

Hyaluronan as a Versatile Biomaterial for Surface Treatment of Medical Devices


Djoerd Hoekstra
President and CEO, Biocoat, Incorporated



Introduction

Interest in hyaluronan has increased dramatically since the early 1980's with major clinical applications in ophthalmology, in the treatment of degenerative joint disease, and in adhesion prevention, combined with production of the polymer on an industrial scale.

In recent years, Biocoat, Inc. has pioneered the use of hyaluronan in hydrophilic coatings for a variety of medical device applications, taking advantage of the unique biocompatibility and versatility of the material. This presentation reviews hyaluronan's basic properties, its biology and chemistry, its medical applications and the techniques for improving the biocompatibility of medical devices by modifying synthetic materials surfaces with hyaluronan.



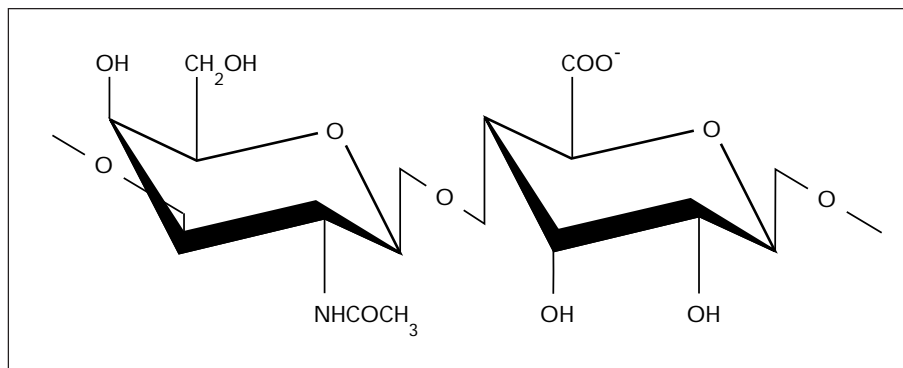


Figure 1. Hyaluronan molecule.
(Illustration courtesy of TSG Communications, Elkins Park, PA)

What is hyaluronan?

Hyaluronan, a polysaccharide, is a unique biopolymer.

It was first isolated from the vitreous body of the eye by Karl Meyer who called it hyaluronic acid, in 1934.^{1,2} The term *hyaluronan* was proposed by Dr. Endre A. Balazs in the 1980's to encompass the different forms the molecule can take, e.g., the acid form, hyaluronic acid, and the salts, such as sodium hyaluronate, which form at physiological pH.³

Now, 65 years after Meyer's initial publication, a lot more is known about the structure of the hyaluronan molecule, how it behaves, its occurrence in different tissues and body fluids, how it is synthesized by the cells, metabolized and cleared from the body and some of the functions it performs.

Hyaluronan and related polysaccharides are called *glycosaminoglycans*. Glycosaminoglycans are made up largely of repeating disaccharide units, containing a derivative of an aminosugar. The most abundant glycosaminoglycans in the body are chondroitin sulfates; others are keratan sulfate, heparin and heparan sulfate, and dermatan sulfate.

Figure 1 shows the disaccharide unit of hyaluronan, consisting of alternating glucuronic acid and N-acetylglucosamine units, which is repeated over and over to form long chains. Each repeating disaccharide unit has one carboxylate group, four hydroxyl groups and an acetamido group. Hyaluronan differs from the other major glycosaminoglycans in that it does not have sulfate groups, as Meyer discovered.

Hyaluronan is synthesized by many types of cells and extruded into the extracellular space where it interacts with the other constituents of

the extracellular matrix to create the supportive and protective structure around the cells. It is found as a constituent of all body fluids and tissues, in higher concentrations in the vitreous humor of the eye and in synovial fluid in the joints; the highest reported concentration in mammals is found in the umbilical cord.

What makes hyaluronan unique?

Hyaluronan is used in medical applications for its unique physical/chemical properties. It is very hydrophilic; its viscous solutions have most unusual rheological properties and are exceedingly lubricious.

