# Hyaluronic acid, the everywhere biopolymer

## New chemistry and expanding markets mean this workhorse ingredient is just getting started

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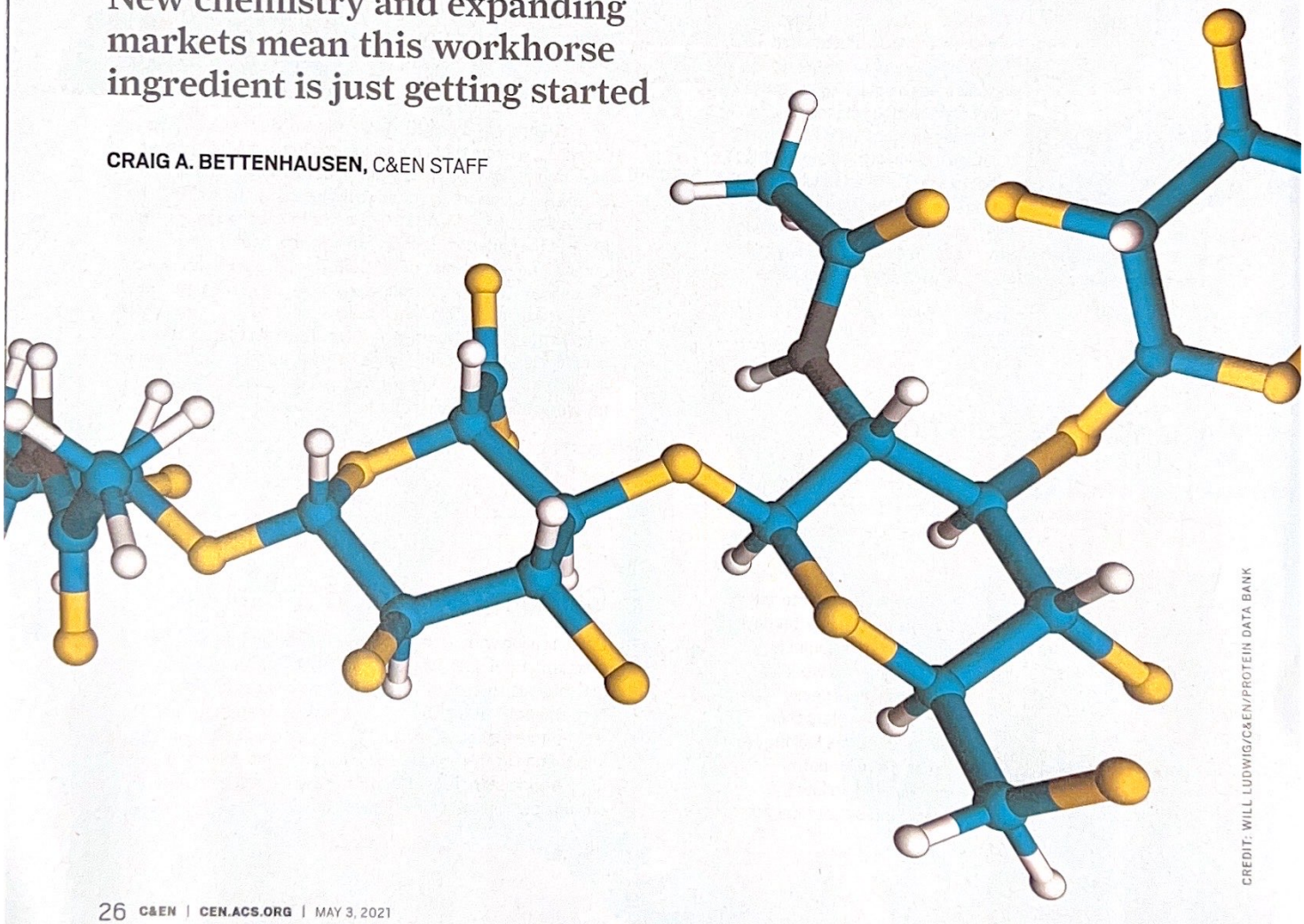

