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Review

Elastomeric recombinant protein-based biomaterials

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ABSTRACT

Elastomeric protein-based biomaterials, produced from elastin derivatives, are widely investigated as promising tissue engineering scaffolds due to their remarkable properties including substantial extensibility, long-term stability, self-assembly, high resilience upon stretching, low energy loss, and excellent biological activity. These elastomers are processed from different sources of soluble elastin such as animal-derived soluble elastin, recombinant human tropoelastin, and elastin-like polypeptides into various forms including three dimensional (3D) porous hydrogels, elastomeric films, and fibrous electrospun scaffolds. Elastin-based biomaterials have shown great potential for the engineering of elastic tissues such as skin, lung and vasculature. In this review, the synthesis and properties of various elastin-based elastomers with their applications in tissue engineering are described.

Contents

1. Introduction

Elastomeric biopolymers are promising biomaterials for engineering elastic tissues due to their unique physical and biological properties. One of the main elastomeric proteins in natural extracellular matrix (ECM) is elastin. This structural protein is the essential component of the elastic fibers that provides elasticity to different tissues and organs such as blood vessels, skin, and

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lung [1,2]. For example, the presence of elastic fibers in blood vessels enables the vessel to stretch and relax more than a billion times during life time [2]. Elastin is one of the most stable proteins in the body with a half-life of 70 years [3]. Elastic fibers are highly crosslinked in native tissues and are extremely insoluble. This persistent insolubility prevents the processing of elastin-based biomaterials from intact elastic tissues. Therefore, various forms of soluble elastin including animal-derived hydrolyzed soluble elastin (*e.g.* α-elastin and κ-elastin) [4,5], elastin-like polypeptides (ELPs) [6,7] and recombinant human tropoelastin (rhTE) [8,9] have been synthesized and utilized to engineer synthetic elastin-based tissue constructs. These elastin-derived molecules have the potential to self-assemble or coacervate under physiological conditions similar to natural elastin protein, and have been used to generate biomimetic elastic biomaterials for the regeneration of various elastic tissues. This review will first describe the *in vivo* synthesis of elastin, native elastic fiber morphology, and the biological function of elastin. Then, various approaches for the synthesis of elastin sequence-based materials, including ELP synthesis and recombinant protein technology, will be discussed. Finally, the use of elastin derivatives to engineer biomimetic elastic biomaterials for various tissue engineering applications will be reviewed. Current techniques for the fabrication of these elastomers, their physical and biological properties, and potential applications will be discussed.

2. Biosynthesis of elastin

Elastin is formed *in vivo* through the process of elastogenesis, which involves a number of important steps (Fig. 1a). In the first step the tropoelastin monomer is transcribed and translated from a single elastin gene by elastogenic cells, including endothelial cells (ECs) [10], chondroblasts [11], fibroblasts [12], mesothelial cells, keratinocytes [13], and smooth muscle cells (SMCs) [14]. Regulation of tropoelastin transcription is controlled at the post-transcriptional level with mRNA deadenylation proposed as a contributory mechanism [15]. The primary transcript of tropoelastin undergoes developmentally regulated alternative splicing, which leads to the translation of multiple heterogeneous tropoelastin isoforms. The most frequently observed human tropoelastin isoform lacks exon 26 A. Following translation and removal of the signal sequence mature intracellular tropoelastin, an unglycosylated ~60–70 kDa protein, is chaperoned to the cell surface through association with the elastin binding protein (EBP) [16], which prevents tropoelastin intracellular self-aggregation and premature degradation. Released tropoelastin monomers aggregate on the cell surface through coacervation [17] to form protein-dense spherules [18]. Following transportation of these spherules to the microfibrils, the monomer is converted to the insoluble elastin polymer through enzyme-mediated crosslinking by the lysyl oxidase family of proteins [19,20].

Tropoelastin monomers are characterized by alternating hydrophobic and hydrophilic domains, which are encoded in separate alternating exons (Fig. 1b). The hydrophobic domains of tropoelastin are implicated in tropoelastin coacervation while the hydrophilic domains are involved in crosslinking of the monomers [21]. The non-polar residues glycine, valine and proline dominate the hydrophobic domains. While the hydrophilic domains are characterized by a high content of either lysine and alanine or lysine and proline residues. Coacervation is a crucial step in elastin fiber formation as tropoelastin monomers align and concentrate during this process to facilitate the formation of crosslinks between closely spaced lysines [17,21]. Coacervation is a reversible temperature transition process where the hydrophobic domains

