The Potential Role of Topically Applied Heparan Sulfate in the Treatment of Photodamage

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ABSTRACT

Heparan sulfate is an essential glycosaminoglycan that plays important roles in development, homeostasis, and disease. As a group, the glycosaminoglycans provide mechanical strength to skin, as they can absorb water and occupy the space between elastin fibers and collagen. Heparan sulfate is also a key participant in cell proliferation, cell migration, collagen fiber formation, basement membrane regeneration, granulation tissue formation, and cell adhesion associated with wound healing. A variety of dermatological disorders are associated with changes in glycosaminoglycans or their associated proteoglycans. A new topical formulation of low molecular weight heparan sulfate glycosaminoglycan has been shown to penetrate the epidermis, basement membrane, and dermis within 24 hours of application. In an 8-week study, 15 patients using this new formulation showed improvement in skin hydration, skin firmness, skin elasticity, skin barrier function, and global fine lines and wrinkles. Incorporating low molecular weight heparan sulfate into topically applied formulations may represent a new approach to improving the appearance of photodamaged skin.

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INTRODUCTION

The advent of cosmeceuticals has revolutionized skin care. Dermatologists can now use topically applied compounds to address a variety of skin concerns with the goal of improving the signs of photodamage, such as uneven pigmentation, fine lines, tactile roughness, and skin tone. The benefits of both over-the-counter retinol and prescription retinoids such as retinoic acid (tretinoin) are well documented, as are the benefits of topical antioxidants such as vitamin C. Although certainly popular in cosmeceuticals, other agents such as peptides and growth factors have not been the subject of similar rigorous studies. Over the past several years, it has become apparent that cosmeceuticals can contribute to overall skin health by targeting certain issues that procedures cannot, namely oxidative stress and DNA repair. An extensively studied component of skin, heparan sulfate (HS), may be one of the most exciting and fascinating compounds that we now have available in the cosmetic arena. This report summarizes what we know about low molecular weight heparan sulfate (LMWHS) not only from a scientific perspective but also from initial clinical studies exploring its effectiveness on the skin.

In order to understand HS, we must first look at the skin and the building blocks that play a crucial role in skin health. These include the full spectrum of glycosaminoglycans (GAGs) besides HS, and molecules known as proteoglycans (PGs), which contain the GAG as a covalently attached side chain. PGs, with their ability to bind and alter enzymatic activity and protein-protein interactions, help to determine cellular responsiveness in development, homeostasis, and disease.[1] PGs and GAGs are vital to life and have major roles in tissue remodeling, cell adhesion, growth factor responsiveness, and immune function.

Glycosaminoglycans and Proteoglycans

A GAG chain consists of repeating disaccharide pairs that usually include an acidic sugar alternating with a hexosamine. The acidic sugar may be iduronic acid or a glucuronic acid, and the hexosamine may be a glucosamine or galactosamine. GAG chains are linear and may contain up to several thousand disaccharides.[2] Important GAGs and their paired disaccharide constituents in skin are shown in Table 1.

Except for hyaluronic acid, the GAGs in Table 1 are sulfated and covalently attached to core proteins. All must be enzymatically modified to become functional in skin. Dermatan sulfate (chondroitin sulfate B) is the major GAG in skin. Hyaluronic acid (hyaluran) in skin is neither sulfated nor covalently attached to a

TABLE 1.

Compositions of Glycosaminoglycan Side Chains[1]	
Glycosaminoglycan	**Disaccharides**
Heparan sulfate	Iduronic or glucuronic acid alternating with N-acetylglucosamine
Chondroitin sulfate	Glucuronic acid alternating with N-acetylgalactosamine
Keratan sulfate	Galactose alternating with N-acetylglucosamine
Dermatan sulfate	Iduronic acid alternating with N-acetylgalactose amine
Hyaluronic acid	Glucuronic acid alternating with N-acetylglucosamine.

core protein. Its high water-absorbing capacity is one of several properties that contribute to its success in soft tissue augmentation.[3]

