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Processing of recombinant spider silk proteins into tailor-made materials for biomaterials applications

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Spider silk has extraordinary mechanical properties, is biocompatible and biodegradable, and therefore an ideal material for biomedical applications. However, a drawback for any application is the inhomogeneity of spider silk, as seen for other natural materials, as well as the low availability due to the cannibalism of most spiders. Recently, developed recombinant spider silk proteins ensure constant material properties, as well as scalable production, and further the processing into morphologies other than fibres. Biotechnology enables genetic modification, broadening the range of applications, such as implant coatings, scaffolds for tissue engineering, wound dressing devices as well as drug delivery systems.

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Introduction

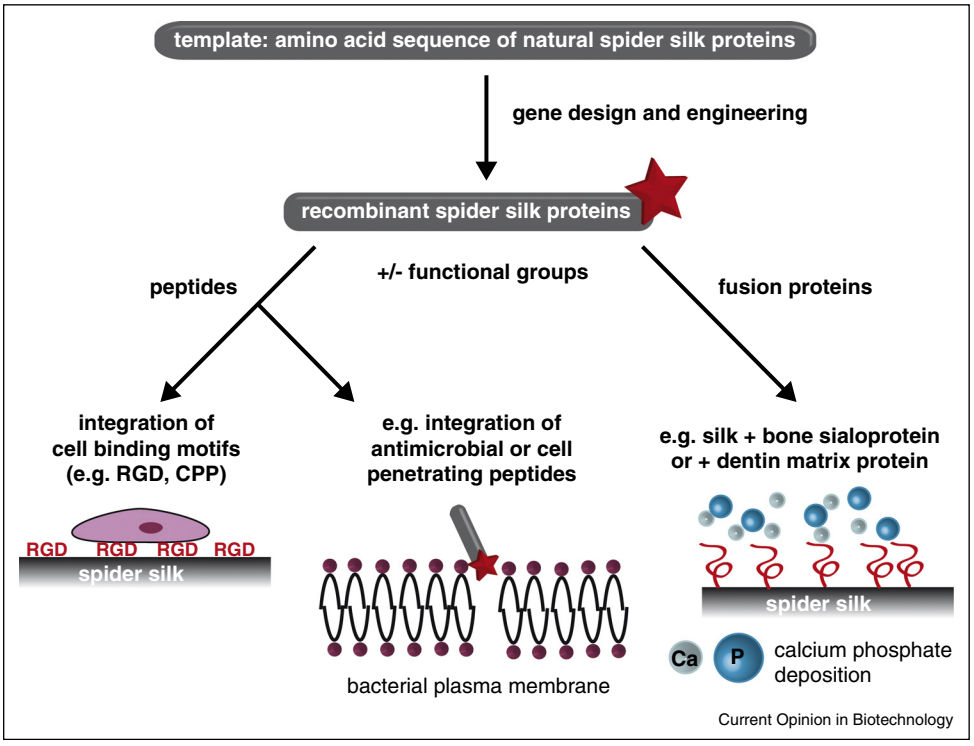
Spider silk fibres fascinate scientists especially due to their extraordinary mechanical properties [1]. The combination of strength and elasticity provides a toughness no other natural or synthetic fibre can achieve [2]. Additionally, spider silk is biocompatible, biodegradable and shows hypoallergenic properties suitable for biomedical applications [3,4].

Importantly, spider silk reflects an entire class of materials with different properties, since spiders can produce several types of silk (for an overview see Heidebrecht and Scheibel [5••]). The best characterised spider silk is the *Major Ampullate* (MA)/Dragline silk, constituting the outer frame of orb webs, serving also as a lifeline for the spider and which will be exclusively discussed herein [6]. Two classes of *Major Ampullate* spidroins have been identified in dragline fibres, called MaSp1 and MaSp2, which differ in proline content and hydrophobicity [7]. All *Major Ampullate* spidroins consist of a highly repetitive core domain, flanked by non-repetitive termini [8]. In the core domain distinct amino acid motifs (glycine-rich repeats and polyalanine blocks) enable secondary structures (random coil/helical and β-sheet) accounting for the mechanical properties of the fibre [8–11]. The terminal domains play an important role during storage of spidroins as a spinning dope in the gland and during the initiation of fibre assembly in the spinning duct [12–15].

