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Active Peptides and Motifs within Collagen and Other ECM Proteins

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Abstract

Collagens are the most abundant proteins in mammals and form an extracellular matrix (ECM) with other components as the structural support of muscle, skin, corneas and blood vessels etc. Other than providing structural support, the ECM exhibits active communication with cells and influences many cellular processes including migration, wound healing, differentiation and cancer metastasis. Though collagen proteins contain highly repetitive primary sequences and defined tertiary structures, more and more studies have shown that many short peptides/motifs within collagen proteins play key roles in various biological processes. These short sequences are effective within triple helical structures or independently as stand-alone molecules resulting from proteolytic degradation. Besides endogenous ECM-derived peptides, many more functional peptides have been produced by tissue processing, chemical synthesis, and recombinant protein production. In this review, we summarize different peptides/motifs identified in collagen and other ECM proteins and discuss their potential for medical, personal care, and cosmetics applications.

Keywords

Collagen, Extracellular Matrix, Peptides, Recombinant Protein

1. Introduction

Structural proteins such as collagens, laminins, and elastin form the backbone of mammal tissues. Collagens, the most abundant proteins in the human body, comprise 28 different types with 42 individual procollagen genes [1]. Each collagen gene encodes a polypeptide *α*-chain containing a unique repeated sequence, "GXY", where X is often proline and Y is hydroxyproline. These repeats form characteristic homo- or hetero-triple helical structures such as collagen type I

(heterotrimer) and type III (homotrimer). Other than GXY repeat regions, procollagen genes also code for non-collagenous domains that are essential to collagen production and function. Besides triple helical domains, collagens contain non-triple helical domains that are used as building blocks by other extracellular matrix proteins.

In addition to structural properties, the identification of the biological importance of collagen in cell signaling and regulation of ECM function is increasing. The collagen triple helical regions include distinct domains and motifs composed of only a few amino acids, and interact with numerous molecules that trigger biological events [1] [2] [3]. Interestingly, proteolytic collagen degradation products arising from the cleavage by matrix metalloproteinases (MMP) display novel biological activities such as angiogenic, antimicrobial and mitogenic properties not exhibited by the intact parent proteins [2] [4] [5]. Additionally, collagen peptides that are produced by chemical hydrolysis or exogenous enzyme treatment also display anti-freezing, antioxidative and anti-tumorigenic properties. Many recombinant collagen proteins and collagen mimetic peptides have been produced and evaluated for bioactivity as well. For example, fragment SMM located within the COL1A2 alpha chain can upregulate collagen type I and elastin synthesis in dermal fibroblast cells [6]. Another recombinant collagen type III alpha chain produced in yeast shows stimulation of endogenous collagen III synthesis [7]. A fully synthetic peptide (Matrixyl) derived from collagen sequence is widely used as a collagen-boosting cosmetic ingredient [8]-[16].

Other ECM proteins, including laminins, elastin, and fibronectin, are important for various biological functions. Laminins are a major constituent of the basement membrane, a thin layer of specialized extracellular matrix that supports many human tissues. Elastin, as one of the dominant proteins in extensible tissue and primarily present in the lungs, aorta, and skin, provides resilience and elasticity to tissues and organs [17]. Fibronectin, secreted as a soluble, covalently bound dimer, mediates cell-ECM interaction during fundamental events such as development, wound healing, fibrosis, and tumor progression [18].

2. Motifs within Collagen Type I

GHK—Type I collagen is the most abundant and best-investigated type of collagen that is expressed in almost all connective tissues. In most cases, it is composed of two α1 chains (COL1A1) and one α2 chain (COL1A2), although an α1 homotrimer exists as a minor form. The tripeptide GHK is present in the human collagen COL1A2 alpha chain. Original studies indicated that GHK isolated from human plasma caused old human liver tissue to synthesize proteins like younger tissue [19]. Subsequent studies established that this peptide has a strong affinity for copper suggesting that GHK functions as a complex with Cu^{2+} [19] [20] [21] [22]. GHK is involved in many biological processes by regulating the expression of more than 2000 genes with a change greater than or equal to 50% [23]. The presence of a GHK triplet in the type I collagen COL1A2 alpha chain

suggests that the tripeptide might be liberated by proteases at the site of a wound or probably during normal tissue turnover and serve as a built-in modulator of dermal repair [24]. It is hypothesized that GHK is involved in wound healing and tissue repair processes and stimulates biological events in tissue repair such as angiogenesis, nerve outgrowth, and chemoattraction of cells critical to healing [25]. GHK also modulates cancer metastasis genes by suppressing RNA production in 70% of 54 human genes overexpressed in patients with an aggressive metastatic form of colon cancer. GHK peptide is one of two ingredients of Matrixyl® 3000, a commercial cosmetic product widely used to improve skin condition.