PGs consist of a core protein and one or more GAG chains covalently linked to the core protein.[2] A diagram is shown in Figure 1. The protein core acts as a scaffold for the spacing and immobilization of GAG chains.[4] The core protein also determines whether the PG is located within the cell, on cellular surfaces, or in the extracellular matrix (ECM).[5] In the skin, syndecan-1 and syndecan-4 are expressed in large amounts on the cell surface of the epidermis[6] while perlecan, an ECM PG, is plentiful in the basement membrane.[7] In the dermis, syndecan-1 and glypican-1 are expressed on the cell surface of fibroblasts, which also produce decorin and versican.[8]

Although PGs are expressed in all tissues,[2] this review will focus on PGs in the epidermis, dermis, and basement membranes of the skin. PGs provide mechanical strength to skin, as they can absorb water and occupy the space between elastin fibers and collagen. PGs also play roles in cell proliferation, cell migration, collagen fiber formation, basement membrane regeneration, granulation tissue formation, and cell adhesion.[4,9] In wound healing, abnormal scars may result if the level of PGs is not adequate.[4]

A variety of skin conditions have been linked to abnormal synthesis or disposition of PGs and GAGs (Table 2). These data suggest that HS, the most widely studied GAG, may play a major role in skin health and disease, and thus may be useful as a topical skin care ingredient to improve the overall appearance of the skin.

Heparan Sulfate

HS chains are assembled in vivo on core proteins by using nucleotide sugars from the cytoplasm and enzymes in the Golgi apparatus.[21] The chains consist of regions of N- and O-sulfated sugar residues alternating with areas of low sulfation. The pattern of these sulfation areas is believed to determine the protein-binding properties of the chain.[1,21] Proteins bound by HS chains include fibroblast growth factors and their receptor tyrosine kinases, bone morphogenetic proteins, transforming growth factors, chemokines and interleukins, Wnt proteins, enzyme and enzyme inhibitors, proteins of the ECM and plasma, lipases, and apolipoproteins.[21]

The anticoagulant heparin is a type of HS. Like HS, heparin has regions of high sulfation. The resulting high negative charges are responsible for heparin's anticoagulant properties.[1] When the highly negative heparin interacts with the positively charged amino acid residues of antithrombin, a conformation change occurs in the protein. This change increases the inactivation of proteases associated with coagulation.[21] Van der Vaal's forces and hydrophobic interactions may also play a role in heparin-protein interactions.[22] Unlike HS, heparin has few regions of low sulfation and is synthesized only in connective tissue mast cells.[23]

A recent study of 96 patients[24] showed that a topical formulation of heparan sulfate (1% cream) relieved signs (edema, disability, color of the lesion) and symptoms (pain) of hematomas and subcutaneous hematic extravasations induced by trauma or surgery. The chemical characteristics and method of preparation of the cream were not reported, and its use as a topical skin care preparation has not been reported.

FIGURE 1. Basic structure of a proteoglycan (PG) with glycosaminoglycan (GAG) side chains. The biological functions of PGs and GAGs are encoded in the amino acid sequences of core proteins, the disaccharide sequences in the GAG chains, and enzymatic modifications that PGs and GAGs must undergo to become functional. A GAG chain consists of repeating disaccharide pairs that usually include an acidic sugar alternating with a hexosamine. The acidic sugar may be iduronic acid (star) or a glucuronic acid (diamond) and the hexosamine may be a glucosamine (square) or galactosamine (circle).[2]