• Rheological properties

In solution, the very large linear polymer chains takes on the form of expanded random coils and these chains entangle with each other at very low concentrations, which may contribute to the unusual rheological properties. At higher concentrations, solutions have an extremely high but shear-dependent viscosity. A 1% solution is like jelly, but when it is put under pressure it moves easily and can be administered through a small bore needle. It has therefore been called a "pseudo plastic" material.

The term *viscoelastic* is used to denote the elasticity of a gel combined with the viscosity of a solution.

• Lubricity

The extraordinary rheological properties of hyaluronan solutions make them ideal as lubricants. There is evidence that hyaluronan separates most tissue surfaces that slide along each other. Solutions of hyaluronan are

extremely lubricious and have been shown to reduce postoperative adhesion formation following abdominal and orthopedic surgery.

- **Hydrophilicity**

In solution, the polymer takes up a stiffened helical configuration, attributed to hydrogen bonding between the hydroxyl groups along the chain, forming a coil structure that traps approximately 1000 times its weight in water.^{4,5}

Overview of Chemistry and Biology

There are a couple of excellent compilations of these and other subjects, both of them edited by Prof. Laurent. The first one contains the papers presented at the 1988 Ciba Foundation Symposium in England, *The Biology of Hyaluronan*. The more recent book, *The Chemistry, Biology and Medical Applications of Hyaluronan and its Derivatives* was published in connection with the Wenner-Gren Symposium in Stockholm in 1996.

Commercial availability

Hyaluronan is isolated either from mammalian tissues or from cultured streptococci.

I have heard that, at one time, the material was isolated from human umbilical cords collected in hospitals. Pharmacia AB (Uppsala, Sweden), then developed a special strain of roosters with very luxurious combs; they isolated it from these combs, and still do, to my knowledge.

More recently, submerged cell culture techniques using certain strains of streptococci were developed to grow hyaluronan.

Commercially available material ranges from under one million to 6 million in average molecular weight.

There are a large number of producers around the world. Biomatrix Inc. (Ridgefield, NJ), a U.S. company, operates a plant that produces hyaluronan from mammalian sources in Canada. Anika (Woburn, MA), Genzyme Corp. (Framingham, MA), and Lifecore Biomedical (Chaska, MN) are other domestic suppliers. Pharmacia produces hyaluronan in Sweden, Fidia Advanced Biopolymers (Brindisi) in Italy, Bio-Technology General Corp. (Iselin, NJ) in Israel, and a number of companies, including Kibun Food Chemifa Co. and Seikagaku Corp. (both Tokyo), in Japan.

Medical Applications

Dr. Balazs and his co-workers have written extensively on medical applications of hyaluronan and its derivatives.⁶

The major application in this country has been the use as a viscoelastic in ophthalmic surgery, mainly the implantation of intraocular lenses in people with cataracts.⁷

Japan supports a large market for hyaluronan because it is used there as an injectable for arthritis. Recently, Anika, Biomatrix, and Fidia obtained FDA approval for that use in the U.S.⁸

Drug release is another interesting application and formulations of hyaluronan and derivatives have been developed as topical, injectable and implantable vehicles for the controlled and localized delivery of biologically active molecules.⁹

With the advent of crosslinked gels and films of hyaluronan, there are now a number of products on the market for the prevention of adhesions after abdominal surgery. Genzyme developed the Seprafilm[®] and Sepracoat[®] line of products, based on crosslinked hyaluronan, to prevent adhesions after abdominal surgery. Lifecore has developed Lubriccoat, a gel form for similar indications.¹⁰

And then there is the application of hyaluronan that is closest to our heart, its use in hydrophilic coatings for a variety of medical devices, including catheters, guidewires, sensors, to improve biocompatibility, lubricity, and to reduce fouling and tissue abrasion.

An interesting application not yet been commercialized - although some human clinical trials have been completed - is the injection of a hyaluronan gel, compounded with thrombin and other materials, for percutaneous embolization.¹¹



Hyaluronan-based coatings are used on a variety of medical devices such as guidewires. (Photos courtesy of Lake Region Mfg. Inc.; Chaska, MN.)

