GLYCOSAMINOGYCAN-BASED BIOMATERIALS USED IN WOUND HEALING

BOGDAN-MIHAI NEAMȚU[1], ANDREEA BARBU[2]

[1,2]Pediatric Clinical Hospital, Sibiu, Research and Telemedicine Center in Neurological Diseases in Children – CEFORATEN, Sibiu,
[1] "Lucian Blaga" University of Sibiu, [2]University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca

Keywords: *biomaterials, glycosaminoglycan, heteroglycan, wound healing*

Abstract: *The following review is focused on a very important wound healing biomaterial class, i.e. glycosaminoglycans, a heteroglycan-based compound. Every biomaterial has its own advantages and disadvantages, but these disadvantages are usually surpassed by blending through different methods with various other compounds. The main advantages of glycosaminoglycans include their water solubility that allows them to maintain the wound environment moist and to absorb the wound exudate. If they are mixed with other materials they can help hemostasis, they do not adhere to the wound bed and thus prevent pain when they are removed from the injured site. They are also known to have antibacterial and antioxidant properties, and low cytotoxicity. This review underlines the benefits glycosaminoglycan application has on wound healing, including the use of several blends that enhance its characteristics.*

INTRODUCTION

A traditional wound healing dressing (i.e. based on lint, honey, herbs) is cost effective but will cause local foreign body reaction and fast dehydration, hence pain and bleeding, because of the cotton fibers.(1–4) Because of this, researchers have developed new biomaterial-based dressings for wound care and healing. A biomaterial is "a natural or synthetic substance that is not a drug, which can be introduced into body tissue as part of an implanted medical device or used to replace, treat, or augment a tissue, an organ or a bodily function".(3)

An ideal wound dressing has to meet several criteria such as biocompatibility, biodegradability, elasticity, reasonable price, non-adherence to the wound bed that will allow a pain free removal, and semi-permeability while allowing gas exchange but no micro-organisms transfer. It would also be ideal if the wound dressing would exhibit debridement, high excess exudate absorption and pain relief activity, non-antigenic and non-toxic properties, provide bacterial, infectious, mechanical, and thermic protection, compatibility with therapeutic agents, and healing/re-epithelialisation capability. Biopolymers sum up most of these benefits while being biodegradable and bioactive, they promote tissue regeneration and wound healing through cell migration and proliferation.(1,3,5-9)

AIM

The wound healing process would be faster and leaner if the used biomaterial would assist the autolytic debridement while modulating the moisture of the wound environment by moisturizing the dry wounds and/or absorbing liquids and exudates from the wet ones. Moreover, a faster and better healing will be observed when angiogenesis is promoted, the immune cells are modulated, and the fibroblasts' and keratinocytes' migration is enhanced while preventing or treating an infection.(8)

In this article we summarize the benefits of using glycosaminoglycas as biomaterials for enhancing wound healing.

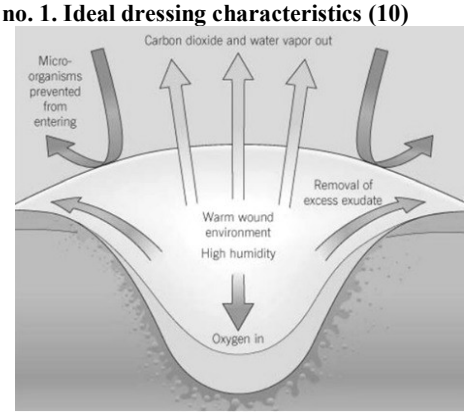
MATERIALS AND METHODS

We searched in Academia.edu, Elsevier, Google Scholar, Pubmed, Researchgate, and Springer databases for relevant articles related to glycosaminoglycans used in wound healing. The keywords used were glycosaminoglycans, biomaterials, hyaluronic acid, wound healing, heparin and heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate. The citations and reference list were made using the Mendeley® software.

RESULTS AND DISCUSSIONS

Biomaterial-based wound dressings can hold drugs and antibiotics because they are able to change their properties and their release kinetics, making them ideal for wound care/healing. Wound healing can also be stimulated by recruiting precursor cells by an ideal regenerative or responsive biomaterial to form new and viable tissues *in vivo* (6), as seen in figure no. 1.

Figure no. 1. Ideal dressing characteristics (10)



Biopolymers are used as biomaterials for wound healing in many forms, either as standalone or in various combinations with other ingredients to maximize the benefits and lower the associated risks. Polymers can be either natural or

[1]Corresponding author: Andreea Barbu, B-dul. Mihai Viteazul, Nr. 11B/13, Sibiu, Romaânia, E-mail: ing.andreea.barbu@gmail.com, Phone: +40748 063335
Article received on 29.07.2019 and accepted for publication on 28.08.2019
ACTA MEDICA TRANSILVANICA September;24(3):84-91

artificial /synthetic. Natural polymers can be classified based on their structure as it follows: polysaccharides, glycolipids, proteoglycans, peptides and proteins and were used until 1993 for suturing, grafting blood vessels or as a tissue substitute.(11) Their degradation rate is fast, so a crosslink between them is made. Crosslinking is the attachment between two polymer chains with chemical agents that make the bond stronger. Another concern is the probability of undesired side effects because most of these biomaterials are obtained from animal sources. Protein-based biomaterials usually have animal and human origins and are represented by bioactive molecules that resemble the extracellular environment, while polysaccharide-based biomaterials derive from algae or from microbial sources.(12) These polymers are modified for therapy in various degradable nontoxic forms such as (injectable) hydrogels, particles, porous scaffolds or thin membranes. The degradation kinetics is not easy to foretell but the topical effects are remarkable.(12)

Polysaccharide-based biomaterials used for wound healing can either be classified as homoglycans and heteroglycans or based on their pH: acidic, basic, neutral, and sulfated like heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulphate.(3,13)