KTTKS—The pentapeptide KTTKS is a portion of type I collagen COL1A1 C-propeptide and dramatically stimulates type I collagen, type III collagen, and fibronectin production in fibroblasts in a dose- and time-dependent manner without affecting total protein synthesis [8] [9]. It is hypothesized that the stimulatory effect of KTTKS at a dose as low as 0.5 mM (0.3 ppm) relates primarily to the biosynthetic pathway, rather than the export or degradation pathways [10] [11] [12]. KTTKS is often modified with a palmitoyl group for better penetration and stability, and formulated with other ingredients, such as ascorbic acid, for cosmetic applications [14] [15] [16].

GVMGFO—A GVMGFO motif in fibrillar collagens (I-III) binds three unrelated proteins: von Willebrand factor (VWF), discoidin domain receptor 2 (DDR2), and the extracellular matrix protein SPARC/osteonectin/BM-40. SPARC (Secreted Protein Acidic and Rich in Cysteine), also referred to as BM-40 or osteonectin, is a calcium-binding protein expressed in various tissues. High expression is associated with morphogenesis, tissue repair, and remodeling. SPARC is a counter-adhesive and anti-proliferative protein that regulates the activity of growth factors, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor, and fibroblast growth factor-2. It interacts with the fibrillar collagens (I, II, III, and V), basement membrane collagen IV, vitronectin, thrombospondin 1, albumin, hydroxy-apatite, and fibrinogen fragments. The binding of SPARC to collagens *in vivo* may regulate collagen assembly through sequestration and complex formation with collagen. SPARC is also essential for embryo development in invertebrates and may act as a chaperone for basement membrane collagen IV [26] [27].

VWF and DDR2 bind to similar domains as SPARC. The minimal sequence for VWF binding, RGQOGVMGF, is also found in collagen III [28]. VWF plays a key role in primary hemostasis through platelet and subendothelial collagen adhesion and the interaction of collagen with VWF requires unique structural properties in both proteins. The optimal hemostatic function requires multimerization of up to 50 VWF monomers in circulating plasma and higher-order multimers bind collagen more tightly than smaller assemblies of VWF [28].

DDR binding motifs—The GVM-GFO motif is the main binding site for DDR1 and DDR2. DDR activation by collagen is strictly dependent on the native, triple-helical conformation of collagen, though many studies demonstrated

that synthetic short motif peptides could be functional as well. The ability of collagen to stimulate DDR tyrosine phosphorylation could be due to a direct association of collagen with the extracellular domain of the receptor, or it could represent an indirect effect of collagen, for example, on the clustering of cell surface molecules [29] [30].

DDRs are receptor tyrosine kinases that function as collagen receptors independent of integrin receptors [30] [31] [32]. DDR1 is typically expressed in epithelial cells and DDR2 is restricted to skeletal muscle, heart, and connective tissues [33] [34]. Both receptors control developmental processes, such as mammary gland development (DDR1) and the growth of long bones (DDR2). Alternatively, they are associated with fibrotic diseases of the liver, kidney, and lung; atherosclerosis; osteoarthritis; and several types of cancer. High levels of DDR1 and DDR2 are seen in fast-growing invasive mammary, ovarian, and lung tumors in keeping with the increased proliferative rates and MMP production in these tumors. The recognition of collagen by DDR2 results in the regulation of cell proliferation and migration [35] [36] [37]. In addition, DDR2 controls extracellular matrix remodeling by upregulating both the expression and activity of matrix metalloproteinases.

P-15—The P-15 peptide is a highly conserved peptide that consists of 15 amino acids (GTPGPQGIAGQRGVV) found in the collagen COL1A1 alpha chain, but rarely found in non-collagen proteins [38]. This particular region within collagen I may be a site of conformational fluctuation that could be exposed for cell binding. Chain separation would lead to the relaxation of conformation, facilitate the generation of specific molecular perspectives for recognition, and provide needed conformational flexibility for allosteric events comprising interactions. Without an RGD domain, P-15 is nearly 45 times more potent than RGD-containing peptides for cell binding [38].