Principal Inventors	Assignee	Subject	Issued
Balazs	Biomatrix	Ultrapure HA	1979
Balazs	Biomatrix	Polymeric Articles	1984-'85
Balazs	Biomatrix	PEO Compositions	1986
Balazs	Biomatrix	Cross-Linked Gels	1986-'87
Leshchiner	Biomatrix	Embolization	1989
Balazs	Biomatrix	Drug Delivery	1992
Beavers	Biocoat	Coated Lens	1987
Halpern	Biocoat	Hydrophilic Coating	1989-'91
Beavers	Biocoat	Free Acid	1998
della Valle	Fidia	Esters	1989-'90
Hamilton	Genzyme	Insoluble Derivatives	1990
Burns	Genzyme	Insoluble Derivatives	1991-'96
Burns	Genzyme	Platelet Function	1996
Giusti	Italian Govt.	IPN's	1997
Guire	SurModics	Photoimmobilization	1988-'93
Larm	Carmeda	Conjugates	1986-'89
Narayanan	Cordis	Stent Coating	1994
Rowland	Cordis	Stent Coating	1994
Yannas	M.I.T.	Collagen Composites	1981

Table I. Hyaluronan-Related U.S. Patents

Patents

The patent literature is extensive and evidences of a great deal of development work in this field in recent years. Of course, it is not always possible to know whether a particular patented invention has practical uses and in some patents hyaluronan is mentioned as only one of a class of compounds (e.g. glycosaminoglycans) that can be used. The major inventors in this field and their corporate affiliations are summarized in Table I.

Balazs, Leshchiner, et al.

Balazs, Leshchiner and their co-workers invented and patented many different processes and uses for hyaluronan.

There is the important Balazs patent, issued in 1979 and now expired, on hyaluronan isolated from animal tissue that does not cause an inflammatory response when tested in the eye of the owl monkey.¹² The process involves extracting

hyaluronan from the blood, deproteinizing the extract, and then treating it with chloroform. The result (marketed by Pharmacia as Healon®) is described by Balazs as a sterile, pyrogen-free, non-antigenic, and non-inflammatory, high molecular weight fraction of hyaluronan, essentially free of proteins, peptides, and nucleic acid impurities. He also describes various therapeutic uses for this material, including improvement of pathological joint function, prevention of post-operative adhesion of tissues, tendons and their sheaths, and various uses in the eye.

Cross-linking

The goal of cross-linking the hyaluronan molecule is usually to enhance rheological properties or to produce forms of hyaluronan less soluble in water, such as solids or gels.

There are many ways in which this can be done.⁶ The Balazs patent on chemically-modified hyaluronan describes cross-linking

with small amounts of an aldehyde, such as formaldehyde to produce a unique soluble polymer fluid with very high viscoelastic properties.¹³ Also described is cross-linking of hyaluronan with divinyl sulfone to obtain a jellylike material. Both of these cross-linkers preferentially react with the hydroxyl groups of the hyaluronan molecule. Balazs uses the term *hylan polymers* for hyaluronan cross-linked through the hydroxyl functionality. The biocompatibility of hylan polymers is reported to be virtually identical to that of hyaluronan.¹⁴

Other methods of cross-linking utilize the carboxylate functionality of the hyaluronan molecule.

In a 1990 U.S. patent assigned to Genzyme, Hamilton discloses water-insoluble derivatives of polysaccharides, activated with carbodiimides and reacted with an amino acid.¹⁵

Tomihata and Ikada also reported on cross-linking of hyaluronic acid with a water-soluble carbodiimide to produce water-insoluble films.¹⁶

Cross-linking can also be achieved with epoxides¹⁷, hydrazides¹⁸, polyvalent cations (ferric, aluminum, etc.) and aziridines (e.g. crosslinker CX-100).

Derivatives

In addition to cross-linking, various chemical modifications of the hyaluronan polymer have been reported and patented over the years.

According to Balazs, the earliest synthesized derivative of hyaluronan was its sulfate ester, which showed resistance to hyaluronidase and anticoagulant activity.⁶

More recently, a group of researchers at the University of Siena, including Abatangelo, Barbucci, and Magnani have published extensively on sulfated hyaluronic acid. They report that introducing sulfate groups in the hyaluronan molecule converts it to a heparin-like material with anti-thrombogenic properties and makes it resistant to enzymatic digestion.^{19,20}

There are several patents assigned to Fidia S.p.A. in which Della Valle describes esters of hyaluronic acid, in which all or only a portion of the carboxylic groups of the acid are esterified by treatment of the free hyaluronic acid with alcohols in the presence of a catalyst.^{21,22,23}

These patents disclose many different types of alcohols that can be used, the use of salts of the partial esters with metals and with pharmaco-

logically active organic bases, and differing degrees of esterification.

