Acta Biomaterialia 9 (2013) 7081–7092

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Review

Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment

A. Fakhari [a], C. Berkland [a,b,c,*]

[a] Bioengineering Graduate Program, University of Kansas, Lawrence, KS 66045, USA
[b] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA
[c] Department of Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS 66045, USA

ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 7 March 2013
Accepted 7 March 2013
Available online 15 March 2013

Keywords:
Hyaluronic acid
Crosslinking
Dermal filler
Viscosupplement

ABSTRACT

Hyaluronic acid (HA) is a naturally occurring biodegradable polymer with a variety of applications in medicine, including scaffolding for tissue engineering, dermatological fillers and viscosupplementation for osteoarthritis treatment. HA is available in most connective tissues in body fluids such as synovial fluid and the vitreous humor of the eye. HA is responsible for several structural properties of tissues as a component of extracellular matrix and is involved in cellular signaling. Degradation of HA is a stepwise process that can occur via enzymatic or non-enzymatic reactions. A reduction in HA mass or molecular weight via degradation or slowing of synthesis affects physical and chemical properties such as tissue volume, viscosity and elasticity. This review addresses the distribution, turnover and tissue-specific properties of HA. This information is used as the context for considering recent products and strategies for modifying the viscoelastic properties of HA in tissue engineering, as a dermal filler and in osteoarthritis treatment.

1. Introduction to hyaluronic acid

Hyaluronic acid (HA), also named hyaluronan, is a high molecular weight (10^5–10^7 Da), naturally occurring biodegradable polymer. HA is an unbranched non-sulfated glycosaminoglycan (GAG) composed of repeating disaccharides (β-1,4-D-glucuronic acid (known as uronic acid) and β-1,3-*N*-acetyl-D-glucosamide) (Fig. 1) [1–3]. HA can include several thousand sugar molecules in the backbone. HA is a polyanion that can self-associate and can also bind to water molecules (when not bound to other molecules), giving it a stiff, viscous quality similar to gelatin [4].

HA is one of the major elements in the extracellular matrix (ECM) of vertebrate tissues. It is available in almost all body fluids and tissues, such as the synovial fluid, the vitreous humor of the eye, and hyaline cartilage (Table 1) [5–8]. This biopolymer functions as a scaffold, binding other matrix molecules, including aggrecan [2]. It is also involved in several important biological functions, such as regulation of cell adhesion and cell motility, manipulation of cell differentiation and proliferation, and providing mechanical properties to tissues [6]. Several cell surface receptors, such as CD44, RHAMM and ICAM-1, have been shown to interact with HA, influencing cellular processes, including morphogenesis, wound repair, inflammation and metastasis [9–12]. Moreover, HA is responsible for providing the viscoelasticity of some biological fluids (synovial fluid and vitreous humor of the eye) and controlling tissue hydration and water transport (Table 1) [4]. In addition, HA has been found during embryonic development in the umbilical cord, suggesting that materials composed of HA may persuade favorable conditions for tissue regeneration and growth [13–17].

HA's characteristics, including its consistency, biocompatibility and hydrophilicity have made it an excellent moisturizer in cosmetic dermatology and skin-care products [4]. Moreover, its unique viscoelasticity and limited immunogenicity have led to its use in several biomedical applications, such as viscosupplementation in osteoarthritis (OA) treatment, as an aid in eye surgery and for wound regeneration [4,5]. In addition, HA has recently been explored as a drug delivery agent for different routes such as nasal, oral, pulmonary, ophthalmic, topical and parenteral [4,18–22].

2. History of HA

In 1934, Karl Meyer and his colleague John Palmer were the first investigators who discovered and isolated HA from the vitreous body of cows' eyes [2,4]. In the 1950s, the chemical structure of HA was solved by this group. They found that HA is composed of two sugar molecules (D-glucuronic acid (known as uronic acid) and D-*N*-acetyl glucosamine) and called it hyaluronic acid (hyalu-

* Corresponding author. Address: 2030 Becker Drive, Lawrence, KS 66045, USA. Tel.: +1 (785) 864 1455; fax: +1 (785) 864 1454.
E-mail address: berkland@ku.edu (C. Berkland).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.03.005

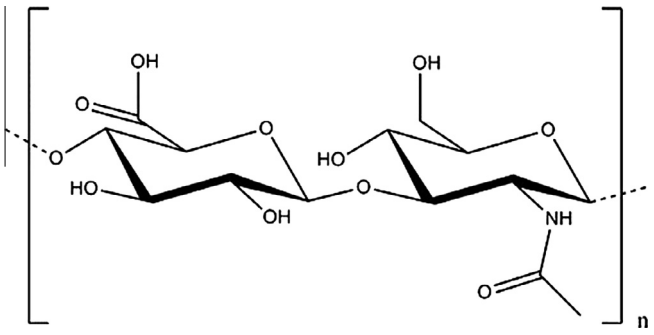

Fig. 1. Chemical structure of HA, which is made of disaccharide repeats of *N*-acetylglucosamine and glucuronic acid.

ronan). This name is derived from "hyalos" (the Greek word for glass + uronic acid). Initially, they isolated HA as an acid, but it behaved like a salt in physiological conditions (sodium hyaluronate) [2,4,5]. Several years after them, in 1942, Ender Balazs patented the first application of HA as a substitute for egg white in bakery products [4].

