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PRACA POGLĄDOWA
REVIEW

Glycosaminoglycans – types, structure, functions, and the role in wound healing processes

Glikozoaminoglikany – rodzaje, struktura, funkcje i rola w procesach gojenia ran

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ABSTRACT

Glycosaminoglycans (GAGs) are a group of heteropolysaccharides, which include: chondroitin sulfates, dermatan sulfates, heparan sulfates, heparin, keratan sulfates, and hyaluronic acid. GAGs are composed of negatively charged polysaccharide chains composed of repeating disaccharide units, which include N-acetylated hexosamine residues – D-glucosamine or D-galactosamine – or N-sulfated D-glucosamine and hexuronic acid residues – D-glucuronic or L-iduronic acid – or galactose. All GAGs, except for hyaluronic acid, have a sulfate group and form proteoglycans (PGs) when attached to the core proteins.

GAGs have many important biological functions influencing PGs functions. PGs are present in all types of tissues and participate in cell migration, proliferation, and differentiation. They occur mainly in the extracellular matrix (ECM), where they participate in ECM organization, structure formation and mechanical properties. They play an important role in maintaining homeostasis and also influence metabolic processes, such as bone mineralization and blood coagulation. PGs (due to the strongly negative charge of the glycan chains) are involved in the selective permeability of cell membranes. Components of the ECM, including GAGs, play a structural and functional role during the healing of tissue damage. They regulate the healing process by acting as a reservoir and modulator for cytokines and growth factors and perform structural functions by filling tissue defects during the repair process.

KEYWORDS

glycosaminoglycans, extracellular matrix, proteoglycans, wound healing

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STRESZCZENIE

Glikozoaminoglikany (*glycosaminoglycans* – GAGs) są grupą heteropolisacharydów, w której skład wchodzą: siarczany chondroityny, siarczany dermatanu, siarczany heparanu, heparyny, siarczany keratanu oraz kwas hialuronowy. GAGs zbudowane są z ujemnie naładowanych łańcuchów polisacharydowych, złożonych z powtarzających się jednostek disacharydowych, do których należą reszty N-acetylowanej heksozoaminy – D-glukozoaminy lub D-galaktozoaminy – albo N-siarczanowanej D-glukozoaminy oraz reszty kwasu heksuronowego – D-glukuronowego lub L-iduronowego – albo galaktozy. Wszystkie GAGs, z wyjątkiem kwasu hialuronowego, posiadają grupę siarczanową oraz tworzą, po przyłączeniu do białek rdzeniowych, proteoglikany (*proteoglycans* – PGs).

GAGs pełnią wiele ważnych biologicznych funkcji, determinujących funkcje PGs. Te ostatnie są obecne we wszystkich rodzajach tkanek, uczestniczą w procesach migracji, proliferacji i różnicowania komórek. Występują głównie w macierzy pozakomórkowej (*extracellular matrix* – ECM), biorąc udział w organizacji ECM, kształtując jej strukturę i właściwości mechaniczne. Pełnią istotną rolę w utrzymaniu homeostazy, a także wywierają wpływ na szereg procesów metabolicznych, takich jak mineralizacja kości i krzepnięcie krwi. PGs (ze względu na silnie ujemny ładunek łańcuchów glikanowych) biorą udział w selektywnej przepuszczalności błon komórkowych. Składniki ECM, w tym GAGs, odgrywają rolę strukturalno-czynnościową podczas gojenia się uszkodzeń tkankowych. Regulują proces gojenia poprzez stanowienie rezerwuaru i modulatora dla cytokin i czynników wzrostu oraz pełnią funkcje strukturalne poprzez wypełnianie ubytków tkankowych podczas procesu naprawczego.

SŁOWA KLUCZOWE

glikozoaminoglikany, macierz pozakomórkowa, proteoglikany, gojenie ran

Structure, occurrence, and functions of glycosaminoglycans

Glycosaminoglycans (GAGs) are unbranched, linear, negatively charged heteropolysaccharides, built of repeating disaccharide units, found in all animal tissues. These units, depending on the class of glycan, consist of N-acetylated hexosamine residues – D-glucosamine (GlcNAc) or D-galactosamine (GalNAc) – or N-sulfated D-glucosamine and hexuronic acid residues – D-glucuronic acid (GlcUA) or L-iduronic acid (IdUA) – or galactose. In most cases, disaccharides are linked by a 1,3-β or 1,4-β-glycosidic bond [1,2,3,4]. The presence of L-fucose, D-mannose, D-xylose and N-acetylneuraminic acid residues has also been demonstrated in some GAGs [5]. The presence of a significant number of sulfate and carboxyl residues in the disaccharide units that build the polysaccharide backbone of GAGs determines the highly negative charge of the polysaccharide chain. GAGs, especially hyaluronic acid (HA), show hygroscopic properties, binding large amounts of water in tissues. This phenomenon is intensified as a result of the binding of cations present in the extracellular matrix (ECM) [2,3,4,6].

GAGs are natural components of blood vessel walls. Dermatan sulfate predominates in the venous wall, and chondroitin sulfate dominates in the arterial wall. These macromolecules are also found in the lungs, intestinal mucosa, liver, kidneys, and nervous tissue [2]. They also occur in the skin, where the role of these macromolecules is to maintain the structure and hydration of the skin, bond the epidermis with the dermis, regulate the transport of nutrients and metabolites, regulate the osmotic pressure, and maintain the proper turgor of this structure. GAGs play a fundamental role in supporting and connecting cells into tissues, tissues into organs, and then – as a result of further tissue organization – into individual parts of the body. The structure and properties of GAGs play an important role in the physiology and pathology of single cells and tissues [4].

GAGs (except for HA) occur in the body in the form of covalent bonds with proteins, forming proteoglycans (PGs) [2]. PGs occur both extracellularly, creating components of the ECM, and inside cells. Intracellular PGs are found in secretory granules, rarely in the cell nucleus, on the surface of cell membranes, or inside them – as so-called transmembrane PGs. Moreover, PGs bind many ligands, including ECM proteins, growth factors, enzymes, and their inhibitors, as well as elements of the cytoskeleton. They participate in the processes of cellular migration, proliferation, and differentiation, as well as in the organization of the ECM, shaping the structure and mechanical properties of the ECM as a result of interaction with fibrous proteins such as collagen and elastin [2,5,7,8,9]. In addition, they play an important role in maintaining homeostasis, e.g. by controlling and participating in the wound healing process or cell-cell interactions [10]. They also influence several metabolic processes, such as bone mineralization and blood coagulation. PGs (due to the strongly negative charge of the glycan chains) are involved in the selective permeability of cell membranes [2,7,8]. Components of the ECM play a structural and functional role in the healing process, participating in the repair process through the formation of a provisional matrix, granulation tissue, and then a scar [11]. Both free GAGs and their complexes with proteins (PGs) are distinctive and richly represented components of the ECM [4].

