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Engineering Spider Genes for high-Tensile Silk and Patenting Challenges

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Abstract

This paper is a techno- legal review of existing scientific research, which explores the exciting field of genetic engineering and its potential in enhancing the tensile strength of spider silk through targeted modification of spider silk genes. Spider silk is renowned for its remarkable strength and flexibility, rendering it a highly coveted substance for many applications encompassing textiles, medical devices, and construction materials. However, its production on a large scale has been limited by the challenges of obtaining sufficient spider silk quantities. To overcome this limitation, researchers have turned to genetic engineering techniques to modify the genes responsible for spider silk production. This research delves into the development of novel techniques that enable precise and targeted modifications of these genes, aiming to enhance the tensile strength of spider silk even further. Additionally, this paper also addresses the legal challenges associated with patenting genetic modifications of spider silk genes. The unique nature of genetic engineering raises questions regarding patent eligibility, ownership, and potential infringement issues. This study discusses the current legal landscape surrounding patenting genetic modifications, including the criteria for patentability and the ethical considerations. By combining scientific advancements with legal insights, this research aims to contribute to the growing body of knowledge in the field of genetic engineering and provide a comprehensive understanding of the potential challenges and opportunities in enhancing spider silk's tensile strength and protecting intellectual property rights.

Keywords: *Genetic Engineering, Spider Silk, Tensile Strength, Targeted Modification, Patenting, Legal Challenges*

1. Introduction

Background on spider silk and its unique properties: Spider silk is a unique material with remarkable properties that make it applicable in various fields. It has a complex structure and composition, and its assembly and disassembly can be triggered by environmental factors. Spider silk can be used to create Fibers, films, and composite materials, and advancements in cloning and expression of spider silk proteins are being explored for industrial use (Singh et al., 2023). Different types of spider silk exist, with varying protein structures and functions. Drag silk, produced by the main ampullate silk gland, is a particularly strong and biocompatible type of spider silk that has potential applications in aerospace and biomedical industries (Joel, 2023) Spider silk threads possess remarkable mechanical characteristics, including high toughness and flexibility, rendering them highly appealing for utilisation in diverse domains of material advancement. Spider silk is also environmentally friendly and has superior physical and chemical properties compared to other natural and synthetic fibers, making it a potential alternative for textile materials (Ramezaniaghdam et al., 2022).

Significance of genetic engineering techniques in modifying spider silk genes: Genetic engineering techniques have significant importance in modifying spider silk genes. These techniques simplify the construction and expression strategy of spider silk proteins (Liu et al., 2022). They provide the ribosome with a circular translation template, enabling the expression of extended peptides containing tandem repeats. This revolutionary method accelerates the development of biomaterials composed of fibrous proteins. Furthermore, advancements in cloning and expression of spider silks are a growing area of research and industrial use (Petrou, 2018). Genetic engineering enables the creation of hybrids made of spider silk that combine with organic nanoparticles, expanding the potential applications of spider silk . Additionally, genetic engineering allows for the functionalization of spider silk proteins with mucoadhesive properties, making them suitable for mucosal wound dressings and drug delivery systems. In the realm of scientific inquiry, it is widely acknowledged that genetic engineering techniques assume a pivotal role in the systematic exploration of the practical utility of spider silk and the subsequent achievement of large-scale production of spider silk proteins.

The current legal landscape surrounding patenting genetic modifications: It involves ethical considerations and criteria for patentability. It is imperative to proactively engage in discourse regarding the ethical ramifications associated with gene editing and genetic selection, particularly in light of the increasing accessibility and affordability of gene sequencing and editing technologies.6 The granting of patents on genes has come under scrutiny, with some experts questioning the practice and its impact on research and innovation. The requirements for granting a patent involving the use of human genetic material are discussed in the context of a recent High Court decision. The eligibility for patenting DNA sequences is a topic that needs to be revisited, with some arguing for a more stringent application of patenting criteria, particularly utility.7 The economic efficiency of patent protection on gene-based inventions is also questioned, as excessive proliferation of property rights and wide patent protection can impede research and development in the biotechnological and pharmaceutical fields. The utilisation of genetic data for the purpose of formulating and directing therapeutic interventions gives rise to innovative patent-related concerns, and ongoing legal proceedings pertaining to various instances have the potential to contribute logical and lucid interpretations to the existing legislation (Nelson & Wiles, 2022).