Della Valle describes applications of these compounds in pharmaceutical preparations, cosmetics, medical and surgical devices. It is claimed that these compounds qualitatively possess the same or similar physical-chemical, pharmacological and therapeutic properties, but that they are considerably more stable, especially with regard to enzymatic degradation by hyaluronidase. Also, it is claimed that most of the esters, unlike hyaluronic acid itself, have a certain degree of solubility in organic solvents, such as DMSO (dimethylsulfoxide) and poor solubility in water. This makes it possible to form articles, such as film, sheets, and threads, or sponges, and ophthalmic lenses.

Hydrazide-modified hyaluronan is described by Prestwich.¹⁸ He starts with the observation that attempts to react the carboxylate groups of hyaluronan with primary amines in the presence of the commonly-used water soluble carbodiimide were not successful. Others have reported the same problem and several ways of modifying the reaction have been patented (e.g. Genzyme's use of an amino acid; Fidia's use of a succinimide).

The technique of modifying the hyaluronan with dihydrazides was discovered by Dr. Pouyani. He found that adipic dihydrazide reacts efficiently with hyaluronan in the presence of carbodiimide. A number of advantages are claimed for the hydrazide-modified hyaluronan which can be used for the attachment of drug molecules and/or cross-linking.

Another example of a hyaluronan derivative, a conjugate with the naturally-occurring free radical scavenger superoxide dismutase, was reported to have greater anti-inflammatory activity *in vivo* than hyaluronan or superoxide dismutase. Amino groups of the superoxide dismutase were coupled with carboxyl groups in the hyaluronan molecule using carbodiimide.²⁴

Composites

Hyaluronan lends itself to compounding or complexing with other materials to produce biomedically useful composites, as is illustrated with the following examples.

Several Balazs patents on hyaluronan-modified polymeric articles describe how materials such as polyHEMA, polyurethanes,

polyesters, polyolefins etc. are rendered biocompatible by inclusion of or coating with hyaluronan.^{25, 26}

In the Balazs patent on cross-linked gels, there are descriptions of mixtures of hyaluronan with other hydrophilic polymers, polysaccharides, proteins of various types, synthetic water-soluble polymers, etc.²⁷

A recent patent, issued in 1997, by Giusti et al, describes a biomaterial comprising an interpenetrating polymer network in which one of the components is an acidic polysaccharide or a semi-synthetic derivative thereof and the second component a synthetic polymer. The polysaccharide can be hyaluronic acid, a total or partial ester or a salt of hyaluronic acid with an organic base.²⁸

One of the earlier patents on my list, by Professor Yannas at MIT, issued in 1981 describes composites of collagen and mucopolysaccharides, incl. hyaluronic acid and crosslinking with aldehydes, carbodiimides, azides, and diisocyanates. Also dehydrothermal crosslinking, in which the material is first dehydrated and then heated. Many of the crosslinked composites were found to be have outstanding mechanical properties and biocompatibility, which collagen alone lacks. The collagen and mucopolysaccharide can be either mixed together, or an article can first be coated with collagen and then the mucopolysaccharide applied to it.²⁹

Immobilizing

There are basically two different schemes for immobilizing hyaluronan to produce biomedically useful coatings:

(1) reacting it with or coupling it to functional groups present or introduced on the surface, or

(2) by attaching a photoreactive group to the hyaluronan molecule which then reacts with the surface upon being illuminated, or photoimmobilization.

There are many variations on these two schemes, depending on the nature of the substrate and the functional requirements of the coating.