Types of glycosaminoglycans

Due to the type of monosaccharide subunits and the type of glycosidic bonds between disaccharide units,

GAGs can be divided into four classes: chondroitin--dermatan GAGs – chondroitin sulfates (CSs) and dermatan sulfates (DSs); heparan GAGs – heparins (Heps) and heparan sulfates (HSs); keratan GAGs – keratan sulfates (KSs); hyaluronic acid (hyaluronan – HA; Figure 1) [1,2].

According to another criterion for the division of GAGs, based on the type of hexosamine residue present in the GAG chain, the following are distinguished: glucosaminoglycans (with N-acetyl or N-sulfate-D--glucosamine residues) – Hep, HSs, HA, KSs; and galactosaminoglycans (with N-acetyl-D-galactosamine residues) – DSs, CSs [2,12].

The difference between particular types of GAGs concerns: amino sugar composition, presence of glucuronic or iduronic acid, presence or absence of sulfate groups, type of bond between components of disaccharide units, chain length, type of bond between GAG and the protein core in PGs, distribution in subcellular structures and tissues, function biological [1,4].

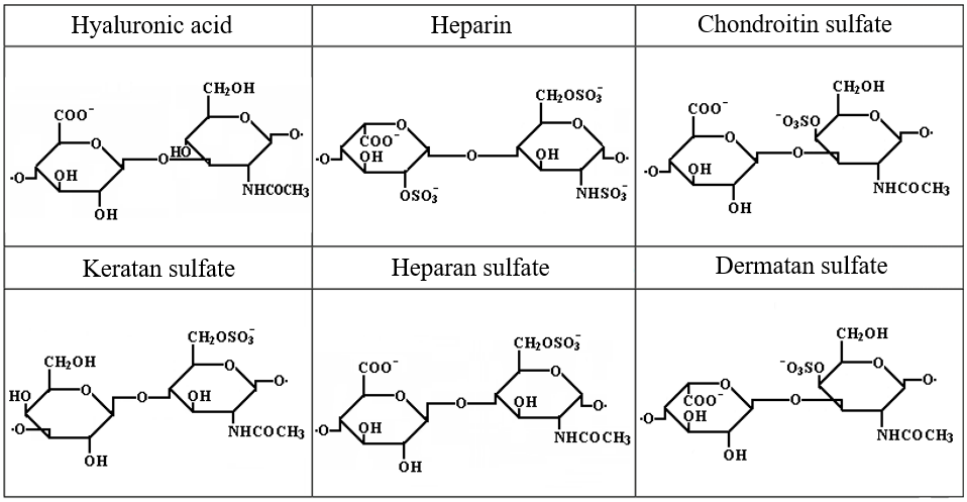

Fig. 1. Glycosaminoglycans classes (author's own study based on [1,2]).

Chondroitin sulfates and dermatan sulfates

Chondroitin A sulfate (chondroitin-4-sulfate, C-4-S), chondroitin C sulfate (chondroitin-6-sulfate, C-6-S), and dermatan sulfate (chondroitin B sulfate) belong to the group of galactosaminoglycans [17,18]. They are composed of N-acetylgalactosamine (GalNAc) residues and glucuronic acid (GlcUA) and L-iduronic acid (IdUA) residues, which are connected by alternating β (1→3) and β (1→4) glycosidic bonds, and undergo sulfation in different positions of the monomeric subunits, i.e. GalNAc and GlcUA. The heterogeneity of the discussed compounds is evidenced by the different ways of their sulfation because the GalNAc residue can be sulfated at the 4- or 6-position of fourth carbon of this monosaccharide, or chondroitin C sulfate, where the sulfation process concerns the GalNAc residue at the sixth carbon position. A single sulfate group is attached to the majority of disaccharide sequences. However, some disaccharides are not subject to sulfation, as well as sequences containing two sulfate groups found, for example, in chondroitin E sulfate (disulfated GalNAc residue) [19,20,21,22]. As a result of further modification of the CS chain, consisting in the epimerization of some residues of GlcUA to IdUA, chondroitin B sulfate, referred to as DS, is formed. It is assumed that if the content of IdUA in the glycan chain exceeds 10% of hexuronic acids, the GAG is referred to as DS. The DS chain is composed mainly of disaccharide units [→4IdUAβ1→3GalNAcβ1→], while to a lesser extent, it consists of doubly sulfated units, i.e. at the fourth carbon position of GalNAc and the second carbon position of IdUA [23].

The process of synthesis of chondroitin-dermatan GAGs consists of three stages – initiation, elongation, and modification [21,24]. The consecutive stages of chondroitin-dermatan GAGs synthesis are presented in Figure 2.