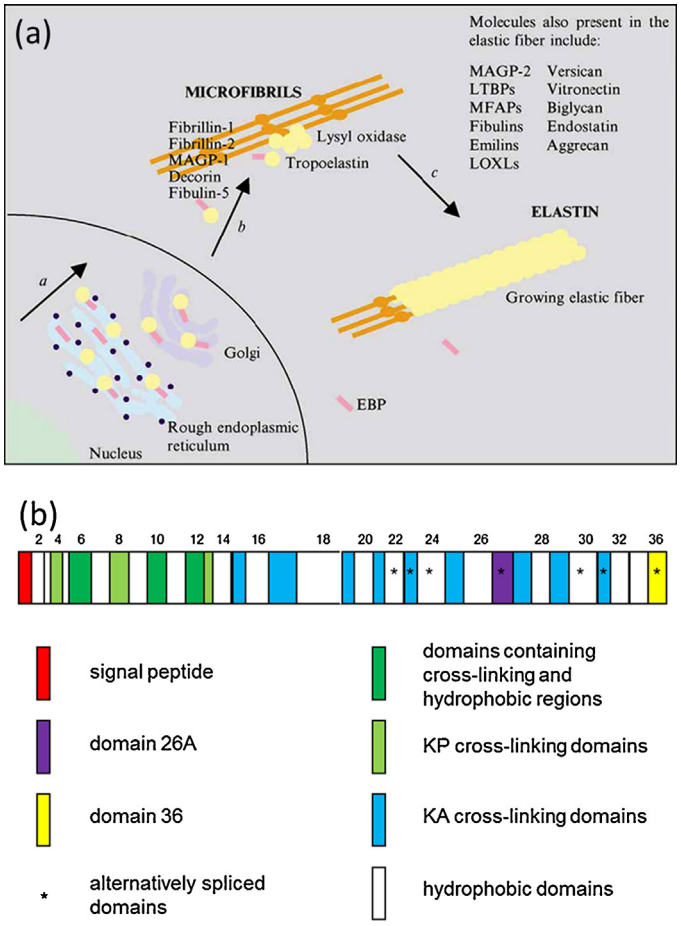

Fig. 1. Schematic of elastogenesis process and structure of human tropoelastin. (a) Elastogenesis process, (b) the human tropoelastin structure is dominated by alternating hydrophobic and hydrophilic regions primarily responsible for coacervation and crosslinking, respectively [1]. Adapted with permission from Elsevier.

of tropoelastin (such as the oligopeptide repetitive sequences GVGVP, GGVP, and GVGVAP) promote protein association [21].

3. Elastin morphology in native tissues

Elastic fibers are composed of two morphologically different components: an elastin core wrapped in a sheath of microfibrils 10–12 nm in diameter [22]. Elastin constitutes approximately 30–57% of the aorta, 50% of elastic ligament, 3–7% of lung, 28–32% of major vascular vessels, 4% of tendons, and 2–5% of the dry weight of skin [1]. The microfibrils consist of a complex array of various molecules such as fibrillins, fibulins, and glycoproteins [23].

Elastin displays different morphology and organization in various elastic tissues (Fig. 2). For example, elastin fibers are presented as parallel-oriented rope-like structures in ligament and tendon, concentric rings of elastic lamellae around the arterial lumen in arteries, 3D honeycomb structures in elastic cartilage, and a delicate latticework throughout the lung [1]. In skin, elastin fibers are arranged into two distinct layers within the dermis. The upper papillary dermis contains elastin fibers that are shaped into small, finger-like vertical projections, which connect the dermis to the epidermis. In contrast, the lower reticular dermis consists of a network of horizontally aligned elastin fibers [24]. In addition, within the medial layer of blood vessels 71% of total elastin is seen as thick continuous elastic lamellae, 27% as a thin protruding network of

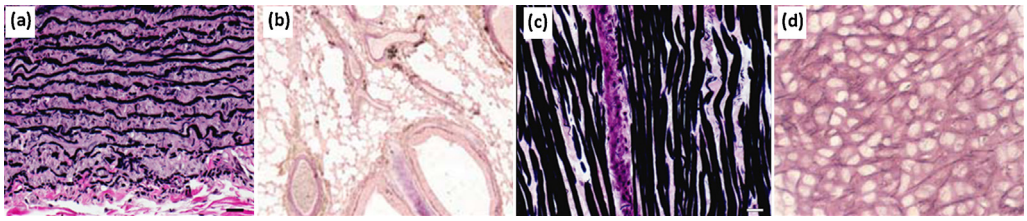

Fig. 2. The morphology and organization of elastin in (a) aorta, (b) lung, (c) ligament, and (d) ear cartilage [1,6]. Adapted with permission from Elsevier.

interlamellar elastin fibers, and 2% as thick radial elastin struts connecting adjacent lamellae [25]. Various morphologies of elastin also exist in the heart valve including sheet-like architecture in the ventricularis, tubular to circumferential in the fibrosa, and sponge-like in the spongiosa [26].

