Hyaluronan in skin wound healing: therapeutic applications

Abstract: Hyaluronan is a vital constituent in effective skin wound healing. This polysaccharide is ubiquitous throughout the human body and has functional significance for tissue repair and remodelling. The importance of hyaluronan in the proliferative phase of healing is diverse, impacting on cell migration, proliferation, modification of the inflammatory response and on angiogenesis. As such, it holds therapeutic potential for a variety of clinical applications that range from facilitating effective wound healing to burns management and scarring. This overview of the multifaceted roles of hyaluronan considers its current applications to clinical practice in plastic surgery as well as the latest advances in research.
Declaration of interest: The authors have no conflicts of interest.

burns • dressings • hyaluronan • hyaluronic acid • scaffold • scar • wound healing

The process of wound healing can be broadly divided into three phases: inflammation, proliferation and remodelling.[1] Wound healing requires a complex series of reactions and interactions between cells and so-called mediators, and the phases of healing are recognised to overlap considerably.[2] Endothelial cells, fibroblasts, platelets, leucocytes and macrophages interact dynamically with a host of growth factors and cytokines which move, synthesise and modify microscopic components throughout the three phases to bring about restoration of tissue 'normality'. An imbalance of these parameters can result in delayed wound healing, which may manifest as hard-to-heal wounds (for instance, diabetic ulcers) or excessive wound healing which causes fibrosis (such as keloid scarring). Within a cutaneous environment, the glycosaminoglycan hyaluronan (HA), has been shown to play a pivotal role in inducing and maintaining normal skin healing.[3]

HA is found as a viscous gel around the body in all tissues and fluids (Table 1). Aside from skin, which accounts for 50% of total body HA, the molecule is also concentrated in articular joints, eyes, lymph, blood and urine.[4] The highest concentrations of HA are found within skin and cartilaginous joints.[5,6] Within skin, the molecule is predominantly based within the interstitial fluid of dermis, just below the basement membrane and around skin appendages. Furthermore, fetal and young skin have yielded higher HA contents compared to older skin.[7]

The linear polysaccharide is built from disaccharide units of N-acetyl-glucosamine and D-glucuronic acid.[8] Based on its chain length, the molecular weight of HA ranges from 2×10^5 to 1×10^7Da.[9] This weight can significantly affect its biological roles and cellular interactions.[10]

High-molecular-weight (HMW) HA is a relatively bioinert molecule that displays anti-inflammatory responses, maintains a highly hydrated environment and regulates growth factor activity.[11]

Low-molecular-weight (LMW) HA has higher bioactivity and exhibits pro-inflammatory responses.[12] This occurs by clustering and activating receptors, including cluster of differentiation 44 (CD44), toll-like receptor 4 (TLR-4), intercellular adhesion molecule-1 (ICAM-1) and receptor for HA-mediated motility (RHAMM).[13]

Over the last decade, numerous studies have utilised the therapeutic potential of HA by developing scaffolds, hydrogels and dressings that have been tested in a variety of cutaneous environments. This review summarises the latest advances in HA and its applications within the context of skin wound healing.

Hyaluronan in skin wound healing

HA is synthesised by a group of cell membrane-bound enzymes called HA synthase (HAS). Three subtypes of this enzyme synthesise HA chains of varying lengths called HAS-1,[14] HAS-2[15] and HAS-3.[16] These enzymes synthesise HA on the inner surface of the cell membrane and then dissociate into the extracellular matrix (ECM).[17] Within skin, the half-life of HA is 12–24 hours, and it is primarily degraded by the hyaluronidase enzyme family.[18,19]

HA can bind to a variety of proteins, known as hyaladherins, within the ECM and cell surface. Three cell surface proteins (or HA receptors) have been identified that interact with HA: CD44, ICAM-1 and RHAMM. These receptors are highly expressed in skin and are involved in cell adhesion and migration of fibroblasts, keratinocytes and endothelial cells.[6,20,21]

Throughout cutaneous wound healing, emerging evidence highlights the involvement of HA in each step. This is characterised by its interactions with multiple hyaladherins and changes in the equilibrium between its synthesis and degradation.

Ian CC King,[1] MA (Oxon), MBBS, MRCS, FRCS (Plast), Extremity Reconstruction Fellow*; **Parviz Sorooshian**,[2] BMBS, MRes, MRCS, Core Surgical Trainee*
***Corresponding author email:** parviz.sorooshian@doctors.org.uk
1 Department of Plastic & Reconstructive Surgery, St Mary's Hospital, Paddington, London W2 1NY. **2** Department of General Surgery, East Sussex Healthcare Trust, Conquest Hospital, St Leonards-on-Sea, East Sussex TN37 7RD.