Under the heading of using functional groups:

(a) Halpern and Beavers describe the patented "bi-laminar graft" configuration which

can be used to immobilize hyaluronan (and other mucopolysaccharides) when suitable functional groups are not present on the substrate. An adhesive polymer coating is first applied to the surface; this first coat provides functional groups (for example, diisocyanates) on its surface which can then be used to covalently bind the second coat of hyaluronan.^{30, 31, 32} In this process, the polysaccharide molecules, depending on their length and shape, may be tied down at multiple points along the chain and probably also through entanglement and interaction between polymer chains.

A recently issued U.S. patent by Beavers et al. describes a process for producing the acid form of hyaluronan which readily undergoes chemical reactions with substances such as epoxides, aziridines, and alcohols.³³

(b) The two Larm patents, issued in 1986 and '89 describe the Carmeda process for the covalent coupling of conjugates of substances, such as polysaccharides, with specific reference to heparin, (partially deacetylated) hyaluronic acid, dermatan sulfate and chitosan. In this process, fragments of the substance are created having reactive terminal aldehyde groups. These aldehyde groups are then reacted with amino groups on the substrate to form unstable Schiff's bases which are converted to stable secondary amines with a suitable reducing agent, such as cyanoborohydride.

In connection with hyaluronic acid, Larm states: "By covalent coupling of hyaluronic acid to plastic implants for for example eye surgery the implants can acquire better tissue affinity. In this manner one avoids complementary activation and activation of the mononuclear cell system..."^{34, 35}

(c) Larsson reported on the biocompatibility of surfaces prepared by immobilized heparin and hyaluronate. In creating the immobilized hyaluronate surfaces he used carbodiimide chemistry to react carboxyl groups on the hyaluronate molecule with primary amine groups on the substrate surface.³⁶

(d) In an international patent application assigned to Fidia Advanced Biopolymers, Morra and several co-inventors describe two different methods for producing hyaluronan-based coatings. In the first method, hyaluronan is reacted in solution with an alkoxy silane coupling

agent; this reaction takes place in the presence of a water-soluble carbodiimide and a succinimide; in a second step, the resultant reaction product is applied in the form of a solution to the substrate surface, which may be plasma-treated.

In the second method, the surface is first treated with plasma and then exposed to a solution of polyethyleneimine to create amino groups on the surface. The amino groups are then reacted with hyaluronan in the presence of the water-soluble carbodiimide and a succinimide. It is claimed that the use of the succinimide prevents the formation of intermediate reaction products which prevent the reaction from being completed.³⁷

(e) Two recently issued patents, assigned to Cordis, describe immobilization of polysaccharides to metallic surfaces, as would be used for stents. In the first of these patents, by Rowland, an organic polysilane coating with amine functionality is applied, followed by the application of the biomaterial, using carbodiimide as the coupling agent.³⁸ In the second patent, by Narayanan, first a coat of hexafluorobutylmethacrylate is applied by RF plasma deposition, followed by RF plasma treatment with water vapor to create functional groups on the surface; carbodiimide chemistry can then be used to tie down the biomaterial. Although in the examples heparin is used, these processes may also be applicable to hyaluronan.³⁹

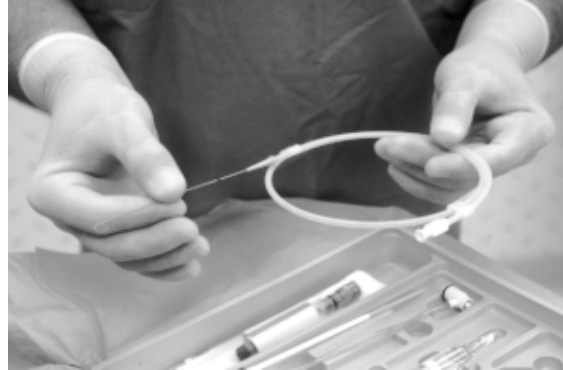
Under the heading of photoimmobilization:

(a) There are a number of patents, assigned to SurModics, which describe methods of attaching all kinds of molecules, including heparin and hyaluronic acid, to substrates using spacer molecules with photochemically and thermochemically reactive groups.^{40,41} One of the patents, issued in 1990, describes biocompatible coatings for solid surfaces, where the biocompatible agents, incl. hydrophilic polymers such as hyaluronic acid, are covalently bonded to the solid surface.⁴²

