proliferation, migration and extracellular matrix production (89).

One mechanism by which TFGβ1 regulates elastin is through PKC-mediated mRNA stabilization, which increases mRNA levels without activating transcription (26). Activation of a TGFβ-specific, open reading frame element in the promoter region of elastin may work in concert with this mRNA stabilization (90, 91). These two molecular mechanisms, taken with data indicating TGFβ and hyaluronan (HA) treatment up-regulates elastin in adult cells, are clear indications that TGFβ plays an important role in elastin remodeling. From a therapeutic standpoint, however, TGFβ is a less than ideal candidate, as it has broad effects on tissue homeostasis (92).

Steroid hormones: aldoterone, glucocorticoids, dexamethasone

Though SMCs and skin fibroblasts are the primary cell source for elastin studies, the importance of elastin in cardiac tissue should not be overlooked. Hinek et al. have extensively studied the role of aldosterone in elastin regulation in fetal cardiac fibroblasts and reported an interesting non-canonical mechanism of action. They find that aldosterone activates the IGF-R1 and Gα13 G-proteins on the cell surface, which initiates a signaling cascade involving c-Src and PI3kinase/Akt. This mechanism is unusual for aldosterone signaling in other arenas, and indicates yet another factor regulating elastin in response to stress (93). The glucocorticoids dexamethosone (DEX) and triamcinolone (TMC) were shown to up-regulate an elastin reporter construct, both in the transgenic mouse and in derived cells (94). It would be interesting to learn the mechanism of DEX-mediated up-regulation in comparison to the aldosterone pathway, particularly because DEX regulates elastin without affecting transcription (95, 96).

Focus on adult cells – the next challenge in elastic tissue engineering

As previously outlined, a significant challenge in the elastin biology field has been the production of elastin from adult cells. As elastin expression ceases during childhood, elastin is only produced in response to injury or disease in adults. Importantly, the resulting elastin

Table 3 Factors impacting elastin expression.

Factor	Effect on elastin	Mechanism of action
IGF-1 (insulin-like growth factor)	↓ in SMCs, No change in lung fibroblasts	Possible release of negative regulator (Sp1) from promoter region of elastin gene (76)
Heparin	↑	Protein kinase C (PKC) induction (80) possibly through direct tropoelastin/heparin interaction (74, 92)
TNFα (tumor necrosis factor α)	↓	Inhibition of transcription initiation (81)
IFNγ (interferon γ)	↓	Posttranslational mechanism
IL-1β (interleukin 1β)	↓	De-differentiation through NFκB induction
bFGF (basic fibroblast growth factor)	↓	MAPK pathway activation, resulting in c-jun/AP1 binding (82)
TGFβ (transforming growth factor β)	↑	mRNA stabilization (26)
Aldosterone	↑	IGF-R1 activation (88)

is disorganized and lacks the characteristic mechanical properties of developmentally produced elastin. In a recent set of papers, members of the Ramamurthi lab have aimed to address this challenge (96). They have found that the addition of copper ions and by implication increased lysyl oxidase activity, in conjunction with hyaluronan, induce increased elastin deposition and crosslinking from adult rat SMCs. Though the amount of Cu^{2+} was higher (0.1 M) than physiological levels, the results point to LOX insufficiency being one of the missing links in adult elastin formation. They also demonstrated that the simultaneous addition of TGFβ and HA leads to an increase in elastin deposition, without the concurrent increase in proliferation that is sometimes seen with TGFβ treatment (43).

Recent work from our lab has demonstrated that adult SMCs and fibroblasts, when treated with heparin, produce an extensive extracellular elastin network. This has also been demonstrated in fetal cells, further supporting the role of heparin-elastin interactions during elastogenesis (78, 79).

Together, these studies suggest that ECM components likely play critical roles not just in the organization of elastin, but in its regulation at the transcriptional level. This increased focus on the manipulation of adult cell-mediated elastogenesis will enhance tissue engineering of elastic fibers, as tissues targeted for replacement are predominantly adult.

Recent advances in the induction of elastin from adult cells illuminate several avenues for future research. Though some hypotheses as to the mechanisms of action of heparin, hyaluronan and copper ions, have been proposed in the respective articles, much work remains. Clarification of downstream signaling pathways activated or inhibited in response to these factors could show common targets for direct intervention.

Tropoelastin as a material for tissue-engineering applications is an exciting area for future research because it specifically provides the primary material that is otherwise missing in these constructs (97, 98). In addition to the innate mechanical properties of tropoelastin, it can be combined with other biomaterials (such as silk) for synergistic properties (99). Further optimization of cellular responses through inclusion of biochemical components

Table 4 Tissue engineering approaches to elastin assembly.

Approach	Advantages	Disadvantages	Potential applications
Adult SMCs; 0.1 M Cu^{2+} with hylauronan (43)	Robust elastin expression from notoriously recalcitrant adult SMCs	Nonphysiological Cu^{2+} concentrations	Chemical modification of devices to promote elastin formation (e.g., vascular equivalents)
Neonatal (85), Adult cells (79) Heparin treatment	Robust elastin protein expression, heparin widely used in clinic with few side effects	Short half-life of heparin, mechanism unknown	Chemical modification of devices to promote elastin formation (e.g., vascular equivalents)
Fabrication using recombinant tropoelastin (48)	Excellent mechanical properties, chemical modification possible, biochemically active in body	Requires overexpressed material	Fabrication of numerous elastomeric constructs (skin, blood vessel)

listed in Tables 3 and 4 has the potential to lead to the development of viable biomaterials for the treatment of multiple human diseases.

Summary

Due to increased interest in tissue-engineered vascular constructs, significant progress has been made in understanding factors regulating elastin expression from SMCs. This knowledge of the mechanisms regulating elastin deposition during normal growth helps to inform tissue-engineering approaches by recapitulating key developmental processes. The discoveries that heparin, copper ions, hyaluronic acid and TGFβ can all induce elastin expression from adult SMCs provides clues into the physiological mechanism of elastin regulation, and are useful tools in the design of novel tissue-engineered constructs.

Acknowledgements: We thank the NIH (EB002520) Tissue Engineering Resource Center for support of the project, NIH grant HL046773 (JJC), the American Heart Association Predoctoral Fellowship (0815680D) to CBS and the Australian Research Council and National Health & Medical Research Council (ASW).

Received September 27, 2012; accepted November 30, 2012

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