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Review

Molecular assembly and mechanical properties of the extracellular matrix: A fibrous protein perspective ☆

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ABSTRACT

The extracellular matrix is an integral and dynamic component of all tissues. Macromolecular compositions and structural architectures of the matrix are tissue-specific and typically are strongly influenced by the magnitude and direction of biomechanical forces experienced as part of normal tissue function. Fibrous extracellular networks of collagen and elastin provide the dominant response to tissue mechanical forces. These matrix proteins enable tissues to withstand high tensile and repetitive stresses without plastic deformation or rupture. Here we provide an overview of the hierarchical molecular and supramolecular assembly of collagens and elastic fibers, and review their capacity for mechanical behavior in response to force. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Composition and complexity of the extracellular matrix

1.1. Introduction

The extracellular matrix (ECM) is a complex and dynamic network that surrounds cells in all tissues, providing structural and mechanical support, and mediating diverse biological processes that are crucial for supporting tissue formation and function. The ECM has long been recognized as an essential provider of tissue integrity (a cellular "glue"), supporting cell cohesion, and the growth and shaping of groups of cells into tissues and organs with defined biological and mechanical functions [1]. Major structural contributors to vertebrate ECM are the fibrous proteins collagen and elastin. Collagen provides tissues with essential tensile strength, enabling resistance to plastic deformation and rupture [2], while elastin imparts the properties of extensibility and reversible recoil, enabling tissues to withstand repetitive mechanical stress [3]. These fibrous protein networks lie in a viscous interstitial milieu rich in glycoproteins, proteoglycans (PGs), glycosaminoglycans (GAGs), and a complex composition of growth factors, cytokines, chemokines and proteases [4]. The matrix further serves as an intercellular space-filling medium that is rich in water sequestered by PGs/GAGs, which cushions tissues from the effects of mechanical forces such as crush from compressive loading [5]. Moreover, matrix–cell interactions are highly coordinated and dynamic. Far from being an inert structural support, ECM macromolecules are bioactive and play an important role in mechanotransduction, in part mediated through interactions with cell surface receptors such as integrins, which are coupled to the intracellular actin cytoskeleton [6]. Thus, the ECM actively contributes to fundamental cellular processes as diverse as differentiation, proliferation, adhesion, migration and apoptosis [6,7].

This review will focus on the molecular assembly of fibrous ECM proteins collagen and elastin, and describe the mechanical properties imparted by collagen and elastic fibers to the ECM network.

1.2. Fibrous proteins of the ECM

The physical response of tissues to mechanical forces is dominated by collagen and elastic fibers in the ECM. This section provides an overview of collagen as well as elastin and fibrillin elastic fiber proteins that play a key role in tissue mechanics.

1.2.1. Collagen

Collagen is the most abundant (~30%) protein in the body, and the largest component of the ECM, where it serves an essential structural role as provider of tensile strength to tissues and organs. It also plays an important role in the elasticity of a limited number of tissues, especially in tendons [8]. Collagen is widespread across many tissues, and forms the structural framework of connective tissues such as bone, tendons and dermis [9]. At least 28 different vertebrate collagens have been reported to date, the product of at least 42 different genes [10], although not all are components of the ECM. Members

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of the collagen superfamily are also found in invertebrates, including basement membrane collagens in *Drosophila melanogaster* and *Caenorhabditis elegans* [10]. Furthermore, collagen-like domains have been identified in more than 100 proteins from bacteria and viruses [11], including a cell-surface protein of streptococcus [12], and in exospore filaments of anthrax [13].

Collagens are characterized by a distinctive amino acid triplet repeat, Gly–X–Y, that gives rise to a signature triple helical structure, where Gly represents glycine, and positions X and Y can be any residue but are commonly proline and hydroxyproline, respectively (Fig. 1). As its name implies, the triple helix is comprised of three strands (α-chains) that are intertwined in helical register. Collagens are differentiated according to their α-chain composition and supramolecular structure. Depending on the repeat length and integrity of the Gly–X–Y repeat motif, collagens may contain substantial amounts of uninterrupted triple helix, for example as featured within the abundant fibrillar collagens. Alternatively, α-chains may contain a variable number of non-collagenous domains that introduce helical interruptions. As such, different collagens give rise to a variety of supramolecular structures including various geometric networks, membrane-spanning fibrils, and beaded-filaments (for review see [14]). In fact, many tissues contain multiple collagen types that may co-distribute with more than one structural class [14,15]. Moreover, with structural heterogeneity comes functional diversity. Different collagens contribute to a range of biological functions including cell adhesion and migration, tissue repair, molecular filtration and tumor suppression [16].

1.2.2. Elastin

The primary role of elastin is to allow tissues to undergo repetitive extension and return to their original dimensions upon removal of the deforming force [2]. Elastin is a more phylogenetically recent protein than collagen, arising concomitant with the evolution of closed circulatory systems, to support the higher pressures of pulsatile blood flow [17,18]. As such, elastin is restricted in distribution to the ECM of vertebrates, with the exception of the agnathans lamprey and hagfish [19,20]. Elastin displays a very low rate of turnover under normal conditions [21,22]. As a consequence, the same elastic fibers laid down during fetal development must sustain up to thousands of millions of cycles of stretch and recoil over a lifetime, as per the normal function of the tissue, without irreversible deformation or failure [23].

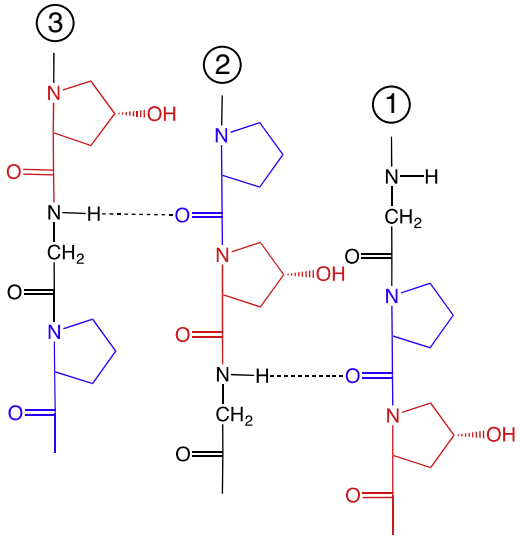

Fig. 1. Gly–X–Y triplet motif of collagen. Representative portions of three individual α-chains (labeled 1, 2, 3) are shown. Each chain is comprised of glycine (black), proline (position "X", blue) and hydroxyproline (position "Y", red). A hydrogen bond is formed between the N–H of glycine on each chain and the carbonyl group of proline on an adjacent chain. The hydrogen bond between chains 1 and 3 is not shown for clarity.

A single gene encodes elastin in mammals, birds and reptiles [24]. The human elastin transcript contains 34 exons and is secreted from the cell as tropoelastin, a highly hydrophobic, ~60 kDa alternately spliced monomer. The primary sequence of tropoelastin is characterized by alternating hydrophobic and cross-linking domains (Fig. 2). Cross-linking domains feature two or three lysine resides that are spaced three or four residues apart. Lysine residues are typically flanked by alanines, such as AAKAAKA (KA-type domains), but may be arranged within proline- and glycine-rich sequence, for example PGAGVKPGKGP (KP-type domains). Hydrophobic domains are rich (>80%) in glycine, proline, valine and alanine residues, commonly arranged in tandem combinations of GV, GVA and PGV motifs. These sequences transiently populate local structural motifs, such as type II β-turns and short polyproline II helices, a flexible left-handed helix that lacks intramolecular hydrogen bonds [25–28]. For review of elastin biochemistry see ref. [29].

1.2.3. Fibrillins

Fibrillins are a family of large (~350 kDa) extracellular glycoproteins and are the major components of microfibrils. Microfibrils are multiprotein fibrils comprising roughly 10% of mature elastic fibers. There are three fibrillin isoforms in humans (fibrillin-1, -2, -3) encoded by different genes, as well as invertebrate homologs [30]. Fibrillin-1 is expressed during fetal development and throughout adult life, while fibrillins-2 and -3 show highest expression in fetal tissue, with limited expression in adult tissue [30–32]. Fibrillins are composed of over 40 tandem calcium-binding EGF domains, interspersed with a small number of TB (TGFβ-binding protein/rich in cysteine) domains, and hybrid domains [33].

Fibrillin-rich microfibrils temporally associate with a range of other matrix proteins during elastic fiber assembly, including elastin, latent transforming growth factor-β binding proteins (LTBPs), matrix-associated glycoproteins (MAGPs), members of the fibulin family and PGs. Fibrillin monomers pack head-to-tail into 10–12 nm thick fibrils with beaded-string morphology, featuring a ~56 nm bead periodicity and a bead shoulder region comprising two symmetrical "arms" [34] (Fig. 3). Correct organization is dependent on the presence of calcium [35]. The cross-sectional microfibril diameter is consistent with about 8 longitudinally arranged fibrillin molecules [34]. Microfibrils are classically described as structural and organizational scaffolds for elastin based on their prior embryonic appearance and co-distribution with tropoelastin [36], and largely peripheral distribution in the mature elastic fiber [37]. Although most commonly associated with elastin, fibrillin microfibrils have important independent functions, including as structural reinforcing fibers in ciliary zonules of the eye [38], and are important in regulating the activity of TGFβ superfamily growth factors. Fibrillin microfibril biogenesis and function are reviewed in ref. [33].

