Recent Advances in Elastin-Based Biomaterials

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ABSTRACT - Elastin is one of the main components of the extracellular matrix; it provides resistance and elasticity to a variety of tissues and organs of the human body, besides participating in cellular signaling. In addition, elastin-derived peptides are synthetic biopolymers with a similar conformation and structure to elastin, but these possess the advantage of solubility in aqueous mediums. Due to their biological activities and physicochemical properties, elastin and related peptides may be applied as biomaterials to develop diverse biomedical devices, including scaffolds, hydrogels, and drug delivery systems for tissue engineering. Likewise, the combination of elastin with natural or synthetic polymers has demonstrated to improve the mechanical properties of biomedical products and drug delivery systems. Here we comprehensively describe the physicochemical properties and physiological functions of elastin. Moreover, we offer an overview of the use of elastin and its derivative polymers as biomaterials to develop scaffolds and hydrogels for tissue engineering. Finally, we discuss some perspectives on the employment of these biopolymers to fabricate new biomedical products.

INTRODUCTION

The use of biomaterials for biomedical applications has progressively increased in the last years, and many biopolymers are growingly employed in the fabrication of hydrogels, scaffolds, and drug delivery systems (1–4). In this regard, the physicochemical and biological properties of some proteins and peptides render them a fascinating option in the development of biopharmaceutical products (5).

Particularly, elastin protein and its derivatives result of singular interest due to their biological activities. Elastin is a significant constituent of the extracellular matrix (ECM); it confers stretch, flexibility, and strength to organs and tissues such as skin, blood vessels, lung, bladder, ligaments, and cartilage (6). Moreover, different studies have shown that elastin participates in cell signaling, regulating processes such as wound healing and vascular morphogenesis (7–9).

Furthermore, elastin-like polypeptides are artificial polymers that mimic the structure and conformation of elastin; however, these are soluble in an aqueous medium (10). Its general structure is composed of a repetitive sequence Val–Pro–Gly–Xaa–Gly, where Xaa may be any amino acid, except proline. Due to their structure and properties, it is expected that elastin and its derivatives exhibit low toxicity and immunogenicity. Thus, elastin and elastin-like peptides possess a strong potential for biomedical purposes, including drug delivery (11–13), wound healing (14–16), and tissue engineering (17–20).

In this review article, we perform a detailed description of the physicochemical properties and physiological functions of elastin. Furthermore, we provide a current outlook on the use of elastin and

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elastin-derivated polymers as biomaterials to develop scaffolds and hydrogels for tissue engineering. Lastly, we mention some perspectives on the utilization of these biomaterials for new biomedical approaches.

Morphogenesis of elastin and elasticity

The elastic fibers are found in the skin, lung, arteries, ligaments, vocal cords, and elastic cartilage. These fibers are structures that provide resilience, ability to length, and snap-back; these allow the tissue to stretch and recoil without loss of structure. They are constituted of two morphologically and chemically distinct components: elastin and microfibrils. Elastin is an insoluble ECM protein that forms the internal core of the elastic fibers; its protein precursor is the tropoelastin. On the other hand, the microfibrils are composed of a complex array of macromolecules, including the structural glycoproteins fibrillin-1, fibrillin-2, and microfibril-associated glycoprotein-1 (MAGP-1). The microfibrils serve as a scaffold for deposition, alignment, and assembly of tropoelastin monomers (21,22). Structurally, elastic fibers are a biomaterial comprising mainly of two distinct parts, an inner core of amorphous cross-linked elastin (~ 90%) and an outer microfibrillar mantle (microfibrils). However, elastic-fiber-associated molecules are also present and play an essential role in the organization of these fibers.

The elastin fibers synthesis involves different and sophisticated processes (Figure 1), which begin with the intracellular transcription and translation of the tropoelastin. It is an approximately 60–70 kDa protein, whose length depends on alternative splicing. Tropoelastin exists as a monomer in solution in two forms: an open globular molecule and a distended. It is secreted from diverse elastogenic cell types such as fibroblasts, endothelial cells, and smooth muscle cells (23–25). Following extensive splicing in the transcript, the mature tropoelastin mRNA is exported from the cell nucleus. The translation is carried out on the surface of the rough endoplasmic reticulum (rER); meanwhile, a signal polypeptide is formed and cleaved at the moment that the protein enters the rER lumen. Then, the protein is transported to the Golgi, traveling through the lumen. In the intracellular space, tropoelastin is likely to be chaperoned by an elastin binding protein (EBP) to prevent self-aggregation, as well as premature degradation (26). Likewise, the peptidyl-prolyl cis/trans isomerase, FKBP65, is also associated with tropoelastin during the secretory pathway (27). Subsequently, the complex composed by tropoelastin and EBP travel through the Golgi, and it is secreted to the extracellular space. Once secreted, the incorporation of tropoelastin into the elastic fiber begins. The EBP delivers the tropoelastin in the fiber formation site and returns to the endosomal compartments to be reassociated with lately synthesized tropoelastin. The delivered tropoelastin is deposited in the microfibrils, covalently interacting through fibrillin-1, this process is facilitated by calcium-dependent binding of MAGP-1 to multiple sites within tropoelastin. MAGP-1 binds to the C-terminal region of tropoelastin and stabilizes it before the enzymatic crosslinking. Thus, the C-terminal of tropoelastin is critical, playing a pivotal role in the deposition of the monomer into growing elastin polymer.

