Hyaluronan, a Ready Choice to Fuel Regeneration: A Mini-Review

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Key Words

Extracellular matrix · Regeneration · Wound healing · Mesenchymal stem cells · Biomaterial

Abstract

Background: Hyaluronan (HA) is present in extracellular spaces and interstitia of many tissue types. As it is capable of binding high amounts of water, HA provides ideal conditions for cell migration and proliferation. Under conditions of cellular crowding, it also permits lineage-specific differentiation and thus promotes many aspects of healing and tissue remodeling. **Methods:** The simplicity of its structure makes it amenable to chemical modifications and/or combined formulations with other bioactive substances. Thus a wide variety of clinical applications have been proposed, several of which are currently being implemented in advanced therapies. **Results:** Known features of HA biology, in particular regarding synthesis and processes related to signaling and control, have been adopted to elaborate specific products in order to support progress in regenerative medicine. Purified HA, HA-based hydrogels or special HA composites have been formulated together with other well-characterized biomaterials and bioactive factors. HA is currently employed in a variety of therapeutic applications both in its pure form and in a biofunctionalized form. **Conclusions:** HA plays an essential role in regenerative processes. Owing to the growing scientific knowledge in this field, medicinal products

based on HA have been devised and are being routinely applied in ophthalmology or in trauma and transplantation surgery. Further areas of application are contemplated, such as the use of HA composite scaffold material in tissue engineering, or refined HA hydrogels enabling controlled release of medication.

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Background

Hyaluronic acid (HA) is present in virtually all human tissues (since HA exists as a polyanionic polysaccharide at physiologic pH, the correct designation is ‘hyaluronan’). The highest concentrations of the polymer are found in highly hydrated connective tissues, such as skin, the umbilical cord or the vitreous. Skeletal structures and supporting tissues (bone, cartilage and synovial fluid) also contain large amounts of HA. Furthermore, the HA content is elevated during periods of rapid tissue regeneration or repair, and the extracellular matrix (ECM) of fetal wounds, which are well known for their ability to avoid scar formation, is rich in HA too.

HA polymers are composed of repeating disaccharides built from β 1,4-D-glucuronic acid- β -1,3-N-acetyl-D-glucosamine that form a linear chain which can easily gain a relative molecular mass of up to several millions (fig. 1). In the past, HA was thought to be a simple space-

Fig. 1. Hyaluronan: chemical structure of poly-[1,4-β-glucopyranosyl uronic acid-(1,3)-N-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl-1,3] better known as hyaluronan (a), transmission electron microscopy of single HA chains spread on mica and subjected to rotary shadowing with carbon (b), viscous solution of high molecular-weight HA (c).

filling compound although it was also considered as a significant constituent of the ground substance and the ECM. In contrast to collagen, HA is extremely hydrated and thus fundamental for biology and a functional determinant in cellular water homeostasis. Since highly hydrated matrices facilitate cell migration and proliferation, HA is also an acknowledged major compound controlling and regulating cell behavior and cell-cell interaction, especially in the course of tissue healing. The latter includes activation and modulation of immune responses, promotion of angiogenesis as well as cell proliferation and migration. In this context, it should be noted that HA is considered to be fully anergic. Its complete lack of antigenicity is due to the fact that HA and its principal receptor, CD44, are functional determinants of the immune response. To give only a few examples, HA is involved in immune cell development, regulation of leukocyte adhesion or immune surveillance. It is also generally accepted now that the polymeric size of HA is decisive in regulating or modulating immune responses: lower molecular-weight forms of HA (<500 kDa), but not the native form (>1,000 kDa), induce inflammatory responses in inflammatory but not in resident macrophages [1]. HA oligomers of tetra- and hexasaccharide size, in contrast to higher molecular-weight HA species, induce immunophenotypic maturation of human monocyte-derived dendritic cells [2]. HA is also sensed through CD44 by regulatory T cells as high molecular-weight HA enhances human CD4⁺CD25⁺ regulatory T lymphocytes to functionally suppress responder cells [3]. It is thus con-

ceivable that high molecular-weight HA provides a potent sensor mechanism linking the state of tissue matrix integrity and the adaptive immune response.