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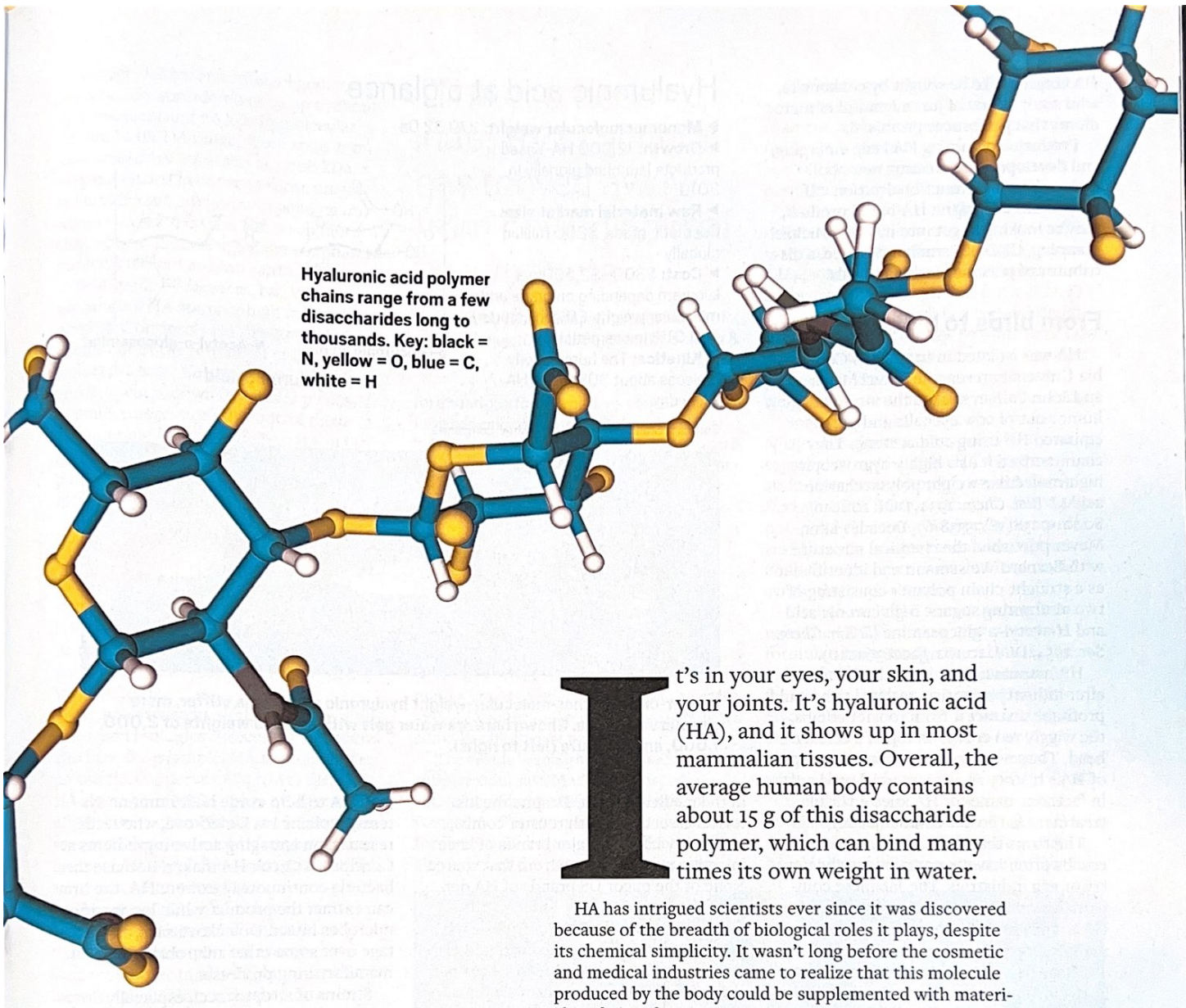