(b) Chen et al., used a photoimmobilization method for sulfated hyaluronic acid in which a water-soluble carbodiimide is first used to attach photoreactive groups to carboxyl groups of the hyaluronic acid. The photoreactive groups then bond directly to a polymeric surface with UV activation.⁴³

Biocompatibility

As one might expect for a material that is ubiquitous in the body, the biocompatibility of hyaluronan-modified surfaces has been well-established. It is found in every mammalian species and in microorganisms and in every tissue in humans. It is interesting that the hyaluronan molecule is identical in all species and all tissues and therefore never recognized as



Benefits of hyaluronan-based coatings include reduced device fouling and tissue abrasion

foreign by the immune system. The only requirement for medically usable preparations is to remove inflammatory fractions, for which processes and techniques have been developed over the years.

An important factor is the fact that large quantities of hyaluronan are metabolized in the human body, on the order of grams per day in a healthy adult. The high capacity for hyaluronan turnover implies that small quantities applied do not add significantly to the metabolic burden.¹⁴

To quote Balazs and Laurent: "Non-inflammatory hyaluronan is ... inert compared to other biomaterials used in medicine in that it does not elicit inflammatory, foreign body or immune reactions when implanted."

Although there is an extensive literature reporting clinical data for solutions and gels of hyaluronan and hyaluronan derivatives in the established therapeutic uses, not much has been published on the biocompatibility of surfaces that have been modified by attaching hyaluronan.

Larsson used various cellular systems and blood *in vitro* to compare the immobilized heparin surfaces with immobilized hyaluronate.

He concluded that the two surfaces were

indistinguishable when evaluated for short-term cellular compatibility, i.e. activation of platelets and cell adhesion in contact with blood. However, the heparin surface could be clearly distinguished from the hyaluronate surface on the basis of its capacity to adsorb and inactivate thrombin.³⁶

It is interesting to compare this finding with Burns, who postulates that hyaluronan is capable of interfering with the interaction of von Willebrand factor with platelets and components of the subendothelial matrix to inhibit platelet aggregation and adhesion. Burns also mentions the coating of devices with hyaluronan to inhibit the interaction of platelets with the surface with a substantially reduced risk of affecting overall hemostasis, unlike heparin and warfarin.⁴⁴

Lowry and Beavers studied the resistance of hyaluronate coatings to hyaluronidase.⁴⁵ They report that coatings prepared by covalent binding with diisocyanates, following the teachings of the Beavers and Halpern patents, are not degraded by the enzyme hyaluronidase, in contrast with hyaluronan in solution, which is rapidly degraded by hyaluronidase.

A recently published study of various photochemically-immobilized polymeric coatings on silicone rubber compared hyaluronic acid with

synthetic materials such as polyacrylamide, polyethylene glycol, and polyvinyl pyrrolidone.⁴⁶ Among the reported findings: the surfaces coated with hyaluronan showed a 92% reduction in *in vitro* adsorption of the protein fibrinogen (as good as or better than the other coatings) compared to uncoated silicone rubber; this is thought to be important because protein adsorption is a critical initial step in the bad things that can happen when blood is in contact with synthetic surfaces. The hyaluronan-coated surface showed nearly complete inhibition of *in vitro* fibroblast growth, as did most of the other coatings. None of the coated surfaces significantly inhibited *in vitro* leukocyte adhesion compared to uncoated silicone rubber. In the *in vivo* implantations in rat, none of the coatings triggered significant inflammation and fibrous capsules and the coated implants were not significantly different from uncoated silicone rubber.

Various unpublished *in vitro* and *in vivo* tests conducted over the years by Biocoat, Inc. and its licensees have demonstrated the biocompatibility of hyaluronan coatings. It has been shown that coated surfaces exhibit a marked reduction or absence of cellular attachment and fouling and of bacterial growth, compared with uncoated surfaces.

Conclusion

Hyaluronan is a unique biomaterial which lends itself to cross-linking and immobilizing in various ways to produce hydrophilic, lubricious and biocompatible surfaces. The ability to derivatize and complex hyaluronan with other substances makes it possible to create a range of bioactive surfaces. Device applications might include using such surfaces, for example, to impart antithrombogenic and antibacterial properties, or to interact preferentially with certain proteins and cells.

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