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Taking Aim at Poaching with Tissue Engineering

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Some researchers think that lab-grown rhinoceros horn and elephant ivory could reduce demand for the real thing.

A market for powdered rhino horn and carved horn objects has fueled a rise in rhinoceros poaching. Credit: Jonathan Pledger/Shutterstock.

Growing on a plastic dish, the stem cells don't look like much. Widely spaced, with jagged borders, they resemble distantly separated islands under the bright light of a microscope. But Garrett Vygantas believes cells like these could save the rhinoceros from extinction.

Induced pluripotent stem (iPS) cells have the ability to mature into any type of cell in the body. Vygantas's company, Ceratotech, is experimenting with growing rhino horns from iPS cells. He's among a handful of scientists who are biofabricating mimics of rhino horn and elephant ivory to drive down the black market price of these animal parts and discourage poaching. Many conservation groups oppose this approach, however, concerned that it may worsen the illegal hunting of rhinos and elephants. For instance, they worry that poachers will try to escape law enforcement by claiming that the horns they harvested from butchered rhinos are biofabricated mimics.

The number of rhinos poached in Africa in 2015 rose to a record 1,338, according to the International Union for Conservation of Nature. A continent-wide survey estimates that poachers kill about 27,000 African savanna elephants each year. Recent economic expansion in East Asia has fueled the growth, precipitating the emergence of a nouveau riche class with a taste for animal parts, prized as status symbols with purported medicinal benefits. Black market dealers tout powdered rhino horn as a cure for everything from hangovers to cancer, and artisans often carve rhino horn and elephant tusk into jewelry and other decorative objects.

A United Nations treaty called the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) prohibits global commercial trade of rhino horn and ivory. But participating countries can decide how to regulate trade within their borders. Conservationists argue that the only way to end poaching is to lower demand through education and awareness campaigns. But Vygantas and other scientists believe these efforts alone won't save endangered species in time.

GROWING RHINO HORN

A physician by training, San Francisco-based Vygantas has a passion for animals that led him at one time to consider veterinary school. After graduating college in 1998, he and his friends hopped in a Toyota Land Cruiser and embarked on a "DIY safari" in Zimbabwe, Botswana, Mozambique, and South Africa. The awe of seeing a rhino up close stayed with Vygantas. Meanwhile, rhino poaching rose dramatically. "It was horrifying that somebody could poach a majestic animal for just a [single] piece of their body", he says.

At the same time, advances in regenerative medicine were allowing scientists to grow skin, cartilage, and other tissues in the laboratory. Vygantas wondered whether he could apply a similar approach to growing rhino horn. "If poachers and consumers are just after the horn, and we've been able to create human tissue using genetic and stem-cell engineering, why shouldn't we give this a try using rhinoceros stem cells?"

Rhino horn is composed almost entirely of the protein keratin, which also makes up human hair and fingernails.

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Building on the Nobel-Prize-winning work that made the same thing possible with human cells, as well as CRISPR gene-editing technology, Vygantas has developed a method to reprogram rhino skin cells into iPS cells and then coax them to mature into keratinocytes, the cells that produce keratin. Now, his company, Ceratotech, is trying to grow keratinocytes onto a three-dimensional-printed, rhino-horn-shaped scaffold.

Garrett Vygantas prepares to do a skin biopsy on a rhinoceros at a private wildlife reserve. Stem cells generated from the biopsy will be used to grow keratinocytes needed to make biofabricated rhino horn. Credit: Courtesy of Garrett Vygantas.

Granted, rhino horn imitations—frequently made of water buffalo horn—already pervade the market. But by making Ceratotech's product identical to wild rhino horn at a genetic level, Vygantas hopes consumers will accept it as the real thing. To allow law enforcement to distinguish lab-grown from poached horn, Ceratotech envisions incorporating a genetic fingerprint into the keratinocytes' DNA sequence or impregnating the keratin with biomarkers, as well as developing hand-held devices for detecting them. Ceratotech plans to market its horn mimic as a replacement for the horn used to make trinkets. Still, Vygantas says, even though he doesn't intend for the faux horn to be used as medicine, "I'm not so naive as to believe it won't end up being used for human consumption". Ultimately, he hopes to build on Ceratotech's technology to biofabricate pangolin scales, shark fins, and other often-poached animal parts.