Heteroglycans (heteropolysaccharides) are high molecular weight polymers containing more than one type of monosaccharide residue. They usually bind to proteins or lipids and form proteoglycans or glycolipids and are usually found in the connective tissue, cartilage, plant gums, cell walls or some algae. Because several heteroglycans are biocompatible, biodegradable, can form electrostatic networks and have thickening and gel- or film-assembling properties, they are used in biomedical or therapeutic applications. The most commonly used heteroglycans for therapy are alginates, agarose, carrageenans, fucoidans, gums, glycosaminoglycans and pectins.(3,14)

Glycosaminoglycans (GAGs) are the major organic components found in the extracellular matrix. The most common mentioned GAGs are hyaluronic acid/hyaluronan (HA), heparin and heparan sulfate (HSGAGs), chondroitin sulfate (CS) and dermatan sulfate (DS) (CSGAGs), and keratan sulfate (KS) and they exhibit high water absorption and swelling properties.(15,16)

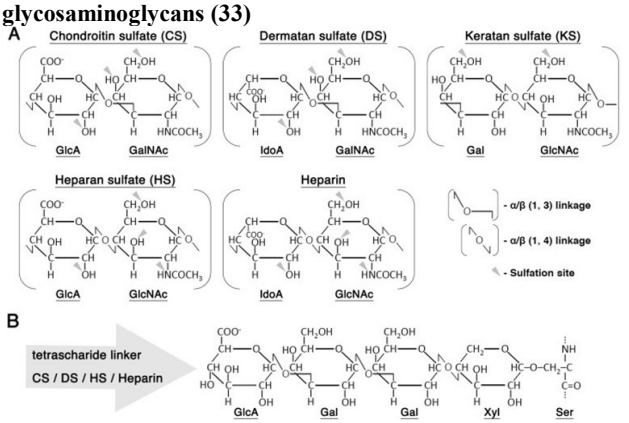
Structure and synthesis of glycosaminoglycans

GAGs are long, linear, unbranched repeated polysaccharides composed of a hexosamine (D-galactosamine, GalNAc, or D-glucosamine, GlcNAc) linked by glycosidic bonds to galactose or an uronic acid (D-glucuronic acid, GlcA or L-iduronic acid, IdoA). Chemical modifications of the saccharide subunits, chain length, composition, glycosidic linkage, sequence, or sulfation develop the heterogenic structure of GAGs. HSGAGs, KS and HA contain glucosamine and CSGAGs contain galactosamine, as seen in figure no. 2 (A). The uronic acids as IdoA/GlcA can be found in HSGAGs and CSGAGs, Gal (β-d-galactose) in KS, and GlcA in HA. GAGs are either sulfated (HSGAGs, CS, DS, KS) or nonsulfated (HA). KS is the only GAG without a carboxyl group, with a molecular weight range between 5-25kDa. If a GAG chain has uronic acids or sulphate groups in its structure, then a negative charge will be observed. The sulfated ones usually have a covalent link to a protein core and result in proteoglycans, hence HA will not become a proteoglycan. Glycosaminoglycans and proteoglycans display an intense remodeling behavior due to their dynamic molecule properties and those found in the bone or in skin suffer quantitative and qualitative changes because of UV irradiation and aging. More information about these GAGs is available on Pubchem (HA ID – 24759 or 24728612, HS ID – 53477714, Heparin ID – 772, CS – 24766, D4S – 32756, KS ID – 446715) (15,17–25) and the most relevant characteristics are summarized in table no. 1.

HA (21,22) is composed of B-D-glucuronic acid and N-acetyl-D-glucosamine (2-acetamide-2-deoxy-α-d-glucose) linked by alternating b-1,4 and b-1,3 glycosidic bonds and is involved in scarless wound healing, enhanced mitotic epithelial cells' division, and macrophage regulation for phagocytosis, and has a bacteriostatic effect.(3,26,27) Wound healing after HA-based treatment can also be seen through enhanced angiogenesis, subcellular tissues regeneration, granulation tissue formation, epidermal thickness, and keratinocytes proliferation.(28) Heparin (25) and Heparan sulfates (23) are negatively charged linear polysaccharides with various degrees of sulfation, composed of L-iduronate (many with 2-sulfate) or D-glucuronate (many with 2-sulfate) and N-sulfo-D-glucosamine-6-sulfate, the linkage is at α-(1, 4) for IdoA, or at β-(1, 4) for GlcA. Heparins contain more overall sulfate and iduronic acid than heparans and are used as antithrombotic drugs. HS is considered to be "the king of the GAG family" because of its variety of clinical applications and biological roles.(16,29) The biosynthesis of HS, CS and DS was discussed in several reviews.(16,30-32)

HSGAGs and CSGAGs are synthetized in the Golgi apparatus and form proteoglycans when the protein cores from the rough endoplasmic reticulum are modified by glycosyltransferases with O-linked glycosylation. HA is not synthetized in the Golgi apparatus, but by transmembrane proteins in the integral plasma membrane synthases, called HA synthases, that secrete elongated disaccharide chains. HA does not form proteoglycans. KS is a sulfated GAG found in the bone, cartilage, central nervous system, and cornea and can modify core proteins through proteoglycan N- or O-linked glycosylation (15,16), as seen in figure no. 2 (B).

Figure no. 2. Chemical structure (A) and synthesis (B) of glycosaminoglycans (33)



The main structural difference between CS and DS consists in the different residue contained by the GAG, DS having the iduronic ones and CS, glucuronic.(16) Chondroitin 4- and 6-sulfates (19) are composed of alternating D-glucuronate (GlcA) and GalNAc-4- or 6-sulfate with a linkage at β-(1, 3).(16) Dermatan sulfates are composed of L-iduronate (IdoA) or D-glucuronate (GlcA) with a GalNAc-4-sulfate, the GlcA and IdoA are sulfated and the linkage is made at β-(1,3) for GlcA or at α-(1, 3) for IdoA.(20)

Keratan sulfates (24) are composed of galactose and GlcNAc-6-sulfate with the linkage at β-(1, 4) and no contain uronic acid residue, with a N-linkage or O-linkage possibility of binding to a protein core.(15,16) Proteoglycans linked to KS can be found in the cornea, cartilage, brain, and bones in three distinct forms (KS I, KS II and KS III), each differing in

structure and location.(16,18,34)

