The role of hyaluronan in wound healing

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Hyaluronan; Clinical application; Dermatology; Wound healing; Wound dressings

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Introduction
The biology of wound healing is an innate science. Broadly speaking, normal wound healing involves three key phases: inflammatory, proliferative and remodelling (1), with each phase exhibiting overlapping processes of coordinated cellular activities (2). Cytokines mediate these cellular processes, enabling wound healing cells to produce the necessary structural proteins and polymers required for wound healing (3).

One extracellular polysaccharide involved in wound healing is the glycosaminoglycan (GAG) hyaluronan (HA). HA is found in every human tissue and body fluid (4), displays unique physiochemical and biological properties (5) and its function can change depending on its size (6). HA is involved in each phase of wound healing and has been studied extensively (6–9) with a few of these studies focussing on the role of HA in one component of wound healing, that is, Borgnoni et al. (1996) (8) examined the role in angiogenesis, whereas David-Raoudi et al. (2008) (6) studied fibroblasts and HA. The key aspects of HA’s role in wound healing as well as its influence on clinical practice and its use in wound dressings will be reviewed.

HA – structure, properties and physiology
In order to properly appreciate the function of HA in wound healing, an overview of HA’s basic structure and physiology is required. HA exists in vivo as a negatively charged, disaccharide polymer of repeating d-glucuronic acid and d-acetylgalactosamine (10) (Figure 1). HA polymers exist in varying lengths, with each molecular size uniquely functioning on each wound healing phase (Table 1). In short, large HA molecules are space-filling molecules with regulatory and structural functions, whereas small HA fragments are involved in angiogenesis, inflammation and immunostimulation (11).

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Abstract
The polysaccharide hyaluronan (HA) (synonyms – hyaluronic acid, hyaluronate) is a versatile, polymorphic, glycosaminoglycan with vast biological functions. HA is found throughout the body, primarily residing in skin, thus playing an important role in wound healing. Research regarding HA’s function has changed over the years, primarily focussing on a particular aspect or function. The contribution of HA in each stage of normal wound healing as well as its clinical wound dressing applications will be examined.

Key Messages
• HA is involved extensively in all phases of wound healing
• understanding the structure and physiology of HA allows us apply its function to wound healing
• the role of HA in wound healing can be understood in a clinical context when carefully applying research
• HA-based dressings show promising clinical applications
The role of HA in wound healing

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Figure 1 HA molecule (12).

Table 1 Summary of hyaluronan function during wound healing

<table>
<thead>
<tr>
<th>Phase</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory phase</td>
<td>• Binds to fibrinogen to commence clotting pathway.</td>
</tr>
<tr>
<td></td>
<td>• Allows inflammatory cell migration.</td>
</tr>
<tr>
<td></td>
<td>• Creates oedema to allow cell infiltration.</td>
</tr>
<tr>
<td></td>
<td>• Inhibits neutrophil migration to dampen inflammatory response.</td>
</tr>
<tr>
<td>Proliferative phase</td>
<td>• Draws fibroblasts to wound site.</td>
</tr>
<tr>
<td></td>
<td>• ‘Fills in gaps’ of newly formed ECM, creating cushioning and structural organisation.</td>
</tr>
<tr>
<td></td>
<td>• Stimulates MMPs for angiogenesis.</td>
</tr>
<tr>
<td></td>
<td>• Promotes keratinocyte migration and proliferation.</td>
</tr>
<tr>
<td>Remodelling phase</td>
<td>• Contributes to normal and pathological scarring.</td>
</tr>
</tbody>
</table>

This differentiates HA from other GAGs as it does not bind to a core protein (13). So too, while other GAGs, such as heparan sulphate, are manufactured within the cell and then released via exocytosis (13), HAS manufacture HA on the outside of the cell wall, enabling it to interact readily with other cells and HA-binding proteins, hyaladherins (12). HA catabolism primarily occurs in two main ways, through six hyaluronidases (HYALs) and reactive oxygen species (ROS). HYALs are responsible for altering the various HA sizes needed during each wound healing phase (11). HYAL-induced breakdown is quite systematic in contrast to the ROS catabolism, which is often induced extrinsically by UV (14) or intrinsically by neutrophils (15). However, it is unknown whether the results of ROS-induced HA catabolism create functional HA fragments (16). HA’s antioxidant and free-radical scavenging properties have been used in various HA-based wound dressings.