4. Biological properties of elastin

Elastin plays a crucial biological role in the regulation of various cellular functions including promotion of cellular attachment, proliferation, differentiation, phenotype preservation, chemotaxis, and migration. For example, it has been demonstrated that ELPs facilitate the migration and proliferation of ECs to enhance angiogenesis and form vascular networks [27]. In addition, elastin is a chemoattractant for ECs, SMCs, and monocytes [28,29]. Furthermore, tropoelastin, ELP, and elastin promote the *in vitro* attachment and proliferation of skin fibroblasts [30,31]. In wound healing processes, elastin can alter the cell phenotype and function by controlling the differentiation of phenotypically proliferative dermal fibroblasts into contractile myofibroblasts to help close the wound by contraction [32]. The VGAPG sequences in elastin induce the migration and terminal differentiation of epidermal keratinocytes to assemble and build the epidermis layer [33].

Several cell-surface receptors have been identified for elastin and its derivatives. These receptors include EBP [34], glycosaminoglycans (GAGs) [35], and the integrin $\alpha_v\beta_3$ [30]. EBP is one of the receptors for elastin, which is expressed by various cells and localized on the cell surface. It binds to peptides of elastin possibly following degradation due to disease or injury, and activates intracellular signaling in various cell types including SMCs, ECs, monocytes, mesenchymal stem cells (MSCs), and leukocytes [36]. A major interaction between tropoelastin and cells occurs through the integrin $\alpha_v\beta_3$ and the residues GRKRK at the C-terminal of the protein. This cell-surface receptor facilitates the attachment of cells to tropoelastin. Glycosaminoglycans on the cell surface also interact with the C-terminus of tropoelastin. Other molecules that have been shown to interact directly with tropoelastin include members of the lysyl oxidase family of enzymes, fibrillin-1 [37] and fibulin-5 [38,39].

5. Production of elastin-sequence derived materials

An increasing appreciation of various biological roles of elastin has highlighted the potential usefulness of this protein and its derivatives to the tissue engineering field. This in turn has led to the development of technologies for the synthesis and purification of elastin-based molecules. Elastin can be obtained from the elastin-rich tissues in animals (*e.g.* bovine ligament) by partial hydrolysis of some peptide bonds in insoluble elastin. Tissues are treated with oxalic acid and potassium hydroxide to yield α-elastin and κ-elastin, respectively [6]. These soluble forms of elastin have shown properties similar to the native tropoelastin, such as ability to coacervate as well as alteration of cell signaling *via* elastin receptors. However, animal-derived elastin is a heterogeneous partially crosslinked mixture of peptides with inadequate cell binding sites [40]. Elastin derivatives can also be produced *via* peptide synthesis to generate ELPs, or biosynthetically to form recombinant proteins.

5.1. Synthesis of synthetic elastin-based peptides

Elastin-like polypeptides that incorporate repetitive amino acids sequence found in tropoelastin have been produced through peptide synthesis. For example, Urry et al. were the first to synthesize ELP with tunable physical properties based on the amino acid compositions. The synthesized polymer was composed of the repeating sequence of Val-Pro-Gly-Xaa-Gly (VPGXG) and exhibited an inverse phase transition similar to human tropoelastin [41]. Lee et al. also fabricated various elastin-based materials (*e.g.* $(Gly\text{-}Val\text{-}Gly\text{-}Ile\text{-}Pro)_{260}$, $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_n$, $(Gly\text{-}Val\text{-}Gly\text{-}Val\text{-}Pro)_{251}$) through the polymerization of pentapeptides and subsequently crosslinked them by γ-irradiation to form elastin-based biomaterials [42,43]. To provide better control over the crosslinking of these pentapeptides, McMillan et al. replaced a Val residue of the elastin sequence $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_n$ with Lys in every seven repeats to fabricate transglutaminase-crosslinked hydrogels for chondrocyte growth [44]. In other studies, electrospun fibers and non-woven scaffolds were formed from 39 repeats of $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_4(Val\text{-}Pro\text{-}Gly\text{-}Lys\text{-}Gly)$ sequence and used as vascular constructs [45–47]. Hydrogels and fibers were also produced by chemically crosslinking of a $(Gly\text{-}\mathbf{Val}\text{-}Gly\text{-}Val\text{-}Pro)_n$ where the bold residue was substituted with Lys or Glu per six pentapeptides. The hydrogels were formed when 50% of Lys and 50% of Gly peptides crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [43]. In addition, elastin-mimetic copolymers with controlled mechanical properties were made through the incorporation of a varying central hydrophilic sequence among identical hydrophobic sequences [48]. These ELPs have been used to fabricate hydrogels for drug delivery and soft tissue engineering applications [49].