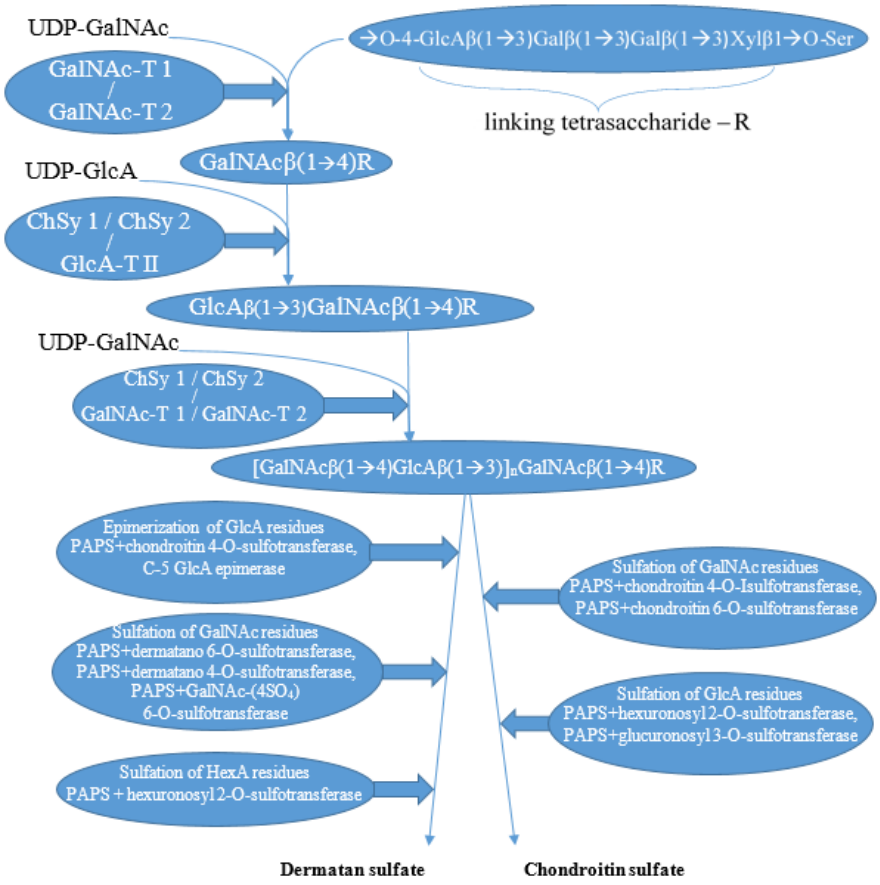

Fig. 2. Biosynthesis of chondroitin sulfate and dermatan sulfate (author's own study based on [13]); ChSy 1 – chondroitin synthase 1; ChSy 2 – chondroitin synthase 2; GalNAc – N-acetylgalactosamine; GalNAc-T 1 – chondroitin β1,4-N-acetylgalactosaminyltransferase 1; GalNAc-T 2 – chondroitin β1,4-N--acetylgalactosaminyltransferase 2; GlcA – glucuronic acid; GlcA-T II – βl,3-glucuronosyltransferase II; HexA – hexuronic acid (glucuronic or iduronic); PAPS – 3'-phosphoadenosine-5'-phosphosulfate; UDP – uridinediphosphate.

In the initial stage (initiation), the synthesis of the core protein takes place in the endoplasmic reticulum. The biosynthesis of chondroitin-dermatan chains occurs as a result of the action of an enzyme (xylosyltransferase), which catalyzes the transfer of a xylose molecule to a serine residue of the core protein. Then, in the Golgi apparatus, with the participation of galactosyltransferase I and galactosyltransferase II, the synthesis of the glycan chain takes place – two galactose residues are added to the existing protein (at the non-reducing end). The GlcA residue (fourth monosaccharide molecule) is added to the growing sugar sequence using glucuronyltransferase I [21,24,25,26]. The above description of the synthesis of the tetrasaccharide fragment – the connecting region – applies to both CSs and DSs but also characterizes heparan glycans. Differences in the subsequent stages of glycan chain biosynthesis (elongation and modification) will determine the formation of a particular type of GAG [24,26].

During the process of elongation of chondroitin--dermatan GAG chains, the appropriate N-acetylhexosamine residues and GlcA residues are alternately attached to their non-reducing end. And so, the transfer to the third carbon atom of the acid residue – N-acetyl--D-galactosamine (GalNAc) by N-acetylgalactosaminyltransferase I (GalNAc transferase I), leads to the formation of chondroitin-dermatan GAGs. In the further stages of the elongation process of the discussed glycans, CS polymerase is involved, showing the activity of N-acetylgalactosaminotransferase and glucuronosyltransferase [14,20,21,26].

The last stage of biosynthesis (modification) consists of epimerization of GlcUA residues to its C5 epimer – IdUA, catalyzed by C5-epimerase as well as sulfation – mainly of GalNAc residues, catalyzed by N-acetylgalactosamine 4-6 sulfotransferase (GalNAc 4-6 ST). The location of sulfate groups as well as their number in the chondroitin-dermatan glycan chains influences the role of these GAGs in acquiring the binding properties of signaling molecules [2,24,27]. The final stage of the transformation of chondroitin-dermatan PGs is their degradation. The degradation process of chondroitin-dermatan GAGs is presented in Figure 3.

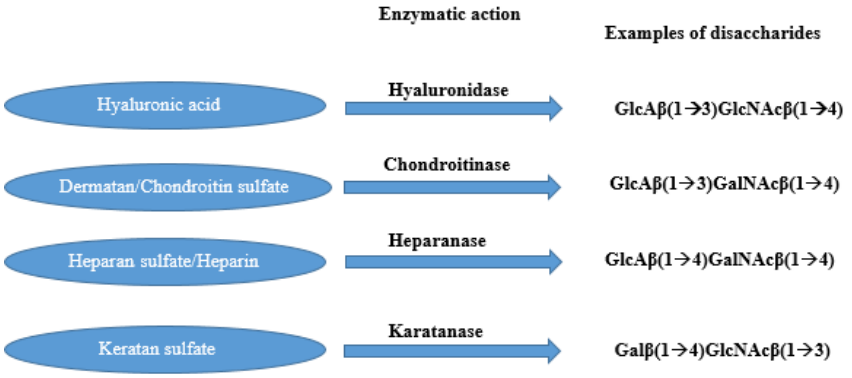

Fig. 3. Glycosaminoglycans degradation (author's own study based on [13]).

In the extracellular space, with the participation of endoglycosidases and proteolytic enzymes, a partial CS/DS disintegration takes place [6]. In turn, complete degradation occurs only intracellularly, in lysosomes, where PGs or their fragments undergo complete hydrolysis. The degradation of the described PGs is influenced by lysosomal proteases. On the other hand, the degradation of glycan chains is dependent on acidic hydrolases – endoglycosidases (hyaluronidases) as well as exoglycosidases and sulfatases. Exoglycosidases include β-glucuronidase and α-L-iduronidase, which cleave terminal acid residues, and α-N-acetylglucosaminidase and β-N-acetylhexosaminidase – which cleave terminal hexosoamine residues. Enzymes that hydrolyze sulfate esters within hexuronic acid residues and hexosamine belong to sulfatases [3,28].

