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REVIEW ARTICLE

Collagenolytic Enzymes and their Applications in Biomedicine

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Abstract: Nowadays, enzymatic therapy is a very promising line of treatment for many different diseases. There is a group of disorders and conditions, caused by fibrotic and scar processes and associated with the excessive accumulation of collagen that needs to be catabolized to normalize the connective tissue content. The human body normally synthesizes special extracellular enzymes, matrix metalloproteases (MMPs) by itself. These enzymes can cleave components of extracellular matrix (ECM) and different types of collagen and thus maintain the balance of the connective tissue components. MMPs are multifunctional enzymes and are involved in a variety of organism processes. However, under pathological conditions, the function of MMPs is not sufficient, and these enzymes fail to deal with disease. Thus, medical intervention is required. Enzymatic therapy is a very effective way of treating such collagen-associated conditions. It involves the application of exogenous collagenolytic enzymes that catabolize excessive collagen at the affected site and lead to the successful elimination of disease. Such collagenolytic enzymes are synthesized by many organisms: bacteria, animals (especially marine organisms), plants and fungi. The most studied and commercially available are collagenases from *Clostridium histolyticum* and from the pancreas of the crab *Paralithodes camtschatica*, due to their ability to effectively hydrolyse human collagen without affecting other tissues, and their wide pH ranges of collagenolytic activity. In the present review, we summarize not only the data concerning existing collagenase-based medications and their applications in different collagen-related diseases and conditions, but we also propose collagenases from different sources for their potential application in enzymatic therapy.

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1. INTRODUCTION

Non-specific proteolytic enzymes are widely used in medicine. There are several disorders involving excessive collagen accumulation (such as fibrous skin transformations [1], scars and strictures) or the formation of necrotic connective tissues at chronic wounds, burns and ulcers. The use of specific collagenolytic enzymes – collagenases (*e.g.*, microbial collagenase, EC 3.4.24.3) – would seem to be appropriate in these cases.

A large-scale family of matrix metalloproteases is known to play an important role in collagen catabolism in humans and other mammals. However, their application in the pharmaceutical industry is limited due to the difficulties of extraction and purification. Therefore, collagenase preparations isolated from bacteria and other organisms are commonly used.

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Medicines based on collagenase isolated from *Clostridium histolyticum* have been effectively used for more than 60 years. Since the 1990-s, there has been a development in medicines based on collagenases from aquatic organisms (mainly crab hepatopancreas). Nevertheless, collagenolytic enzymes from many other organisms, from bacteria to plants are being studied extensively [2]. Due to their unique properties, these enzymes are proposed as active compounds for novel effective drugs.

In the present review, we analyse the existing data devoted to the mechanisms of different types of collagenases obtained from different sources as well as collagenase-based applications as therapeutic agents for the treatment of chronic wounds, burns, ulcers and diverse fibrous-scars disorders in various fields of medicine.

2. OVERVIEW OF COLLAGEN AND COLLAGENASES

2.1. Collagen

Collagen (more specific, the family of collagens) is the major protein of extracellular matrix (ECM) for different types of connective tissue comprising about 30% of the overall protein mass in the human body. The collagen macromolecule consists of three intertwined polypeptide chains (α-helices) (Fig. **1**) and is synthesized in fibroblasts, fibrocytes and in their specialized forms (chondro-, osteo-, tenno- and keratinocytes); collagen is synthesized in small amounts in endothelial, smooth muscle and in some epithelial cells [3, 4]. Nowadays, based on molecular organization, the molecule size, and special functions in supramolecular structures formation, 29 types of collagen have been identified. The most important are fibrillar collagen types I and III, which pass the stages of fibrillo- and fibrogenesis outside the cell (primary filaments, subfibrils, fibrils, fibres and fibre bundles); fibrillar collagen type II in cartilage, which does not form fibre; and non-fibrillar collagen type IV located in basement membranes. The other (minor) types of collagen are involved in fibrillogenesis, intermolecular and interfibrillar binding of the major types of collagen, fusion of collagen structures and the other elements of ECM and cells. All collagen structures play an important role in the mechanical skeleton of the body (bones, cartilages, tendons and ligaments), and serve as the inner skeleton of the interorgan and intraorgan connective tissue. Collagens, along with other components of ECM, participate in the regulation of the metabolism and the functions, intercellular interactions, proliferation, differentiation, and apoptosis of the cells. It is worth mentioning the reparative function of collagens especially during skin wound healing and the reparation of inner organ and muscle defects.

Altogether, collagen and other ECM components, such as glycoproteins and proteoglycans, form the basis of structures such as the skin, cartilage, tendons and ligaments, serous and mucous membranes, the aponeurosis, the meninx, inner organ capsules, sclera and cornea of the eyes, the structures of the middle and the inner ear, and vascular adventitia.

2.2. Collagenases

In general, the catabolism of collagens in mammals is performed by specific collagenolytic enzymes – matrix metalloproteases (MMPs). These enzymes are synthesized by the cells of connective tissue such as macrophages and fibroblasts. They are also present, in small amounts, in the endothelium of vessels, and in some epithelial cells. During inflammation, MMPs are produced by neutrophils. Major members of MMP family, their substrates (collagen and ECM components) and functions are summarized in Table **1**.

MMPs are extracellular (Zn^{2+})-dependent neutral endopeptidases and together can degrade all the ECM components. Dysregulation and the loss of control in the metabolism of ECM components lead to many diseases: accelerated turnover of ECM induces atherosclerotic plaque rupture; rheumatoid arthritis; osteoarthritis; aortic aneurysms; periodontitis; autoimmune blistering disorders of the skin; dermal photoaging; and chronic ulcerations. MMPs are also involved in the pathogenesis of different conditions such as glomerulonephritis, tumour cell invasion and metastasis [5, 6]. MMPs may play a role in cancer cell survival [7].

Collagen accumulation (especially collagen type I) occurs because of ECM components metabolism dysregulation. It leads to the development of keloid scars of the skin, fibrosis and sclerosis of inner organs (liver, kidneys, lungs and heart), the formation of contractures, ankylosis, commissures, and occlusions of the lumen of tubular organs, *etc.*

Twenty-nine MMPs have been identified in vertebrates: 27 different metalloproteases and two copies of MMP-23 [8]. MMPs are present in humans, but are also found in other organisms (*e.g.*, chicken, *Xenopus* [9], *Hydra* [10], sea urchins [11] and *Arabidopsis* [12]). MMPs generally possess similar structures and include a prodomain, catalytic domain, a hinge region and a