Hyaluronic acid polymer chains range from a few disaccharides long to thousands. Key: black = N, yellow = O, blue = C, white = H

## In brief

**Hyaluronic acid has come a long way** from its discovery in eyeballs. Scientists have since found the disaccharide biopolymer throughout human and animal bodies and around the cell walls of some bacteria. Meanwhile, cosmetic and medicinal chemists have found numerous ways to use manufactured versions of hyaluronic acid in skin care, joint health, wound healing, eye surgery, and scalpel-free cosmetic procedures. As academic researchers work out the material's complex biochemistry, industrial scientists are exploring the synthetic options afforded by the sugar's side groups. Both lines of work could yield even more uses for this ubiquitous substance.

It's in your eyes, your skin, and your joints. It's hyaluronic acid (HA), and it shows up in most mammalian tissues. Overall, the average human body contains about 15 g of this disaccharide polymer, which can bind many times its own weight in water.

HA has intrigued scientists ever since it was discovered because of the breadth of biological roles it plays, despite its chemical simplicity. It wasn't long before the cosmetic and medical industries came to realize that this molecule produced by the body could be supplemented with material made in a factory.

Today, topical HA is a workhorse of cosmetics that moisturize skin and reduce the prominence of wrinkles. In medicine, plastic surgeons inject HA as a dermal filler to lift sunken areas in the face. Ophthalmologists use it to protect and lubricate eyes during surgery. And HA injections into joints can provide months-long relief from osteoarthritis.

Although hyaluronic acid is central to many cosmetics and has been studied extensively, there's still a lot unknown about this ubiquitous polymer. Its biochemistry is particularly complex, as it interacts differently with various cell receptors depending on its molecular weight. Chemically, the monomer offers three different handles ripe for functionalization, and chemists are just scratching the surface on what properties those modifications could yield.

As a result, hyaluronic acid is a big business with the potential to get bigger. The global market for finished HA products was around $20 billion in 2019, according to the market research firm Grand View Research. And unlike cosmetic ingredients that have had their stars rise and fall,

HA continues to be sought by consumers, who see it as one of just a handful of ingredients that perform as promised.

Products containing HA keep emerging, and developers are creating new applications based on functionalization. "If they're not making an HA-based product, they're looking to get into it," says Michael Manning, CEO of Paradigm Science, a distributor of personal care chemicals.

## From birds to biotechnology

HA was isolated in 1934 when Columbia University researchers Karl Meyer and John Palmer sucked the vitreous humor out of cow eyeballs and precipitated HA using cold acetone. They characterized it as a highly hygroscopic, high-molecular-weight polysaccharide acid (*J. Biol. Chem.* 1934, DOI: 10.1016/S0021-9258(18)75338-6). Decades later, Meyer published the chemical structure with Bernard Weissmann and identified it as a straight-chain polymer consisting of two alternating sugars, D-glucuronic acid and *N*-acetyl-D-glucosamine (*J. Am. Chem. Soc.* 1954, DOI: 10.1021/ja01636a010).

HA manufacturing began in the 1970s after industry scientists realized they could profitably extract it from rooster combs, the wiggly red crown on top of a chicken's head. That avian source dominated most of HA's history as a commercial product—in fact, one name for HA knee arthritis treatment is rooster-comb injections.

The firms that make HA from rooster combs often have connections to the poultry or egg industries. The Japanese company Kewpie, for instance, expanded into HA in 1983 from its roots as a mayonnaise manufacturer.

These days, though, most investment in HA is in newer fermentation-based production processes. In March, for example, DSM, a pioneer of the rooster-comb product, launched three HA ingredients made via sugar fermentation for the cosmetic industry.

"With the growing demand for vegan beauty products, synthetic and fermented ingredients have become a very attractive product to use and make new marketing claims around," says Volker Rosenberger, marketing manager for skin care at DSM.