DS owes its name to the dermis, in which it constitutes about 0.3% of the dry weight. Mentioned GAGs, apart from the previously mentioned dermis, are found mainly in cartilage, tendons, umbilical cord, walls of blood vessels, and heart valves. DS is also present in the cornea of the eye (as a PGs decorin part), which ensures its transparency, and in the sclera, is responsible for maintaining the correct shape of the eyeballs, as well as in the bone tissue (as a PGs biglycan part) and the aortic wall. PGs containing CSs are present in the aorta, skeletal muscles, eye, lungs, and brain [2,19,29,30,31,32].

CSs perform many important biological functions in the body. Their most important role is to maintain the structure of the ECM. These compounds are also present in the central nervous system, where PGs containing CSs (agrecan, versican, neurocan, brevican, phosfacan, neuroglycan C), DS and HS (syndecan-2, syndecan-3) participate in the formation of neural network in developing mammalian brains. The discussed PGs regulate such processes as proliferation, differentiation, cell adhesion, neuronal migration, and synaptogenesis. The presence of CSs can also be observed in the calcification process during endochondral bone formation. In addition to the previously mentioned functions, CS complexes low-density lipoproteins (LDLs) and very low-density lipoproteins (VLDLs), and accelerates the formation of fibrin [2,7,19,29].

DS plays a role in the processes of blood coagulation, cell growth, protection against pathogenic factors, as well as in the repair process of skin damage. In the case of tissue damage, a local intensification of syndecan-4 synthesis and accumulation of this PG on the surface of endothelial cells and hyperproliferating keratinocytes was observed. This phenomenon was accompanied by an increase in decorin content. Mice lacking syndecan--4 synthesis were shown to have a longer wound healing time compared to mice capable of syndecan-4 synthesizing [19,33,34]. Chondroitin-dermatan glycans additionally have anti-inflammatory and antioxidant effects [3,17,35].

Heparan sulfates and heparins

The group of heparan GAGs includes HSs and Heps. The division into HSs and Heps depends on the scope of post-synthetic modifications, including differences in the content of N-acetylated and N-sulfated D--glucosamine residues, as well as residues of GlcUA or its epimer – IdUA, which are combined by glycosidic bonds such as β (1→4) [2,36]. In HS chains, GlcUA residues dominate quantitatively, while D-glucosamine residues are N-sulfated and N-acetylated in variable proportions. On the other hand, heparin chains are dominated by rich sulfated fragments, which are built of N-sulfated D-glucosamine residues, and 90% of hexuronic residues are iduronic residues [23,27,37]. The initial stage of biosynthesis of heparan GAGs (the process of synthesis of the linking region) is common for the discussed glycans and chondroitin-dermatan glycans, which were described in an earlier subsection of this paper. The consecutive stages of HS/Hep GAGs synthesis are presented in Figure 4.