Tropoelastin crosslinking requires that the molecules be associated and aligned to facilitate the generation of crosslinks between closely spaced lysines. The molecular mechanism through which tropoelastin concentration and ordering occur is known as coacervation. Coacervation plays a crucial role in elastogenesis because the inhibition of this mechanism leads to a decrease in elastin formation (28). Coacervation is an endothermic and entropically-driven process that involves the interactions between the hydrophobic domains, triggering destabilization of the clathrate-like water shielding these regions. Tropoelastin is soluble in solutions at low temperatures, and water forms a clathrate-like structure around its hydrophobic regions, keeping the protein unfolded. At increasing temperature, tropoelastin molecules begin to aggregate and order by interactions between hydrophobic domains; also, the clathrate water is disrupted, allowing the interaction of hydrophobic regions (28,29). These regions are rich in non-polar amino acids, including valine, glycine, proline, and alanine, which are often arranged in repeats of three to six amino acids peptides, such as GVGVP, GGVP, and GVGVAP. The needed conditions for an optimal coacervation process of human tropoelastin are 37 °C, 150 mM NaCl, and pH 7-8; thus, the physiological environment of the ECM is adequate for this process.

After deposition and alignment, tropoelastin molecules are crosslinked into elastin via enzymatic reactions. This crosslinking is carried out by the action of the lysyl oxidase, a copper-dependent amine oxidase. This enzyme catalyzes the oxidative deamination of ε-amino groups on lysine residues within tropoelastin to form the α-aminoadipic-δ-semialdehyde, allysine. In this respect, allysine is the reactive precursor to a variety of inter- and

intramolecular crosslinks found in elastin. The spontaneous condensation of lysin and allysine results in the formation of elastin-specific crosslinks named *desmosines* and *isodesmosines,* which play an essential role in elastic fibers. Furthermore, these crosslinks are stable in structure, conferring a high tough to the elastic fibers.

The mechanical study at the micro- and macro-mechanical levels of elastic fibers confirms its remarkable elastic and resilient properties. Concerning this, Aaron et al. (30) determined Young's modulus of single elastin fibers isolated from bovine ligamentum nuchae, values were found in the range of 0.4 – 1.2 MPa. Similarly, Koenders et al. (31) determined Young's modulus of elastic fibers in the presence and absence of fibrillin–microfibrils, the measurements were 0.90 ± 0.23 MPa and 0.79 ± 0.17 MPa, respectively. At the macro mechanical level, studies in elastic fiber-rich tissue from the dog, sheep, and pig aorta determined Young's modulus to be in the range of 0.1–0.8 MPa (31,32). However, the more outstanding mechanical property of elastin is the linear elastic extension (103 – 150%), the largest of any known biological material (32). Several hypotheses have been proposed to explain the elastic properties of elastin, being hydration, and consequently, changes in entropy the basis for understanding its elastic behavior.