P-15 enhances cell attachment to bone substitutes and upregulates ECM production. P-15 upregulates gene expression of alkaline phosphatase (ALP), BMP-2 and BMP-7 when added in scaffold material indicating that P-15 may promote osteoblastic activity in human osteoblast cells [38] [39]. P-15 was also found to stimulate the proliferation and differentiation rate as well as the growth factor production of osteoblasts *in vitro*. Preclinical results show that P-15-containing bone graft substitutes could facilitate bone healing and regeneration [38]. In bone defects, the application of P-15 containing bone substitutes increased the rate of bone growth compared to the defects left empty or filled with bone substitute alone.

LAIR binding motifs—Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1) is a member of the immunoglobulin superfamily and expressed in almost all immune cells, including NK cells, T cells, B cells and monocytes, monocyte-derived dendritic cells, eosinophils, basophils and mast cells [40] [41] [42] [43]. It recognizes multiple collagens with conserved GPO collagen repeats. Binding of collagen to LAIR-1 directly inhibits immune cell activation *in vitro*.

In addition, collagens could play a role in the regulation, differentiation, and proliferation of CD34+ progenitor cells by interacting with LAIR-1 in the bone marrow. Collagen type I fragments, generated by MMP1 or MMP9 in cancer, mediate T cell immunosuppression by binding and triggering LAIR-1. These LAIR-1 triggered by collagen I fragments also inhibit CD3 signaling and IFN-g secretion in a T cell line. Proteomic and RNA profiling revealed increased collagen and LAIR-1 levels resulting in lung tumors resistant to PD-1/PD-L1 blockade [44]. Elevated collagen level correlates with decreased total CD8+ T cells and increased exhausted CD8+ T cell subpopulations in lung tumors [45].

Other than LAIR-1, LAIR-2 protein is expressed as a soluble receptor with high affinity for various collagen molecules in a hydroxyproline-dependent manner. Soluble LAIR-2 may function as a natural competitor for LAIR-1 and regulate its inhibitory activity. Indeed, LAIR-2 prevents binding of human LAIR-1 to collagens and LAIR-1 cross-linking *in vitro*, suggesting that the protein has an immunoregulatory function *in vivo* [46] [47].

Heparin-binding motif—the glycosaminoglycan chains of cell surface heparan sulfate proteoglycans regulate cell adhesion, proliferation, and extracellular matrix assembly through their interactions with heparin-binding proteins. HSPGs, heparan sulfates, and heparin can bind to type I collagen fibrils [48] [49]. Heparin-binding sequences consist of nine basic amino acids, six of them contributed by the α1 chains (KGHRGF) and three by the α2 chain (KGIRGH). The interaction is completely disrupted by high salt, suggesting a primarily electrostatic basis for the binding. It has also been demonstrated that the collagen triple helix structure is required for high-affinity HSPG/heparin-type I collagen binding. The relationships between the cell-attachment sites of type I collagen fibrils and heparin-binding sites are of great interest because heparin and syndecan-1 block cell adhesion to type I collagen *in vitro*. It is proposed that proteoglycans have this effect because they bind extracellular matrix molecules at sites that are adjacent to cell-attachment sites and thereby sterically hinder the binding of cell surface integrin receptors.

Integrins binding motifs—integrins are the largest family of cell adhesion receptors that are composed of one α and one β subunit—two transmembrane proteins noncovalently linked together. Fibrillar collagens support cellular adhesion primarily through a subset of collagen-binding integrins, $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$, which are shown to recognize a series of similar sequences. These contain GXX'GEX" motifs where X is a hydrophobic residue, X' is usually O (hydroxyproline) and X" is often R. Additionally, the E is important for recognition where substituting to D displays a loss of recognition. Peptides with a sequence of GFOGER, when in triple-helical conformation, readily support $\alpha2\beta1$-dependent cell adhesion and exhibit divalent cation-dependent (Mg^{2+}) binding to collagen [50]. The same sequence binds integrin $\alpha1$ A-domain and supports integrin $\alpha1\beta1$-mediated cell adhesion.