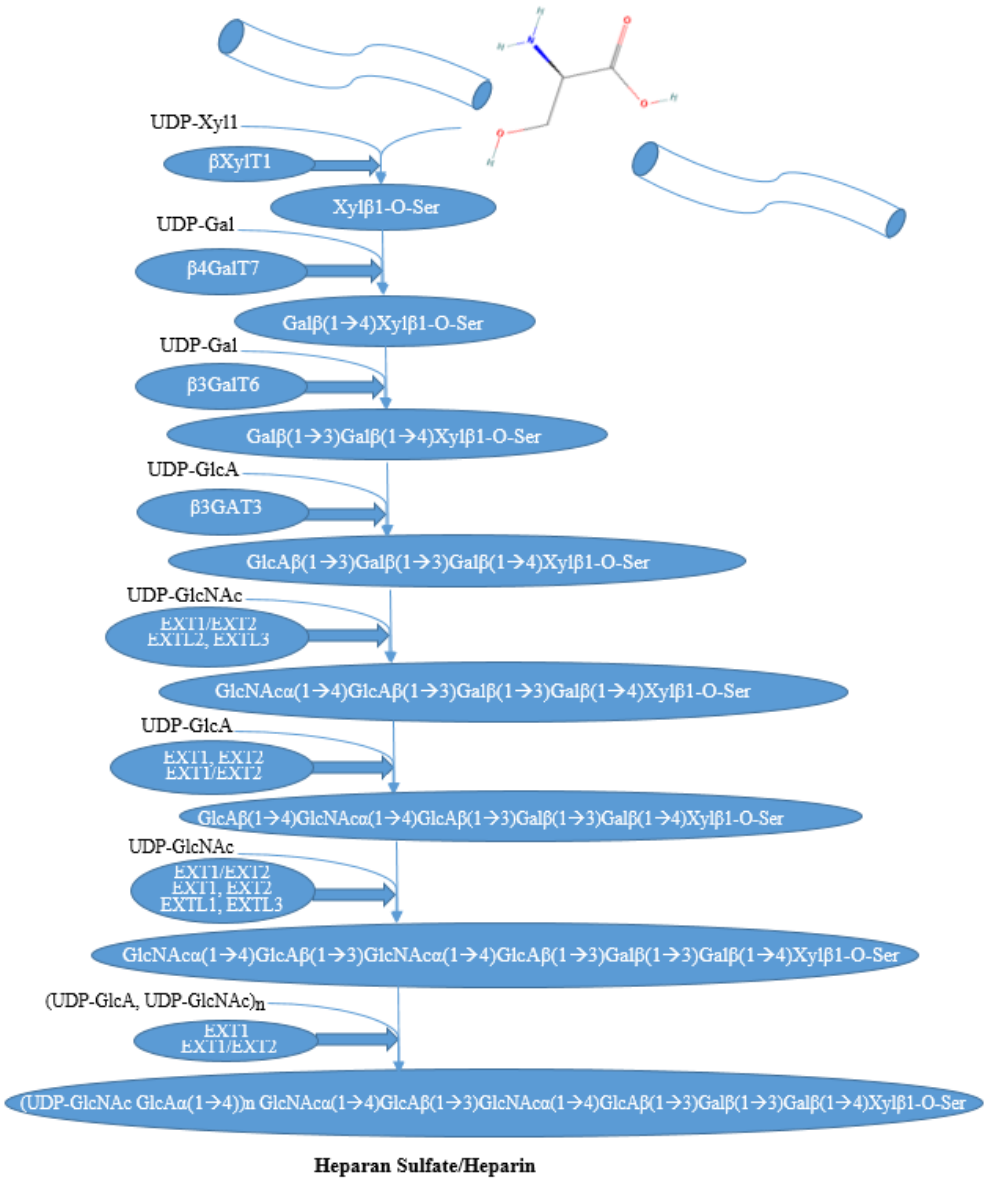

Fig. 4. Biosynthesis of heparan sulfate/heparin (author's own study based on [14,15]); UDP-Xyl1 – UDP-xylose; βXylT1 – beta--1,2-xylosyltransferase; UDP-Gal – UDP-galactose; β4GALT7 – beta-1,4-galactosyltransferase; β3GALT6 – beta-1,3-galactosyltransferase; UDP-GlcA – UDP-glucuronic acid; β3GAT3 – beta-1,3-glucuronyltransferase; UDP-GalNAc – UDP-N-acetylgalactosamine; EXT1 – exostosin glycosyltransferase 1; EXT2 – exostosin glycosyltransferase 2; EXTL2 – exostosin like glycosyltransferase 2; EXTL3 – exostosin like glycosyltransferase 3; UDP – uridinediphosphate.

The tetrasaccharide connecting region initiates the next stage of GAGs biosynthesis – elongation. During the process of elongation of GAG chains, alternate attachment to the non-reducing end of the growing chain of GlcNAc and GlcUA takes place. The beginning of the HS/Hep chains is formed by the attachment of GlcNAc to the fourth carbon atom of the glucuronide residue [14,26,38]. The process of elongation of the discussed GAGs is catalyzed by enzymes such as: N-acetylglucosaminotransferase I and heparan polymerase – a heterodimer, being a product of expression of two genes, i.e. exostosin I (EXT1) and exostosin II (EXT2). Heparan polymerase has the catalytic activity of glucuronosyltransferase II and N-acetylglucosaminotransferase II [14,24,38]. During elongation, the glycan chain undergoes numerous modifications. These modifications include sequential reactions – N-deacetylation of GlcNAc residues followed by their sulfation, epimerization of adjacent glucuronide residues to iduronate residues, 2-O-sulfation of hexuronic acid (usually IdUA) and 6-O-sulfation of glucosamine residues [22,25]. Other O-sulfated derivatives of sugar residues, e.g. 3-O--sulfated-N-sulfated glucosamine or 2-O-sulfated GlcUA are rare [39]. Not all areas of the heparan chain undergo the modifications described above, which means that they remain unchanged [24,27]. The degradation of heparan GAGs initially takes place in

the extracellular space and then in the lysosomes. It occurs with the participation of an endoglycosidase specific for heparan and heparin sulfates – heparan glucuronidase and has been presented in Figure 3. The scheme of the degradation process of the above GAGs is analogous to the scheme of degradation of chondroitin-dermatan GAGs [3,6,28,40].

HSs occur in the form of PGs, forming heparan sulfate proteoglycans (HSPGs), both on the cell surface, in secretory granules, and the extracellular space. Free HSs, which are formed as a result of the degradation of HSPGs, are also present in lysosomes and the cell nucleus, and they co-create the basic substance of the ECM, including basement membranes [1,2,5,19,23,30, 37,41,42]. In the human body, a particularly high content of these PGs is found in the lungs, liver, kidneys, nervous tissue, and the walls of blood vessels. The main places of occurrence of Heps are basophils and mast cells of the lungs, intestines, and liver. Heparin PGs present in mast cell granules can form complexes with other molecules (e.g. enzymes) by combining with antithrombin III and serine protease, they determine the anticoagulant effect [2,7,19,23,35, 43,44,45].

HSPGs have a regulating effect on the permeability of basement membranes. They participate in interactions between cells, as well as between cells and the ECM, resulting in the migration and adhesion of cells to ECM components such as fibronectin, laminin, collagen type I, III, V, XV or XVIII [27,36]. HSPGs are involved in the accumulation of leukocytes at the site of injury or infection, which initiates the process of tissue repair [41].

HSs, through their presence, protect the vascular endothelium against its damage. With the increase in blood glucose concentration, the HSs content in the endothelial cells decreases, which promotes their damage, contributing to the development of diabetic nephropathy, retinopathy, and macroangiopathy resulting from the development of atherosclerosis [2,46,47].

HSs perform a similar function to heparins – they have anticoagulant and antiproliferative effects and also stimulate the activity of lipoprotein lipase (LPL) [7]. Heparan GAGs act as co-receptors or receptors for extracellular ligands, and growth factors, regulating the processes of cellular proliferation, angiogenesis, embryogenesis, or carcinogenesis processes [21,39].

Both heparan sulfate and heparin PGs play a role in the development of inflammation that occurs with tissue damage. They are an indicator of the effectiveness of the wound-healing process. In the initial phase of wound healing, their content increases, which is crucial in the early phase of tissue damage repair [48].

Keratan sulfates

KSs are composed of repeating disaccharide units containing D-galactose residues and GlcNAc residues connected by alternating glycosidic bonds [→3Galβ1→4GlcNAcβ1→] [19,27]. In the discussed glycans, the hexuronic acid residue is replaced by a galactose one. KS chains derived from femoral head cartilage contain fucose residues β (1→3) linked to GlcNAc residues and N-acetylneuraminic acid residues β (1→4) linked to D-galactose residues. Due to the different binding mode to the core protein for the other glycans and the lack of hexuronic acid residues in the KS molecule, KS belongs to the group of galactosaminoglycans [49,50].

The structure of the region connecting KSs with the core protein is responsible for the division of KS into three types: KS I – corneal, KS II – skeletal, KS III – cerebral [50].

The biosynthesis of keratan GAGs, similarly to the CS, DS, HS and Hep synthesis described above, starts in the cytosol, as a result of the activation of dinucleotide derivatives, and subsequently takes place in the Golgi apparatus. The consecutive stages of keratan sulfate GAGs synthesis are presented in Figure 5.