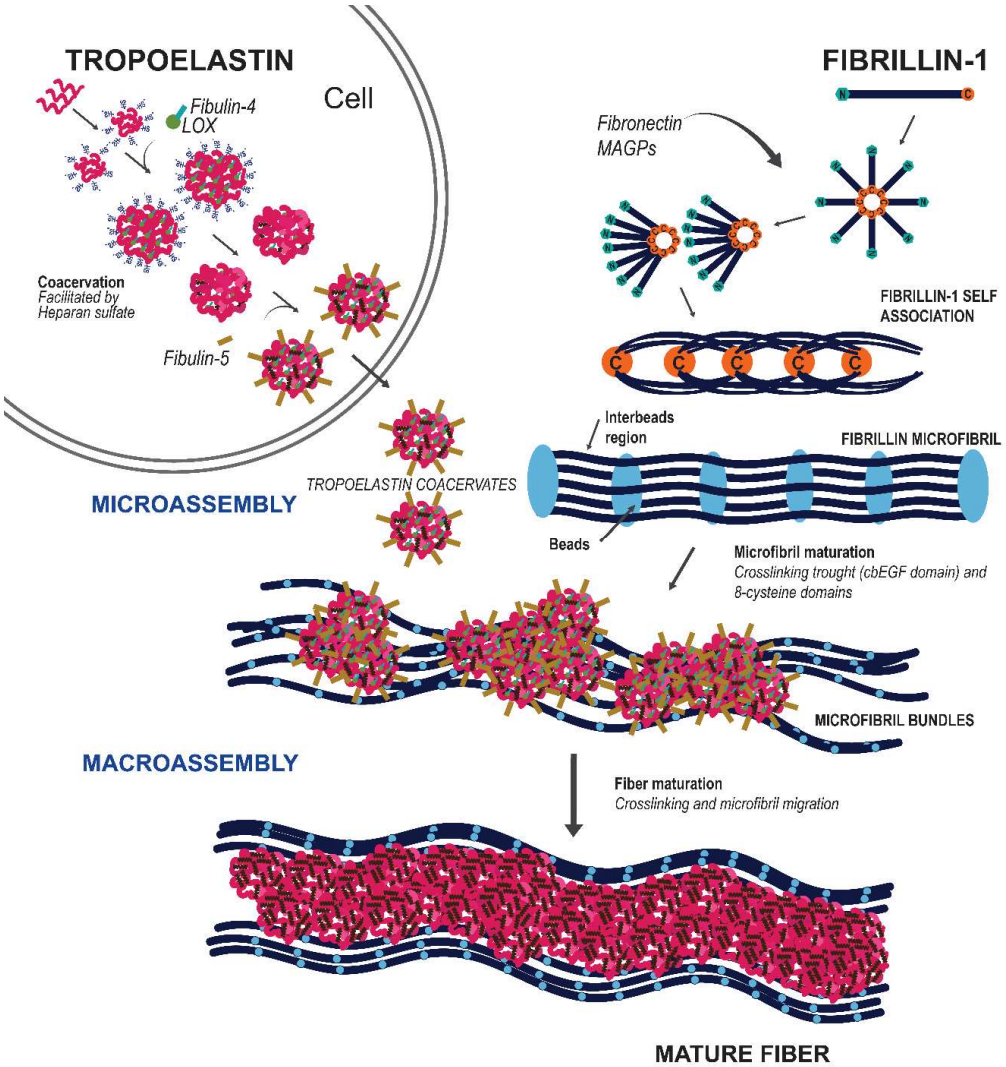

Figure 1. Diagram of the stages of elastic fiber formation. First, the micro-assembly of tropoelastin in coacervates and then, in aggregates released on the cell surface, whereas LOX associated with Fibulin-4 produces the initial crosslinking of tropoelastin. At the same time, Fibulin-5 helps in transport and fixation of the aggregates to microfibrils rich in fibrillin. Elsewhere, fibrillin-1 assembles into multimers through C-terminal-N-terminal interactions and then into cross-linked supramolecular fibrils via cbEGF and 8-cysteine domains. The tropoelastin accumulates in the microfibrils providing a direct deposition of elastin. Finally, the macro-assembly is completed with the crosslinking of the tropoelastin mediated by LOX, to give rise to mature elastic fibers.

The hydration of the polypeptide backbone of elastin is essential to keep the structural flexibility and grade of order (or disorder), considering the high percentage of non-polar amino acids in its structure. Li et al. (33) suggested that hydration in the hydrophobic domains is an essential source of the entropy-based elasticity of elastin. The changes in hydrophobic hydration of elastin tend to reorder itself to form a hydrophobic globule when it is held in its extended state, indicating that the hydrophobic effect also contributes to the holding process. On the other hand, restriction in the number of conformations available for the elastin due to the high elastin chain disorder opposes both stretching and tight packing (32).

THE PHYSIOLOGICAL FUNCTION OF ELASTIN

ECM molecules are relevant regulators of cellular function, and it is known that many types of diseases are strongly correlated with a disruption in ECM homeostasis (34). As previously mentioned, elastin is a vital constituent of the ECM that presents a specific chemical nature and strong crosslinking, making it a very stable molecule.

In physiological conditions such as growth, wound healing, and tissue remodeling during aging, several ECM components are degraded and have biological activity, for example, as chemoattractants or pro-inflammatory chemokines involved in signaling pathways. For this reason, they are termed matrikines (35). It has been demonstrated that matrikines can modulate essential cell functions such as cell proliferation, migration, invasion, protease production, and angiogenesis (36), either positively or negatively. In some cases, alterations in those functions suggest a relevant role of matrikines in the development of tumor invasion and metastasis (37). In particular, matrikines originated by a chemical or enzymatical degradation process of elastin, are known as elastin derived peptides (EDP) or elastokines (38). During physiological aging, there is an increment in the activity of enzymes that catalyze the process of elastin degradation; such enzymes are named elastases (39). Also, during several pathological and physiopathological conditions, elastases and other enzymes with elastase activity like several metalloproteinases (MMPs) (MMP-2, MMP-7, MMP-9, and MMP-12) (40), are up-regulated, causing an increase in the number of released elastin peptides.

Since elastin cleavage is considered a pathological process, most studies have focused on investigating the adverse effects of EDP release (Figure 2). Nevertheless, it has also been shown that EDP also has an essential biological role in the human body (41), mainly in organs or tissues with high elastin content such as skin, arteries, and lungs, as well as in its interaction with the immune and nervous system (42–45).

Biological activity of EDP is mediated by several cell surface receptors such as galectin-3, $\alpha_V\beta_3$, $\alpha_V\beta_5$ integrins, and its main receptor, the elastin receptor complex (ERC). The ERC is a heterotrimeric receptor (26) composed of a peripheral EBP subunit associated with the protective protein/cathepsin A (PPCA) and neuraminidase-1 (Neu-1), a membrane-associated protein. The EBP is the part of the complex that interacts with EDP and has a binding site for EDP and other for a galactosugar.

Once bound to EBP, EDP elicits the transduction of signals from the extracellular space to the cytoplasmic zone. Regarding this point, Duca et al. (46) demonstrated that EDP-EDP interaction stimulates the Neu-1 sialidase activity. Additionally, it was suggested that sialic acid residues cleaved from glycosugars and glycolipids act as second messengers, leading to the regulation of different signal transduction pathways in several cell types interacting with EDP.

Galactosugars (mainly lactose or chondroitin sulfate) are antagonists of EDP as they promote the release of EBP from the complex, inhibiting the biological effects of EDP-EBP interaction (47). Besides, Robinet et al. (48) reported that it is possible to block EDP binding onto EBP using the V14 peptide (VVGSPSAQDEASPL), which can trap elastin derived peptides, preventing its adverse effects. Likewise, deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA) is another antagonist molecule that inhibits the sialidase activity of Neu-1, necessary for signal transduction (46). Interestingly, Neu-1 activation involves signaling events such as the activation of phosphoinositide-3-kinase γ (PI3Kγ) pathway related to atherosclerosis. In this inflammatory disease, foam cells (lipid-laden macrophages) contribute to plaque formation within the artery wall (49). CD36 receptor is also modulated by Neu-1, which activates an intracellular signaling cascade that increases the uptake of oxLDL by macrophages, thus, contributing to atherosclerotic plaque formation (50).