The use of peptide synthesis to produce elastin-mimetic molecules has several advantages. This technique allows control over amino acid sequence and chain length, and the production of new proteins with varying properties (pH or temperature responsiveness). In addition, peptide synthesis enables easy incorporation of specific sequences in the protein chain such as Arg-Gly-Asp (RGD) for improving cell-interactive properties or non-natural amino acids for modification or crosslinking reactions. However, the *in vivo* biocompatibility of ELPs is still unknown. It is important to investigate the *in vivo* response to engineered polypeptide proteins but to date just a few ELPs have been tested *in vivo*. Unlike polypeptide synthesis, recombinant technologies result in the formation of highly homogenous long protein chains with defined length, sequences, and compositions.

5.2. Recombinant protein technology (biosynthetic approach)

Using biosynthetic approaches, recombinant elastin sequence containing proteins are expressed in hosts such as *Escherichia coli* [50], plants [51–53], and yeast [54,55]. In most studies, the protein is obtained from expression in a bacterial host following the

construction of genes encoding for tropoelastin or tropoelastin-derived sequences.

Recombinant human tropoelastin (rhTE) was first expressed in a recombinant bacterial system in very low yield by Indik et al. after the construction of an expression vector containing the cDNA sequence of an isoform of human tropoelastin [56]. To improve the yield of rhTE production, 5 years later Martin and Weiss created a synthetic gene (2210-bp synthetic human TEL-encoding gene (SHEL)) for tropoelastin, which contained codons optimized for maximum expression in *E. coli*. The developed synthetic gene supported substantial expression of recombinant sequences and provided commercial yields [57]. The production of rhTE in a bacterial host is a valuable tool in obtaining individual isoforms of purified human tropoelastin in high quantity through a controllable process. The increased availability of rhTE allowed extensive studies on tropoelastin function and structure [21]. In addition, this recombinant protein has been processed into a variety of promising biomaterials for different tissue engineering applications [33].

The application of recombinant technologies to the synthesis of ELPs has allowed for the production of polypeptides containing an array of alternating functional motifs including derivatives of the elastin containing pentapeptide VPGVG as well as hydrophobic and/or hydrophilic amino acid blocks, crosslinking and cell recognition sequences. This exquisite control over the amino acid sequence has allowed for the design of proteins with specific mechanical, physical and cell interactive properties. Interestingly, incorporation of the inherent temperature dependent reversible phase transition ELP sequences confers the added benefit of enhancing purification yields of recombinant proteins [58]. This feature is being exploited to great advantage through the development of ELP fusion proteins, using a technology referred to as ELPylation, including most recently in plant based expression systems [51,53,59,60]. This technology has substantial potential for the large scale and cost competitive production of recombinant proteins [61,62]

6. Elastin as a biomaterial for tissue engineering

The recent increase in elastin derivatives synthesis has led to the formation of a range of elastin-based biomaterials for various tissue engineering applications (Table 1).

6.1. Animal-derived elastin-based constructs

Hydrolyzed soluble elastin has been used to generate various forms of biomaterials such as electrospun fibers, 3D hydrogels, and crosslinked 2D sheets. Due to the importance of vascularization in the field of tissue engineering [63], the use of these elastic biomaterials for vascular network formation has been studied. For example, Leach et al. crosslinked α-elastin with a diepoxy crosslinker to generate elastic films with controlled mechanical properties. Although the fabricated scaffolds supported attachment of SMCs, cell proliferation on these materials decreased during culture [5]. Electrospun α-elastin scaffolds with enhanced elasticity were also produced for vascular tissue engineering [64]. It was shown that the fabricated fibrous scaffolds containing elastic fibers with diameter similar to native elastin fibers regulated SMC phenotype [64]. In another study, it was found that crosslinked porous scaffolds containing soluble elastin promoted angiogenesis, elastin fiber formation, and deposition of collagen [6]. In addition, scaffolds containing hydrolyzed soluble elastin did not cause calcification in contrast to those made from insoluble elastin presumably due to higher purity [6].