Table no. 1. General characteristics of glycosaminoglycans

Aspect	Description	References
Type	Linear polysaccharides - alternating monosaccharide residues	(18, 35)
Source	Organic extracellular matrix	(17)
Structure	N-acetylated hexosamine in a repeating disaccharide unit (uronic acid or galactose). The disaccharides involved alternate.	(15, 16)
Molecular weight (g/mol)	High and variable: CS 463.363; DS 475.374; HA 776.651; Heparin 1134.899; HS 637.504; KS 1052.914	(19–25)
Functional groups	N-acetylated hexosamine and 1,3- or 1,4-linkages of acetylglucosamine or acetylgalactosamine. Either sulfated or non-sulfated	(15, 16, 36, 37)
Solubility	HA – soluble, visco-elastic solution Heparin – water soluble (200 mg/mL), insoluble in acetone, alcohol, benzene, chloroform, and ether, pH 5.0-7.5 (in 17% sol)	(18, 25)
Gelation factors	Temperature, hygroscopicity, shear stress, chemical structure	(12, 15, 37–39)
Swelling factors	The negative charge attracts Na+ ions and they swell due to hydrostatic pressure; pH	(38, 40)
Dissolution factors/time	Type, temperature, molecular weight, log P	(19–25, 37, 39)

Local properties

GAGs are involved in the remodeling processes of skin regeneration and bone formation because they regulate protein action, gene expression and precursor cells attraction and differentiation, but GAGs specific action is influenced mostly by their structure (polymer length and sulfation degree). GAGs are also involved in extracellular cation and water homeostasis, various proteins' (such as chemokines, cytokines, enzymes, and growth factors) and adhesion molecules' function interaction and modulation. Also, GAGs modulate the adhesion, differentiation, migration, and proliferation of diverse bone or skin cells, and the collagen's matrix structure in scaffolds.(17) Because of their polyanionic nature GAGs bind to divalent cations (Ca2+, Mg2+, Na2+) and result in a high hydrodynamic volume with low compressibility media, which acts as a size-dependent filter that only allows small molecules to pass through (17). The biological activities in which glycosaminoglycans are involved in are summarized in table no. 2.

HA, HS, CS, and DS form the "ground substance", also known as hyaloplasm, an amorphous gel which aids in collagen fibers' aggregation and accumulation. Collagen is produced during the fibroblast proliferation phase for about 3 weeks, it increases the wound tensile strength, and then the degradation rate will be equal to the production. After cross-linking and a 4:1 type I to type III collagen ratio is achieved, the scaring process and GAGs' degradation will stop, because they will come to a normal concentration.(41) Integra®, a collagen-GAG scaffold, has shown its ability to help capillary growth, endothelial and fibroblast invasion, and epithelialization even though there is no areal vascularisation.(9,17) Angiogenesis or neovascularization involve fibroblastic proliferation that will help synthetize type III collagen and proteoglycans. Type III collagen also creates a fibrous capsule found in various biomaterials.(42)

GAGs' ability to bind with IL-8, bFGF and TGF-β has a wound healing advantage, because it makes the monocytes/macrophages, inflammatory mediators, toll-like receptors and neutrophils migrate towards the injured area. A skin injury will produce changes of fibronectin, laminin, and other ECM components. Keratinocytes lead to antimicrobial peptides release that will provide an anti-bacterial protection. HAS1 and HAS3 enzymes initiation will lead to a higher HA synthesis at the dermal and epidermal layers, but if their level lowers then wound healing will be impaired.(17) If GAG matrices are UV cross-linked to collagen they exhibit a low toxicity level when compared to other dermal substitutes based on glutaraldehyde.(8)

Hyaluronic acid is the main GAG found in skin, and CS and HA are immune-suppressants. If HA is cross-linked to a cable-like structure then a highly adhesive network will be formed that will allow pro-inflammatory mediators to be isolated and leukocytes to attach, a useful process in chronic inflammation.(17,43) Also, propolis was shown to have beneficial roles on burned skin wounds as it follows: chondroitin/dermatan sulfate structure might be modified, GAGs such as HA will accumulate and keratinocyte proliferation will increase at the animal wound site.(2) A critical review made by Hussain et al in 2017 analyzed HA alone or in various combinations (collagen, curcumin, fibrin, growth factors, silk fibroin, silver etc) and showed its beneficial role in treating several acute or chronic wound types such as articular, cartilage, corneal, skin and tracheal ones under different forms like anti-adhesive sheets, cultured dermal substitutes, dermal matrix grafts, films, hydrogels, scaffolds, sponges or thin membranes.(28)

HA-based wound healing products such as Hyaff®, Hyalograft®, Hyalogran®, Hyalo4® and Laserskin®, and HA-based hydrogel scaffolds direct tissue regeneration because their angiogenesis support and neuritis repair ability are being used with the FDA approval. HA and its derivatives are also used in arthrology, cancer therapy, drug delivery systems, odontology, ophthalmologic and plastic surgery, tissue engineering and anti-wrinkle injections.(18,44) HA's anti-inflammatory effect is directly linked to its molecular weight or its interaction with CD44 from the fibroblasts. Because HA scaffolds cannot easily be electrospun due to their high viscosity and surface tension that increases the water intake, the hot air blowing technique is preferred in making HA nanofibers because after water evaporation homogenous nanofibers can be used for wound healing, showing better results compared to vaseline-based gauge or adhesive HA bandages in a study made on pigs.(3,14,18,27,45) An experimental rat study showed a significantly reduced wound size after a composite polyurethane foam with HA and silver sulfadiazine impregnation was applied. Other stable HA-based hydrogels showed encouraging *in vitro* cortical cell growth results by crosslinking HA with glycidyl methacrylate groups and DNA or by functionalizing HA with thiol cross-linking sites.(27)

From the FDA approved commercially available HA-based products Hyalogran® is used for pressure wounds, ischemic and diabetic wounds, and foot ulcers, especially those that are covered with necrotic tissue; Hyalomatrix® can be used on abrasions, chronic vascular, diabetic, pressure or venous ulcers, partial or full thickness wounds, second degree burns, and post-surgery wounds, and Jaloskin® is indicated for superficial exudative wounds such as chronic vascular, diabetic, pressure or venous ulcers, post-surgery wounds, first and second degree burns, and abrasions.(18,46-48)