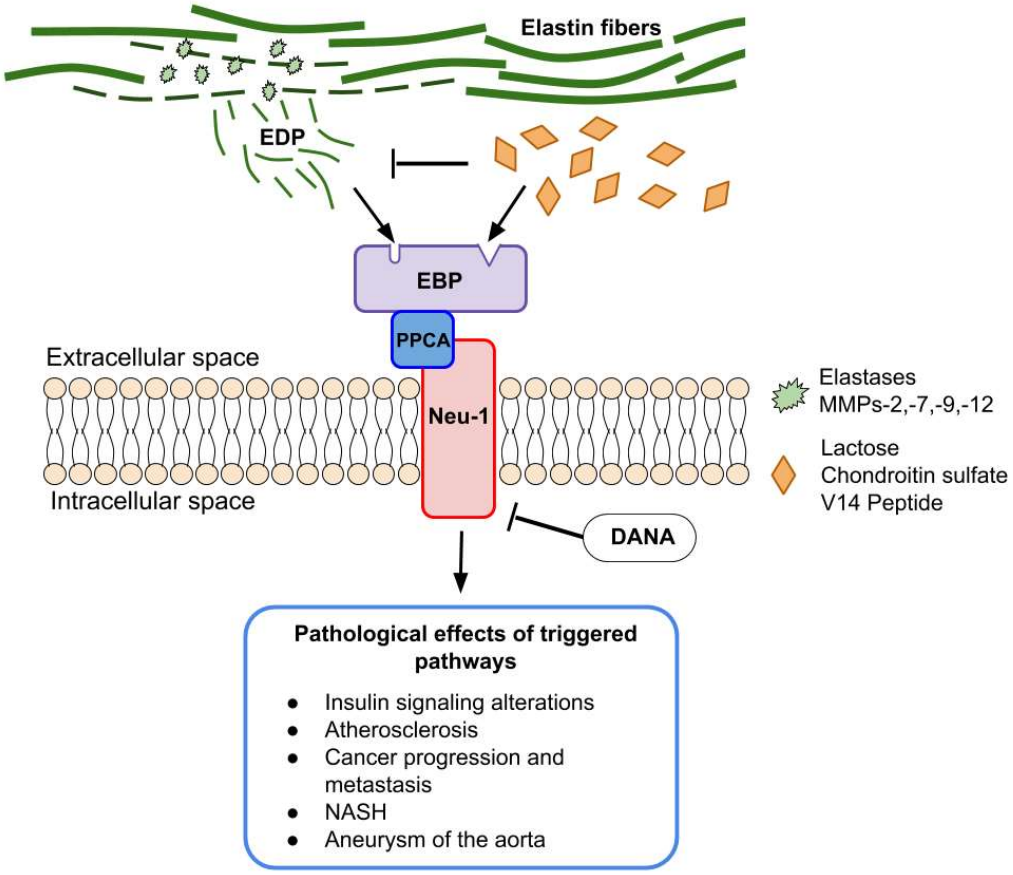

Figure 2. Pathological role of elastin derived peptides (EDP). Elastin is cleaved by elastases and matrix metalloproteinases (MMPs) during either physiological or pathophysiological aging, producing EDP. Binding of these peptides to the elastin receptor complex (ERC) triggers several signaling pathways modulated by Neuraminidase-1 (Neu-1) sialidase activity. Various of the activated signaling events have an important role in the development of pathological conditions. Lactose, chondroitin sulfate, V14 peptide, and deoxy-2,3-dehydro-N-acetylneuraminic acid (DANA) inhibit the effects of the interaction between EDP and ERC. EBP, elastin binding protein; PPCA, protective protein/cathepsin A; NASH, non-alcoholic steatohepatitis.

EDPs have been shown as regulators of insulin resistance development (51), chemotactic attractors to cancer cells (52), and contributors to lipid accumulation in hepatocytes in patients with non-alcoholic steatohepatitis (NASH) (53). It has been demonstrated that, besides physiological aging, several genetic alterations also lead to connective tissue disorders related to elastin degradation. Marfan syndrome is an example of a heritable disorder linked to mutations in fibrillin genes (fibrillin-1 is the main component of the microfibrils in which tropoelastin is deposited and crosslinked to form elastic fibers). Some patients also presented an up-regulation of MMP-1, MMP-2, MMP-3, and MMP-9, implicated in the cleavage of fibrillin-1 (54). This disease may be life-threatening because of its manifestations in the cardiovascular system. One of the most common is aneurysm formation, having aortic wall rupture as a consequence (55). Finally, due to all physiological and pathophysiological implications of EDP, these peptides are suggested as biomarkers of the progression of diseases that, in certain stages, could be lethal. Therefore, the development of therapeutic alternatives in which EDP adverse effects may be, at least, partially reduced, is of great interest.

ELASTIN-BASED BIOMATERIALS

The growing interest in elastin as a biomaterial is due to its properties of elasticity, self-assembly, long-term stability, and biological activity. Notably, the attributes of elasticity and biological activity of elastin in combination with natural or synthetic polymers are utilized to model and improve the mechanical properties of biomedical products and drug delivery systems.

Elastin-based cell scaffolds

The exceptional properties of elastin make it an attractive candidate for a range of tissue-engineering applications. Many autografts, allografts, and xenografts contain elastic fibers. Well-known examples are split-skin autografts for burn wounds (56), autologous blood vessels allografts for coronary graft reconstruction (57), and aortic heart valve xenografts (58). Biofabrication methodologies provide access to numerous variants from elastin-based cell-scaffolds. Therefore, elastin-mimetic protein polymers represent suitable candidates for use as scaffolding materials in tissue engineering, in which critical properties such as mechanical response, cell–scaffold interactions, and biodegradability may be controlled through modification of the biomaterial properties.

