Chapter 8

Biomedical Applications of Hyaluronic Acid

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Hyaluronic acid (HA), also named hyaluronan, is a high molecular weight polysaccharide found in the body in pericellular matrices, various bodily fluids, and in specialized tissues such as the vitreous humor of the eye and cartilage. Hyaluronic acid possesses both biological activities and physical properties that add to the uniqueness of this ubiquitous polysaccharide. The uniqueness of HA and its importance both biologically and physically apparently accounts for its identical structure when synthesized in bacteria, birds, and mammals. Because of this property, HA has been purified from chickens or bacteria for use as a biomaterial in medical devices in humans or other mammals. Hyaluronic acid is unique because of its viscoelastic and hydrodynamic properties, its assembly into extracellular and pericellular matrices, and its effects on cell signaling. Its use as a biomaterial has been driven largely by its physical properties and viscoelastic behavior. The biological properties of HA and its fragments have largely been ignored in its use as a biomaterial. As HA is more widely used and studied, these properties are becoming increasingly apparent and important in its use in medical devices.

Hyaluronic acid (HA) is a component of the extracellular matrix and is a ubiquitous substance found abundantly in nature. Many types of cells synthesize HA; it interacts with other constituents (proteins) of the extracellular matrix including the cell surface to create the supportive and protective structure around the cells. HA has also been shown to provide extracellular signals to cells related to locomotion and gene expression. It is a constituent of all body fluids and tissues, and it is found in higher concentrations in the vitreous humor of the eye, hyaline cartilage, and the synovial fluid. It was initially isolated from the vitreous of the eye.

Structurally, it is a polydisaccharide containing D-glucuronic acid and D-N-acetylglucosamine with repeating β(1-3), β(1-4) saccharide linkages. Three isozymes of hyaluronan synthases have been identified in bacterial and animal cells (for review, see reference(*1*)). These enzymes produce HA of variable molecular weights depending on the tissue. It is a most unusual polymer in that the same polysaccharide is produced in bacteria, birds, and mammals although the molecular weights may differ depending on its source. The highest molecular weight HA is found in cartilage. Hyaluronic acid being an extracellular and pericellular polymer is catabolized by receptor-mediated endocytosis and lysosomal hydrolysis in various tissues (*2*) or after transport to lymph nodes (*3*). Hyaluronidases are broadly distributed enzymes involved in tissue invasion and remodeling, (*4*) and are found in plasma (*5*).

Of special interest is that HA can be degraded by and fragmented to smaller oligosacharides via reactive oxygen (*6*) such as that produced by inflammatory cells, supporting a role for HA fragments in wound healing and inflammation (*7*) (for review of HA homeostasis in the body see reference (*1*)).

Hyaluronic acid has been purified in quantity from cartilage and bacteria owing to the relative abundance of HA in those materials. Once HA was purified its rheological properties as a viscoelastic polymer became apparent. The physical properties of HA point to its pivotal role in the extracellular and pericellular matrix in tissues. Hyaluronic acid, in solution, is viscoelastic and the rheological properties depend on concentration, ionic strength, pH, and molecular weight (*8, 9*). These rheological properties have been exploited for use in some biomedical applications. Solutions of high molecular weight HA are viscous, cohesive, lubricious, and hydrophilic. These properties have found use as viscoelastic adjuncts in ophthalmic surgery, viscosupplementation in the synovium of arthritic joints, and as covalently bound lubricious hydrophilic coatings for medical devices, e.g., stents, catheters (*10,11*). For medical applications that require extended residence time in situ, HA can also be crosslinked by a variety of chemical methods. Cross-linking HA dramatically increases the elastic component of the overall modulus of HA causing the polymer gel to behave more like an elastic solid and less like a viscous fluid in response to deformation. Crosslinked HA products have found use as surgical adhesion barriers, synovial viscosupplements, and more recently as materials for augmentation of the dermis (*12-14*).

This review will focus on the biological and physical properties of HA that have been successfully exploited for its use as a biomaterial.

Biological Properties of Hyaluronic Acid

Hyaluronic acid is found both in the extracellular matrix between cells in tissues and associated with cell surfaces, in the pericellular matrix. Furthermore, HA is not an inert material. It is recognized specifically by several proteins in the extracellular matrix that give rise to important biological functions (*15*).

The first two proteins discovered to interact with HA are the link protein and the protein aggrecan found in the extracellular matrix of cartilage (*16*). The association of these two proteins with HA contributes to the formation of proteoglycan aggregates in cartilage, which are responsible for the bulk elastic and hydrodynamic properties of cartilage. A second type of interaction is with receptor proteins found on cell surfaces (for review see (*17*)). Two of these proteins are CD 44, a receptor found on many cell types (*18*) and RHAMM, a receptor for hyaluronan-mediated motility (*19*).

CD 44 is a transmembrane receptor that connects the pericellular matrix on the outside of cells with cytoskeletal proteins (*17*). The close association of HA with the cell pericellular matrix suggests an explanation for the role of CD44 in leukocyte migration, neoplasia, and in wound healing (*17*). In order for cells to migrate in tissues they must be capable of disrupting connections to the extracellular matrix. As cells migrate they are exposed to HA that is present in the extracellular matrix. A protein that is also implicated in cell mobility is RHAMM which is present in several intracellular compartments and is also exported to the cell surface where it interacts with HA (*17*). RHAMM was shown to be involved in fibroblast locomotion (*20*) and cell mobility (for review, see reference (*17*)).