The KS biosynthesis consists of the two stages: the formation of the region connecting the core protein with the GAG chain (there are three types of connections) and the elongation and modification of the chain. There are three types of the above connections [38,49,50]:

- Type I – the glycan chain is connected to the asparagine residue of the protein core (previously synthesized). Two GlcNAc residues and a mannose residue are then attached to the amino acid residue in question. In the next step, two mannose residues, which are responsible for the branched structure of KS, are added simultaneously to the aforementioned terminal residue at positions 3 and 5. Three carbohydrate residues – GlcNAc, D-galactose and N-acetylneuraminic acid form the first chain. In the second chain, the N-acetyl-6-O-sulfate-D-glucosamine residue and the 6-O-sulfate-D-galactose residue are added alternately. The end of KS I biosynthesis occurs after the addition of the N-acetylneuraminic acid residue [27,38,50].
- Type II – the glycan chain is connected to the serine or threonine residue of the core protein through the GalNAc residue. Then, the residue of GlcNAc and the residue of D-galactose join the O-glycosidically attached sugar residue. In the next stage, disaccharide units are added, which are formed by N-acetyl-6-O-sulfate-D-glucosamine as well as 6-O--sulfate-D-galactose; a D-galactose residue to which

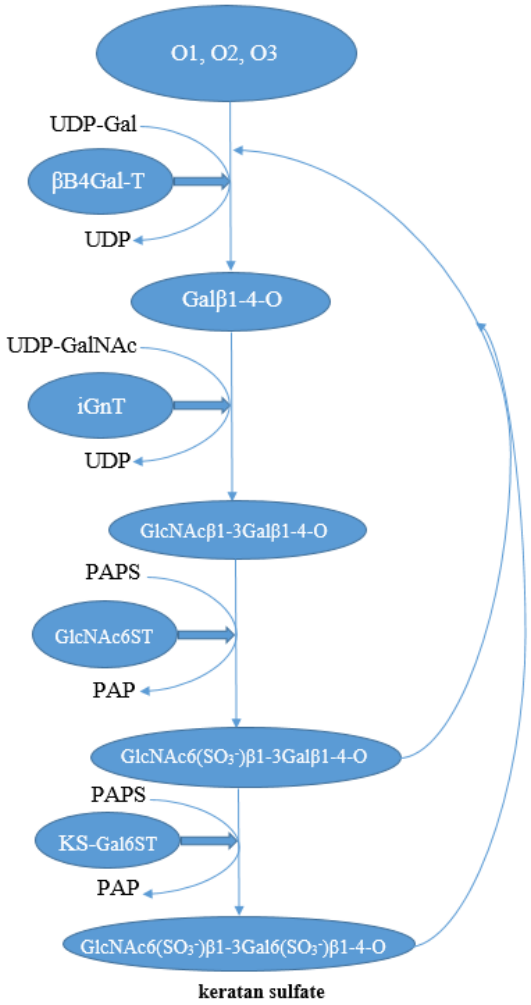

Fig. 5. Biosynthesis of keratan sulfates (author's own study based on [13]); βB4Gal-T – beta-1,4-galactosyltransferase; UDP-Gal – UDP-galactose; GlcNAc – N-acetylglucosamine; GlcNAc6ST – N-acetylglucosaminyl-6-transferase; iGnT – β1,3-N-acetylglucosaminyltransferase; KS-Gal6ST – galactosyl-6-sulfotransferase keratanosulfate; PAP – 3'-phosphoadenosine-5'-phosphate; PAPS – 3'-phosphoadenosine-5'-phosphosulfate; UDP – uridinediphosphate; O1 – N-glycosidically linked oligosaccharide of the complex type connecting the KS chain to the core protein; O2 – N-glycosidically linked oligosaccharide type 2 connecting KS chain to core protein; O3 – N-glycosidically linked mannose residue connecting KS chain to core protein.

an N-acetylneuraminic acid residue is added. Similarly as in KS I – the biosynthesis of the polysaccharide chain of KS II ends with the attachment of the N-acetylneuraminic acid residue [38,50].

- Type III – the glycan chain is connected through the mannose residue to the serine or threonine residue of the protein core by means of an O-glycosidic bond. The binding of N-acetylneuraminic acid terminates the biosynthesis of KS I and KS II, while the terminal residues of KS III are unknown [21,50].

Keratan sulfate GAGs are initially degraded in the extracellular space, then in the lysosomal compartment (see Figure 3). As a result of the acidic hydrolases of N-acetylglucosaminidase, β-galactosidase and sulfatases, successive parts of the glycan chain are gradually removed. First, the sulfate group is removed from the galactose residue at the end of the KS chain, then the galactose residue is detached. The next step is the hydrolysis of the sulfate group attached by an ester bond to the GlcNAc residue, preceded by the detachment of the hexosamine residue from the glycan chain. The above reactions are repeated until the complete degradation of the keratan sulfate GAG chain [2,3].

These glycans occur in the ECM as well as on the surface of cell membranes as PGs, but not as commonly as other GAGs. Keratan GAGs occur primarily within the structures that are exposed to loading forces, i.e. bones, cartilage, tendons. They are also present in the peripheral and central nervous system, cornea and epithelial tissue [1,2,50].

KSs, like other GAGs, perform many important functions in the body. KSs are involved in the repair of tissue damage; in cartilage, on the other hand, they give the tissue the properties of load transfer and counteracting compression forces. In turn, KSs present in nerve cells are involved in the development and metabolism of nervous tissue, they are responsible for the proper conduction of signals. These glycans are the key components of the cornea, where they are located between collagen fibers and give it transparency. KSs also participate in the process of embryo implantation [2,19,49].

Hyaluronic acid

HA is a naturally occurring, unbranched polysaccharide chain composed of repeating disaccharide units containing GlcUA and GlcNAc linked by glycosidic β-1,3 and β-1,4 linkages [2,51,52,53,54]. Unlike other GAGs, it does not form complexes with proteins, so it does not form PGs. However, it can non-covalently bind to other proteins, which leads to the formation of proteoglycan aggregates (HA is the axis to which PG molecules bind). The disaccharide components of HA are not subject to such modifications as sulfation or epimerization. Due to the presence of carboxyl residues, HA exhibits a polyanionic character [2,55,56,57]. The synthesis of HA – unlike other GAGs – does not require the presence of a protein core. The synthesis of HA is presented in Figure 6.