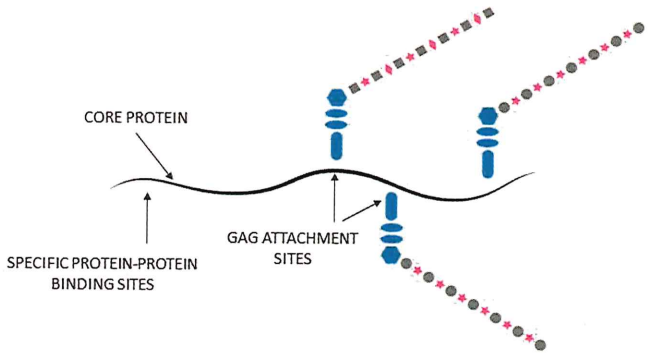


TABLE 2

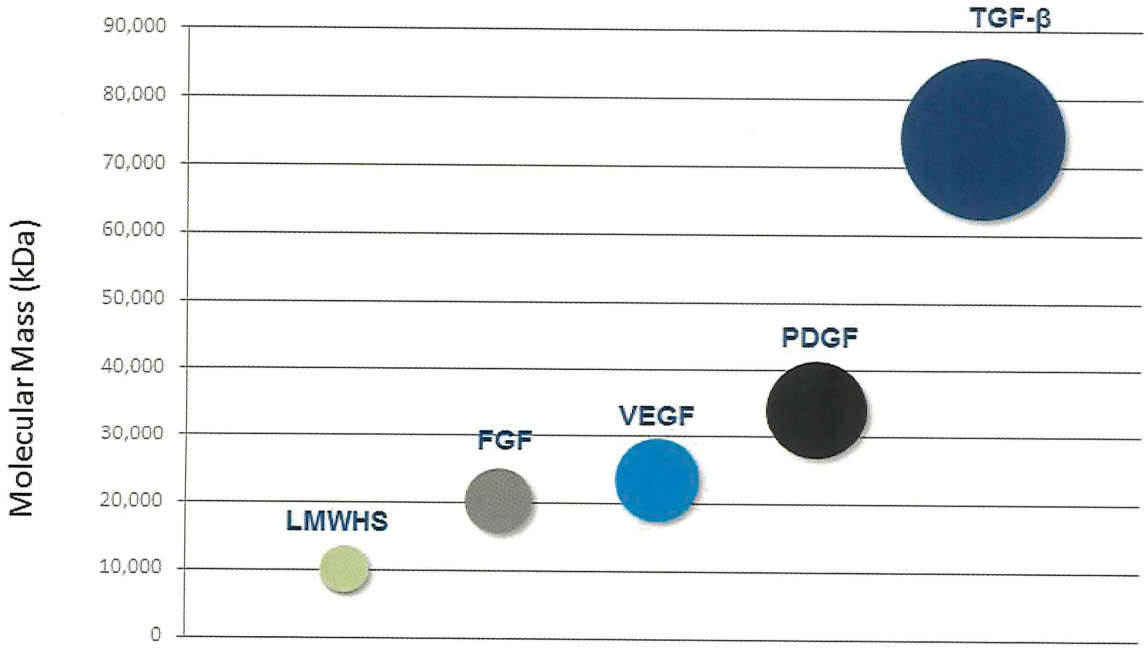
Cutaneous Abnormalities Associated With Alterations in Proteoglycan or GAG

Disorder	Role of PG or GAG	Reference
Ehlers-Danlos syndrome	Deficiency of decorin core protein	Wu et al.[10]
Skin injury	Expression of syndecan-1, CD-44 in keratinocyte migration and differentiation	Oksala et al. [9]
Pseudoxanthoma elasticum	Changes in PG metabolism in affected fibroblasts	Passi et al.[11]
Systemic sclerosis	Increased amount of dermatan/chondroitin sulfate in fibroblasts, enhanced expression of decorin core protein	Kuroda et al.[12]
Psoriasis	Altered expression of heparan sulfate PGs	Seyger et al. [13]
Lichen myxedema	Increased production of GAG	Turakainen et al. [14]
UV-irradiated skin	Increase in PG content in irradiated skin	Margelin et al.[15]
Chronic ulcers	Changed expression pattern of glypican and syndecan-1 and -4	Lundqvist et al.[16]
Nevus mucinosus	Large amounts of acid PGs in dermis	Brakman et al.[17]
Aging skin	Decrease in hyaluronan, increase in dermatan sulfate	Edward[18]
Solid tumors	Hyaluron chondroitin/dermatan sulfate increased in tumor area	Prathiba et al.[4]
Invasive squamous cell carcinoma	Syndecan-1 lost or reduced	Maata et al. [19]
Skin cancers	Loss of syndecan-1	Stepp et al.[20]

PG = proteoglycan, GAG = glycosaminoglycan, SCE = subcutaneous hematic extravasations.

