Why low molecular weight HA in wound healing?

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Hyaluronic acid (HA), also called hyaluronan, is a natural anionic, non-sulphated glycosaminoglycan (GAG) that is distributed widely throughout the body. Structurally, HA is a linear polymer composed of repeating polymeric disaccharides units of D-glucuronic acid and N-acetyl-D-glucosamine, and has the unique capacity to retain a large amount of water. In addition to its high hygroscopic nature, HA has a wide variety of functions and beneficial properties (**Figure 1**): in addition to antioxidant, anti-inflammatory, viscoelastic, bacteriostatic and anti-oedematous properties, it is highly biocompatible and completely non-immunogenic[1]. For these reasons, HA has a wide range of applications in medicine, including wound repair.

As a major component of the extracellular matrix and found in high concentrations in the epidermis, HA is considered one of the key players in the tissue regeneration process[1]. It has been proven to modulate, via specific HA receptors, processes involved in inflammation, cellular migration and proliferation, and angiogenesis, which are the main phases of wound healing[2]. Interestingly, most of

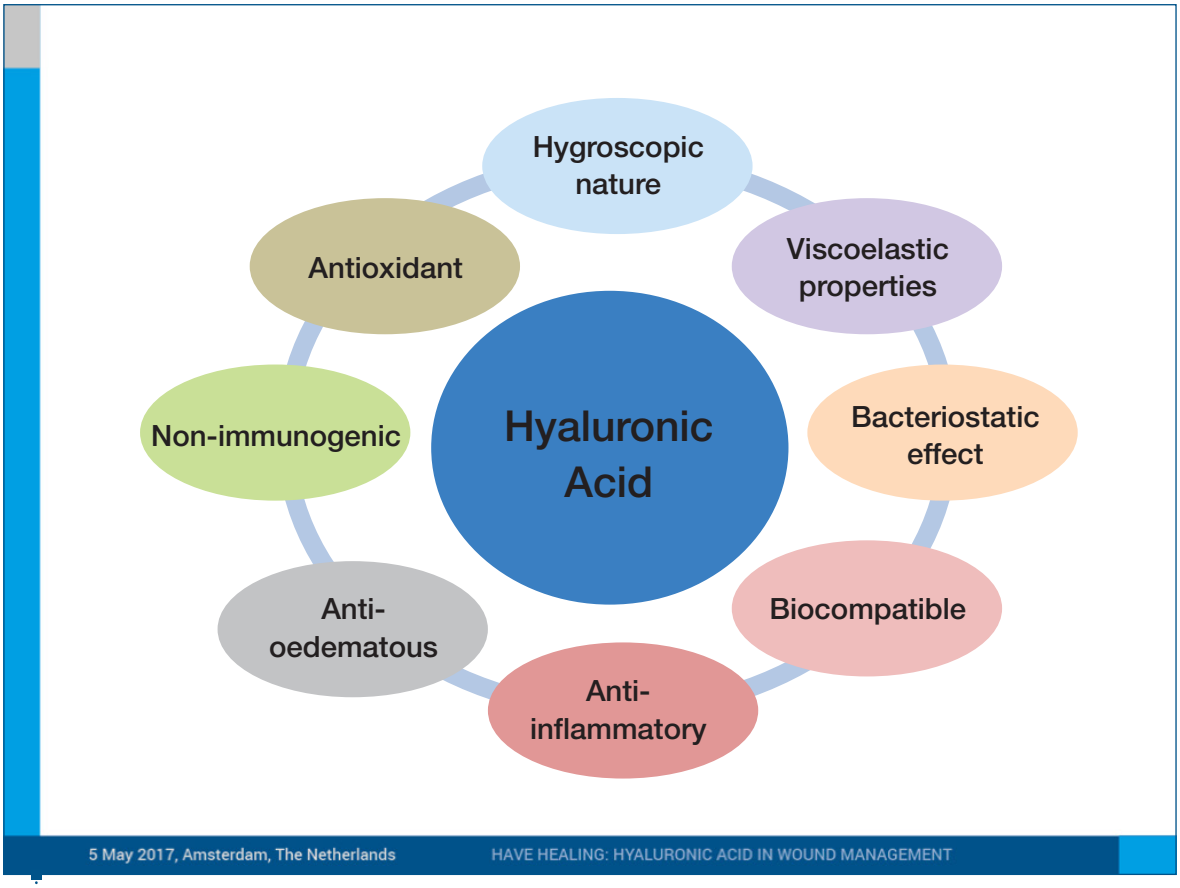

Figure 1. Key properties of HA.