Inflammation

HA is present in high concentrations during the initial phase of wound healing.[22] Furthermore, increasing literature suggests that its fragments (HMW and LMW HA) regulate cellular and molecular signalling cascades.[23]

At a cellular level, the high concentration and hydrophilic properties of HA facilitate the passive diffusion of water into the interstitial space, resulting in oedema.[24] This promotes the migration of inflammatory cells into the wound, including neutrophils and monocytes, which trigger further pro-inflammatory cascades.[25]

At a molecular level, HMW HA is metabolised from platelets via hyaluronidase-2 activity following a cutaneous injury.[26] These fragments stimulate the extrinsic clotting cascade by binding to tissue factor and fibrinogen.[27] This contributes towards the formation of the initial fibrin plug during inflammation.

HA also acts by stimulating pro-inflammatory cytokines. These include tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-8.[28] These molecular signalling molecules promote vasodilation, allowing further cell migration into the wound.

As well as promoting inflammation, HA can express anti-inflammatory capabilities when modulating the inflammatory response. Several studies suggest that this property is facilitated through its interaction with TNF-stimulated-gene-6 (TSG-6) protein.[29] TSG-6 is a hyaladherin that is produced by fibroblasts in response to the increased expression of TNF-α and IL-1. It initially binds to inter-alpha-inhibitor (IαI) to form a TSG-6/IαI complex that is stable in nature. HA then binds to this complex, which facilitates and prolongs anti-inflammatory properties across wound healing and lung models.[30.31] This has been shown to be via a cascade of matrix metalloproteinases and proteinases which brings about the inhibition of plasmin and neutrophil migration.[32]

Proliferation

The proliferative phase is characterised by fibroblast migration, granulation tissue formation, wound contraction and re-epithelialisation with neovascularisation.[33] The importance of HA throughout the proliferative phase is diverse.

Several *in vitro* studies suggest that HA regulates fibroblast migration via the receptors of CD44, ICAM-1 and RHAMM,[34,35] yet the effect of different HA fragment lengths on this activity remains controversial. David-Raoudi et al. noted that migration was affected by the length of HA in culture media.[3] This contradicts the findings of Ferguson et al. who found that different fragments of HA had little influence on migration.[36] Instead, they found a correlation with its concentration rather than molecular size, whereby lower concentrations inhibited fibroblast migration. It is suspected that this difference lies in the hydration and therefore special properties conferred by higher concentrations of HA.

HA also has a structural role within newly developed granulation tissue. In particular, HMW HA integrates into the gaps between the fibrous scaffolds composed of collagen and elastin.[32] This gives granulation tissue its elasticity and malleability, which allows scar tissue to remain intact, particularly over mobile areas of skin (i.e., joints).

The role of HA in angiogenesis is two-fold in that it has an inhibitory or stimulating function depending on fragment size.[37,38] HMW HA inhibits angiogenesis, whereas LMW HA stimulates it.[32] LMW HA has been shown to promote these angiogenic mechanisms through matrix metalloproteinases (MMPs).[39] MMPs lyse the basement membrane of wounds, allowing new capillaries to sprout from existing ones.[11]

The pro- and anti-angiogenic effects of different HA fragment lengths have been demonstrated in rat skin models of full-thickness skin autograft revascularisation following sub-lethal cryoinjury as well as primary (sutured) and secondary (open) full-thickness skin wounds.[40,41] Comparing the effect of different molecular weights of HA (1–4kDa versus 33kDa) with a control group, Lees et al.[40] demonstrated that LMW fragments increased blood flow and increased vessel growth in the graft, whereas the larger fragments had no such effects. In the absence of injury however, LMW HA had no effect on either blood flow or vessel growth, suggesting that the presence of substrates within active wounds is essential for these LMW HA fragments.

Remodelling

The re-modelling phase usually commences after approximately three weeks, when an equilibrium is struck between collagen synthesis and degradation within the wound.[42] Furthermore, wound edges contract through fibroblast activity and ECM interactions. Over time, the cells, structures and HA within the wound are degraded, which leaves an avascular, collagenous scar.

Several studies suggest that the level of HA in the papillary dermis may affect the extent of scarring. This includes an ex vivo study which showed that normal mature scar tissue held most HA in the papillary

Table 1. Reported quantities of hyaluronan in human tissue and fluid

Soft tissue (nanograms per gram of tissue)		Interstitial fluid (nanograms per millilitre of fluid)	
Articular cartilage	500,000–2,500,000	Synovial fluid	2,000,000–3,000,000
Skin*	400,000–500,000	Vitreous humour	200,000
Other organs	1000–100,000	Aqueous humour	1000
		Lymph fluid	100–18,000
		Breast milk	200–800
		Urine	100–300
		Blood	10–100

* mostly confined to dermis

dermis.[43] However, in keloid scar tissue, the glycosaminoglycan was much less prevalent in the papillary dermis and more prominent in the epidermis.[44]

Low HA levels have also been identified in elderly and fetal subjects, which are two patient cohorts known for developing minimal scar tissue.[45] While it is reasonable to postulate that HA levels change with age, Meyer and Stern found the levels and distribution to remain consistent regardless of age but that its activity at different ages may vary.[46] This could be attributed to the level of binding to hyaladherin.

