Collagen in Wound Healing

Subjects: Others | Cell & Tissue Engineering | Others

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Collagens are the most abundant protein found throughout the body. In the healing wound, these collagens are synthesized by cells such as fibroblasts and modified into complex morphologies . The type, amount and organization of collagen changes in the healing wound and determines the tensile strength of the healed skin. Collagen III is the first to be synthesized in the early stages of wound healing and is replaced by collagen I, the dominant skin collagen. The initial random deposition of collagen during the granulation tissue formation is further enhanced by lysyl oxidase enzyme-induced covalent cross-linking. This process matures the collagen into complex structures that are reoriented for tensile strength restoration. Collagen remodeling continues for months after wound closure and the tensile strength of the repaired tissue increases to about 80–85% of normal tissue if all processes proceed without any perturbations.

In the skin, the fibrillar collagens types I, III and V are the most common, followed by fibril-associated collagens type XII, XIV, XVI, and VI. The non-fibrillar collagens type IV, XVIII are found in the basement membrane of the skin

collagen wound healing

1. Processing of Collagen in the Skin and Wound

1.1. Biosynthesis and Cross-Linking

In the healing wound, cells such as fibroblasts (resident, and myeloid cell converted fibroblasts) [1] are the main source of newly synthesized collagen. The biosynthesis activities of fibril-forming collagens are the most extensively studied among all the collagens and involve multiple complex steps requiring the temporal and spatial coordination of several biochemical events [2][3]. Following transcription, the nascent/pre-pro-collagen is post-translationally modified in the endoplasmic reticulum into pro-collagen with the removal of the signal peptide on the N-terminus. Hydroxylation and glycosylation of amino acid residues results in the formation of the triple-helical structure characteristic of collagens. Supported by chaperone proteins, the pro-collagen triple-helical structure is stabilized for further processing and maturation in the Golgi apparatus and assembled into secretory vesicles that are extruded into the extracellular space where the pro-collagen is enzymatically modified into tropocollagen. The final collagen fibril assembly occurs by covalent cross-linking. The mechanical properties (elasticity and reversible deformation) of fibrillar collagens are dependent on this cross-linking process. Some of these cross-links include: (1) disulfide bonds; (2) reducible and mature cross-links produced via the lysyl oxidase pathway; (3) transglutaminase cross-links; and (4) advanced glycation end (AGE) product-mediated cross-links, among others. The nuances of cross-linking vary with the type of collagen and the tissue context and creates a multi-layered

hierarchical structure [4]. Mature cross-links add resistance to shear stress. AGE-specific cross-links contribute to increased stiffness of collagens in aged tissues.

1.2. Degradation

Collagen degradation is involved in inflammation, angiogenesis, and re-epithelialization in the wound regulated by complex molecular pathways [5]. During the inflammation phase, soluble fragments from collagen degradation recruit immune cells such as macrophages that patrol the wound for removal of microbes and devitalized tissue. This aids in the transition to the proliferative phase. During this stage, collagen fragments serve as potent angiogenic signals to promote the development of new blood vessels. Keratinocyte migration is also promoted by collagen and contributes to wound re-epithelialization [6][7][8]. Degradation is regulated by extracellular and intracellular pathways. The former involves membrane-bound and secreted proteolytic enzymes. The latter involves internalization of intact collagen fibrils and fragmented collagen (through phagocytosis, macropinocytosis or endocytosis), followed by enzymatic breakdown. Defects in the regulated turnover of collagens results in pathological conditions such as fibrosis [9][10][11][12].

The actions of proteolytic enzymes at different stages in the wound healing process guides the remodeling of the repaired tissue. Two important enzyme families are the matrix metalloproteinases (MMPs) and serine proteases. The production and secretion of these enzymes are tightly regulated and are associated with specific cellular subtypes [13][14]. Among the MMPs, collagenases and gelatinases, which degrade intact and damaged fibrillar collagen respectively, are key for collagen turnover during wound healing. Collagens I and III are preferentially cleaved by MMP-1 (also called collagenase-1) and MMP-8 (collagenase-2) while collagen IV is degraded by the gelatinase MMP-9. Extensive research has determined that collagenolytic enzymes can recognize, bind, unwind and cleave the individual strands of the triple helix. It is speculated that this high specificity could be driven by the primary and super-secondary structures of collagen. MMPs drive physiological (development and tissue repair) and pathological (tumorigenesis and metastasis) processes. They also contribute to the release of bioactive fragments (also termed matricryptins) such as endostatin and tumstatin from full-length collagens [15]. These fragments specifically guide blood vessel pruning that in turn enables the re-establishment of the tissue architecture during healing [16][17][18][19][20]. Neutrophil elastase is a serine protease that aids in the same process. A balance of enzyme activity and inhibition is required for normal wound healing and is under tight regulatory control. Imbalances in the levels of these enzymes are a factor in wound chronicity. Wounds infected with microbes that produce these collagen-degrading enzymes add to the imbalance, leading to chronic wounds.

1.3. Receptor-Mediated Signaling

Collagen in all its forms, triple-helical, matrix-incorporated and degraded fragments, are cognate ligands of diverse families of cell surface receptors including integrins, receptor tyrosine kinases and immunoglobulin type receptors [21][22]. In the wound environment, collagens mediate several key steps such as platelet aggregation, inflammation modulation, angiogenesis, granulation tissue formation and re-epithelialization in a integrin signaling-dependent manner [11][12][22]. Receptor tyrosine kinases such as Discoidin Domain Receptors (DDR-1 and DDR-2) bind matrix-

incorporated collagen and regulate key wound healing processes. Loss of function of these signaling molecules inhibits keratinocyte proliferation and collagen remodeling during wound healing, resulting in wounds with low tensile strength [23]. Abnormal signaling induced by collagen is observed in pathological conditions such as scar formation [12].

