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Advances in biomimetic regeneration of elastic matrix structures

Balakrishnan Sivaraman, Chris A. Bashur, and Anand Ramamurthi

Department of Biomedical Engineering, The Cleveland Clinic, 9500 Euclid Avenue, ND 20, Cleveland, OH 44195, USA

Anand Ramamurthi: ramamua@ccf.org

Abstract

Elastin is a vital component of the extracellular matrix, providing soft connective tissues with the property of elastic recoil following deformation and regulating the cellular response via biomechanical transduction to maintain tissue homeostasis. The limited ability of most adult cells to synthesize elastin precursors and assemble them into mature crosslinked structures has hindered the development of functional tissue-engineered constructs that exhibit the structure and biomechanics of normal native elastic tissues in the body. In diseased tissues, the chronic overexpression of proteolytic enzymes can cause significant matrix degradation, to further limit the accumulation and quality (e.g., fiber formation) of newly deposited elastic matrix. This review provides an overview of the role and importance of elastin and elastic matrix in soft tissues, the challenges to elastic matrix generation in vitro and to regenerative elastic matrix repair in vivo, current biomolecular strategies to enhance elastin deposition and matrix assembly, and the need to concurrently inhibit proteolytic matrix disruption for improving the quantity and quality of elastogenesis. The review further presents biomaterial-based options using scaffolds and nanocarriers for spatio-temporal control over the presentation and release of these biomolecules, to enable biomimetic assembly of clinically relevant native elastic matrix-like superstructures. Finally, this review provides an overview of recent advances and prospects for the application of these strategies to regenerating tissue-type specific elastic matrix structures and superstructures.

Keywords

Elastin; Elastic fibers; Induced elastogenesis; Extracellular matrix; Matrix assembly; Regenerative tissue repair

Introduction

Regenerative medicine is broadly defined as the “repair, replacement, or regeneration of cells, tissues, or organs, to restore impaired tissue function resulting from disease, trauma or aging” [1, 2]. Technologies pertaining to tissue engineering and biomaterials afford promise to accomplishing these goals. Regenerative tissue repair provides an alternative to organ transplantation, which is impeded by the limited availability of donor tissues and the need for donor–recipient tissue matching to prevent immune rejection of the transplants. However, the true potential of these technologies has not yet been fully realized, on account of numerous challenges. These include (a) the limited ability of adult cell types, particularly diseased cells, to recapitulate the processes of extracellular matrix (ECM) and tissue

assembly that occurs during development and (b) the insufficiency of biomolecules (e.g., growth factors and other cytokines or chemokines) alone, and lack of appropriate biomaterial scaffolds, to re-create the healthy cellular microenvironment which coaxes repair and regeneration of functional tissues replicating native tissue composition, biology, and mechanics. Additional challenges involve (c) control of spatiotemporal patterns of matrix assembly, and (d) adapting in vitro tissue engineering technologies to effect in situ localized tissue repair at sites of impaired native tissues.

The ECM is a key component of the local cellular microenvironment. It is composed of structural proteins (e.g., elastin and collagen), and proteoglycans and glycosaminoglycans (GAGs), which in combination with cells make up the tissues in the human body. The ECM serves as a reservoir of growth factors and other biomolecules, enabling their controlled spatio-temporal bioavailability to the cells, and thereby plays a role in cell signaling, tissue homeostasis, and tissue regeneration [3, 4]. While matrix generation or regeneration implies de novo cellular matrix synthesis and assembly, it must be noted that matrix repair implies positive cellular remodeling of existing, disrupted matrix structures in vivo for their functional restoration. ECM components are synthesized, secreted, and remodeled by cells in most tissues, in response to perceived biochemical and biomechanical transductive cues. This response is typically highly specific to tissue-type and state of health. Among the different cell-types, smooth muscle cells (SMCs) and fibroblasts are examples of cells that produce significant ECM.

A variety of biomolecules and growth factors serve as cues for cells during development and growth, which have been adopted in strategies to induce matrix and tissue generation and regeneration by adult cells [3]. Precise control over their delivery and subsequent signaling is key to the tissue regeneration process [3–5]. For example, the signaling of certain biomolecules like epidermal growth factor may be limited to the length-scale of a single cell [6], while others such as insulin-like growth factor-1 (IGF-1) may act within a tissue or through the circulation [7], depending on the ratio of factor molecules to their cell surface receptors. Furthermore, regulation of local growth factor concentration is also important. The excess bioavailability of TGF- β 1, a cytokine which has been shown to promote elastic matrix synthesis [8–12], can cause cellular apoptosis and trigger cellular processes leading to enhanced matrix mineralization [13, 14]. Hence, the cellular microenvironment is also a determinant of the ability for and nature of tissue repair and regeneration.

Biomaterial scaffolds [15–22] can be used to direct cell phenotype and behavior [23–26], by providing a defined local cellular microenvironment [4, 5] that mimics the characteristics of native ECM [27]. This includes the spatio-temporal availability of typical ECM-bound cell signaling growth factors [4]. Scaffolds can be modified to promote and control cell adhesion, for example, with the use of an Arg-Gly-Asp (RGD) peptide sequence which binds to integrins of many different cell types [28–31] including the $\alpha_v\beta_3$ integrin on SMCs and fibroblasts. Other important considerations/parameters involve the sustained and controlled release of pro-regenerative biomolecules and agents which prevent proteolytic degradation of the ECM, while maintaining their stability in the regenerative tissue microenvironment. Nanoparticles encapsulating these active agents [32–35] are useful for this purpose and can be delivered standalone or tethered to biomaterial scaffolds [36–41]. Ideally, biomaterial scaffolds used to engineer or regenerate tissues must be biodegradable, with their degradation rate coordinated with deposition of new matrix by cells. The new matrix would then be expected to assume the primary load-bearing role of the tissue, after the scaffold degrades [17, 19, 42–47]. Predictable release of biomolecules from the nanoparticles delivered or tethered on the scaffold is also another important consideration [33, 48, 49]. More recently, studies have also examined ‘smart’ biomaterials [5, 23], which respond to altered, disease-specific microenvironmental conditions that are characteristic of

a disease by releasing therapeutic agents. Specific triggers include chronic overexpression of proteolytic enzymes [50, 51], pH changes [52], and perceived tissue stresses [53].

Despite advances in the development of scaffolds and systems for controlled delivery of biomolecular factors, repair and/or regeneration of the complex ECM architecture has been a challenge. This is the case particularly with regards to elastic matrix structures, as adult cells are poorly capable of synthesizing and assembling elastin precursors. The elastic matrix plays a critical role in maintaining the native structure of soft tissues, enabling them to recoil following elastic deformation upon encountering stresses, and serving to regulate the cell responses in healthy and injured tissues via biomechanical transduction [54–57]. The latter aspect is mediated by elastin protein, fibrillin glycoproteins, and at least 30 other components that compose elastic fibers [58]. Although cells are the building blocks in tissue engineering, the inherently poor ability of most adult cells to synthesize elastin and remodel elastic matrix structures has limited the ability to engineer soft elastic tissue replacements. Possible exceptions are SMCs in healthy bladder [59] and vaginal tissue [60]. The lack of cell-synthesized elastic matrix potentially can be circumvented with incorporation of recombinant elastin and synthetic elastin-like peptides into tissue-engineered scaffolds [61–65], thereby imparting them with mechanical/structural properties similar to that of native elastin, as well as the ability to mediate cell adhesion and signaling [66, 67]. Biomaterials assembled from elastin and elastin peptides have been hypothesized to enhance long-term elastin synthesis by a variety of cell types, on account of the interactions of elastin peptides with elastin receptors on cells [64, 68]. However, tissue homeostasis may be impacted adversely at the same time, specifically due to the absence of other non-elastin components of the native elastic fibers within these elastomers, which mediates cell interactions with these fibers and their subsequent behavior [69]. Hence, further development of biomaterial technologies based on an intimate understanding of the process of elastogenesis is necessary. This will enable cellular synthesis of elastin and elastic fiber proteins and their assembly into a three-dimensional (3D) functional elastic matrix. This applies both in vitro within tissue-engineered constructs and also in vivo at sites of proteolytic elastic matrix degradation due to disease or injury.

This review focuses on technologies to enable biomimetic elastic matrix assembly or regenerative repair, with special emphasis on vascular tissue engineering/regeneration that our lab has specific expertise in. The review specifically seeks to (a) outline the role and importance of elastin and elastic fibers to tissue homeostasis and unique facets of its architecture, (b) summarize the biocomplexity of the elastic matrix assembly process and key steps involved therein, (c) highlight intrinsic and disease-induced factors that cause aberrant elastic matrix assembly and/or impede its structural instability, and (d) evaluate different biomolecules that may be harnessed to enhance elastin synthesis and matrix deposition by cells. The review further discusses biomaterial-based strategies (e.g., scaffolds and nanoparticles) for presentation and/or controlled delivery of elastogenic biomolecules and provides an overview of specific challenges in and approaches to tissue engineering of different target elastic tissue-types. Finally, the review concludes with an overview of the future directions in elastic matrix generation/regeneration required for developing clinically functional elastic tissue constructs.

Distribution, synthesis, and biological roles of the elastic matrix

Elastin is a primary structural ECM protein component of elastic tissues such as blood vessels, heart valves, lungs, bladder, pelvic floor tissues, vocal fold, and skin. On a dry tissue weight basis, elastin constitutes ~9 % in vocal folds, 30 % in lungs, and up to 50 % in elastic arteries [70, 71]. The elastic matrix is not as prevalent as collagen, which determines

tissue stiffness, but it provides unique mechanical properties to tissues and regulates cell behavior within.

The elastin precursor molecule is tropoelastin, which is encoded by a single-copy gene *ELN* in humans. Tropoelastin is a ~72 kDa protein that is synthesized and secreted by a variety of cells, including SMCs [72] and fibroblasts [73]. The formation of different isoforms of tropoelastin occurs with alternative splicing of the transcripts, the expression of which is controlled at a post-transcriptional level [74]. These molecules are composed mostly of hydrophobic amino acids (i.e., ~75 %), with hydrophilic domains containing lysine and alanine residues forming the remainder. The hydrophilic lysine residues play an important role in the crosslinking of tropoelastin molecules [75, 76], which is a critical step of the elastic matrix assembly process.