Julie Bianchini, a principal scientist for Johnson & Johnson's facial moisture and treatment group, says the move away from chicken-derived HA is part of a broader shift toward synthetic, botanical, and fermented ingredients. "The cosmetic industry in general has moved away from having animal by-products," she says.

Rooster-comb HA is also losing ground in the medical market. Despite the historical association with rooster combs, today only half the major brands of knee injection products come from that source. None of the major US brands of HA dermal filler are avian.

The French firm HTL describes itself as the largest pure-play medical-grade HA maker, producing several metric tons per year via fermentation exclusively for the medical market.

Deputy CEO Charles Ruban says that some osteoarthritis injections use rooster-comb HA simply because those products already have regulatory approval. "You know how difficult it is to make changes in pharmaceutical products," Ruban says. But animal-derived ingredients are falling out of favor in many product categories, including medicine, though that trend is less pronounced in parts of Asia. Avian ingredients are more accepted in Japan than in other parts of the world, for example, he says.

Despite rooster-comb HA's foothold in musculoskeletal treatments, Ruban expects that fermentation-derived HA will capture all the substantial growth in HA demand and capacity from here on out.

Fermented HA comes from bacterial cultures. The bacteria coat their cell walls with HA to help evade host immune systems, explains Iva Dolečková, who leads research on antiaging active ingredients at Contipro, a Czech HA maker. Because the bacteria continuously excrete HA, the firm can extract the product while leaving the microbes intact, Dolečková says, an advantage over some other microbial chemical manufacturing processes.

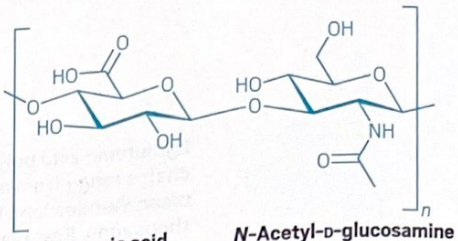
Strains of streptococci, especially *Streptococcus equi*, are the microbes of choice for most HA fermentation because they naturally produce HA in reasonably large amounts. Other species from the broader lactic acid bacteria group also make HA, and firms such as Givaudan are exploring genetically engineered bacteria and yeast in hopes of higher yields, easier isolation, and tailored polymer chain length.

## Molecular weight matters

The length of an HA polymer chain profoundly affects its physical, chemical, and biological properties. Most people who work with HA talk about it in terms of molecular weight and place it in one of three categories: oligomer, low molecular weight (LMW), and high molecular weight (HMW).

## Hyaluronic acid at a glance

- **Monomer molecular weight:** 379.32 Da
- **Growth:** 12,000 HA-based products launched globally in 2019.
- **Raw material market size:** Cosmetic grade, $200 million globally
- **Cost:** $300–$2,500 per kilogram depending on grade and molecular weight. Efficacy starts at 0.01% in cosmetics.
- **Kinetics:** The human body replaces about 30% of its HA every day.




D-Glucuronic acid *N*-Acetyl-D-glucosamine

**Hyaluronic acid**

**Sources:** Grand View Research, Mintel, companies.

**Longer-chain, higher-molecular-weight hyaluronic acid forms stiffer, more viscous water gels. Shown here are water gels with average weights of 2,000, 1,000, and 500 kDa (left to right).**

CREDIT: CHARLES CRIE/HTL

Though definitions differ, oligomers are generally considered to be up to about 20 monomers and 7.6 kDa in molecular weight. LMW HA ranges from 20 to 300 kDa, and HMW HA starts at 1,000 kDa. Polymer chains in between those ranges fall in different categories depending on whom you ask. The size can reach more than 25,000 kDa, though most commercial products top out around 4,000 kDa.

Be it made by roosters, bacteria, or humans, all HA starts at high molecular weights. Contipro's HA, for example, comes out of the fermenter at 2,300 kDa, Dolečková says. Enzymes, acid hydrolysis, and heat cut it down to smaller pieces. Manufacturers carefully control those HA-snipping factors to deliver HA in targeted molecular-weight ranges. Dried and powdered, it's stable for up to 2 years at most molecular weights.