Heparan sulfate proteoglycans (HSPGs) have been extensively studied in wound repair.[2,4,9,25-33] For example, Gallo and colleagues[25] discovered that an antimicrobial peptide (PR-39) in wound fluid induces the expression of cell surface syndecans, the major cell-surface HSPG, and that PR-39 kills bacteria as well. The ability of PR-39 to induce cell surface expression of HS is associated with an enhanced response of cells to components in their microenvironment that participate in wound repair. In

FIGURE 2. The molecular mass (kDa) of low molecular weight heparan sulfate (LMWHS) compared to that of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGF-ß).



their study of healing of human mucosal wounds, Oksala and colleagues[9] reported increased expression of cell membrane-associated CD44 and syndecan-1 in migrating keratinocytes. McGrath and Eady[28] reported that HSPGs may influence inflammation, cell migration and attachment, and growth factor binding during wound healing. Gallo[2] suggested that antimicrobial peptide-induced syndecans can control responsiveness of growth factors during wound healing. In their review of wound healing, Fears and Woods[29] reported that syndecans are involved in cell motility, angiogenesis, fibroblast and endothelial proliferation, and organization of the extracellular matrix.

HS is found in the epidermis, basement membrane, and dermis.[4] As a group, PGs and GAGs have been shown to change in a variety of dermatological conditions (Table 2). Since HS is a major GAG and is found in many PGs, it may be inferred that HS undergoes alterations in these disorders and that the addition of HS may have therapeutic benefit in skin disease. This is supported by studies in which HS is involved in skin tissue maintenance and repair. Gheduzzi and colleagues[34] showed that HS interactions with elastin may be involved in tissue elastin fibrogenesis and may modulate elastin stability in diseases. In addition, HS mimetic polymers (RGTAs) may modulate collagen synthesis in smooth muscle cells, protease activities in ECM remodeling, and collagen production in burned skin.[35] This concept has been reviewed in detail.[36]

Clinical Evaluation of Low Molecular Weight Heparan Sulfate

A formulation of low molecular weight HS (LMWHS, Laboratori Derivati Organici, Milan, Italy) was studied to determine whether when topically applied, the LMWHS would penetrate the skin and exert therapeutic effects. The molecular mass of LMWHS (10 kDa) compared to that of four growth factors is shown in Figure 2.

To evaluate penetration into skin, HS was covalently labeled with a fluorescent tag (fluorescein isothiocyanate, FITC) and incorporated into a cream at a 0.5% concentration. The mixture was applied to murine skin. The results (Figure 3A and B) show that the HS penetrated the skin within 48 hours of once-daily application. A subsequent study on human skin (Figure 3C and D) showed penetration to the epidermis and dermis at 24 hours by the same-labeled LMWHS applied at 12-hour intervals.

Once it was shown that LMWHS could penetrate the epidermis and dermis, evaluation of the functional potential of this preparation in human subjects was the next logical step. In a preliminary open-label study (BioScreen Testing Services, Inc., Phoenix, AZ), the effects of topical LMWHS on skin hydration and moisturization, skin firmness, skin elasticity, and skin barrier function were examined, as was its effect on the appearance of fine lines and wrinkles of the face.

FIGURE 3. Fluorescent micrographs (magnification 200x) showing penetration of low-molecular-weight heparan sulfate (LMWHS) into murine skin. The skin was treated once daily for 48 hours with vehicle (A) and a cream of LMWHS (0.5%) labeled with fluorescein isothiocyanate (FITC) (B). The dotted line is the basement membrane between the epidermis and dermis. The bright green fluorescence is LMWHS and the blue fluorescence is cellular nuclei labeled with fluorescent DAPI (4',6-diamidino-2-phenylindole). C and D are fluorescent micrographs of human skin treated with vehicle and FITC-labeled LMWHS (0.5%) cream every 12 hours for 24 hours. Bright green fluorescence was present in the skin treated with HS-FITC and absent in the vehicle-treated skin, indicating the HS had penetrated epidermis and dermis of human skin.