Further north along the Pacific coast, entrepreneur Matthew Markus's Seattle-based firm, Pembient, is also working on manufacturing rhino horn. Originally, Pembient team members engineered yeast cells to produce keratin, which they made into powder for shaping into a rhino horn with a 3-D printer. But the horn's material properties—its texture, for instance, or the way it sheared when cut—didn't exactly replicate those of real rhino horn. Now, like Ceratotech, Pembient has adopted a tissue-engineering approach. The team hopes to coax stem cells from a rhino or other animal to mature into keratinocytes, which they would then implant onto a scaffold.

During a visit to Vietnam in 2015, Markus had a hunch that spoons, vases, and other bric-a-brac carved from rhino horn played a bigger role in driving poaching than horn-based medicines, made with the shavings from these carvings. "Those are waste products", he says. "What's really valuable are the carvings." While powdered rhino horn may look indistinguishable from powdered water buffalo horn, it's hard for carved objects—especially larger ones—from animals other than rhino to fool anyone. Rhino horn is solid, whereas horns from other animals, like water buffalo and cow, are hollow.

That realization led Markus to focus on synthesizing horn cylinders, marketed to carvers, artisans, and designers. Many objects, like jewelry and eyeglasses, though now made of plastic, were once carved from horn. Because keratin is a biopolymer, it once served as "a great, biodegradable plastic", Markus says. "My real goal is to bring horn back to its origins as a plastic." Pembient has raised a projected $20,000 in the form of digital currency to fund its efforts, offering 1 g of its horn for every virtual coin donated, with delivery set for November 2022.

But amid worries from conservationists that biofabricated horn could increase poaching, Markus is now exploring the possibility of making horn composed of cow cells and DNA (trademarked as Cowino horn) but grown as a solid like rhino horn, perhaps in the form of cylinders or sheets. "It's not as exciting to me [as rhino horn]", but conservationists may find it more acceptable, he says, since it wouldn't raise the same concerns as biofabricated rhino horn or other mimics of illegal wildlife products.

■ IMITATING IVORY

At the University of Oxford, professor of zoology Fritz Vollrath has undertaken the even more daunting task of synthesizing the ivory that makes up elephant tusks, also a symbol of wealth. Once used to make piano keys and joint replacements, today it's carved into bangles, figurines, and other trinkets. Unlike rhino horn, which is made almost solely of keratin, ivory consists of two materials: a highly ordered scaffolding made of collagen—the protein that gives

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structure to skin, tendons, and other tissues—embedded with hydroxyapatite, a hard, calcium-based mineral also found in bone.

Poached elephant ivory gets carved into trinkets and decorative objects, which are symbols of wealth. Credit: Jo Crebbin/Shutterstock.

Much of Vollrath's research investigates the properties of spider silk, but he also studies elephant behavior and chairs the conservation nonprofit Save the Elephants; his efforts to synthesize ivory bridge these realms of expertise. In addition to characterizing ivory, Vollrath and his team, which includes Zhengzhong Shao and Ruixin Mi of Fudan University, have begun experimenting with mixing hydroxyapatite into liquid silkworm silk, which they then set into solid, ivory-like blocks. They found that silk allowed much more flexibility than collagen to shape and form the composite material. So far, his team has made small, polished pieces of ivory. They plan to make bigger pieces and optimize the process using feedback from carvers.

Eventually, Vollrath wants to perforate biofabricated ivory with microscopic channels like those in natural ivory, except angled slightly differently. With that small adjustment, law enforcement officials could easily spot the difference between lab-grown and poached ivory, using just a magnifying glass.

■ MARKET FORCES

Vygantas and Markus believe flooding the market with lab-grown substitutes will drive down the price of rhino horn—which hit a record $65,000 per kilogram in 2012—making poaching less lucrative. Vygantas believes the market for poached products would decrease because consumers could easily buy a lab-grown version, and consequently, poachers would no longer view slaughtering animals as worth the risk. Markus agrees, adding that his goal to make Pembient's horn a bioplastic used in everyday objects instead of a luxury good would also lower the price of rhino horn.

Vollrath envisions taking a slightly different approach. Ivory commands a much lower price than rhino horn—the price fell below $1,000 per kilogram in China in February—and lacks the same mythical aura. So rather than flooding the market with biofabricated ivory, he plans to simply present it as an alternative for carvers. "If a good carver can make wonderful necklaces with this stuff and increase the value because it comes from carver A or B, then I think we've done what we set out to do", he says.