the properties of HA, including those in wound healing, are dependent not only on its concentration, but also upon its molecular weight (MW) (**Figure 2**).

The smallest polymers of HA are involved in neo-angiogenesis and inflammation, while larger polymers, in the range of 150-300mers, increase migration and proliferation of fibroblasts and aid in formation of granulation tissue. Increasingly larger polymers of HA, from ≤1500mers to >2000mers, have a role in the assembly of the extracellular matrix and in water retention/viscosupplementation, respectively. Importantly, a fundamental aspect for all these activities *in vivo*, is that in order to exert these biological activities, each fraction needs to be highly "pure", meaning each type of activity is associated with a specific and most appropriate range of HA polymer chains: HA preparations containing polymers that are "out-of-range" will not show the same biological activity. This is clearly shown in studies where only the 200 kDa fraction of HA (LMW-HA) induces fibroblast proliferation[3] and in a keratinocyte model of wound healing wherein migration of cells is clearly mediated through HA of this molecular weight [2]. In wound repair, LMW-HA further increases the self-defense of skin epithelium by releasing the natural antimicrobial β-defensin-2 peptide via the TLR2 and TLR4 receptors, highlighting the role of HA in early activation of tissue repair. This is clinically important as insufficient upregulation of β-defensin-2 in both diabetic foot and venous calf ulcers has pointed to a pathological role of this protein in the chronicity of ulcers[4]. Thus, upregulation of β-defensin-2 by has the capacity to ameliorate the skin self-defense mechanism against infection by microorganisms[5].

As mentioned, it has been shown that LMW-HA induces the migration of keratinocytes. This has been most clearly demonstrated using a so-called scratch test, where petri dishes containing