The HA hydrogel that encapsulates amniotic fluid stem cells could retain and disperse cytokines and growth factors that were discharged from those cells long after their presence was observed. Nanoparticles enhanced with HA and vitamin E inside a thin film discharges the vitamin in a controlled manner and reduces the water loss. Wound healing was enhanced in a diabetic mouse model if matrix metalloproteinase enzyme (MMP) degradable HA hydrogels were enriched with VEGF plasmids. Porous nanoparticles based on HA that contained PDGF-BB had better results regarding rat excisional wound healing when compared to the control

group.(8) Another study centered upon creating freeze-dried collagen-GAG scaffolds with a mean pore size of 85 to 325 μm.(49)

Because HA can bind to CD44, and their interaction is thought to be involved in malignant tumor invasion, caution is advised. On the other hand, if their interaction takes place in the cartilage it can improve homeostasis because it modulates chondrocytes' life cycle and metabolism and it is also involved in cell migration and leukocyte activation.(16,26)

Heparin and **Heparan sulfate** display anticoagulant properties and anti-cancer potential (50), but many other studies regarding heparin's effect are currently performed. These human and animal studies are centered on acquired HIV, adult respiratory distress syndrome, allergic encephalomyelitis and rhinitis, arthritis, asthma, inflammatory bowel syndrome, interstitial cystitis, and transplant rejections. Heparin and HS showed HIV1 and 2 reduced ability to adhere to T4 cells, it neutralized cytotoxic cell products improving animal's lung function, reduced cell activation and gathering, inflammatory cell transport, collagen destruction, metastasis, tumor growth, increased animal survival time, and prolonged allograft survival time in animal studies.(51-53) New drugs that are structurally similar to heparin are also being developed, with various biological activities against angiogenesis, allergies, adhesion, inflammation, and metastasis, while being orally active, non-immunogenic, non-anticoagulant, and heparanase inhibiting.(51) Several molecular biological assays rely on heparin's ability to hinder DNA to RNA polymerase bonding because heparin can occupy the DNA binding sites. Low molecular weight heparins have longer half-life (4 hours compared to 1-2 hours), high bioavailability and estimable anticoagulant response, with a reduced risk of heparin-induced thrombocytopenia and osteoporosis compared to unfractionated heparin.(54,55) Heparin interacts with antithrombin III and enhances the inhibition of factor Xa and IIa, and with annexin V enhance the protein oligomerization.(15,54)

HS interacts with tropoelastin, is involved in the formation of elastic fibers while being present in human dermis elastic fibers and as proteoglycan cell surface receptors. Its proteoglycans are involved in adhesion between cells and matrix, growth factor binding, immunomodulation (against parasites and viruses), protease and early embryonic development modulation, hemostasis regulation, and lipoprotein metabolism.(16,29,56) Scaffolds based on heparin-coated aligned nanofibers showed promising results for full-thickness dermic remodeling in regards to an endothelial cell infiltration increase, and a mixture of HS and OTR4120 polymer stimulated angiogenesis and collagen maturation while decreasing the inflammation, and accelerated the wounds and burns regeneration process.(3,14,52) Heparin/HS acts through several pathways like cell proliferation, anticoagulation pathway, and virus entry with enzymes like N-deacetylase/N-sulfotransferase, glucuronyl C5-epimerase, uronosyl 2-O-sulfotransferase, glycosaminyl 6-O-sulfotransferase, glucosaminyl 3-O-sulfotransferase changing their structure and, hence, their biological applications.(16) HS is also involved in anti-viral protection because it prevents virus-to-cell attachment due to the GAG's covalent link to a protein core at the cell surface, GAG's negative charge and high sulphate level that all block virus particles from interacting with the host cell.(57) Propylene glycol alginate sodium sulfate, a sulfated ALG derivative, is used in China for more than 30 years as an oral heparinoid for its blood thinning properties.(58,59)

Chondroitin sulfates downregulate MMPs, COX-2, IL-1β, NOS2, and TNFα expression by impeding the NF-κB (proteins that modulate DNA transcription, cytokine production and cell survival) signaling pathways, hence providing anti-inflammatory conditions.(60) Because the neuronal system is loaded with all CS proteoglycans, they inhibit excessive growth, and cell and neurite motion but if these proteoglycans are upregulated then this may lead to gliomas. CS binds to glycoprotein C through the E unit and may inhibit the *Herpes simplex* virus access. CS obtained from shark cartilage consumed with glucosamine helps arthritis and osteoporosis treatment and if CS is combined with chitin and chitosan an increased proteoglycan production will improve cartilage deficiency treatment, mainly because of DS and CS ability to form proteoglycans that contain aggrecan, biglycan, decorin, and versican.(16,61,62) Aggrecan also binds to KS, as well as ephrin and semaphoring.(18) More effects of CS use in wound healing are listed in table no. 2, such as ternary scaffolds of silk fibroin-CS-HA that were tested on mice and displayed an accelerated wound healing, with a regenerated dermis and granulation tissue, and better angiogenesis and collagen deposition.(28)

HA- and CS-based hydrogels were made, after cross-linking PEG propion-dialdehyde to their adipic dihydrazide derivatives, and both of them were used in full-thickness wounds on a rat model. The clear and flexible gels formed in a matter of minutes, at a neutral pH and at room temperature, to which Tegaderm® was added, and showed increased re-epithelialization after 5 and 7 days and more fibro-vascular tissue after 10 days since the injury was made. Other skin healing evidences were present, such as cell migration, differentiation and growth factors, and other matrix elements aggregating, suggesting that this hydrogel might be considered, in fact, a bio-interactive scaffold.(37)