Hydrolyzed elastin has also been used in dermal replacements because of the advantageous properties it imparts compared to other elastin-free biomaterials used for skin repair and wound healing [65]. For instance, a collagen scaffold containing α-elastin (MatriDerm) significantly enhanced skin elasticity [66]. We have previously shown that porous crosslinked α-elastin hydrogels supported dermal fibroblast infiltration, adhesion, and proliferation *in vitro* [4,67].

Hydrolyzed soluble elastin has been combined with various materials including collagen [68], glycosaminoglycans [69], calcium phosphate [70], fibrin [71], rhTE [72], and polyethylene glycol terephthalate (PET) [73] to make composites with improved properties for different tissue engineering applications. In our previous study, we found that the addition of rhTE to α-elastin significantly improved the physical properties of rhTE/α-elastin composite scaffolds [72]. The pore sizes of the fabricated composites were significantly enhanced by performing crosslinking reaction under dense gas CO_2, which resulted in an improvement in cellular penetration within the 3D structure of the hydrogels (Fig. 3) [72]. Although biomaterials based on animal-derived soluble elastin exhibited remarkable properties as tissue engineering scaffolds, some of their limitations include batch-to-batch variations, risk of pathogen transfer and immunological rejection, and less cell signaling properties compared to recombinant human protein.

Table 1
Elastin-based biomaterials and their physical properties.

Sample	Treatment	Shape	Young's modulus (kPa)	Swelling ratio (g PBS/g protein)	Ref.
Native elastin	Naturally crosslinked tropoelastin	Fiber structure	300–600	0.46	[106]
rhTE	BS3	Gel	220–280	6.8	[79]
rhTE	GA	Gel	32.7	6.1	[72]
rhTE	GA/high pressure	Gel	46.7	7.3	[72]
rhTE	Electrospun/GA vapor	Scaffold	265	–	[81]
rhTE	*Pichia pastoris* lysyl oxidase	Gel	8–12	5.4	[75]
rhTE	Electrospun/HMDI	Scaffold	111	–	[107]
rhTE	Electrospun/DSS	Scaffold	150–910	–	[108]
α-elastin	EGDE	Gel	40–120	8.4–24	[5]
α-elastin/rhTE	GA/high pressure	Gel	13.9–46.7	4.6–6.8	[72]
α-elastin	HMDI/high pressure	Gel	4–8.6	18.6	[4]
ELP	TSAT	Gel	1.5–16	0.2–0.6	[91]
ELP	Genipin	Film	400	2.31	[109]
ELP	BS3	Film	70–190	–	[110]
ELP	THPP	Injectable gel	5.8–45.8	4.2	[89]
ELP	GA	Film	99–321	–	[94]

BS3: bis(sulfosuccinimidyl) suberate; GA: glutaraldehyde; HMDI: hexamethylene diisocyanate; DSS: disuccinimidyl suberate; EGDE: ethylene glycol diglycidyl ether; HMDI: hexamethylene diisocyanate; TSAT: tris-succinimidyl aminotriacetate; THPP: β-[tris(hydroxymethyl)phosphino]propionic acid.