Overview of Spider Silk Genes

Types of spider silk genes and their functions: The genetic information responsible for the production of spider silk is stored within the spidroin gene family, encompassing spidroins and spidroin-like (SpL) genes. Spidroins exhibit conserved terminal regions and a repetitive central region, whereas SpL genes showcase repetitive sequences and amino acid compositions that bear resemblance to spidroins, albeit lacking the terminal domains. The aforementioned genes play a crucial role in the process of silk production and exhibit expression primarily within silk glands. Notably, certain genes demonstrate an upregulation in male silk glands in comparison to their female counterparts (Correa, 2021). Distinct gene expression profiles are observed in various silk gland types present within an individual spider. Notably, the major ampullate and minor ampullate silk glands exhibit comparable expression profiles (Chaw et al., 2021). Spider mites, known for their ability to produce silk fibres, possess fibroin genes that bear resemblance to the aciniform or cylindrical spidroins found in spider silk. This similarity is believed to play a role in the spider mites' notable high Young's modulus. The essentiality of the terminal regions of spidroins in the process of silk self-assembly is well-established. Despite variations in their sequence, it is noteworthy that the majority of spidroins exhibit comparable mechanisms for stabilisation, dimer formation, and tertiary structure (Arakawa, 2021).

Structural components of spider silk genes: Spider silk genes consist of various structural components, primarily spidroins, which are the main proteins found in spider silk fibers (Babb, 2017). Spidroins represent a distinctive assemblage of proteins that constitute the predominant constituents of spider silks. The synthesis of these proteins occurs within the silk glands and is accountable for the wide array of physical characteristics exhibited by various spider silk variants (Huemmerich, 2004). In addition to spidroins, there are other proteins associated with silk synthesis that have been identified through proteomic analysis (Frandsen, 2013). These proteins are encoded by differentially expressed genes in silk glands and are involved in silk production . The primary structure elements of spider silk proteins, such as repetitive regions, play a role in determining protein solubility and aggregation, which are important for silk assembly. The observation of significant diversity in silk genes, encompassing variations in both length and the order of repeat motifs, has been documented within individual arthropods. This finding implies the existence of shared mechanisms responsible for the creation and preservation of genes encoding structural proteins.

Importance of specific gene regions for tensile strength: Spider silk tensile strength is influenced by specific gene regions. The repetitive central region of spidroin genes, such as the H-fibroin repetitive domain, plays a role in silk tensile strength. Additionally, the amino acid composition of spidroins, including the presence of glycine and proline, can affect silk mechanics (Kono et al., 2021). The presence of particular motifs, such as poly-A and GA motifs, in the fibroin gene also contributes to the mechanical properties of silk. Furthermore, the terminal domains of spidroins, such as the amino- and carboxyl-terminal regions, are important for silk self-assembly and mechanical properties (Zhu, 2020). Overall, the combination of repetitive regions, specific amino acids, and terminal domains in spidroin genes are key factors in determining spider silk tensile strength.

Traditional genetic engineering techniques used for spider silk modification: Traditional genetic engineering techniques used for spider silk modification include the use of large DNA templates composed of many tandem repeats to produce proteins containing long tandem repeats. An alternative methodology entails the utilisation of genetic engineering to precisely modify the surface characteristics of spider silk particles, thereby imparting distinct charges or peptide tags that cater to particular applications such as drug delivery or cell docking (Weiss, 2020). Additionally, genetic engineering can be used to functionalize spider silk proteins with mucoadhesive properties, allowing for the development of mucoadhesive materials for wound dressings and drug delivery systems[5] Moreover, the application of genetic engineering involves the manipulation of the fundamental arrangement of genetic material, thereby facilitating the introduction of spider silk protein coding sequences into diverse organisms, such as bacteria, yeast, plants, mammalian cells, and silkworms, to achieve exogenous expression. The utilisation of conventional genetic engineering methodologies has facilitated the advancement and manipulation of spider silk proteins, thereby enabling their diverse applications in the realms of industry and biomedicine.