In addition to HA’s unique structural properties, these cell surface interactions are crucial to understand HA’s role in wound healing, as HA’s size and shape dictates its differing roles and interactions at each wound stage.

HA and the inflammatory phase

During the inflammatory phase of wound healing, HA synthesis increases rapidly (7). Upon breach of the skin, large, heavy molecular size HA fragments are synthesised from platelets and from available HA in the blood stream (11). These HA fragments are able to bind to fibrinogen to commence the extrinsic clotting pathway (11). Because of the large amounts of HA released at wounding, the wound site is saturated with fluid leading to oedema (11). HA’s hydrophilic properties cause swelling of the tissue surrounding the wound (17), creating a porous framework for cells to migrate to the injury site (12).

Oedema can be observed macroscopically in this initial stage of wound healing, allowing for microscopic chemotaxis of inflammatory cells (18). This inflammatory process is driven by the primary cytokines tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and IL-8 and is stimulated when concentrations of HA are high (5). These inflammatory cytokines increase blood vessel dilation, allowing increased cellular recruitment to the wound (18). This vasodilation presents as heat and redness, serving as a clinical indicator of wound inflammation and progression (19). Inflammation and its associated symptoms are essential for successful wound repair (11).

Conversely, HA also has a role in reducing and moderating the inflammatory response, through its interaction with the hyaladherin TNF-stimulated gene-6 (TSG-6) (20). TSG-6 is stimulated by inflammatory cytokines IL-1 and TNF-α, causing fibroblasts and other inflammatory cells to express the TSG-6 protein (21). Once expressed by cells, TSG-6 proteins are retained in HA-rich environments, binding to high-molecular-weight HA polymers that form heavy chains (22). These heavy HA chains prevent inflammation by inhibiting neutrophil migration (23) and inhibition of plasmin through negative feedback loops (21). This mechanism was tested in a murine pouch study by Wisniewski et al. (1996) (24) where inflammation was induced by IL-1 or carrageenan. Following administration of TSG-6, reduction of inflammation was achieved, comparable with the corticosteroid dexamethasone. Although this study shows TSG-6s anti-inflammatory properties, the laboratory conditions in the study are not typical for normal human wound healing. The short timeframe used to induce inflammation (4 hours), the use of corticosteroids and...
the narrow spectrum of wound proteins examined may not reflect clinical conditions.

As the wound progresses out of the inflammatory phase, the inflammatory signal reduces. A delay or lengthening in this phase can render an acute wound chronic, halting its progress to healing (25). The cellular changes associated with reduction of the inflammatory phase are visible, as the classic inflammatory signs recede and granulation tissue begins to form in preparation for the proliferative phase (18). This phase is too heavily influenced by HA’s multifaceted properties.

**HA and the proliferative phase**

Following on from the late inflammatory phase, the proliferative phase of wound healing marks the arrival of fibroblast migration (19). These dermal cells are drawn to wound tissue by small HA fragments (6–20 saccharides) (11) and growth factors. Fibroblasts create collagen and GAGS (including HA), constructing and anchoring the newly formed extracellular matrix (ECM) (2).

In their study, David-Raoudi et al. (2008) (6) tested the differential effects of HA fragments of varying size (small, medium and large) on human dermal fibroblasts (HDF). The researchers found that multiwell plates coated with different lengths of HA produced greater HDF adhesion than the control group. Additionally, David-Raoudi et al. also found in an assay to examine proliferation that varying lengths of HA and in particular native HA promoted HDF proliferation at statistically significant levels compared with the control group ($P < 0.05$). These results reflect physiological conditions with altering HA lengths at each wound healing stage (11).

In contrast, Ferguson et al. (2011) (26) found that differing HA fragments did not significantly influence fibroblast migration and its subsequent collagen production. Additionally, Ferguson et al. found that the molar concentrations of HA influenced fibroblast proliferation more so than molecular size, with low molar concentrations of HA, inhibiting fibroblast migration. This may explain the discrepancies between Ferguson et al. and David-Raoudi et al. and suggests potential pharmacological wound healing applications, with lower HA concentrations acting as a delivery system for wound therapies.