Decellularized cell scaffolds containing elastin

Decellularization is defined as a process to obtain natural matrices frequently used as scaffolds to replace tissues and organs. This process implies the removal of the allogeneic or xenogeneic antigens from cells in the source tissue or organ that lead to an immune response in the host body (59,60). Thus, decellularization produces a natural three-dimensional scaffold containing the main elements of the ECM, which can be repopulated with host cells (61). One of the main advantages of decellularized matrices is the preservation of native architecture of the source tissue or organ in contrast to purified-component processes, e.g., we have obtained a human-decellularized artery with well-preserved elastin (Figure 3). However, some approaches had used decellularization to get a bio-ink for the 3D manufacturing process, disrupting the native architecture, and taking advantage of the biochemical composition and biological cues of the ECM. Nevertheless, it is challenging to retain all-natural components of the ECM (62).

On the other hand, this process has some unsolved challenges, such as the hazard of incomplete decellularization or the uncontrollable variability in the attributes of the final matrix (59). Thus, decellularization is performed with a wide variety of intricated protocols, using diverse decellularization agents (physical, chemical, and biological) such as detergents (e.g., Sodium deoxycholate, SDS) and enzymes (e.g., trypsin) (63). However, decellularization by SDS or trypsin could change the ECM composition (63). Finally, it is difficult to compare results obtained from different approaches since each protocol will result in its own set of residual ECM components. Besides, it is unknown which molecule causes a specific effect due to the complex composition of the ECM.

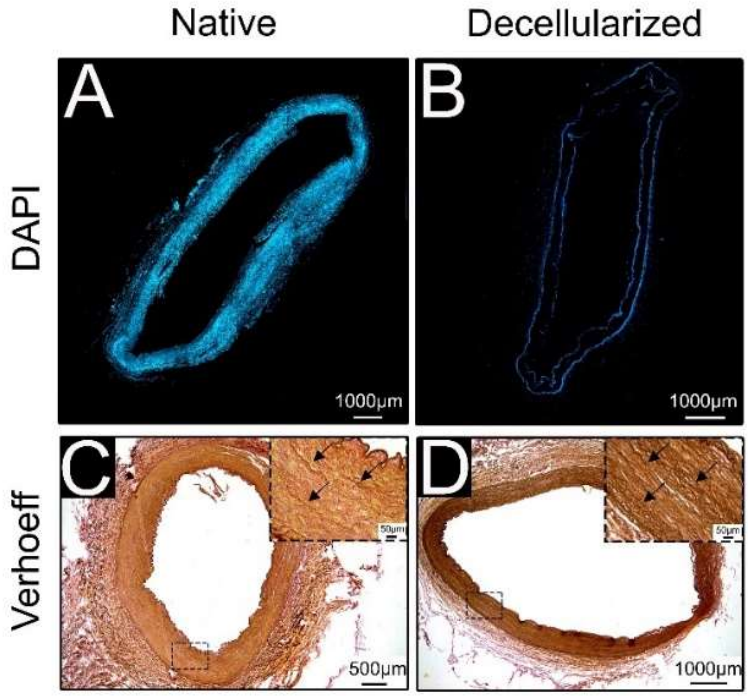

Figure 3. Elastin-based scaffold obtained by the perfusion decellularization process (human artery). The method removes the cells in the native ECM (A), leaving a scaffold without cells (B). The process retains essential fibrous protein like elastin (D). The arrows show the preservation of elastin from native tissue (C) to the decellularized matrix (D).

Purified elastin cell scaffolds

Purification of a target protein is essential when are necessary all effects of mature extracellular elastin in fibrous form. In this respect, due to the intermolecular cross-links in elastin, the protein is highly insoluble, and it can only be dissolved hydrolyzing some peptide bonds, which may lead to degradation of the final properties of the purified product. Thus, this insolubility is often used for isolation of elastin from tissues. Sources rich in insoluble elastin include bovine and equine ligaments because that protein comprises a high percentage of its dry weight. Another standard method to obtain elastin-mimetic biomaterials is a phase transition, which may be applied for purification recombinant proteins as fusions to the elastin sequence (64). This technique has the advantage over sophisticated chromatographic techniques that the purification can be achieved through the relatively benign method of inverse temperature cycling, which can provide high purity fusions (65).

Scaffolds

One of the predominant applications of elastin in tissue engineering is as a 3D scaffold to biomimetize tissues (Table 1). The combination of elastin with collagen is one of the most frequent strategies to simulate the ECM of soft tissues. The type of interaction of both proteins can start from a simple approach such as blending and casting at room temperature or freeze-drying to induce crosslinking, until the use of crosslinkers such as genipin, or via the 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) route (66). For scaffolds that require a higher mechanical response, stability, and resistance to degradation, chemical crosslinking is a suitable alternative to improve their performance (67). Although the freeze-drying technique induces pore formation during the sublimation process, the control of tortuosity can be increased and enhanced by injection of CO_2 at controlled pressure and temperature (68).