The process of elastic matrix assembly occurs primarily during development and is virtually complete by adolescence. It is a complex, hierarchical process as illustrated in Fig. 1. The process of elastin synthesis and elastic matrix assembly is regulated at multiple levels during the assembly process, the details of which are beyond the scope of this review but are described elsewhere [74, 77]. Initially, the tropoelastin molecule is secreted from the cell and binds to the elastin binding protein (EBP) on the cell membrane; this protects it from degradation [78, 79]. The EBP also enables both the interaction of tropoelastin with highly aligned groups of microfibrillar glycoproteins and its dissociation from its complex with the EBP on the cell membrane. This allows the tropoelastin to coacervate on the microfibrils and crosslink to form amorphous elastin [80]. The crosslinking process is initiated by enzymatic oxidation of lysine residues on adjacent elastin molecules by a copper ion (Cu^{2+})-dependent amine oxidase enzyme, lysyl oxidase (LOX). Adjacent elastin molecules become covalently bound by desmosine and isodesmosine moieties in this manner [81]. The resulting growth of elastin deposits ultimately forms a mature elastic fiber. These fibers contain a central core of cross-linked, amorphous elastin surrounded by highly aligned microfibrils. They are typically 300 nm to more than 1 μm in diameter [82, 83]. The highly aligned microfibril scaffold, laid down prior to elastin deposition, is just as important to the mechanics of the elastic fibers as the elastin component itself. This is partially because the microfibril scaffold contributes to the anisotropic mechanics required for many tissues [84]. In conclusion, all of the steps in elastic fiber assembly are necessary and impairment in even one of them can alter tissue mechanics and function (Fig. 2).

The unique mechanical properties of elastic fibers are a result of the covalent intermolecular crosslinks (i.e., desmosine and isodesmosine). After a load is released, chains return to their original conformation, accounting for the recoil properties they impart to tissues. In tissues, elastic fibers contribute to the low-slope, initial region of the stress–strain curve while collagen contributes to the high-slope region at higher strains [85].

Another important property of elastic fibers is their ability to regulate cellular phenotype, as well as matrix and cytokine production [54–57]. One mechanism by which this is accomplished is the storage of growth factors in the ECM and the regulation of their activity [58, 86]. Latent TGF- β binding protein (LTBP) present in microfibrillar components of elastic fibers has been shown to bind and store TGF- β [87]. More specifically, the LTPB protein interacts with fibrillin-1, the primary glycoprotein component of microfibrils. Therefore, the lack of fibrillin-1 prevents the binding and storage of TGF- β [87], thus increasing its bioavailability. The bioavailability of TGF- β can critically influence the behavior of contacting cells, including the induction of cell apoptosis and matrix mineralization at high concentrations [14], as mentioned earlier. This is one characteristic of certain genetic disorders (e.g., Marfan Syndrome) and other pathological tissue environments (e.g., neointima of vascular diseases) [88]. Additionally, elastic fiber

components (e.g., fibrillin-1, fibrillin-2, microfibril-associated glycoprotein-1 (MAGP-1), and fibulins) also impact cell binding and cell signaling through mechanotransductive cues [58, 89]. For example, fibrillin-1 possesses an RGD motif capable of binding integrins [89]. Finally, these microfibrillar components enable interactions with the surrounding ECM. MAGP-1 has been shown to interact with decorin, dermatan sulfate groups of proteoglycans, and collagen VI [90], and fibulins have been shown to impact elastin deposition and crosslinking by binding both LOX and tropoelastin [91]. Additionally, the microfibril scaffold has also been shown to sequester TGF- β and regulate its bioavailability and activity [92–94]. Thus, microfibrillar glycoprotein components of elastic fibers critically impact cell signaling and elastic fiber assembly. This is an important consideration in developing strategies for induced elastic matrix regeneration.

Considerations for growing tissue-type-specific elastic matrices

The content and predominance of elastin/elastic matrix can vary greatly between different tissue-types. As mentioned earlier, adult cells generally have limited elastogenic potential, due to downregulation of tropoelastin and fibrillin genes in the post-neonatal period. As a result of this, these cells are inherently poor in recruiting, crosslinking, and organizing elastin precursors into fibers [83]. Likewise, the elastic fibers in different tissue-types are organized into different superstructural architectures that provide the mechanics dictated by tissue location and function. These superstructures include discrete fiber networks found in elastic ligaments, lung, and skin, denser fibrous membranes found in the aortic valves, and sheets or lamellae found in arteries [58].

Mimicking such superstructural organization of elastin is critical to create functional tissue replacements, since inappropriate superstructures (e.g., different fiber orientation) and aberrant or deficient elastic fiber formation can shift mechanics of the engineered construct away from that of native tissue. As an illustrative example, tissue engineered vascular grafts have been found to lack sufficient elastic matrix deposition, proper fiber formation, and superstructural organization [95]. These are factors that contribute to local wall weakening and lead to expansion resulting in aneurysm formation. The incorporation of microfibrillar components within the cell-generated elastic matrix is also critical, as described in the preceding section. Additional considerations are age and tissue-type specific variations in microfibrillar composition and the potential impacts on tissue function [96]. For example, it is known that fibrillin-2 is found primarily in embryonic tissue, but fibrillin-1 is found primarily in adult tissue [58]. In addition, LTBP-1 has been found to associate with microfibrils in tendon and vascular tissues but is present in limited amounts in other tissues (e.g., skeletal muscle and lung) [90]. Tropoelastin precursors are laid down on microfibrillar pre-scaffolds to form amorphous deposits that grow radially and axially to form mature fibers. Hence, controlling spatial patterns and orientations of microfibrillar components is also mandated, which has been virtually unstudied until the present.

Consideration must also be given to the expression of elastolytic enzymes such as matrix metalloproteinases (MMPs), which occurs as a routine cellular response to tissue-engineering scaffolds in the culture microenvironment. This response can be more pronounced due to interaction of the cultured cells with unnatural scaffolding materials in vitro and the recruitment of inflammatory cells to these scaffolds in vivo, especially at sites of tissue injury or disease. In such cases, attenuation of proteolytic enzyme activity may be necessitated to shift the balance between de novo elastin synthesis and matrix disruption in favor of net accumulation of new elastic matrix.

In vivo regenerative repair of existing ECM

The key challenges to de novo elastin synthesis in vitro also apply to its regeneration in vivo at sites of proteolytic tissue disruption; however, there are additional considerations. The first challenge is the need to promote regenerative repair of existing elastic matrix structures (e.g., fiber growth and reforming of disrupted crosslinks) [97, 98] in addition to de novo elastin deposition. The second challenge is that cells within such diseased microenvironments exhibit an abnormal phenotype frequently characterized by much lower elastogenic potential relative to their healthy counterparts [99]. This mandates elastogenic induction of these cells. The third and perhaps most important aspect is the difference in the tissue microenvironment relative to a healthy state, which includes accumulation of matrix components that impede elastin deposition (e.g., proteoglycans) [100] and the presence of inflammatory cells and their release of inflammatory cytokines [101]. The accumulation of proteoglycans that lead to impeded nucleation of elastin precursors on the EBP (e.g., versican and biglycan) is higher in adult tissues compared with embryonic tissues [102] and is further increased in diseased microenvironments [100]. Inflammatory cytokines in disease tissues can also trigger chronic local upregulation and overexpression of elastolytic enzymes including MMP-2, MMP-9, MMP-12, and neutrophil elastase. The increased expression and activation of MMP-9, in particular, has been found to be significantly increased in aortic aneurysms [103] and after lung injury [104]. These cytokines can be produced by both parenchymal cells (e.g., SMCs) and recruited inflammatory cells. Thus, increased cytokine production potentially has the greatest critical impact on the quality of regenerative elastic matrix repair.

Studies have shown that intact elastic fibers act as nucleation sites for deposition of new tropoelastin and are essential for proper fiber growth [105]. Therefore, degradation of elastic fibers due to chronic overexpression of proteolytic enzymes leading to attenuation of net elastic matrix accumulation can adversely impact this aspect of matrix assembly. A significant concern is the positive feedback to this degradation process provided by elastin degradation products (e.g., soluble elastin peptides or EPs), which elicit a very different cellular response than intact elastin. This pathological cell response to EPs includes MMP overexpression by cells (e.g., SMCs), increased chemotactic activity, and matrix calcification [106, 107]. Thus, in such diseased microenvironments, parallel strategies to limit elastic matrix disruption (e.g., MMP inhibition or reduction in their activity) are necessary to improve quantity and quality of elastic matrix regeneration.

Biomolecular strategies to enhance elastin synthesis and matrix deposition

Improving elastic fiber synthesis and maturity have been sought via strategies that target enhancement of individual steps in elastic fiber assembly, including tropoelastin synthesis, its crosslinking, and higher-level elastic fiber organization and alignment. Specific strategies attempt to improve mature elastic fiber formation by altering different aspects of the cellular microenvironment (e.g., the presence and spatiotemporal presentation of elastogenic factors, topographic cues, and biomechanical stimuli). The focus of this review is on the controlled spatio-temporal presentation of elastogenic factors, since other strategies are covered in more detail in pertinent literature [108–109].