HA also exerts a pivotal function in controlling water homeostasis in the vitreous and synovial fluid. In conjunction with specific HA-binding proteins, it reinforces the biomechanical properties of connective tissues: for instance, the hyaline matrix of cartilage greatly depends on the interlinking capabilities of this biopolymer. Thus, HA is also an important constituent of the nucleus pulposus of intervertebral disks. Notably, during tissue repair, augmented production of HA efficiently supports the entrapment of water molecules and thereby sequentially triggers the loosening and relaxation of the pericellular space. Consequently, the constitution of such highly hydrated environments in combination with tightly regulated expression of HA-binding receptors greatly sustains and complements cell proliferation and differentiation. Comparable patterns of HA expression and accumulation were observed in embryonic tissues and tumors, consistent with massive rates of cell division and migration further corroborating the essential role of HA in cell biology, and tissue formation and remodeling [4].

About one half of the overall HA content of the adult human body is located within the skin. The synovial fluid is also rich in HA. With advancing age, the HA content largely decreases in the epidermis and synovial fluid. The decline is more pronounced in the latter when osteoarthritic alterations emerge as well. Late in life, the human body contains about one half to two thirds less HA. This

deficit, together with a significant change in other matrix components, such as collagens, elastin and glycosaminoglycans other than HA, is thought to have physiological consequences, in particular in the case of HA, such as impaired cellular proliferation and a steady dwindling of the water content of tissues [5].

Brief Summary of Hyaluronan Molecular Biology

HA is produced at the inner side of the plasma membrane and extruded into the extracellular space; it is catalyzed by a single enzyme: HA synthase (HAS). Three HAS isoenzymes are known to date: HAS1, HAS2 and HAS3, which exhibit distinct kinetic characteristics and polymeric weights [recently reviewed in ref. 6], and it is well established by now that growth factors such as the epidermal growth factor (EGF) or the keratinocyte growth factor increase the rate of HA synthesis. HA synthesis appears to be inhibited through naturally occurring antisense mRNAs complementary to HAS2, HASNT (HA synthase 2 antisense) [7]. However, mannose also impedes the activity of the enzyme [8]. Synthetic HAS inhibitors, such as the coumarin 4-methylumbelliferone, have been approved for clinical use by the FDA [9]. Recently, inhibition of HAS2 in human aortic smooth muscle cells was reported following treatment with adenosine monophosphate-activated protein kinase activators such as 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside or by the antidiabetic drug, metformin [10]. Based on the cases reported so far, interference with HA biosynthesis led to reduced mitosis and motility while overproduction of HA as a result of ectopic expression of HAS accelerated cellular motility and resulted in partial deregulation of contact-mediated inhibition of cell growth. In addition, specific pathways via two major HA receptors, i.e. the receptor for HA-mediated motility, RHAMM, and CD44, have been suggested to be key factors in HA-induced cell motility. Both HA receptors are well known to be involved in wound repair and it has been demonstrated in numerous studies that aberrant regulation of cell motility contributes to a variety of diseases including arthritis and cancer [11].

Application in Regenerative Medicine

In 1934, HA was purified from bovine vitreous by Karl Meyer and John Palmer, and its molecular structure could be unraveled. Meyer and Palmer suggested naming

the novel uronic acid that contained glycosaminoglycan 'hyaluronic acid', from *hyalos* (Greek for glass). Since then, numerous articles describing the potential use of HA in medical applications have been published, and several of the previously proposed scientific concepts have successfully been implemented in various clinical procedures and standards [12].

Given that HA is a major constituent of the eye, the compound purified from various other sources progressively found its way into ophthalmology, ranging from sodium hyaluronate eye drops to treat eye dryness to HA-containing elastoviscous materials used when operating on delicate tissue structures (fig. 1c). By now, highly elastic HA solutions are also routinely employed as viscosurgical agents in transplantation surgery to avoid damage caused during implantation/transplantation surgery as well as to prevent mechanical injury, desiccation but also to clear a space for surgical manipulation and instruments. HA-containing gels are also used for viscoaugmentation of the vitreous after retinal detachment surgery. A recent report employing cell replacement therapy describes the beneficial effects of an HA hydrogel-based support material to deliver retinal stem-progenitor cells in retinopathy [13]. Furthermore, in vitrectomy, injectable cross-linked HA hydrogels are used as vitreous substitutes. Such modified gels are nontoxic and usually they are well tolerated and provide refractive indices very similar to the natural vitreous [14]. Decreased HA levels were also found in the vitreous and in the aged human eye, most likely as a consequence of aging [15].

