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Review Article

The Evolution of Biosteel ™

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Abstract

Biosteel™ is a spider silk based high strength fibre material made of recombinant spider silk obtained from the milk of transgenic goats. The name Biosteel™ was first used by a Canadian company "Nexia Biotechnology", founded in 1993. Biosteel is reported to have materialistic properties of toughness and stress resistance, which make it a unique Bio-material for many useful purposes. With support from the US Army and Canadian Department of Defence, Nexia Biotechnology mainly focused on making medical, commercial and military utilized products like artificial tendon ligaments, biodegradable fishing nets and bullet-proof vests with spider silk. Later in 2009, due to failure in large scale silk production, Nexia Biotech went bankrupt. Now, the commercial Biosteel™ is produced by a German company "AMsilk". AMsilk mainly focuses on large scale production of spider silk to make medical devices, tiers, shoes etc. In the article we attempt to track down the amazing evolution of Biosteel™ followed by its invention, utilization, failures, present and future prospects and the real science behind the Biosteel's amazing strength and durability.

Introduction

Spiders are an evolutionary success story for 380 million years. There are over 41,000 species of spiders in the world, among which the golden orb weavers (*Nephila sp.)* are famous for their large size and web spinning ability [1]. The most researched spider silk is the "Dragline" silk produced from their Major Ampullate gland. The dragline silk is used for web's main structure like rims and spokes. The silk of *Nephila sp.* can hold up to 12.71 GPa tensile stress with its high strength and toughness [2]. One single strand of silk is 4 times thinner than a normal human hair but strong as steel per unit weight. The strength of a spider net can be guessed by looking into how the web nets are used by spiders to catch prey, suspend themselves or by how the web holds water droplets after rain.

The spider silk Biosteel™ was produced from the genes of spider species *Nephila clavipes* also known as the golden orb weaver or the giant wood spider [3].

Composition of the Dragline Silk and the Man Behind it

Dragline major silk is the most researched spider silk protein (Spidroin) for its extreme properties like toughness and high tensile strength though being thinner than a strand of human hair. Professor Randolph V. Lewis from University of Wyoming first worked with the dragline spider silk and analyzed the mechanical and chemical properties of the fibre. Later, Professor Lewis cloned the key protein's gene from four different types of spiders and

Fig 1: A female *Nephila clavipes* in its web

licensed them to Nexia Biotechnology. The major ampullate (Ma) silk is the combination of Glu, Pro, Gly, and Ala amino acids, which constitute 80% of the silk. The first cDNA sequence for spider silk protein from the major ampullate of *Nephila clavipes*, MaSp1,was published in 1990. The partial sequence contains similar but non identical repeats with motifs of poly-Ala up to seven residues alternating with (GGX)*n* sequences, where G is Glycine and the X residues are Tyrosine (Y), Leucine (L), and Glutamine (Q) in that sequence order. The second cDNA of the major ampullate silk protein sequenced later was called MaSp2. The motif of MaSp2 consists of GPGXX (where G is Glycine and P is Proline). The motif of the MaSp2 has β-turns in its protein structure. The repetitive sequence of this protein is responsible for the elasticity and high tensile strength of the spider silk. The properties of dragline silk include strength of 4x109(N m-2) and elongation up to 35%. Another useful property of spider silk is its super contraction in water which suggests that at high humidity there will be contraction making the spider silk much tighter giving it an amazing property to be used as Biosteel [4].

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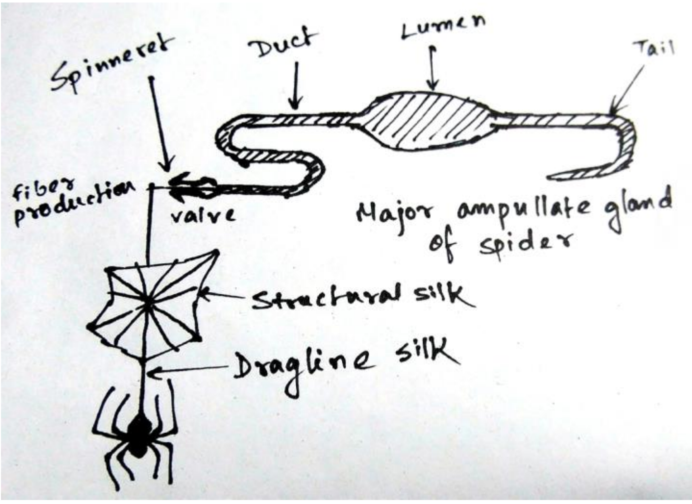

Fig 2: Dragline silk is produced from major ampullate gland.