Since HA is found in pericellular matrix it is not surprising that cell migration, mobility and wound healing may be influenced by HA. Of interest is the finding that fragments of HA, presumably resulting from its degradation, are more active in interaction with these cell surface receptors than native intact HA(21) leading to a role for fragments of HA in acute (*22*) and chronic inflammation (*7*). HA fragments can induce genes coding for inflammatory mediators (*22, 23*). These observations suggests that HA fragments, resulting from degradation of high molecular weight HA, are capable of cell signaling through interaction of receptor-mediated control pathways, followed by alterations in cell mobility and gene expression. Support for this pathway is that fragments of HA are capable of inducing cytokine expression in macrophages (*24*).

The finding that HA interacts specifically with proteins in the body serving both structural and cell signaling functions points to a potentially complex response by tissue when HA is injected into the body in the form of a medical device. For example, as HA is degraded it may have effects on leukocyte mobility and therefore inflammation and wound healing.

Physical Properties of Hyaluronic Acid

HA Structure in Solution and Rheology

The structure of uncrosslinked HA in dilute and concentrated solution has been extensively studied (for a review see reference (*25*)). The original work envisioned that uncrosslinked HA is a high molecular weight unbranched polysaccharide that behaves as a stiffened random coil in solution. The molecule occupies a large hydrated volume and shows solute-solute interactions at unusually low concentration. Since HA is a polyelectrolyte, the solution properties are greatly affected by ionic strength (*25*). The conformation of HA in solution has been studied at neutral pH and at physiological concentration, using nuclear magnetic resonance and circular dichroism. These studies support a model incorporating dynamically formed and broken hydrogen bonds that contribute to the semi-flexibility of the polymer chain (*26*).

Hyaluronic acid that is not crosslinked is water soluble, rapidly resorbed, and has a short residence time in situ that limits its utility for use in biomedical applications. HA in solution is subject to degradation via ultrasound, UV irradiation, thermal, and free radicals (*25*). Uncrosslinked HA in solution behaves as a pseudoplastic shear thinning fluid and the zero shear viscosity (η_o) correlates to the solution concentration multiplied by the molecular weight (*27*). Figure 1 describes the relationship of the log η_o to the log of the (concentration *molecular weight) for a series of HA solutions prepared from HA of three molecular weights, 1800 kg mol^{-1}, 680 kg mol^{-1}, and 350 kg mol^{-1}, at several concentrations, between 10-90mg/ml, in phosphate buffered saline. Also included in Figure 1 are seven commercial preparations of uncrosslinked HA used as medical devices. The data for these HA medical products are included in Table 1. The plot of the log of the solution η_o vs. the log of the solution (concentration*MW) is linear. This means that the viscosity of an HA solution can be controlled by adjusting polymer molecular weight and/or the solution concentration.

The data in Figure 1 also imply that the properties of two uncrosslinked HA solutions of a given viscosity can be quite different. Consider two HA solutions both with a η_o of ~170 Pas. Solution A is a 16mg/ml solution of HA 1800 kg $mol^{-1}$and solution B is a 70mg/ml solution of HA 350 kg mol^{-1}. The η^* at low frequency, 0.0628 rad/s, low deformation rates, or low shear is 172 Pas for both materials. The viscoelastic properties of these two solutions are quite different and this is shown in Figure 2. This Figure describes the complex viscosity (η^*) and the storage viscosity (η"), the elastic component of the complex viscosity vs. frequency for HA solutions A and B. At low frequency both solutions have

the same η*; as the frequency is increased, the η* of the high molecular weight HA solution decreases more rapidly than the η* of lower molecular weight HA. At high frequency, the η* of the concentrated low molecular weight HA is greater than 10 times higher than the dilute solution of high molecular weight HA. The data in Figure 2 also show that the elastic component of the η*, η", at low frequency for the solution of high molecular weight HA is much higher than that of low molecular weight HA. At low deformation rates, the high molecular weight HA solution with an entangled or aggregated chain structure responds elastically to deformation. Under these conditions the concentrated low molecular weight solution responds in a primarily viscous manner. The high molecular weight HA solution, up to a frequency of ~ 1 rad/s, is more elastic than the solution of low molecular weight HA. Both materials have the same viscosity at low deformation rates, but solution A is a dilute entangled cohesive solution ideally suited for bulk removal during eye surgery, while solution B is a concentrated non-cohesive tissue adhesive material possibly better suited for tissue coating and lubrication medical applications. The higher viscosity and elasticity of low molecular weight HA at high frequency also indicate that it may be a better tissue coating and lubricating material under conditions of high deformation rates.

Cohesive Properties of Hyaluronic Acid: Cohesion-Dispersion Index (CDI)

The cohesive nature of HA is of prime importance to its use as a viscoelastic adjunct in eye surgery (*28*). The more cohesive the material is the more likely it is to be readily removed from the anterior chamber of the eye during cataract surgery. Cohesion is a result of intermolecular entanglement of the HA polymer chains that imparts a bolus-like behavior to the viscoelastic solution. A quantitative dynamic aspiration method that measures the cohesion of viscoelastic agents has been developed (*29*). The principle of the technique is to measure the amount of sample aspirated with increasing vacuum applied to the sample; vacuum levels of 127, 254, 381, 533, and 711 mm of Hg were used. At each vacuum level, the weight of material aspirated through a 0.5mm pipette tip in two seconds is measured. The data are plotted as the percent aspirated vs. vacuum level and the slope of the steepest portion of the curve is determined. The cohesive-dispersive index (CDI) is equal to the slope at steepest portion of the curve. Materials that are more cohesive are removed in bulk at a lower vacuum than materials of lesser cohesiveness. Table I list the CDI results for a series of HA viscoelastic agents that have been used in eye surgery. From these data it was concluded that the cohesive nature of HA increases with increasing molecular weight (*29*). The study was extended to include the effect of concentration and molecular weight on the cohesive properties of uncrosslinked HA (*27*). It was found that the CDI correlates very well to solution concentration

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