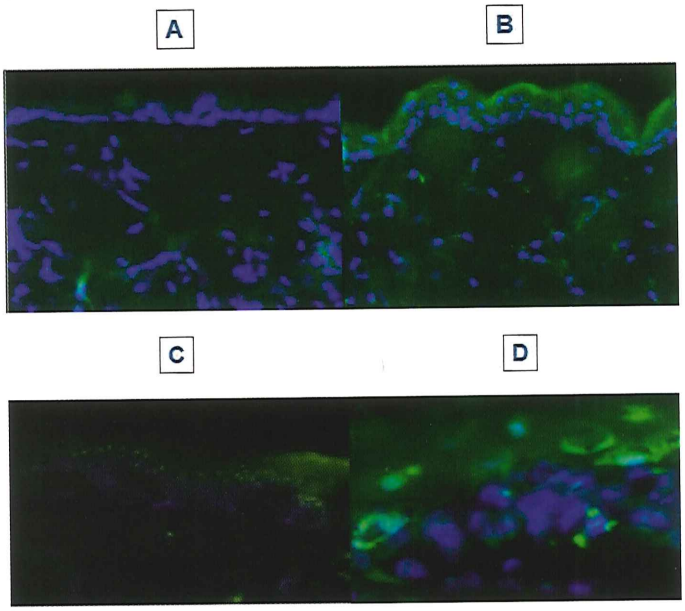


TABLE 3.

LMWHS Product Results			
Skin characteristic (method)	Mean percent difference from baseline (% of subjects improved)		
	Week 2	Week 4	Week 8
Hydration (Corneometer)	29 (100) (s)	28 (100) (s)	31 (100) (s)
Firmness (Cutometer)	1 (53)	2 (53)	18 (93) (s)
Elasticity (Cutometer)	6 (40)	3 (40)	26 (93) (s)
Barrier function (TEWL)	27 (87) (s)	32 (100) (s)	34 (100) (s)
Fine lines, wrinkles (Image analysis)	6* (s) (73)	4 (93) (s)	3** (s) (73)

s = statistically significant ($P \leq 0.05$) for both mean percent difference from baseline and percent of subjects improved.
LMWHS = low-molecular-weight HS; TEWL = transepidermal water loss.
*Significant ($P \leq 0.05$) only for mean percent difference from baseline.
**Directional significance ($P \leq 0.10$) only for mean percent difference from baseline.

Healthy subjects (n=15 females) aged 35 to 55 years of age and without dermatological or systemic disease that could affect the study results were recruited for this pilot study. All subjects included in the study had mild to moderate fine lines and wrinkles of the face. The subjects agreed to not use personal care products (lotions, creams, serums) and cosmetics (other than those provided for the study) during the washout period and for the 8 weeks of the study. They also agreed to avoid sun exposure as much as possible for the entire period of the clinical evaluation. Grounds for exclusion included (but were not limited to) pregnancy, severe wrinkles, insulin-dependent diabetes, medical cosmetic procedures on the face within the past 12 months, and recent treatments for photo aged skin or fine lines and wrinkles. Subjects provided signed informed consent prior to participation in this clinical evaluation.

Subjects began with a washout period in which they cleansed their faces regularly for at least 5 days with a neutral soap provided by the study site. When the washout was complete, subjects returned to the facility for baseline (pretreatment) photography and measurement of skin hydration, firmness, elasticity, and transepidermal water loss (TEWL) at two sites on their facial skin. Subjects were given the LMWHS product with instructions for daily use during the study. Subjects were also given a different soap (Dove) to cleanse their faces during the study period.

Subjects visited the test facility at 2, 4, and 8 weeks for photography, the aforementioned measurements, and to complete self-assessment questionnaires.

The results are summarized in Table 3.

The results from the clinical trial showed significant improvement in all the aforementioned parameters. Among the five skin characteristics evaluated, the earliest improvements (2 weeks) were observed in skin hydration and skin barrier function, while skin firmness and elasticity improved significantly at 8 weeks. Most subjects (73% to 93%) showed an improvement in fine lines and wrinkles throughout the study period. Adverse events were not observed.