Application to clinical practice

The therapeutic potential of HA has emerged over the last few years. In addition to its wound healing properties, the polymer yields good biocompatibility and minimal cytotoxicity within soft tissues, regardless of its concentration, when compared to other polymers, including carbomer and sodium alginate.[47,48]

Wound healing

Several HA-based dressings have emerged over the last decade that can be applied to a variety of acute and hard-to-heal wounds (Table 2). Although they have demonstrated satisfactory outcomes,[49–52] emerging research has focused on combining HA with other biomaterials and growth factors to improve wound healing outcomes.

Scaffolds are micro-/nanoscopic porous networks that can be composed of organic and/or synthetic materials. Within the field of skin healing, methods have been developed to synthesise scaffolds, including decellularisation,[53] electrospinning[54] and freeze-drying.[55] Pig peritoneum is a popular source for decellularised scaffolds.[56] Within bovine *in vivo* models, Wu et al. have incorporated HA into these scaffolds along with the growth factors of basic fibroblast growth factor (bFGF)[57] and epidermal growth factor (EGF).[58] While the HA-based scaffolds were beneficial, the addition of EGF led to significantly faster wound healing rates and greater dermal thickness when compared to bFGF. Similarly, Su et al. demonstrated similar outcomes using the same scaffold with EGF, with healing rates of 70% at 20 days.[53] Although these results are promising, the outcomes have not been measured past 20 days and no comparisons have been drawn against split skin grafts (SSGs) or full-thickness skin grafts (FTSGs).

Electrospinning has also been used to produce HA scaffolds with good tensile strength and to stimulate collagen synthesis.[59] Wang et al. recently incorporated EGF into electrospun HA-polycaprolactone (PCL) scaffolds.[54] While the growth factor improved wound healing compared to controls (healing by secondary intention), scaffolds without EGF yielded slower wound healing rates than the controls. Similarly, Shin et al. found that electrospun HA-poly(lactic-co-glycolic) acid (PLGA) scaffolds incorporated with the pro-angiogenic factor, epigallocatechin-3-O-gallate (EGCG), led to significantly faster wound healing rates in diabetic rats compared to the scaffold without any growth factors.[60] These outcomes can be attributed to the need for fast vascularisation.[61] This enables nutrients and pro-inflammatory cells to infiltrate and initiate wound healing processes, including collagen deposition. As such, pro-angiogenic growth factors are crucial for HA scaffolds within the context of cutaneous wound healing.

Freeze-drying has been employed for the creation of HA–gelatin scaffolds,[55,62] with wound closure of over 75% at 14 days. As such, the method by which HA scaffolds are produced appears independent of its wound-healing benefits and is more reliant on the scaffold adjuncts.

Although the benefits of different growth factors in HA scaffolds have not been directly investigated, Lai et al. demonstrated that the addition of multiple pro-angiogenic factors to collagen–HA scaffolds significantly improves wound closure of hard-to-heal wounds in diabetic rats.[63] Specifically, the combination of bFGF and EGF demonstrated greater wound closure rates, while the combination of these two growth factors with vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) led to significantly higher rates. While this may suggest that future scaffolds should incorporate a combination of these factors, the benefits of individual factors within an HA scaffold requires further exploration.

While no HA-based scaffolds have been manufactured as dressings for clinical trials, there is emerging evidence that pro-inflammatory adjuncts have similar beneficial effects in human trials as *in vivo* studies. This includes a randomised control trial (RCT) where autologous fibroblast–HA dressings were applied to treat diabetic foot ulcers.[64] The biodegradable dressings accelerated wound healing by 12 days on average when compared to a non-adherent foam dressing (36.4 versus 48.4 days).

Burns

The main aims of burn scar management centre on dermal preservation and accelerating wound healing, while minimising excessive collagen deposition in scar tissue, thereby reducing the risk of contractures, hypertrophy and keloids.[65] The regulatory activity of HA has led to the development of HA-based dressings, including Hyalomatrix (Anika Therapeutics, Italy), Hyiodine (Contipro, Czech Republic) and Sorelex (Contipro, Czech Republic).

Two double-blinded RCTs with a total of 144 patients assessed the use of HA with silver sulfadiazine dressings in patients with superficial burns.[66,67] Although adverse events were not reported, silver sulfadiazine dressings infiltrated with 0.2% HA cream led to complete burn healing by 8–9 days compared to 13–14 days with a silver sulfadiazine dressing alone. Despite yielding a low risk of selection and attrition bias, a systematic review of both studies concluded that more evidence was required to support the use of HA in treating superficial burns.[68]

Deeper burns have proved to be more difficult to treat with HA dressings. A multicentre retrospective national

survey assessed the efficacy of Hyalomatrix in 57 patients with deep partial and full-thickness burns.[49] This study found that complete wound closure was achieved in 32.7% of patients after 29 days and in 85.7% of patients at 37 days. While this study did not implement a control group, the normal healing time of deep partial-thickness burns has been reported to be between 14–21 days and over 21 days for full-thickness burns.[65]

These results could be attributed to the high risk of infection within deeper burn wounds that could hinder the healing process. As such, several *in vivo* studies are continuing to incorporate antimicrobial agents with HA to improve outcomes with promising results, including zinc oxide,[69] copper oxide[70] and sanguinarine.[71]

Further work should explore the combination of antimicrobials and HA-based dressings to optimise wound healing in deeper burn wounds.