1.3. A dynamic tissue-specific matrix

Cells regulate the synthesis and secretion and help direct the assembly of their own ECM components, thus coordinating the deposition of a tissue-specific matrix during embryogenesis. Once the ECM is laid down, environmental stimuli (including biochemical signals and mechanical stress) continue to modulate the secretion of matrix proteins to support tissue maintenance and dynamic remodeling throughout a lifetime [39,40]. For example, the ability for normal mechanical stretching of tissues is dependent on matrix composition and correctly formed architecture, yet may lead to matrix degradation (as part of turnover) triggered by cell deformation. Indeed, matrix turnover is required for biological process such as cell migration [41], highlighting the dynamic nature of the ECM, and the importance of

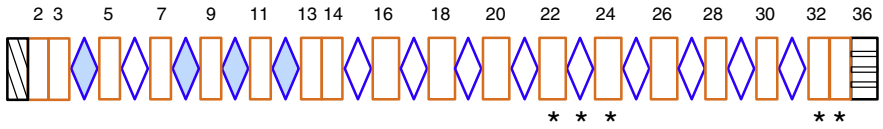

Fig. 2. Schematic domain structure of the human tropoelastin monomer. The elastin monomer is comprised of alternating hydrophobic domains (orange rectangles) and cross-linking domains (diamonds). Domains are numbered above the diagram. Genomic human tropoelastin has lost sequence corresponding to exons 34 and 35 compared to other mammalian species. KP-type cross-linking domains are shaded blue, KA-type cross-linking domains are white. Domain 1 (diagonal stripes) is a signal sequence. The C-terminal domain 36 (horizontal stripes) is highly basic and contains the only two cysteine residues in the protein. Domains with alternative exon splicing are marked with an asterisk. Domains are not to scale.

continual feedback between cells and their matrix for tissue homeostasis [7,40].

The composition and three-dimensional architecture of the ECM is directly related to the specialized function of the tissue of which it is a part. In the case of fibrous proteins, their supramolecular structure is largely dependent on the mechanical function of the tissue, and is optimized for the magnitude and direction of force the tissue must handle. Many connective tissues are rich in type I collagen, including bone (~90% of bone matrix) [42], tendon (60–85%) [43] and skin (~75% by dry weight) [9]. Collagen fibers of the Achilles tendon are aligned in thick bundles parallel to the long axis of the tendon, providing high unidirectional tensile strength [44], while collagen in skin forms an isotropic network of loose wavy bundles that are able to cope with multidirectional forces [45]. Type II collagen within articular cartilage (~60% by dry weight) also forms a loosely woven fibrillar network. However, fibers are orientated parallel to the tissue surface, or are randomly or radially distributed depending on their depth within the tissue, providing optimal load distribution in joints [43]. Network collagens, such as type IV in basement membranes, form open mesh-like structures with various geometries. The linear and lateral assembly of collagen type IV multimers results in a chicken-wire-like three-dimensional lattice that contributes to the anchoring of epithelial cells to underlying connective tissue [14,46].

Although less abundant than collagen, elastin is present in large amounts within highly elastic tissues. The elastic nuchal ligament is one of the richest (up to 80%) sources of elastin, and is located along the spinal chord where it provides important head and neck support to large grazing mammals [47]. Within this ligament, elastin forms a filamentous network that orients parallel to the direction of stretching [48]. Vascular tissue must extend and recoil under the high pulsatile pressures of blood flow. Accordingly, major arteries contain a high (up to 50% by dry weight) proportion of elastin, layered in concentric sheets interspersed with smooth muscle cells in the aorta wall [37,49]. Moreover, throughout the lung (up to ~30% elastin by weight) elastic fibers form a fine lattice-like mesh, in addition to concentrating at alveolar junctions and openings, which experience greater mechanical stress [29,50].

2. Assembly: from monomers to fibers to three-dimensional matrix

While the proportion of fibrillar proteins and their supramolecular architecture in the ECM is strongly influenced by the mechanical requirements and biological functions of individual tissues, assembly of the major fibrous structural proteins proceeds via a common strategy that is underpinned by the polymerization of extracellular monomeric "units" into fibers. For collagen, the basic unit is the triple helix, while for elastin, fibers polymerize from tropoelastin monomers deposited onto a scaffold of fibrillin-rich microfibrils. Here we describe the main features of collagen and elastin self-assembly into extracellular fibers, and outline the contributions of matrix macromolecules, cells and mechanical forces to the formation of three-dimensional networks.

2.1. Collagen assembly

2.1.1. Sequence features and modifications of polypeptide α-chains

The collagen triple helix is a super-coiled, right-handed structure comprised of three parallel α-chains, each with polyproline II helical conformation [9]. The key feature of collagen, and precondition for triple helix formation, is a repeating amino acid triplet motif Gly–X–Y within composite α-chains. The super-coiling of three α-chains necessitates glycine, lacking a side chain, in every third position of the triplet, where it packs tightly into the central helical axis. Indeed, mutation of a single glycine residue within the triple helical repeat region of fibrillar collagens disrupts helix integrity and frequently causes disease, including osteogenesis imperfecta, a bone fragility disorder, with mild to lethal effects [51].

Imino acids, commonly proline (28%) and hydroxyproline (38%), are favored in triplet positions X and Y, respectively. Together, glycine–proline–hydroxyproline is the most common (~10%) collagen triplet motif [9]. Hydroxyproline arises from the post-translational modification of prolines in the Y position by the enzyme prolyl 4-hydroxylase, and occurs before triple helix formation [52]. Hydroxyproline confers substantial thermal and structural stability to the triple helix largely by restricting main-chain torsion angles [9]. Further stabilization of the

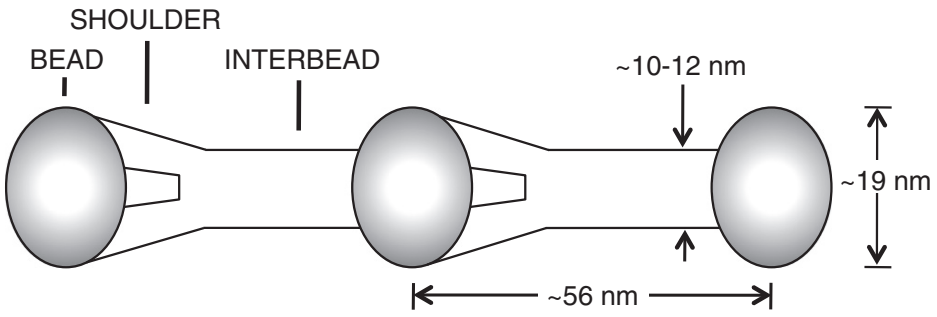

Fig. 3. Schematic diagram of fibrillin microfibril "beaded-string" morphology. Fibrillin monomers pack into ~10–12 nm diameter fibers with average beaded periodicity of ~56 nm. Adapted from [34].

helix arises from the formation of one inter-strand hydrogen bond per Gly–X–Y motif, between the N–H of glycine and carbonyl group of the residue in position X (often proline), which effectively creates a "ladder" of hydrogen bonds extending the length of the triple helical region [9] (Fig. 1). Additional post-translational modifications include hydroxylation of certain lysine residues and glycosylation of polypeptide α-chains, as well as the cross-linking of collagen fibrils through lysine and hydroxylysine residues by lysyl oxidase [10].

2.1.2. Triple helix formation and processing

Collagen is a highly processed molecule synthesized in the form of a soluble precursor, termed procollagen (Fig. 4). Procollagen synthesis is initiated within the lumen of the endoplasmic reticulum [10] and continued within narrow infolds of the plasma membrane that are continuous with the extracellular space [44,53]. Fibrillar procollagen molecules typically contain an uninterrupted ~300 nm central Gly–X–Y region of roughly 1000 residues that is flanked by non-helical (i.e. non Gly–X–Y) N- and C-terminal propeptide ends [9]. C-terminal propeptide domains contain recognition sequences important for the initial alignment of polypeptide α-chains, as well as cysteine residues that facilitate inter-chain disulfide bonding to secure the α-chains during assembly [10]. Helical assembly proceeds slowly [51] in a zipper-like manner from the C-terminus to the N-terminus [54]. Alpha-chains may be homotrimeric (three identical α-chains) or heterotrimeric (two or even three different α-chain types) within a single triple helical molecule, where their molecular composition influences the assembly architecture and mechanical properties of the resulting collagen [15].

Propeptide end sequences facilitate the initial linear interaction of procollagens, while limiting lateral association to about 5 molecules, equivalent to a thin fibril of ~4 nm diameter [44]. The cleavage of non-helical propeptide sequences results in a shortened triple helical product known as tropocollagen. Tropocollagen is typically defined as the extracellular "monomeric unit" of collagen, and undergoes hierarchical tissue-specific assembly into supramolecular structures such as thick fiber bundles in tendon [44].

2.1.3. Supramolecular assembly

Collagen assembly most commonly involves the alignment and cross-linking of tropocollagen triple helices into extended fibrillar structures within tissues. However, collagen architectures are as diverse as thick parallel bundles, open weave hexagonal networks, chicken-wire lattices, thin membrane-bound fibrils, and beaded fibrillar strings (for reviews see [14,16]) (Fig. 5). Architectures depend on many factors, including composition of α-chains and the combination of collagen types present, and are integral to tissue function. Supramolecular assembly is directed by a combination of tissue-specific matrix macromolecules, notably fibronectin and PGs, cell surface integrins, and intracellular forces [15]. Here we limit discussion to fibrillar collagens, of which the most widespread and well-studied is collagen type I.