The first biomedical application of HA took place in the late 1950s, when HA was used for a vitreous substitution/replacement during eye surgery. For medical applications, HA was initially isolated from umbilical cords and shortly afterward, from rooster combs [4,5]. Later, HA was isolated from other sources, and the structural/biological characteristics of this polysaccharide were investigated more deeply in several laboratories [4].

3. Properties of HA

3.1. Chemical properties of HA

Structural studies showed that the two sugar molecules, D-glucuronic acid and D-*N*-acetyl glucosamine, in the HA disaccharide structure are connected together through alternative β-1,4 and β-1,3 glycosidic bonds (Fig. 1) [2,4]. The HA backbone is stiffened in physiological solution via a combination of internal hydrogen bonds, interactions with solvents and the chemical structure of the disaccharide. HA molecular investigations suggested that the axial hydrogen atoms form a non-polar face (relatively hydrophobic) and the equatorial side chains form a more polar face (hydrophilic), which led to a twisted ribbon structure for HA called a coiled structure [4].

HA's structural characteristics hinge on this random coiled structure in solution. At very low concentrations, chains entangle each other, leading to a mild viscosity (molecular weight dependent). In contrast, HA solutions at higher concentrations have a higher than expected viscosity, owing to greater HA chain entanglement that is shear-dependent. For instance, a 1% solution of high molecular weight HA (M_w >∼1000 kDa) can behave like jelly, but when shear stress is applied, it will easily shear thin and can be injected via a thin needle [4]. As such, HA is known as a "pseudoplastic" material. This rheological property (concentration and molecular weight dependent) of HA solutions has made HA ideal for lubrication in biomedical applications [4].

In addition to the unique viscosity of HA, the viscoelasticity of HA is another characteristic resulting from entanglement and self-association of HA random coils in solution [2]. It was suggested that the molecular self-association of HA occurs by forming anti-parallel double helices, bundles and ropes. Further experiments verified that HA chain–chain association indeed occurred in solution. Moreover, studies proposed that hydrogen bonding between adjacent saccharides occurred alongside mutual electrostatic repulsion between carboxyl groups, thus stiffening HA networks [4,7,23]. Viscoelasticity of HA can be tied to these molecular interactions, which are also dependent on concentration and molecular weight.

Electrostatic and ionic effects on HA have also been evaluated as a function of counter-ion type and valency. Studies suggested that these greatly affect rheological and hydrodynamic properties of HA. In one study, the effect of electrostatic and ionic interactions was investigated by comparing HA solution properties in deionized (DI) water, 0.5 M NaCl, and 0.5 M NaOH. The role of hydrogen bonds was investigated by comparing concentration-dependent solution properties. This revealed the effect of electrostatic shielding and also the profound effect of alkaline pH on HA chain stiffness. The study also showed that solution properties affect the hydrogen bonding and electrostatic interaction between the solution and HA, resulting in a change in HA chain stiffness [2,24]. Moreover, the hydrodynamic radius of HA was found to be greater in DI water than in 0.5 M NaCl or 0.5 M NaOH (DI water >0.5 M NaCl >0.5 M NaOH). The high pH shrank the volume occupied by HA (i.e., the apparent size of the polymer chains). The volume occupied by HA chains was decreased by more than 100 times when changing from DI water to 0.5 M NaOH. This dramatic change was attributed to increased electrostatic interactions and hydrogen bond formation, resulting in the reduction of the hydrodynamic radius of HA [2].

3.2. Biological properties of HA

HA performs several structural tasks in the ECM, as it binds with cells and other biological components through specific and non-specific interactions. Several ECM proteins are stabilized upon binding to HA. Specific molecules and receptors that interact with HA are involved in cellular signal transduction; molecules such as aggrecan, versican and neurocan, and receptors including CD44, RHAMM, TSG6, GHAP, ICAM-1 and LYVE-1 are examples of cell components that bind to HA [4]. Between these receptors, CD44 (cell surface glycoprotein) and RHAMM (receptor for HA-mediated motility) seem to have received more attention, since they have been found to be involved in cancer metastases [25–27]. CD44 is

Table 1
Examples of body tissues/fluids that contain HA; table reproduced with permission [92].

Tissue or body fluid	Concentration (μg g^{-1}; μg ml^{-1})	Remarks
Umbilical cord	4100	High molecular weight HA
Joint (synovial) fluid	1400–3600	Decreasing HA concentration occurs due to increasing the synovial fluid volume under inflammatory conditions
Vitreous body	140–500	During tissue maturation, HA concentration increases
Cartilage	–	HA works as a scaffold for binding other matrix molecules such as aggrecan
Dermis	200–500	HA is used as a "rejuvenating" agent in cosmetic dermatology
Epidermis	100	High HA concentration was observed around cells that synthesized dermis
Thoracic lymph	0.2–50	HA molecular weight affects inflammatory response and cell binding

ID	Title	Pages
508	Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment	12