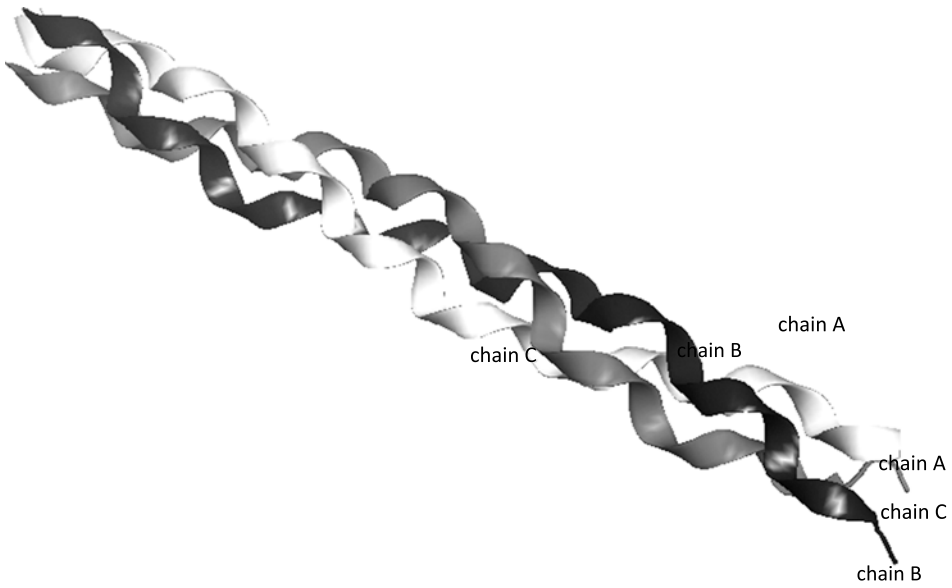

Fig. (1). Structure of collagen (collagen-like peptide, PDB 1CAG).

haemopexin domain [13]. Proteases of this family contain the cysteine switch motif PRCGXPD that allows pro-MMPs to maintain their zymogen form, and Zn^{2+}-binding motif HEXGHXXGXXH in the catalytic domain. This motif participates in the catalysis of MMP reactions by allowing the zinc ions to assume a quasipenta coordinated state through dissociation. Glutamic acid residue oxygen atoms, the carbonyl oxygen atom of substrate, and two histidine residues coordinate the zinc ion resulting in the reversible electron donor state of the glutamic acid oxygen atom. In this oxyanion transition state, a water molecule completes the hydrolysation of the substrate scissile bond [14].

According to substrate specificity, sequence homology and domain organization, MMPs are typically divided into six groups (Table **1**): collagenases (MMP-1, MMP-8, MMP-13); gelatinases (MMP-2, MMP-9); stromelysins (MMP-4, MMP-10, MMP-11); matrilysins (MMP-7, MMP-26); membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, MMP-25); and others (MMP-12, MMP-19, MMP-20, MMP-22, MMP-23(A and B) and MMP-28). The evolutionary analysis of MMP family members revealed that all MMPs can be divided into four evolutionary distinct groups: 1) MMP-19; 2) MMP-11, MMP-14, MMP-15, MMP-16, MMP-17; 3) MMP-2 and MMP-9; and 4) all other MMP proteins [15].

Collagenases cleave interstitial triple-helical collagen (types I, II and III) into three-quarter and one-quarter fragments from the N-terminus generating tropocollagens A and B, and appear to be major participants in collagen catabolism. Collagens are the major components of dentin, bone and cartilage: interstitial MMPs perform their collagenolysis. Interstitial collagenases also cleave a large number of other ECM substrates, summarized in Table **1**. For example, the cleavage of protease activated receptor 1 (PAR1) by MMP-1 promotes the invasion and growth of breast carcinoma cells [16]. Gelatinases A (MMP-2) and B (MMP-9) digest denaturated collagens type IV, V and XI, and gelatines. MMP-2 digests collagens I, II and III, but MMP-9 does not. The absence of MMP-2 in humans results in the autosomal recessive form of multicentric osteolysis leading to the destruction of bones, which suggests the importance of MMP-2 in osteogenesis [17]. MMP-2 and MMP-9 are thought to be very important for metastasis [18]. Stromelysins are like collagenases but do not cleave interstitial collagen and mainly participate in pro-MMP activation. MMP-11 cleaves serpins, and its isoenzyme was found in placenta [19, 20]. Matrylisins are characterized by the absence of the haemopexin domain. MMP-7 and MMP-26 are called endometases and mainly cleave surface molecules such as E-cadherin, pro-TNF-α, heparin-binding epidermal growth factor (EGF), RANK ligand, Fas ligand, and pro-α-defensin [13]. Endometases are widely associated with different types of tumours [21, 22]. Four type 1 transmembrane proteins (MMP-14, MMP-15, MMP-16 and MMP-24) and GPI-anchored proteins (MMP-17 and MMP-25) represent membrane-type MMPs. Besides MMP-17 they all activate pro-MMP-2 by proteolysis [13]. MMP-14 cleaves collagen types I–III and plays an important role in angiogenesis and embryogenesis [23, 24]. Other MT-MMPs are expressed in different parts of the human organism: in the

Table 1. Table summarizing major matrix metalloproteases (MMPs) and their roles in the cellular activity. Adapted from [5, 6, 13, 35, 40-43].

