

By any measure silk is special. Technically, it is nature's only continuous filament, and the only natural fibre that is spun, not grown. Add to these unique traits the high tensile strength and elasticity of the special type of silk that spiders spin and you have a highly coveted material. Biotech laboratories around the world are making progress in reproducing this wonder silk and some may even be close to commercialisation, all involving genetic engineering.

Beyond biomimicry

We'll call it the second round for spider silk. The first would have been in the early 1990s, when Dr Randy Lewis published his first papers on the structure of spider dragline silk. By the end of the decade, spider silk made from genetically modified (GM) silkworms or extracted from the milk of GM goats was thought to be imminent. Canadian biotech firm Nexia was specifically working on extracting spider silk proteins from goat's milk to make Biosteel, before closing. In the late 2000s, a new generation of start-ups in the US, Europe and Japan began to look at the idea and may now be closer to making the miracle material a commercial reality.

Like many biotech industries, laboratories specialising in spider silk have attracted generous funding. This September, Tokyo-based sportswear manufacturer Goldwin invested €22.4 million to acquire a 12% share of Yamagata-based Spiber to help it develop and commercialise a synthetic spider silk it is calling Qmonos. In June this year, Bolt Threads, based in Emeryville, California, raised close to $40 million from Silicon Valley investors to back its bacteria-based spider silk making method. More modestly, German start-up AMSilk received €5 million in funding in 2011 to pursue work on its bacteria-based spider silk that is – coincidentally or not – called Biosteel. It's worth noting that in this micro-sector, two companies operate under the same name, Spiber, the previously mentioned Japanese company and a Swedish start-up, based in Stockholm, Spiber Technologies, also working on a bacteria-based spider silk manufacturing process.

Spiders are capable of producing several types of silk for different uses.

www.shutterstock.com

COPYRIGHT

The world of silks

What makes spider silk so covetable? In its natural state, the quasi-immaterial web of fine elastic filaments the insect spins is capable of capturing insects in flight and resists wind, rain and sunlight. Its properties are often compared to those of Kevlar and steel. Arachnids produce not one but up to six different types of filaments (and adhesives) in different glands, such as dragline silk, capture silk, scaffolding or cocoon making filaments.

Silkworms secrete only one type of filament, which they use to craft their cocoons during metamorphosis. Honeybees are said to make silk for thermal insulation and to buttress their hives, and certain ants do too.

A protein fibre, like wool, traditional silk made by mulberry silkworms contains two proteins, sericin and fibroin. Only one protein, spidroin, is found in spider silk. This large protein is the secret of the material's remarkable mechanical properties: it is its combination of high tensile strength and extensibility that enables it to absorb a lot of energy before breaking. Spider silk is said to be as strong as steel (hence the references to Biosteel), but much lighter thanks to its lower density. It is also said to possess roughly the same characteristics as Kevlar or high molecular weight polyethylene (HMWPE). "But depending on how it is tested, it can present superior performance levels," says Dr Lewis, now a professor at Utah State University.

Unlike silkworms – the Bombyx mori genus, considered entirely domesticated – farming spiders to collect their silk is near impossible as they become cannibalistic when kept in captivity. This is the main reason that most attempts to produce spider silk have taken alternative routes to obtain the precious protein. Of the various genetically engineered processes in development, the most logical – if not the most natural – involves transferring spider silk-making genes into the genetic make-up of the Bombyx mori, the species the world relies on for natural silk. Silkworms possess an energy efficient spinning process that takes place at ambient temperatures and low pressures, says Oxford Silk Group professor, Fritz Vollrath.

Kraig BioCraft, based in Michigan, chose silkworms to make its spider silk. "Silkworms are very good at producing protein fibres," says the company's chief operating officer, Jon Rice, and he believes this method "is the most powerful" compared to others technologies. Sigma Life Sciences, a division of US biotech firm Sigma Aldrich, developed the spider gene transfer technique (ZFN). The process involves "thousands of microinjections of a plasmid containing our target protein sequence," says Mr Rice and "acceptance rates are very low." A test determines which larvae contain the new genetic material and can be reared. This is the most haphazard stage of the operation. The easy part is harvesting the cocoons. "Silkworms have a high production capacity, about 40% of the larvae's weight is dedicated to producing silk," explains Mr Rice. The filaments Kraig's GM silkworms produce measure the same size as conventional silk, from 4 to 10 microns, he adds. Protective materials specialist Warwick Mills, based in New Hampshire, has made a fabric with the company's fibres.