Several biomolecules have been shown to positively impact elastic matrix synthesis in different tissues (Table 1). They include growth factors, matrix GAGs, nucleotides, exogenous enzymes, vitamins, and steroids. These elastogenic biomolecules can impact different steps in the assembly of elastic fibers, as also described in Table 1. For example, IGF-1 has been shown to upregulate tropoelastin expression by neonatal vascular SMCs [110]. Proteoglycans such as versican have been shown to inhibit elastic matrix synthesis. The chondroitin sulfate (CS) side-chains of versican engage the EBP and result in premature

release of EBP-bound tropoelastin [78, 79]. This prevents proper interaction with the fibrillin scaffolds. Thus, treating cells with V3, a splice variant of versican not containing the CS-bearing domain, improves engagement of fibrillin and fiber formation. The role of other proteoglycans is less clear, with long-chain hyaluronan (HA; MW>10⁴ Da) shown to have no effect on tropoelastin synthesis but to enhance tropoelastin recruitment and crosslinking [102, 111]. This likely occurs by physical coacervation induced by opposite charge interactions, since GAGs are highly anionic [105]. However, HA can interfere with elastic fiber formation and matrix assembly by binding versican. Very differently, oligomeric forms of HA (HA-o), specifically HA tetramers, have been shown to enhance tropoelastin synthesis and matrix formation [9, 112–116]. This has been hypothesized to occur on account of HA-o competing with long-chain HA to bind to cell surface receptors, thereby interfering with versican binding and thus its engagement of the EBP [117]. As mentioned in an earlier section, crosslinking of the elastic fibers is mediated by LOX [118]. Kothapalli et al. supplemented cultures with exogenous LOX [119], as well as added inorganic Cu²⁺ [116] to enhance synthesis and functional activity of LOX. This resulted in improved synthesis and crosslinking of tropoelastin in healthy rat aortic SMC cultures. Furthermore, the impact of several of these biomolecules have been shown to be dose dependent (e.g., TGF- β 1 [13] and ascorbic acid/ascorbate [120–125]), demonstrating the importance of controlled delivery and the need to understand their mechanisms of action. Some biomolecules appear to act through common pathways (e.g., aldosterone acts via IGF-1 receptor signaling) [126]. Many of these elastogenic biomolecules will be discussed in later sections, detailing their delivery to cells.

In addition to stimulating elastogenesis, attenuating elastolytic matrix degradation by MMPs is key to quantitatively improving elastin deposition. MMPs can be inhibited endogenously by tissue inhibitors of MMPs (TIMPs) and exogenously via antibodies and drugs which act via indirect and direct mechanisms. Technical issues with TIMP production and usage [127], coupled with undesirable effects, including upregulation of MMP-2 activity [128] and apoptosis [129] limit their usefulness. Anti-MMP antibodies inhibit MMP function but are expensive [130], and none have reached clinical trials. Drugs such as non-steroidal anti-inflammatories, glucocorticoids, and statins have also been shown to disrupt upstream signaling pathways at the transcriptional level as well as subsequent downstream processes associated with MMP production and secretion [130]. Direct MMP inhibition has typically been accomplished using agents that consist of a group that binds to the catalytic Zn²⁺ ion in the active site of the MMPs, such as tetracycline-based drugs. Among these, doxycycline (DOX) and minocycline are two MMP inhibitors approved for clinical use by the Food and Drug Administration in the United States. However, there are concerns with the use of DOX, including its non-specificity that can lead to undesirable sideeffects [131]. Furthermore, a recent study suggested that, at certain high doses, DOX negatively impacts elastic matrix deposition [132], thereby potentially limiting its use in elastin regenerative milieu. As an alternative approach at the RNA level, recent studies have demonstrated that MMP-2 and MMP-9 activity is enhanced by overexpression of microRNA-29b (miRNA-29b) [133] in a mouse abdominal aortic aneurysm (AAA) model. Their inhibition using anti-miRNA-29b leads to reduction of aneurysmal expansion of the abdominal aorta. It has also been shown that inhibiting microRNA-29a causes increased elastin gene expression and matrix synthesis in bioengineered human vessels and vessels in elastin haplosufficient conditions [134]. Furthermore, in a study by Maegdefessel et al., they demonstrated that expression of miRNA-21 slowed/inhibited AAA growth in a similar mouse model of AAA [135]. These results illustrate the ability to identify and target-specific miRNAs as therapeutic targets for mediating/promoting vascular remodeling towards inhibition of proteolysis of elastic matrix.

Biomaterial-based strategies for elastic matrix generation

As elucidated in the “Introduction,” biomaterials play a critical role in tissue engineering and regenerative medicine by controlling and directing cell function and behavior [23–25], while enabling a degree of spatio-temporal control over the availability of biomolecules to these cells [4, 32, 33, 35]. This control is necessary for functional tissue generation and/or regeneration. Biomaterials for delivery of biomolecules may be derived from natural or synthetic sources, with these biomolecules either being adsorbed, chemically immobilized, or physically encapsulated within the biomaterials. Scaffolds and nanocarriers are the two primary modes for biomolecular delivery (Fig. 3).

Tissue-engineering scaffolds and nanocarriers are chosen based on several frequently inter-dependent inherent characteristics such as degradability, chemistry, topography, and compliance. All of these properties have been shown to mediate the cellular response. For example, the chemistry, topography, and compliance of a scaffold impact cell attachment to the surface and integrin-mediated mechanotransductive signaling [136, 137]. The presence of degradation products can also influence cellular phenotype. Badylak et al. [138] found that small intestinal submucosa (SIS) scaffolds without crosslinking promote a pro-remodeling macrophage phenotype (i.e., M2), while crosslinked SIS scaffolds promote a pro-inflammatory (M1) phenotype. These responses are considerations when deciding between using natural (e.g., collagen, elastin, and fibrin) and synthetic (e.g., polyesters and segmented polyurethanes) materials [108, 139]. Composite materials such as a poly(ϵ -caprolactone (PCL)-collagen blend uniquely proffer the advantages of both natural and synthetic polymers [140]. Biomaterial choice and the impact of different scaffold or nanocarrier properties is an extensive area of discussion and have been covered in more detail elsewhere [108, 136, 137, 139]. This review focuses on scaffold properties (i.e., degradation rate, macrostructure, and the ability for surface modification) that directly impact the spatio-temporal delivery or presentation of elastogenic biomolecules.

Scaffolds

Scaffolds provide initial mechanical structure to the construct and cellular binding sites while the scaffold is intact. Upon breakdown, they generate degradation products that can serve as cues to modulate cell behavior. These may represent biomolecules that are either structural components (e.g., collagen and decellularized xenogeneic elastin) or growth factors and other biomolecules incorporated specifically to control cellular response. One common approach to incorporate biomolecules into a scaffold is to adsorb them on the surface of the scaffold. An alternative approach is covalent tethering, which has the potential to increase the time that the biomolecules are presented to the cells prior to degradation [141]. This strategy has been shown to lead to improved response of cells to the tethered factors [142]. Despite the above advantage of covalently tethering biomolecules, there are relevant concerns. These include either the potential loss of biomolecule activity upon chemical modification or derivatization or the incorporation of too many crosslinks and an associated conformational change or crosslinking of cell adhesion amino acid sequences [3]. A final approach is the incorporation of biomolecules (e.g., natural/synthetic blended scaffolds and encapsulated growth factors) and not just on the surface. A significant benefit of incorporating biomolecules through the bulk of the scaffold is that their biological effects on cells can be maintained in the interior even after the outer surface layer of the scaffold is degraded. However, a unique concern for physical incorporation of factors is the use of solvents and temperatures that can induce protein denaturation. For example, studies have suggested that dissolving collagen in hexafluoro-2-propanol can remove its crosslinks, cause denaturation, and increase its solubility in water [143]. Through careful control of the processing and the use of alternative solvents, this effect can be reduced but not completely prevented [144]. Growth factors are even more prone to denaturation and careful control

over processing is critical to preserving activity [145]. For this reason, Sahoo et al. encapsulated bFGF within PLGA nanofibers by using an aqueous buffer for the growth factors and HFIP for the PLGA [146]. In conclusion, scaffolds have shown promise for presenting biomolecules at the implant site, but require careful control over their processing and adaption for the specific tissue application. For localized or systemic in vivo delivery, the use of nanocarriers is an attractive option.

Nanocarriers

Nanocarriers (e.g., polymer nanoparticles and liposomes) can be formulated and functionalized to target cells localized at the tissue site of delivery and also distal sites. They are especially efficient as vehicles for localized delivery of therapeutic agents to diseased tissue microenvironments characterized by enhanced permeability (i.e., due to matrix disruption or enhanced vascularity) [32, 33, 147–149]. Their large surface area per unit volume and predictable diffusion characteristics and degradation kinetics also enable efficient and controllable delivery of therapeutic agents at the target sites. The biomolecules are typically encapsulated [33, 53, 150], although covalent tethering of biomolecules to the surface has also been performed [3, 53]. Encapsulation of biomolecules is typically preferred over their surface presentation, as it enhances the half life of the biomolecules in vivo leading to enhanced accumulation in target tissues [151, 152]. However, nanoparticles can potentially be functionalized with suitable moieties to bind elastin, targeting specific amino acid sequences or hydrophobic domains in the elastin molecules or fibers. In addition, antibodies [153], peptides [153], and more elastic fiber-specific recognition sequences can be tethered to nanocarriers. Nanocarriers exhibit several properties that are important for their function.

For nanocarriers, the therapeutic loading density, nanocarrier concentration, and carrier properties, such as molecular weight and composition, are important factors which mediate the extent and rate of release. Precise control over nanoparticle size is important, as it is a critical determinant of their localization in cells or tissues and also plays a role in their phagocytosis and subsequent immune response. Phagocytic uptake of nanoparticles has been found to be enhanced for nanoparticles greater than 500 nm [154], while sizes less than 100 nm have been found to be immunogenic [155]. On the other hand, smaller nanoparticles (i.e., ~100 nm) have been shown to exhibit an approximately threefold greater uptake in the arterial wall compared with larger nanoparticles (i.e., ~275 nm) in an ex vivo canine carotid artery model [156]. Furthermore, drug release and uptake by cells/tissues has also been found to be improved with increased nanoparticle concentration and decreased drug loading [33]. The surface charge or zeta potential of the nanoparticles has been shown to determine their intracellular trafficking, with particles having a net positive surface charge exhibiting enhanced cellular uptake and thereby enabling efficient intracellular delivery of encapsulated therapeutic agents [157]. At the same time, high positive surface charges on nanoparticles have been shown to lead to increased interactions with the cell membrane, which is generally negatively charged. This leads to increased depolarization, disruption, and subsequent cell rupture and death [158–160]. However, the ideal nanocarrier charge is tissue-type-dependent. For example, the mucosal lining of tissues such as vocal folds and vagina serves as a physical barrier for the delivery of elastogenic agents to these tissues [161, 162]. The mucus layer has a net negative charge, on account of the carboxyl and sulfate groups on the mucin proteoglycans. Therefore, carriers/nanoparticles should ideally be neutral for efficient drug delivery across mucosal membranes. This would enable their rapid diffusion through this layer, without being trapped in the mucin network either on account of their size or via ionic interactions due to their charge [161].