The cellular response of elastin-collagen scaffolds is usually governed by the protein that predominates in concentration. Generally, these types of structures have excellent biocompatibility, favoring cell proliferation, and acting as a high-quality ECM alternative. Because elastin exhibits biological properties, the proposals where it is used as a scaffolding coating agent to promote cell induction are also attractive. Even the combination strategy of elastin with poly-ε-caprolactone (PCL) exhibits suitable applications in tissue engineering. Considering that PCL is a polymer widely known for its medical applications, it favors the acceptance of the resulting elastin scaffolds, in addition to reducing the cost of the final product and exhibiting better mechanical properties (69). In general, scaffolds from elastin, among other aspects of practicality and accessibility, seek to increase the robustness of biomaterials in tissue engineering, in contrast to the low reproducibility of decellularized tissues.

Electrospinning

The use of electrospinning allows us to have small diameter fibers. Depending on the diameter and length of the fiber, it is possible to modulate the elastic response. The collection of fibers allows different types of orientation to confer diverse geometries that may favor cell infiltration. One electrospinning feature is pore size control, similar to Bottom-up constructs in the nanotechnology area (70). Furthermore, the set of fibers or mesh may have a subsequent surface functionalization, the interior of the fibers may contain a drug or protein, and the sum of the functional factors confers a high potential for application to this technique. The predominant compositions are elastin with collagen, or sometimes with PCL. While crosslinking after fiber formation via EDC/NHS is a common strategy to confer adequate mechanical properties (71). The elastin-collagen mesh from electrospinning can be deposited in its formation on an implant tube to favor its grafting. Other scaffolding structures consist of double or triple layers of interlocked mesh (71). Even though electrospinning is a technique that is not recent, its peak is evident in literature today. As with traditional scaffolds, there is little exploration of other polymers for medical use to confer adequate mechanical properties and improved cellular compatibility.

Hydrogels

The manufacture of hydrogels for tissue engineering has been aimed at non-specific applications in most cases, with descriptions of mechanical resistance rather than cellular interaction. Hydrogels may function as a scaffold, exhibiting a biological response; it can be applied as a reservoir for the controlled release of drugs or proteins that affect the cell lines of interest (72). Besides, they can present a response to external stimuli such as changes in viscosity as a function of temperature variation. These aspects facilitate injection into anatomical regions and subsequent gelling as a tissue engineering scaffold.

The composition that has predominated is collagen and elastin to simulate ECM. Since elastin or collagen alone does not offer an increased stiffness to hydrogels, in almost all studies, a chemical crosslinking is employed to generate covalent bonds that allow greater rigidity and structural support in cell seeding (73). The control of pore formation is crucial, as with scaffolds and nanofiber formation. In this regard, the CO_2 injection at controlled pressure and temperature is an attractive strategy.

Some limitations to consider in the use of elastin as a biomaterial is its low solubility in water, the possible nucleation for calcification in tissues, and supplier quality controls to offer a sample with specific physicochemical properties. However, the use of elastin in biomaterials is attractive due to its mechanical resistance and biological response, which has allowed the biomimetization of scaffolds for tissue engineering.

Table 1. Selected examples of elastin biomaterials for tissue engineering

Protein	Polymer	Interaction	Application	Evaluation	Reference
SCAFFOLD					
Bovine elastin	Bovine type I collagen	Blended and crosslinked with 1,4-butanediol diglycidyl ether	Extrahepatic islet transplantation	Promotion of angiogenesis and ECM deposition. Preserving the integrity, viability, and function of the islet.	(74)
Bovine elastin	Bovine type I collagen	Blended and lyophilized	Heart valve	Bi-layer scaffold with anisotropic bending moduli with adequate cell distribution.	(75)
Elastin	Type I collagen	Blended and crosslinked with 1,4-butanediol diglycidyl ether	Biomimetic collagen/elastin meshes for ventral hernia	Biomechanical effect for hernia repair, increased tissue restoration, neovascularization, and gene expression associated with de novo matrix deposition, angiogenesis, adipogenesis, and skeletal muscles.	(67)
Bovine elastin	Bovine type I collagen	Blended	Promote the cellular interaction of composites	The predominant cellular interaction is due to the protein in the larger composition.	(76)
Equine elastin	Bovine type I collagen	Casting, heparin-EDC/NHS cross-linking, freezing and freeze-drying	Vascular grafts resembling the native blood vessel	Practical use in anastomosis.	(66)
Bovine elastin	Bovine type I collagen	Blended and freeze-drying	Biomimetic scaffold for cardiovascular tissue engineering	Modulation of smooth muscle cells towards a contractile state via reduced proliferation and increase of α-SMA.	(77)
Bovine elastin	Bovine type I collagen	EDC/NHS cross-linking and freeze-drying	Scaffold for soft tissue engineering	Structural stability, strength, and degradation resistance. Highly porous structures were able to support cell infiltration and growth.	(78)
Bovine elastin	Silk fibroin	Blended, crosslinked with genipin, and freeze-drying	Wound dressing	Adequate re-epithelialization and the fastest wound closure.	(79)
Equine elastin	Bovine type I collagen, chondroitin sulfate	EDC/NHS cross-linking and freeze-drying	Scaffolds for tissue engineering	Adequate mechanical resistance and elasticity.	(80)