Another finding of David-Raoudi et al. was that differing HA lengths stimulate the production of specific collagen types, which can promote or inhibit scar formation. One important function that fibroblasts exert on the developing ECM is collagen synthesis (27). Collagen, which is the body’s main connective protein, is crucial to the developing ECM, as it provides tensile strength to the healing wound (13). Various collagen types are produced by fibroblasts, each with its own structure and function (2).

The developing ECM is visibly identifiable as granulation tissue, displaying the structural properties of HA. With collagen and elastin providing the fibrous scaffolding, HA ‘fills in the gaps’ to form a cushioning gel (5). As shown during the inflammatory phase of wound healing, long, heavy HA chains form this gel because of HA’s hydrophilic nature. Upon saturation, HA displays the elastic recoil properties similar to cartilage (12). This malleability is an important clinical feature of granulation tissue, as wound healing often occurs in areas of high movement or pressure that is joints, plantar surface of feet. Without HA’s microscopic, hydrophilic and porous networking abilities, the macroscopic granulation tissue would not able to hold its shape to allow for normal wound healing.

Although granulation tissue is elastic, sharp trauma causes bleeding (2). This hallmark feature of granulation tissue is primarily because of angiogenesis (27). New blood capillaries visibly form within granulation tissue, because of increased metabolic demands from the cells present in the wound. HA contributes to this process by its short-chain lengths called oligomers, which are as small as 6–20 molecules in length (11). These oligomers bind to the hyaladherin CD44 and act as stimulating fragments for matrix metalloproteinases (MMPs) (23). The MMPs are essential for new capillary sprouting by breaking down the basement membrane of the wound. This allows for new capillary buds to sprout from existing ones (28). Borgognoni et al. (1996) (8) studied low-weight (short chain) HA and its effects on angiogenesis. In their study, primary (sutured) and secondary (open) wounds were created in rats. Both groups were treated with HA gel. The results showed an increased microvasculature compared with control groups, but only wounds healing by secondary intention demonstrated accelerated healing.

Although this study helps to elucidate the contribution of HA to angiogenesis, its clinical influence is unclear. Of significance, the group with primary wounds and HA treatment showed delayed healing, suggesting negative wound healing outcomes when topical HA is used on primary wounds. Conversely, the group with secondary wounds and HA treatment showed accelerated healing overall. This hastening of healing may positively influence clinical practice, as accelerated healing has potential time and money reductions to both patient and practitioner, thereby promoting the use of HA-impregnated dressings.

However, HA’s direct role in accelerated secondary wound healing in the study by Borgognoni et al. (1996) is questionable for a number of reasons. The authors used a low concentration (0.2%) of HA, in a sodium alginate dressing. This raises two main problems; first, at such a low concentration, HA might not be therapeutic and more importantly, sodium alginate is considered a wound dressing and resembles HA structurally and physically (29). This casts doubt over which component may have stimulated angiogenesis. Thus, it is difficult to clearly determine the effect of sodium alginate alone on angiogenesis, as the results were not analysed separately.

The final component of the proliferative phase of wound healing is epithelialisation, which begins very early after wounding (27). The skin contains most of the body’s HA, which is concentrated in the deeper, intercellular layers (stratum basale) of the epidermis as well as in the dermis (30). The main cell type in this basal layer is the keratinocyte (31), which expresses hyaladherin CD44 in large amounts (23). In the skin, HA functions to hydrate the stratum basale, creating the aforementioned porous structures for nutrient channeling (5).

Upon wounding, keratinocytes and their HA structures are torn apart, commencing the inflammatory phase of wound healing (11). Through CD44 interactions, keratinocytes
migrate to the wound site, collecting at wound edges (5). Keratinocytes then form a delicate cover over the new wound from the wound borders. These cells then 'leapfrog' over each other to form a cover of epithelial cells over the new wound (2). These new cells then differentiate to create the various epidermal skin layers, providing a protective barrier against infection and fluid loss (1).

Successful epithelialisation is not only merely a protective function but also morphological. Kaya et al. (1997) (9) produced transgenic mice whose cells expressed an antisense CD44. This genetic change impaired keratinocyte migration, producing gross morphological changes such as reduced skin elasticity and wound healing delay. This early study highlighted the requirement of the presence of HAs for effective epithelialisation, for without its signalling and physiochemical properties, excessive scarring and delayed wound healing may occur.