Fibrillar collagens are the most abundant collagens of the ECM [15]. The primary fibrillar collagens are type I, which is widespread throughout non-cartilagenous connective tissues including skin, bone, tendon, ligament and cornea, and type II, found in cartilage and the vitreous humor of the eye [51]. Fibrillar collagens assemble via the lateral association of tropocollagen triple helices first into fibrils (in the order of ~10–300 nm diameter) and then fibers (~1–20 μm). Fibers may further associate into large bundles, such as in tendons, with diameters of up to ~500 μm [44]. The semi-crystalline packing of collagen fibrils forms the basis of the tensile strength of tissues [55]. Fibrils assemble from the parallel alignment of tropocollagen molecules, and feature a 67 nm periodic banding pattern arising from the precisely staggered overlap of adjacent molecules. This distinctive banding runs perpendicular to the long axis of the fibril [15]. Tropocollagen end sequences (known as telopeptides)

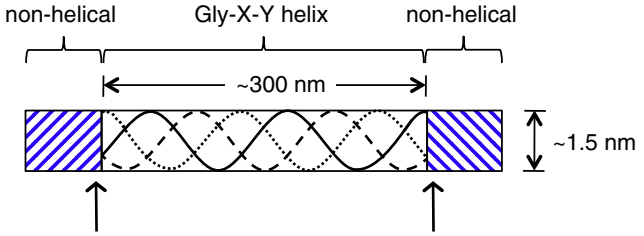

Fig. 4. Schematic diagram of the fibrillar procollagen molecule. The procollagen molecule is comprised of three polypeptide α-chains (solid, dashed, dotted lines) supercoiled into a right-handed helix spanning ~300 nm. Each α-chain is a left-handed polyproline-II helix containing a central Gly–X–Y repeat region. The triple helical region is flanked by non-helical N- and C-terminal ends (propeptides) (diagonal shading). Cleavage (at arrows) of the majority of the non-helical sequence produces the triple helical "monomeric unit" of collagen, tropocollagen. Very short non-helical ends remain (telopeptides). Domains not drawn to scale.

play a role in directing the staggered arrangement, and also contribute sites for cross-linking between molecules. Covalent cross-linking of lysine and hydroxylysine residues through the action of lysyl oxidase stabilizes collagen fibrils, and provides integrity essential for both structure formation and mechanical function [55].

While collagen I is capable of spontaneously forming fibrils *in vitro* in the absence of cells [56], assembly *in vivo* requires the presence of fibronectin and fibronectin-binding integrins (e.g. α5β1), as well as collagen-binding integrins (e.g. α2β1), thus enabling cellular control over assembly and organization [57]. Fibronectin is an abundant glycoprotein of the ECM that forms a branched fibrous network from secreted dimers. It is a modular protein with discrete binding sites for integrins, matrix proteins such as collagen, heparin, and itself [58]. The intracellular actin cytoskeleton supplies tension to integrin-bound fibronectin, exposing cryptic binding sites required for multimerization [59]. The molecular role of fibronectin in collagen assembly is not defined, but through direct interactions it may serve to concentrate collagen monomers or specify the location of assembly [57]. Potentially, the binding of collagen to integrins may induce a conformational change upon cell-mediated stretching to promote collagen fibrillogenesis [57]. Separately, proteolytic events may be required to expose cryptic binding sites on the collagen fibril surface for integrins and/or other ligands [60].

Collagens I and II co-distribute with ancillary collagen types to form heterotypic fibers. Collagen I associates with fibrillar collagens III and V, and the so-called fibril-associated collagen with interrupted triple helices (FACIT) type XII in different tissues, while collagen II is commonly distributed with small amounts of FACIT type IX and fibrillar type XI collagens. Indeed, assembly of collagens I and II is dependent on collagens V and XI, respectively, likely for nucleation [61,62]. Moreover, ancillary

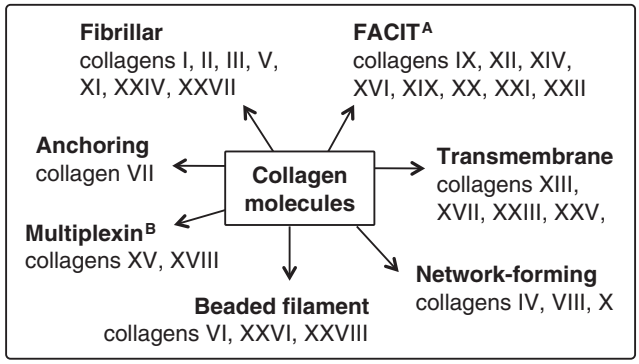

Fig. 5. The diversity of supramolecular assembly architectures of collagens I–XXVIII. [A]Fibril-associated collagen with interrupted triple helices. [B]Multiple triple helix domains and interruptions. Compiled from [9,16].

collagens contribute to the overall fiber organization and regulation of fiber diameter, and mediate interactions with other matrix molecules [15]. For example, some ancillary collagens, such as type IX on collagen II fibrils, stabilize architectures by facilitating interfibril interactions. Others, such as type III on collagen I fibrils, regulate fibril diameters through partial retention of bulky N-terminal propeptide domains, whose surface localization interferes with further lateral assembly [44]. These features in turn modulate supramolecular structure and mechanical properties.

The surface of collagen fibrils and fibers is an important interaction site for matrix macromolecules. Collagen I interacts with over 50 ligands *in vivo* including a range of PGs [60,63]. The large range of collagen ligands testifies to its wide role in biological processes in health and disease. PGs including perlecan, decorin, biglycan, fibromodulin and lumican contribute to the regulation of collagen fibrillogenesis [57,60,64]. PGs help to hold fibrils in register through bridging via anionic GAG chains, which aggregate between fibrils [15]. Furthermore, small PGs sequester water to collagen in joints, where properties of high viscosity and low compressibility facilitate joint lubrication.

2.2. Elastin assembly

Tropoelastin is secreted as a monomer principally from fibroblasts and smooth muscle cells into the extracellular space where it undergoes phase-separation into protein-rich globules and subsequently, the formation of elastic fibers [29,65].

2.2.1. Self-association of monomers (coacervation)

The early stage of elastin assembly in the extracellular space is dominated by an endothermic, primarily entropy-driven phase separation known as coacervation, which corresponds to the self-association of monomers through hydrophobic domains [65] (Fig. 6). *In vitro* this process is initially reversible. However, if left to mature, it leads to the formation of a protein-rich viscoelastic phase and a protein-poor solution phase [66]. Coacervation has long been described as serving to concentrate monomers, while potentially aligning lysine residues for cross-linking [37]. In recent years, the description of these events has advanced through a combination of microscopic and rheological approaches, and cell-based immunohistochemistry studies.

Coacervation is an intrinsic property of hydrophobic elastin sequences (cross-linking domains alone do not coacervate) [67]. This process can be modeled *in vitro* using synthetic full-length tropoelastin and elastin-mimetic polypeptides, where it is measured by spectrophotometry as a sharp increase in absorbance concomitant with heating to a critical temperature. Coacervation proceeds as a cooperative reaction that is highly dependent on solution conditions such as temperature, pH and salt concentration [66,68], and on the concentration, molecular weight, contextual domain arrangement and sequence of the protein [67,69,70].

In vivo, tropoelastin monomers are secreted into the extracellular space, where they spontaneously coalescence into roughly spherical globules of ~1–6 μm diameter at the cell surface in a concentration dependent manner, corresponding to coacervation [1,71]. Stable droplet formation can also be modeled *in vitro* using preparations of tropoelastin or elastin-like polypeptides in the absence of all other ECM components [72,73]. Few intermediate droplet states are detected by dynamic light scattering, concomitant with a rapid initial assembly process [73,74].

During this initial "microassembly" phase, coacervate droplets remain attached to the cell surface through interactions with a cell-surface protein(s), whose identity is not yet conclusively established. Several candidates include highly negatively charged heparan sulfate or chondroitin sulfate PGs that bind to tropoelastin via a unique cluster of charged and hydrophobic residues at the elastin C-terminus, potentially further triggering intracellular signaling pathways [75]. Heparan sulfate, in particular, is a significant component of cell-surfaces [76], is associated with mature elastic fibers [77], and promotes coacervation [78] by lowering the critical concentration required for droplet formation [74]. Alternatively, droplets at the cell-surface may interact with a G protein-coupled receptor [79]; with integrin $\alpha v\beta 3$ via a non-RGD interaction site [80]; or with a membrane-bound elastin binding protein complex (EBP, a 67 kDa inactive splice variant of β-galactosidase), which recognizes short VPG-type motifs found in hydrophobic elastin domains [81].

Droplets function as optimized delivery vehicles for the transport and deposition of monomers to the growing fiber. The colloid-like spherules preserve a hydrated interior of concentrated elastin [73] that likely contributes to both the prevention of hydrophobic collapse as monomers massively associate, and at least partial retention of conformational disorder, consistent with molecular dynamics simulations of $(GVPGV)_7$ aggregation [28] and NMR studies showing backbone mobility within the mature elastic fiber [82]. Moreover, the interaction of coacervate droplets with negatively charged cell-surface GAGs suggests that lysine residues may partition at the surface of the drop to facilitate exposure to GAGs. Any lysine residues not involved in tethering coacervate droplets to the cell surface may contribute to the final droplet size on the basis of charge repulsion at higher concentrations [65], although this remains to be confirmed.

2.2.2. Fiber formation

Formation of elastic fibers involves the deposition of coacervate droplets onto fibrillin-rich microfibrils and the cross-linking of elastin through lysine residues (Fig. 7). Temporal and spatial details of these events and the contribution of cells to fiber assembly are becoming better understood with advanced imaging techniques and molecular tracking experiments. However, interactions between the large numbers of elastic fiber proteins appear complex and, for the most part, molecular details remain poorly elucidated.

