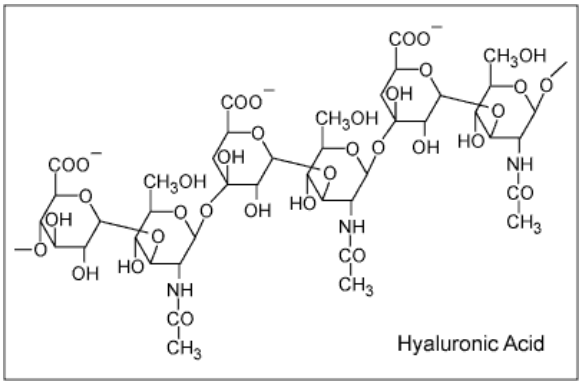
Hyaluronic acid.

The hyaluronan molecule

Hyaluronan is a linear polysaccharide comprising alternating glucuronic acid and N-acetyl-glucosamine residues, repeated approximately 2000 times,[1,2] and belongs to a group of substances known as glycosaminoglycans.



The hyaluronan molecule was first described in the 1930s, and was linked with other members of the glycosaminoglycans group, including chondroitin sulphate, keratan sulphate and heparin sulphate.[3] Hyaluronan is synthesised on the cell membrane via the activity of hyaluronan synthase, and secreted directly into the extracellular space. The newly synthesised molecule protrudes directly into the extracellular environment, promoting a highly hydrated microenvironment because of its hygroscopic properties.[5] Hyaluronan is broken down by hyaluronidases, four of which have been identified.[6] It is removed from the plasma via the lymphatics and liver by the activity of specific endocytic receptors.[7]

Currently, it is thought that hyaluronan has three functions :

- It expands the extracellular space by binding to salt and water — its hygroscopic properties attract water, resulting in expansion
- It interacts with a variety of extracellular molecules to form the extracellular matrix — this is rich in glycosaminoglycans, and hyaluronan provides stability and elasticity[8]
- It activates intracellular signalling pathways via the activity of cell surface receptors, such as CD44 and RHAMM.[9]

Hyaluronan is normally found in the inter-cellular spaces of the epidermis, although not in the upper granular layer nor the stratum corneum. In the dermis it is mainly situated below the basement membrane and around skin appendages: the skin normally contains the highest concentration of hyaluronan within the body,[10] with further high levels found in the vitreous humour in the eye and articular cartilage. Hyaluronan is metabolised rapidly in humans, and around one third (5g) of its total amount is replaced per day.[11] Hyaluronan levels within the blood are relatively low. This is thought to be because most hyaluronan is cross-linked with collagen, so free hyaluronan concentration remains low.[8]

However, during normal cell division hyaluronan synthesis rises, helping the dividing cell to disassociate from its substratum, thereby permitting cell movement. Hyaluronan's ability to do this is directly linked to its hygroscopic properties, which allow it to attract large amounts of water to an area.[1] After the cell has undergone

mitosis and dissociation and the epithelial cells mature and migrate, hyaluronan levels gradually return to normal.[8]

High circulating levels of hyaluronan are not thought to be beneficial as it binds easily with fibrin, promoting blood clot formation. The higher the concentration of free hyaluronan, the faster the clotting time,[12] and therefore the greater the likelihood of thrombus formation, with subsequent pathological implications.

Hyaluronan levels also rise during the earlier phases of wound healing.[13,14] Oksala et al. demonstrated this using in vitro human tissue biopsied from healing mucosal wounds.[15] Results suggested that levels rose in early wound healing, before reducing again by day seven, post-injury. Higher levels were also found in newly forming granulation tissue. As this was a human wound model, it could be inferred that these findings have more value and are more reflective of normal wound healing than studies carried out using in vitro animal models.

Hyaluronan is implicated in a number of processes during early wound healing, including

- Cell migration
- Cell proliferation
- Organisation of granulation tissue
- Moderation of the inflammatory response
- Angiogenesis.[4]

Hyaluronan and cell migration

Studies using exogenous hyaluronan demonstrated that it affects the migration of fibroblasts to a wound site as illustrated by Ellis and Schor.[9] This in vitro study used human adult and foetal fibroblasts obtained from the culture of skin biopsies to monitor the level of glycosaminoglycan production, particularly hyaluronan, and to compare levels of two varieties of fibroblast (fibroblasts synthesise collagen). Results suggested that in both the adult and foetal groups fibroblast migration increased in the presence of hyaluronan and TGF-b1 in a dose-dependent fashion — the higher the levels of hyaluronan, the greater the cell migration. However, later work suggested that adult fibroblast migration is not affected by the presence of TGF-b1, but instead by other cytokines such as EGF, PDGF, aFGF and bFGF,[19] suggesting that hyaluronan is key in the mediation of cell migration.