Dermatan sulfates and CS are involved in cartilage function, cell and protein interaction, perception and response, coagulation, fibroblast growth factors, hepatocyte growth factor, hemostasis regulation at the subluminal spaces, heparin cofactor II binding, pathogen receptors interaction, tenascin-X interaction, and neurotransmission and are present on the cells' surface or in the ECM.(16) Single and repeated doses of intramuscular DS were use on healthy human subjects and showed a linear relation between plasma the activated partial thromboplastin time and thrombin clotting time response and DS concentration. The DS was absorbed slower after the single shot and faster after the repeated dose. The terminal half-time of lower molecular weight DS was 12 hours after repeated i.m. injections.(63) Vascular DS might help regulate the antithrombotic effect of Heparin Cofactor II (HC II) in a mouse study, maybe because DS binds specifically to HC II in the carotid artery adventitia. By modulating heparin's cofactor II activity, DS inhibits thrombin, but not as well as heparin, and can link itself to activated protein C with a factor V inhibition effect, both leading to an anticoagulation effect (64). Ultrathin polymer coating application for two weeks of chitosan, DS, and poly 2 that delivered siRNA with MMP-9 gene as a target improved the diabetic mouse's model wound healing.(8)

Keratan sulfates levels decrease after an injury while HS and CS levels increase, but if the tissue is not harmed these components show a different status, where the KS level is high but the HS and CS levels were decreased, these observations leading to the idea that, in bovine and human studies, GAGs play a differential regulatory role in cell migration for injured and non-wounded tissue.(18,65) If the cornea is wounded then the KS synthesis is low and usually nonsulfated, but keratocytes will divide and will have a fibroblastic behavior similar to corneal fibroblasts that were cultured. KS will come to a normal level after the cell migration stops.(34)

Table no. 2. Biological characteristics of glycosaminoglycans

Properties	Description	References
Formulations	CS: micro-, nanocapsules, lyophilized, films, nanofibers, coating, hydrogel, HA: Biomimetic hydrogels, creams, coating, foams, gauze, hydrofibers, hydrogels, injections, nanofibers, nanoparticles, scaffolds Heparin: IV, solutions HS: porous scaffolds and mesh	(18, 40, 49, 66–68)
Topical biocompatibility	Confirmed. CHI/CS/AgSD films: not toxic to Vero cells. Caution with over-sulphated CS and i.v. heparin.	(15, 40, 64)
Local properties	CHI/CS/AgSD and CHI/CS films promote cell proliferation CS: pain relief, anti-inflammatory, antirheumatic, anticoagulant, HA: cicatrizer, slowing diffusion barrier at intercellular spaces, shield for fibroblasts, matrix interactions, mesothelial cells, and some stem cells Heparin and DS: Antithrombotic, Heparin helps neovascularization HS: cell adhesion, cell growth and proliferation regulator, collagen maturation, developmental processes, cell surface binding of lipoprotein lipase and other proteins, angiogenesis, chondrogenesis regulator	(3, 14, 52, 61, 69, 70, 15–18, 28, 32, 40, 45)
Mechanisms	1. CS targets "intrinsic tenase complex with low FXII activation", lowers COX-2, IL-1β, MMPs, NOS2, and TNFα expression 2. DS interacts with fibroblast growth factor FGF-2 and FGF-7 for cellular proliferation and wound repair, 3. HS and CS targeted by Bleomycin in cancer cells, 4. HS interacts with dimeric cytokines & binds to/regulates interferon γ, 5. Heparin – binds to FGF-2; liver reticuloendothelial system metabolism, antithrombin-dependent.	(5, 13, 34, 35, 37, 44, 46, 56, 57, 61, 63, 64, 14, 71–74, 15, 16, 20, 23, 25, 30, 32)
Immunogenicity	Block the virus-host cell receptor interaction HA: non-immunogenic HS: prevents viral invasion, and tumor metastasis HSGAGs: prevent tumor metastasis	(14–16, 26, 42, 64, 67, 75)
Anti-infectious properties	CHI/CS/AgSD films against *P. aeruginosa* and *S. aureus* CHI/HA- antibacterial	(18, 40)
Anti-inflammatory properties	CHI/ GAG: anti-inflammatory CS suppress the nuclear translocation of NF-κB Heparin and CS disrupt the chemokine-GAG interaction HSGAGs and OTR4120 show anti-inflammatory effects	(3, 16, 18, 42, 50)
3D scaffolds	Collagen-glycosaminoglycan scaffolds, Chitosan-CS hydrogels, Silk fibroin-DS-CS ternary scaffolds, Electrospun CS/polyvinyl alcohol nanofibers, Silver sulfadiazine (AgSD) loaded chitosan/chondroitin (CHI/CS) sulfate films, CS+collagen II+grafted porous PCL, Heparin-coated aligned nanofiber	(12, 18, 77, 78, 27, 40, 49, 66–68, 75, 76)
Elimination	Physiological, Not in milk (heparin doesn't transfer into milk in nursing women) HA: Gastro-intestinal tract, hepatic, pulmonary and renal; through enzymatic or non-enzymatic reactions, made through the canal of Schlemm Heparin: Renal clearance 10% (active fragments) and 40% (active and non-active fragments) of the dose	(79) HA: (26) Hep: (80)

Decorin, a proteoglycan derived from CS and DS, has an important role in collagen fibrillo-genesis and in keeping the skin's integrity because of its ability to regulate collagen structures.(6) Fibrillar collagen's thickness and new matrix accumulation rely on the proteoglycan chains (ionic) interaction within fibril spaces, where its protein binds to the collagen fibrils and create an anionic GAG bridge and gives a mechanically stable structure. Anionic GAGs, CS, DS, and KS if linked to decorin will space the collagen fibrils at 65 nm in a non-covalent manner and will make thinner collagen fibril if this proteoglycan has an increased concentration. If it is absent, the degree and rate of collagen fibrillo-genesis will be influenced by the thicker and unorganized fibril huddle. Anionic GAGs display compressive features because they are able to swell and to make spaces between the elastic collagen fibrils and it is thought they are able to transform local compression into tensile strength through a sliding model of proteoglycan-collagen filament.(6,33) Decorin absence will lead to abnormalities in collagen morphology, higher skin fragility, inferior and irregular fiber structure, and improper fibrotic evolution after a myocardial infarction.(6) **Lumican**, a keratan sulfate proteoglycan, modulates the toll-like receptor 4 signaling pathway from macrophages that are lacking lumican and helps reduce the pro-inflammatory reaction.(18)

CONCLUSIONS

Glycosaminoglycans have a wide variety of sources and applications, with proven antibacterial, antiviral, antioxidant, antitumor, and anti-thrombotic properties, low cytotoxic level against healthy cells and a hydrophilic character. When mixed with other substances, compounds and/or materials all these benefits are enhanced. Because they are checking nearly all the characteristics of an ideal wound dressing, and the possibilities of creating new blends are infinite, the research centered upon creating new or improved GAG-based wound healing biomaterials must be continued.