**HA and the remodelling phase**

Although pathological scar formation can be a reality of wound care, normal wound healing does result in scar formation (25). As tissue continues to repair itself, wound edges contract from fibroblast and ECM interactions and collagen is continuously synthesised and degraded, equilibrating at 3 weeks (2). Wound strength increases progressively over time, albeit still less than the original unwounded tissue (2). Gradually, the wound-specific cells, structures and HA are degraded and replaced by an avascular, collagenous scar (2). The collagen produced in wound scarring never reaches the same strength as injured skin due to its disorganised structure (1).

The associated physiological changes of remodelling are also seen within the wound as its appearance gradually normalises. The previously raw, granulated wound is now a healing scar, functioning concurrently with surrounding healthy skin, steadily and subtly improving over time.

Clinically, minimal scar tissue also occurs with advancing age because of HA being present primarily in the upper dermis, as is also seen in foetal subjects (30). This might lead to the assumption that HA skin concentrations change at various ages. However, Meyer and Stern (1994) (32) found that the distribution and size of HA remain consistent throughout a life time. This consistency was seen in tissue samples of foetal, middle-aged and elderly skin. Meyer and Stern (1994) suggest that although the amount of HA remains the same, the amount of extractable HA changes with age because of hyaladherin binding. This shows that while HA is present in human skin of all ages, its location and the extent of hyaladherin binding influences HA’s contribution to scarring (30).

The research by Meyer and Stern (1994) in addition to other work in scarless (6,33) and foetal wound healing (34) has shown promising real-life applications.

**HA in wound dressings**

Aside from its role in biology of wound healing, HA’s properties have recently been successfully used in a number of wound dressings. HYALOFILL (Anika), HYALOMATRIX (Anika), IALUSET (Laboratoires Genévrier) and HYIODINE (Contipro) are current examples featuring HA, which have been studied and have been shown to be clinically effective in a variety of wounds (35).

Dereure et al. (2012) (36) found that Ialuset resulted in faster wound healing and reduced pain in venous leg ulcers compared with control in a double-blinded, randomised control trial (RCT). However, the rate of complete healing was found to be similar in both groups at the secondary end point of 60 days. Moseley et al. (2003) (15) compared the antioxidant properties of HA-based dressings (HA benzyl ester), low- and high-molecular weight HA and AQUACEL (Convatec) in vitro. The results showed that HA benzyl ester, Aquacel and high-molecular-weight HA exhibit free-radical scavenging properties, with HA benzyl ester showing the highest antioxidant properties. Although consistent with HA’s known antioxidant properties, Moseley et al. research may not have yielded the same results in vivo. Other studies have shown success with the use of hyiadine (37–39), yet none of these have been RCTs. Therefore, future research comparing to use a variety of HA-based dressings in RCTs may be of further clinical benefit.

**Conclusion**

Research into HA’s role in wound healing is an evolving science. Much of the early research reviewed appeared to focus on HA’s role in scar formation and its presence in skin. As scientific research of each phase and process of wound healing has progressed, understanding of HA’s roles has deepened, implicating it in every major wound healing event.

HA’s functions during wound healing change with its size. Large, heavy and long HA chains appear to have structural functions such as porous networks during inflammation and as a space filler in granulation tissue. This contrasts with the small, light and short HA fragments that have stimulatory and attracting properties such as fibroblast migration and collagen production (40).

The influence of HA on clinical practice can be viewed in differing ways. From a purely educational perspective, HA gives the clinician insight into the physiological depth of wound healing because of HA presence in many wound healing processes. When this cellular understanding is applied to HA research, the clinical context of wound treatment is promoted from a series of biological events to a real-time science.

Care must be taken when applying the reviewed HA research clinically, as some of the research reviewed does not replicate clinical conditions. Wound research, however, is challenging to perform in real-life conditions. Apart from the ethical and financial considerations, due to the complex nature of wound healing, consistency in human wound healing research can be difficult to attain (41). These limitations aside, many clinical applications such a scarless healing and HA-based dressing can be drawn from the reviewed research, with promising future developments.
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References