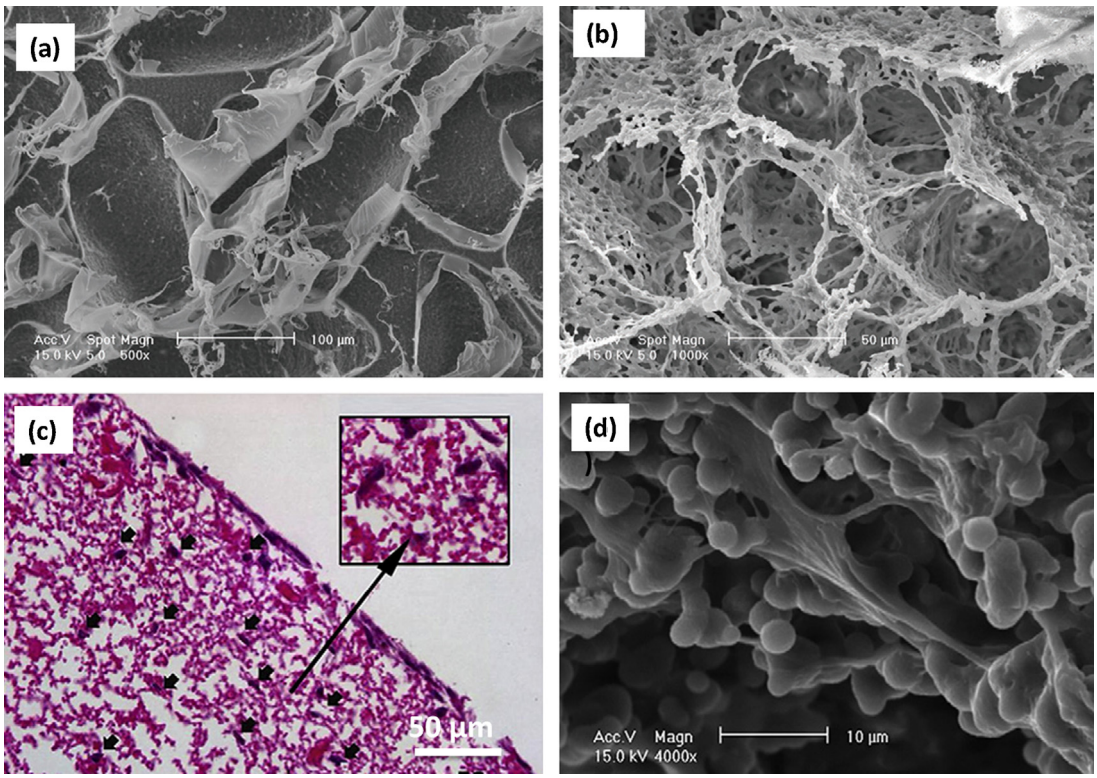

Fig. 3. rhTE/α-elastin composite hydrogels fabricated using (a) atmospheric pressure, (b) dense gas CO_2, (c and d) skin fibroblast cell penetration and growth within porous 3D hydrogels [72]. Adapted with permission from Biomaterials.

6.2. Elastic Biomaterials derived from recombinant human tropoelastin (rhTE)

Various types of elastic biomaterials with remarkable mechanical and cell interactive properties have been engineered from rhTE. These elastic biomaterials have been formed through chemical crosslinking of rhTE [72,74], enzymatic crosslinking by a yeast lysyl oxidase (PPLO) [75] and a fungal copper amine oxidase [76], and physical crosslinking by increasing the pH of rhTE solution [9]. Using these three approaches, highly elastic materials with excellent cell adhesion properties were fabricated in forms of 3D hydrogels, porous membrane, and electrospun synthetic elastic constructs. In addition, rhTE has been used as a coating agent to improve cell attachment and proliferation on the surfaces of medical implants [77,78].

rhTE has been chemically crosslinked with bis(sulfosuccinimidyl) suberate (BS3) [79] and glutaraldehyde (GA) [72] to form hydrogels with different structures including elastic sponges, sheets, and tubes. The fabricated hydrogels displayed stimuli-responsive characteristics toward temperature where they absorbed 63 and 33 g H_2O/g protein when swelled at 4 °C and 37 °C, respectively [79]. The elastic moduli of the GA crosslinked rhTE gels was around 47 kPa, which was 4.3-fold higher than pure α-elastin hydrogels (11 kPa) [72]. In addition, rhTE hydrogels exhibited remarkable biological properties both *in vitro* and *in vivo*; these materials supported *in vitro* penetration, attachment, and growth of dermal fibroblasts within the 3D structure of the hydrogels [72]. It was shown that the incorporation of GAGs [80] and the use of high pressure CO_2 [72] increased hydrogel porosity and consequently cellular infiltration within the 3D constructs. *In vivo* studies on BS3 crosslinked rhTE hydrogels also indicated that the fabricated gels were biocompatible and well-tolerated in subcutaneous implantation studies in guinea pigs for up to 13 weeks [79]. Enzymatically crosslinked rhTE was also fabricated by using LO purified from the yeast strain *Pichia pastoris* to mimic the process of elastin fiber formation *in vivo* [75]. The crosslinking efficiency in this process was lower than that of *in vivo* enzymatic crosslinking by mammalian LO. As a result, the LO crosslinked hydrogel exhibited a high swelling ratio and elastic modulus of ~10 kPa, which is 30–60 fold lower than that of natural elastin [75].

Highly elastic rhTE hydrogels were also fabricated by increasing the pH of rhTE solution to facilitate the coacervation and self-assembly of rhTE spherules through a sol–gel transition in the absence of chemical or enzymatic crosslinking [9]. The resulting hydrogels were highly porous, could be molded in a variety of shapes *in vitro*, and used as injectable gels *in vivo*. These physically crosslinked gels supported *in vitro* attachment and proliferation of dermal fibroblast and persisted for at least 2 weeks following intradermal injection into Sprague–Dawley rats [9].