Hypertrophic and keloid scarring

Hypertrophic and keloid scarring occur when pro-inflammatory signalling persists within a wound and cannot be countered by sufficient anti-inflammatory signalling, leading to fibrosis. This signalling imbalance is also present in patients with scleroderma, where the pro-inflammatory properties of LMW HA are believed to impede wound healing.[72] As such, the anti-inflammatory properties of HMW HA give it potential as a therapeutic agent. HMW HA treatment has been shown to reduce fibroblast hyper-proliferation and collagen production, which reflects reduced fibrosis.[73]

While promising, no subsequent studies have evaluated the effects of HMW HA on keloid scarring *in vivo* and in clinical trials due to the benign nature of established scar tissue. As such, there has been a recent shift towards targeting the upstream regulators of HMW HA expression. This includes HAS-2, which has been shown to be over-expressed in keloid keratinocytes.[74] Inhibition of this enzyme has been achieved by 4-methylumbelliferone and reduced TGF-β1 activity,[75] both of which may reduce keloid keratinocyte migration and activity.

Several *in vitro* studies have explored the interaction between HA and fetal fibroblasts to understand the physiology behind scar-free fetal wound healing. Mast et al. observed that fetal fibroblasts exposed to HA exhibited reduced fibroblast proliferation while maintaining collagen synthesis stimulation when compared to adult fibroblasts.[76] This may result in adequate collagen deposition with minimal inflammation, which is characteristic of scar-free wounds.[77] This response could be attributed to higher

Table 2. The properties and applications of current Hyaluronan-based dressings

Dressing name	Manufacturer	Hyaluronan component	Adjunct	Useful properties	Suitable wound depth	Suitable wound exudate	Applications
Hyalosafe®	Anika Therapeutics, Italy	HYAFF*	None	• Transparent dressing	Superficial	Moderate	• Donor sites • First and second degree burns
Hyalogran®	Anika Therapeutics, Italy	HYAFF*	Sodium alginate	• Moulds to the wound • Eases removal of necrotic tissue	Deep	High	• Leg ulcers • Pressure ulcers • Ischaemic wounds • Diabetic wounds
Hyalomatrix®	Anika Therapeutics, Italy	Hyaluronan derivative	Silicone membrane	• Biodegradable inner layer • Facilitates cellular invasion and capillary growth	Superficial and deep	Low and high	• Second degree burns • Trauma wounds • Venous leg ulcers • Pressure ulcers
Hyalofill®	Anika Therapeutics, Italy	HYAFF*	None	• Different shapes available for specific wound types	Deep	High	• Sinuses and fistulae • Venous leg ulcers • Pressure ulcers • Traumatic wounds
Hylodine®	Contipro, Czech Republic	Hyaluronan	Iodine	• Antimicrobial effect	Deep	High	• Infected wounds • Diabetic wounds • Sinuses and fistulae • Venous leg ulcers • Pressure ulcers
Sorelex®	Contipro, Czech Republic	Hyaluronan	Octenidine	• Antimicrobial effect	Superficial	High	• Venous leg ulcers • Diabetic wounds • Donor sites

*A total benzyl ester of hyaluronan

concentrations of the CD44 receptor that are found on fetal fibroblasts compared to adult fibroblasts.[78] Therefore, the greater expression of CD44 receptors may allow greater uptake of HA and reduce scarring. Conversely, Shatirishvili et al. demonstrated that the inhibition of CD44 receptors reduced epidermal thickening and stiffness within normal mice.[79] As such, an anti-CD44 antibody may attenuate the activity of keloid keratinocytes.[80] Further work is required to evaluate the effects of regulating HA receptors on keloid scarring.

Conclusion

The diverse roles of HA within cutaneous tissues gives it significant potential for treating many pathologies surrounding skin wound healing. While being a well-established agent within cosmesis and hard-to-heal wound healing, further work is required to unearth its potential when combined with pro-angiogenic adjuncts, such as growth factors and antimicrobial molecules. This will ultimately allow hostile wound environments of diabetic ulcers and deep burns to heal at accelerated rates and optimise clinical outcomes. **JWC**

Reflective questions

- Should hyaluronan (HA)-based scaffolds be used as routine dressings for acute and hard-to-heal wounds?
- Could the individual properties of high and low molecular weight HAs be used to promote or limit the rate of skin wound healing?
- Should HA-based agents be used in current day-to-day practice to promote skin wound healing?

