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Spider Silk in Tissue Engineering

Biotech Blueprint

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Introduction

The biomedical applications of spider silk can be traced back to ancient Roman times, where silk fibre meshes were used to treat skin lesions.[1] Today, spider silk is commonly used as a suturing material in eye, intraoral, and lip surgeries due to its strength and extensibility.[2] However, new applications for spider silk have been identified in the field of tissue engineering, with potential uses ranging from meshes and coatings to scaffolding for tissue regeneration.[3] These applications take advantage of spider silk's biocompatibility and high tensile strength to promote cardiac tissue regeneration, peripheral axon myelination, bone regeneration, and cartilage growth.[3] In this article, the latest large-scale production methods and several promising applications of spider silk are reviewed.

Large-Scale Production Methods

Farming spiders for large-scale silk production is not feasible due to their territorial and cannibalistic behaviors.[4] As a result, silk-production systems generally utilize other organisms including bacteria (e.g. *Escherichia coli*), mammalian and insect cell lines, and even transgenic "spider-goats" that produce silk proteins in their milk.[5-7] Currently, the most common method for producing spider silk uses recombinant *E. coli* bacteria which express the genes for spider silk proteins.[6] Since these bacteria have shorter doubling times and do not require very sophisticated laboratory setups, this production method can be easily scaled-up.[6] However, the use of *E. coli* bacteria and other similar methods of artificial silk production must also utilize costly, patented manufacturing technologies to assemble silk fibres once secreted.[6] Because of this barrier, the only natural fibre currently produced on a large scale for commercial use is produced by the silkworm, *Bombyx mori*.[7] The similarity in high molecular weights and repetitive structures of silkworm proteins (i.e. FibH) and spider silk proteins (i.e. MaSp1) has prompted researchers to investigate the effects of replacing the *FibH* gene in *B. mori* with a synthetic gene containing the *MaSp1* gene sequence under the *FibH* promoter.[7] This process successfully produced MaSp1-containing silk, though it had significantly different mechanical properties from regular spider silk.[7] Although this study only replaced one gene segment to yield a hybrid spider silk fibre, the results introduce a potential approach for future production methods of purified spider silk.[7]

Peripheral Nerve Axon Regeneration

A large body of research has focused on the use of spider silk in axon regeneration following peripheral nerve injury and tumor infiltration.[8] Since donors for nerve transplants are rare and autologous transplantation can induce complications, artificial nerve grafting has become a logical solution to support the regeneration and remyelination of peripheral nerve axons.[8] However, artificial grafts made from non-resorbable materials can often lead to long term complications and chronic pain if not removed.[8] Moreover, other graft materials, though resorbable, cannot viably support the full length of the axon, disqualifying them for use for peripheral nerve regeneration procedures.[8,9] Spider silk's unique characteristics resolve the issue of biocompatibility and it has been successfully used to regenerate full peripheral nerve axons in large animals.[3,8,10] Furthermore, when effectively adhered to the silk graft, Schwann cells proliferated and remyelinated peripheral axons, an essential step in the conduction of peripheral nerve impulses.[9-11] These studies highlight spider silk's ability to enhance peripheral nerve regeneration and restore electrophysiological function, introducing it as a viable alternative that minimizes patient trauma.[11]

Bone and Cartilage Regeneration

It is estimated that at least 1 in 3 women and 1 in 5 men will suffer from an osteoporotic fracture, due to weak and brittle bones, in their lifetime.[12] Bone is naturally composed of both inorganic (e.g. calcium phosphate) and organic materials (e.g. collagen).[3] Though collagen has previously been studied for bone tissue engineering, it generally lacks mechanical stability when processed *in vitro* and loses integrity over time.[13] Spider silk has since gained interest in the field of bone regeneration due to its ability to bind and facilitate mineralization of calcium phosphate, which is necessary for bone growth and regeneration.[14] One study found that spider silk functionalized with bone sialoprotein is able to accelerate calcification, allow good adhesion, improve differentiation of human mesenchymal stem cells, and promote bone remodelling and osteoblast differentiation.[15] Silk has also been studied for its potential applications in inducing the proliferation of chondrocytes for cartilage regeneration.[16] Chondrocytes in articular cartilage help mechanically distribute loads across joints by secreting extracellular matrix to maintain and sustain the cartilage.[17] Scheller et al. found that the growth and proliferation of chondrocytes was similar when grown on spider silk and natural collagen.[16] Compared to collagen, spider silk has a relatively higher mechanical stability and tensile strength, as well as a similar chondrocyte regeneration rate. Thus, it may one day be used to treat osteoarthritis and other joint disorders.[3]

Heart Muscle Tissue Regeneration

Cardiovascular disease (CVD) is the second leading cause of death in Canada, with patients reporting a myocardial infarction (MI) having a death rate four times higher than healthy individuals.[18] Of all human organs, the heart is the least regenerative, meaning its capacity for repair and regeneration is extremely limited, particularly following an episode of MI.[19] Thus, a solution to improve tissue regeneration or compensate for the loss of cardiomyocytes is needed. Research surrounding the application of spider silk protein eADF4(κ16) as a scaffold for cardiac tissue engineering shows promising results.[20,21] Not only can cardiomyocytes bind to and proliferate effectively on this fibre, but spider silk also provides the benefits of high tensile strength, low immunogenicity, and biodegradability —key considerations for scaffold implantation.[21] Importantly, cardiomyocytes grown on eADF4(κ16) can effectively contract with natural frequency and rhythm without affecting their response to extracellular modulators (e.g. hormones).[20] Additionally, the biocompatibility and flexibility of spider silk allow for its effective application as a coating for carbon nanotubes used in tissue engineering. This use of carbon nanotubes allows for increased electrical activity, while the spider silk coating provides an extracellular matrix which is soft and can facilitate cell adhesion.[22] Together, the silk-coated nanotubes can have a variety of applications, such as being a cardiomyocyte scaffold for regenerative purposes or a component of a biosensor.[22] Ultimately, the use of spider silk provides a promising range of applications in regenerating cardiomyocytes and restoring heart function, which could help lower the burden of CVD and MI once tested and perfected.

Future Directions

Despite the many bioapplications of spider silk, *in vivo* studies of spider silk-based materials are still relatively limited. Studies exploring the characteristics and safety of spider silk show its ability to stimulate angiogenesis without amplifying inflammation —a vital quality in wound healing.[3] Meanwhile, *in vivo* studies of rats confirm the non-toxic degradation of silk proteins by macrophages; a process that can be adjusted via genetic engineering depending on the persistence of the application.[23,24] Following recent developments, further applications for spider silk are continuing to surface, including its use in coating breast implants, epidermal wound healing, and incorporation into films, foams, and hydrogels.[3] Currently, scalability poses the largest barrier to the wide-spread clinical use of spider silk. However, as researchers continue to explore new production methods, the age of silk-based therapeutics may not be far off.[3]

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