The Role of Low Molecular Weight Heparan Sulfate in Skin Rejuvenation and Wound Healing

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Abstract

Low molecular weight Heparan Sulfate is the active ingredient found in Senté Dermal Repair Cream (San Diego, California). A biologically active glycosaminoglycan, Heparan Sulfate facilitates important wound healing and skin rejuvenation processes in the body including growth factor and stem cell enablement, collagen regulation, anti-oxidation, thrombolysis, skin-soothing and water-binding properties. Given the vast scientific evidence on the role that Heparan Sulfate plays in the skin, incorporation of the molecule as an active ingredient is both merited and beneficial to skin repair and health.

Introduction

A burgeoning body of literature has accrued that demonstrates wound healing to be a complex, yet orderly process that is essential to maintain the integrity of the largest organ in the body, the skin. In the absence of wound healing, even the slightest compromise of the skin could be potentially fatal. Fortunately, wound healing and regeneration processes exist, at the center of which reside Heparan Sulfate, a glycosaminoglycan (GAG) – a linear polysaccharide consisting of repeating disaccharide units of variable length – which when bound to a core protein form Heparan Sulfate proteoglycans (HSPGs) (Figure 1).

Figure 1: Heparan Sulfate Molecule
HSPGs are located in all tissues, and may be classified as either membrane-bound, secretory, or basement membrane (extracellular matrix) associated (Table 1).

Table 1: Heparan Sulfate Proteoglycans

<table>
<thead>
<tr>
<th>TYPE</th>
<th>EXAMPLES</th>
<th>LOCATION</th>
<th>FUNCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Membrane–Bound</td>
<td>- Syndecans, Glypicans,</td>
<td>- Epithelial cells,</td>
<td>- Cell - ECM attachment</td>
</tr>
<tr>
<td></td>
<td>Betaglycan, CD44v3</td>
<td>Fibroblasts, Leukocytes</td>
<td>- Cell - cell interactions</td>
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<td></td>
<td></td>
<td></td>
<td>- Cell motility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Co-receptors</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Ligand binding and processing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Cytokine, Chemokine, Growth Factor regulation</td>
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<tr>
<td>- Secretory vesicles</td>
<td>- Serglycin</td>
<td>- Mast cells, Hematopoietic cells</td>
<td>- Packaging and maintenance of granular contents</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Process regulation after release (i.e. inflammation and wound repair)</td>
</tr>
<tr>
<td>- Basement membrane</td>
<td>- Perlecan, Agrin, Collagen</td>
<td>- Basement membranes,</td>
<td>- Organize basement membrane structure and function</td>
</tr>
<tr>
<td></td>
<td>XVIII</td>
<td>Epithelial cells</td>
<td>- Facilitate cell migration</td>
</tr>
</tbody>
</table>

HSPGs serve integral functions in the numerous and complex pathways that contribute to wound healing including, but not limited to, cellular adhesion, migration, and differentiation, regulation of growth factor expression, organization and maintenance of the basement membrane structure and function and facilitation of stem cell recruitment and differentiation (Figure 2).

Figure 2: Functions of Heparan Sulfate Proteoglycans

Unlike other proteoglycans, HSPGs are located throughout all layers of the skin. In addition, improvements in diabetic skin ulcers and retardation of the aging process further support the importance of HSPGs.

Role of Heparan Sulfate in Wound Healing

In order to understand Heparan Sulfate’s role in wound healing, it is essential to be familiar with its critical role in maintaining skin health. HSPGs have the unique ability to bind and interact with many components of the extracellular matrix. Further, HSPGs are responsible for maintaining the structure of the basement membrane, regulating many interactions with growth factors and other extracellular matrix proteins, and for directing cellular adhesion, migration and differentiation.

Following injury, normal wound healing usually undergoes an orderly process of hemostasis and inflammation, a proliferative phase consisting primarily of re-epithelialization, angiogenesis, and collagen formation, and tissue remodeling culminating in formation of a scar. HSPGs are integral throughout the entire wound healing process with the ultimate goal being restoration of skin health. Certain features of HSPGs (Table 2) may explain their ability to coordinate many of the complex mechanisms that contribute to wound healing (Table 3).

Table 2

<table>
<thead>
<tr>
<th>Features of Heparan Sulfate Proteoglycans</th>
<th>Contributions of HSPG to Wound Healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Polyanionic – attract and retain water</td>
<td>• Attract and retain water</td>
</tr>
<tr>
<td>• Variable GAG chain size and number -</td>
<td>• Organize basement membrane</td>
</tr>
<tr>
<td>depending on source and growth</td>
<td>• Mediate cell migration, proliferation and differentiation</td>
</tr>
<tr>
<td>conditions</td>
<td>• Mediate growth factor synthesis and expression</td>
</tr>
<tr>
<td>• Not limited to interact with only HS</td>
<td>• Mediate chemokine and chemokine</td>
</tr>
<tr>
<td>– modifiable</td>
<td>receptor expression and function</td>
</tr>
<tr>
<td>• Number and sulfation state variability</td>
<td>• Regulate enzyme function</td>
</tr>
<tr>
<td>• Dynamic ligand binding sites</td>
<td>• Regulates inflammatory and</td>
</tr>
<tr>
<td>• Properties dependent on cell type</td>
<td>hematopoietic response</td>
</tr>
<tr>
<td></td>
<td>• Regulates hemostasis</td>
</tr>
</tbody>
</table>

Throughout the wound healing process HSPGs essentially facilitate similar processes; however, the components involved differ. For example, HSPGs primarily address hemostasis and inflammation in the early phase of wound healing while they primarily address the tensile properties of the healing wound at later stages.
Effect of Aging and Disease on Wound Healing

The effects of aging and disease on wound healing are complex and varied depending on the model used for assessment \(^{11,22,23}\). As noted in (Figure 3) as skin ages there is atrophy of the extracellular and cellular matrix, loss of vascularization, alteration in collagen organization, and thinning of the epidermis.