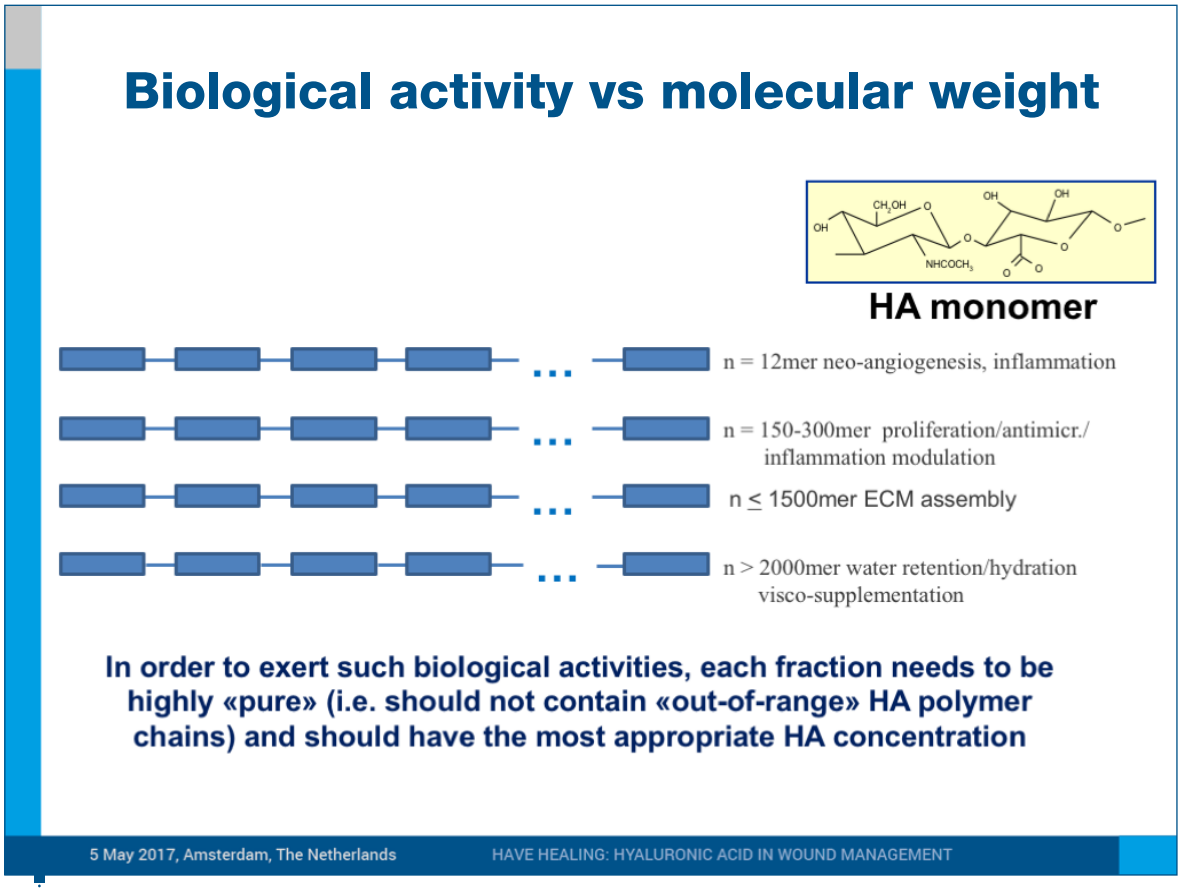

Figure 2. Biological activities vs. molecular weight of HA polymers.

keratinocytes are physically “scratched” to mimic a wound (**Figure 3**). In this model, keratinocyte migration is influenced only by LMW-HA, and not by other HA polymers of lower or higher size [2,5]. Considering the role of HA in the extracellular matrix, LMW-HA also has the ability to induce expression of fibrillar matrix components. In an in *vitro* model of TGF-β1-driven induction and maintenance of myofibroblasts, both HA and fibronectin were increased and co-localized in the extracellular matrix following myofibroblast induction by TGF-β1[6]. This implies that the matrix HA can interfere with the assembly of fibrillar components of the extracellular matrix. In addition, and importantly during the process of wound healing, greater cellular adhesion through HA in the matrix has an impact on cytoskeletal organisation, by directly affecting deposition of extracellular fibronectin and collagen. Skin atrophy is a common manifestation of aging and is frequently accompanied by ulceration and delayed wound healing.

Of interest, atrophic skin shows decreased HA content and expression of the major cell-surface HA receptor, CD44[7]. Treatment of primary keratinocyte cultures with LMW-HA, but not with smaller or larger HA polymers is able to induce wild-type (wt) but not CD44-deficient (CD44-/-) proliferation of keratinocytes. The same study also demonstrated that LMW-HA is able to penetrate the epidermal barriers, significantly increasing the HA content in both the epidermal and dermal layers. This provides direct evidence that HA acts specifically through a CD44-dependent mechanism to induce HA-induced keratinocyte proliferation; as such, topical application of HA may be viewed as an attractive therapeutic option in human skin atrophy.