Acknowledgement:

This work has been conducted in the Pediatric Clinical Hospital Sibiu, within the Research and Telemedicine Center in Neurological Diseases in Children - CEFORATEN project (ID 928 SMIS-CSNR 13605) financed by ANCSI with the grant number 432 / 21.12.2012 through the Sectoral Operational Programme "Increase of Economic Competitiveness". This study is part of the doctoral thesis of the PhD student Andreea Barbu, under the supervision of Professor Vioara Miresan.

Conflict of interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

1. Sezer AD, Cevher E. Biopolymers as Wound Healing Materials: Challenges and New Strategies. In: Rosario Pignatello, editor. Biomaterials Applications for

Nanomedicine. InTech; 2011. p. 383–414.
2. Pereira RF, Bártolo PJ. Traditional Therapies for Skin Wound Healing. Adv Wound Care. 2016;5(5):208–29. From: http://online.liebertpub.com/doi/10.1089/wound.2013.0506
3. Aramwit P. Introduction to biomaterials for wound healing. In: Wound Healing Biomaterials. Elsevier Ltd; 2016. p. 3–38. From: http://dx.doi.org/10.1016/B978-1-78242-456-7.00001-5.
4. Shah JB. The history of wound care. J Am Col Certif Wound Spec. 2011;3(3):65–6. From: http://dx.doi.org/10.1016/j.jcws.2012.04.002
5. Branski LK, Herndon DN, Celis MM, Norbury WB, Masters OE, Jeschke MG. Amnion in the treatment of pediatric partial-thickness facial burns. Burns. 2008;34(3):393–9.
6. Mathur AB. Regenerative Wound Healing via Biomaterials. In: Gefen A, editor. Bioengineering Research of Chronic Wounds: A Multidisciplinary Study Approach. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. p. 405–24. From: https://doi.org/10.1007/978-3-642-00534-3_18
7. Wiegand C, Hipler UC. Polymer-based biomaterials as dressings for chronic stagnating wounds. Macromol Symp. 2010;294(2):1–13.
8. Das S, Baker AB. Biomaterials and Nanotherapeutics for Enhancing Skin Wound Healing. Front Bioeng Biotechnol. 2016;4(October):1–20. From: http://journal.frontiersin.org/article/10.3389/fbioe.2016.00082/full
9. Liu J, Zheng H, Dai X, Sun S, Machens HG, Schilling AF. Biomaterials for Promoting Wound Healing in Diabetes. J Tissue Sci Eng. 2017;08(01):8–11. From: https://www.omicsonline.org/open-access/biomaterials-for-promoting-wound-healing-in-diabetes-2157-7552-1000193.php?aid=85945
10. Derm 8 - Wound Healing Flashcards | Quizlet [Internet]. From: https://quizlet.com/78192547/derm-8-wound-healing-flash-cards/.
11. Ratner BD. New ideas in biomaterials science - a path to engineered biomaterials. J Biomed Mater Res. 1993;27:837–50.
12. Chen FM, Liu X. Advancing biomaterials of human origin for tissue engineering. Progress in Polymer Science. 2016;53:86–168.
13. F. Kennedy J, Knill C, Thorley M. Natural polymers for healing wounds. In: Recent Advances in Environmentally Compatible Polymers. 2001. p. 97–104.
14. Chandran S, Seetharaman A, Rajalekshmi G, Pandimadevi M. Potential wound healing materials from the natural polymers - A review. Int J Pharma Bio Sci. 2015;6(3):1365–89.
15. Sasisekharan R, Raman R, Prabhakar V. Glycomics Approach To Structure-Function Relationships of Glycosaminoglycans. Annu Rev Biomed Eng. 2006;8(1):181–231. From: http://www.annualreviews.org/doi/10.1146/annurev.bioeng.8.061505.095745
16. Jones CL, Liu J, Xu D. Structure, Biosynthesis, and Function of Glycosaminoglycans. In: Comprehensive Natural Products II. Elsevier; 2010;6:407-27. [cited 2018 Mar 19]. From: http://linkinghub.elsevier.com/retrieve/pii/B9780080453828001325.
17. Salbach J, Rachner TD, Rauner M, Hempel U, Anderegg U, Franz S, et al. Regenerative potential of glycosaminoglycans for skin and bone. J Mol Med. 2012;90(6):625–35.
18. Köwitsch A, Zhou G, Groth T. Medical application of glycosaminoglycans: A review. J Tissue Eng Regen Med. 2017.
19. Chondroitin sulfate [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=24766. From: https://pubchem.ncbi.nlm.nih.gov/compound/24766
20. Dermatan, 4-(hydrogen sulfate)| C14H21NO15S-2 - PubChem [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=32756. From: https://pubchem.ncbi.nlm.nih.gov/compound/32756.
21. Hyaluronic acid 1 [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=24759. From: https://pubchem.ncbi.nlm.nih.gov/compound/24759.
22. Hyaluronic acid 2. National Center for Biotechnology Information. PubChem Compound Database. p. CID=24728612.
23. Heparan sulfate [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=53477714. From: https://pubchem.ncbi.nlm.nih.gov/compound/53477714.
24. Keratan [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=446715. From: https://pubchem.ncbi.nlm.nih.gov/compound/446715.
25. Heparin [Internet]. National Center for Biotechnology Information. PubChem Compound Database. p. CID=772. From: https://pubchem.ncbi.nlm.nih.gov/compound/772.
26. Necas J, Bartosikova L, Brauner P, Kolar J. Hyaluronic acid (hyaluronan): A review. Vet Med (Praha). 2008;53(8):397–411.
27. Chaudhari AA, Vig K, Baganizi DR, Sahu R, Dixit S, Dennis V, et al. Future prospects for scaffolding methods and biomaterials in skin tissue engineering: A review. Vol. 17, International Journal of Molecular Sciences; 2016.
28. Hussain Z, Thu HE, Katas H, Bukhari SNA. Hyaluronic Acid-Based Biomaterials: A Versatile and Smart Approach to Tissue Regeneration and Treating Traumatic, Surgical, and Chronic Wounds. Polymer Reviews. 2017;57:594-630.
29. Garron ML, Cygler M. Structural and mechanistic classification of uronic acid-containing polysaccharide lyases. Glycobiology. 2010;20(12):1547–73.
30. Esko JD, Selleck SB. Order Out of Chaos: Assembly of Ligand Binding Sites in Heparan Sulfate. Annu Rev Biochem. 2002;71(1):435–71. From: http://www.annualreviews.org/doi/10.1146/annurev.biochem.71.110601.135458.
31. Silbert JE, Sugumaran G. Biosynthesis of Chondroitin / Dermatan Sulfate. IUBMB Life. 2002;54:177–86.
32. Salmivirta M, Udholt K, Lindahl U. Heparan Sulfate : a Piece of Information. FASEB. 1996;10(11):1270–9.
33. Mihov D, Spiess M. Glycosaminoglycans: Sorting determinants in intracellular protein traffic. Int J Biochem Cell Biol. 2015;68:87–91. From: http://dx.doi.org/10.1016/j.biocel.2015.08.019.
34. Funderburgh JL. Keratan sulfate: structure, biosynthesis, and function. Glycobiology. 2000;10(10):951–8.
35. Smith AM, Moxon S, Morris GA. Biopolymers as wound healing materials. In: Ågren M, editor. Wound Healing Biomaterials. 1st ed. Woodhead Publishing; 2016. p. 261–87.
36. Jiao G, Yu G, Zhang J, Ewart HS. Chemical structures and