HA's viscosity increases with molecular weight. HMW gels can be stiff enough to form self-supporting structures, whereas solutions of HA oligomers can have the viscosity of ice-cold vodka. Though all HA is hydrophilic, HMW samples can absorb up to 1,000 times their weight in water, much more than what lower molecular weights hold.

Molecular weight similarly makes a difference in biological processes. At around 400 kDa, for example, HA stimulates the production of nerve cell growth factors. HA closer to 120 kDa spurs proliferation of cytokines, chemokines, and other messenger molecules involved in growth and healing (*ACS Appl. Bio Mater.* 2020, DOI: 10.1021/acsabm.0c01364).

That dependence of behavior on molecular weight is more chemical detail than you might expect an average cosmetic customer to absorb, but people are increasingly willing to do the homework, DSM's Rosenberger says. "Consumers have become more educated on the role of the molecular size to deliver specific benefits," he says. And consumer education efforts such as the nonprofit Environmental Working Group's Skin Deep database or the newly launched Science of Beauty Collective are making vetted scientific information available.

## Scientific skin care

Most HA is used in either medicine or cosmetics. In 2019, global sales of finished medical products containing HA were $9 billion; the figure for cosmetic products was $8 billion.; human and animal nutrition had the remaining $3 billion, according to Grand View.

Cosmetic firms have used HA in their formulations for the past 15–20 years as a moisturizing ingredient. Today, the global market for cosmetic-grade bulk HA is worth around $200 million, according to a recent report from the consulting firm Kline. But a more sophisticated understanding of the roles that HA plays at different molecular weights is opening new applications.

> "The cosmetic industry in general has moved away from having animal by-products."
>
> —**Julie Bianchini,** principal scientist for facial moisture and treatment, Johnson & Johnson

Above roughly 300 kDa, HA stays on the skin without being absorbed (*Skin Res. Technol.* 2015, DOI: 10.1111/srt.12228). There it forms a barrier, locking in moisture and, in some conditions, attracting water from the air. In the LMW and oligomer range, HA penetrates the skin to carry and hold water in deeper layers and trigger some key biological pathways.

The result is shallower wrinkles and suppler skin, says Mathias Fleury, the active ingredient category manager for Givaudan. The Swiss firm is known mostly for flavor and fragrance ingredients, but it also supplies the personal care industry.

Givaudan got into the HA business in 2014 when it acquired Soliance, a French cosmetic active ingredient firm that has made HA by fermentation since the early 1990s. HA is a major part of Givaudan's cosmetic business, Fleury says.

When applied to the skin, HA stimulates cells to produce their own HA. One theory is that the cell membrane–bound receptor CD44 monitors the local concentration of lower-molecular-weight HA fragments, Fleury says. When that hits a certain threshold, CD44 signals the cell to synthesize new HA. Because HA breaks down into smaller pieces over time, HA of any molecular weight will eventually trigger that cascade.

Some studies have also shown that *N*-acetyl-D-glucosamine, one of the two sugars that make up the HA monomer, stimulates production of new HA (*Arch. Dermatol. Res.* 2009, DOI: 10.1007/s00403-009-0932-z). So any HA breakdown process that snips sugar molecules off the end of the polymer chain can lead to HA synthesis by nearby cells.

Though early HA skin-care products used the HMW version, many modern formulations include several sizes. The J&J brand Neutrogena uses molecular weights from 50 to 1,700 kDa in its Hydro Boost line of HA moisturizers. Formulating with multiple sizes of HA provides hydration both on and within the skin's surface, Bianchini says.

Some skin-care experts question the rampant proliferation of HA. Though HA is generally a safe substance, some users have reported irritation. On the surface or

CREDIT: GIVAUDAN

**A lab worker fills hyaluronic acid bottles at Givaudan's facility in Pomacle, France.**

in deeper layers of the skin, critics say, the hygroscopic polymer can pull water out of the skin. As it penetrates into deeper layers, LMW HA can drag along fragrances and other molecules meant for the surface.