References

1 Broughton G 2nd, Janis JE, Attinger CE. The basic science of wound healing. Plast Reconstr Surg 2006; 117(7 Suppl):12S–34S. https://doi.org/10.1097/01.prs.0000225430.42531.c2
2 Broughton G, Janis JE, Attinger CE. Wound healing: an overview. Plast Reconstr Surg 2006;117(7 Suppl):1e-S-32e-S. https://doi.org/10.1097/01.prs.0000222562.60260.f9
3 David-Raoudi M, Tranchepain F, Deschrevel B et al. Differential effects of hyaluronan and its fragments on fibroblasts: relation to wound healing. Wound Repair Regen 2008; 16(2):274–287. https://doi.org/10.1111/j.1524-475X.2007.00342.x
4 Reed RK, Lilja K, Laurent TC. Hyaluronan in the rat with special reference to the skin. Acta Physiol Scand 1988; 134(3):405–411. https://doi.org/10.1111/j.1748-1716.1988.tb08508.x
5 Holmes MW, Bayliss MT, Muir H. Hyaluronic acid in human articular cartilage: age-related changes in content and size. Biochem J 1988; 250(2):435–441. https://doi.org/10.1042/bj2500435
6 Tzellos TG, Sinopidis X, Kyrgidis A et al. Differential hyaluronan homeostasis and expression of proteoglycans in juvenile and adult human skin. J Dermatol Sci 2011; 61(1):69–72. https://doi.org/10.1016/j.jdermsci.2010.10.010
7 Tzellos TG, Klagas I, Vahtsevanos K et al. Extrinsic ageing in the human skin is associated with alterations in the expression of hyaluronic acid and its metabolizing enzymes. Exp Dermatol 2009; 18(12):1028–1035. https://doi.org/10.1111/j.1600-0625.2009.00889.x
8 Meyer K, Hobby GL, Chaffee E, Dawson MH. The hydrolysis of hyaluronic acid by bacterial enzymes. J Exp Med 1940; 71(2):137–146. https://doi.org/10.1084/jem.71.2.137
9 Yu H, Li Q, Zhou X et al. Role of hyaluronan and CD44 in reactive oxygen species-induced mucus hypersecretion. Mol Cell Biochem 2011; 352(1-2):65–75. https://doi.org/10.1007/s11010-011-0740-6
10 Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. J Intern Med 1997; 242(1):27–33. https://doi.org/10.1046/j.1365-2796.1997.00170.x
11 Pardue EL, Ibrahim S, Ramamurthi A. Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. Organogenesis 2008; 4(4):203–214. https://doi.org/10.4161/org.4.4.6926
12 Prevo R, Banerji S, Ferguson DJ et al. Mouse LYVE-1 is an endocytic receptor for hyaluronan in lymphatic endothelium. J Biol Chem 2001; 276(22):19420–19430. https://doi.org/10.1074/jbc.M011004200
13 Evanko SP, Johnson PY, Braun KR et al. Platelet-derived growth factor stimulates the formation of versican-hyaluronan aggregates and pericellular matrix expansion in arterial smooth muscle cells. Arch Biochem Biophys 2001; 394(1):29–38. https://doi.org/10.1006/abbi.2001.2507
14 Shyjan AM, Heldin P, Butcher EC et al. Functional cloning of the cDNA for a human hyaluronan synthase. J Biol Chem 1996; 271(38):23395–23399. https://doi.org/10.1074/jbc.271.38.23395
15 Spicer AP, Augustine ML, McDonald JA. Molecular cloning and characterization of a putative mouse hyaluronan synthase. J Biol Chem 1996; 271(38):23400–23406. https://doi.org/10.1074/jbc.271.38.23400
16 Spicer AP, Olson JS, McDonald JA. Molecular cloning and characterization of a cDNA encoding the third putative mammalian hyaluronan synthase. J Biol Chem 1997; 272(14):8957–8961. https://doi.org/10.1074/jbc.272.14.8957
17 Prehm P. Release of hyaluronate from eukaryotic cells. Biochem J 1990; 267(1):185–189. https://doi.org/10.1042/bj2670185
18 Csóka AB, Scherer SW, Stern R. Expression analysis of six paralogous human hyaluronidase genes clustered on chromosomes 3p21 and 7q31. Genomics 1999; 60(3):356–361. https://doi.org/10.1006/geno.1999.5876