Experiments show that the GROGER-containing peptide was able to bind

both $\alpha 1$ and $\alpha 2$ with high affinity and effectively inhibit the binding of $\alpha 1$ and $\alpha 2$ to type III and I collagens, whereas the GAOGER-containing peptide was less effective. Using 57 synthetic peptides in a cell attachment experiment, the identification of two more integrin $\alpha 2\beta 1$ binding sites (GROGER and GLOGEN) within human collagen type III and a third motif (GLKGEN) display intermediate activity [51]. Similar motifs are also found in other than fibril-forming collagens suggesting their importance, especially in collagens that are homotrimers while, in collagens composed of two or three different α chains, the binding mechanism might be more complex.

DGEA—the DGEA tetra-peptide within collagen type I sequence binds to $\alpha 2\beta 1$ integrin and inhibits cell adhesion to collagen and laminin substrates. Deletion of the alanine residue or substitution of alanine for either the glutamic or aspartic acid residues resulted in a marked loss of inhibitory activity [52] [53]. DGEA may promote cell adhesion, spreading, and osteogenic differentiation.

RGD—RGD is the minimal recognition sequence within collagen type I and fibronectin which is required for cell attachment. The RGD motif is presented in slightly different ways in different proteins, making it possible for the many RGD-binding integrins to selectively distinguish individual adhesion proteins. For example, integrin $\alpha v\beta 3$ binds to denatured collagen type I through RGD [54] [55] [56]. Evidence suggests that RGD enhances cell attachment and spreading of osteoblasts onto scaffolds and graft material, whilst increasing cellular proliferation [38]. Further, it promotes osteoblast differentiation and mineralization. RGD-coated implants increase peri-implant bone formation and enhance direct bone apposition, even in areas of poor surrounding bone, which significantly increases the bone-to-implant contact. Finally, RGD-containing scaffolds used to deliver growth factors, such as BMP-2 to promote bone regeneration in experimental fracture models, exhibit favorable results [38].

Other peptides/motifs within collagen I include a decorin binding motif with a sequence of KXGDRGE or AKGDRGE. Decorin is the archetypal small leucine-rich repeat proteoglycan of the vertebrate extracellular matrix and one molecule of decorin interacts with four to six collagen molecules [57]. The osteoclast-associated receptor (OSCAR) is a collagen-binding immune receptor with important roles in dendritic cell maturation and activation of inflammatory monocytes as well as in osteoclastogenesis. OSCAR binds to a defined consensus recognition sequence, GXOGPXGFX, and the orphan motif GAOGASGDR [58] [59]. The C-propeptide of human COL1A1 procollagen was found to stimulate the production of both collagen and fibronectin by 6-8-fold in a cell culture assay [8]. A small domain within the human COL1A2 alpha chain is produced as a recombinant protein and promotes fibroblast cell proliferation and collagen type I synthesis. This human COL1A2-derived peptide also enhances wound healing and elastin production [6].

So far, almost 50 molecules have been found to interact with type I collagen and about half of their binding sites on this collagen have been identified [60]. In

this work, all the ligand-binding sites and mutations of type I collagen were mapped and several hotspots for ligand interactions were revealed. This "interactome" reveals the existence of several hot spots for ligand interactions. At the same time, two exclusively lethal regions seem to align with the major ligand-binding regions (MLBR). Interestingly MLBR2 is known to contain high-affinity binding sites for $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins [40]. Similar linear maps of collagen type III and type IV have also been constructed with major structural landmarks, known and predicted ligand-binding sites, and missense mutations to demonstrate potential functional domains and ligand interactions and explain genotype-phenotype variation in inherited disease [61] [62].

3. Motifs within Collagen Type III

GG motif—the fibrillar collagen type III is the second most abundant collagen and consists of only one collagen alpha chain that forms a homotrimer. A distinctive sequence feature of type III collagen is the glycine pair (GGY and GXG) motifs. Glycine pairs may enable chain flexibility to the rigid and tightly packed collagen triple helix as glycine is the smallest amino acid. This flexibility could potentially affect the structural and biological functions of type III collagen [63].

KOGEOGPK—several platelet receptors interact with type I and type III collagens, including GP Ia/IIa and GP VI. A platelet receptor (TIIICBP) specific binding sequence, the KOGEOGPK octapeptide, is a type III collagen-related primary binding motif. This binding is Ca^{2+} and Mg^{2+} independent, not inhibited by an antibody against GP VI, and triggers platelet protein tyrosine phosphorylation [64].