Electrospun rhTE-based scaffolds with different biological and physical properties have been fabricated and show great potential for skin [81,82] and vascular (Fig. 4) [83] tissue engineering applications. The electrospun biomaterials were generated in forms of highly elastic films, containing random or aligned fibers, and hollow tubular constructs. In addition, the scaffold architecture (*e.g.* fiber diameter, pore size, and porosity) and physical properties could be modulated by changing electrospinning parameters and the polymer composition [65,81,82]. *In vitro* studies demonstrated that electrospun rhTE scaffolds supported attachment and proliferation of various cell types including ECs [82,83], dermal fibroblast cells [81], and embryonic palatal mesenchymal cells [84].

rhTE-derived scaffolds possess unique mechanical and cell-interactive properties [8] and have shown potential advantages over elastomers made from animal-derived soluble elastin and ELP. For example, unlike animal-derived soluble elastin, rhTE exhibits no batch-to-batch variations as it is produced from bacteria using a well-controlled and highly reproducible process and carries

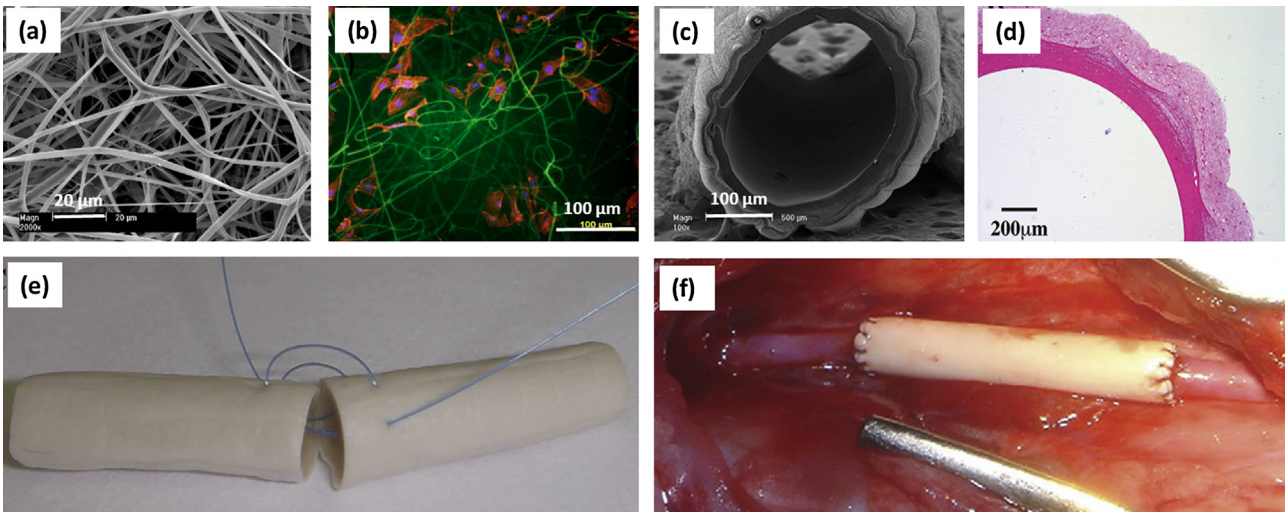

Fig. 4. Electrspun rhTE-based tissue engineered constructs. (a) SEM image of electrospun rhTE fibers, (b) fluorescence image of rhodamine phalloidin/DAPI stained ECs on rhTE fibers, (c) SEM image of an electrospun rhTE/polycaprolactone (PCL) graft, (d) histology of the graft stained with hematoxylin and eosin, (e) rhTE-based graft with multiple 6-0 prolene sutures, and (f) image from the graft *in situ* [82,83]. Adapted with permission from Elsevier.

little risk of immunological rejection upon implantation as evidenced by the aforementioned animal studies. In addition, rhTE-based gels have superior cell-interactive properties compared to other elastin-based materials due to the presence of integrin-based cell-binding sites on rhTE molecules [30]. Animal-derived elastin and ELPs do not replicate the full functionality of rhTE as they lack the full protein sequence (particularly the cell-binding C-terminus) amongst other sequences.

6.3. Elastomers derived from elastin-like polypeptides (ELPs)

ELPs are promising polymers for the formation of elastic, tissue engineered scaffolds. This class of polymers has shown remarkable properties for tissue engineering applications including similarity to native ECM, controllable degradation rates and material properties (*e.g.* chain length, architecture, and number of crosslinking sites), and the potential for incorporation of bioactive peptide moieties within polymer chains during synthesis. Due to these unique properties, ELPs have been widely used to fabricate fibers, hydrogels, and films for the regeneration of various tissues such as cartilage, liver, vascular, ocular, and soft tissues [7,85].