19 Reed RK, Laurent UB, Fraser JR, Laurent TC. Removal rate of [3H] hyaluronan injected subcutaneously in rabbits. Am J Physiol 1990; 259(2):H532–H535. https://doi.org/10.1152/ajpheart.1990.259.2.H532
20 Song HF, Chai JK, Lin ZH et al. Expression of hyaluronic acid and its receptor in the process of wound healing in different skin tissues and its significance [Article in Chinese]. Zhonghua Yi Xue Za Zhi 2003; 83(12):1070–1074
21 Groves RW, Ross E, Barker JN et al. Effect of in vivo interleukin-1 on adhesion molecule expression in normal human skin. J Invest Dermatol 1992; 98(3):384–387. https://doi.org/10.1111/1523-1747.ep12499816
22 Oksala O, Salo T, Tammi R et al. Expression of proteoglycans and hyaluronan during wound healing. J Histochem Cytochem 1995; 43(2):125–135. https://doi.org/10.1177/43.2.7529785
23 de la Motte C, Nigro J, Vasanji A et al. Platelet-derived hyaluronidase 2 cleaves hyaluronan into fragments that trigger monocyte-mediated production of proinflammatory cytokines. Am J Pathol 2009; 174(6):2254–2264. https://doi.org/10.2353/ajpath.2009.080831
24 Frost SJ, Weigel PH. Binding of hyaluronic acid to mammalian fibrinogens. Biochimica et Biophysica Acta (BBA) - General Subjects 1990; 1034(1):39–45. https://doi.org/10.1016/0304-4165(90)90150-U
25 Tavianatou AG, Caon I, Franchi M et al. Hyaluronan: molecular size-dependent signaling and biological functions in inflammation and cancer. FEBS J 2019; 286(15):2883–2908. https://doi.org/10.1111/febs.14777
26 Menezes GB, McAvoy EF, Kubes P. Hyaluronan, platelets, and monocytes: a novel pro-inflammatory triad. Am J Pathol 2009; 174(6):1993–1995. https://doi.org/10.2353/ajpath.2009.081138
27 Kim J, Nafiujjaman M, Nurunnabi M et al. Effects of polymer-coated boron nitrides with increased hemorheological compatibility on human erythrocytes and blood coagulation. Clin Hemorheol Microcirc 2018; 70(3):241–256. https://doi.org/10.3233/CH-170307
28 Kobayashi H, Terao T. Hyaluronic acid-specific regulation of cytokines by human uterine fibroblasts. Am J Physiol Cell Physiol 1997; 273(4):C1151–C1159. https://doi.org/10.1152/ajpcell.1997.273.4.C1151
29 Wisniewski HG, Vilček J. TSG-6: an IL-1 /TNF-inducible protein with anti-inflammatory activity. Cytokine Growth Factor Rev 1997; 8(2):143–156. https://doi.org/10.1016/S1359-6101(97)00008-7
30 Tan KT, McGrouther DA, Day AJ et al. Characterization of hyaluronan and TSG-6 in skin scarring: differential distribution in keloid scars, normal scars and unscarred skin. J Eur Acad Dermatol Venereol 2011; 25(3):317–327. https://doi.org/10.1111/j.1468-3083.2010.03792.x
31 Stober VP, Johnson CG, Majors A et al. TNF-stimulated gene 6 promotes formation of hyaluronan–inter-α-inhibitor heavy chain complexes necessary for ozone-induced airway hyperresponsiveness. J Biol Chem 2017; 292(51):20845–20858. https://doi.org/10.1074/jbc.M116.756627
32 Chen WJ, Abatangelo G. Functions of hyaluronan in wound repair. Wound Repair Regen 1999; 7(2):79–89. https://doi.org/10.1046/j.1524-475X.1999.00079.x
33 Schultz GS, Davidson JM, Kirsner RS et al. Dynamic reciprocity in the wound microenvironment. Wound Repair Regen 2011; 19(2):134–148. https://doi.org/10.1111/j.1524-475X.2011.00673.x
34 Stern R, Asari AA, Sugahara KN. Hyaluronan fragments: an information-rich system. Eur J Cell Biol 2006; 85(8):699–715. https://doi.