**Figure 3: Aging of the Skin**

![Aging of the Skin](image)

Whereas older animals may still have relatively normal healing, albeit delayed in one model \(^{22}\), regeneration may be the primary mechanism of wound healing in young skin \(^{10,11}\). It is clear that there is altered expression of skin proteoglycans, which may play a role in the physical properties and response to injury of skin \(^{24}\).

Altered expression of HSPGs also occurs in disease states, such as diabetes and smoking that lead to suboptimal wound healing. In both of these disease states, normal wound healing may be restored through the delivery of Heparan Sulfate analogues \(^{23}\). Of note, restoration of the extracellular matrix milieu towards a more normal one has been show to improve wound healing towards normal.

**Heparan Sulfate in Tissue Regeneration**

One of the more exciting areas in skin biology is that of regeneration. Consider the potential benefits of being able to regenerate normal skin (i.e. scar less skin). As discussed wound repair in fetal skin does just that. Heparan Sulfate is involved in and regulates several aspects of stem cell physiology that make it a prime candidate to assist with regeneration of skin following injury.
Heparan Sulfate has been shown to mediate hematopoietic stem cell homing\textsuperscript{25}. It also has been shown to facilitate growth factor mediated mesoderm differentiation of embryonic stem cells\textsuperscript{3}. Importantly, Heparan Sulfate exerts control over the skin’s stem cell niche\textsuperscript{7}. Taken together, these and other data support the possibility of tissue regeneration following injury with targeted use of Heparan Sulfate and HSPGs.

**Clinical Potential of Heparan Sulfate for Wound Healing, Tissue Rejuvenation and Tissue Regeneration**

An understanding of the critical role that Heparan Sulfate plays in maintaining skin health and promoting wound healing, has lead to the development of a topical low molecular weight formulation of Heparan Sulfate (Senté Dermal Repair Cream). The low molecular weight HS formulation allows for robust penetration in the epidermis. Although the HS molecule is 10k Dalton’s, penetration is thought to be facilitated by the long thin, rod-like molecular structure and it’s negative charge (Figure 4).

**Figure 4: Penetration of the epidermis by low molecular weight Heparan Sulfate in mouse skin with two applications 1% HS**

![Figure 4](https://via.placeholder.com/150)

(Courtesy of Jun Mato M.D. Ph.D, UCSD)

- 10 kDa HS applied in 0.5% cream q day x 48hr to mouse skin
- Dotted line = basement membrane zone between epidermis and dermis
- Green = FITC-heparan sulfate, Blue = DAPI staining of nuclei
- Magnification 200X
- Note: Auto-fluorescence of collagen in vehicle only control

Low molecular weight Heparan Sulfate is significantly smaller than proteins in topically applied growth factor products whose molecular weights vary from 18kd-140kd. Further the low molecular weight Heparan Sulfate was shown to induce various effects, including a profound rehydration of the skin, potential anti aging effects, and a beneficial effect on the combined endpoint of subjective symptoms and objective signs (hematoma color) following a surgical procedure (Figure 5). The effect on
tissue bruising is thought to be mediated by thrombinogenesis and promotion of the fibrinolytic process through both intrinsic and extrinsic pathways.

Heparan Sulfate has been shown to activate proactivants, antagonize plasmin inhibitors, produce anti-Xa and anti-complement activity and activate anti-thrombin III\(^{26}\). Although these results are promising, further studies on the efficacy of this agent in other populations need to be performed before it can be widely adopted.

**Figure 5: Effect of Heparan Sulfate Cream on Hematomas and/or Subcutaneous Hematic Extravasations**

![Graph showing effect of Heparan Sulfate Cream on percent of subjects with complete disappearance at day ten.]

*Statistically significant

(Polieri, T. Et al, 2012)

**Summary**

Heparan Sulfate is a ubiquitous glycosaminoglycan that exists in all tissue types in its free or protein bound forms. Heparan Sulfate regulates numerous processes critical to skin homeostasis, repair, and regeneration. A proprietary low molecular weight Heparan Sulfate has been developed to exploit the beneficial effects of naturally occurring Heparan Sulfate. This low molecular weight Heparan Sulfate is the active ingredient in Senté’ Dermal Repair Cream, which has shown significant promise in this regard.
References

4. ’Hirano K, Van Kuppevelt TH, Nishihara S. The transition of mouse pluripotent stem cells from the naive to the primed state requires Fas signaling through 3-O sulfated heparan sulfate structures recognized by the HS4C3 antibody. Biochem Biophys Res Commun 2013;430:1175-81.
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