Professor Lewis thereafter created transgenic goats (spider goats) having genes of spider silk for large scale silk production. But after the closure of Nexia Biotechnology, the goats were brought back to Lewis's lab and presently Professor Lewis is working in University of Utah with spider goats.

Spider Silk Gene Expression in Transgenic Goats

Spiders are wild creatures and it is tough to keep these in lab. The population cannot be controlled as the female spiders kill and eat the male spiders (It is known as spider cannibalism). It was never possible to produce spider silk in large scale for commercial use by harvesting spiders in the lab. Scientists of Nexia Biotechnology trasfected the spider silk gene into bovine mammary cells(milk producing cell line),which resulted in secreting spider silk proteins outside the cells [5]. Later the gene of spider silk was cloned into goat's milk protein gene (**α**–casein). This helped to create transgenic female goats which were able to produce spider silk in their milk. The inserted transgenic contained a His-tag which helped in easy separation of the spider silk from goat milk. The transgenic goats were called "spider goats" [6].

The spider goats were created by taking eggs of one female goat and replacing their nuclei with the nuclei of BELE goats (breed early, lactate early). Then the spider silk gene was transfected (by electroporation and nucleofection gene transfer process) into an unfertilized BELE egg. Later the egg was fertilized and the new born transgenic goat was allowed to mature. It can be guessed that BELE goats were taken to induce the chance of successful trangene transfer [7].

Methods

The goats were created by two methods (A)TALEN targeting assembly and (B) pBC1 insertion.

(A) **Gene Insertion by Talen Targeting**

Transcription Aactivator-like effectors nucleases (**TALEN**s) are restriction enzymes those can be engineered to cut specific sequences of DNA. The required TALEN enzymes and TALEN cut sites of **α**–casein were determined by using online tools (Golden Gate as-sembly protocol and TAL Effectors Nucleotide Targeter 2.05). After that, the TALENs were expressed in mammalian expression vector pCDNA3.1 and transfection of spider silk gene into goat was finally carried out using LONZA 4D Nucleofector TM System. The result showed 70% trasfection efficiency [6].

Final α**–casein Sequence after TALEN assembly :**

CCTTTTAGGAAGCAAGGACCAAGTAAACATGAAGTT
CT-TCATTTTTACCT**GCCTTTTGGCCGTTG**CCCTTGC
A**A88---Spider Silk Gene plusHis-Tag---700T**GAGGTAC**CT**
TTAAGATTCTTGAATTAACT-GCTTCTACCTGGTTATGG
TTGGACTGGAAAATCTATCTTCTACAGTTTCAGATC-TA
CCACTTTACTTCATACAACCAGCATGTTTGGGGTGGC
GCATCAATAAGA-CAAATCGCAGAGTATTTCCTGAATT
ATTTATACTCTCCTGGTTAGTTTATATT-GAATATACTG
GGTTTATATGGTGGTTTCATACAAACTTAGCTGATGA
TTATTTA-AAATCCTTTCCCTAATCTTTCTGAGTTATAG-
AAATATTTTTCTTTCCCTTTG

Fig 3: Between 88th and 700th nucleotide positions of the casein gene, the 612bp long spider silk gene was inserted (with his-tag attached). The sequence of two sides are some part of the original caesin sequence into which enzyme has cut and green colored parts indicate TALEN recognition sites. (Ref 6)

(B) **Pbc1 Insertion**

The pBC1 vector was treated with restriction enzyme (Xhol) and the spider silk gene was inserted into the vector. After insertion the new construct was inserted into a goat via somatic cell nuclear transfer [6].

Schematic diagram of creating transgenic Spider goat & Biosteel silk from the milk of the goat.

Silk Fibre from the Transgenic Goat Milk

Specifically West African dwarf goats were used by Nexia to create transgenic goats because they were sexually active all year round. The goats were only able to produce milk containing the spider silk, they were not able to spin webs naturally like spiders. At first milk of goat was centrifuged so that the components could be separated based on their density. In this way the fats and creams were taken out first; then by adding salts the proteins were precipitated in solid form. The silk protein was then dissolved in aqueous solu-tion and turned into a golden-tinged syrup. This silk concentrate was known as ''spin dope'' and was identical to what was inside a spider's belly. The lab of Nexia had many machines to spin silks.