org/10.1016/j.ejcb.2006.05.009
35 Qian Y, Li L, Jiang C et al. The effect of hyaluronan on the motility of skin dermal fibroblasts in nanofibrous scaffolds. Int J Biol Macromol 2015; 79:133–143. https://doi.org/10.1016/j.ijbiomac.2015.04.059
36 Ferguson EL, Roberts JL, Moseley R et al. Evaluation of the physical and biological properties of hyaluronan and hyaluronan fragments. Int J Pharm 2011; 420(1):84–92. https://doi.org/10.1016/j.ijpharm.2011.08.031
37 Feinberg R, Beebe D. Hyaluronate in vasculogenesis. Science 1983; 220(4602):1177–1179. https://doi.org/10.1126/science.6857242
38 Sattar A, Rooney P, Kumar S et al. Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. J Invest Dermatol 1994; 103(4):576–579. https://doi.org/10.1111/1523-1747.ep12396880
39 Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. Physiol Rev 2011;91(1):221–264. https://doi.org/10.1152/physrev.00052.2009
40 Lees VC, Fan TP, West DC. Angiogenesis in a delayed revascularization model is accelerated by angiogenic oligosaccharides of hyaluronan. Lab Invest 1995; 73(2):259–266
41 Borgognoni L, Reali UM, Santucci M. Low molecular weight hyaluronic acid induces angiogenesis and modulation of the cellular infiltrate in primary and secondary healing wounds. Eur J Dermatol 1996; 6(2):127–131. (http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=3000532)
42 Frenkel JS. The role of hyaluronan in wound healing. Int Wound J 2014; 11(2):159–163. https://doi.org/10.1111/j.1742-481X.2012.01057.x
43 Meyer LJ, Egbert BM, Shuster S et al. Reduced hyaluronan in keloid tissue and cultured keloid fibroblasts. J Invest Dermatol 2000; 114(5):953–959. https://doi.org/10.1046/j.1523-1747.2000.00950.x
44 Jumper N, Paus R, Bayat A. Functional histopathology of keloid disease. Histol Histopathol 2015; 30(9):1033–1057. https://doi.org/10.14670/HH-11-624
45 Juhlin L. Hyaluronan in skin. J Intern Med 1997; 242(1):61–66. https://doi.org/10.1046/j.1365-2796.1997.00175.x
46 Meyer LJ, Stern R. Age-dependent changes of hyaluronan in human skin. J Invest Dermatol 1994; 102(3):385–389. https://doi.org/10.1111/1523-1747.ep12371800
47 Guo X, Huang S, Sun J, Wang F. Comparison of the cytotoxicities and wound healing effects of hyaluronan, carbomer, and alginate on skin cells in vitro. Adv Skin Wound Care 2015; 28(9):410–414. https://doi.org/10.1097/01.ASW.0000467303.39079.59
48 Dereure O, Czubek M, Combemale P. Efficacy and safety of hyaluronic acid in treatment of leg ulcers: a double-blind RCT. J Wound Care 2012; 21(3):131–139. https://doi.org/10.12968/jowc.2012.21.3.131
49 Gravante G, Sorge R, Merone A et al. Hyalomatrix PA in burn care practice: results from a national retrospective survey, 2005 to 2006. Ann Plast Surg 2010; 64(1):69–79. https://doi.org/10.1097/SAP.0b013e31819b3d59
50 Esposito G, Gravante G, Filingeri V et al. Use of hyaluronan dressings following dermabrasion avoids escharectomy and facilitates healing in pediatric burn patients. Plast Reconstr Surg 2007; 119(7):2346–2347. https://doi.org/10.1097/01.prs.0000261076.40549.cf
51 Colletta V, Dioguardi D, Di Lonardo A et al. A trial to assess the efficacy and tolerability of Hyalofill-F in non-healing venous leg ulcers. J Wound Care 2003; 12(9):357–361. https://doi.org/10.12968/jowc.2003.12.9.26530
52 Brenes RA, Ajemian MS, Macaron SH et al. Initial experience using a hyaluronate-iodine complex for wound healing. Am Surg 2011; 77(3):355–359. https://doi.org/10.1177/000313481107700329
53 Su Z, Ma H, Wu Z et al. Enhancement of skin wound healing with decellularized scaffolds loaded with hyaluronic acid and epidermal growth factor. Mater Sci Eng C 2014; 44:440–448. https://doi.org/10.1016/j.msec.2014.07.039
54 Wang Z, Qian Y, Li L et al. Evaluation of emulsion electrospun polycaprolactone/hyaluronan/epidermal growth factor nanofibrous scaffolds for wound healing. J Biomater Appl 2016; 30(6):686–698. https://doi.org/10.1177/0885328215586907
55 Quan R, Zheng X, Xu S et al. Gelatin-chondroitin-6-sulfate-hyaluronic acid scaffold seeded with vascular endothelial growth factor 165 modified hair follicle stem cells as a three-dimensional skin substitute. Stem Cell Res Ther 2014; 5(5):118. https://doi.org/10.1186/scrt508
56 Xu H, Wan H, Zuo W et al. A porcine-derived acellular dermal scaffold that supports soft tissue regeneration: removal of terminal galactose-alpha-(1,3)-galactose and retention of matrix structure. Tissue Eng Part A 2009; 15(7):1807–1819. https://doi.org/10.1089/ten.tea.2008.0384
57 Wu Z, Fan L, Xu B et al. Use of decellularized scaffolds combined with hyaluronic acid and basic fibroblast growth factor for skin tissue engineering. Tissue Eng Part A 2015; 21(1-2):390–402. https://doi.org/10.1089/ten.tea.2013.0260
58 Wu Z, Tang Y, Fang H et al. Decellularized scaffolds containing hyaluronic acid and EGF for promoting the recovery of skin wounds. J Mater Sci Mater Med 2015; 26(1):59. https://doi.org/10.1007/s10856-014-5322-1