Combinatory strategy of co-incorporation of nanoparticles within scaffolds

Nanoparticles can also be combined with scaffolds to enable dual active agent delivery, while protecting and prolonging their release to promote functional tissue generation/regeneration. This is especially important since most synthetic polymers exhibit bulk degradation/erosion [163], as a result of which the encapsulated agent may also undergo degradation before it is released in the extracellular space. For this reason, polymers exhibiting surface erosion (e.g., poly-orthoesters and poly-anhydrides) are used for protection of the drug within the scaffold [163]. In addition, Sarkar et al. used the differences in melting points and degradation rates [164] of PLGA and PCL to incorporate PLGA NPs into thin micropatterned PCL scaffolds [165] and to form a biodegradable composite scaffolding for vascular applications.

Non-tissue-specific delivery of biomolecules for elastogenic applications

Delivery of pro-elastogenic biomolecules

As listed in Table 1, a number of growth factors and other biomolecules have been found to stimulate elastin synthesis and elastic matrix deposition and structural assembly. These can potentially have a more potent effect in influencing elastic matrix synthesis and assembly when present in a scaffold or delivered locally using nanocarriers than when provided exogenously. This is something that our own studies with tethered HA-o [117] and exogenous HA-o [9, 112, 114–116] have shown.

The scaffold material itself, without any additional modification, has been shown to impact elastogenesis. This is particularly true for natural biomaterials and their degradation byproducts. Degradation products can elicit either a positive (e.g., fibrin) [166] or negative (e.g., elastic peptides) elastogenic response [68, 167]. Ahmann et al. [166] have shown that fibrin degradation products stimulate the generation of both collagen and elastin. Another consideration is that with reduced or slowed scaffold degradation the parenchymal cells can exhibit frustrated phagocytosis and release additional proteolytic enzymes and reactive oxygen species [168], which can lead to cell apoptosis and elastic matrix damage. Nonstructural biomolecules, including elastogenic factors, such as bFGF and IGF-1 [169], and other small molecules, such as dexamethasone, have been encapsulated within hydrogels for delivery [170]. Ibrahim et al. prepared hydrogels based on methacrylated, photocrosslinked high-molecular-weight HA, which were used to present elastogenic HA-o to SMCs embedded within [171]. This study found that the incorporated HA-o induced cellular elastic matrix deposition, although, like most hydrogels, the scaffolds did not have sufficient mechanical strength. Electrospun polymer fibers [145, 146] and particulate-leached scaffolds [172] have also been used for controlled delivery of encapsulated biomolecules (e.g., bFGF). However, few biomolecules that are known to impact elastic fiber formation have been encapsulated within such scaffolds, nor have those elastogenic biomolecules (e.g., IGF) been employed with the goal of improving elastogenesis.

Scaffolds containing solubilized elastin and elastin peptides have been hypothesized to enhance long-term elastin synthesis by a variety of cell types due to interactions of elastin peptides with elastin receptors on cells [64, 68]. For example, a proteolytic digest of bovine elastin has been found to enhance elastin generation in vitro in human dermal fibroblast cultures, ex vivo in human skin explants, and in vivo in the skin of nude mice [173]. Implantation of scaffolds comprised of solubilized elastin incorporated within a collagen matrix in vivo in rats [174] was shown to enhance elastic fiber formation compared with control collagen scaffolds. Furthermore, the scaffolds containing solubilized elastin did not show any calcification, unlike collagen scaffold controls [174]. The exact nature of cellular interactions with biomaterials containing elastin, and its derivatives, however, needs further examination. Elastin-based biomaterials have been shown to promote attachment and growth

of cardiac myoblasts and bone marrow stromal cells [175], as well as aortic SMCs and dermal fibroblasts and endothelial cells [176]. On the other hand, they have also been shown to inhibit vascular SMC growth [65, 177–179], in an elastin concentration-dependent manner [178], promoting a more contractile, quiescent phenotype. Thus, incorporation of elastin derivatives within biomaterials may alter the cell–biomaterial interface significantly, causing changes in phenotype of attached cells [66]. Furthermore, elastin peptides interact with cells very differently from intact elastin. They have been shown to behave as ‘matrikines’ [68], triggering an inflammatory response. This leads to cell activation and undesirable vascular matrix remodeling outcomes on account of upregulation of proteolytic enzymes [167, 180]. Future studies will hopefully elucidate the nature of cellular interactions with elastin-based biomaterials and offer deeper insights into the development of elastic biomaterials.

Components of the elastic matrix (e.g., tropoelastin, fibrillin-1, and fibulin-5) have also been adsorbed onto scaffolds (i.e., poly(urethane)/PCL) to regulate SMC attachment and function [181]. The scaffold surface chemistry influences microfibril morphology and functionality on biomaterial surfaces [182, 183]. Microfibrils adsorbed on PLGA assume dimensions and morphology similar to that exhibited in their hydrated form. Similarly, short elastin peptides [184] assemble into fibrillar structures on hydrophobic substrates on account of hydrophobic interactions [185]. With these adsorbed elastic matrix components and with other biomolecules, the conformation is critical. For example, the conformation of the ECM glycoprotein fibronectin impacts its ability to bind integrins and mediate mechanotransductive signaling [186]. In addition, growth factors (e.g., IGF) can bind to fibronectin [186], and conformational changes in the fibronectin molecule can expose other binding sequences that are undesirable for elastic fiber generation (e.g., an epidermal growth factor binding sequence) [187]. Covalent tethering of growth factors (e.g., TGF- β) and other elastogenic biomolecules (e.g., fibronectin and collagen) is also a common strategy in the development of bioactive scaffolds [188, 189]. As an example, a study by Joddar et al. [112] has shown that surface immobilization of HA-o not only maintains their elastogenic effects but also improves elastic matrix crosslinking compared with the exogenous delivery of the same biomolecules. This was suggested to be on account of more intimate, non-transient HA-o interactions with cells [112].

With carrier delivery, several elastogenic biomolecules have been encapsulated, although most of these applications did not involve elastic fiber generation or repair. TGF- β 1 is one growth factor that has been encapsulated in several types of particles, including PLGA-PEG microparticles [190–193] and liposomes [194]. However, these microparticles have been used for the non-elastogenic applications of stimulating the osteoblastic differentiation of marrow stromal cells in vitro and enhancing repair of cartilage defects at 12 weeks post-surgery in an in vivo rabbit model. It is noteworthy that the optimal TGF- β 1 concentration of 1.0 ng/mL used for optimal osteoblast differentiation is similar to that which has been found to be elastogenic by our group with in vitro SMC cultures [9, 10, 195, 196]. This is in agreement with the varied impacts of this growth factor in different cell types and its potential interaction with other microenvironmental parameters. Controlled release of IGF-1 from PLGA nanoparticles [197] and microspheres [198] and release of HA-o from PLGA/PEG microparticles [199] have also been obtained in vitro. In addition, All-trans-retinoic acid has been delivered from liposomes [200, 201]. However, the elastogenic effects of all of these encapsulated biomolecules have not been studied in detail.

Overall, these different scaffold and nanocarrier-based strategies have demonstrated the feasibility of incorporating elastogenic biomolecules, although very few studies have used these strategies to improve elastic fiber generation and maturation. Further research into the impact of the specific incorporation methods on the elastogenicity of these biomolecules is

required before these strategies can be adopted for targeted and controlled delivery of elastogenic factors for enhancing functional elastic matrix generation/regeneration or repair by cells.

Delivery of MMP-inhibitors

As elucidated earlier in this review, the inhibition of elastolysis by MMPs concurrent with stimulation of elastin production by cells is important to enhance new elastic matrix accumulation and improve the quantity and quality of elastic matrix structures (e.g., fibers) deposited. MMP overexpression by cells can occur in vitro, especially when cells are grown on unnatural substrates or microenvironments, but it is more likely at sites of proteolytic matrix disruption in vivo. MMP-inhibitors including DOX and minocycline among others have, to the authors' knowledge, been delivered using micro- and nanocarriers and not scaffold based approaches. DOX has been encapsulated in PLGA [202, 203], PLGA-encapsulated hydroxyapatite microspheres [204], and PLGA-PCL microspheres [205] for controlled release in vitro and in vivo [205]. DOX has also been encapsulated in liposomes [206]. To date, nanocarrier-based encapsulation and delivery of MMP inhibitors, however, has not been examined in the literature. In addition, the potential use of DOX for elastic matrix generation is likely limited, since in vitro cell culture studies indicate that the micrograms per milliliter dose range that is useful from MMP inhibition also inhibits elastic matrix deposition by adult vascular cells [132] and ECM synthesis by other adult cell types [207, 208]. However, in vivo as well as clinical studies have shown that aortic tissue level concentrations of DOX in the nanograms per milliliter range [209, 210] inhibits MMP-2 and MMP-9 and suppresses aneurysm growth [211, 212]. The above example highlights the need for localized and sustained delivery systems for DOX and other such MMP inhibitors.