Self-assessment questionnaires indicated that most or all subjects felt that the product improved the look and feel of their skin, reduced skin redness, and moisturized their skin. More than 85% of subjects would recommend the product to a family member or friend.

These results show that the topical formulation provides improvement in at least five skin characteristics within 8 weeks. Clinical examples are shown in Figures 4 through 7.

FIGURE 4. A 43-year-old female at baseline (left) and after 8 weeks of treatment (right) with the heparan sulfate (1%) cream. Fine lines are noticeably improved, especially on the forehead.

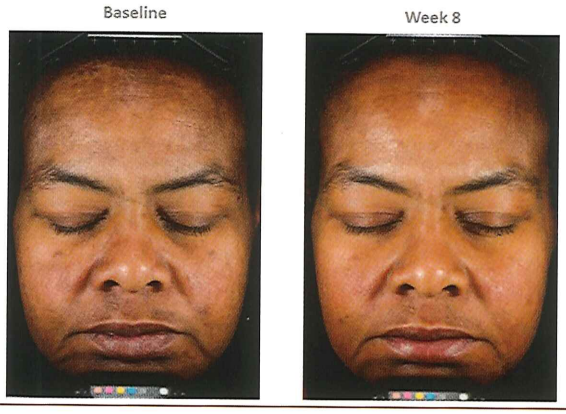


FIGURE 5. A 41-year-old female at baseline (left) and after 8 weeks of treatment (right) with the heparan sulfate (1%) cream. Fine lines are noticeably improved on the forehead.

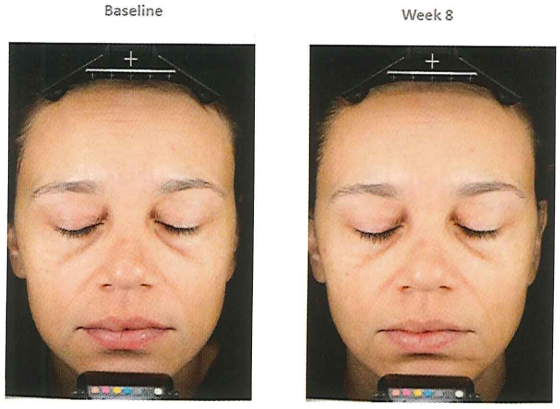
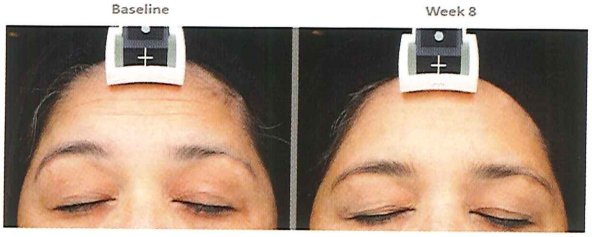


FIGURE 6. A female approximately 40 years of age at baseline (left) and after 8 weeks of treatment (right) with heparan sulfate (1%) cream. Fine lines are noticeably improved on the forehead.

FIGURE 7. A female approximately 40 years of age at baseline (left) and after 8 weeks of treatment (right) with heparan sulfate (1%) cream. Fine lines are noticeably improved on the forehead.



LMWHS cream represents a new treatment modality for skin care. Unlike other anti-aging skin care products, HS chains regulate skin health by binding fibroblast growth factors and their receptor tyrosine kinases, bone morphogenetic proteins, transforming growth factors, chemokines and interleukins, Wnt proteins, enzyme and enzyme inhibitors, proteins of the ECM and plasma, lipases, and apolipoproteins.[21] Because HS production declines with age, a topical formulation of HS that penetrates the epidermis and dermis will have widespread application for improving skin hydration, firmness, elasticity, barrier function, and fine lines and wrinkles, particularly in the skin of aging patients, and without adverse effects. Additionally, HS plays a role in inflammation,[28,30,37,38] which declines with advancing age,[39] as HS may attenuate the decline of the inflammatory response that normally occurs with advancing age. For these reasons, the authors recommend LMWHS as a part of a complete skin care program.

