The Benefits of *Bacillus*-derived Hyaluronic Acid
in Adding New and Improved Attributes to Existing Drug Formulations
and Offering Opportunities for New Delivery System Development

Hans Ole Klingenberg is Director for the Global Marketing group in Novozymes Biopharma.

1. Can you explain the potential of Hyaluronic Acid for drug delivery?

Hyaluronic acid (HA or hyaluronan) is a natural, high molecular weight, linear polysaccharide composed of alternating N-acetyl glucosamine and glucuronic acid disaccharide units. HA is ubiquitous in human and animal tissues. It is found in the vitreous body, in the extracellular matrix of soft connective tissues, and in the synovial fluids. By supplementing the body’s level of glycosaminoglycan and providing water-binding, viscoelastic and biological properties, HA serves as a major ingredient in medical devices and pharmaceuticals.

HA exhibits significant structural, rheological, physiological, and biological functions. These, coupled with its lack of immunogenicity and toxicity, make HA a very attractive carrier for drug delivery applications:

1. Due to its amphiphilic nature, HA forms a large coiled meshwork even at low concentrations that can be used for the solvation, stabilisation and entrainment of smaller molecules.
2. Specific HA cell receptors, namely CD44 and RHAMM, are upregulated on malignant tissues, where activation of the CD44 receptors within the tumoral environment mediates HA internalisation.
3. The relatively simple chemical structure allows HA to be modified to create a wide range of possible biocompatible and bioresorbable materials for use as drug delivery carriers.
4. HA is non-toxic and non-immunogenic and considered as biologically inert material by the regulatory authorities.

The potential applications of HA in drug delivery can be segmented based on needs. These needs include targeted therapies, localisation of drugs, controlled release of drugs and finally also use of HA for stabilisation of labile drugs.

2. The FDA, EMA and other regulatory bodies expressed concern about commercially available HAs traditionally used in osteoarthritis applications. Could you explain how *Bacillus*-derived HA, or Hyasis, overcomes these issues?

The popularity of animal-free ingredients is continuously growing. For example, regulatory authorities are beginning to enforce tighter quality controls on products used in ophthalmic applications to improve safety and minimise risk to patients. Traditional commercial sources of HA are primarily derived from either rooster comb extraction or various attenuated strains of *Streptococcus* bacteria. However, these sources can potentially result in contamination risks from animal-derived proteins, viruses or endotoxins. In addition, these sources of HA are purified using organic solvents, which pose further health issues to patients and make them a concern for regulatory agencies. Until recently, the course of a specific HA product has not been considered to be clinically important. However, with its growing use in health technologies and interventions, and the spotlight on potential contamination risks, demand is increasing for safer alternatives.

*Bacillus*-derived fermentation process for the production of HA has been developed to overcome both the manufacturing and safety challenges associated with animal-derived sources. Through this we use minimal medium, no animal-derived raw materials and a water-based technique, which removes the use of organic solvents at any stage during the manufacturing process. The *Bacillus*-derived fermentation process allows HA to be released from the cell naturally so it is able to achieve high levels of purity, as well as controlling the molecular weight of the end product. When HA is torn from a cell to extract it, as with the *Streptococcus* fermentation process, it results in a heterogeneous mix of polymers without the ability to distinguish between high and low molecular weight. From the outset, it was essential that a technique was found to modify all elements of filtration including flow, process times and temperature, to enable a highly viscous polymer such as HA to be moved through a system, while at the same time removing impurities.

It is true that in current clinical trials, the data in support of efficacy for HA in treatment of osteoarthritis does not show a very substantial effect versus placebo. However, there are likely to be many reasons for this, including the fact that the placebo treatments used are in fact not placebo (placebo is often a saline injection treatment, which is believed to help flush the knee joint of synovial fluid and inflammatory cytokines, thereby reducing inflammation and pain at least temporarily).

It also follows from this that we today do not have any data to suggest that *Bacillus*-derived HA, or Hyasis, can improve or change the treatment paradigm here, when it comes to efficacy. We do however believe that the high purity of *Bacillus*-derived HA can support the opportunity to reduce the number of adverse events occurring in knee treatments, where there in some cases have been reports of increased swelling and sensitivity post HA injection (which may stem from the use of HA materials that are not pure enough).

3. What are the advantages of Hyasis in combination with active pharmaceutical ingredients (APIs)?

HA can be combined with most APIs, including both small molecules and peptides or proteins. However, the compatibility will depend on the hydrophilicity of the API, HA being most compatible with hydrophilic compounds. One specific advantage of formulating HA with suitable APIs is the resulting slower-release time in an HA concentration-dependent manner.
In the case of diclofenac, a non-steroidal anti-inflammatory drug (NSAID) used in conditions such as acute injury and arthritis, experiment results showed the formulation without HA exhibits a complete release after ten minutes, which reflects a reasonable water solubility and a quick distribution in the dissolution system. By adding Hyasis to the formulation, the release time was extended to three, six and nine hours in an HA concentration-dependent manner. The slower release time is attributed to the increased viscosity of the formulations containing HA, resulting in a slower diffusion of the API. As a result, the diclofenac containing 3% HA showed a steady release profile with a 50 times slower release compared to formulation without HA.