N-propeptide—kinetic binding studies show that a synthetic peptide containing a COL3 cysteine-rich (CR) domain found within its N-propeptide binds in a dose-dependent manner to TGFβ1. The CR peptide attenuates TGFβ signaling in fibroblasts and 4T1 breast cancer cells, and suppresses fibroblast activation and contraction, as assessed by smooth muscle actin staining, cell wrinkling of deformable silicone, and stressed-fibroblast populated collagen lattice contraction assays. CR peptide treatment of orthotopically injected breast cancer cells (4T1) suppresses intratumoral fibroblast activation and inhibits primary tumor growth compared to CR control [65].

50 kDa protein—A recombinant 50 kDa protein is produced in yeast that is 100% identical to the N-terminus of the human type III collagen helical domain. This protein was shown to stimulate both collagen type I and type III production and secretion by primary human dermal fibroblasts in an *in vitro* study. This final protein product was also shown to be safe for general applications to human skin and compatible with common formulation protocols, including ethanol-based formulations [7].

4. Motifs within Other Collagens

Type II collagen is a fibrillar collagen, and the main component of cartilage and

its stability and strength provide the tissue with integrity and resiliency to stress [1]. A fibronectin-binding region resides in the sequence of collagen type II. Solid-state binding assays on the chimeric collagen showed that the 6-triplet type II collagen sequence, GLAGQRGIVGLPGQRGER, was the minimal requirement for FN binding in both triple-helical and denatured conditions. It is not clear if the native protein binds fibronectin with the same primary sequence [66].

Type IV collagen is a main component of the basement membrane extracellular matrix. Numerous matrilines are described to derive from the helical domain of α(IV) chains and promote tumor cell adhesion or spreading [61] [67] [68]. The adhesion of various cancer cells to the α1(IV) 531-543 peptide is mediated by the $\alpha 3\beta 1$ integrin and triggers an intracellular transduction pathway, which involves the phosphorylation of p125FAK and induces the secretion of active proteases, such as MMPs.

Canstatin from collagen IV is an endogenous anti-angiogenic and anti-tumor factor, which also has an anti-lymphangiogenic effect [69]. It is abundantly expressed in the heart tissue and exerts various biological activities in cardiac cells by modulating voltage-dependent calcium channel activity in cardiomyocytes.

5. Other Collagen Binding Partners

Many other proteins have been reported to interact with various collagen molecules via different motifs [70] [71]. Aegyptin binds to VWF binding motif RGQOGVMGF with high affinity and inhibits platelet aggregation. Additionally, aegyptin interacts with linear RGQPGVMGF peptide and heat-denatured collagen, suggesting that the triple-helix and hydroxyproline may not be required for binding [72]. A platelet protein of 47 kDa, isolated from human bone, binds to type III collagen but not type I collagen and inhibits type III collagen-induced platelet aggregation in a dose-dependent manner [73]. The Yersinia adhesin YadA binds to many peptides spanning the sequences of type II and III human collagens and mediates the adhesion of the human enteropathogen Yersinia enterocolitica to collagens and other components of the extracellular matrix [74]. GPR56, an orphan G protein-coupled receptor (GPCR) from the family of adhesion GPCRs, plays an indispensable role in cortical development and lamination. The collagen type III COL3A1 chain was found to bind GPR56 through an *in vitro* biotinylation/proteomics approach. Functional studies suggest that the interaction of collagen III with its receptor GPR56 inhibits neural migration *in vitro*. By acting as the major ligand of GPR56, collagen III may regulate the proper lamination of the cerebral cortex in the developing brain [75]. Pigment epithelium-derived factor (PEDF) is a multifunctional extracellular protein. In addition to its known anti-angiogenic and neurotrophic roles in collagen-rich tissues, PEDF is thought to be involved in collagen fibril assembly due to its sequence-specific binding to the collagen fibril and high expression in regions of active bone formation [76]. A sequence located between 929 and 938 amino acids of COL1A1 (IKGHRGFSGL) was identified as PEDF binding site. The com-

ponents of the extracellular matrix influence the biological activity of several growth factors and cytokines including PDGF. Its isoforms (AA, BB, AB) can specifically bind to type I, II, III, IV, V, and VI collagens. The biological activity of collagen-bound PDGF was demonstrated by an increased stimulation of the proliferation of human fibroblasts and mouse 3T3 cells [77].