And HA has demonstrated both prebiotic and antimicrobial activity in different experiments, according to entrepreneur and skin scientist Elsa Jungman. But exactly which microbes HA might be feeding or killing remains poorly characterized.

Another source of uncertainty goes back to molecular weight. HMW and oligomer HA seem to suppress inflammation and immune response. LMW HA, however, causes inflammation, at least in wounds. The balance of biological response, molecular breakdown, and the body's synthesis of HA differs by person and changes over time.

"As you age, HA synthesis slows down, and its degradation increases," J&J's Bianchini says. "When your skin is subjected to things like UV damage from the sun, that also increases the degradation of HA." Supplementing the body's HA with creams and other products seems to reduce wrinkles and plump skin for most people, but a better understanding of the cases where supplementation goes awry could lead to more sophisticated and effective antiaging products.

## A base in medicine

Among HA's attributes is its activity in wound healing. The body naturally delivers HA to injured skin, where it plays several roles as healing progresses. HMW HA helps form clots by binding with fibrinogen, a clotting factor that circulates in blood. And at the wound site, HA forms a gel that provides a medium in which immune cells can circulate.

Later in the wound-healing process, the HA breaks down to molecular weights around 120 kDa, which promote inflammation—useful at that stage of healing—and blood vessel rebuilding. In the final stages of healing, HA is cut down further to oligomers, which suppress inflammation and help recruit and proliferate new skin cells and collagen.

Seeing opportunity in wound care, Contipro and other companies are marketing gels, films, and other wound dressings to supplement our bodies' supply at injury sites.

The most established medical use of HA is to treat osteoarthritis of the knee. Cartilage and the synovial fluid that sits between bones are rich in HA, which serves as a lubricant and shock absorber, says Mohammed Emam, a professor and orthopedic physician at Johns Hopkins Medicine. As a person ages, the concentration of HA in joints often decreases, leading to pain, stiffness, and restricted ranges of motion.

In a typical course of treatment, a physician injects 2–3 mL of solution containing 20–30 mg of HA into one or more locations in a patient's knee once a week for 3–5 weeks. Although the injected HA is gone from the injection site within 2–3 days after each treatment, Emam says, patients can expect relief from their symptoms for 6 months to a year. That mismatch of timelines suggests that, as in the skin, externally added HA stimulates internal HA production in joints.

Adding HA by injection began as a treatment for horses. Doctors started using it on humans about 50 years ago. In the 1990s, the injection gained US Food and Drug Administration approval, and insurance companies began covering the procedure.

In 2013, however, the American Academy of Orthopaedic Surgeons (AAOS) stopped recommending HA injections as a treatment for osteoarthritis, saying that the available data didn't show it to be more effective than saline injections. "Although a few individual studies found statistically significant treatment effects, when combined together in a meta-analysis the evidence did not meet the minimum clinically important improvement thresholds," David S. Jevsevar, then chair of the AAOS's evidence-based practice committee, said in a press release at the time.

The clinical orthopedic community does not stand firmly behind the AAOS's recommendations.

Critics of the AAOS contend that the group's methodology was flawed. A 2020 paper in the *Journal of Bone and Joint Surgery* argues that saline is an effective treatment and therefore a poor placebo and that studies using HA larger than 1,600 kDa show clinically meaningful effects (DOI: 10.2106/JBJS.19.00272). In a commentary in response, Jevsevar notes that one of the authors worked for an HA injection maker; he also suggests that the studies that show HA injections to be effective are biased.

All that debate assumes that HA injections can be studied as a cohesive category. But a 2018 study of HA for orthopedic injections concludes that different major brands have such different molecular weights and viscosities that it makes more sense to evaluate them individually.

Technique also matters, Emam says. He uses ultrasound imaging to target recesses above or under the kneecap. Less-precise injections might not deliver the HA where it's needed.