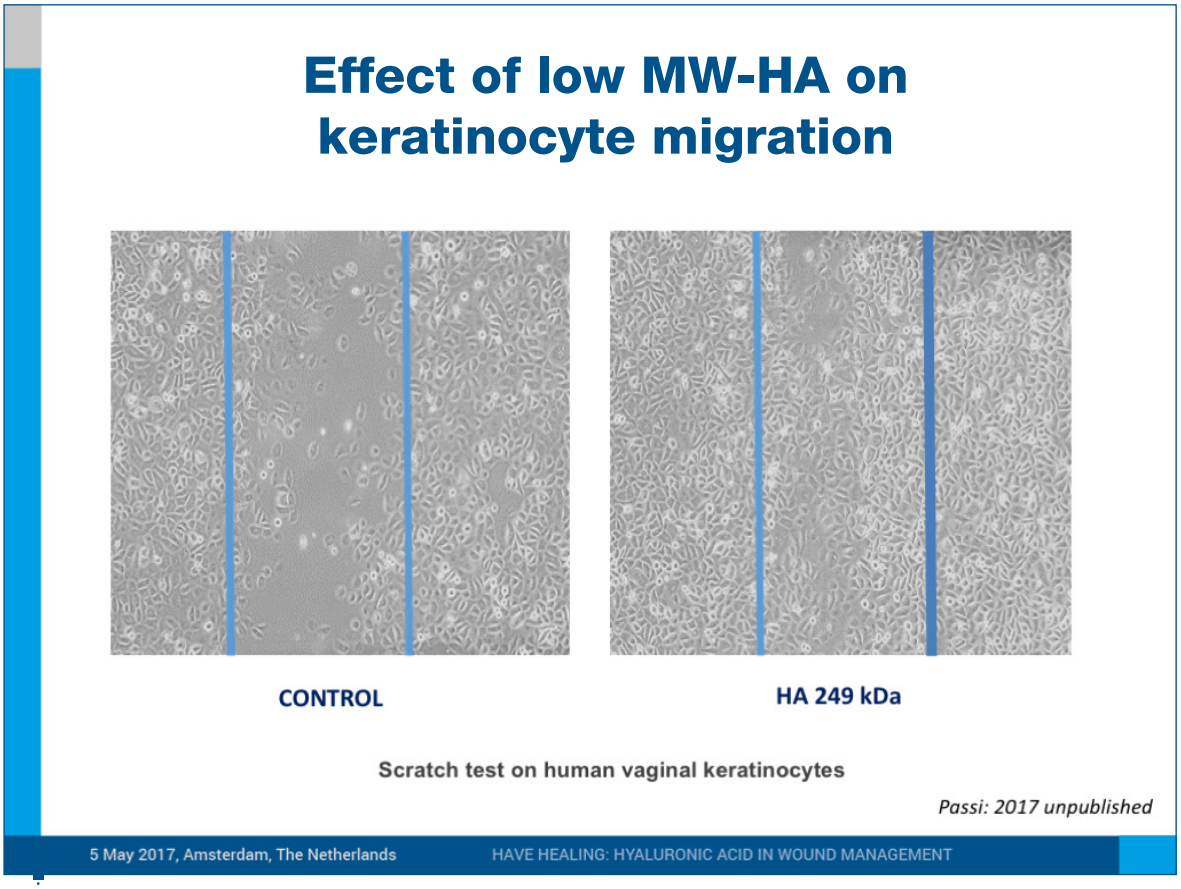

Figure 3. Scratch test of human vaginal keratinocytes after 24 hours incubated with control medium or medium containing LMW-HA [4].

Another important aspect in wound healing is angiogenesis. HA, and in particular LMW-HA with much less influence by high MW-A, has also been shown in *vitro* in a human endothelial umbilical cell model to increase angiogenesis to the same extent as IL-1β (**Figure 4**)[8].

Thus, from this brief overview, it should be apparent that HA plays a fundamental role in wound healing though biological actions mediated by specific HA receptors and precisely defined molecular weights. In all processes, but especially in wound healing, it should be remembered that HA fractions need to be highly homogeneous in terms of molecular weight in order to exert their specific actions. LMW-HA (200 kDa) is the most appropriate fraction for wound healing, exerting a synergistic effect with other biologically active substances in the healing process.

