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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

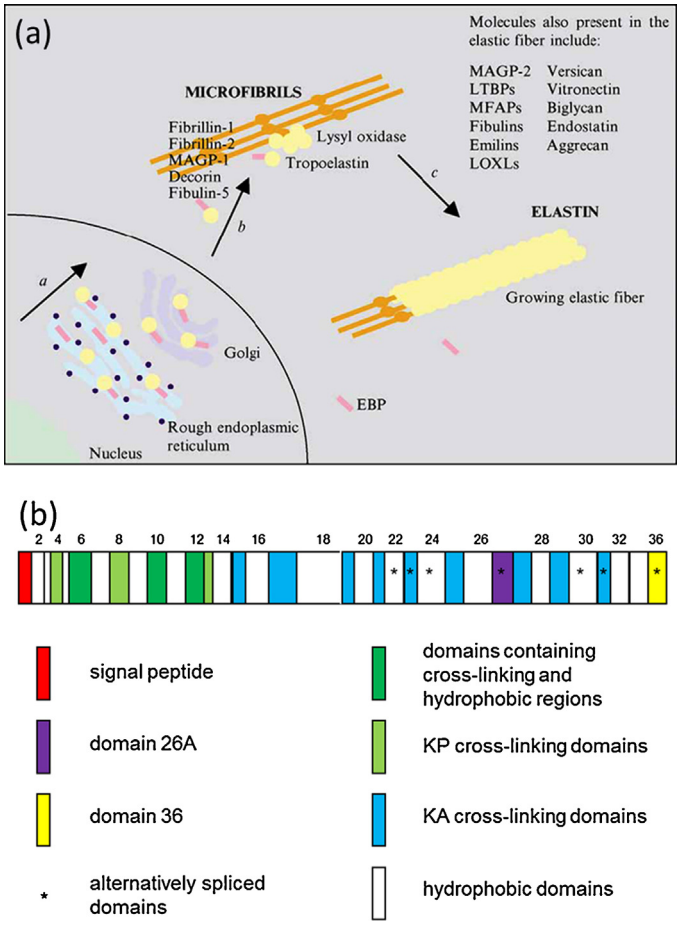

Fig. 1. Schematic of elastogenesis process and structure of human tropoelastin. (a) Elastogenesis process, (b) the human tropoelastin structure is dominated by alternating hydrophobic and hydrophilic regions primarily responsible for coacervation and crosslinking, respectively [1]. Adapted with permission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