Other more selective MMP-inhibitors have also been encapsulated within nanocarriers. Ro 28-2653, a highly selective synthetic inhibitor of MMP-2 and MMP-9, has been encapsulated and released from liposomes [213]. The use of miRNAs (e.g., miRNA-29b) as therapeutic targets for inhibition of MMPs towards potential inhibition of elastic matrix degradation is a fairly recent discovery [133, 135]. Most current studies have delivered nano-sized liposomes containing miRNAs/anti-miRNAs for anti-cancer therapy [214, 215]. For example, Anand et al. used liposomes encapsulating anti-miRNA-132 functionalized with cyclic RGD peptides to suppress angiogenesis in a mouse model of human breast cancer [214]. While delivery of miRNA will likely have potential elastogenic benefits, the current knowledge is insufficient and additional research is required. Agents which indirectly inhibit MMPs such as dexamethasone, a glucocorticoid steroid, have also been widely encapsulated in PLGA microspheres [216, 217], nanoparticles [218, 219], and liposomes [220] for controlled, sustained release. Dexamethasone released from the microspheres suppressed the inflammatory response at subcutaneous implant sites in rats [216], while localized release of dexamethasone from liposomes has been shown to attenuate inflammation in the lungs of mice [220]. These results from representative studies point to the feasibility of encapsulation of indirect MMP inhibitors for controlled release.

Nanoparticles can also potentially be formulated to include suitable moieties to bind elastin, targeting specific amino acid sequences or hydrophobic domains in the elastin molecules or fibers. Elastin is composed of a network of highly hydrophobic polypeptide chains [221], on account of which long-chain hydrophobic compounds such as sodium dodecyl sulfate (SDS) have been found to bind to it [221, 222]. The anionic groups on SDS lead to an increase in the net anionic character of the elastin–SDS complex, stimulating elastolysis due to increased binding to the positively charged groups on cationic elastase [223]. In an extension of this work, Kagan et al. demonstrated that the hydrophobic fatty acid moieties of cationic, long-chain hydrophobic amphiphiles such as dodecyltrimethyl ammonium bromide and dodecylamine hydrochloride bind with affinity to elastin structures and also stimulate

activity of LOX, the enzyme that plays a critical role in elastin crosslinking [224, 225]. This has been suggested to be on account of the (a) electrostatic repulsion of positively charged elastase by the cationic elastin–amphiphile complex, (b) concurrent electrostatic attraction of LOX, which is negatively charged at physiological pH, and (c) induction of conformational changes in elastin molecules, which enhances access of their lysine side chains for LOX-mediated crosslinking [226]. Thus, it can be hypothesized that functionalization of nanoparticles with cationic, long-chain hydrophobic compounds can potentially stimulate elastin crosslinking towards the synthesis and deposition of functional elastic matrix structures in vitro and in vivo (Fig. 4).

Surface modification of nanocarriers with elastin-binding proteins [79] or peptides, such as RGD and other amino acid sequences that bind to cell surface receptors of SMCs and fibroblasts [227, 228], can also enhance their targeting and consequent therapeutic efficacy. However, the targeted binding of moieties or compounds may sometimes have deleterious effects. Imidazoles [229] and rofecoxib (a non-steroidal anti-inflammatory drug also known as Vioxx) [230, 231] have been shown to bind to elastin via its allysine residues. However, their binding to allysines has been found to cause dose-dependent elastin degradation and disruption of elastic lamellae on account of impairment of new cross-linking of elastin in the absence of available allysine residues [231]. Thus, careful design is essential while developing agents for targeted binding to elastin/elastic matrix.

Finally, surface modification of the nanocarriers can be used to design “smart” drug delivery systems that respond to physiological stimuli such as changes in pH, temperature, or protease activity to trigger the release of encapsulated biomolecules. Studies have utilized MMP-sensitive linkages to conjugate biomolecules to nanocarriers, enabling them to be released in response to proteolytic activity at the target site [50, 232, 233]. For example, capitalizing on the elevated MMP activity within tumors, studies have demonstrated the feasibility of MMP-sensitive peptides incorporation on liposomes for tumor targeting to promote the release of liposomal contents upon contact with MMPs secreted by tumor cells [234–238]. Similarly, MMP activity is elevated in many other pathological environments where elastic matrix generation/regeneration is desired. Thus, MMP production and/or activity, which is undesirable for net elastic matrix accumulation, can be utilized for releasing therapeutic biomolecules for MMP inhibition and also potentially concurrent elastogenic induction. Since the functional requirements and considerations of scaffolds are highly target-tissue-dependent, the ideal scaffold and delivery method would differ between tissue-types.

Progress towards engineering/regenerating tissue-type-specific elastic matrices

As elucidated earlier in this review, elastin and elastic fibers provide tissues with the critical property of elasticity and elastic recoil, while mediating tissue homeostasis by biomechanical transduction [69]. This section seeks to provide an overview of the role and structure of elastic matrix in various elastic tissue-types. We review tested and potential strategies of presenting or delivering biomolecular agents to stimulate elastogenesis while attenuating elastic matrix disruption within these tissues.

Vascular tissues

Elastin and elastic fibers are present in all three layers (i.e., intima, media, and adventitia) of the arterial wall of blood vessels. The internal and elastic laminae along with the medial elastic lamellae are vital for elastic recoil in blood vessels. The elastic lamellae are arranged in the form of concentric sheets of elastic fibers, with some collagen fibers and

proteoglycans interspersed. The type and function of the blood vessel determines the number of elastic lamellae, with the aorta and other conduction arteries having between 40–70 lamellae, while coronary, peripheral, and other arteries possess 3–40 elastic lamellar layers [95]. Elastic fiber fragmentation within blood vessels can occur due to impaired matrix assembly on account of genetic abnormalities (e.g., fibrillin-1 defects in Marfan Syndrome, elastin gene defects in Williams-Buren Syndrome, etc.) [239] or as a secondary effect of local trauma and inflammation. In both cases, elastic fiber fragmentation occurs when recruited inflammatory cells chronically overexpress proteolytic enzymes such as MMPs. Disruption and loss of the vascular elastic matrix leads to wall thinning, dilatation, weakening, and potentially fatal rupture, typical of aneurysms. Restoration of these lost matrix structures by auto-regenerative mechanisms is severely limited, since diseased vascular cells are poorly elastogenic compared with healthy vascular cells [195]. Thus, in the absence of suitable autografts for surgical repair, elastogenic stimulation of such cells is the only recourse for reinstatement of a healthy or near-normal elastic matrix structure [69].

As described earlier, ECM deposition in the vascular wall principally occurs during late fetal development and the neonatal stage. It is mediated by vascular SMCs which are closely appositioned to and bind to elastic fibers and lamellae in the media [240]. Our lab has extensively examined the role of different exogenous biomolecules in stimulating elastogenesis in vascular SMC cultures in both two-dimensional [9, 10, 112, 195, 241] and 3D microenvironments [196]. Molecules associated with the elastic fiber/matrix assembly process can also be utilized to direct elastic fiber deposition and crosslinking, including in the vascular context. As mentioned earlier, fibrillin microfibrils which serve as the framework for fiber formation can adhere and self-assemble on biomaterial surfaces [182–184], thereby directing the matrix assembly process. Recombinant tropoelastin [95], fibrillin-1 [242], and fibulin-5 [243] coatings on vascular scaffolds have been found to enhance SMC attachment and mediate their function. Thus, biomolecules associated with elastic fiber assembly can be attached to biomaterial surfaces towards developing functional engineered vascular elastic-fiber-rich constructs.

Tissue-engineered blood vessels have been fabricated from elastomeric, biocompatible synthetic biomaterials with appropriate degradation properties, such as polyurethane [244, 245]. They are populated ex vivo or in vitro with endothelial cells to provide a non-thrombotic, vasoactive endothelium and SMCs to secrete an elastic fiber-rich, functional vascular ECM [246–249]. However, they are limited by their poor compliance. This is due to the lack of suitable elastin fibers and elastic matrix deposited by the SMCs seeded within and can be overcome using biomaterials which incorporate elastin or components of elastic matrices [64].

Recombinant human tropoelastin can be crosslinked to form elastic tubes, sheets, and sponges with elastic properties similar to that of native elastin [67]. Alternatively, decellularized blood vessels may be used, since they retain their elastic lamellar structure and demonstrate burst strengths similar to native vessels [250, 251]. Biocompatible vascular grafts have been directly fabricated from electrospun elastin blends containing collagen and PLGA, with mechanical properties similar to native tissue [176]. Despite the tissue constructs grown on such synthetic scaffolds being able to fulfill mechanical roles and closely approach the compliance of native vascular tissues, there are two major limitations to the use of such elastomers. The first limitation is the lack of interaction of SMCs with intact elastic fibers in vitro and in vivo when synthetic elastomers are used [252], which can alter their phenotype. The second limitation is that elastomers assembled from tropoelastin or elastin peptide precursors and decellularized vascular elastic matrices do not include the microfibrillar components that are integral to the elastic fiber structure and mediate interactions with vascular SMCs [253]. Moreover, cell activation and adverse vascular

matrix remodeling outcomes [167, 180] are a potential concern, as elastin peptides have been shown to behave as matrikines [68], triggering an inflammatory response, as elucidated earlier. However, recent studies have used recombinant tropoelastin [66] to form electrospun crosslinked tropoelastin microfibers [254, 255], which promote the attachment and growth of SMCs and endothelial cells. Thus, further studies into the nature and mechanisms of cellular interactions with synthetic tropo-elastin microfiber-based materials are essential for progress in its use as an elastomeric biomaterial.

Scaffolds can also be functionalized with motifs derived from elastin, such as RGD and VGVAPG, to enhance vascular cell adhesion and overall biocompatibility [256]. Elastin-based motifs, as well other biomolecules associated with fiber assembly (e.g., fibrillin), can also be incorporated into scaffolds to direct cell behavior and ECM deposition within [257, 258]. In addition, fibrillin regulates the delivery of TGF- β 1 [58], which plays a critical role in vascular cell signaling [259], and has been found to stimulate elastogenesis by our group [9, 10, 98, 196]. Fibrin-based hydrogels have also been shown to improve elastic fiber deposition by SMCs in vitro [260]. They hold significant promise as small-diameter vascular grafts, as evidenced by the fact that a fibrin hydrogel populated with ovine SMCs and endothelial cells and implanted in vivo in the lamb jugular vein remained patent and demonstrated blood flow rates similar to native vessels. These grafts exhibited deposition of significant amounts of elastic matrix and SMC orientation perpendicular to the direction of blood flow [261].