Metalloprotease	Substrates	Function and Localization
MMP-1 (collagenase 1)	Collagens I, II, III, VII, VIII, X and XI; aggrecan; entactin/nidogen; fibronectin; myelin basic protein; gelatine; insulin-like growth factor-binding proteins (IGFBPs); laminin; link protein; perlecan; tenascin; vitronectin; α1-antitrypsin; α1-antichymotrypsin; casein; C1q; fibrin; fibrinogen; IL-1b; monocyte chemoattractant protein-1, -3 and -4; pro-TNF-a; pro-MMP-1; pro-MMP-2; α2-macroglobulin; kallikrein; chymase; PAR1.	Breakdown of extracellular matrix proteins during tissue remodelling in normal physiological processes; PAR1 activation; anti-inflammatory and pro-inflammatory activity depending on substrate; apoptosis (amnion epithelial cells); cell migration; keratinocyte migration and reepithelialization; degradation and remodelling of collagen.
MMP-8 (collagenase 2, neutrophil collagenase)	Collagens I, II and III; aggrecan; α1-antitrypsin; α2-macroglobulin; C1q; fibrinogen; substance P; pro-MMP-8; LIX.	Breakdown of extracellular matrix proteins during tissue remodelling in normal physiological processes; apoptosis (amnion epithelial cells); degradation and remodelling of collagen; expression in keratinocytes at the trailing membrane edge during wound healing.
MMP-13 (collagenase 3)	Collagens I, II, III, IV, IX, X and XIV; gelatine; fibronectin; laminin; large tenascin C; versican; fibrillin; osteonectin; aggrecan; α2-macroglobulin; casein; C1q; factor XII; fibrinogen; monocyte chemoattractant protein-3; stromal cell-derived factor-1; pro-MMP-2; pro-MMP-9; pro-MMP-13; perlecan.	Breakdown of extracellular matrix proteins during tissue remodelling in normal physiological processes; anti-inflammatory activity; apoptosis (amnion epithelial cells); release of basic fibroblast growth factor (bFGF); osteoclast activation; degradation and remodelling of collagen; overexpression in keratinocytes delays reepithelialization.
MMP-2 (gelatinase A)	Gelatine; collagen I, IV, V, VII, X and XI; laminin; aggrecan; fibronectin; tenascin; stromal cell-derived factor 1α; adrenomedullin; big endothelin; decorin; monocyte chemoatractant protein-3; chondroitinsulphate proteoglycan.	Neuronal apoptosis leading to neurodegeneration; conversion of vasodilator to vasoconstrictor; increased bioavailability of transforming growth factor (TGF)-β; anti-inflammatory activity; cell migration; neurite outgrowth; osteogenesis; metastasis.
MMP-9 (gelatinase B)	Gelatine; collagen I, III, IV, V, VII, X and XI; elastin; aggrecan; fibrillin; galactin-3; precursor of TGF-β; IL-2Rα; intracellular adhesion molecule (ICAM)-1.	Hypertrophic chondrocytes apoptosis and recruitment of osteoclast; thymic neovascularization; bioavailability of TGF-β; reduced IL-2 response; anti-inflammatory activity and pro-inflammatory activity depending on substrate; tumour cell resistance; metastasis.
MMP-3 (stromelysin-1)	Collagen II, III, IV, V, IX and X; fibronectin; elastin; laminin; gelatine; aggrecan; nidogen; fibrillin; E-cadherin; pro-MMP-1; pro-MMP-7; pro-MMP-9; decorin; monocyte chemoatractant protein-3; perlecan.	Breakdown of extracellular matrix proteins during tissue remodelling in normal physiological processes and in arthritis, tumour initiation and metastasis; wound repair; progression of atherosclerosis; transcription control; disrupted cell aggregation and increased cell invasion; increased bioavailability of TGF-β; anti-inflammatory and pro-inflammatory activity depending on substrate; release of bFGF; cell migration.
MMP-10 (stromelysin-2, transin-2)	Collagen IV, V, IX and X; fibronectin; elastin; gelatine; laminin; aggrecan; nidogen; E-cadherin.	Expressed in synovium; autoimmune responses in chronic inflammatory forms of arthritis.
MMP-11 (stromelysin-3)	Serine protease inhibitors; 1-proteinase inhibitor; serpins.	Physiopathological tissue remodelling.
MMP-7 (matrilysin)	Elastin; fibronectin; laminin; nidogen; collagen IV; tenascin; versican; 1-proteinase inhibitor; E-cadherin; pro-TNF; heparin-binding epidermal growth factor (EGF); RANK ligand; Fas ligand; decorin; pro–α-defensin.	Vasoconstriction and cell growth; osteoclast activation; pro-inflammatory activity; Fas-receptor mediated apoptosis; disrupted cell aggregation and increased cell invasion; increased bioavailability of TGF-β; adipocyte differentiation.
MMP-12 (metalloelastase)	Collagen IV; gelatine; fibronectin; laminin; vitronectin; elastin; fibrillin; 1-proteinase inhibitor; apolipoprotein A.	Expression in macrophages (no expression in epithelial cells); macrophage migration.
MMP-14 (MT1-MMP)	Collagen I, II and III; gelatine; fibronectin; laminin; vitronectin; aggrecan; tenascin; nidogen; perlecan; fibrillin; 1-proteinase inhibitor; α2-macroglobulin; fibrin; pro-MMP-2; MUC1; cell surface tissue transglutaminase; monocyte chemoatractant protein-3; CD44.	Embryo attachment to uterine epithelia; reduced cell adhesion and spreading; anti-inflammatory activity; kidney tubulogenesis; cell migration; angiogenesis.

(Table 1) contd….

Metalloprotease	Substrates	Function and Localization
MMP-15 (MT2-MMP)	Fibronectin; laminin; aggrecan; tenascin; nidogen; perlecan; pro-MMP-2; cell surface tissue transglutaminase.	Reducing of cell adhesion and spreading.
MMP-16 (MT3-MMP)	Collagen III; fibronectin; gelatine; casein; laminin; α2-macroglobulin; pro-MMP-2; cell surface tissue transglutaminase.	Reducing of cell adhesion and spreading.
MMP-17 (MT4-MMP)	Fibrin; fibrinogen; TNF precursor; EGFR.	Expressed primarily in cerebrum, lung, spleen, intestine and uterus; driver of cancer cell proliferation and tumour progression.
MMP-24 (MT5-MMP)	Pro-MMP-2.	Brain-specific and is mainly expressed in the cerebellum.
MMP-25 (MT6-MMP, leukolysin)	Gelatine; pro-MMP-2.	Expressed almost exclusively in peripheral blood leukocytes and in anaplastic astrocytomas and glioblastomas but not in meningiomas.
MMP-19 (RASI-1)	Gelatine; aggrecan; cartilage oligomeric matrix protein; collagen IV; laminin; nidogen; large tenascin; entactin.	Expressed on the surface of activated peripheral blood mononuclear cells and is detected as an autoantigen in rheumatoid arthritis and associated with it joint tissue destruction.
MMP-20 (enamelysin)	Amelogenin; aggrecan; cartilage oligomeric matrix protein.	Teeth development.
MMP-28 (epilysin)	Furin; casein.	Tissue haemostasis and wound repair.

brain (MT5-MMP) [25]; and peripheral blood leukocytes (MT6-MMP) [26]. Other MMPs are involved in different processes such as macrophage migration (MMP-12) [27], gastrointestinal diseases and the development of fibrosis (liver MMP-19) [28, 29], teeth formation (MMP-20) [30] and tissue formation (MMP-28) [31]. MMP-23 is expressed in reproductive tissues and involved in testicular development [32].

MMPs are specifically inhibited by endogenous α2-macroglobulin and tissue inhibitors of metalloproteases (TIMPs) in a 1:1 stoichiometry [13]. In vertebrates, four types of TIMPs have been identified. They are active during development and tissue remodelling. TIMPs are possible candidates for using as therapeutic tools for cardiovascular diseases and cancer [33, 34].

However, despite the variety of human MMPs and their substrates, they are not used as therapeutic agents. TIMPs and synthetic inhibitors are applied in the regulation of MMP activity such as doxycycline for treatment of periodontal disease, minocycline, marimastat (broad-spectrum inhibitor) and cipemastat (selective to MMP-1 inhibitor) [35]. First, there is a complex network of relationships between MMPs in humans: one pro-MMP may be activated by many MMPs and *vice versa*, many MMPs may activate the same pro-MMP. In addition, every human MMP has many non-matrix substrates, the cleavage of which leads to the regulation of vital activity for the whole organism. In fact, overexpression of a single MMP within the circulation leads to an immediate counterbalance between ECM components and MMP inhibitors or to an accelerated turnover of ECM molecules which, as mentioned above, inevitably results in different pathological processes and, most frequently, contributes to cancer progress [36]. Secondly, MMPs are encoded by large eukaryotic genes containing introns, and it is difficult to obtain recombinant enzymes. However, expression of the full-length recombinant MMP-9 in *Escherichia coli* and *Drosophila* S2 cells has been attempted [37, 38]. MMP-7 was also expressed in *E. coli* [39]. Proteins aggregate in inclusion bodies and are then refolded retaining proteolytic activity. Nevertheless, except for ECM substrates, every MMP cleaves non-ECM substrates, which makes them non-directed. Thirdly, different MMPs are expressed in different tissues and cell types, and overexpressed MMP needs to be delivered to the target area.