bioactivities of sulfated polysaccharides from marine algae. Mar Drugs. 2011;9(2):196–233.
37. Kirker KR, Luo Y, Nielson JH, Shelby J, Prestwich GD. Glycosaminoglycan hydrogel films as bio-interactive dressings for wound healing. Biomaterials. 2002;23(17):3661–71.
38. Cramer GD, Bakkum BW. Microscopic Anatomy of the Zygapophysial Joints, Intervertebral Discs, and Other Major Tissues of the Back. In: Cramer GD, Darby SA, editors. Clinical Anatomy of the Spine, Spinal Cord, and ANS. Third Edit. Elsevier Inc.; 2013. p. 586–637. From: http://dx.doi.org/10.1016/B978-0-323-07954-9.00014-1
39. Biomaterials for health. A Strategic Roadmap for Research and Innovation HORIZON 2020 1. :1–65.
40. Fajardo AR, Lopes LC, Caleare AO, Britta EA, Nakamura CV, Rubira AF, et al. Silver sulfadiazine loaded chitosan/chondroitin sulfate films for a potential wound dressing application. Mater Sci Eng C. 2013;33(2):588–95. From: http://dx.doi.org/10.1016/j.msec.2012.09.025.
41. Stadelmann WK, Digenis AG, Tobin GR. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg. 1998;176(Suppl 2 A):26S-38S.
42. Anderson J, Cramer S. Perspectives on the Inflammatory, Healing, and Foreign Body Responses to Biomaterials and Medical Devices [Internet]. Host Response to Biomaterials. Elsevier Inc.; 2015;1:13-36. From: http://www.sciencedirect.com/science/article/pii/B9780128001967000025.
43. Singh S, Young A, McNaught CE. The physiology of wound healing. Surg (United Kingdom). 2017;35(9).
44. Fallacara A, Baldini E, Manfredini S, Vertuani S. Hyaluronic acid in the third millennium. Polymers (Basel). 2018;10(7).
45. Pan H, Jiang H, Chen W. The Biodegradability of Electrospun Dextran/PLGA Scaffold in a Fibroblast/Macrophage Co-culture. Biomaterials. 2008;29(11):1583–1592.
46. Dhivya S, Padma VV, Santhinia E. Wound dressings – a review. Biomedicine. 2015;5(4):24–8. From: 10.7603/s40681-015-0022-9
47. Norouzi M, Boroujeni SM, Omidvarkordshouli N, Soleimani M. Advances in Skin Regeneration: Application of Electrospun Scaffolds. Adv Healthc Mater. 2015;4(8):1114–33.
48. Carella S, Maruccia M, Fino P, Onesti MG. An atypical case of Henoch-Shönlein purpura in a young patient: Treatment of the skin lesions with hyaluronic acid-based dressings. In Vivo (Brooklyn). 2013;27:147–52. From: http://iv.iiarjournals.org/content/27/1/147.full.pdf+html%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=2013053553.
49. Haugh MG, Murphy CM, O'Brien FJ. Novel Freeze-Drying Methods to Produce a Range of Collagen–Glycosaminoglycan Scaffolds with Tailored Mean Pore Sizes. Tissue Eng Part C Methods. 2010;16(5):887–94. From: www.liebertonline.com/doi/abs/10.1089/ten.tec.2009.0422.
50. Severin IC, Soares A, Hantson J, Teixeira M, Sachs D, Valognes D, et al. Glycosaminoglycan analogs as a novel anti-inflammatory strategy. Front Immunol. 2012;3(OCT):1–12.
51. Lever R, Page CP. Novel drug development opportunities for heparin. Nat Rev Drug Discov. 2002;1(2):140–8.
52. Tong M, Tuk B, Hekking IM, Vermeij M, Barritault D, Van Neck JW. Stimulated neovascularization, inflammation resolution and collagen maturation in healing rat cutaneous wounds by a heparan sulfate glycosaminoglycan mimetic, OTR4120. Wound Repair Regen. 2009;17(6):840–52.
53. Coombe DR, Kett WC. Heparan sulfate-protein interactions: Therapeutic potential through structure-function insights. Cell Mol Life Sci. 2005;62(4):410–24.
54. Eikelboom JW, Hankey GJ. Low molecular weight hearins and heparinoids. Med J Aust. 2002;177(7):379–83.
55. Martel N, Lee J, Wells PS. Risk of heparin induced thrombocytopenia with unfractionated and low molecular weight heparin thromboprophylaxis: a meta-analysis. Blood. 2005;106(8):2710–6. From: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15985543
56. Daamen WF, Veerkamp JH, van Hest JCM, van Kuppevelt TH. Elastin as a biomaterial for tissue engineering. Biomaterials. 2007;28(30):4378–98.
57. De Jesus Raposo MF, De Morais AMB, De Morais RMSC. Marine polysaccharides from algae with potential biomedical applications. Mar Drugs. 2015;13(5):2967–3028.
58. Zeng Y, Yang D, Qiu P, Han Z, Zeng P, He Y, et al. Efficacy of Heparinoid PSS in Treating Cardiovascular Diseases and beyond - A Review of 27 Years Clinical Experiences in China. Clin Appl Thromb. 2016;22(3):222–9.
59. Szekalska M, Pucilowska A, Szymanska E, Ciosek P, Winnicka K. Alginate : Current Use and Future Perspectives in Pharmaceutical and Biomedical Applications. Int J Polym Sci. 2016;2016.
60. Franz S, Rammelt S, Scharnweber D, Simon JC. Immune responses to implants - A review of the implications for the design of immunomodulatory biomaterials. Biomaterials. 2011;32(28):6692–709.
61. Hileman RE, Fromm JR, Weiler JM, Linhardt RJ. Glycosaminoglycan-protein interactions: Definition of consensus sites in glycosaminoglycan binding proteins. BioEssays. 1998;20(2):156–67.
62. Venugopal V. Marine Polysaccharides: food applications. Taylor and Francis Group, editor. Boca Raton, FL: CRC Press; 2011.
63. Saivin S, Cambus J-P, Thalamas C, Lau G, Boneu B, Houin G, et al. Pharmacokinetics and Pharmacodynamics of Intramuscular Dermatan Sulfate Revisited. Clin Drug Investig. 2003;23(8):533–43. From: http://www.scopus.com/inward/record.url?eid=2-s2.0-0041380738&partnerID=tZOtx3y1
64. He L, Giri TK, Vicente CP, Tollefsen DM. Vascular dermatan sulfate regulates the antithrombotic activity of heparin cofactor II. Blood. 2008;111(8):4118–25.
65. 65. Gordon SR. Cell Migration Along the Basement Membrane During Wound Repair. The Corneal Endothelium as a Model System. In: Gefen A, editor. Bioengineering Research of Chronic Wounds: A Multidisciplinary Study Approach. Springer; 2009. p. 43–84.
66. Cunha L, Grenha A. Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Mar Drugs; 2016.
67. Boateng J, Catanzano O. Advanced Therapeutic Dressings for Effective Wound Healing - A Review. Journal of Pharmaceutical Sciences. 2015;104.
68. Mogoşanu GD, Grumezescu AM. Natural and synthetic polymers for wounds and burns dressing. Int J Pharm. 2014;463(2):127–36.
69. Karunanithi P, Murali MR, Samuel S, Raghavendran HRB,