2. Roles for Collagen in the Skin and Wound

Collagen contributes to the mechanical strength and elasticity of tissues and acts as a natural substrate for cellular attachment, proliferation, and differentiation (Figure 1) [6][2][7][8]. Biofilm-mediated upregulation of MMP-2 via microRNAs creates a collagenolytic environment in the wound, sharply decreasing the collagen I/collagen III ratio and compromising the biomechanical properties of the repaired skin, possibly making the repaired skin vulnerable to wound recurrence [24]. A recent mapping study of collagen structure and function suggested that in normal, injured tissue the collagen fibril is in a closed conformation that upon exposure to blood following injury exposes cell- and ligand-binding sites that could promote the wound healing process [9]. Several recent reviews detail roles of collagen in the skin and wounds [21][25][26][27][28][29][30][31][32][33].

2.1. Role in Inflammation

The inflammatory phase of wound healing includes hemostasis and inflammation [34]. Collagen exposure due to injury activates the clotting cascade, resulting in a fibrin clot that stops the initial bleeding. Collagen I and IV fragments can be mediators of inflammation by acting as potent chemoattractants for neutrophils, enhancing phagocytosis and immune responses and modulating gene expression [35][15]. Inflammation is a critical step in the normal process of wound healing and drives the proliferation of fibroblasts which synthesize collagen and ECM [36]. The resolution of inflammation in a timely manner is equally important in normal wound healing. Resolution of inflammation is an active process that is driven by balanced pro and anti-inflammatory responses. A study using a stabilized collagen matrix showed that collagen mounts a robust and sharp inflammatory response that is transient and resolves rapidly to make way for wound healing to advance [37]. Furthermore, an important role for collagen in promoting an anti-inflammatory, pro-angiogenic wound macrophage phenotype via microRNA signaling has also been demonstrated [38][39].

2.2. Role in Angiogenesis

Angiogenesis, a critical component of physiological (development, wound healing) and pathological (cancer) processes, is tightly regulated by the balanced activity of stimulators and inhibitors. ECM remodeling provides critical support for vascular development and collagens play an important role in this process [38][40][41][42][43]. Depending on the type of collagen, the role might be as a promoter or inhibitor of angiogenesis. A live analysis via multiphoton microscopy of neovessel formation in vitro identified a dynamic modulation of collagen I that showed early stage remodeling of collagen fibrils progressing to collagen condensation in later stages of development [44]. Collagen I is known to potently stimulate angiogenesis in vitro and in vivo through engagement of specific integrin receptors. Specifically, the C-propeptide fragment of collagen I recruits endothelial cells, potentially triggering

angiogenesis in the healing wound [13]. By contrast, proteolytic collagen fragments of collagen IV and XVIII (e.g., endostatin, arresten, canstatin, tumstatin) show anti-angiogenic properties [16][17]. Studies have shown a role for these fragments in inhibiting proliferation and migration of endothelial cells and inducing endothelial cell apoptosis. These fragments are of interest in curbing angiogenesis in several pathological conditions [13][35][15].

2.3. Role in ECM Remodeling

Collagens are a structural component of the ECM that contribute to skin flexibility in addition to stabilizing growth factors and regulating cell adhesion and signaling between cells and ECM. In the process of wound healing, as the wound tissue undergoes remodeling over years, the adult wound heals with the formation of a 'normal' scar. The scar tissue regains anywhere from 50–80% of the original tensile strength of normal skin but may be functionally deficient [45]. The main difference between the scar and unwounded skin appears to be the density, fiber size and orientation of the collagen fibrils [7].

Abnormalities in the ECM reconstitution during wound healing result in hypertrophic and keloid scars. Scarring is a consequence of altered levels of the same molecules that typically make up the ECM, i.e., collagen I and III, fibronectin and laminin are abnormally high in scar tissue [45]. Collagen fiber orientation in scars (normotrophic, hypertrophic and keloid) are parallel to the epithelial surface unlike that of normal skin where the fibers form a three-dimensional basketweave-like network [46]. There are structural and compositional differences between these types of scars. Keloid scars are characterized by abnormally thick bundles of collagen that are poorly organized with fewer cross-links that are found in the deep dermis compared to superficial dermic. Hypertrophic scars have thinner collagen bundles than keloid or normotrophic scars [47][48][49]. The ratio of collagen I to III is higher in keloids than normotrophic scars. Even within the keloid scar, there is a heterogeneity to the collagen I/III ratio [50].

3. Effect of Aging on Collagen in the Skin and Wound

The aged skin has lower collagen density that is increasingly cross-linked and fragmented [51][52]. Together with senescence, collagen fiber remodeling results in increased stiffness. Furthermore, the aging skin has a higher percentage of collagen III [53]. Collagen organization visualized through Fourier transformed infrared imaging, scanning electron microscopy and histological staining showed fragmented, clustered and coarse fiber bundles that are oriented parallel to the skin surface in aging skin [54][55][56]. Age-induced alterations (reduced collagen deposition and increased non-enzymatic cross-linking) in collagen impact the mechanical environment of the skin and predispose it to wound healing impairments [57][58][59].

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