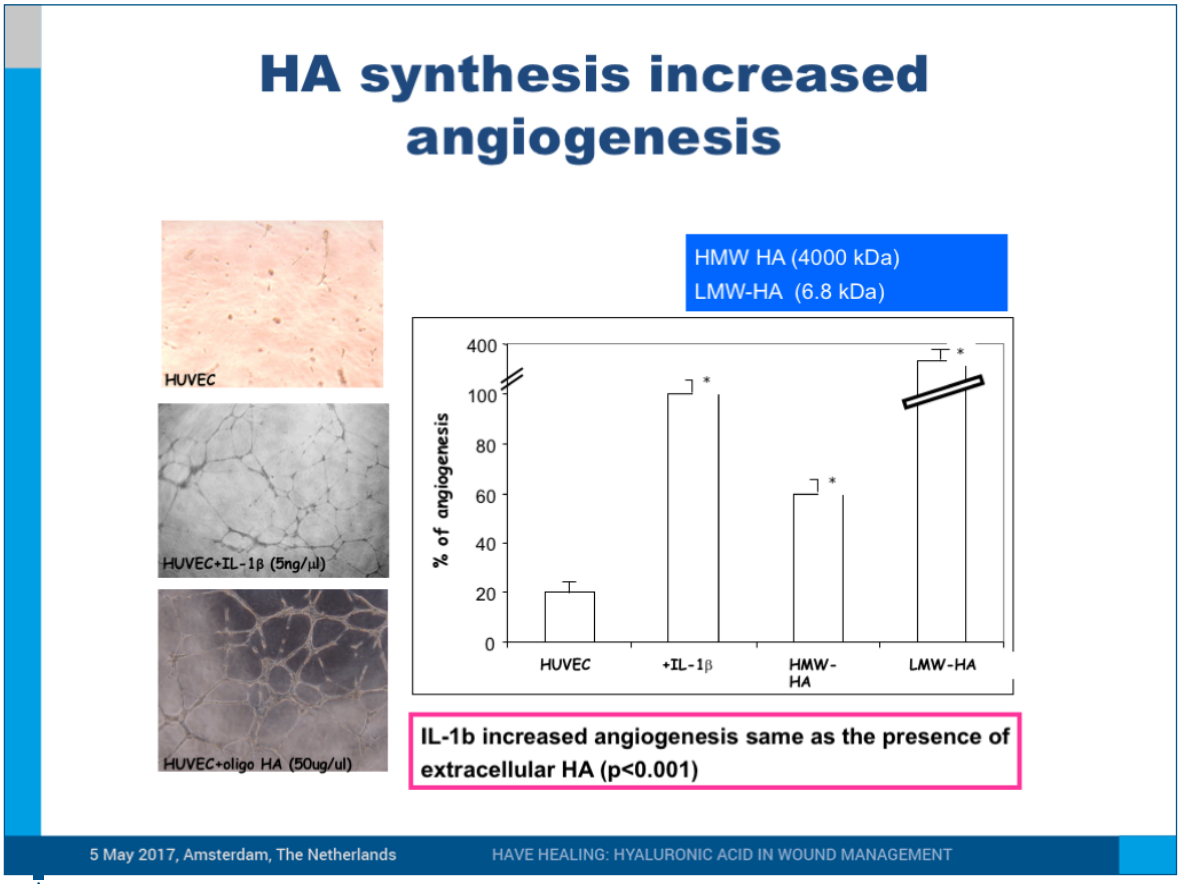

Figure 4. Effects of LMW-HA and HMW-HA on angiogenesis in a human umbilical endothelial cell model[8].

REFERENCES

1. Litwiniuk, et al. Wounds. 2016;28(3):78-88. **2. Ghazi K, et al.** PLoS One. 2012;7(11):e48351. doi: 10.1371/journal.pone.0048351. **3. Gariboldi S, et al.** J Immunol. 2008;181(3):2103-10. **4. Galkowska H, et al.** Folia Histochem Cytobiol. 2005;43(3):133-6. **5. Passi, et al.** Unpublished data. **6. Evanko SP, et al.** Matrix Biol. 2015;42:74-92. **7. Kaya G, et al.** PLoS Med. 2006;3(12):e493. **8. Passi A.** Unpublished data.