Thus, collagenases from other organisms need to fill the limitations of endogenous MMPs with high specificity and directivity. In addition to MMPs from vertebrates, different collagenolytic enzymes were identified in a variety of living organisms: bacteria, fungi, larvae of worms, crabs and plants (Tables **2**, **3** and **4**). These collagenases form a wide base for the development of novel therapeutic agents, which are used, or could potentially be used, in modern biomedicine.

Table 2. Table, summarizing acidic collagenolytic enzymes from different organisms.

Collagenases	Optimal pH and Temperature	Substrates	Refs.
Cardosin A from *Cynara cardunculus*	pH 5.0, 25˚C,37˚C	Collagen type I, Synthetic peptide (Leu–Ser–Phe(NO2)–Nle– Ala–Leu–oMe), k-casein, α-Lactalbumin, β-Lactoglobulin	[59], [60]
FhCL2 from *Fasciola hepatica*	pH 5.5-7 28-37˚C	Collagen type I and substrates with Pro at P2 position	[61]
FhCL3 from *Fasciola hepatica*	pH 5.5-7.0 28-37˚C	Type I collagen, more effective than FhCl2	[61]
Collagenase from *Rhizoctonia solani*	pH 5, 40˚C	Collagen I, casein, elastin	[62]
Triticain-α from *Triticum aestivum*	pH 3.6-4.6 6.0-6.5, 37˚C	Bovine dermis collagen	[63, 64]
GP2 and GP3 from *Zingiber officinale*	pH 5.5-7.0, 21-23˚C	Collagen type I	[65, 66]

Table 3. Table, summarizing alkaline collagenolytic enzymes from different organisms.

Collagenases	Optimal pH and Temperature	Substrates	Refs.
Collagenase from *Chionoecetes opilio*	pH 8.0-8.5, 40˚C	Collagen I	[67]
Cathepsin L-like protease *Dermestes frischii*	pH 8.0, 37˚C	Azocollagen, oxidized insulin chains A and B and melittin	[68]
Serine protease from *Gadus morhua*	pH 7.8-(8.1), 25˚C	Native collagen types I, III, IV and V, sAAPFpna, succinyI-L-Ala-L-Aia-L-Pro-L-Leu-p-nitroanilide	[69]
Collagenase from *Penicillium aurantiogriseum* URM 4622	pH 9, 37˚C	Collagen I, Gelatin, Azocasein	[70]
Plantagolisin from *Plantago maior*	pH 8.1, 40˚C	Collagen type I from human placenta, low digestion of bovine serum albumin, ovalbumin, azoalbumin, thrombin, azocasein	[71]
Alkaline metalloproteinases from *Sebastes sp.*	pH 8.0, 20-24˚C	Collagen type I, gelatin, collagen fibrils (low)	[72]
SGT from *Streptomyces griseus ATCC* 10137	pH 8.0-9.0, 37˚C	Collagen I	[73]
SOT from *Streptomyces omiyaensis*	pH 8.0-9.0, 37˚C	Collagen I and IV, gelatin, casein	[73]
VPM from *Vibrio parahaemolyticus*	pH 8.0, 37˚C	Collagen I, II, III, and IV, gelatin, casein, synthetic peptides	[74]
VVP from *Vibrio vulnificus*	pH 8.0, 37˚C	Collagen I and IV	[75]

3. HUMAN PATHOLOGICAL CONDITIONS THAT CAN BE TREATED WITH COLLAGENASES

In medical research, collagenolytic enzymes (*C. histolyticum* collagenase) are used for the separation of cells, and for their isolation from tissues and further cultivation of fibroblast stem cells of lungs, liver cells, adrenal gland cells, pancreatic islets cells, *etc.* [44, 45]. This may be achieved through the release of the cells from the ECM and from collagen fibres. Meanwhile, the cells remain intact.

The possible application of collagenase in the treatment of a range of widespread diseases are the interest of our review. In Table **5**, we show the data, detailing pharmaceuticals based on collagenases that are used for the treatment of different conditions. It is important to note that the aim of our review is to cover diseases and pathological conditions that are not directly caused by mutations in collagen-encoding genes [46], but are secondarily associated with collagen, *e.g.*, with its excessive accumulation. Described conditions are not only diseases, but also symptoms and complications of

Table 4. Table, summarizing neutral collagenolytic enzymes from different organisms.

Collagenases	Optimal pH and Temperature	Substrates	Refs.
Collagenase from *Aspergillus aculeatus*	pH 7, 35˚C	Collagen I, casein, elastin, gelatine, p-nitrophenol caprylate	[76]
Collagenase from *Aspergillus flavus*	pH 7.0, 37˚C	Azocasein, Type I Collagen, Elastin	[77]
CNA1 from *Bacillus cereus*	pH 7.0, 45˚C	Collagen I	[78]
Col-J from *Bacillus pumilus*	pH 7.5, 45˚C	Collagen I	[79]
Collagenase from *Carcinus maenas*	pH 7.4, 30˚C	Collagen I and III	[80]
Collagenase A from *Clostridium perfringens*	pH 7.2, 42˚C	Collagen I, azocoll, gelatine,	[81]
E-a and E-b from *Erimacrus isenbeckii,*	pH 7.0-8.0, 37˚C	Calf skin collagen	[82]
Pancreatic Juice enzyme B from Dog	pH 7.4, 37˚C	Tritiated tendon collagen, gelatine, casein	[83]
Collagenase from *Hypoderma lineatum*	pH 7.2-7.6, 37˚C	Collagen I, Casein, Bz- Arg-OEt, Bz- Tyr-OEt, Acid-soluble collagen, (C14) Acetilated collagen, oxidized insulin B chain	[84, 85]
CNL3 from *Klebsiella pneumoniae*	pH 6.0, 40˚C	Collagen I	[78]
Collagenase from *Litopenaeus vannamei*	pH 7.5, 37˚C	Native porcine type I collagen, casein	[86]
Collagenase from *Novoden modestrus*	pH 7.0-8.0, 37˚C	Type I collagen	[87]
Proteases A2 and B from *Pandalus eous*	pH 7.5, 25˚C	Native porcine collagen type I, gelatine, DNP-peptide	[88]
Collagenase from *Panulirus japanicus*	pH 7.5, 25˚C	Calf skin collagen	[82]
Serine protease from *Parasilurus asotua*	pH 7.5, 37˚C	Acid soluble type I collagen, gelatine, casein, hemoglobin and elastin	[89]
Collagenases from *Penaeus monodon, P. japonicus* and *P. penicillatus*	pH 7.6-8.0, 25˚C	Soluble type I, IV collagen, insoluble fibrils of type I collagen, carp skin, guinea pig skin, insoluble collagen fibril isolated from rat tail tendon	[90]
PrtC from *Porphyromonas gingivalis ATCC 53977*	pH 7.8, 22˚C	Reconstituted type I collagen, heat-denatured type I collagen, azocoll	[91]
Collagenase from *Pseudopleuronectes americanus*	pH 7.4, 37˚C	Bovine Achilles tendon collagen	[92]
Collagenases A and B *Pycnopodia Helianthoides* (starfish)	pH 7.8, 24˚C	Pig skin collagen, native fibrillar collagen	[93]
Collagenase from *Rattus rattus* (uterus and skin)	pH 7.4, 37˚C	Native collagen I, II and III	[94, 95]
Serine collagenase from *Scomber japonicus*	pH 7.5, 37-55˚C	Native collagen types I, II, III and V	[96]
Collagenase from *Scylla serrata*	pH 7.5, 25˚C	Collagen type I, gelatine, hemoglobin, casein, BSA	[97]
Collagenase from *Seriola quinqueradiata*	pH 7.6, 25-37˚C	Carp and rat skin collagen	[98, 99]
Collagenase from *Streptococcus gordnii*	pH 7.0, 37˚C	Human placental type IV collagen, gelatine, α chain of fibrinogen, synthetic peptides	[100]
Collagenase from *Streptomyces exfoliatus CFS 1068*	pH 7.0, 40˚C	Azocoll	[101]
Collagenase from *Thunnus thymus*	pH 7.5, 37˚C	Collagen types I, II, III and V	[102]
Collagenase from *Treponema denticola*	pH 7.5, 22˚C	Collagen I, II, III	[103]
Collagenase from *Vibrio alginolyticus*	pH 6.3–8.8, 37˚C	Synthetic peptides	[104]
MMP-18 (collagenase 4) from *Xenopus laevis*	pH 7.5, 25˚C	Collagen I, II, III, and X	[105]