Single strand of artificial spider silk produced under laboratory conditions.

Peterpacz1/CC BY-SA 4.0

Kraig Biocraft has created three types of 'hybrid' spider silks, so called because they contain fibroin as well as spidroin. Initially developed in 2011 and in pilot production since 2013, Monster Silk is the company's first composite filament. It is made by a large-sized silkworm, hence its name, and the silk it produces is elastic, said to stretch by 38%. Big Red, the second product, was "designed" to offer more strength rather than elasticity. The newest hybrid, Dragon Silk, is engineered for both strength and elasticity. "Some samples exceed the performance of natural dragline spider silk," says Mr Rice. The ultimate goal is to achieve a higher content of spider silk. Sigma continues research with the aim of creating "pure" spider silk by inserting spider silk genes into the silkworm genome while simultaneously removing the native silkworm genes. The company has also envisioned expanding production of the mutant caterpillars. A Memorandum of Understanding was signed

COPYRIGHT

with the Vietnamese administration in January 2015. The country was chosen for its "expertise in rearing silkworms and processing silk," says Mr Rice.

Biotech proteins

The method used by Kraig BioCraft is partly based on Dr Lewis' research. His team at Utah State University has succeeded in expressing the spidroin protein in silkworms, bacteria (Escherichia Coli), alfalfa (medicago) and goats. It has also developed a spinning process. Of the four techniques, the E.coli method is the most advanced, and a pilot scale facility is currently in operation. Its 1,000-litre fermentation capacity can produce from 100 grammes to 1 kilo of proteins, says Dr Lewis. A university spin-out, Araknitek, Inc. has been set up to license the technology.

The genetically-modified Escherichia coli has been chosen by several biotech companies, including, in Europe, German start-up AMSilk. Founded in 2008 and based in Planegg, near Munich, the spin-off from the Technische Universitaet Muenchen (TUM) has developed a process called Biosteel that is the result of research by Thomas Scheibel, currently Professor of Biomaterials at the University of Bayreuth. Its spider silk biopolymers are being used to create cosmetics and pharmaceutical products. The company launched its own cosmetic line under the brand name Spidersilk in 2013 and claims to have made the "first silk of non-animal origin."

In Stockholm, Spiber Technologies, also founded in 2008, has cloned a gene sequence it calls mini-spidroin, also in E.coli. The bacteria is also the vehicle chosen by MIT professor of civil and environmental engineering Markus Buehler, who published his most recent findings in the journal *Nature Communications* this May.

Japanese company Spiber, founded in 2007, similarly uses a microbial fermentation process to produce fibroin, which is spun into the Qmonos fibre. The brand name comes from the Japanese for spider's web, 'kumo-no-su'. The company announced the launch of a pilot plant this year, which could be scaled up to reach mass production of "fibres, films, gels, sponges, powders, and nano-fibres." The pilot plant is expected to produce 10 tonnes of the Qmonos fibre per year.

The method developed by Bolt Threads, in California, also calls on mutant microorganisms to produce silk protein. The company claims to be able to "tune" the spidroin solution to obtain different qualities (stronger, more elastic or waterproof), but does not provide detailed data. Sam Hudson, a third-party advisor to the company, interviewed in *Wired* magazine in June, declared that the "fermentation system developed is unique in that large quantities of silk protein can be isolated. The fibres are 100% silk protein." In 2015 Bolt Threads partnered with biotech accelerator Michigan Biotechnology Institute (MBI) to scale up production. The start-up has also teamed up with Unifi and says that the first commercial textiles could be available in 2016.



Regular silk cocoon next to Kraig BioCraft's Monster silk cocoon, seen under UV filter.
Kraig BioCraft

Back to the farm

Bolt Threads had initially begun producing spider silk by milking actual spiders. It subsequently abandoned this method, but not everyone has given up on spider-made silk.