These dose-dependent effects of hyaluronan on cell migration are mediated by its interaction with hyaluronan receptors (CD44, ICAM-1 and RHAMM). A higher receptor population will bind more hyaluronan, mediating further fibroblast migration. It follows, therefore, that the higher numbers of fibroblasts in the wound area can contribute to the increased production of the extracellular matrix and new collagen.

As well as providing structure, the newly-deposited extracellular matrix itself affects fibroblast behaviour by modulating the synthesis of matrix macromolecules and controlling cellular response to cytokines such as EGF and PDGF.[19] Additionally,

Hyaluronan also supports cell migration due to its physiochemical properties, providing a hydrated matrix that facilitates easier cell movement.[20]

Hyaluronan and cell proliferation

Once cells have migrated to the wound site it is essential that they can proliferate. While hyaluronan is important for fibroblast migration, there is no evidence it acts directly on mitogenic activity. However, it has been shown that high levels of hyaluronan are present during cell mitosis , and several studies have shown that hyaluronan synthesis and hyaluronan synthase activity varies with the proliferative state of cells.[21,22] Analysis of these studies also suggests that inhibition of hyaluronan synthesis leads to the prevention of cell mitosis and proliferation — when hyaluronan was removed from the culture cell, proliferation decreased. It is thought that hyaluronan does not produce the mitogenic activity, but instead promotes the hydrated environment in the extracellular space which aids cell detachment.[20] Therefore, an increase in production of hyaluronan and its protrusion into the extracellular space, in turn, increases the area's water content.

Hyaluronan and the modification of the inflammatory response

A further role for hyaluronan during proliferation is moderation of the inflammatory response. Inflammation initiates the healing process, but the inflammatory response needs to be moderated, otherwise tissue repair cannot proceed normally and granulation tissue cannot be stabilised.[4] As discussed, hyaluronan is important in mediating cell migration and proliferation, both essential in promoting the

inflammatory response. However, in a somewhat contradictory role, it can moderate inflammation in a number of ways.

Effect on free radicals

First, hyaluronan has an effect against free radicals. A free radical is any molecular species that contains one or more unpaired electrons. They are produced by oxidation or reduction reactions, where the molecule gives up an electron, or when a bond is broken.[23] Many free radicals are highly reactive due to the electrons' tendency to 'seek' electrons from other molecules and pair with them.[24] If they come into contact with cells they can damage both the cell membrane and DNA, resulting in poor cell function or even death.[25] Oxygen free radicals tend to remove electrons from their target molecule (oxidation), pairing the donated electron with their own free electron.

Oxygen free radicals impair wound healing because they reduce the tensile strength of the wound, and may be implicated in wound leakage, as described by Foschi et al.[26] In this study, rat wounds were treated with different preparations of hyaluronan, and it was noted that the strength of healing wounds was significantly improved. However, it was not made clear whether the hyaluronan was acting in a free radical scavenging role or had a different function. Further research on rat models had similar results, with use of topical low molecular weight hyaluronan increasing wound tensile strength when compared with a placebo, although, again, the specific role of hyaluronan was not made clear.[27]

Interactions with constituents of the inflammatory response

In addition to its effect on free radicals, hyaluronan is thought to moderate the inflammatory response through its specific interactions with constituents of the inflammatory response. During inflammation the cytokine TNF-a is produced, which stimulates fibroblasts to produce TNF-stimulated gene6 (TSG-6), a hyaluronan-binding protein. TSG-6 forms a complex with inter-alpha-inhibitor (IaI), a serum proteinase inhibitor. Together, they affect the action of plasmin, which activates the cascade of matrix metalloproteinases and other proteinases that lead to inflammatory tissue damage.[4]

It appears that the TSG-6/IaI complex is stabilised by binding to hyaluronan, when it moderates inflammation and stabilises granulation tissue.[28] This was demonstrated in vitro when TSG-6 was administered into an induced inflammation model, resulting in a reduced inflammatory response.[29] As with all non-human experimental models, it can never be assumed that these findings would be exactly the same as those from a human in vivo study due to environmental and experimental control factors. But it is hoped that the experimental design makes the results representative of the activity of hyaluronan in this situation, with a strong inference that the same would occur in the 'normal' situation.

Clinical importance of hyaluronan

Can hyaluronan influence clinical practice? Hyaluronan is used in the management of articular and ophthalmic procedures because of its highly viscoelastic properties.[8]

In the field of wound healing, hyaluronan has already been used, and there is much interest in using it to stimulate healing in chronic wounds.[1] Hyaluronan has been used as a pre-autograft wound bed preparation in the Vivoderm system, and as a wound treatment on its own as Hyalofill (ConvaTec). Here, the dressing breaks down in contact with the wound, liberating hyaluronan, which encourages the development of a highly hydrated environment, thought to aid the healing process. Hyaluronan's role in cell migration and proliferation may also make it beneficial in the management of chronic wounds as this might help stimulate the wound to move out of chronicity. Currently, hyaluronan-based products are designed to be used as medical devices because of its physicochemical properties, but as its biological role becomes better understood, products could be developed that support these processes as well.

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