59 Ji Y, Ghosh K, Li B et al. Dual-syringe reactive electrospinning of cross-linked hyaluronic acid hydrogel nanofibers for tissue engineering applications. Macromol Biosci 2006; 6(10):811–817. https://doi.org/10.1002/mabi.200600132
60 Shin YC, Shin DM, Lee EJ et al. Hyaluronic acid/PLGA core/shell fiber matrices loaded with EGCG beneficial to diabetic wound healing. Adv Healthc Mater 2016; 5(23):3035–3045. https://doi.org/10.1002/adhm.201600658
61 Guo A, Owens WA, Coady M et al. An in vivo mouse model of human skin replacement for wound healing and cell therapy studies. J Plast Reconstr Aesthet Surg 2012; 65(8):1129–1131. https://doi.org/10.1016/j.bjps.2011.12.008
62 Wang HM, Chou YT, Wen ZH et al. Novel biodegradable porous scaffold applied to skin regeneration. PLoS One 2013; 8(6):e56330. https://doi.org/10.1371/journal.pone.0056330
63 Lai HJ, Kuan CH, Wu HC et al. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. Acta Biomater 2014; 10(10):4156–4166. https://doi.org/10.1016/j.actbio.2014.05.001
64 You HJ, Han SK, Rhie JW. Randomised controlled clinical trial for autologous fibroblast-hyaluronic acid complex in treating diabetic foot ulcers. J Wound Care 2014; 23(11):521-530. https://doi.org/10.12968/jowc.2014.23.11.521
65 Rowan MP, Cancio LC, Elster EA et al. Burn wound healing and treatment: review and advancements. Crit Care 2015; 19(1):243. https://doi.org/10.1186/s13054-015-0961-2
66 Koller J. Topical treatment of partial thickness burns by silver sulfadiazine plus hyaluronic acid compared to silver sulfadiazine alone: a double-blind, clinical study. Drugs Exp Clin Res 2004; 30(5-6):183–190
67 Costagliola M, Agrosì M. Second-degree burns: a comparative, multicenter, randomized trial of hyaluronic acid plus silver sulfadiazine vs. silver sulfadiazine alone. Curr Med Res Opin 2005; 21(8):1235–1240. https://doi.org/10.1185/030079905X56510
68 Dalmedico MM, Meier MJ, Felix JV et al. Hyaluronic acid covers in burn treatment: a systematic review. Rev Esc Enferm USP 2016; 50(3):522–528. https://doi.org/10.1590/S0080-623420160000400020
69 Hadisi Z, Farokhi M, Bakhsheshi-Rad HR et al. Hyaluronic acid (HA)-based silk fibroin/zinc oxide core–shell electrospun dressing for burn wound management. Macromol Biosci 2020; 20(4):1900328. https://doi.org/10.1002/mabi.201900328
70 Thanusha AV, Dinda AK, Koul V. Evaluation of nano hydrogel composite based on gelatin/HA/CS suffused with asiatic acid/ZnO and CuO nanoparticles for second degree burns. Mater Sci Eng C 2018; 89:378–386. https://doi.org/10.1016/j.msec.2018.03.034
71 Zhu Q, Jiang M, Liu Q et al. Enhanced healing activity of burn wound infection by a dextran-HA hydrogel enriched with sanguinarine. Biomater Sci 2018; 6(9):2472–2486. https://doi.org/10.1039/C8BM00478A
72 Gualdi G, Monari P, Cammalleri D et al. Hyaluronic acid-based products are strictly contraindicated in scleroderma-related skin ulcers. Wounds 2019; 31(3):81–84. https://www.woundsresearch.com/article/hyaluronic-acid-based-products-are-strictly-contraindicated-scleroderma-related-skin-ulcers (accessed 18 November 2020)
73 Hoffmann A, Hoing JL, Newman M, Simman R. Role of hyaluronic acid treatment in the prevention of keloid scarring. J Am Coll Clin Wound Spec 2012; 4(2):23–31. https://doi.org/10.1016/j.jccw.2013.06.001
74 Hahn JM, Glaser K, McFarland KL et al. Keloid-derived keratinocytes exhibit an abnormal gene expression profile consistent with a distinct causal role in keloid pathology. Wound Repair Regen 2013; 21(4):530–544. https://doi.org/10.1111/wrr.12060
75 Hahn JM, McFarland KL, Combs KA, Supp DM. Partial epithelial-mesenchymal transition in keloid scars: regulation of keloid keratinocyte gene expression by transforming growth factor-β1. Burns Trauma 2016; 4(1):1–17. https://doi.org/10.1186/s41038-016-0055-7
76 Mast BA, Diegelmann RF, Krummel TM, Cohen IK. Hyaluronic acid modulates proliferation, collagen and protein synthesis of cultured fetal fibroblasts. Matrix 1993; 13(6):441–446. https://doi.org/10.1016/S0934-8832(11)80110-1
77 Kennedy CI, Diegelmann RF, Haynes JH, Yager DR. Proinflammatory cytokines differentially regulate hyaluronan synthase isoforms in fetal and adult fibroblasts. J Pediatr Surg 2000; 35(6):874–879. https://doi.org/10.1053/jpsu.2000.6869
78 Alaish SM, Yager D, Diegelmann RF, Cohen IK. Biology of fetal wound healing: hyaluronate receptor expression in fetal fibroblasts. J Pediatr Surg 1994; 29(8):1040–1043. https://doi.org/10.1016/0022-3468(94)90275-5
79 Shatirishvili M, Burk AS, Franz CM et al. Epidermal-specific deletion of CD44 reveals a function in keratinocytes in response to mechanical stress. Cell Death Dis 2016; 7(11):e2461. https://doi.org/10.1038/cddis.2016.342
80 Maytin EV. Hyaluronan: more than just a wrinkle filler. Glycobiology 2016; 26(6):553–559. https://doi.org/10.1093/glycob/cww033