6. Motifs in Other ECM Proteins

Laminins are a family of heterotrimeric extracellular matrix glycoproteins in the basement membrane of different tissues. Laminins consist of a unique combination of three subchains, α, β and γ chains, that assemble into 15 different laminins. The peptides that originate from the human laminin α4 and α5 chains exhibit a dose-dependent antimicrobial activity against gram-positive and gram-negative bacteria. Other laminin-derived peptides are shown to accelerate wound healing [78]. The 12-mer peptides C16 and A13 bind to integrins $\alpha v\beta 3$ and $\alpha 5\beta 1$ and exhibit angiogenic activity. A13 increases wound re-epithelialization and C16 increases coverage. Both peptides stimulated fibroblast migration in Boyden chamber assays, and downregulated expression of MMP2 collagenase activity in foreskin fibroblasts indicating their role in collagen accumulation. Finally, a bioactive RGD-containing peptide from laminin α1 chain, A99 (AGTFALRGDNPQG), was found to promote strong cell attachment and demonstrated utility in cell culture and tissue engineering [78].

Elastin is a key ECM protein that provides resilience and elasticity to tissues and organs, where elastin is roughly 1000 times more flexible than collagens. It is the dominant protein in extensible tissues and is primarily present in the lungs, aorta, and skin. Elastin degrades over time and degradation products, termed elastin peptides (EPs) and bioactive EPS are named elastokines [79] [80]. The sequence "VGVAPG" within tropoelastin exhibits most of the biological activities of elastin. EPs have been shown to regulate a plethora of biological activities such as cell chemotaxis, proliferation, proteinase production, tumor invasion, angiogenesis, cell survival, reactive oxygen species production, ion flux, and vasomotricity. Some of their biological activities are very beneficial, such as protection of the heart against ischemia/reperfusion injury or tissue repair [79] [80].

The peptide PHSRN is found in the 9th type III domain of fibronectin, adjacent to the 10th domain that contains the RGD peptide. PHSRN is a synergy ligand that enhances the spreading of cells that are attached to substrates presenting the RGD peptide, though PHSRN alone can support the attachment of cells and accelerate wound healing [81] [82] [83].

7. Cryptic Peptides

Other than motifs and peptide with highly conserved sequences in human collagen and ECM proteins, there are numerous peptides with less conserved or unknown sequences distinct functions. For example, GFRGTIGLVG, GPAGPAG, and GFPSG are short peptide sequences from acid-soluble collagen extracted

from scales of croceine croaker [84]. These peptides have antioxidative properties and showed good scavenging activities on hydroxyl radical, DPPH radical, superoxide radical, and ABTS radical. GFPSG is effective against lipid peroxidation in the Lipid Peroxidation Inhibition Assay [84].

8. Medical Implications

The ECM-cell interactions are mediated via cell surface receptors either directly or indirectly with cooperative molecules and undergo continuous remodeling processing that influences cell-signaling pathways. The fragmentation of ECM macromolecules provides even further complexity for the intricate environment of the cells. A healthy body maintains a good balance between the ECM environment and the cells. Oppositely, dysregulation in these interactions can lead to pathological processes and various diseases. More and more attention has been focused on therapeutic applications based on optimizing ECM-cell interactions. So, ECM-cell interaction become a promising target for potent pharmacotherapies development. At the same time, many ECM-derived peptides have been shown to have specific activity in diseases including cancer development [2].

As discussed earlier, many ECM-derived peptides promote wound-healing responses. Peptides containing the GFOGER and GLOGEN motifs present a high affinity for SAF-1 cell adhesion and induce the expression of genes encoding proinflammatory molecules suggesting that specific collagen motifs are involved in the regulation of the inflammatory and healing responses [85]. COL3 deficiency (COL3+/–) in aged mice resulted in accelerated wound closure with increased wound contraction, increased myofibroblast density in wound granulation, and significantly more scar tissues on day 21 post-wounding compared to wild-type mice. Additionally, MCPs (marine collagen peptides) promoted scratch closure in *in vitro* scratch assays [86]. ECM-derived peptides targeted to myocardial infarct site induced angiogenesis and altered the negative remodeling seen after an acute myocardial infarction, suggesting a potentially new strategy for repairing damaged tissue [87]. In combination, this suggests that both fibronectin and collagen affect both myofibroblast differentiation and scar formation in tissue regeneration and repair [88]. ECM-derived peptides cause cellular activation and chemotaxis of a variety of cells through the liberation of bioactive fragments. These fragments and their cellular activities are important in the development and progression of tissue injury seen in chronic lung disease.