Emam is among the physicians not dissuaded by the AAOS's concerns. "There might be a little bit of debate in the literature if it really works or if it does not work. But from what I know, it does work. It does help patients as long as you select the right patient"—namely, people with mild to moderate cases, he says. "Does it work for osteoarthritis? Yes."

Commercial hyaluronic acid is extracted from rooster combs or fermented.

CREDIT: SHUTTERSTOCK

## Hyaluronic acid's next act

Though it is already well established in its existing uses, HA still has growth ahead. On the medical side, dermal filler injections are the fastest-growing noninvasive cosmetic treatment in the US, according to the American Society of Plastic Surgeons, and they are gaining popularity in Asia. HA is used in 78% of those operations.

HTL's Ruban says that dermal fillers are "extremely dynamic," a trend he is happy with because one of every two dermal filler syringes contains the firm's HA, he says.

Johns Hopkins's Emam says he's seen increased patient interest in HA injections as a way

to stave off or avoid knee replacement surgery. Unlike corticosteroid injections, another treatment for osteoarthritis, patients can return repeatedly for HA if it is providing relief.

HA injections are also showing promise for treating nerve and muscle pain. Those uses are still experimental, but use in hip joints may get FDA approval and insurance coverage soon, Emam says.

Improvements in manufacturing are also reducing the cost of HA, says Nikola Matic, director of the chemical practice at Kline. This development could open up new markets, such as nutritional supplements. HA is already appearing in some edible products marketed as helping with joint pain, for example. Contipro has HA jellies for dogs and horses and just added a variety for humans.

Manufacturers say they can take HA even further with functionalized forms of the polymer. HA's side groups offer three sites—a carboxylic acid, a primary alcohol, and an amide—for chemical modification.

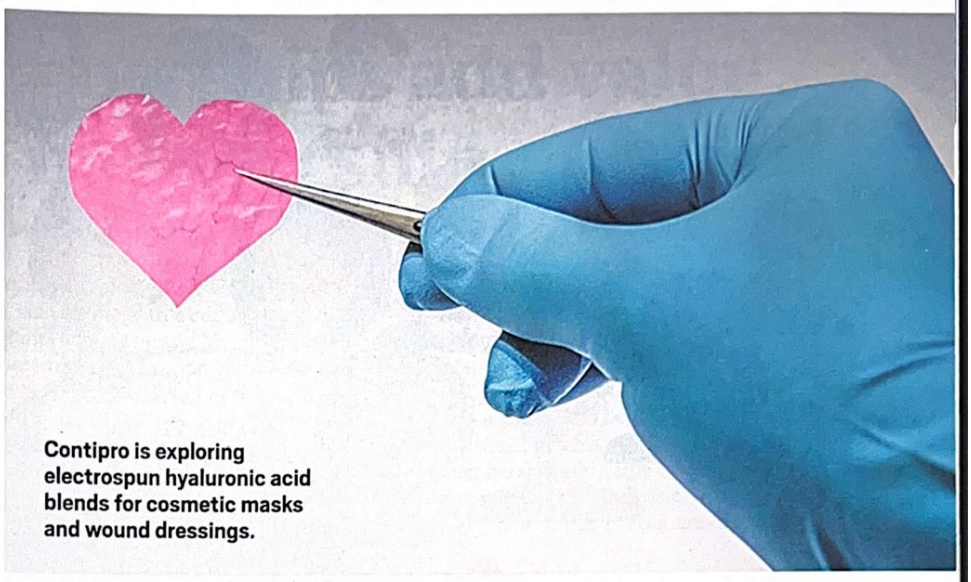
The carboxylic acid has received the most attention. HTL, for example, wants to alter HA's degradation kinetics via reactions at the carboxylic acid. Additions there seem to hide HA from the enzymes that evolved to break it down. HA modified that way already appears in some injected medical products, which promise longer effects than their nonfunctionalized competitors.