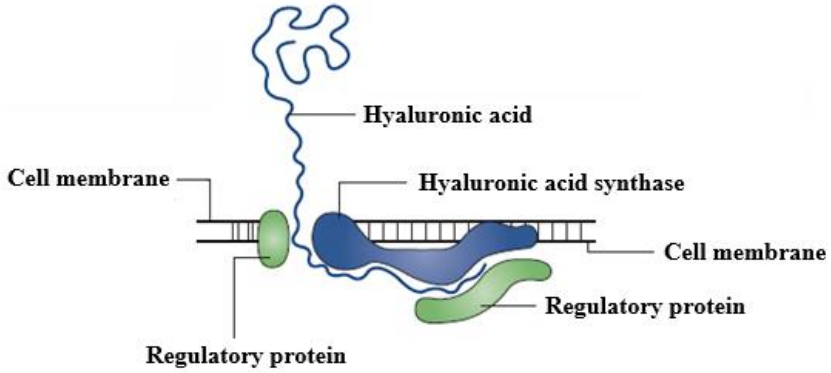

Fig. 6. Hyaluronan biosynthesis (author's own study based on [16]).

It occurs on the inside of the cell membrane and is catalyzed by three isoforms of an enzyme called hyaluronan synthase (HAS-1, HAS-2 and HAS-3) [51,55,57,58]. This enzyme catalyzes the transglycosylation reaction of GlcUA and GlcNAc from the appropriate nucleotide precursors, i.e. UDP-GlcUA (uridine-5'-diphosphoglucuronic acid) and UDP--GlcNAc (uridine-5'-diphospho-N-acetyl-D-glucosamine). The discussed monosaccharide subunits are attached alternately, with glycosidic bonds, to the reducing end of the growing chain, resulting in disaccharide sequences of HA, which are not subject to modifications [2,27,38,55]. The newly synthesized polymer, growing on the inner, cytoplasmic side of the cell membrane, is translocated outside the cell membrane from the side of the non-reducing end. The synthesis of HA is continued, which allows the polymer to grow and obtain molecules of large size, and consequently of high molecular weight, in approximately 107 Da [35,55,57,59].

Systemic degradation of HA is an integral part of its metabolism (see Figure 3). Degraded molecules are replaced by newly synthesized molecules. About 1/3 of the entire amount of human hyaluronan is exchanged daily in the body [55,60]. Depending on the type of tissue, HA is catabolized at different rates. It is most rapidly removed from the blood, where the half-life of HA is 2–5 minutes, in the skin the half-life is 12 hours, 1–3 weeks in cartilage and up to 70 days in the vitreous body of the eye [55,60,61]. Part of the biopolymer in question is decomposed at the site of biosynthesis and in the tissue, while part is transported with the lymph to the lymph nodes, where it is degraded. The remaining HA, which has not been degraded in the above--mentioned lymphatic structures, come into the systemic circulation, from which it is removed primarily by the endothelial cells of the sinusoidal vessels of the liver, and small amounts are excreted by the kidneys [55,57,60]. Elimination of HA from tissues occurs via CD44 (membrane glycoprotein), followed by endocytosis, often preceded by extracellular degradation, which is catalyzed by extracellular hyaluronidases or the influence of reactive oxygen species (ROS), the amount of which is influenced by support of the body's natural antioxidant defenses. HA is sensitive to ROS, which can cause local inflammation. Non-enzymatic HA degradation can also occur under the influence of pH, ultrasound, and temperature. Under the influence of hyaluronidase and exoglycosidases, such as β-glucuronidase or hexosaminidase, HA is degraded within the lysosomes in the cell [55,60].

In the human body, HA is present in all body fluids as well as in tissues. Tissues of mature organisms contain HA in small amounts. On the other hand, HA is very abundant during embryonic development, and in inflamemation, during healing, and within cancerous tumors. The largest amounts of HA are located in the dermis, cartilage and synovial fluid, the vitreous body of the eye, umbilical cord, heart muscle, kidneys, muscles, lymphatic system, mucous membranes, and also in the brain [2,5,51,53,55,62,63].

The basic function of HA is to bind and retain water in intercellular space, which determines the tissue resistance to mechanical loading. Together with CS, HA provides tissue strength as well as elasticity [19].

HA has a significant degree of hygroscopic properties because 1 g of this glycan can bind up to 6 l of water (i.e. about 250 water molecules), thus increasing its volume up to 1000 times [57,64]. The result of these properties is the ability to form a free and durable network, creating highly viscous and flexible solutions that fill the intercellular space. Thanks to the ability to bind water, HA is characterized by viscoelastic (viscoplastic) properties, responsible for maintaining proper hydration, turgor, and tension of the ECM [65,66]. The discussed glycan, in the form of high molecular weight chains, is involved in the processes of cell adhesion and migration, exhibits anti-inflammatory properties and plays an antioxidant role. Small-molecule fragments of HA, however, show the ability to induce (in endothelial, epithelial and dendritic cells, fibroblasts and macrophages) inflammatory genes expression [2,3,23,35,55,59,67]. HA also has the properties of a signal molecule HA (HA fragmentation intensifies its ability to activate signal transduction pathways). In the body, HA interacts with specific binding proteins, the so-called hyaladherins [55,57,64]. Research by Volpi et al. [68] showed the participation of hyaluronan in many processes related to ovulation and fertilization. The presence of this glycan facilitates the release of the oocyte during ovulation. The above studies showed a relationship between the ability of sperm to penetrate a highly viscous solution, resulting from the presence of HA, and sperm motility and fertilization efficiency. In addition, by showing the ability to inhibit the activity of platelet-derived growth factor (PDGF), HA exhibits antiproliferative activity against smooth muscle cells involved in the pathogenesis of atherosclerosis [2,52]. In medicine, it has been used as a marker of some diseases (e.g. rheumatic or cancer) [2,23,67]. It is used in surgery, aesthetic medicine, ophthalmology, dermatology, orthopedics, gynecology, urology, neurology, and tissue engineering [2,57,58].