Table 5. Table summarizing collagenase-based medications already used for treatment of collagen-associated conditions.

Condition	Clinical effect	Composition of Pathological Tissues	pH in Affected Site	Collagenases already used in Biomedicine (pH range)		
				Clostridium histloliticum Collagenase (pH Optimum 6.3-8.8)	Aquatic Organisms Collagenases (pH Optimum 7.0-8.0)	Plant Collagenases (pH Optima: Papain 3.0-12.0 and Bromelain 5.5-8.5 and Ficin 5.0-7.5)
Wounds and burns	Enzymatic debridement, lysis of necrotic tissue, accelerated healing	Collagen I and III	5.4-8.5	Iruxol, Collagenase, Santyl, Solosite, Novuxol	Collagenase CC 250 U , Polikollagenasa-K, Kolladiosorb, Morikrol, Fermenkol	Debridace, Panafil, NexoBrid
Keloid scar	Softening, involution of scar tissue	Collagen I, III and IV	6.0-7.2	Collalysin	Collagenase CC 250 U , Polikollagenasa-K, Kolladiosorb, Morikrol, Fermenkol	
Peyronie's disease	Penile deformity improvement	Collagen I and III	7.2	XIAFLEX, Xiapex		
Dupuytren's contracture	Pain and itch elimination, involution of fibrotic tissue	Collagen I and III	7.2	XIAFLEX, Xiapex		NexoBrid
Uterine myoma	Volume reduction	Collagen I, III and IV	7.0-7.5	*Clostridium histloliticum* collagenase solution		
Glaucoma (lacrimal path stricture)	Widening of lacrimal path lumen	Collagen I and III	7.5	Collalysin	Collagenase CC 250 U	
Corneal, retinal and eyelid skin scars	Involution of scar tissue	Collagen I, III and IV	7.2	Collalysin		
Urethral stricture	Lumen expansion	Collagen I and III	7.2	XIAFLEX	Collagenase CC 250 U	
Herniated disks	Hernia lysis	Collagen I and II	7.5	*Clostridium histloliticum* collagenase solution		Chymopapain solution
Fibrotic cellulite	Lysis of fibrotic and body fat depositions	Collagen I, III, IV and V	6.0-7.0	XIAFLEX, Promoitalia Proshock Shape	Kollagenasa	
Nipple pain	Pain elimination	Collagen I and III	7.2	*Clostridium histloliticum* collagenase solution		
Oral healthcare	Plaque removal, dentin deproteinizing, periodontitis treatment	Collagen I, III and IV	<5.0-7.0		Collagenase CC 250 U	Papacarie, Carie Care
Placenta detachment delay	Placenta detachment	ND*	6.0-7.0	*Clostridium histloliticum* collagenase solution		

*ND – not defined

non-collagen-associated diseases that can be relieved by the action of exogenic collagenases.

Such secondary conditions can be divided into two major groups: wound processes and fibrotic, scarring processes and other diseases, which are not included in these groups: opacification of the eye vitreous and cornea; glaucoma; herniated discs; uterine fibroids; nipple pain; and the detachment of the placenta after childbirth. The major target of proteolytic activity of collagenases in these diseases is the collagen of necrotic tissue and excessive collagen of scars. It is worth mentioning that, due to the inflammatory process, the pH of wound environments lowers from 7.3 to 5 and even lower [47]. In scar tissue, the pH is close to normal (7.2–7.5). This circumstance needs to be considered when prescribing medication containing *C. histolyticum* collagenase: the pH range of the enzyme activity is from 6.3 to 8.0 [48], or crab collagenase (pH range 7.0–7.8) [49]. The environmental temperature of the affected tissue is also important because it is 1–1.5 degrees higher in inflamed tissue than in normal tissue. Collagen of the skin dermis and scar tissues are mainly represented by collagen type I, with a little by collagen type III, and small amounts of collagen types IV, V, VII, IX and XIII. They are the substrates of *C. histolyticum* and crab collagenases and correspond with the same pH ranges.

The most studied, efficient and readily available are collagenases from *C. histolyticum* [48]. This collagenase was first discovered in 1953, and since then it has been widely studied and used in the treatment of diseases [50]. Clostridial collagenase is a metalloprotease, and is structurally and functionally close to the human matrix metalloproteases. Two distinct genes encode collagenase: *ColG* and *ColH*. The *ColH* gene shares 26–39% and *ColG* gene shares 21–37% sequence identities with human interstitial MMP-1, -8 and -13, and gelatinases MMP-2 and -9. The transcription and translation of these genes result in the synthesis of seven distinct but structurally and functionally similar isoforms of the enzyme, named α, β, γ, δ, ε, ξ, and η. Clostridial collagenases are divided into two groups: one group is designated class I (*ColG*) and the other is class II (*ColH*). They possess different substrate specificity and evolutionary origins: class I (AUX-I) collagenases cleave intact triple helical collagen affecting N- and C-terminal domains, whereas class II (AUX-II) collagenases preferentially cleave small peptides, denaturated collagen and internal peptide domains of collagen molecule [51]. All the enzymes hydrolyse peptide bonds between Gly-Pro and the X residue of the tri-peptide unit: X is often Pro or hydroxyproline [52]. The optimum pH of clostridial collagenases is pH 7.4, with a temperature of 37˚C, but they are active from pH 6.3–8.0. Collagenases from *C. histolyticum* form the basis of several medications such as Iruxol (Pliva, Croatia; Knoll, Germany), Collalysin (St. Petersburg Research Institute of Vaccines and Serums and Enterprise for Production of Bacterial Preparations, Russia), XIAFLEX (Auxilium Pharmaceuticals, Inc., USA), Xiapex (Pfizer, Europe), collagenase Santyl (Smith & Nephew, USA), Solosite gel (Smith & Nephew, USA), and Novuxol (Turkey), Promoitalia Proshock Shape (Promoitalia, Italy).