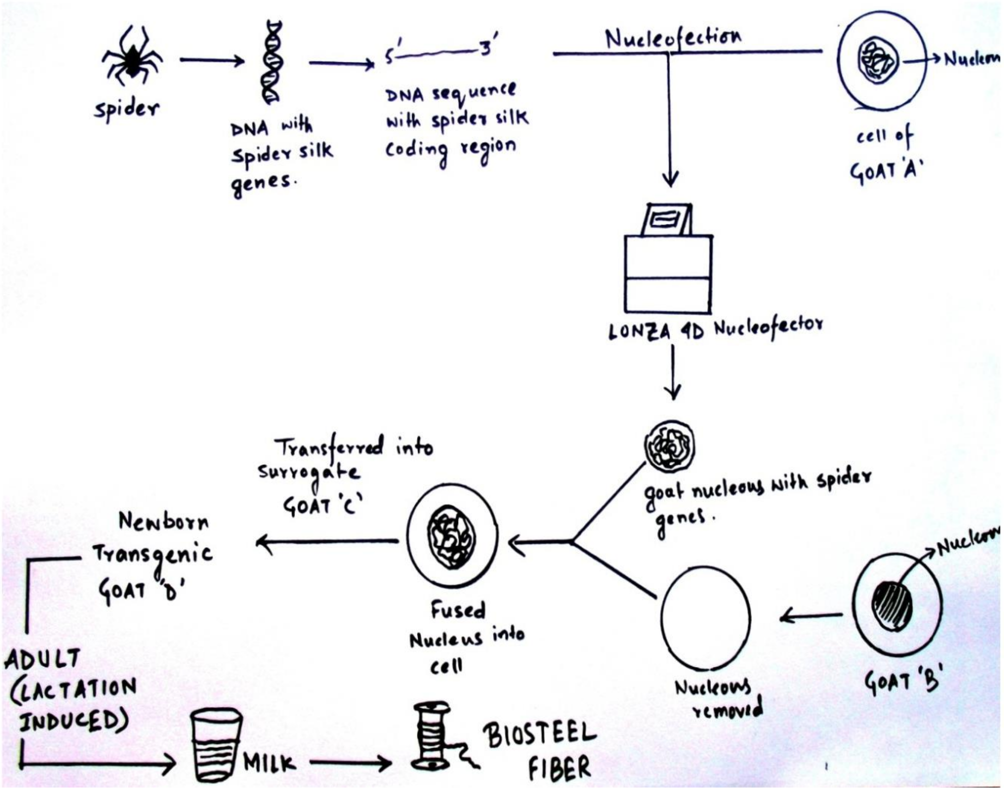

Fig 4: Schematic diagram of creating transgenic Spidergoat & Biosteel silk from the milk of the goat.

Fig 5: A Biosteel goat and milking the Biosteel goat (Ref 16).

Through an extrusion machine the wet silk came out as a little jet, in presence of air the fibre solidified which produced a continuous strand of Biosteel [8].

Uses of Biosteel™ Focused by Nexia Biotech

1. Spider silk is not harmful to human. It is very fine and strong. So, Biosteel was used to create artificial ligament and tendons for medical treatment.
2. Nexia Biotech tried to develop a bullet-proof vest for military use with the Biosteel keeping in mind the properties of the dragline silk and how a web can convert 70% of its kinetic energy into heat when struck by any object without being torn.
3. Nexia biotech also planned to commercialize fishing nets and few other products with Biosteel [9].

Due to the winding up of the company in 2009, the fate of Biosteel and spider goat changed. The transgenic goats from the Nexia farm were brought back to the lab of Professor Lewis. Currently the testing of bullet-proof vests with spider silk is going on and may have positive results in future. Professor Lewis donated one of his original Nexia Biosteel goat to a museum called 'PostNatural History' in 2013. That is the only Biosteel goat on a public display in the world, has been mentioned in figure 5.

Tests to be Done in Future and Alternative Sources of Spider Silk

One important fact is spider's natural silk is not very fine or smooth as the diet of the wild spiders cannot be controlled. The silk obtained from spider goat is very fine and smooth in structure as the diet and transgenic expression can be controlled. The natural spi-der silk can hold their strength below –40°C and up to 220°C and is highly contractile in nature in presence of moisture. It is an ad-vantage that the spider goats can produce high amount of silk but more research is needed to ensure the durability of the spider silk at higher temperature, in presence of water and ultra-violet rays [9]. Spider dragline silk gene has been also expressed through trans-genic plants like tobacco and potato. In future plants can be also used as source of Biosteel [10].