Novel Genetic Engineering Techniques for Targeted Modification

CRISPR-Cas9 system: Principles and applications in spider silk gene editing, the CRISPR-Cas9 system is a gene editing technology that has been widely used in various fields such as molecular biology, biomedicine, and medicine. It has also been applied in the editing of spider silk genes in silkworms, allowing for the production of high-performance fibers (Zou, 2021). The CRISPR-Cas9 system exhibits distinct advantages in comparison to alternative gene editing techniques, namely zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Saifaldedeen, 2020). Nevertheless, the prevalence of off-target effects poses significant constraints on its practical utility, prompting scientists to actively explore novel methodologies to enhance the precision of genome editing. One such approach involves the utilisation of CRISPR-FokI dead Cas9 (fdCas9), which is currently being investigated by researchers. The fdCas9 system is an innovative approach that involves the fusion of an inert Cas9 protein with a catalytic domain of FokI endonuclease. This unique combination yields a gene editing tool that exhibits remarkable specificity in its ability to precisely modify targeted genes.

Gene stacking and gene fusion strategies for enhancing tensile strength: The investigation of gene stacking and gene fusion methodologies has been undertaken to augment the tensile potency of spider silk. Jansson investigated the functionalization of the recombinant spider silk protein 4RepCT using coating and genetic fusion methodologies. The system devised by Xu et al. involved the manipulation of Bombyx mori, a species of silkworm, to enhance the production of spider silk on a large scale. This was achieved by substituting the gene responsible for the heavy chain of silkworm fibroin with the gene encoding major ampullate spidroin-1, a key protein found in spider silk. As a result, notable alterations in the mechanical properties of the silk fibre were observed (Xu, 2018). Liu et al. successfully expressed the dragline silk gene from Araneus ventricosus in both prokaryotic and eukaryotic systems, demonstrating the potential for spider silk protein production through genetic engineering (Wenli, 2011). The authors, Thatikonda et al., have presented a novel approach for the covalent conjugation of basic fibroblast growth factor (bFGF) to a partial spider silk protein. This innovative strategy has led to the formation of self-assembling silk-like fibres that exhibit preserved bioactivity (Thatikonda et al., 2018). Tang et al. synthesized a spider silk crystal protein gene that can be effectively expressed in fibrous plants, enhancing the strength of plant fibers.

Synthetic biology approaches in spider silk gene modification: The utilisation of synthetic biology enables the manipulation of spider silk genes through the implementation of diverse methodologies, including computational techniques, directed evolution methodologies, and a range of expression platforms (Poddar et al., 2020). These methodologies facilitate the examination of spider silk proteins, enabling researchers to advance their efforts in the field of synthetic spider silk engineering and production (Kucharczyk, 2018). By modifying the sequence of spider silk proteins, their affinity for drugs can be controlled, enabling them to be used as carriers for drug delivery.[1] Additionally, synthetic biology can be used to create hybrids of spider silk with organic nanoparticles, expanding the potential applications of spider silk in nanotechnology (Michalczechen, 2014). Moreover, it has been demonstrated that synthetic biology methodologies have achieved notable accomplishments in the efficient synthesis of extensive spider silk proteins within Escherichia coli. Consequently, this has resulted in the economically viable generation of silk proteins on a substantial scale (Yang et al., 2018). In general, the field of synthetic biology presents a formidable array of techniques and methodologies that can be employed to manipulate the genetic composition of spider silk and exploit the distinctive characteristics of this remarkable biomaterial for diverse purposes.

Enhancement of Tensile Strength through Genetic Modifications

Case studies on successful genetic modifications for enhanced tensile strength of spider silk: The application of genetic modifications has yielded positive outcomes in augmenting the tensile strength of spider silk. Recombinant spider major ampullate spidroin 1 (sMaSp1) variants featuring distinct repeat modules (24mer, 48mer, 72mer, and 96mer) were synthesised to exhibit enhanced water solubility. Consequently, these modifications resulted in notable enhancements in the mechanical characteristics of collagen-based films (Peng, 2022). The presence of several repetitive units and terminal domains in recombinant spidroins was found to be necessary for optimal tensile properties.[16] The influence of distinct amino acids within the elastic motif and temperature on the quantity of β-sheet structures exhibited by proteins was demonstrated (Taule et al., 2007). The fibres derived from preassembled films consisting solely of silk-like proteins demonstrated superior fibre alignment and increased tensile strength in comparison to fibres produced through spinning techniques. The optimisation of mechanical properties in spider silk, specifically the yield strain, has been the subject of discourse surrounding structural modifications (Brown et al., 2011).