A mixture of four collagenolytic enzymes from the Kamchatka crab hepatopancreas are highly active against different types of collagen, especially against calf skin collagen type III and bovine lens capsule collagen type IV at pH 7.5 and temperature 42˚C [53]. Collagenolytic proteases A and C with different substrate specificity from the crab *Paralithodes camtschatica* have been reported. Both proteases reveal high efficacy in hydrolysis of types I and III collagen at multiple sites. It also cleaves gelatine and fibrinogen. The optimum pH for protease A is 8.0 and for protease C is 9.0 [54-56]. Collagenases from *P. camtschatica* form the basis of such medications as Polikollagenasa-K (Technologiya, Russia), Kolladiosorb, Kollagenasa (St. Petersburg Research Institute of Vaccines and Serums and Enterprise for Production of Bacterial Preparations, Russia), liniment Morikrol (Trinita, Russia), Collagenase CC 250 U (E.G. Elyakov Pacific Institute of Bioorganic Chemistry, Russia) and Fermenkol (Fermenkol, Russia). These preparations are used for wound and burn debridement, and the healing of keloid scars, *etc*. (Table **5**). Trypsin-like serine protease I and protease II are isolated from the fiddler crab *Uca pugilator*. Protease I is homologous to mammalian trypsin-like proteases, while protease II is homologous to crayfish trypsin [57]. Protease I is able to cleave a wide range of low-molecular weight non-collagenous substrates and collagen types I–III at pH 8.0 [57]. Native type V collagen might be partially cleaved by an enzyme. Collagenase II cleaves the native triple helix of collagen types I, II, III, IV and V at multiple loci under physiological conditions [58].

Open wounds of different etiologies, including festering wounds, chronic wounds, thermal and chemical burns, frostbites, trophic and radiation ulcers, diabetic foot, pressure ulcers, and osteomyelitis fistulas, all refer to wound processes. Wound healing has several stages: the inflammatory stage, reparative (granulation

tissue growth and epithelization) stage, scarring of a defect and remodelling of scar tissue. In a festering wound, the inflammation stage is prolonged due to infection that results in the need for wound debridement. Chronic wounds that include pressure wounds, leg ulcers, diabetic ulcers, non-healing or indolent wounds, osteomyelitis fistulas, necrotic form of erysipelas, *etc.*, are characterized not only by enhanced exudation, but also by the accumulation of necrotic, devitalized tissue, which slows down wound healing. To activate the abruption of necrotic tissue and stop the adsorption of toxic products caused by bacteria and tissue breakdown, enzymatic debridement as well as surgical, mechanical, and chemical methods, are used for wound debridement [106]. Collagen is the major component of devitalized tissue, and thus the application of collagenolytic enzymes is justified. *C. histolyticum* collagenase is effective in the enzymatic debridement of chronic wounds, trophic and diabetic ulcers, and burns in several papers [107-111]. In the treatment of burns by *C. histolyticum* collagenase, it was shown that the ointment possesses advantages compared with surgical debridement [112]. It is worth mentioning that enzymatic debridement leads to the release of peptides from endothelial and fibroblast-derived extracellular matrices. Matrix-derived peptides produced during collagenase proteolysis stimulate cellular proliferation; released matrix fragments activate endothelial morphogenesis *in vitro*, whereas ECM-derived peptides produced by collagenase promote tissue repair *in vivo* [113].

Acceleration in the purification of wounds, ulcers and burns, and in the growth of granulation tissue and epithelialization was also shown for collagenase from the *P. camtschatica* crab [49, 106, 114]. Collagenolytic serine protease PC is used for medical applications such as Morikrol, a moricrase-containing ointment for wound healing in the treatment of trophic ulcers and postoperative scars [67, 114]. Treatment with Morikrol may be effectively applied to new-scar therapy as well as in other cases when skin regeneration may occur. The efficiency of Morikrol is comparable to clostridiopeptidase preparations [115]. Crab collagenase was shown to have the greatest debriding ability *in vivo* in experimental wounds covered with necrotic tissue and/or fibrinous slough [49, 116]. Collagenolytic enzymes used for the treatment of surface wounds and ulcers are produced in the form of ointments, gels and draining powders.

Plants are also the source of collagenases that are used in the treatment of collagen-related disorders. Papain is extracted from *C. papaya* latex and is active at pH range 3.0–12.0. It possesses gelatinolytic activities and can be used for the treatment of keloid and hypertrophic scars [117]. Strong debridement of ulcerating wounds and non-infected crusts by papain solution application without affecting healthy tissue has been shown [118]. Papain- and urea-based ointment Debridace (Virchow Healthcare, India) or Accuzyme (Healthpoint, Ltd., USA) and the composition of papain-urea-chlorophyll Panafil (Healthpoint, Ltd., USA) are used for the healing of wounds and burns. However, it is not safe for large deep burn wounds [119]. Another plant protease bromelain from *Ananas comosus* is used for burn debridement and wound healing without any toxic effects [120]. Bromelain-based debriding enzyme NexoBrid® is undergoing clinical trials [121]. One more plant protease ficin, derived from the latex of a fig tree, possesses collagenolytic activity: it is active at pH range 5.0–7.5, and forms the basis of Debricin®, which performs fast and effective digestion of second-degree burns [122], and management of open wounds and ulcers by necrotic tissue debridement [123].

The modulation of fibrotic, sclerotic and scar processes is another possible application for collagenases. They include pathological scars of skin (encompassing eyelid skin), conjunctiva, eye cornea and retina, Peyronie's disease, Dupuytren's contracture, strictures (lumen constrictions) of the urethra, bronchi and lachrymal tract, symblepharon (scar fusion of eyelid conjunctiva and eyeball conjunctiva), adhesions in abdominal and pleural cavity, post-acne scars, and fibrotic cellulite. The excessive accumulation of collagen type I, and the expansion of the volume and density of connective tissue characterize all these diseases and results in the malfunction of the corresponding organs. Collagen accumulation is caused not only by enhanced protein biosynthesis in proliferative fibroblasts, but also by insufficiency of enzymes of collagenolysis.

Peyronie's disease (fibroplastic induration of penis) is a consequence of focal fibrosis of protein envelopes, which leads to the deformation of the penis. Proliferation of fibroblasts and chronic inflammation cause enhanced production of collagen types I and III. Injections of *C. histolyticum* collagenase solution (XIAFLEX) [124-127] induce degradation of collagen and decrease deformation.

Dupuytren's contracture (palmar fibromatosis) is a non-inflammatory disease, which causes the development of fibrotic transformation of the palmar tendons. Collagen type I prevails in scar tissue. However, there

is a significant amount of collagen type III, which leads to flexion contracture of the fingers and to their function loss. Injections of *C. histolyticum* collagenase solution (XIAFLEX) was more effective than surgical treatment of the disease [51, 128]. At a cellular level, *C. histolyticum* collagenase inhibited the spreading, attachment and proliferation of fibroblasts in a dose- and time-dependent manner. At a transcriptional level, collagenase also showed dose-dependent inhibition of several ECM components, cytokines and growth factors, and when it was removed, the cellular processes recovered in nodules, cords and skin, but not in fat [129]. In addition, bromelain debriding pharmaceutical agent (NexoBrid®) was modified into an injectable Bromelain Solution (IBS) and shown to be a rapid eschar-specific, deep burn debridement agent for dissolving the Dupuytren's cords in Dupuytren's disease patients [130].