Elastin and elastic fibers closely interact with cells, whose migration exerts mechanical stress on the surrounding matrix and is a major contributor to fiber assembly [1,83]. The migration of cells during early tissue development brings small globules of tropoelastin at the cell surface into close proximity. Extracellular elastin globules aggregate when they come within ~10 μm of each other, creating an increasing number of stable, but randomly oriented, short fibrils [83].

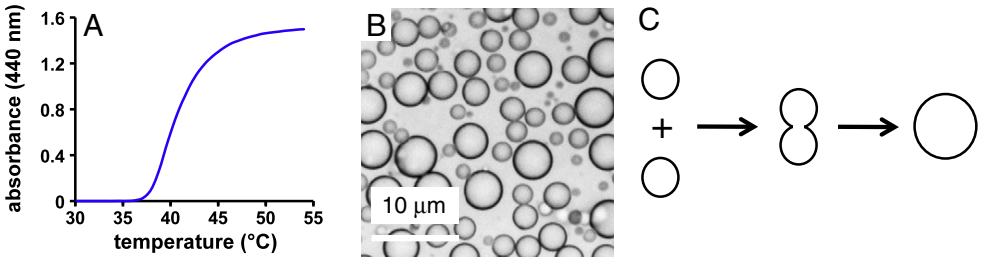

Fig. 6. Coacervation of tropoelastin. A. Representative coacervation curve illustrating a sharp increase in absorbance once a critical temperature is reached. B. Light microscopy image of coacervate droplets *in vitro*. C. Schematic shows droplet growth by coalescence.

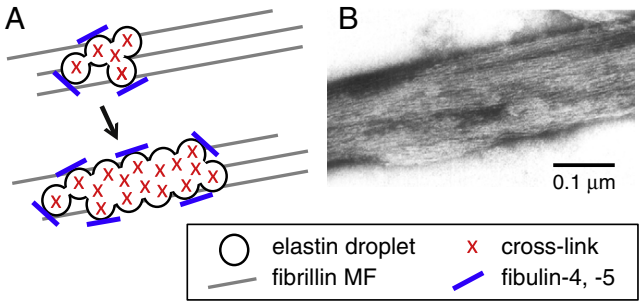

Fig. 7. Elastic fiber formation. A. Elastin coacervate is deposited onto fibrillin microfibrils (MF) and cross-linked by lysyl oxidase. Elastic fibers are associated with many matrix macromolecules, including fibulin-4 and fibulin-5. B. Fibrillar elastin aggregate. Micrograph reproduced from [114] with permission from Elsevier.

The concerted, directional elongation of these fibrils is coupled to the progressive coordination of cell motion. By late-phase assembly, the motions of cells and associated elastic fibers are correlated over distances of greater than 120 μm (i.e. multiple cell diameters), so that elastic fibers extend directionally within a given local environment and form interactions with multiple cells [83]. Newly secreted tropoelastin globules continue to be deposited onto this growing elastic fiber network [1].

Mature elastic fibers are comprised of ~90% elastin and ~10% fibrillin-rich microfibrils, which are located mainly peripherally but also sparsely throughout the fiber. Tropoelastin is found co-localized with fibrillin-1 in avian embryos as early as 23 h of development at the notochord and somites [36], and these proteins remain associated throughout fiber assembly [84]. Fine-mapping studies of fibrillin-1 interactions with tropoelastin have largely relied on a set of overlapping fibrillin fragments due to the immense size (~350 kDa) of the protein. A major binding site for tropoelastin lies within a region spanning 12 central domains of fibrillin (encoded by exons 18–30), potentially centered on the TB3 domain [85]. This region forms the solvent exposed bead shoulder of microfibrils. A second binding site of more moderate affinity is identified around the amino-terminal domains of fibrillins-1 and -2, extending from the proline-rich and glycine-rich regions, respectively, to the TB2 domain [85,86]. Tropoelastin binds to this region of fibrillin through positively charged lysine residues [86]. More specifically, a transglutaminase cross-link is described between the TB2 domain of fibrillin and the KP-type cross-linking domain 4 of elastin that is potentially a key stabilizer of initial elastin deposition [85,87].

C-terminal domains 29–36 of tropoelastin, particularly domain 30, are essential for the incorporation of tropoelastin into growing elastic fibers [84]. Hydrophobic domains in this region are enriched in glycine, such as GGLGV motifs, and correspondingly poor in proline residues. A depletion of proline particularly within hydrophobic domains 28 and 30 is suggested to induce the formation of β-structure by these sequences [27,84,88,89]. Indeed, domain 30 in isolation aggregates to form tightly packed amyloid-like fibrils, rich in β-structure [84,88]. Thus in the context of the full-length monomer, domain 30 may mediate specific protein–protein interactions through structural motifs during fiber assembly, including elastin interactions with matrix molecules and itself [27]. Separately, the C-terminal domain 36 of tropoelastin is highly basic and contains the only two cysteine residues in the monomer, which create a highly charged pocket upon disulfide bonding. This domain is among the most conserved across species [24] and is an important site for matrix protein and cell interactions [71,90–92].

Deconstruction of the complexity of interactions between elastic fiber proteins is ongoing. Elastic fibers are associated with more than 30 matrix macromolecules including tropoelastin, fibrillins, fibulins, matrix-associated glycoproteins (MAGPs) and latent transforming growth factor-β binding proteins (LTBPs) (for review see [35]). Some, such as fibrillins and LTBP-2, are structural components of microfibrils, while others co-localize to the microfibril surface, including tropoelastin and MAGPs, or the elastic fiber–cell interface, such as fibulin-5. Many matrix macromolecules display tissue-specific patterns of expression and likely serve as stabilizing or bridging molecules between elastic fiber proteins [39]. Indeed, the presence of matrix proteins fibrillin-1, fibulin-5 or MAGP-1 substantially restricts the growth of elastin coacervate droplets *in vitro* by stabilizing droplet surfaces, and promotes drop clustering [72]. Given that these proteins each interact directly with elastin, and collectively form a major component of elastic fibers, they likely contribute to the stabilization of elastin globules and their interaction with microfibrils during assembly.

Functional fiber formation is dependent on fibulin-4 and fibulin-5 (reviewed in [93]). Fibulin-5 is required for the organization and maturation of elastic fibers *in vivo* [94,95], and contains an integrin-binding RGD site that may mediate the interaction of elastic fibers with the cell surface. Fibulin-5 null mice have loose skin and display severe abnormalities in vascular and lung tissue commensurate with disorganized and fragmented elastic fibers [94,95]. Fibulin-4 is essential for elastin fibrillogenesis. Elastic fiber assembly is abolished in fibulin-4 null mice, which die prematurely, although irregular elastin aggregates are still observed [96]. Fibulins-4 and -5 differentially bind elastin, fibrillin-1 and lysyl oxidase, and co-localize to elastic fibers, suggesting a possible role for these fibulins in mediating tropoelastin deposition and/or interaction with the growing elastic fiber [97]. More subtly, fibulins-4 and -5 may regulate tropoelastin substrate specificity and the distribution of globules on the fiber [93].

Tropoelastin is covalently cross-linked via lysine residues during fiber assembly through the action of the copper-dependent enzyme lysyl oxidase [98]. Cross-linking provides elastic fibers with structural integrity and durability, and contributes to their high insolubility. Lysyl oxidase is found localized with tropoelastin coacervate globules at the cell surface [99] and with elastic fibers [39]. Lysine cross-linking involves the oxidative deamination of ε-amino groups followed by spontaneous condensation events, and results in formation of tetrafunctional desmosine and isodesmosine linkages, and bi-functional allysine-aldol and lysinonorleucines. The contribution of specific lysine residues to cross-links remains largely unknown due to the highly insoluble nature of mature fibers and the repetitive sequence of elastin, making identification of fragments difficult. A suggested cross-linking hub involves the formation of a desmosine from specific lysines within domains 19 and 25, and two lysinonorleucines that bridge domain 10 to this region, however the number of individual monomers involved in this coupling is not defined [100]. Separate studies show lysines within domains 19 to 25 are hot-spots for cross-linking by synthetic reagents [101].

3. Tissue responses to mechanical forces

Mechanical forces exerted on the ECM vary greatly in magnitude and direction, and may be sporadic, sustained or repetitive. Highly cross-linked collagen and elastic fibers provide tissues with structural integrity and provide the ability to withstand a range of mechanical stresses, from large-scale coordinated motions generated during breathing and walking to molecular stress associated with fundamental cell processes and tissue homeostasis. This section will discuss the tensile strength and energy storage capacity of collagen-rich tissues, and the extensibility and recoil properties of elastin-rich tissues, and describe the entropic mechanism of elasticity.

3.1. Definition of mechanical properties

Mechanical measurements are commonly made on pieces of fabricated biomaterials, such as a sheet of cross-linked synthetic elastin, or tissue samples, such as extracted collagen fibers, mounted in a mechanical testing apparatus. The extension (strain) exhibited by a

given material is measured upon the application of a force (stress), resulting in the collection of a stress–strain curve from which physical properties can be determined (Fig. 8). The applied force is commonly tensile (i.e. stretching) and less often, compressive.