ECM-derived peptides impact neurological functions demonstrated in numerous studies. Hydrolyzed collagen peptides enhanced hippocampal neurogenesis and exerted emotional behavior in adult mice [89]. A dipeptide PO significantly decreased depression-like behavior, and significantly increased the gene expression of brain-derived neurotrophic factor and nerve growth factor in the hippocampus which promoted proliferation of neural progenitor cells. PO also increases the dopamine concentration in the prefrontal cortex. [90]. Collagen hydrolysates (CH) impact language cognitive function and brain structure, as

shown by increased brain activity [91].

Bone formation and density are also impacted by ECM-derived peptides. Investigations in rodents as well as *in vitro* experiments suggest an anabolic influence of specific collagen peptides on bone formation and bone mineral density. Specific collagen peptides increased bone mineral density and increased bone formation markers in postmenopausal women with primary, age-related reduction of bone mineral density [92]. Additionally, oral administration of bovine collagen peptide combined with calcium citrate inhibits bone loss [93].

Finally, more and more evidence shows that ECM-derived peptides impact diseases such as cancer. In tumors, collagens and many other ECM molecules are mainly produced by fibroblasts, and recent evidence points towards the role of tumor-derived collagens in tumor progression and metastasis. Specifically, collagen type III induces tumor dormancy in both *in vitro* and *in vivo* mouse models [94]. Collagen also regulates immune evasion and affects the function and phenotype of various types of tumor-infiltrating immune cells, such as tumor-associated macrophages (TAMs) and T cells. This suggests that tumor-associated collagen could have important immune modulatory functions within the tumor microenvironment, affecting cancer progression as well as the efficacy of cancer immunotherapy. Collagens could serve as prognostic markers for cancer patients, and therapeutic strategies targeting the collagen ECM have the potential to prevent tumor progression and metastasis [94] [95] [96].

9. Cosmetics

Several human studies demonstrated the occurrence of two major collagen peptides, PO and OP, in human peripheral blood. Many *in vitro* studies have demonstrated that PO and OP exert chemotaxis on dermal fibroblasts and enhance cell proliferation. Additionally, PO enhances the production of hyaluronic acid by dermal fibroblasts [97]. These findings suggest that the amounts of PO and OP in blood are important factors in showing the efficacy of collagen hydrolysates on skin health which leads to the improvement in facial skin conditions, including facial skin moisture, elasticity, wrinkles and roughness by topical application [97] [98] [99] [100] [101]. While uneven skin pigmentation is a significant cosmetic concern, the tetrapeptide PKEK was found to exert skin whitening effects based on one *in vitro* and four double-blinded vehicle-controlled *in vivo* studies [102].

10. Perspectives and Recombinant Collagen Technology

Animal-derived collagen products are widely used but with their own concerns and limitations. Mad cow disease, a brain disease caused by abnormal proteins, is a particularly worrisome concern. Other concerns about using natural collagen products are associated with their potential antigenicity, interaction with secreted antibodies, and immunogenicity, elicitation of the immune response. Meanwhile, researchers suggested that cryptic epitopes, generated by enzymatic

degradation or denaturation of triple helices of the collagen-based materials, may also interact with antibodies [103]. Scientists believe that primary exposure to exogenous collagen is dietary and about 2% to 4% of the total population has an inherent immunity, *i.e.*, allergy, to bovine collagen I. In general, biomedical products manufactured using animal-derived collagens do not present any significant danger to human recipients. However, some concerns still exist about the potential negative impacts of non-collagenous molecules that co-purify with animal-derived collagen.

The application of recombinant human collagens in research and medicine, as well as drug, food, and cosmetic industries, offers an attractive alternative to the use of the animal-derived collagen materials. Despite the overall safety of the animal collagens, the human collagens offer ultimate biocompatibility and safety. Moreover, technologies to produce recombinant collagens would potentially provide quantities of less abundant collagen types that would be impossible to isolate from tissues. Furthermore, technologies to produce recombinant collagens may be utilized to produce unique collagenous proteins that correspond to those from other animal groups, including avian and marine species. By understanding the various motifs within collagen and ECM molecules, it is possible to design and produce these recombinant collagens for cosmetic, personal care and biomedical uses.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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