Abbas AA, Kamarul T. Three dimensional alginate-fucoidan composite hydrogel augments the chondrogenic differentiation of mesenchymal stromal cells. Carbohydr Polym. 2016;147:294–303. From: http://dx.doi.org/10.1016/j.carbpol.2016.03.102.

70. Yue K, Trujillo-de Santiago G, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials. 2015;73:254–71. From: http://dx.doi.org/10.1016/j.biomaterials.2015.08.045.
71. Li J, Li S, Yan L, Ding T, Linhardt RJ, Yu Y, et al. Fucosylated chondroitin sulfate oligosaccharides exert anticoagulant activity by targeting at intrinsic tenase complex with low FXII activation: Importance of sulfation pattern and molecular size. Eur J Med Chem. 2017;139:191–200. From: http://dx.doi.org/10.1016/j.ejmech.2017.07.065.
72. Trowbridge JM, Rudisill JA, Ron D, Gallo RL. Dermatan sulfate binds and potentiates activity of keratinocyte growth factor (FGF-7). J Biol Chem. 2002;277(45):42815–20.
73. Chargé SBP, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev. 2004;84(1):209–38.
74. Li X, Lan Y, He Y, Liu Y, Luo H, Yu H, et al. Heparan Sulfate and Chondroitin Sulfate Glycosaminoglycans Are Targeted by Bleomycin in Cancer Cells. Cell Physiol Biochem. 2017;43(3):1220–34.
75. Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: A review. J Pharm Sci. 2008;97(8):2892–923. From: http://dx.doi.org/10.1002/jps.21210.
76. Lim YM, Gwon HJ, Choi JH, Shin J, Nho YC, Jeong SI, et al. Preparation and biocompatibility study of gelatin/kappa-carrageenan scaffolds. Macromol Res. 2010;18(1):29–34.
77. Chang KY, Hung LH, Chu IM, Ko CS, Lee Y Der. The application of type II collagen and chondroitin sulfate grafted PCL porous scaffold in cartilage tissue engineering. J Biomed Mater Res - Part A. 2010;92(2):712–23.
78. Bhardwaj TR, Kanwar M, Lal R, Gupta A. Natural gums and modified natural gums as sustained release carriers. Drug Dev Ind Pharm. 2000;26(10):1025–38.
79. Richter C, Sitzmann J, Lang P, Weitzel H, Huch A, Huch R. Excretion of low molecular weight heparin in human milk. Br J Clin Pharmacol. 2001;52(6):708–10.
80. Sutapa BM, Dhruti A, Priyanka G, Gopa RB. Absorption, distribution, metabolism and elimination (ADME) and toxicity profile of marine sulfated polysaccharides used in bionanotechnology. African J Pharm Pharmacol. 2018;12(1):1–10. From: http://academicjournals.org/journal/AJPP/article-abstract/E14B77655630.