A typical stress–strain curve is J-shaped, where the initial "softer" response is due to entropic elastic deformation and the latter stiffer response correlates with molecular deformation (changes in internal energy), which is followed by plastic deformation and rupture of the material [3,102,103]. The stress–strain curve for elastin is dominated by an initial linear entropic response, while curves for collagen exhibit a classic J-shape with a small entropic response (typically below strains of ~3%) and a substantial molecular stretching component, which adopts linearity at higher strains [2,102–104]. The stress and strain at the point of rupture correspond to the strength and maximum extensibility of the material, respectively. The elastic (Young's) modulus is an important measure of material stiffness (or "stretchiness"), and is calculated as the gradient of the stress–strain curve within the linear region. A more extensible material will exhibit a lower elastic modulus than a stiffer material under the same force. Elastomeric tissues are commonly subject to hysteresis upon cycles of stretch and relaxation (i.e. the stress–strain curve for relaxation will follow a lower trajectory than that for extension). Elastic hysteresis occurs because the tissue is viscoelastic (combines viscous and elastic components), and is typically indicative of energy lost to heat. Energy loss is calculated as the difference in energy (integration under the stress–strain curve) required to stretch a material and the energy that is restored upon relaxation. A more resilient elastomeric tissue will lose less energy to heat (will exhibit smaller hysteresis). Thus, resilience (as a percentage) is the energy required to stretch a material minus the energy lost to heat, normalized by the energy required for stretching [2].

3.2. Mechanical contributions of collagen and elastin

Collagen and elastin each form extensive fibrous networks within the crowded extracellular space where they contribute significantly to tissue integrity and provide mechanical stability in response to biomechanical forces. While supramolecular assembly of both these fibers requires interactions with many of the same matrix macromolecules (e.g. PGs, lysyl oxidase, emilin), each mature network primarily remains structurally independent of the other. Indeed, while interfacial interactions between collagen and elastic fibers in many connective tissues are likely to be mediated via a range of matrix macromolecules, including PGs, it is somewhat surprising that so few physical interactions (or cross-links) have been documented between these abundant networks. Limited interactions include collagen VI with some microfibrils, collagen XVI with some dermal microfibrils, collagen VIII with some elastic fibers, and endostatin (a collagen XVIII product) with vascular elastic fibers (reviewed in [35]).

Collagen and elastic fibers combine to provide tissues with composite strength and elasticity. Notwithstanding, tissues rich in either collagen fibers or elastic fibers often face distortions of different magnitudes and durations, and confer mechanical resistance through separate mechanisms. Collagen strength is based on the well-packed semi-crystalline molecular structure of the triple helix, while elasticity is due primarily to the high entropy (disorder) of polypeptide chains in the relaxed (non-stretched) state. Mechanical properties of collagen fibers (tendon) and elastic fibers (ligament, aorta, and synthetic) are shown in Table 1.

3.2.1. Mechanical responses of collagen-rich tissues

Collagen provides tissues with strength and stiffness, which serve to protect against fracture under an applied force. Collagen in mammalian tendon has a strength of ~0.12 GPa, just one order of magnitude lower than high tensile steel [2,104], and an elastic modulus of ~1.2 GPa, demonstrating substantial material stiffness (Table 1). Indeed, collagen fibers have remarkable capacity for energy storage (show high (90%) resilience with little energy lost to heat) but stretch only minimally (~13%) [2,44]. These properties are a consequence of structural features, including the supercoiling of triple helices and the close parallel alignment of collagen molecules within fibrils, both of which contribute to semi-crystalline packing. However, kinks at the molecular and fibrillar levels remain due to helical interruptions by a variety of non-collagenous domains.

Collagen extension first results from the straightening of kinks within collagen fibrils and within the triple helix [102]. Further extension is due to shear, including "gliding" of molecules within fibrils, and between fibrils in the matrix. Response depends on the rate and magnitude of strain, although the molecular details are not well understood. Intra- and interfibril shear, and molecular distortion with stretching also contribute to the storage of elastic energy as changes in internal energy, where recoil is driven by a return to a state of lowest potential energy (characteristic of stiff materials) [3,102]. Interfibril shear is further dependent on the level of PG decoration of fibril surfaces, which influences the extent of GAG entanglement and electrostatic interactions between fibrils and the crowded matrix [15].

The distribution of collagen fibril thicknesses is a modulator of tissue strength [45]. In decorin null mice, irregular collagen fibril diameters result in decreased skin tissue strength [105]. Collagen III is typically thinner and more flexible than collagen I. These collagens form a substantial proportion of tissue scaffolding in certain tissues, thus, mixing at different ratios may be one way to provide diversity of mechanical properties [7]. FACIT collagen XII associates with the surface of collagen I fibrils, where it facilitates fibril fusion (thickening) when fewer FACIT molecules are present but inhibits fusion at higher decorations [14]. Interestingly, collagen XII is upregulated in tissue in response to mechanical loading [44].

Tissues face differential magnitudes of force *in vivo*, which reflected their range of collagen architectures. Wavy bundles of

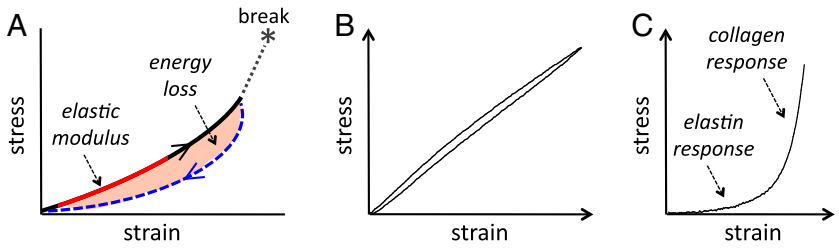

Fig. 8. Measuring mechanical properties. A. Schematic stress–strain curve showing mechanical properties measurable upon tensile stretching (bold line) of a material. Arrowheads indicate direction of loading (black) and unloading (blue). Stretch to break is indicated by dotted line. Asterisk denotes point of rupture. If the stress is removed before plastic deformation and material rupture, a stress–strain curve can be obtained for relaxation (blue dashed line). Elastic modulus (red line) is the gradient of the curve in the linear region. % Energy lost to heat is the hysteresis (shaded area) between stretch and relaxation curves. B. Typical stress–strain curve for elastin showing linearity of elastic extension and minimal hysteresis upon relaxation. C. J-shaped stress–strain curve representative of tissues containing both collagen and elastin. Elastin is responsible for the initial "softer" response and collagen is responsible for the latter stiffer response.

Table 1
Mechanical properties of collagen and elastin. Proteins of tissue origin refer to excised (tendon) or extracted (elastin) fibers. Elastin of synthetic origin refers to a biomaterial cast from recombinant human tropoelastin and cross-linked with bis(sulfosuccinimidyl) suberate. NR = not reported.

Protein	Origin	Elastic modulus	Strength (stress at break)	Extensibility (strain at break)	Resilience	Reference
Collagen	Mammalian tendon	1.2 GPa	120 MPa	13%	90%	[104]
Elastin	Bovine nuchal ligament	1.1 MPa	2 MPa	150%	90%	[2]
	Porcine aorta	0.81 MPa	1.02 MPa	103%	77%	[115,116]
	Synthetic	0.22–0.28 MPa	~0.4 MPa	200–370%	NR	[103]

collagen, for example in skin, experience smaller forces than tendons and predominantly provide strength for reinforcement. These tissues often contain a small (~3% in skin) but important elastic fiber component that provides most of the elasticity [2,37]. In contrast, the Achilles tendon functions at strains approaching that of breaking [2] and contains collagen arranged in thick linear bundles, which provides substantially increased elastic storage capacity [106].

During embryonic development, and with onset of animal locomotion, the tensile strength and capacity for elastic energy storage of tendons increases dramatically in response to collagen fiber elongation [44]. In mature tendons, elastic energy stored on the basis of extension of flexible kinks within collagen triple helices is highly dependent on the length of fibers, i.e. the number of collagen molecules cross-linked end-to-end in series [44,102]. Mineralization of tendons increases tensile strength while limiting maximal extension, perhaps due to mineral deposition within more flexible regions of the triple helix (reviewed in [44]).

3.2.2. Mechanical responses and elastic mechanisms of elastin-rich tissues

Elastin displays remarkable elastic extension as indicated by an elastic modulus of ~0.3–1 MPa (Table 1). In fact, elastin can undergo substantial extension with minimal force, and notably, exhibits the greatest (at least 150%) linear elastic extension of any known biological material [103]. It is also very (~90%) resilient and shows extreme durability, as underscored by the minimal turnover of elastin during a lifetime [21,22]. However, elastin has low (~2 MPa) tensile strength. Separately, microfibrils exhibit elastomeric properties independent of elastin. However, the elastic modulus of eye zonule microfibrils is around 78–96 MPa, roughly two orders of magnitude stiffer than elastin [38]. Thus, microfibrils within elastic fibers largely provide reinforcement to elastomeric tissues.

Importantly, elastic fibers are only elastic when hydrated — when dry, elastin is hard and brittle [107]. Hydration of the polypeptide backbone is an essential contributor to the maintenance of structural flexibility and disorder (high entropy) within the tropoelastin monomer, a key driver of elastic recoil [3,28,108]. Indeed, in what at first may appear paradoxical, tropoelastin monomers massively aggregate yet remain disordered [28]. An extremely non-polar amino acid composition is classically expected to render a protein highly susceptible to the formation of an ordered, water-excluding hydrophobic core, in which the close packing of secondary structure shields non-polar side-chains from the surrounding polar environment. Remarkably, in the case of elastin, the highly (~80% non-polar amino acids) hydrophobic monomer is substantially disordered and flexible in solution [26,109–111], and retains backbone mobility even in the aggregated state [28] and in mature cross-linked fibers [82].