Nanoparticles have been used for therapeutic delivery to the vascular wall [156, 262, 263], with cationic surface functionalization being shown to enhance their arterial uptake and retention, as mentioned earlier [262]. Based on the role of cationic surfactants that are chemically analogous to DMAB, in upregulating LOX activity and inhibiting elastase activity [224, 225], this cationic modification of the nanoparticles is a promising strategy toward stimulating elastin production and preservation of elastic fiber and matrix integrity.

Overall, elastic fiber synthesis and deposition can be enhanced in the vessel wall by adsorption, conjugation, or encapsulation of suitable exogenous biomolecules on a biomaterial scaffold or encapsulation within nanoparticles for their controlled, sustained presentation or delivery.

Pulmonary tissues

Elastic fibers mediate the extension and recoil of blood vessels, airways, and alveoli in the lungs [264]. Chondroblasts, myofibroblasts, and SMCs synthesize and secrete elastin, which accounts for ~30 % of the total lung tissue protein on a dry weight basis [71] and are typically organized as a latticework of elastic fibers [265]. As in vascular tissues, the rate of elastin synthesis tends to also be the highest during fetal and neonatal development [266] in pulmonary tissues and diminishes with age. Elastic fiber damage has been found to occur in acute and chronic lung diseases [267], with upregulation of MMP-2, MMP-9, and MMP-12, leading to reduction in pulmonary function [268, 269]. Although the damaged elastic fibers can be repaired to some extent [270], the tissue may not regain its normal physiological function, particularly since the reconstruction of the highly complex alveolar structure is difficult [264].

Lung transplants are not an attractive option on account of the poor success rates, prohibitive costs, and shortage of donor organs [271]. Therefore, the development of tissue-engineered lungs has been pursued as an alternative. Although these tissue-engineered constructs replicate some facets of alveolar architecture, they do not participate in the critical function of gas exchange [272–275]. In a recent study, Petersen et al. populated decellularized rat lung tissue with neonatal rat lung epithelial cells to produce a tissue-engineered lung capable

of mediating gas exchange for short durations of time following in vivo implantation in rats [276]. This is the first step towards the development of a functional tissue-engineered lung, and its clinical application will be enhanced with the identification of suitable cell sources for efficient scaffold re-population [277–279]. However, few studies have thus far addressed the issue of reduced elastic matrix stability and its increased degradability when designing tissue-engineered lung tissue constructs. For example, Price et al. demonstrated reduced compliance of their decellularized lungs, which they attributed to decreased elastin content due to the decellularization process [275]. The lack of suitable compliance of tissue-engineered lungs can lead to conditions similar to those typically observed in pulmonary fibrosis [280], illustrating the critical role played by elastin and the elastic matrix in the maintenance of normal lung function.

On account of MMP upregulation in pulmonary diseases [281], their inhibition is an attractive therapeutic option to prevent further matrix loss and tissue deterioration. Vandenbroucke et al. reviewed the potential for using MMP inhibitory agents towards treating these lung diseases and restoring elastic matrix, but concluded that their non-specificity and broad-spectrum inhibitory effects need to be better understood before being used clinically [282]. Retinoic acid is a biomolecule of interest in restoring pulmonary ECM architecture [264], as it has been shown to cause a significant increase in alveoli number in vivo in rats [283] and to reverse lung damage and symptoms after 25 days in an experimental rat model of emphysema [284]. Other biomolecules such as growth factors and glucocorticoids [285] upregulate components associated with the elastic fiber assembly process, thus playing a critical role in elastic matrix deposition in the lung. The challenge however lies in the delivery of these agents to the site of injury in the lungs for therapeutic effects.

Review articles [286, 287] have provided significant insights into the use of polymeric nanoparticles and liposomes for pulmonary delivery of therapeutic biomolecules. These proffer the advantages of localized, sustained release over oral dosage systems, coupled with the availability of the large alveolar surface area for drug absorption. Aerosol-based delivery of therapeutic agents via particulate carriers [288] can be used for local and systemic delivery [289, 290]. The particle size/aerodynamic diameter mediates delivery, with aerodynamic diameters between 1 and 5 μm exhibiting maximum uptake in vitro in a deep lung model system used for examining particle deposition [291]. The aerosolized particles, however, may often be trapped in the mucus layer coating the lumen of the bronchial airways. These trapped particles can potentially be cleared via the cilia on this mucosal layer towards proximal airways where they may be either swallowed or ejected from the throat or airways [287]. Particles which overcome the mucociliary clearance mechanisms must be smaller than 260 nm in order to avoid uptake by alveolar macrophages in vivo [292, 293]. However, studies have also shown that particles sizes between 12 and 200 nm adversely affect cytoskeletal functions of alveolar macrophages in vitro by impairing their phagocytic ability and intracellular transport processes [294]. Based on contrasting results obtained from in vitro cytotoxicity experiments in cell cultures and in vivo pulmonary toxicity studies in rats, Sayes et al. concluded that further research must be done towards determining the relative toxicity of nanoparticles for targeted drug delivery to pulmonary tissues [295].

Drug release in the lungs can be prolonged through control of the nanoparticle properties. For example, anti-tuberculosis therapy delivered using PLGA nanoparticles from a nebulizer in guinea pigs has been found to prolong drug release in the lungs for between 9 and 15 days [296, 297]. This illustrates the potential for long-term pulmonary drug delivery from nanoparticles. Furthermore, in vitro studies have demonstrated the cationic functionalization of polystyrene microspheres to enhance their uptake by human pulmonary epithelial cells

[298]. Nanoparticles have also been found to be effective for in vivo gene transfer following intrapulmonary delivery in mice [299].

Thus, nanocarriers can be formulated and functionalized suitably to bind elastic fibers in pulmonary tissue and release the therapeutic agents in a sustained manner for arresting or preventing elastic fiber degradation and stimulating elastin regeneration. However, future studies will provide deeper insight into the optimal nanoparticle size for their uptake and retention in the lungs, while avoiding any potential cytotoxicity.

Pelvic floor tissues

Elastin and collagen present in the endopelvic fascia mediate the structural properties of pelvic connective tissue [300]. They have different fiber orientations, enabling significant stretching under strain without undergoing rupture [300]. These two structural proteins are synthesized by SMCs in the endopelvic fascia of the vaginal wall and play a critical role in vaginal support. Unlike their counterparts in the vascular wall and other tissues, vaginal SMCs continue to produce elastin and elastic matrix well into adulthood [60], as mentioned previously in this review. Studies have demonstrated enhanced elastin fiber production and crosslinking [301] in the vaginal wall postpartum, on account of its distension during the delivery process. However, this is also accompanied by increased elastin remodeling by MMP-2 and MMP-9 [297]. This leads to weakening of the pelvic tissue and the development of a condition called pelvic organ prolapse (POP). Genetic impairment of the elastic fiber assembly machinery also appears to be linked to the susceptibility to develop POP [302, 303]. The primary risk factors for this condition are repeat vaginal delivery and aging [60]. Although the exact mechanisms by which vaginal childbirth leads to this condition remain unclear [304], studies have demonstrated that mice with null mutations in genes encoding for fibulin-5 [301] and LOXL1, a protein homologue of LOX [305, 306] develop this condition, especially upon vaginal distension [55]. Since both proteins are critical in elastic fiber formation and cross-linking [307, 308], their absence likely leads to impaired elastic fiber synthesis and regenerative repair by vaginal SMCs following vaginal delivery. Together with the MMP-mediated degradation of elastic matrix structures, this has a cascade effect leading to POP development. However, very little is known about whether elastin homeostatic disturbances are a cause of or differently, a result of POP. Elucidating these aspects would be critical to developing specific regenerative strategies that target overcoming deficient elastic matrix assembly.

At this time, there are no pharmacologic or regenerative therapies for POP, and very little has been published in this regard. A key requirement is the probable need to circumvent deficiency or aberrations of specific elastic matrix assembly proteins. For example, the impaired elastic fiber formation on account of LOXL1 deficiency can potentially be overcome by stimulating LOX enzyme activity to enhance tropoelastin crosslinking and fiber formation. As in other disease systems and tissues, concurrent inhibition of proteolytic MMPs along with functional elastic fiber regeneration is essential for restoration of elastic matrix homeostasis in pelvic floor tissues following postpartum changes, to prevent or at least delay their propensity to prolapse.

Few studies have examined nanoparticle delivery to the vagina [309]. The tissue architecture of the cervicovaginal canal is complex, with the cervical mucus layer behaving as a barrier for nanoparticulate delivery from the canal lumen to the underlying epithelial layer. Nanoparticles larger than 200 nm are too large to diffuse rapidly through this mucus layer on account of their size being greater than the pore size of the mucus network mesh [310]. Mucolytic agents can be used as adjuvants to disturb the mucus, but this strategy does not enhance nanoparticle penetration and delivery to the epithelium [309]. More recently, polystyrene nanoparticles of 200 and 500 nm diameter that would normally have been

trapped by the mucus layer due to their size were coated with PEG to impart them with a neutral charge. These neutrally charged nanoparticles showed enhanced transport through human cervicovaginal mucus compared with uncoated (i.e., negatively charged) nanoparticles with diameter of 100 nm [310]. This illustrates the key role played by the surface charge of the nanoparticles in mediating transport through the mucus for effective localization in the underlying epithelium. At the same time, mucoadhesive nanoparticles have been formulated using poloxamers, polyacrylates, and chitosan and its derivatives, for retention in the mucosal layer, although this renders them inadequate for intracellular delivery [311]. They can also potentially be injected directly into the vaginal wall, for optimal therapeutic effect, with their mucoadhesive properties enabling retention within and preventing their outflow through the mucus layer. Thus, surface properties of nanoparticles, particularly their charge and size, can be fine-tuned to enable effective localization and delivery of therapeutic agents in the vaginal wall as well in the vaginal mucosa.