CONCLUSION

The complex role of HS in skin biology has been well documented for nearly two decades. The results of the pilot study presented here suggest that this LMWHS may improve skin hydration, skin barrier function, skin firmness, skin elasticity, and the appearance of facial lines and wrinkles. Further clinical evaluations are warranted.

DISCLOSURES

Dr. Gallo is Chief Scientific Advisor to Senté, Inc. Drs. Bucay, Shamban, Lima-Maribona, Lewis, Ditre, Mayoral, and Gold are members of Senté's Clinical Advisory Board.

REFERENCES

1. Gallo RL, Trowbridge JM. Proteoglycans and glycosaminoglycans of skin. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, eds. *Fitzpatrick's Dermatology in General Medicine*. 6th ed. New York, NY: McGraw-Hill. 2003:210 – 216.
2. Gallo RL. Proteoglycans and cutaneous vascular defense and repair. *J Investig Dermatol Symp Proc*. 2000;5:55-60.
3. Beer K. A randomized, evaluator-blinded comparison of efficacy of hyaluronic acid gel and avian-sourced hylan B plus gel for correction of nasolabial folds. *Dermatol Surg*. 2007;33:928-936.
4. Prathiba V, Gupta PD. Cutaneous wound healing: significance of PGs in scar formation. *Current Science*. 2000;78:1-5.
5. Kjellén L, Lindahl U. Proteoglycans: structures and interactions. *Annu Rev Biochem*. 1991;60:443-475.
6. Gallo R, Kim C, Kokenyesi R, et al. Syndecans-1 and -4 are induced during wound repair of neonatal but not fetal skin. *J Invest Dermatol*. 1996;107:676-683.
7. Noonan DM, Fulle A, Valente P, et al. The complete sequence of perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein-receptor, and the neural cell adhesion molecule. *J Biol Chem*. 1991;266:22939-22947.
8. Iozzo RV, Murdoch AD. Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. *FASEB J*. 1996;10:598-614.
9. Oksala O, Salo T, Tammi R, et al. Expression of proteoglycans and hyaluronan during wound healing, *J Histochem Cytochem*. 1995;43:125-135.
10. Wu J, Utani A, Endo H, Shinkai H. Deficiency of the decorin core protein in the variant form of Ehlers-Danlos syndrome with chronic skin ulcer. *J Dermatol Sci*. 2001;27:95-103.
11. Passi A, Albertini R, Baccarani Contri M, et al. Proteoglycan alterations in skin fibroblast cultures from patients affected with pseudoxanthoma elasticum. *Cell Biochem Funct*. 1996;14:111-120.
12. Kuroda K, Shinkai H. Decorin and glycosaminoglycan synthesis in skin fibroblasts from patients with systemic sclerosis. *Arch Dermatol Res*. 1997;289:481-485.
13. Seyger MM, van den Born J, Schalkwijk J, et al. Altered distribution of heparan sulfate proteoglycans in psoriasis. *Acta Derm Venereol*. 1997;77:105-109.
14. Turakainen H, Välimäki M, Penttinen R. Synthesis of glycosaminoglycans and collagen in skin fibroblasts cultured from a patient with lichen myxedematosus. *Arch Dermatol Res*. 1985;277:55-59.
15. Margelin D, Fourtanier A, Thevenin T, et al. Alterations of proteoglycans in ultraviolet-irradiated skin. *Photochem Photobiol*. 1993;58:211-218.
16. Lundqvist K, Schmidtchen A. Immunohistochemical studies on proteoglycan expression in normal skin and chronic ulcers. *Br J Dermatol*. 2001;144:254-259.
17. Brakman M, Starink TM, Tafelkruyer J, Bos JD. Linear connective tissue naevus of the proteoglycan type ('naevus mucinosus'). *Br J Dermatol*. 1994;131:368-370.
18. Edward M. Proteoglycans and glycosaminoglycans. In: Priestley, GC, ed. *Molecular Aspects of Dermatology*. Chichester, England: John Wiley & Sons. 1993:89-110.
19. Maata A, Jaakkola P, Jalkanen M. Extracellular matrix-dependent activation of syndecan-1 expression in keratinocyte growth factor-treated keratinocytes. *J Biol Chem*. 1999;274: 9891-9898.