Impact of gene modifications on the structural properties of spider silk : The empirical evidence suggests that alterations in genetic composition have a discernible impact on the inherent characteristics of spider silk. In a particular investigation, the genetic sequence encoding the heavy chain of the fibroin protein in silkworms (known as FibH) was substituted with the genetic sequence encoding the major ampullate spidroin-1 protein in spiders (referred to as MaSp1) within the species Nephila clavipes. This genetic manipulation led to the synthesis of a hybridised MaSp1 protein within the transformed cocoon shells. This replacement significantly changed the mechanical characteristics of the silk fiber, particularly the extensibility. Another investigation centred around the repetitive core regions of silk genes and ascertained that distinct amalgamations of genes yielded proteins exhibiting diverse mechanical characteristics, such as elevated elastic moduli and β-sheet content (Jaleel, 2020). Additionally, gene modifications in bacteria were found to impact the amount of β-sheet structures present in spider silk proteins, with temperature also playing a role in β-sheet formation. These findings highlight the potential for gene modifications to alter the structural properties of spider silk and provide insights for the development of new biomaterials (Brown et al., 2011).

Evaluation of spider silk tensile strength enhancement using various techniques: The evaluation of tensile strength enhancement in spider silk has been conducted using a multitude of techniques. In a particular investigation, a customised solid-state (SS) technique was employed, utilising a low molecular weight compound. The objective was to generate a water-soluble variant of a recombinant spider dragline silk (RSDS) protein. Wet spinning was utilised as a means to replicate the intricate process of spider spinning, yielding assemblies of spidroins exhibiting a fibril structure. This approach led to enhancements in the alignment, crystalline arrangement, and melting behaviour of the hierarchical structure (Marzouki et al., 2017). Furthermore, an investigation was conducted on the viscoelastic characteristics of dragline silk, encompassing both linear and non-linear behaviours. This analysis was performed utilising a Micro-Extension Rheometer. The silk material displayed a phenomenon known as strain-softening, where its response to strain resulted in a decrease in stiffness. This was followed by strain-stiffening, where the material's stiffness increased as the strain continued. Additionally, it was observed that the time required for stress relaxation increased as the strain applied to the silk increased (Dubey, 2020).

Future Directions and Challenges

Potential applications of genetically modified spider silk: Genetically modified spider silk has potential applications in various fields. It can be used in nanotechnology to create fibers, films, and composite materials by combining spider silk with organic nanoparticles. The functionalization of spider silk can be achieved through the implementation of modular strategies, thereby enabling the embellishment of silk with diverse motifs including fluorescent proteins, enzymes, and cell-binding ligands.[34] Within the realm of the biomedical sector, the utilisation of recombinant spider silk has exhibited considerable potential in the field of tissue engineering. This includes its application in the regeneration of skin, as well as its use in the engineering of various bodily tissues such as cartilage, tendon, bone, cardiovascular, and neural tissues (Nateghi et al., 2021). Spider silk proteins can also be used as conduit constructs, medical sutures, and bioinks for 3D printing. Additionally, genetically modified spider silk can be combined with magnetic iron oxide nanoparticles to create composite materials for drug delivery and hyperthermia-based cancer treatments. The controllable degradation of recombinant spider silk protein makes it advantageous for various applications, including biomedical use (Chun et al., 2016).

Ethical considerations and regulatory aspects of genetic engineering: Genetic engineering raises several ethical considerations and regulatory aspects. The development of biotechnology has the potential to fundamentally modify human functioning, which can disturb the equilibrium of nature for millions of years (Vermeersch, 2017). The ethical assessment of biotechnology requires a different approach to ethics due to its potential to cause harm and unanticipated consequences. Science and technology are not socially, politically, and ethically neutral, and certain technologies may require state intervention and have differential accessibility (Peng, 2022). The application of genetic engineering within the realm of conservation necessitates the evaluation and control of potential risks, while simultaneously inducing modifications to established methodologies, principles, and ethical considerations within the field of conservation. This consequently gives rise to a wider range of ethical apprehensions (Taule et al., 2007) The assessment of germline alteration and genetic enhancement necessitates the establishment of societal dispositions, wherein gene therapy is employed in a manner that upholds the inherent worth of the human species, while concurrently implementing appropriate measures of precautionary nature as dictated by the collective (Brown et al., 2011).