Vocal fold tissues

Vocal folds or vocal cords located within the larynx play a critical role in voice production and quality through their vibration, for which their elasticity is critical. The mechanical properties of the vocal folds are determined by the ECM in their lamina propria, which are part of the vocal mucosa and vocal ligament. The lamina propria, which is a constituent of the vocal fold mucosa, is rich in elastin, collagen, and HA [312, 313], with elastin accounting for ~9 % of the total tissue protein on a dry weight basis [71, 314]. Fibroblasts in the lamina propria of vocal folds synthesize elastin and collagen actively in newborns and have been hypothesized to mediate post-natal development of the vocal ligament structure [315, 316]. Vocal fold elasticity is lost with age, with RT-PCR studies in rats indicating decreased tropoelastin, LOX, and TIMP mRNA expression in aged rats compared with neonatal levels [317]. Injury to the vocal folds through overuse or abuse leads to changes in its tissue architecture, with enhanced elastic matrix fragmentation. This leads to reduced elastic matrix density and organization [318, 319] and can cause changes in tissue biomechanics which affect vocal quality adversely. Currently, there is no optimal strategy for vocal fold regeneration and restoration of their function. Therefore, further research into the role of elastin in vocal fold pathologies is necessary [71, 320, 321].

Transplantation of autologous fibroblasts into a canine model of lamina propria injury demonstrated increased collagen density but decreased elastin content compared with uninjured controls [322]. As an alternative approach, exogenous delivery of biomolecules can be used for treating a variety of laryngeal conditions [323]. For example, steroid injections have been used for the reduction of vocal fold scarring post-surgery [324]. Hepatocyte growth factor (HGF) is a biomolecule with elastogenic properties (see Table 1) and has been detected in the epithelial and gland cells of healthy vocal folds, as well as in injured lamina propria [325]. HGF enhanced HA and elastin synthesis with decreased collagen synthesis in vitro in vocal fold fibroblast (VFF) cultures [326–329]. However, Hirano et al. showed that injection of HGF into rabbit vocal folds did not lead to a significant change in elastin or HA, compared with uninjured controls in vivo [330]. Hence, in order to improve the bioavailability and prolong the efficacy of the therapeutic agents, while avoiding repeated injections, they can be encapsulated in nanoparticles for sustained release. Kolachala et al. have demonstrated the efficacy of laryngeal therapy involving HGF delivered from PLGA nanoparticles over repeated injections [323] in decreasing procollagen mRNA, the production of which is undesirable in vocal fold regeneration. This illustrates the utility of nanoparticles for controlled delivery of therapies for vocal fold regeneration.

Recombinant elastin-like polypeptides have been shown to improve wound healing in vocal folds when combined with HA hydrogels [71, 331], as well as synthetic elastin-mimetic polymers which permit growth of VFFs [332]. Recent studies have also demonstrated the

clinical potential of adipose-derived stem cells (ASCs) in fibrin-based constructs as options for vocal fold regeneration [333, 334]. The tissue-engineered construct composed of fibrin and ASCs [333] demonstrated an indentation modulus, microstructure, and vibration properties similar to that of human vocal folds, while fibrin–HA and fibrin–collagen hydrogels exhibited increased ASC proliferation, differentiation, and elastin production [334]. This study confirmed the role of fibrin gels in enhancing elastin expression by cells [260]. Therapeutic efficacy can potentially be enhanced by combining nanoparticles within these fibrin- or HA-based hydrogels for sustained release of elastogenic biomolecules within these constructs.

Overall, most studies have focused on the individual therapeutic strategies for vocal fold regeneration. However, recent studies listed above that involve a synergistic approach of combining biomaterials and biomolecules have demonstrated significant potential in improving regenerative outcomes. The use of elastin-based biomaterials particularly affords promise in enhancing the biomechanical properties of vocal folds. It is important to note, however, that therapeutic intervention is carried out immediately after vocal fold injury in the majority of recent studies. Since many vocal fold pathologies progressively develop over time, it would also be important to evaluate these regenerative strategies in models of chronic vocal fold injury.

Skin

Elastin accounts for approximately 2–4 % of total tissue protein in skin on a dry weight basis [71]. It is primarily distributed in the lower layer of the dermis as well organized, horizontal mesh-like layers of thick, discrete fibers. Thinner elastic fibers are present in the uppermost layer of the dermis which merge into the fibrillin-rich microfibrils at the dermal–epidermal junction [58]. As in other elastic tissues, the microfibrils in skin also play a critical role in elastic fiber formation, as is evidenced by the fact that LTBP-1 [335] and fibulin-2 [336] have been found to co-localize with microfibrils and fibrillin-1. Microfibril-associated protein-1, protein-3 and protein-4 have also been shown to localize with elastic fibers in skin [337–342].

Scar formation is an inevitable outcome of wound healing in the skin, with the scar tissue being inelastic and lacking in flexibility compared with the normal dermis [343, 344]. This is largely due to elastin disruption and its inadequate regeneration during the repair process [345] coupled with upregulation of collagen synthesis and accumulation. Although elastin/elastic fibers are synthesized within scar tissue, they are disordered functionally and spatially. They appear thin and fragmented and less mature than those observed in normal, healthy skin [345–348]. Thus, a critical problem in elastic matrix restoration in skin appears to be lack of mature fiber formation and crosslinking, rather than production and recruitment of tropoelastin precursors as in vascular tissues. In human skin, fibroblasts are the primary elastin-generating cell-type [73, 349], which synthesize tropoelastin quite robustly and crosslink the precursors into an alkali-insoluble matrix. However, in vitro cell culture studies of dermal fibroblasts have demonstrated their decreased ability to synthesize elastin with increasing age of tissue donors, like cells in vascular tissue and vocal folds [73]. This has been a limiting factor for the development of tissue-engineered constructs for skin substitutes [350]. In fact, in epithelial autografts composed of sheets of cultured epithelial cells implanted at burn sites, sufficient deposition of elastic matrix has been found to take 4–5 years post-implantation [351]. Even when elastic fibers are deposited, as observed in a skin substitute consisting of a type I collagen gel seeded with human neonatal foreskin dermal fibroblasts and keratinocytes, they tend to be highly disorganized [352].

Elastin has often been overlooked in favor of collagen in designing dermal substitutes [353]. However, in the last two decades, processed xenogeneic or recombinant elastin and its

derivatives have been incorporated into biomaterials for effective skin substitutes, on account of their increased availability. Type I collagen scaffolds coated with 3 % solubilized α -elastin alone [354] and in combination with meshed split skin grafts [355] have been shown to enhance wound healing and tissue regeneration in porcine excised wound models. The elastin incorporated in the scaffold has been hypothesized to attenuate scar tissue formation and decrease wound contraction by inhibiting the differentiation of proliferative fibroblasts into a contractile myofibroblast phenotype [356]. Results from an in vivo study in rats involving sub-cutaneous implantation of these collagen–elastin scaffolds [174] suggest that the incorporation of solubilized elastin within the collagen scaffold upregulated elastic fiber formation, compared with control collagen scaffolds. Co-localization of fibrillin-1 with the newly synthesized elastin fibers was also found. These concur with results from an earlier study by Raghunath et al. [357] which indicated that fibrillin-1 microfibrils deposited serve as templates for elastic fiber deposition in wound repair of skin. Furthermore, the scaffolds containing solubilized elastin did not show any calcification, unlike control collagen scaffolds [174]. Cutometer measurements done in clinical studies have shown such collagen–elastin skin substitutes to improve skin elasticity in 31 patients with acute burn injuries [358], while being effective in restoring the full range of hand motion in ten patients with severe burn injuries [359]. Overall, the inherent biomechanical transducing and cell signaling properties of elastin must be harnessed in the design and development of novel scaffolds for skin substitutes that replicate the native elastic properties of healthy skin.

Induced elastic matrix synthesis via incorporation of elastogenic biofactors is also a potential strategy to enhance skin regeneration and wound healing. As in other disease systems, nanoparticle-based release of such factors affords their controlled and predictable delivery. Since the skin acts as a natural diffusion barrier, delivery of such nanoparticulate carriers or the biomolecules themselves to the dermis or epidermis can be limited. However, owing to a less dense matrix and more porous skin structure, this barrier can be overcome in the case of aged or diseased skin [360]. Solid lipid nanoparticles (SLNs) have been widely used for drug delivery to the epidermis [361–363]. SLNs encapsulating corticosteroids have been shown to permeate into the epidermis in excised human skin in ex vivo studies, but the amounts of drug ultimately delivered represented 1 % of the total encapsulated amount [364]. A more recent study carried out ex vivo using porcine skin demonstrated that dendrimer-based nanoparticles (20–30 nm) are superior to SLNs (150–170 nm) as vehicles for active agent delivery to skin. The dendrimer-based NPs were found to enhance penetration of a fluorescent dye, Nile red into the epidermis, by two- to fourfold. SLNs have been used to encapsulate and deliver the elastogenic biomolecule retinoic acid for topical drug delivery [361, 365–367]. While these systems enable controlled, sustained release of the retinoic acid [366], only approximately 5 % of the total retinoic acid dose from the SLNs, was found to have permeated and localized in the dermis of abdominal rat aortic skin ex vivo [366, 367].

Thus, although elastin-based scaffolds for skin substitutes enable improved elastin regenerative outcomes, nanocarrier-based delivery of elastogenic therapeutics is limited by their poor permeation into skin. Overall, these systems must be optimized by engineering the size, surface chemistry, and other parameters to optimize delivery of therapeutic agents into the epidermis and dermis.

Bladder tissues

Bladder ECM is composed of proteins (primarily collagen I and III), GAGs, and proteoglycans. It provides structural support to bladder cells and mediates cell signaling [368, 369]. Elastin and elastic fibers are sparsely distributed in the lamina propria, a constituent of the mucosal layer in the bladder wall [370]. However, they critically enable

expansion of the urinary bladder to more than 15 times its original volume during filling and recoil to its initial shape following micturition [371]. Unlike other cell-types, such as vascular SMCs, bladder SMCs have been shown to be prolific in their ability to produce elastin [59]. Studies have demonstrated that the average elastin content of the bladder wall in humans (expressed as a percentage of the total weight of the bladder) increased from 1.1 % in 4-year-old children to 2.4 % in 63-year-old adults [372].