For centuries, spider's webs have been successfully used to stop bleeding and to promote wound healing [16]. Recently, spider silk has been used as an artificial support for nerve regeneration [17,18]. Defects of peripheral nerves can be repaired by a composite nerve graft made of acellularized veins, spider silk fibres and Schwann cells (SC) mixed with matrigel (a solubilized tissue basement membrane matrix rich in extracellular matrix proteins). In adult sheep, spider silk enhanced Schwann cell migration, axonal-regrowth and remyelination including electrophysiological recovery in a 6.0 cm tibial nerve defect [19••]. Further, native spider silk fibres were tested as a braided microsurgical suture to substitute conventional materials in microsurgery and neurosurgery [20,21]. It was shown that the mechanical properties of braided spider silk sutures were superior to those of nylon, the current clinical gold-standard [21]. However, one major drawback of natural spider silk sutures is the inhomogeneity of the fibres, as seen with other natural materials, since differences in silk properties occur between individual spiders and even within single individuals upon environmental changes. Another drawback is the low availability of natural material due to problems in farming based on the cannibalistic behaviour of spiders [22,23].

Biotechnological production of spider silk proteins, as well as the development of silk processing techniques enabled the supply of engineered silk materials for biomedical applications, such as implant coatings, drug delivery systems or scaffolds for tissue engineering, which are reviewed herein.

Figure 1



Genetic engineering to achieve functional spider silk proteins.

Recombinant production of engineered spider silk proteins

In the last decades, several prokaryotic and eukaryotic hosts have been tested concerning recombinant production of spider silk proteins, as recently summarized in Heidebrecht and Scheibel [5••].

The benefits of recombinant spider silk proteins (RSSP) are the homogeneity of the starting material as well as the controllable processability into different morphologies, like films, hydrogels, particles or non-woven meshes for various applications [24–29]. Further, biotechnology enables genetic engineering to directly incorporate functional groups into the RSSPs (Figure 1) [24,25,30•].

The simplest genetic modification is the incorporation of individual amino acid residues with chemically specific side chains, like cysteine residues comprising thiol groups. A cysteine variant of the RSSP eADF4(C16) (based on the dragline silk protein ADF4 of *A. diadematus*) allowed the covalent coupling of peptides, enzymes or particles before and after silk processing into materials, demonstrating its potential for a broad range of applications [25,30•].

Engineered spider silk proteins comprising functional peptide sequences

For biomaterials applications, specific interactions between cells and the surface of a material are essential. Spider silk proteins can be exemplarily modified with cell adhesive peptides to improve cell binding, such as the integrin-binding motif RGD (Arg-Gly-Asp) (Figure 1) [28,30•,31,32]. Films made of eADF4(C16)-RGD showed a significantly improved attachment and proliferation of fibroblasts (BALB/3T3) in comparison to unmodified eADF4(C16) films [30•]. Another RSSP, 4RepCT, genetically functionalized with RGD or the cell binding peptides IKVAV, naturally found in the laminin α1 chain, or YIGSR, present in the β1 chain, were processed into fibres, foams and films [33,34]. The adhesion of all tested cell types (fibroblasts, keratinocytes, endothelial and Schwann cells) was significantly improved on RGD-modified in comparison to unmodified 4RepCT films. While only Schwann cells adhered better on matrices comprising the IKVAV-motif, no clear effect of YIGSR could be detected on any of the selected cell types [35].

Functionalizing spider silk proteins or silk hybrids with antimicrobial peptides could be a new approach to