Overcoming technical challenges and limitations in gene modification: The main technical challenges and limitations in gene modification include the need for tissue-specific promoters to target specific cell types (Jaleel, 2020). The use of adenoviral vectors expressing Cre recombinase can alleviate the need for tissue-specific promoters, but the efficiency of infection of different cell types can vary. Another challenge is the lack of detailed knowledge of gene repair mechanisms at the molecular level, which affects the frequency of repair in oligonucleotide-directed gene repair. This technology has shown variations in repair frequencies and a lack of reproducibility in early experiments.[43] Additionally, the conventional genotype-environment interaction analysis is limited in detecting genotypes with ideal sensitivity in different environments (Jiang, 2023). Overall, these challenges and limitations hinder the development and application of gene modification techniques in various fields, including human gene therapy, crop improvement, and functional genomics.

3. Results and Discussion

The primary obstacles associated with patenting the genetic modification of spiders to enhance the tensile strength of spider silk encompass the requirement for further advancements and cooperative endeavours within the domains of biochemistry, molecular biology, and material science. These disciplines are crucial for attaining a comprehensive comprehension of the molecular attributes, functionalities, and spinning mechanism underlying spider silks (Bakhshandeh et al., 2021). The production of recombinant silk proteins is relatively easier compared to turning them into genuine silk fibers, which is proving to be very challenging (Chun, 2019). However, progress has been made in spinning synthetic spider silk fibers using recombinant proteins, although they are not as good as natural dragline silk (Vermeersch, 2017). The investigation of recombinant DNA techniques for the cloning of spider silk genes with the intention of expressing them in transgenic organisms is currently being undertaken as a potential substitute for traditional spider silk farming practises (Fait, 2019). The overarching objective entails the expression and purification of substantial quantities of recombinant silk proteins, alongside the development of a spinning methodology for synthetic silks that can be utilised in diverse industrial contexts (Sandler, 2020).

Summary of key findings and advancements in the field of spider gene modification for spider silk: Researchers have made significant progress in the field of spider gene modification for spider silk. By harnessing the power of genetic engineering, scientists have been able to produce spider silk in other organisms like bacteria and goats, expanding its potential applications. This breakthrough has paved the way for mass production of spider silk, which is known for its exceptional strength and versatility.

Implications of genetically modified spider silk for various industries: The applications of genetically modified spider silk are vast and promising for multiple industries. In the field of medicine, spider silk can be used as a biocompatible material for tissue engineering, wound healing, and drug delivery systems. In the textile industry, it can be used to create stronger and more durable fabrics. Additionally, the aerospace and defense sectors can benefit from spider silk's lightweight and high tensile strength properties for applications such as bulletproof vests and lightweight aircraft components.

Future prospects and potential impact on material science and biotechnology: The future prospects for genetically modified spider silk are exciting and hold immense potential for material science and biotechnology. Researchers are actively exploring ways to improve the production efficiency and scalability of spider silk, aiming for large-scale commercialization. As advancements continue, we can expect to see spider silk-based materials revolutionize industries such as construction, automotive, and even consumer goods. Furthermore, spider silk's biodegradable nature offers a sustainable alternative to conventional synthetic materials, contributing to a more environmentally friendly future.

4. Conclusion

The current legal landscape surrounding patenting genetic modifications, particularly those involving spider genes for spider silk, is a complex and evolving area. The criteria for patentability in this context are often cantered around novelty, inventiveness, and industrial applicability. To qualify for a patent, the genetic modification must demonstrate a significant departure from what is already known in the field. The modification should be inventive and not obvious to a skilled person in the relevant scientific community. Additionally, the modification should have a practical use or application, ensuring that it has industrial applicability. Ethical considerations also play a crucial role in the patenting of genetic modifications.

The potential benefits of spider silk, such as its exceptional strength and versatility, make it an attractive target for patenting. However, it is important to navigate the ethical implications carefully. One ethical concern revolves around the potential impact on biodiversity. Patenting spider genes for silk production may lead to the commercialization of these naturally occurring materials, potentially disrupting the delicate balance of ecosystems. Therefore, it is important to strike a balance between innovation and environmental sustainability. Another ethical consideration is access to the benefits of genetic modifications. Patents can create barriers to access and affordability, limiting the potential benefits for society.

It is crucial to ensure that patented genetic modifications do not hinder scientific progress, impede research, or prevent the development of life-saving therapies. The legal landscape surrounding patenting genetic modifications, particularly spider genes for spider silk, requires careful consideration of both patentability criteria and ethical implications. Balancing innovation, environmental sustainability, and accessibility will be key to fostering a future where genetic advancements can benefit humanity while respecting ethical boundaries..

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