TABLE 1 Continued

Equine elastin	Bovine type I collagen	EDC/NHS cross-linking	Scaffolds for tissue engineering	Decrease in the calcification phenomenon associated with the presence of elastin in the scaffold.	(81)
Bovine elastin	Polyethylene oxide/chitin/chitosan matrix	Crosslinked with genipin	Cartilage tissue engineering	Induces a high amount of regenerated bovine knee chondrocytes, glycosaminoglycans, and type II collagen; all these are cartilaginous components.	(82)
Bovine elastin	Polyethylene oxide/chitin/chitosan matrix	Crosslinked with genipin	Cartilage tissue engineering	Adequate cryopreservation, synthesis of glycosaminoglycans and collagen, high proliferation of chondrocytes.	(83)
Bovine elastin, human serum albumin, and poly-L-lysine	Chitosan/γ-poly(glutamic acid) scaffold	Blended and surface crosslinked with genipin	Cartilage tissue engineering	Promotes the growth of chondrocytes, secrete ECM and improve the regenerative ability of cartilaginous tissues.	(84)
Bovine elastin	PCL	Blended	3D composite	Highly porous foam.	(68)
Bovine elastin	PCL	Crosslinked with glutaraldehyde	3D composite	High porous elastic scaffold with high swelling capacity. The 3D structure support primary articular cartilage chondrocyte adhesion and proliferation	(69)
ELECTROSPINNING					
Bovine elastin	Collagen type I from calfskin	Blended, electrospinning, and EDC/NHS cross-linking	Mesh for tissue engineering	Growth of smooth muscle cells as a confluent layer after 14 d of culture.	(70)
Bovine elastin	Bovine type I collagen, silk fibroin, and PCL	Blended, electrospinning, and EDC/NHS cross-linking	Tri-layered arterial grafts	Burst strength adequate and four-week degradation *in vitro*	(71)
Bovine elastin	Rat collagen type I, PCL	Electrospinning	Dermal substitute scaffold	Low stiffness and high elasticity. Promotion of keratinocyte and fibroblast proliferation, tissue integration, and accelerated angiogenesis.	(85)
Bovine elastin	Bovine type I collagen, PCL	Blended, electrospinning, and EDC cross-linking	Three-layered matrix to mimic the arterial architecture	Similar mechanical response as a native artery.	(86)

TABLE 1 Continued…

Elastin	Collagen hydrolysate type I and III, PCL functionalized	Electrospinning	Scaffolds for tissue engineering	Morphological stability and good water uptake.	(87)
rhT	PCL	Electrospinning	Synthetic vascular conduit	Mechanical properties optimized, permeability, compliance, elastic modulus, burst pressure.	(88)
Elastin	PLGA	Blended, electrospinning	Salivary gland tissue regeneration	High modulus of elasticity with apicobasal polarization of salivary gland epithelial cells.	(89)
Bovine elastin	Polydioxanone	Electrospun blend	Bioresorbable scaffolds	Minimal effects in innate and acquired immune response assays.	(90)
Bovine elastin	Polydioxanone	Cross-linking of electrospun elastin on tubes of polydioxanone blended with soluble elastin	Vascular tissue engineering	High systolic pressure, similar to the native artery. Ideal for replacement applications.	(91)
rHT	Bis-sulfosuccinimidyl and glutaraldehyde as cross-linkers	Electrospinning on hydrogel	Dermal substitute scaffold	Fibroblasts attached and proliferated for at least 14 days and deposited fibronectin and collagen type I.	(92)
rHT	-	Electrospinning and cross-linking with glutaraldehyde	Dermal tissue engineering	Suitable elastic module with high porosity to promote cell persistence. Induction de novo collagen synthesis and angiogenesis.	(93)
HYDROGEL					
Porcine elastin	Rat collagen	Crosslinked hydrogel with squaric acid	Hydrogel for tissue engineering	Stiffer hydrogel and more resistant to enzymatic degradation.	(73)
Bovine elastin	Rat type-I collagen	Blended	Lung tissue engineering	Stiffness equal to the theoretical value for a single alveolar wall. Lung fibroblast adhered to the hydrogel.	(94)

TABLE 1. Continued….

Bovine elastin	Hyaluronic acid functionalized with ethylenediamine and ethylene glycol diglycidyl ether	Crosslinked hydrogel	Dermal and vascular tissue engineering	Adequate swelling capacity, improved attachment, viability, and proliferation of dermal fibroblasts.	(95)
Bovine elastin	rHT	Crosslinked hydrogel with glutaraldehyde	Hybrid hydrogels	Adequate mechanical properties, high porosity that allowed the migration of human skin fibroblast cells.	(96)
Bovine elastin	Hyaluronic acid, HA-EDA-g-α-elastin	Crosslinked hydrogel with EDC/NHS	3D culture of vascular endothelial cells	Control release of vascular endothelial growth factor, proliferation of human vascular endothelial cells and formation of tubular structures.	(72)
Bovine elastin	Alginate	Crosslinked hydrogel and films with calcium chloride	Hydrogel and films for tissue engineering	Biocompatible material	(97)
Bovine elastin	PNPHO	Crosslinked hydrogel with succinimide	Thermoresponsive hydrogel	High compression moduli, stable in physiological conditions, bioabsorbable, cell encapsulation for at least 5 days.	(98)
Bovine elastin	-	Crosslinked hydrogel with hexamethylene diisocyanate	Hydrogel for tissue engineering	High porosity that induced the cellular penetration and growth throughout the matrices	(99)
Bovine elastin	-	Crosslinked hydrogel with glutaraldehyde	Hydrogel for tissue engineering	High porosity, tortuosity facilitated fibroblast penetration and proliferation	(100)

rHT: recombinant human tropoelastin, PCL: poly-ε-caprolactone, ECM: extracellular matrix, PLGA: poly(D-lactide-co-glycolide), EDC: N-(3-dimethylaminopropyl)-N'-ethylcarboiimide hydrochloride, NHS: N-hydroxysuccinimide, HA-EDA-g-α-elastin: α-elastin grafted to hyaluronic-(2-aminoethyl)-carbamate acid

ELASTIN-LIKE PROTEIN-BASED BIOMATERIALS

Polymers science has revealed that macromolecules can be excellent candidates to create highly functional biomaterials. Many macromolecules have specific applied functionality, such as the ability to self-assemble, definite recognition, and monodispersity. These features are found enhanced in protein-based biomaterials, and they are based on a complex and strictly defined primary structure (101,102).