The basis for structural disorder within hydrophobic elastin domains, and indeed, for hydrophobic sequences from a diverse range of elastomeric proteins including insect resilin, mussel byssus thread and spider silks, lies with their high combined composition of flexible glycine and rigid proline residues for a minimum sequence threshold of (2Pro + Gly) > ~60% [28]. These residues combine to prevent the formation of extended secondary structures (such as α-helix and β-sheet) due to the high entropic penalty for structural confinement of glycines, and the fixed ϕ dihedral angle and lack of amide hydrogen of prolines. Maintenance of structural disorder is further provided by an average proline spacing of every 4–8 residues within the majority of hydrophobic elastin domains [27]. An average proline spacing of greater than eight leaves hydrophobic elastin sequences susceptible to β-sheet aggregation [27], including the formation of amyloid-like fibrils [84,88,112]. Highly disordered monomers and aggregates are consistent with a dominant entropic driving force for elastic recoil, and rubber-like elasticity [113].

Two types of entropic forces combine to drive the elastic recoil of stretched elastin molecules: the hydrophobic effect, which describes solvent entropy, and a high structural disorder of the polypeptide chain, which describes solute entropy. High polypeptide chain disorder opposes both stretching and tight packing, each of which restricts the number of conformations available to the protein. The hydrophobic effect favors protein collapse, but the high glycine and proline content resists formation of extended secondary structure. Thus, chain entropy remains substantial (reviewed in [3,28]).

4. Conclusions

Collagen and elastin assemble into fibrous extracellular networks that fulfill crucial structural and mechanical roles supporting tissue function. Although both collagen and elastin are rich in glycine and proline residues, fundamental differences in sequence arrangement lead to profound differences in structural organization at both the molecular and fibrillar level. Moreover, while both proteins form cross-linked fibers from monomeric units, these fibers exhibit remarkably different mechanical properties and mechanisms of mechanical response. The result is a resilient matrix that enables tissues to cope with a range of forces spanning biological relevance.

Due to their impressive suite of mechanical properties, these proteins present a tempting direction for biomedical applications, including wound healing, tissue regeneration, and the rational design of mimetic biomaterials. Such design requires recapitulation of mechanical properties, which necessitates a thorough understanding of the relationship between sequence, structure and assembly as they apply to function.

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References

[1] B.A. Kozel, B.J. Rongish, A. Czirok, J. Zach, C.D. Little, E.C. Davis, R.H. Knutsen, J.E. Wagenseil, M.A. Levy, R.P. Mecham, Elastic fiber formation: a dynamic view of extracellular matrix assembly using timer reporters, J. Cell. Physiol. 207 (2006) 87–96.
[2] J. Gosline, M. Lillie, E. Carrington, P. Guerette, C. Ortlepp, K. Savage, Elastic proteins: biological roles and mechanical properties, Philos. Trans. R. Soc. Lond. B Biol. Sci. 357 (2002) 121–132.
[3] S. Rauscher, R. Pomes, Structural disorder and protein elasticity, Adv. Exp. Med. Biol. 725 (2012) 159–183.
[4] T.N. Wight, S. Potter-Perigo, The extracellular matrix: an active or passive player in fibrosis? Am. J. Physiol. Gastrointest. Liver Physiol. 301 (2011) G950–G955.
[5] E.M. Culav, C.H. Clark, M.J. Merrilees, Connective tissues: matrix composition and its relevance to physical therapy, Phys. Ther. 79 (1999) 308–319.