But many conservationists believe the emergence of lab-grown mimics will only exacerbate poaching. They worry, for instance, that these products could create a smokescreen for poachers, allowing them to pass off their contraband as biofabricated and therefore animal friendly. Although Vygantas and others have proposed genetic fingerprinting and other forms of verification technology, "the reality is further off", says Tanya Sanerib, senior attorney for the Center for Biological Diversity, a nonprofit organization that advocates for protection of endangered species.

Biofabricated rhino horn and ivory may also simply open up a more affordable market that exists in parallel with the market for poached products. That's much like what happened with lab-grown diamonds, developed to avoid the environmental and human rights abuses associated with mining diamonds. But that hasn't stopped the desire for the real deal, Sanerib says.

Such parallel markets could even boost the value of the real thing. Poached products can raise a person's social cachet, showing that the customer has enough wealth to not only buy them but also afford to risk getting caught owning them. "The fact that you have something growing in the lab won't remove the allure of the real thing", she says. What's more, lab-grown products could be used for products not currently in the market, creating new demand, says John Baker, managing director of programs at WildAid, a non-profit organization working to lessen demand for rhino horn and other wildlife products. "Once you let the genie out of the bottle, you don't know what people are going to do", he says.

To sidestep these thorny issues, Ceratotech will be transparent about its stem cell approach, Vygantas says. He remains confident that consumers will perceive the firm's horn as "real", as they already do many lab-grown human tissues. "The same way that transplanted hair grows like hair, that's what we envision happening with keratinocytes", he says.

"There's always a danger of a parallel market", Markus says. But "that's not under our control." Although Pembient plans to market its horn as biofabricated, whether artisans

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choose to disclose that information when selling the objects they carve from it is up to them, he says.

THE FATAL FLAW?

Unlike many scientists and conservation groups, Frederick Chen, an economist at Wake Forest University, doesn't see biofabricated horn as an either-or question. Rather, "it can work with the right conditions." In a paper published in the journal *Ecological Economics* in November, he argues that for biofabricated rhino horn to end poaching, it should closely, but not perfectly, mimic wild horn. Manufacturers should incorporate a hard-to-detect flaw into their product—an ingredient that triggers a mild stomachache when swallowed, for instance—to sow distrust of rhino horn in general, including the poached product, among consumers. Of course, this "gets into an ethical question", Chen says. Demand for all rhino horn would decline, since consumers would fear buying a "lemon", which would, in turn, lower prices and—if the decrease in price is large enough—eliminate the incentive for poaching. "I don't want to shift consumption a little bit", Chen says. "I want to destroy the whole rhino horn market."

Of course, making a product that doesn't measure up to real horn would go against for-profit companies' interests. "Profit motives and conservation motives don't go hand in hand. If we care about saving the rhinos, we shouldn't care about making a profit", Chen says.

If rhino horn contained a medically validated active pharmaceutical ingredient, then incorporating a defect, like omitting an ingredient, would reduce demand, Vygantas says. But since it doesn't, "there is no obvious defect we can incorporate." Markus says Pembient doesn't necessarily have to demonstrate its horn's inferiority through the horn itself. For instance, the company could publish its products' DNA fingerprints after two years, but consumers wouldn't know at the time of purchase whether the horn would turn out to bear one of those fingerprints.

In contrast to rhino horn, ivory is used only for carving into various objects, which means the material properties of synthetic ivory need to resemble those of real ivory as closely as possible, Vollrath says. In fact, if its material properties surpassed those of real ivory—rather than fell short of them, as Chen argues they should—then that would further ensure its success in replacing the real thing. "I might be wrong", he says, "but let's get the discussion going."

Vygantas also acknowledges the uncertainty inherent in his efforts. The shadiness of the poaching market makes it hard to secure funding to biofabricate horn and ensure success. Still, "it's a noble cause to pursue", he says. He insists that he doesn't seek to become wealthy from his venture, only to ensure that his two children get to see a rhino in the wild in their lifetimes, as he did on safari in Africa. "I would love for them to experience that feeling of awe", he says.

Melissa Pandika is a freelance contributor to Chemical & Engineering News, the weekly newsmagazine of the American Chemical Society.

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