In nature, living cells conduct the protein biosynthesis with complete control of the amino acid sequence, from the first amino acid to the last without randomness (102). Therefore, a basic understanding of amino acid-based peptides is the basis for the current development of peptide-based biomaterials. Many features of elastin-like molecules can be controlled through protein engineering, including amino acid sequence, peptide length, or even block polymer when it is desirable (103). It is for the above that peptides

and high molecular weight polypeptides are emerging as a new class of biomaterials because of their unique chemical, physical, and biological properties. Concerning this, elastin has been used in the biomaterials field in different presentations, including decellularized matrices from various sources (autografts, allografts, and xenografts) and purified preparations. Moreover, insoluble elastin may also be hydrolyzed to obtain soluble elastin preparations, whereas repeated elastin-like sequences can be produced by synthetic or recombinant methods (103).

The development of recombinant DNA technologies to obtain recombinant protein-based polymers has brought the explosion of new peptide-based materials; these polymers are known as recombinamers (104). These materials have functionalities that can be designed in a precise method with selected and advanced properties, such as the integration of selected exogenous bioactivities to develop polymers for cutting-edge in tissue engineering approaches (105).

The basic structure of elastin-like recombinamers (ELRs) involves a repeating sequence based on the recurring steps found in the mammalian elastin (106), drawn on a heterologous host (mainly *Escherichia coli*) (107,108). The biosynthesis of any artificial protein generally includes: (1) the building of a synthetic gene that encodes the protein of interest in a plasmid; (2) the cloning of a recombinant gene with the necessary transcriptional regulatory elements into competent cells; (3) the screening of plasmids containing the desired clones and verification of their DNA sequence; (4) transformation of the chosen plasmids into expression competent host microorganism; (5) the growth of appropriate volumes of host microorganism and induction of protein expression; and (6) purification of the protein of interest from host lysates (109).

The importance of ELRs resides in that these macromolecules exhibit a broad range of interesting properties that are not easily found simultaneously in other polymers, including stimuli-responsive behavior and the ability to self-assemble (102). These features are present because of a molecular transition of the polypeptide chain in the presence of water at temperatures above a certain level; this is the transition temperature T_t. The T_t of polymers structures based on (Val-Pro-Gly-X-Gly)n like elastin could be controlled by the amino acid at the X position; this position modifies parameters in the macromolecule like electrochemical potential (110).

Detailed investigations about the influence of polymer microstructure on physical performance, mechanical behavior, and rheological properties are being conducted with regards to the potential impact in tissue engineering applications. Some investigations demonstrate the possible use of the ELRs as materials to construct resolutive cell-laden tissue matrices. These materials provide an ECM-like environment sufficient to induce cell proliferation and differentiation (111). Another approach has proposed a hybrid cell-scaffold for vascular application made of poly-L-lactic acid/poly(D-lactide-co-glycolide) treated with tropoelastin, where the morphology, expansion, and maturity of the newly formed vessels were examined. The results indicated that the elastin-treated scaffolds developed more vascularization in comparison to the untreated. Moreover, implantation of tropoelastin-treated scaffolds into mouse abdominal muscle resulted in enhanced perfusion of the penetrating vasculature and improved integration (112). Similar approaches provided further evidence of the benefits of the hybrids ELRs-based-materials, such as blended biomaterials made from tropoelastin and silk fibroin with an adequate mechanical and biological performance (113).

Another method to modify the biomaterial features is changing the composition of a complete protein block, as was done in elastin-mimetic protein block copolymers changing the hydrophobic end block sequences and the hydrophilic block. Some examples of this are in hydrogels materials, which can be obtained by aggregation between the hydrophobic segments of multiblock ELR copolymers. Some studies suggest that the resulting amphiphilic blocks are stable *in vivo*. Furthermore, it is common to find protein polymers characterized by alternating blocks of hydrophilic and hydrophobic amino acids (114,115).

CONCLUSION AND PERSPECTIVES

In this article, we summarized the most recent advances in elastin-based biopolymers as biomaterials for tissue engineering applications. Proteins are indispensable for the correct functioning of all the organisms, and elastin accomplishes an essential function in numerous tissues of being humans. Besides its crucial biological role, elastin and its derivated peptides have exhibited a high potential as biomaterials for wound healing, drug delivery, and tissue engineering. A proof of the above is the significant amount of current investigations utilizing

these biopolymers to manufacture scaffolds and hydrogels for medical purposes. Notably, the mixture of elastin with other biopolymers such as collagen has shown promising results in the fabrication of biopharmaceutical products. Likewise, recent technologies allowed to develop ELPs and ELRs, which may possess a variety of functions and properties present in any natural peptide. Moreover, these functional peptides can self-assemble; thus, they can be mixed with other materials to improve the efficacy of virtually any biomedical device.

However, despite the enormous potential of current technologies, these have generated high expectations and goals that have not been entirely achieved in tissue engineering. A possible explanation for this lack of satisfaction is the difficulty of mimicking the complex environment of human organs. Therefore, existing elastin-based systems can still be improved. In this context, new developments will need to be thoroughly tested in vivo to optimize their physicochemical characteristics and maximize their therapeutic effects.

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INTEREST CONFLICT

The authors declare no conflict of interest

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