Hydrogels

Since HA is found in the ECM throughout the body, biomaterials based on HA derivatives constitute a promising clinical application, notably for manufacturing containers for cell delivery or drug reservoirs. Many chemical modifications have been achieved and used to devise synthetic biogels [for a recent review, see ref. 16]. Modifications include cross-linking via adipic hydrazide, tyramide, benzyl ester, glycidyl methacrylate, thio-propionyl hydrazide or bromoacetate either at the carboxylic acid of the glucuronic acid or the C-6 hydroxyl group of the N-acetylglucosamine. By now, thiolated HA (e.g. Extracel™, Glycosan BioSystems, Inc.) is a well-established system, in which mechanical properties and rates of biodegradation can be customized [17]. But aldehyde- or tyramide-modified HA hydrogels have been applied as well to generate hierarchical networks suitable for soft-tissue repair [18, 19]. The Huisgen cycloaddition reaction, now better known as 'click chemistry', is a fast

reaction of azides with alkynes, generating triazoles that can be applied to produce synthetic ECM also based on HA. A hydrogel with encapsulated yeast cells was produced with copper chloride as a catalyst [20], but a copper-free approach applying difluorocyclooctyne click chemistry has also been reported recently and represents a nontoxic alternative route for preparing biocompatible HA hydrogels [21].

A most appreciated function and desirable role of such bioartificial microenvironments comprise cell attraction and subsequently cell retention, thereby promoting the natural regeneration process mediated by endogenous cells such as mesenchymal stromal cells (MSCs). For the repair of articular defects, engineered cartilage based on HA hydrogels in combination with MSCs is an attractive goal as it is generally accepted that HA favors chondrogenic differentiation and matrix production. First promising results have recently been obtained when seeding MSCs onto 1% photocross-linked HA hydrogels. In this way, the compressive modulus of the construct, which is a good measure of the capacity to withstand axially directed pushing forces, could be significantly increased, demonstrating the potential of this approach in advancing functional stem cell-based tissue engineering of cartilage [22]. Thus, it is now conceivable that stem cells or precursor cells borne within the mesenchymal stroma can actually be attracted to the site of the defect for de novo tissue regeneration by incorporating, and thus supplying, specific growth factors. In a recent study, hepatocyte growth factor was added in a three-dimensional thiolated HA and thiolated heparin hydrogel scaffold (Heprasil™, Glycosan BioSystems Inc.) to induce migration of MSCs in vitro [23]. In a different report, nanoporous HA hydrogel microparticles with conjugated perlecan domains with bound heparin sulfate chains provided a controlled-release system for bone morphogenic protein BMP 2 and thereby promoted cartilage-specific ECM production of MSCs in vitro [24]. In addition to attracting endogenous cells with drug-eluting synthetic ECM towards injured sites, hydrogels with already encapsulated cells have been developed. MSCs enclosed in cross-linked thiolated HA and gelatin appeared to be a suitable approach to repair full-thickness defects in the patellar groove of rabbit femoral articular cartilage. Tissue was shown to be fully regenerated after 12 weeks [25]. In an osteochondral rat model, chondrogenic cells derived from human embryonic stem cells encapsulated in an HA-based hydrogel greatly enhanced functional tissue regeneration by producing hyaline-like neocartilage that eventually exhibited scarless integra-

tion of the existing cartilage and the regenerating subchondral bone [26].

The development of scaffold-based products for tissue repair and tissue engineering composed either of synthetic substances, such as polylactide-co-glycolide in combination with HA (InQu®, Istotechnologies, Inc.), or materials that closely mimic natural bone, such as β -tricalcium phosphate granules together with sodium hyaluronate (ChronOS putty commercialized by Synthes, Inc.), were further advancements. These approaches offer the advantage of guiding bone repair by means of highly hydrated osteoinductive matrices with pores that provide the proper physicochemical conditions for cell infiltration, thus eventually allowing for full osteoconduction of the wound or defect area, which means that de novo bone synthesis and remodeling are guided and efficiently propagated by adjacent native bone. Furthermore, as the scaffolds are made of resorbable material, the implant is eventually replaced by host bone.