[6] S.H. Kim, J. Turnbull, S. Guimond, Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor, J. Endocrinol. 209 (2011) 139–151.
[7] K.J. Aitken, D.J. Bagli, The bladder extracellular matrix. Part I: architecture, development and disease, Nat. Rev. Urol. 6 (2009) 596–611.
[8] R.E. Shadwick, Elastic energy storage in tendons: mechanical differences related to function and age, J. Appl. Physiol. 68 (1990) 1033–1040.
[9] M.D. Shoulders, R.T. Raines, Collagen structure and stability, Annu. Rev. Biochem. 78 (2009) 929–958.
[10] J. Myllyharju, K.I. Kivirikko, Collagens, modifying enzymes and their mutations in humans, flies and worms, Trends Genet. 20 (2004) 33–43.
[11] M. Rasmussen, M. Jacobsson, L. Bjorck, Genome-based identification and analysis of collagen-related structural motifs in bacterial and viral proteins, J. Biol. Chem. 278 (2003) 32313–32316.
[12] Y. Xu, D.R. Keene, J.M. Bujnicki, M. Hook, S. Lukomski, Streptococcal Scl1 and Scl2 proteins form collagen-like triple helices, J. Biol. Chem. 277 (2002) 27312–27318.
[13] P. Sylvestre, E. Couture-Tosi, M. Mock, Polymorphism in the collagen-like region of the *Bacillus anthracis* BclA protein leads to variation in exosporium filament length, J. Bacteriol. 185 (2003) 1555–1563.
[14] M.K. Gordon, R.A. Hahn, Collagens, Cell Tissue Res. 339 (2010) 247–257.
[15] T.J. Wess, Collagen fibril form and function, Adv. Protein Chem. 70 (2005) 341–374.
[16] K.E. Kadler, C. Baldock, J. Bella, R.P. Boot-Handford, Collagens at a glance, J. Cell Sci. 120 (2007) 1955–1958.
[17] H. Sage, Structure–function relationship in the evolution of elastin, J. Invest. Dermatol. 79 (Suppl. 1) (1982) 146s–153s.
[18] G. Faury, Function–structure relationship of elastic arteries in evolution: from microfibrils to elastin and elastic fibres, Pathol. Biol. (Paris) 49 (2001) 310–325.
[19] E.H. Sage, W.R. Gray, Evolution of elastin structure, Adv. Exp. Med. Biol. 79 (1977) 291–312.
[20] H. Sage, W.R. Gray, Studies on the evolution of elastin—I. Phylogenetic distribution, Comp. Biochem. Physiol. B: Comp. Biochem. 64 (1979) 313–327.
[21] E.C. Davis, Stability of elastin in the developing mouse aorta: a quantitative radioautographic study, Histochemistry 100 (1993) 17–26.
[22] S.D. Shapiro, S.K. Endicott, M.A. Province, J.A. Pierce, E.J. Campbell, Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon, J. Clin. Invest. 87 (1991) 1828–1834.
[23] F.W. Keeley, C.M. Bellingham, K.A. Woodhouse, Elastin as a self-organizing biomaterial: use of recombinantly expressed human elastin polypeptides as a model for investigations of structure and self-assembly of elastin, Philos. Trans. R. Soc. Lond. B Biol. Sci. 357 (2002) 185–189.
[24] M.I. Chung, M. Miao, R.J. Stahl, E. Chan, J. Parkinson, F.W. Keeley, Sequences and domain structures of mammalian, avian, amphibian and teleost tropoelastins: clues to the evolutionary history of elastins, Matrix Biol. 25 (2006) 492–504.
[25] A. Pepe, D. Guerra, B. Bochicchio, D. Quaglino, D. Gheduzzi, I. Pasquali Ronchetti, A.M. Tamburro, Dissection of human tropoelastin: supramolecular organization of polypeptide sequences coded by particular exons, Matrix Biol. 24 (2005) 96–109.
[26] A.M. Tamburro, B. Bochicchio, A. Pepe, Dissection of human tropoelastin: exon-by-exon chemical synthesis and related conformational studies, Biochemistry 42 (2003) 13347–13362.
[27] L.D. Muiznieks, F.W. Keeley, Proline periodicity modulates the self-assembly properties of elastin-like polypeptides, J. Biol. Chem. 285 (2010) 39779–39789.
[28] S. Rauscher, S. Baud, M. Miao, F.W. Keeley, R. Pomes, Proline and glycine control protein self-organization into elastomeric or amyloid fibrils, Structure 14 (2006) 1667–1676.
[29] S.M. Mithieux, A.S. Weiss, Elastin, Adv. Protein Chem. 70 (2005) 437–461.
[30] L. Sabatier, N. Miosge, D. Hubmacher, G. Lin, E.C. Davis, D.P. Reinhardt, Fibrillin-3 expression in human development, Matrix Biol. 30 (2011) 43–52.
[31] G.M. Corson, N.L. Charbonneau, D.R. Keene, L.Y. Sakai, Differential expression of fibrillin-3 adds to microfibril variety in human and avian, but not rodent, connective tissues, Genomics 83 (2004) 461–472.
[32] H. Zhang, S.D. Apfelroth, W. Hu, E.C. Davis, C. Sanguinieti, J. Bonadio, R.P. Mecham, F. Ramirez, Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices, J. Cell Biol. 124 (1994) 855–863.
[33] D. Hubmacher, K. Tiedemann, D.P. Reinhardt, Fibrillins: from biogenesis of microfibrils to signaling functions, Curr. Top. Dev. Biol. 75 (2006) 93–123.
[34] C. Baldock, A.J. Koster, U. Ziese, M.J. Rock, M.J. Sherratt, K.E. Kadler, C.A. Shuttleworth, C.M. Kielty, The supramolecular organization of fibrillin-rich microfibrils, J. Cell Biol. 152 (2001) 1045–1056.
[35] C.M. Kielty, M.J. Sherratt, C.A. Shuttleworth, Elastic fibres, J. Cell Sci. 115 (2002) 2817–2828.
[36] R.P. Visconti, J.L. Barth, F.W. Keeley, C.D. Little, Codistribution analysis of elastin and related fibrillar proteins in early vertebrate development, Matrix Biol. 22 (2003) 109–121.
[37] J. Rosenbloom, W.R. Abrams, R. Mecham, Extracellular matrix 4: the elastic fiber, FASEB J. 7 (1993) 1208–1218.
[38] M.J. Sherratt, C. Baldock, J.L. Haston, D.F. Holmes, C.J. Jones, C.A. Shuttleworth, T.J. Wess, C.M. Kielty, Fibrillin microfibrils are stiff reinforcing fibres in compliant tissues, J. Mol. Biol. 332 (2003) 183–193.
[39] A. Shifren, R.P. Mecham, The stumbling block in lung repair of emphysema: elastic fiber assembly, Proc. Am. Thorac. Soc. 3 (2006) 428–433.
[40] B. Suki, J.H. Bates, Extracellular matrix mechanics in lung parenchymal diseases, Respir. Physiol. Neurobiol. 163 (2008) 33–43.
[41] A.R. Gillies, R.L. Lieber, Structure and function of the skeletal muscle extracellular matrix, Muscle Nerve 44 (2011) 318–331.
[42] B. Batge, J. Diebold, H. Stein, M. Bodo, P.K. Muller, Compositional analysis of the collagenous bone matrix. A study on adult normal and osteopenic bone tissue, Eur. J. Clin. Invest. 22 (1992) 805–812.
[43] L. Cen, W. Liu, L. Cui, W. Zhang, Y. Cao, Collagen tissue engineering: development of novel biomaterials and applications, Pediatr. Res. 63 (2008) 492–496.
[44] F.H. Silver, J.W. Freeman, G.P. Seehra, Collagen self-assembly and the development of tendon mechanical properties, J. Biomech. 36 (2003) 1529–1553.
[45] V. Ottani, M. Raspanti, A. Ruggeri, Collagen structure and functional implications, Micron 32 (2001) 251–260.
[46] C. Knupp, J.M. Squire, Molecular packing in network-forming collagens, Adv. Protein Chem. 70 (2005) 375–403.
[47] B.J. Reinboth, M.L. Finnis, M.A. Gibson, L.B. Sandberg, E.G. Cleary, Developmental expression of dermatan sulfate proteoglycans in the elastic bovine nuchal ligament, Matrix Biol. 19 (2000) 149–162.
[48] I. Pasquali Ronchetti, A. Alessandrini, M. Baccarani Contri, C. Fornieri, G. Mori, D. Quaglino Jr., U. Valdre, Study of elastic fiber organization by scanning force microscopy, Matrix Biol. 17 (1998) 75–83.
[49] D.Y. Li, B. Brooke, E.C. Davis, R.P. Mecham, L.K. Sorensen, B.B. Boak, E. Eichwald, M.T. Keating, Elastin is an essential determinant of arterial morphogenesis, Nature 393 (1998) 276–280.
[50] B.C. Starcher, Elastin and the lung, Thorax 41 (1986) 577–585.
[51] B. Brodsky, A.V. Persikov, Molecular structure of the collagen triple helix, Adv. Protein Chem. 70 (2005) 301–339.
[52] S.M. Krane, The importance of proline residues in the structure, stability and susceptibility to proteolytic degradation of collagens, Amino Acids 35 (2008) 703–710.
[53] D.E. Birk, R.L. Trelstad, Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation, J. Cell Biol. 103 (1986) 231–240.
[54] J. Khoshnoodi, K. Sigmundsson, J.P. Cartailler, O. Bondar, M. Sundaramoorthy, B.G. Hudson, Mechanism of chain selection in the assembly of collagen IV: a prominent role for the alpha2 chain, J. Biol. Chem. 281 (2006) 6058–6069.
[55] K.E. Kadler, D.F. Holmes, J.A. Trotter, J.A. Chapman, Collagen fibril formation, Biochem. J. 316 (Pt 1) (1996) 1–11.
[56] K.E. Kadler, Y. Hojima, D.J. Prockop, Assembly of collagen fibrils de novo by cleavage of the type I pC-collagen with procollagen C-proteinase. Assay of critical concentration demonstrates that collagen self-assembly is a classical example of an entropy-driven process, J. Biol. Chem. 262 (1987) 15696–15701.
[57] K.E. Kadler, A. Hill, E.G. Canty-Laird, Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators, Curr. Opin. Cell Biol. 20 (2008) 495–501.
[58] P. Singh, C. Carraher, J.E. Schwarzbauer, Assembly of fibronectin extracellular matrix, Annu. Rev. Cell Dev. Biol. 26 (2010) 397–419.
[59] C. Zhong, M. Chrzanowska-Wodnicka, J. Brown, A. Shaub, A.M. Belkin, K. Burridge, Rho-mediated contractility exposes a cryptic site in fibronectin and induces fibronectin matrix assembly, J. Cell Biol. 141 (1998) 539–551.
[60] J.P. Orgel, J.D. San Antonio, O. Antipova, Molecular and structural mapping of collagen fibril interactions, Connect. Tissue Res. 52 (2011) 2–17.
[61] R.J. Wenstrup, J.B. Florer, E.W. Brunskill, S.M. Bell, I. Chervoneva, D.E. Birk, Type V collagen controls the initiation of collagen fibril assembly, J. Biol. Chem. 279 (2004) 53331–53337.
[62] R.J. Fernandes, M. Weis, M.A. Scott, R.E. Seegmiller, D.R. Eyre, Collagen XI chain misassembly in cartilage of the chondrodysplasia (cho) mouse, Matrix Biol. 26 (2007) 597–603.
[63] G.A. Di Lullo, S.M. Sweeney, J. Korkko, L. Ala-Kokko, J.D. San Antonio, Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen, J. Biol. Chem. 277 (2002) 4223–4231.
[64] J.E. Scott, Proteodermatan and proteokeratan sulfate (decorin, lumican/fibromodulin) proteins are horseshoe shaped. Implications for their interactions with collagen, Biochemistry 35 (1996) 8795–8799.
[65] G.C. Yeo, F.W. Keeley, A.S. Weiss, Coacervation of tropoelastin, Adv. Colloid Interface Sci. 167 (2011) 94–103.
[66] B. Vrhovski, S. Jensen, A.S. Weiss, Coacervation characteristics of recombinant human tropoelastin, Eur. J. Biochem. 250 (1997) 92–98.
[67] C.M. Bellingham, K.A. Woodhouse, P. Robson, S.J. Rothstein, F.W. Keeley, Self-aggregation characteristics of recombinantly expressed human elastin polypeptides, Biochim. Biophys. Acta 1550 (2001) 6–19.
[68] J.T. Cirulis, F.W. Keeley, Kinetics and morphology of self-assembly of an elastin-like polypeptide based on the alternating domain arrangement of human tropoelastin, Biochemistry 49 (2010) 5726–5733.
[69] P. Toonkool, S.A. Jensen, A.L. Maxwell, A.S. Weiss, Hydrophobic domains of human tropoelastin interact in a context-dependent manner, J. Biol. Chem. 276 (2001) 44575–44580.
[70] M. Miao, J.T. Cirulis, S. Lee, F.W. Keeley, Structural determinants of cross-linking and hydrophobic domains for self-assembly of elastin-like polypeptides, Biochemistry 44 (2005) 14367–14375.
[71] B.A. Kozel, C.H. Ciliberto, R.P. Mecham, Deposition of tropoelastin into the extracellular matrix requires a competent elastic fiber scaffold but not live cells, Matrix Biol. 23 (2004) 23–34.
[72] J.T. Cirulis, C.M. Bellingham, E.C. Davis, D. Hubmacher, D.P. Reinhardt, R.P. Mecham, F.W. Keeley, Fibrillins, fibulins, and matrix-associated glycoprotein modulate the kinetics and morphology of in vitro self-assembly of a recombinant elastin-like polypeptide, Biochemistry 47 (2008) 12601–12613.