Using the carboxylic acid group to cross-link polymer strands changes HA's stiffness and water-holding properties. The direct-to-consumer cosmetic brand the Ordinary, for example, says its cross-linked medium-to-high-molecular-weight HA "tightly binds water and releases it in a timely fashion upon application on the skin."

Changes to the amide group seem to inhibit HA's interaction with its most common cellular receptor, CD44. But other than a few projects, such as Givaudan's alcohol-acetylated HA, which mixes better into oil-based formulations, companies have generally not exploited the amide and alcohol groups.

Micelles made from HA modified with fatty acids are also of interest to researchers, manufacturers, and HA product makers. The hydrophobic ends of the fatty acids cause HA to curl in on itself, creating micelles that penetrate the skin better than unmodified HA of the same chain length, Contipro's Dolečková says. Contipro is testing versions of the micelles made with oleic acid as a vehicle for delivering small molecules, including coenzyme $Q_{10}$, cannabidiol, and, eventually, pharmaceuticals.

In a cosmetic application, micelles made by combining HA with linoleic acid act as a skin-whitening agent by suppressing the action of melanin-producing cells called melanocytes.



**Contipro is exploring electrospun hyaluronic acid blends for cosmetic masks and wound dressings.**

CREDIT: CONTIPRO

Functionalized HA has even attracted research interest as a cancer therapeutic. CD44 is overexpressed in many tumor cells. Because HA is CD44's main ligand, it could carry cancer drugs that are either suspended in micelles or covalently attached to HA's alcohol or carboxylic acid groups (*Biomacromolecules* 2020, DOI: 10.1021/acs.biomac.9b00564).

Companies are also exploring new ways to formulate HA. Contipro has invested in electrospinning equipment that it uses to make nanoscale fibers, thin films, and fabrics from HA blends. These new forms of HA could be used in face masks, wound dressings, and innovative packaging, Dolečková says.

J&J, through Neutrogena and other brands, is launching products containing new forms of HA, such as pressed serums—a type of lotion based on non-Newtonian shear-thinning fluids—and HA combined with other biomolecules.

HTL is looking at producing other biopolymers—such as chondroitin and heparosan, both of which are now only available derived from animals—via fermentation and combining them with HA. In addition to conducting research for itself and customers, HTL runs an incubator program that invests up to $1 million in start-ups working on HA and other biopolymers.

Jungman says HA fits the cosmetic and personal care industry's trend toward shorter ingredient lists that customers can understand with some research. The products in her brand, Dr. Elsa Jungman, which launched last year, have eight ingredients or fewer. "It has, especially in the US, become a thing to be really looking at the actives in a product," she says. "Before, I think consumers would look more at claims."

Matic has seen the same trend at Kline. "The marketing claims would have been enough in the past," he says. Consumers now are less credulous and less content with basic formulations supported with glitzy marketing, he says. They want active ingredients with proven efficacy delivered at appropriate concentrations, and they want to see visible results. This higher standard reinforces demand for ingredients like HA, retinol, certain peptides, and others that have bodies of peer-reviewed research supporting them.

Manufacturers are bullish about HA's future. Later this year, HTL will start production at a second HA fermentation plant it's building at its site in Javené, France. The new facility will have 2.5 times the capacity of the existing plant nearby. The firm has also doubled its R&D staff over the past couple of years.

Similarly, the promising outlook for HA partly motivated Givaudan's purchase of the French biotechnology firm Alderys last year, Fleury says. Alderys has expertise with microbes beyond the lactic acid bacteria that Givaudan relies on for its HA, as well as genetic engineering and cell culture technology. Givaudan is hoping to use the new capabilities to optimize production processes and obtain HA with even more precise molecular-weight distributions, among other projects.

"There are still a lot of things to be done with HA from a chemical point of view," Fleury says, such as functionalizing the amide and alcohol groups and studying how the modified HA behaves on the skin and in other biochemical environments. "This is still a molecule for the future." ■