Setton and Chilkoti produced an injectable ELP-based biomaterial for cartilage tissue engineering [86] where the temperature-triggered coacervation of the ELP was used to encapsulate chondrocytes within the 3D structure of the hydrogels. These cell-laden scaffolds support the viability of chondrocytes and the deposition of cartilage specific-ECM including type II collagen and glycosaminoglycans [86]. In another study, they demonstrated that these injectable materials induced *in vitro* chondrogenic differentiation of human adipose-derived adult stem cells in the absence of chondrocyte growth factors [87]. Although coacervated ELP-based gels provided suitable environments for chondrocyte growth and cartilage formation, the uncrosslinked ELP hydrogels had low structural stability and stiffness, which limited their applications for regeneration of load bearing tissues. ELPs could be enzymatically crosslinked by transglutaminase to form elastic gels with improved mechanical properties but the clinical applications of these gels was hampered due to the length of time required for the crosslinking reaction to take place [88]. To solve this problem, Setton and Chilkoti synthesized an ELP containing lysine, ELP[V_6K_1-224], which could chemically crosslink in less than 5 min by using β-[tris(hydroxymethyl)phosphino]propionic acid (THPP) as a crosslinking agent under physiological conditions [89–92]. The fabricated hydrogel was injected into an osteochondral defect in a goat model. Even though, the injectable ELP hydrogel supported cell infiltration and ECM production, rapid *in vivo* degradation of these materials was an issue [92]. Lysine containing ELPs have also been chemically crosslinked using different types of crosslinkers such as BS3 [93] and tris-succinimidyl aminotriacetate [91] to create stable hydrogels for various tissue engineering applications.

ECM protein sequences (*e.g.* CS5 fibronectin and RGD) have been incorporated into ELP peptide sequences to enhance the cell interactive properties of ELP hydrogels [94,95]. For example, Urry et al. modified an ELP through the incorporation of RGDS peptides to improve the attachment of bovine aortic ECs to ELP gels [95]. Similarly, Welsh and Tirrell developed a chemically crosslinked ELP containing CS5 fibronectin to promote vascular network formation [94].

ELP-based hydrogels are a unique class of elastic biomaterials with controlled biological properties for various tissue engineering applications. In addition, their low toxicity, tunable degradation and mechanical properties, and their potential *in vivo* biocompatibility make them attractive candidates for *in vivo* applications.

Elastin-based materials are promising scaffolds for various tissue engineering applications specifically engineering elastic tissues where elasticity plays an important functional role such as cardiovascular tissues, skin, lung, blood vessel, and ligament. However, prior to clinical applications, certain aspects of these materials should be investigated. For example, comparative *in vivo* studies of various elastin-based biomaterials using different animal models will provide useful information about the biocompatibility of elastin-based biomaterials for tissue engineering applications. In addition, engineered technologies to tailor the architectures and properties of elastin-based biomaterials will advance their potential applications in the field of tissue engineering. It has been demonstrated that the physical properties of elastin biomaterials can be tailored by polymer compositions and crosslinking density; however, more systematic approaches for controlling the scaffold properties are required to be developed. In addition, the use of microfabrication technologies [96] to generate elastin-based biomaterials with controlled architectures and geometries is an emerging topic in elastin biomaterials field. These technologies have been used to fabricate various microfabricated cell-laden hydrogels from gelatin [97–99], hyaluronic acid [100],

pullulan [101], and poly(ethylene glycol) (PEG)/gelatin [102], and gelatin/silk [103]. Recently, we combined a photocrosslinkable cell-laden hydrogel, methacrylated tropoelastin (MeTro) [104] and microfabrication technologies to generate micropatterned elastic films for cardiomyocyte alignment and proper function [105]. Next step could be to generate vascularized elastin-based constructs by using microscale techniques to engineer microchannels in the 3D structure of materials.

7. Conclusion

Elastin is a unique structural protein that confers both physically and biologically active properties. Elastin-based biomaterials exhibit remarkable biophysical, biomechanical, and biological properties for tissue engineering applications. These bioelastomers are formed from various elastin derivatives (animal-derived soluble elastin, rhTE, and ELP) and support the *in vitro* adhesion and proliferation of different cell types. The physical and biological properties of the resulting materials can be modulated by changing the polymer synthesis parameters. Although some *in vivo* experiments have been performed to confirm the biocompatibility of elastin-based biomaterials, more specific and systematic *in vivo* analysis should be conducted in comparative studies of various elastin-based materials to select the most medically useful tissue constructs.

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