Another example of this can be seen in dexamethasone phosphate, which is a corticosteroid with an anti-inflammatory effect. Similar to diclofenac, the formulation without HA exhibits a fast distribution in the dissolution system, and a complete release was obtained after just ten minutes. The inclusion of HA in the formulation is again similar to diclofenac, where a complete release of dexamethasone phosphate is obtained after 9-10 hours when formulated with 3% HA.

Data indicates that the higher the hydrophobicity, the longer the release time using HA in its non-modified form. The exact release mechanism for poorly soluble drugs from an HA carrier is not known. However, factors including local retention of the drug at the site of injection, slow dissolution rate, polymer erosion and diffusion from the HA matrix may all play a role.

As both APIs are used in the treatment of osteoarthritis, could you evaluate the requirement for HA as a treatment option?

Yes, it could certainly be considered to combine HA and diclofenac as a combined treatment. Both are compatible with each other from a formulation perspective. The question is then whether this would lead to an improved therapeutic (the latter would need to be tested in clinical trials).

The exact mechanism by which HA-based therapies for OA are effective is not known. However, use of viscosupplementation with HA is believed to help improve the physiologic environment of the osteoarthritic joint and restore the shock absorption and lubrication properties of the synovial fluid. As a consequence, HA helps to "cushion" the joint and thereby reduce pain. Moreover, some studies have shown that the analgesic effect of viscous HA solutions is due to: (i) the ionic interaction of HA with positively charged Ca2+ channel activators; (ii) a detoxicating effect, i.e. the interaction of HA with the nociceptors or with pain-producing...
molecules, resulting in protection of the receptors by inactivation of these agents and in downregulation of the inflammatory response.

7. Are the 1%, 2% and 3% concentrations of HA (% w/v) convenient for practitioners in offering good injectability?

In general, the meta-analysis studies that have been done on HA osteoarthritis therapies have not been able to show any statistically significant difference between current marketed therapies, based on concentrations, number of injections or molecular weight. Hence it is not clear today that any one formulation is better than another.

8. What benefit does Bacillus-derived HA offer to eye care?

We have published a report highlighting the superiority of Hyasis for topical ophthalmic formulations, based on their physico-chemical and biological properties, tolerance and handling (including at industrial scale). Such solutions are expected to enhance tear film stability, to allow maximum comfort and to exhibit high residence time, while being biocompatible and easy to filter.

As a result of its viscosity-enhancing properties, HA can be used as an efficient carrier for ophthalmic therapeutics. In this respect, Bacillus HA presents good compatibility with a variety of drugs such as ciprofloxacin, diclofenac and dexamethasone. Increased lacrimation and tear turnover following the application of ocular formulations most often lead to short precorneal residence time and, as a result, poor drug bioavailability. According to gamma scintigraphy, the precorneal residence time of Bacillus-derived HA is similar to that of Streptococcus-derived HA of higher molecular weight at the same concentration. Furthermore, the incorporation of HA in drug-containing ophthalmic solutions can increase drug retention in the tear fluid, along with drug contact time with the ocular surface, consequently improving drug bioavailability. The underlying mechanisms for drug retention are believed to be largely related to viscosity, bioadhesion (mucoadhesion) and physical attachment.

9. Looking ahead, what research is currently being conducted in this area, and how do you see the technology moving forward?

There is certainly a lot of research effort to improve the performance of HA-based material for biomedical applications but also for drug delivery. With regard to OS, there is an attempt to increase the residence time of the injected HA in the joint to decrease the frequency of injections. Parameters such as the form of HA (hydrogel vs. particles), concentration, modification technology (e.g. degree of cross-linking), injected volume etc. are being tested to find the optimal combination.

Finally, the use of HA as a drug delivery vehicle has not been fully exploited yet and tremendous research efforts are being conducted in this area and in the coming years will no doubt see an increase in its use.

Hans Ole Klingenberg is Director for the Global Marketing group in Novozymes Biopharma.

Hans Ole has been with Novozymes for more than 10 years and has through his time at Novozymes held various positions in the area of corporate business development, with a focus on establishing new business entities for Novozymes in the biopharmaceutical industry. Hans Ole has since 2007 been working in Novozymes’ Biopharma business with a focus on marketing, and is commercially responsible for establishment of Novozymes’ new Hyaluronic Acid business franchise. Hans Ole has a background from Copenhagen University, with a bachelor in chemistry and master in economics. He is based in Denmark. Email: biopharma@novozymes.com