The best known spider silk is the one used to make a series of fabrics and capes exhibited at the Metropolitan Museum in New York and the Victoria & Albert Museum in London in 2012. The striking gold-hued silk was "spun" by Golden Orb Spiders in Madagascar. The endeavour, led by British art historian and textile expert Simon Peers and American fashion designer Nicholas Godley was the result of a painstaking process involving one million spiders and 80 people over a period of four to five years, beginning in 2004.

A French designer, Thomas Maincent has created what he believes is a "realistic" attempt to produce spider silk without resorting to genetic manipulation. The result of a two-year investigation, the Spider Farm he invented is his attempt to find the ideal balance between natural process and industrial production. A prototype factory was successfully set up and tested using Nephilia spiders for an exhibit in Belgium in 2012 (at the Z33). More than twenty spiders spun silk in individual compartments and were kept in a giant terrarium when not "working". The project included a factory to generate flies to feed them. No other source of energy was necessary. "The farm is nearly autonomous," says Mr Maincent. Biomimicry inspired the project, but he points out

COPYRIGHT



Spider Farm by French designer Thomas Maincent. The working prototype successfully produced spider silk in 2012.

Kristof Vrancken

that "nature produces only what she needs". His Spider Farm would be a seasonal factory and would produce just the required amount. "You can't change nature. A spider's silk-making capacity is limited," he says. He too set up a spider farm in Madagascar but abandoned the project due to political unrest there. The project is currently on hold, but Mr Maincent maintains that it is feasible and believes it could become a reality one day, though at a micro-production level.

Ahimsa silk is another natural silk making method that seeks to abide by nature's way. In this case, the silkworms are not killed but allowed to escape before the silk is unwound from the cocoon. Known as Peace silk – 'ahimsa' is a term derived from Sanskrit meaning 'do no harm' – the patented process was developed in India by Kusuma Rajaiah. The silk produced is said to be slightly less lustrous but softer to the touch than conventional silk. Ahimsa silk capacity is also limited.

The search for applications

Spider silk production itself is far from reaching industrial scales. The actual amount of spider silk proteins that make up the materials in development is in fact unknown (and not much publicised). This suggests that they do not possess the mechanical properties of natural spider silk.

Most companies looking to make spider silk seek to produce fibres, films and foams. Textile uses are one of the primary markets targeted. "It will remain a niche market," says Dr Lewis, and dependent on the ability to produce materials that possess the desired material properties. He is currently optimistic regarding applications in coatings. "The incredible flexibility of spider silk would be great for coatings, and various supplementary functions could be achieved with antimicrobial, reflective, or antibiotic additives." Coatings could also require no more than 5% spider silk protein content.

Goldwin Japan has stated that it intends to develop "next-generation sports clothing" with Spiber's Qmonos fibre. Despite references to many possible applications – ropes, bullet-proof jackets, etc. – Jon Rice at Kraig Biocraft says "the market is still looking for real applications. There is no shortage of potential applications for spider silk technology. What Kraig Labs is focused on is identifying and targeting those markets and applications where our materials measurably outperform existing solutions."

The "protein-producing" factory that silkworms are said to be suggests that other types of proteins – beyond spidroin – could come out of the vats of genetically modified bacteria, particularly for medical or cosmetic uses. Dr Kristina Martinell, production director of Spiber Technologies (Sweden), says the company is targeting applications in regenerative medicine and diagnostics for its spider silk proteins.

No company has yet estimated the possible cost of the spider or the hybrid silkworm/spider silk when it does becomes available. Natural silk is considered the most expensive of the natural fibres. The luxury material is sold at a premium without any tinkering with its genetic make-up. This issue raises its own set of questions as to precautionary principles and safety regarding the evolution of modified species. That said, the textile industry already uses GM bacteria in its manufacturing processes. GM microorganisms synthesise one of the ingredients DuPont uses to make Sorona, a biosourced polyester polymer. Those who work with silkworms point out that the species does not fly and cannot survive in nature. Before spider silk can be sold, it will have to be vetted by official administrations and it may also come under close scrutiny from anti-GMO organisations.

COPYRIGHT