20. Stepp MA, Pal-Ghosh S, Tadvalkar G, et al. Loss of syndecan-1 is associated with malignant conversion in skin carcinogenesis. *Mol Carcinog*. 2010;49:363-373.
21. Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature*. 2007;446:1030-1037.
22. Thompson LD, Pantoliano MW, Springer BA. Energetic characterization of the basic fibroblast growth factor-heparin interaction: identification of the heparin binding domain. *Biochemistry*. 1994;33:3831-3840.
23. Stringer SE, Gallagher JT Heparan sulphate. *Int J Biochem Cell Biol*. 1997;29:709-714.
24. Polieri T, Orsoni E, Saponati G, Castellacci E. Efficacy and tolerability of Clarema 1% Cream and Hirudoid 40000 U.APTT Gel in the topical treatment of haematomas and/or subcutaneous haematic extravasations, *ISRN Orthopedics*. 2012;2012:Article ID 504151. 5 pages.
25. Gallo RL, Ono M, Povsic T, et al. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. *Proc Natl Acad Sci USA*. 1994;91:11035-11039.
26. Meddahi A, Caruelle JP, Gold L, et al. New concepts in tissue repair: skin as an example. *Diabetes Metab*. 1996;22:274-278.
27. Andriessen MP, van den Born J, Latijnhouwers MA, et al. Basal membrane heparan sulphate proteoglycan expression during wound healing in human skin. *J Pathol*. 1997; 183:264-271.
28. McGrath JA, Eady RA Heparan sulphate proteoglycan and wound healing in skin. *J Pathol*. 1997;183:251-252.
29. Fears CY, Woods A The role of syndecans in disease and wound healing, *Matrix Biol*. 2006; 25:443-456.
30. Alexopoulou AN, Multhaupt HA, Couchman JR. Syndecans in wound healing, inflammation and vascular biology. *Int J Biochem Cell Biol*. 2007;39:505-528.
31. Tong M, Tuk B, Shang P, et al. Diabetes-impaired wound healing is improved by matrix therapy with heparan sulfate glycosaminoglycan mimetic OTR4120 in rats. *Diabetes*. 2012; 61:2633-2641.
32. Maltseva I, Chan M, Kalus I, et al. The SULFs, extracellular sulfatases for heparan sulfate, promote the migration of corneal epithelial cells during wound repair. *PLoS One*. 2013;8:e69642.
33. Martino MM, Briquez PS, Güç E, et al. Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. *Science*. 2014; 343(6173):885-888.
34. Gheduzzi D, Guerra D, Bochicchio B, et al. Heparan sulphate interacts with tropoelastin, with some tropoelastin peptides and is present in human dermis elastic fibers. *Matrix Biol*. 2005; 24:15-25.
35. Garcia-Filipe S1, Barbier-Chassefiere V, Alexakis C et al. RGTA OTR4120C a heparan sulfate mimetic, is a possible long-term active agent to heal burned skin, *J Biomed Mater Res A*. 2007;80:75-84.
36. van Neck J, Tuk B, Barritault D, Miao Tong M. (2012). Heparan sulfate proteoglycan mimetics promote tissue regeneration: an overview, tissue regeneration - from basic biology to clinicalapplication, Prof. Jamie Davies (Ed.), ISBN: 978-953-51-0387-5, InTech, Available from: http://www.intechopen.com/books/tissue-regeneration-from-basic-biology-to-clinical-application/heparansulfate-proteoglycan-mimetics-thrive-tissue-regeneration-an-overview. Accessed February 23, 2015.
37. DePrisco G, Bandel C, Cockerell CJ, Ehrig T. Interstitial heparan sulfate in granulomatous inflammatory skin diseases. *J Am Acad Dermatol*. 2004;50:253-257.
38. Taylor KR, Gallo RL. Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation. *FASEB J*. 2006;20:9-22.
39. Gilchrest BA, Stoff JS, Soter NA. Chronologic aging alters the response to ultraviolet-induced inflammation in human skin. *J Invest Dermatol*. 1982;79:11-15.

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