Glycosaminoglycans in wound healing

Wound healing is a complex and dynamic, time-synchronized physiological process, consisting of four overlapping phases: hemostasis, inflammation, proliferation, and remodeling, which are necessary for the proper course of regeneration of the resulting tissue damage. The discussed process is, therefore, the body's reaction to damage to the continuity of tissues and is associated with the activity of such cells as macrophages, leukocytes, keratinocytes, fibroblasts, endothelial cells, or platelets, but also with the activity of numerous biologically active compounds, e.g. cytokines, interleukins, growth factors, and components of the ECM, such as collagen, laminin, vitronectin, fibronectin, PGs and GAGs [69,70,71]. Components of the ECM play a structural and functional role in each of the above-mentioned phases of healing. These components participate in the repair process, creating a provisional matrix, granulation tissue, and then a scar. They participate in signal transduction, stimulate cell adhesion and migration, mediate cell-cell and cell-ECM interactions, also modulate the healing process. ECM components perform functional functions, regulating the healing process by acting as a reservoir and modulator for cytokines and growth factors; they also perform structural functions by filling tissue defects during the repair process [11,72,73]. GAGs are essential components of the ECM involved in the process of tissue repair [11].

One of the GAG fractions, i.e. DSs, dominates in normal skin, constituting 78% of all sulfated GAGs, hence – during the healing process – the DSs content, both in the wound fluid and in the tissue, increases the most [19,74]. The GAG in question is the main glycan that occurs in the wound bed [75]. Its role is to activate leukocyte adhesion to endothelial cells (as a result of ICAM-1 stimulation) [76]. DSs, together with HSs and Hep, have a high affinity for hepatocyte growth factor/spreading factor, which provides growth, motility, and morphogenic stimuli to epithelial, endothelial, and neural cells in the wound healing process [77].

A study by Olczyk et al. [76] showed that GAGs, i.e. CSs, DSs and HA, were isolated in all samples taken from the burn wound bed. In the above studies, it was observed that propolis stimulate significant changes in the content of particular types of GAGs during burn healing. Propolis accelerated the repair of damaged tissue by stimulating the accumulation of GAGs in the burn wound bed needed for granulation, tissue growth, and wound closure. In addition, propolis accelerated the modification of the structure of chondroitin/dermatan sulfates, which are responsible for binding growth factors, and playing a key role in tissue repair.

The above conclusions also correspond to the results of research conducted by Bentley [78], which indicated a systematic increase in the content of DSs and CSs in healing wounds. Siméon et al. [79] also showed, in a study using the tripeptide (gly-his-lys) – Cu^{2+} complex, a gradual increase in the content of CSs and DSs during the healing of tissue damage.

Sulfate residues, present in CSs and DSs, stimulate the transformation of fibroblasts into myofibroblasts, thereby influencing wound contraction. CSs, like DSs, stimulate the production of nitric oxide by the endothelium of blood vessels, which modulates the angiogenesis process and stimulates leukocyte adhesion to endothelial cells [10,74,80].

HS is involved in the process of transformation of fibroblasts into myofibroblasts leading to the wound closure [81]. The initial increase in HS content is crucial in the early stages of wound healing [81,82].

Studies by Belvedere et al. [74] showed a transient increase in HSPG expression during tissue repair. During the study, a significant relationship was

observed between the migration of keratinocytes and fibroblasts during the healing of venous ulcers and the content of GAGs in the wound bed. The discussed experiment confirmed the role of GAGs (including HS) in the wound healing process by modulating the migration, proliferation, and differentiation of inflamematory cells, fibroblasts, and keratinocytes [74,83].

Studies by Olczyk et al. [81] also indicated a short-lived increase in the content of heparan and heparin sulfates in the bed of burn wounds treated with a propolis formulation, which confirmed the beneficial effect of the aforementioned compound of natural origin on the tissue repair process.

HA plays an important role in the healing of wounds of the skin or mucous membranes, influencing inflammation, granulation, and re-epithelialization, as a result of its anti-inflammatory effect, influence on fibroblast migration and stimulation of collagen synthesis and angiogenesis [84,85,86]. This glycan is also involved in tissue repair and serves as an integral part of the ECM, also promoting keratinocyte proliferation and migration in the process of re--epithelialization [74,87].

The study by Siméon et al. [79] showed an initial increase in the content of HA in the skin wound bed of rats, followed by a progressive decrease in the content of HA at the wound site. Hamed et al. [88,89] showed that erythropoietin used in the experiment stimulates an increase in the HA content in the wound bed in the initial phase of the healing process. The consequence of this change was an accelerated wound healing process resulting from the stimulation of angiogenesis, re--epithetlialization, collagen synthesis, and, on the other hand, inhibition of the inflammatory response and apoptosis.

During an experiment conducted by Olczyk et al. [76] assessing the effect of an apitherapeutic – propolis – on changes in the content of GAGs in post-burn wounds, it was shown that in the initial phase of the experiment, there was an increase in the content of HA in the wound bed treated with an ointment containing propolis, and then – in the final phase – reduction of HA content. The authors associated the described tendency of changes in the content of HA with the properties of propolis, which intensifies the expression of TGF-β, stimulating fibroblasts to synthesize HA.

In selected studies conducted by Olczyk et al. [81,90,91] the experimentally induced wound healing was evaluated through quantitative and/or qualitative characterization also ECM components other than GAGs e.g. laminin, vitronectin, collagen or fibronectin in the tissue samples taken from injuries, inflicted on domestic pigs.

The mentioned animal model, according to Hoekstra et al. [92], is known as useful, standard experimental model for the evaluation of wound repair because of many similarities of pig skin to human one.

The other analyses conducted by the above mentioned researchers allowed to identify two multiadhesive glycoproteins – vitronectin and laminin in the bed wound. An increase in both vitronectin and laminin was observed, especially in the initial days of healing of the damaged tissue, as a result of the use of propolis [81].

In addition, it was found that the treatment of thermal damage with propolis increased the expression of collagen type I and III, especially in the initial phase of the study. The assessed therapeutic effectiveness of propolis, through quantitative and qualitative analyses of the expression of type I and III collagen, indicated that the apitherapeutic agent discussed in that study could generate a favorable biochemical environment supporting re-epithelialization [90].

Moreover, it was noticed that treatment of burns with medicinal products containing propolis reduced the release of synthesized fibronectin molecules from healing wounds about tissue damage treated with ointment without that natural product which indicated propolis influence on fibronectin metabolism modification thereby preventing the unnecessary complications of wound healing process [91].

Given the described participation of GAGs in the healing process, the leading role of these macromolecules in tissue repair should be emphasized. At the same time, it is important to highlight the beneficial effect of apitherapeutics on the transformation of ECM components involved in the remodeling of damaged tissue.

Author's contribution

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