How Biosteel™ is Produced by Amsilk

AMsilk is a German biotechnology company(founded in 2008) with aim of high quantity Biosteel spider silk production. In pi-lot scale and in large scale bioreactors, the spider silk is produced with the help of scientists from Technische Universität München (TUM) by spin-off process. The artificial spider silk is made of recombinant spider silk proteins. The researchers of the university have found the molecular mechanism behind the strength of Biosteel **[**11**].** AMsilk focuses on the use of Biosteel in cosmetics (as spider silk protein is good for skin), use of the Biosteel as a medical coating or for other medical purposes. Biosteel can be used also in aerospace, shoe and textile industries. Biosteel can be used also to make tiers of cars or planes, to make airbags of cars [12]**.** The tie-up of AMsilk with sports brand Adidas and launch of their new product, a sports shoe made of Biosteel fibre is a remarkable achievement in this context. The concept represents innovation and functionality of renewable textile. The shoe is natural, 100% biodegradable, 15% lighter than conventional synthetic polymer mesh material and is created to demonstrate that Biosteel products can last long, can be tough and better than the used synthetic ma-terials. [13]

Fig 6: Recombinant spider silk bundle by AMsilk (Ref 11)

How Biosteel Is Manufactured In Labs

AMsilk produces high amount of spider silk fibres in their labs without using any transgenic animals. In labs of AMsilk, big biore-actors of 50,000 litre are used to make Biosteel. At first the spider is selected from which the gene has to be taken for cloning. Then the spider silk genes are identified and sequenced. After that the spider silk genes are cloned into the bacteria by recombinant DNAnology. The bacterial cells with spider silk genes are cultured in big bioreactors to process the proteins in high amount to produce silk fibres. The raw material needs to go through more modifications to produce fine silk filaments. A company named Groz-Beckert, provides optimal knitting-machine needle called Vo-LC™, which creates the fine Biosteel filaments from the raw spider silk. The entire process is carried out in the factory's TEZ (Technology and Development centre) by using automated "Loop Technology" [14].

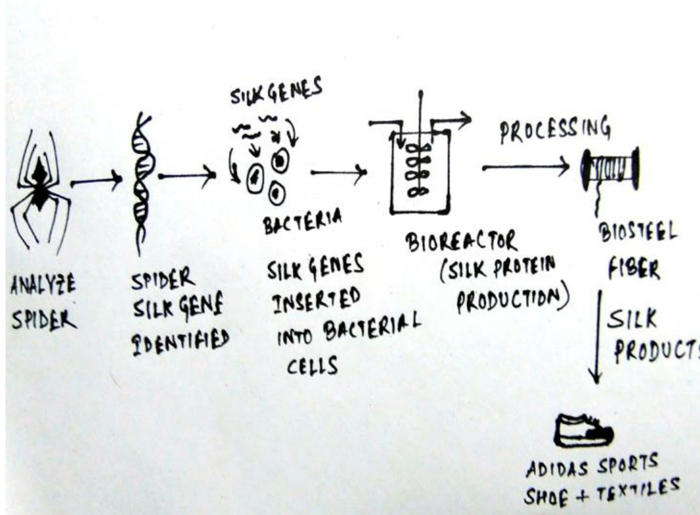

Fig 7: AMSilk's approach to develop Biosteel and silk products.

Conclusion and Future Aspects

It can be concluded that Biosteel has come across a long way since its invention in Nexia Biotechnology to the innovation of sports shoes made of spider silk by AMsilk and Adidas. Keeping in mind all the properties of the spider silk (Biosteel), which makes it a techtougher and stronger material than steel of the same weight, it can be considered as a revolutionary material like Kevlar, for future. It is reported that with high tensile strength a pencil thick Biosteel fibre can hold a big airbus of 380 tons. Biosteel is 100% biodegradable, good for skin and extremely light in weight [15]. It may sound insane but in distant future it might be possible that human keratin can be merged with the spider silk gene to create transgenic super humans with tough skin. Thus with the unbelievable strength of Biosteel the comic character Spiderman will not be a myth any-more, where a man can easily hang and swing from buildings.

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