[73] A.W. Clarke, E.C. Arnspang, S.M. Mithieux, E. Korkmaz, F. Braet, A.S. Weiss, Tropoelastin massively associates during coacervation to form quantized protein spheres, Biochemistry 45 (2006) 9989–9996.
[74] Y. Tu, A.S. Weiss, Transient tropoelastin nanoparticles are early-stage intermediates in the coacervation of human tropoelastin whose aggregation is facilitated by heparan sulfate and heparin decasaccharides, Matrix Biol. 29 (2010) 152–159.
[75] T.J. Broekelmann, B.A. Kozel, H. Ishibashi, C.C. Werneck, F.W. Keeley, L. Zhang, R.P. Mecham, Tropoelastin interacts with cell-surface glycosaminoglycans via its COOH-terminal domain, J. Biol. Chem. 280 (2005) 40939–40947.
[76] N. Perrimon, M. Bernfield, Specificities of heparan sulphate proteoglycans in developmental processes, Nature 404 (2000) 725–728.
[77] D. Gheduzzi, D. Guerra, B. Bochicchio, A. Pepe, A.M. Tamburro, D. Quaglino, S. Mithieux, A.S. Weiss, I. Pasquali Ronchetti, Heparan sulphate interacts with tropoelastin, with some tropoelastin peptides and is present in human dermis elastic fibers, Matrix Biol. 24 (2005) 15–25.
[78] W.J. Wu, B. Vrhovski, A.S. Weiss, Glycosaminoglycans mediate the coacervation of human tropoelastin through dominant charge interactions involving lysine side chains, J. Biol. Chem. 274 (1999) 21719–21724.
[79] S.K. Karnik, J.D. Wythe, L. Sorensen, B.S. Brooke, L.D. Urness, D.Y. Li, Elastin induces myofibrillogenesis via a specific domain, VGVAPG, Matrix Biol. 22 (2003) 409–425.
[80] U.R. Rodgers, A.S. Weiss, Integrin avb3 binds a unique non-RGD site near the C-terminus of human tropoelastin, Biochimie 86 (2004) 173–178.
[81] A. Hinek, M. Rabinovitch, 67-kD elastin-binding protein is a protective "companion" of extracellular insoluble elastin and intracellular tropoelastin, J. Cell Biol. 126 (1994) 563–574.
[82] M.S. Pometun, E.Y. Chekmenev, R.J. Wittebort, Quantitative observation of backbone disorder in native elastin, J. Biol. Chem. 279 (2004) 7982–7987.
[83] A. Czirok, J. Zach, B.A. Kozel, R.P. Mecham, E.C. Davis, B.J. Rongish, Elastic fiber macro-assembly is a hierarchical, cell motion-mediated process, J. Cell. Physiol. 207 (2006) 97–106.
[84] B.A. Kozel, H. Wachi, E.C. Davis, R.P. Mecham, Domains in tropoelastin that mediate elastin deposition in vitro and in vivo, J. Biol. Chem. 278 (2003) 18491–18498.
[85] M.J. Rock, S.A. Cain, L.J. Freeman, A. Morgan, K. Mellody, A. Marson, C.A. Shuttleworth, A.S. Weiss, C.M. Kielty, Molecular basis of elastic fiber formation. Critical interactions and a tropoelastin-fibrillin-1 cross-link, J. Biol. Chem. 279 (2004) 23748–23758.
[86] T.M. Trask, B.C. Trask, T.M. Ritty, W.R. Abrams, J. Rosenbloom, R.P. Mecham, Interaction of tropoelastin with the amino-terminal domains of fibrillin-1 and fibrillin-2 suggests a role for the fibrillins in elastic fiber assembly, J. Biol. Chem. 275 (2000) 24400–24406.
[87] A.W. Clarke, S.G. Wise, S.A. Cain, C.M. Kielty, A.S. Weiss, Coacervation is promoted by molecular interactions between the PF2 segment of fibrillin-1 and the domain 4 region of tropoelastin, Biochemistry 44 (2005) 10271–10281.
[88] A.M. Tamburro, A. Pepe, B. Bochicchio, D. Quaglino, I.P. Ronchetti, Supramolecular amyloid-like assembly of the polypeptide sequence coded by exon 30 of human tropoelastin, J. Biol. Chem. 280 (2005) 2682–2690.
[89] B. Bochicchio, A. Pepe, R. Flamia, M. Lorusso, A.M. Tamburro, Investigating the amyloidogenic nanostructured sequences of elastin: sequence encoded by exon 28 of human tropoelastin gene, Biomacromolecules 8 (2007) 3478–3486.
[90] P. Brown-Augsburger, T. Broekelmann, J. Rosenbloom, R.P. Mecham, Functional domains on elastin and microfibril-associated glycoprotein involved in elastic fibre assembly, Biochem. J. 318 (Pt 1) (1996) 149–155.
[91] U.R. Rodgers, A.S. Weiss, Cellular interactions with elastin, Pathol. Biol. (Paris) 53 (2005) 390–398.
[92] N. Floquet, A. Pepe, M. Dauchez, B. Bochicchio, A.M. Tamburro, A.J. Alix, Structure and modeling studies of the carboxy-terminus region of human tropoelastin, Matrix Biol. 24 (2005) 271–282.
[93] H. Yanagisawa, E.C. Davis, Unraveling the mechanism of elastic fiber assembly: the roles of short fibulins, Int. J. Biochem. Cell Biol. 42 (2010) 1084–1093.
[94] T. Nakamura, P.R. Lozano, Y. Ikeda, Y. Iwanaga, A. Hinek, S. Minamisawa, C.F. Cheng, K. Kobuke, N. Dalton, Y. Takada, K. Tashiro, J. Ross Jr., T. Honjo, K.R. Chien, Fibulin-5/DANCE is essential for elastogenesis in vivo, Nature 415 (2002) 171–175.
[95] H. Yanagisawa, E.C. Davis, B.C. Starcher, T. Ouchi, M. Yanagisawa, J.A. Richardson, E.N. Olson, Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo, Nature 415 (2002) 168–171.
[96] P.J. McLaughlin, Q. Chen, M. Horiguchi, B.C. Starcher, J.B. Stanton, T.J. Broekelmann, A.D. Marmorstein, B. McKay, R. Mecham, T. Nakamura, L.Y. Marmorstein, Targeted disruption of fibulin-4 abolishes elastogenesis and causes perinatal lethality in mice, Mol. Cell. Biol. 26 (2006) 1700–1709.
[97] R. Choudhury, A. McGovern, C. Ridley, S.A. Cain, A. Baldwin, M.C. Wang, C. Guo, A. Mironov Jr., Z. Drymoussi, D. Trump, A. Shuttleworth, C. Baldock, C.M. Kielty, Differential regulation of elastic fiber formation by fibulin-4 and -5, J. Biol. Chem. 284 (2009) 24553–24567.
[98] H.M. Kagan, K.A. Sullivan, Lysyl oxidase: preparation and role in elastin biosynthesis, Methods Enzymol. 82 (1982) 637–650.
[99] L. Thomassin, C.C. Werneck, T.J. Broekelmann, C. Gleyzal, I.K. Hornstra, R.P. Mecham, P. Sommer, The Pro-regions of lysyl oxidase and lysyl oxidase-like 1 are required for deposition onto elastic fibers, J. Biol. Chem. 280 (2005) 42848–42855.
[100] P. Brown-Augsburger, C. Tisdale, T. Broekelmann, C. Sloan, R.P. Mecham, Identification of an elastin cross-linking domain that joins three peptide chains. Possible role in nucleated assembly, J. Biol. Chem. 270 (1995) 17778–17783.
[101] S.G. Wise, S.M. Mithieux, M.J. Raftery, A.S. Weiss, Specificity in the coacervation of tropoelastin, J. Struct. Biol. 149 (2005) 273–281.
[102] P. Fratzl, K. Misof, I. Zizak, G. Rapp, H. Amenitsch, S. Bernstorff, Fibrillar structure and mechanical properties of collagen, J. Struct. Biol. 122 (1998) 119–122.
[103] S.M. Mithieux, J.E. Rasko, A.S. Weiss, Synthetic elastin hydrogels derived from massive elastic assemblies of self-organized human protein monomers, Biomaterials 25 (2004) 4921–4927.
[104] C.M. Pollock, R.E. Shadwick, Relationship between body mass and biomechanical properties of limb tendons in adult mammals, Am. J. Physiol. 266 (1994) R1016–R1021.
[105] K.G. Danielson, H. Baribault, D.F. Holmes, H. Graham, K.E. Kadler, R.V. Iozzo, Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility, J. Cell Biol. 136 (1997) 729–743.
[106] F.H. Silver, J.W. Freeman, D. DeVore, Viscoelastic properties of human skin and processed dermis, Skin Res. Technol. 7 (2001) 18–23.
[107] M.A. Lillie, J.M. Gosline, The viscoelastic basis for the tensile strength of elastin, Int. J. Biol. Macromol. 30 (2002) 119–127.
[108] L.D. Muiznieks, A.S. Weiss, F.W. Keeley, Structural disorder and dynamics of elastin, Biochem. Cell Biol. 88 (2010) 239–250.
[109] L.D. Muiznieks, A.S. Weiss, Flexibility in the solution structure of human tropoelastin, Biochemistry 46 (2007) 8196–8205.
[110] P. Toonkool, D.G. Regan, P.W. Kuchel, M.B. Morris, A.S. Weiss, Thermodynamic and hydrodynamic properties of human tropoelastin. Analytical ultracentrifuge and pulsed field-gradient spin-echo NMR studies, J. Biol. Chem. 276 (2001) 28042–28050.
[111] C. Baldock, A.F. Oberhauser, L. Ma, D. Lammie, V. Siegler, S.M. Mithieux, Y. Tu, J.Y. Chow, F. Suleman, M. Malfois, S. Rogers, L. Guo, T.C. Irving, T.J. Wess, A.S. Weiss, Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4322–4327.
[112] M. Miao, C.M. Bellingham, R.J. Stahl, E.E. Sitarz, C.J. Lane, F.W. Keeley, Sequence and structure determinants for the self-aggregation of recombinant polypeptides modeled after human elastin, J. Biol. Chem. 278 (2003) 48553–48562.
[113] C.A.J. Hoeve, P.J. Flory, The elastic properties of elastin, Biopolymers 13 (1974) 677–686.
[114] G.M. Bressan, I. Pasquali-Ronchetti, C. Fornieri, F. Mattioli, I. Castellani, D. Volpin, Relevance of aggregation properties of tropoelastin to the assembly and structure of elastic fibers, J. Ultrastruct. Mol. Struct. Res. 94 (1986) 209–216.
[115] C.M. Bellingham, M.A. Lillie, J.M. Gosline, G.M. Wright, B.C. Starcher, A.J. Bailey, K.A. Woodhouse, F.W. Keeley, Recombinant human elastin polypeptides self-assemble into biomaterials with elastin-like properties, Biopolymers 70 (2003) 445–455.
[116] M.A. Lillie, G.J. David, J.M. Gosline, Mechanical role of elastin-associated microfibrils in pig aortic elastic tissue, Connect. Tissue Res. 37 (1998) 121–141.