As is the case in other tissues, deficient or aberrant elastic fiber formation and matrix deposition affects bladder function [368, 369, 373]. Excess levels of CS and dermatan sulfate have been implicated in downregulating elastin secretion by bladder fibroblasts [374, 375]. The bladders of LOXL1-deficient mice with POP have also been shown to exhibit hyperdistension and reduced contraction with disorganized elastic fibers, on account of decreased elastin cross-linking [305, 306]. Additionally, increases in MMP-2 and MMP-9 production and activation have been observed in vitro in bladder SMC cultures in response to excessive strains, as well as in vivo in animal models of obstructive bladder disease [376, 377].

Tissue-engineered scaffolds offer promise for functional bladder regeneration on account of the ability to control their physical properties, particularly surface topology. Studies by Pattinson et al. demonstrated that bladder SMCs seeded on nano-dimensional porous PLGA scaffolds exhibited enhanced elastin and collagen synthesis compared with conventional micro-dimensional scaffolds [378]. This was attributed to the enhanced surface area available on account of the nanoscale features present, as well as the increased pore-diameter and porosity compared with conventional micro-scale scaffolds.

Exogenous delivery of biomolecules can be utilized to enhance cell attachment, growth, and elastic matrix synthesis within scaffolds for bladder tissue engineering. For example, increased compliance of the rat bladder following spinal cord injury has been attributed to the upregulation of TGF- β 1 and IGF-1 in the bladder wall [379]. Studies by Parekh et al. have shown that TGF- β 1 can also be a potent regulator of ECM architecture in pathological conditions of the bladder on account of its modulation of SMC function and phenotype [380, 381]. Elastin production has been found to be upregulated in bFGF-treated scaffolds compared with untreated controls under cyclic stretch [382]. This is surprising, considering the fact that bFGF downregulates elastin mRNA expression in aortic SMC cultures [383]. Overall, these studies clearly illustrate the benefits of biomolecular stimuli in enhancing elastogenesis towards developing functional tissue-engineered replacements for the urinary bladder wall.

Nanoparticles formulated from chitosan, gelatin, and poly-L-lysine have been delivered to the bladder for chemotherapy to treat bladder cancer [384–386]. Cationic modification has been shown to enhance their uptake and retention in vivo in the bladder wall on account of strong binding to the negatively charged mucin proteoglycans [311] in the mucosal layer of the bladder wall, as well as enhanced cellular interactions [384, 386]. In another study, negatively charged HA has been conjugated on the surface of cationic PLGA nanoparticles and then incorporated within SIS scaffolds for improving their permeability for use in bladder tissue-engineering applications [387, 388]. These studies illustrate the potential for synergistic applications of scaffolds with nanoparticles towards regenerative repair in the bladder [389].

Conclusions and future directions

In conclusion, there has been significant progress in the last decade toward delineating the mechanisms and role of different biomolecules in mediating elastic fiber assembly. In

addition to the biomolecules listed in Table 1, other growth factors and biomolecules may play a role in stimulating elastin synthesis and elastic matrix assembly. Insights into this can be gained from a comprehensive analysis of the process of elastic fiber deposition and matrix assembly during fetal and neonatal development. The inhibition of proteolytic MMPs is also critical for preventing the degradation of newly synthesized/deposited elastic matrix, and the accomplishment of this at the gene and protein levels offers exciting therapeutic options.

Biomaterial-based strategies using scaffold and nanoparticles for the encapsulation of these agents provides spatiotemporal control over their activity and bioavailability, although the field still needs to optimize strategies for conjugation or incorporation of these agents with minimal or no loss of their bioactivity. There is potential for improving the conjugation of biomolecules associated with the elastic fiber assembly process, such as fibrillin, fibulin, and tropoelastin, on biomaterial surfaces. This would potentially provide a degree of spatial control over their distribution, thereby enabling them to direct elastic fiber and matrix assembly. Nanoparticles and scaffolds can also be functionalized with suitable peptides and other compounds for targeted elastin binding to concurrently enhance the therapeutic effect of their encapsulated agents. Similarly, they can be conjugated with biomolecules using MMP-sensitive linkages to form 'smart' biomaterials which release the therapy in the presence of activated MMPs, thereby harnessing the proteolytic environment for therapeutic benefits. Overall, an optimal balance must be achieved using biomolecules and biomaterials towards developing novel strategies for functional elastic matrix engineering.

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Fig. 1. Schematic demonstrating the steps required for de novo synthesis of elastic fibers. Tropoelastin (*Eln*) and microfibrillar components (e.g., fibrillin-1) are secreted from the cells into the extracellular space, and tropoelastin molecules interact with the EBP. Both the microfibrils and the tropoelastin require crosslinking by activated transglutaminases (TGMs) and lysyl oxidase (LOX), respectively. Coacervation of the tropoelastin occurs when it interacts with the microfibrillar scaffold, and then subsequent crosslinking can occur. The result is complex superstructures that are tissue-type specific. As described in the legend, particular steps of the elastic assembly process have been shown to be impacted by age or genetic diseases and/or to be impacted by the presence of particular biomolecules

Fig. 2. Transmission electron microscope (TEM) images showing the positive impact of particular elastogenic biomolecules on elastic matrix synthesis. Cultured aneurysmal rat aortic smooth muscle cells from an elastase perfusion model both without (**a**) and with (**b**, **c**) the addition of 2 µg/mL HA-o and 10 ng/mL TGF-β1. The *arrows* point to areas of significant elastic fiber formation

Fig. 3. Schematic showing specific considerations for scaffold- and nano-carrier-based strategies, as well as general methods for incorporation of the elastogenic biomolecules into the biomaterial. Although these general strategies are not the focus of this review article, this figure highlights the importance of both scaffold / nano-carrier properties (e.g., chemistry and topography) and the choice of delivery method for elastic matrix generation or regeneration applications

Fig. 4. Schematic representation of active agent-loaded nanoparticles and their proposed role in promoting functional elastic matrix generation. Nanoparticles are surface-modified to enhance their uptake and retention in elastic matrix/tissue. Modification of nanoparticles with cationic surfactants likely enables their binding to hydrophobic domains in elastin, while upregulating lysyl oxidase (LOX) activity and attenuating effects of cationic elastases and matrix metalloproteinases (MMPs). Active agent(s) released in a sustained fashion from the nanoparticles interact with cells to (1) stimulate LOX and tropoelastin secretion and (2) inhibit MMPs to prevent proteolytic degradation of elastin and elastic fibers. LOX mediates crosslinking of tropoelastin molecules into elastic fibers

Table 1**Biomolecular agents influencing elastin synthesis and matrix deposition**

Biomolecule	Specific impacts	Cell-type	References
Agents which stimulate elastin synthesis and matrix deposition			
Cyclic GMP	↑ Elastin synthesis in presence of intracellular Ca^{2+}	Ligament fibroblasts	[390]
FCS	5 % w/v ↑ elastin synthesis	Ligament fibroblasts	[391]
IGF-1	↑ Tropoelastin and matrix assembly	Aortic SMCs	[110, 115, 392]
Dexamethasone	↑ Tropoelastin synthesis	Ligament fibroblasts	[391]
Bleomycin	↑ Tropoelastin synthesis	Ligament fibroblasts	[391]
Aldosterone	↑ Tropoelastin synthesis and fiber formation	Dermal fibroblasts	[126]
Retinoic acid	↑ Tropoelastin synthesis, ↑ fibrillin	Vascular SMCs, dermal fibroblasts	[397, 398]
Lysyl oxidase	↑ Tropoelastin synthesis and matrix assembly	Aortic SMCs	[119]
HA oligomers	↑ Tropoelastin synthesis and matrix assembly	Aortic SMCs	[9, 10, 112–115]
HGF	↑ Tropoelastin synthesis	VFFs	[326–329]
Agents which inhibit elastin synthesis and matrix deposition			
Ascorbic acid	↓ LOX activity, ↓ crosslinking of tropoelastin, ↓ tropoelastin synthesis and matrix assembly	Aortic SMCs, dermal fibroblasts, pulmonary arterial SMCs	[120–125]
bFGF	↓ Tropoelastin synthesis, ↓ elastin mRNA	Lung fibroblasts	[393]
cAMP	↓ Tropoelastin synthesis, cGMP antagonist	Ligament fibroblasts	[390]
Theophylline	↓ Tropoelastin synthesis, dexamethasone antagonist	Ligament fibroblasts	[391]
Monensin	↓ Tropoelastin synthesis	Ligament fibroblasts, chondrocytes, aortic SMCs	[394] [395]
Bafilomycin A1	↓ Tropoelastin synthesis	Ligament fibroblasts, chondrocytes	[394]
Chloroquine	↓ Tropoelastin synthesis	Ligament fibroblasts, chondrocytes	[394]
NH_4Cl	↓ Tropoelastin synthesis	Ligament fibroblasts, chondrocytes	[394]
Versican	Release of EBP from cell surface leading to ↓ elastin fiber assembly	Aortic SMCs	[78]
Agents which stimulate and also inhibit elastin synthesis and matrix deposition			
HA-long chain	↑ Tropoelastin, ↑ tropoelastin crosslinking; ↑ versican binding, ↓ elastin fiber assembly	Aortic SMCs	[113, 114]
TGF- β 1	↑ LOX expression and activity; ↓ MMP-2, MMP-9; ↑ TIMP-1, TIMP-2, TIMP-3; ↑ elastin synthesis	Aortic SMCs	[8–12]
	Matrix mineralization at high doses	Fibroblasts, SMCs	[13, 14]
Cu^{2+} ion	↑ Crosslinking; toxic at high doses	Aortic SMCs	[82, 116, 396]