Urethral fibrosis (urethral strictures) is a disease where local scarring and wall thickening result in the development of urethra lumen constriction and urination delay. Injections of collagenase solution were shown to reduce, and even prevent, urethral fibrosis in experimental models of strictures [131].

Keloid scarring is tumour-like, and an often recidivating excrescence of dense scar tissue (collagen types I and III) at the skin wound site, and less often at the intact skin. Keloid scarring is characterized by enhanced collagen synthesis and tissue collagenase activity compared with normal and hypertrophic scars [132]. Bacterial and crab collagenases are used for the treatment of scars in the form of ointment bandages followed by compression [114, 132-134].

In ophthalmology, collagenases are used to prevent and treat symblepharon (scar fusion of eyelid and eyeball conjunctivas), scars of the eyelid skin, conjunctiva, cornea and retina, after cataracts, stricture of the lachrymal tract, glaucoma, the opacification of the eye vitreous and cornea, iridocyclitis, and vitrectomy resulting from vitreous haemorrhage or its post-operational fibroproliferative changes [135].

In addition, experimental studies of the effect of *C. histolyticum* collagenase on adhesions in abdomen revealed that the Novuxol ointment prevented the development of post-operational adhesions [136]. The use of collagenase injections resulted in a preventive anti-adhesive effect, but they had no effect on present adhesions [137].

Clostridial collagenase was used for injections into the herniated discs (collagen types II and I) for the treatment of spinal osteochondrosis [138]. There was a reduction of hernias and pain. Chymopapain – cysteine protease from *C. papaya* – dissolves herniated nuclear material. It has become the basis for the invention of the chemonucleolysis procedure for treatment of sciatica – the consequence of disc herniation. Chemonucleolysis is a more effective and safer procedure than chymopapain injection and open discectomy. Its only disadvantage is the possibility of an allergic reaction to papain or papaya, which can be easily predicted [139].

Intraplacental injections of bacterial collagenases through umbilical cord arteries were used for placenta detachment after childbirth. The treatment of joint cartilage defects before stem cell transplantation improved the results of cartilage repair [140].

C. papaya protease papain is also used in oral healthcare: dentin matrix is composed of intact type I collagen fibrils that can be partially degraded by papain-gel (Papacarie and Carie Care, India) during the procedure chemomechanical removal of caries [141]. Bromelain acts similarly to papain: it is active at pH range 5.5–8.5 and is used for deproteinizing dentin from the collagen network for decreasing leakage scores of the adhesive system [142].

4. COLLAGENASES FROM DIFFERENT ORGANISMS WITH POTENTIAL APPLICATIONS FOR THE TREATMENT OF COLLAGEN-ASSOCIATED DISEASES

Among the potential collagenases from different organisms for biomedical applications (Table **2**) there are collagenases D and M from the larvae of beetles, *Dermestes frichii* and *Dermestes maculatus,* tested for their ability to remove necrotic tissues from wound and burn areas. The collagenases that cleave type I collagen are active at pH 7.5–8.0. Collagenase D was also very effective against chronic scars: 2-year-old hypertrophic scars, and 2- and 3-year-old keloid scars in comparison with clostridial collagenase and collagenase from the crab *P. camtschatica* [143]. Another potential organism for the commercial production of collagenase are the larvae of *Lucilia sericata* [144], whose exact mechanism of action is not yet known. However, it has been shown to possess not only collagenolytic activity (it can cleave collagen types I and III), but it also has antimicrobial and immunological properties. *Lucilia sericata* collagenase cleaves laminin, fibronectin and is active at pH 8.0, and temperature 37˚C [145]. Another collagenase that can be used in the treatment of keloid scars is the hatching enzyme (HE) from starfish, *Asterias amurensis* [146]. It has been reported that this

Table 6. Table summarizing collagenases with potential medical application.

Collagenases with Potential Application	Optimal pH Range	Observed Effect	Refs.
Collagenase D from *Dermestes frichii*	pH 7.5-8.0	Effective against chronic hypertrophic and keloid scars, able to remove necrotic tissues.	[143]
the Hatching Enzyme from *Asterias amurensis*	pH 4.0-6.0	Collagen I cleavage, keloid scar improvement though MMP gene expression.	[146]
Collagenase from *Lucilia sericata*	pH 8.0	Collagen I and III, laminin, fibronectin cleavage.	[145]
Cardosin A from *Cynara cardunculus*	pH 5.0	Collagen I cleavage, potential post-surgical applications to assist on the ECM remodeling.	[59]
Latex protein fraction (WTLP) from *Wrightia tinctoria*	pH 7.4	Collagen I and IV cleavage, debridement of chronic wounds, necrotic tissue removal.	[151, 152]
Extracts from *Cucumis sativus*	pH 7.4	Collagen I cleavage, potential sap extract application in cosmetics and wound.	[153]
Triticain-α from *Triticum aestivum*	pH 3.6-4.6 and 6.0-6.5	Collagen I cleavage, potential application in wound healing.	[63]
Collagenase from *Sebastes* sp	pH 8.0	Potential application on wound healing, gelatin cleavage.	[72]
Collagenase from *Euphausia Superba Dana*	pH 7.5	Collagen I and V, hemoglobin, casein, γ-globulin and serum albumins, elastin, myosin, fibrinogen and fibrin cleavage. Potent agent in debridement of venous leg ulcers: necrotic tissue removal; able to dissolve fibrin clots.	[147-149]

collagenase can cleave collagen type I and regulate the MMP gene expression contributing to the improvement of the scar and keloid tissue. It is stable at pH 4.0–6.0 and temperature 30–40˚C. Two metalloproteinases from the skeletal muscle of Pacific rockfish (*Sebastes* sp.), similar to human MMP gelatinases and stromelysins, can hydrolyse collagen and gelatine at pH 8.0, producing fragments similar to one-quarter and three-quarters of collagen, but they show very low activity in solubilizing native fibrillar collagen. These two enzymes might be used in wound healing [72]. Serine protease from Antarctic krill (*Euphausia superba Dana*) digests collagen type I and V, and reconstituted fibrils of calf skin collagen at pH 7.5. As for the other substrates, protease from Antarctic krill cleaves haemoglobin, casein, γ-globulin and serum albumins, elastin, myosin, fibrinogen and fibrin [147]. A wound healing effect was observed: in animal models, effective cleavage of necrotic tissue by krill protease preparation was shown [148]. Antarctic krill protease seems to be a potent agent in the debridement of venous leg ulcers, without noticeable side effects, and seems to be enhanced with the ability of protease to dissolve fibrin clots [149]. Besides enzymatic debridement of necrotic skin wounds, reepithelialization of corneal alkali burn and oral removal *in vivo* and *in vitro* by Antarctic krill preparations have been examined with promising results [150].