Anti-Adhesion Material

Modified HA-based solutions (e.g. Seprafilm® Adhesion Barrier, Genzyme Corp.) are routinely employed to reduce postsurgical adhesion formation. One or two days after engraftment, such biocompatible and bioresorbable film barriers between damaged tissue surfaces turn into a hydrated gel that serves as a heavily hydrated insulator that greatly impedes adhesion formation after abdominal or pelvic surgery. To further prevent abnormal joining of anatomical structures as a consequence of scar tissue formation, cross-linked HA-collagen composites have been developed and tested in a rat model [27]. Interestingly, intraperitoneal adhesion prevention was strongly dependent on the applied ratios of HA to collagen. A sprayable liquid barrier composed of sodium hyaluronate and carboxymethylcellulose appeared to be effective in decreasing the overall incidence of postoperative adhesions in a rat model of laparotomy [28]. In contrast, in a study on patients who underwent thyroidectomy, sodium hyaluronate and carboxymethylcellulose solution did not decrease postoperative adhesions when introduced between adhesiogenic tissue surfaces [29], thus showing that further development and engineering in this direction need to be performed to efficiently cope with and surmount unexpected obstacles.

HA-Based Composites

A novel composite biomaterial based on HA-hydrogel containing cross-linked fibronectin has been developed. The aim was to first promote angiogenesis in order to

improve wound healing. Subcutaneous pure HA gels have previously been found to reduce infiltration of inflammatory cells and, notably, the material also promoted angiogenesis to some extent. However, this synthetic matrix was greatly repellent for endothelial cells in culture and also most cells refrained from invading it in vivo [30]. To enhance endothelial cell adhesion, proliferation as well as migration, fibronectin was cross-linked into the HA hydrogel thereby allowing strong binding of integrins. Working along this line, the angiogenic potential of this type of synthetic ECM could be greatly enhanced [31].

In a porcine deep-dermal partial-thickness burn model, novel vitronectin/growth factor complexes were applied to overcome common obstacles in healing complex wounds. Although the beneficial effect of the innovative composite was found to be independent of the HA carrier, the authors concluded that the HA composite material actually confers enhanced absorption, which in the long run results in improved delivery of the vitronectin/growth factor to the wounds [32]. However, in another approach, MeroGel® nasal dressing (Medtronic USA, Inc.) consisting of esterified HA was impregnated with insulin-like growth factor 1. This dressing significantly improved re-epithelialization of nasal mucosal tissue in sheep [33]. In a rat full-thickness skin defect model, cross-linked HA was freeze-dried to form a spongy scaffold and immersed into aqueous solution containing low molecular-weight HA, arginine and EGF. Application of the biofunctionalized material greatly promoted epithelialization [34]. The authors suggest that skin regeneration is improved by topical application of EGF with HA-based spongy sheets which actually enhances early-stage inflammation; this indirectly supports wound healing by subsequent cytokine release from inflammatory cells stimulated by the applied EGF.

Conspicuously, HA accumulates within the transplanted organ at rejection. Therefore serum levels have been investigated in order to find out whether HA may serve as a predictor of organ rejection, which is likely mediated by an unwanted immune response. Indeed, in a rat heart transplantation model, elevated HA concentrations were found in the circulation of the rats that rejected their grafts in contrast to those that did not [35]. Despite this clear evidence indicating that cumulated masses of HA potentially trigger deleterious immune responses, HA-based gels are currently being applied to entirely wrap the organs prior transplantation. The main idea there is that scaffolds rich in HA form highly hydrated soft matrices which protect delicate tissue structures to some extent

and may also prevent unspecific tissue scarring after transplantation. During the healing process, HA is rapidly metabolized and resorbed, which is thought to greatly support tissue regeneration. Moreover, it could also be shown in a murine transplant model that fragmented intragraft HA specifically activates dendritic cells [36], and innate alloimmunity became predominantly activated via the HA receptor CD44, whose function in priming allogeneic T cells is well known [37]. Hence CD44 may powerfully guide the adaptive immune response, which in many real cases will decisively support healing and regeneration.

Conclusion and Perspectives

Particular aspects of HA biology are amenable to advanced therapeutic use [38]. Indeed, HA has made its way into the clinics either as a basic curative agent or as an auxiliary material. Randomized controlled trials have successfully been carried out and are currently in progress to evaluate the application of HA derivatives in healing burns, and epithelial surgical and chronic wounds [39].

Further progress is expected because it has been shown by now in many cases that HA, when specifically combined with other tissue-specific compounds and bioactive factors, synergistically enhances the activity of those substances in supporting healing and regeneration. Hence HA derivatives and HA-based synthetic ECM hydrogels are presently being designed to warrant three-dimensional culture in vitro, in particular to facilitate tissue-engineering approaches, as well as for translational use in vivo [12].

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