Plant collagenases have several potential medical applications. Cardosin A isolated from *Cynara cardunculus* has previously been shown to hydrolyse type I collagen inside the triple helix in a similar to MMP-1 manner at pH 5.0, temperature 37˚C. Cardosin A was suggested as useful in post-surgical applications to assist in ECM remodelling or in other medical applications, where an increase of ECM degradation is required [59]. *Wrightia tinctoria* latex protein fraction (WTLP) could be used in the clinical debridement of clearing chronic, non-healing wounds because of their ability to hydrolyse all subunits of type I and α-fragment of type IV collagen at pH 7.4, temperature 37˚C, forming small molecular weight proteins and to enhance MMPs activity in granulating tissues at the initial phase of wound healing [151]. WTLP removes dead tissues and supports the scavenging of free radicals during the initial stage and the onset of repair [152]. Collagenolytic activity was shown for a multiple protein mixture from cucumber sap extract (*Cucumis sativus*). The proteins are shown to degrade all chains of type I collagen at pH 7.4, temperature 37˚C, without toxic effects such as haemorrhage and oedema that result from cucumber sap extract application in cosmetics

and wound healing [153]. Collagenase activity was also shown for recombinant cysteine protease triticain-α from wheat (*Triticum aestivum*). Triticain-α performs full hydrolysis of type I bovine collagen at temperature 37˚C and at pH ranges 3.6–4.6 and 6.0–6.5. Substrate specificity of triticain-α is seems to be GXXG motif repeats. This enzyme could be used in wound healing [63]. GP2, isolated from ginger rhizomes (*Zingiber officinale*), cleaves the native type I collagen at multiple sites and is unique from other known collagenases in all three chains of collagen at pH 5.5 [65]. Collagenases described above are summarized in Table **6**.

There are many other organisms that synthesize collagenases. They are not well studied yet but have a potential for application in the treatment of collagen-associated diseases. Some of them are active in close to physiological conditions (*i.e.*, temperature 37˚C, pH 6.0–8.0): several *Vibrio* and *Streptomyces* species; fungi *Aspergillus* species; and proteases from different species of crabs, shrimps, insects, *etc.* Some of the proteases act in acidic environments (pH<6.0, Table **2**), some close to neutral (pH 6.0–8.0, Table **4**) and the others in an alkaline environment (pH>8.0, Table **3**).

CONCLUSION

The application of collagenolytic enzymes in medicine is a part of a more extensive field-enzymatic therapy. Data provided in this review suggest that a great number of collagenases of different origin have been isolated and studied.

Proteolytic, fibrinolytic and glycolytic enzymes have been used in the treatment of different diseases, including wound and scar processes, for a long time. The significant advantage of collagenase use is their relative selectivity towards substrates – collagens of different types and different ECM components (mainly glycoproteins and proteoglycans).

Excessive accumulation of collagen and other ECM components underlies numerous disorders associated with fibrous scar transformation of connective tissue. An important point to consider about these disorders is the metabolic imbalance between collagen biosynthesis and catabolism. The use of collagenases has been proven effective in the management of these diseases. It fits the trend towards creating remedies and methods of treatment that lower the number of invasive (surgical) methods. Injective methods of collagenase delivery are usually used for the treatment of scars, although, in case of skin scars, salve dressings moistened with enzyme solution tissues, electrophoresis and phonophoresis are used.

Existing data suggest that the injective method of collagenase therapy is still not used sufficiently. It is necessary to develop new methods of collagenase delivery for the treatment of other fibrous scar disorders, especially scars after blepharoplasty, rhinoplasty, and mammary gland implanting (fibrous thickening prosthesis capsule). As mentioned above, some ophthalmological disorders may be treated with collagenases, and thus it seems reasonable to develop the technologies of scar management in otorhinolaryngology (trachea, larynx, and sinuses), stomatology, and urology. The successful treatment of Dupuytren's contracture with collagenases gives hope for the successful treatment of joint contractures and different fibromatosis conditions. One more application of collagenase therapy may be the reduction of atherosclerotic plaques in the artery at sclerotic stage, but undoubtedly, we need to find the correct collagenase delivery systems for abdominal and pleural adhesions management.

Other medical conditions for collagenase application are chronic wounds and ulcers, where normally wound dressing, draining powders and electrophoresis are used. The main point of this cure is the enzymatic debridement of the wound surface. The fact is that devitalized tissues are mainly composed of collagen, which makes collagenase the agent of choice. Furthermore, it should be noted that collagen degradation leads to the formation of biologically active peptides that affect fibroblast proliferation and collagen synthesis *de novo*. Thus, there is a need to develop novel, more effective collagenase-based drugs and their delivery systems in wounds.

Thus, it is possible to conclude that collagenase therapy is underestimated. One can speculate that the following possible reasons explain the status of collagenase therapy and offer prospective directions for the development of collagenase-based therapeutic applications:

1. Hitherto, most commercial drugs have been based on collagenases obtained from *C. histolyticum* and hydrobionts (hepatopancreas of crabs, krill). At the same time, modern biochemistry provides a variety of collagenolytic enzymes from other organisms (microbes, animals, plants). Pharmacological research may lead to the development of novel, more effective collagenase-based drugs for wounds and scars treatment. The engineering of new recombinant human matrix metalloproteinases can increase the efficiency of collagenase therapy, with sufficient specificity of action on different types of collagen. Considering that most collagenases cleave only tri-

ple helix collagen molecules, it is necessary to combine collagenases with other proteases to cleave formed fragments or use the collagenases that can digest collagen completely.

2. Biochemical studies are mainly aimed at investigating the mechanisms of collagenase action on different types of collagen from different tissues. Nevertheless, collagen is presented in tissues in the form of fibrils and fibres, linked by intermolecular and interfibrillar bonds of different strength, and they are strongly bound to other matrix components. These structural features differ significantly in scar tissues, necrotic wound tissues, in denaturated collagen of thermo- and chemical burns. The mechanisms of collagenase action on intact tissue with the use of modern methods of optical visualization have still not been studied sufficiently. The development of this field may accelerate the creation of collagenase formulations optimal for various tissues and pathologies.
3. The final reason for the underestimation of collagenase therapy is the lack of information given in experimental and clinical studies that do not follow the guidelines of evidence-based medicine. Thus, more rigorous analysis of the reasons for the complications and ineffectiveness of the treatment is required. This will be extremely valuable for the improvement of drugs and techniques.

In conclusion, the data presented in this review suggest the need for further research and development of collagenase-based therapeutic assays as a significant branch of enzyme therapy. The development of novel, and highly efficient, enzyme formulations remains crucial.

LIST OF ABBREVIATIONS

bFGF = Basic fibroblast growth factor

BSA = Bovine serum albumin

ECM = Extracellular matrix

EGF = Epidermal growth factor

EGFR = Epidermal growth factor receptor

HE = Hatching enzyme

IBS = Injectable bromelain solution

IGFBPs = Insulin-like growth factor-binding proteins

MMP = Matrix metalloprotease

PAR1 = Protease activated receptor 1

TGF-β = Transforming growth factor beta

TIMP = Tissue inhibitors of metalloproteases

TNF = Tumour necrosis factor

WTLP = *Wrightia tinctoria* latex protein

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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