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Review

Controlled assembly: A prerequisite for the use of recombinant spider silk in regenerative medicine? ☆

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ABSTRACT

Recent biotechnological progress has enabled the production of spider silk proteins, spidroins, in heterologous hosts. Matrices based on recombinant spidroins support stem cell growth and are well tolerated when implanted in living tissue, thus the material is highly attractive for use in regenerative medicine. However, the matrices made are far from natural silk in terms of mechanical properties and are either spontaneously assembled, which results in heterogeneous products, or spun from harsh solvents with the concomitant risk of harmful remnants in the final products. If we could mimic the spider's aqueous silk spinning process we would likely obtain a material that had reproducible and better characteristics and that more easily could be transferred to clinical practice. Herein, the knowledge of the spiders' silk production system and the prerequisites for artificial spinning and assembly of recombinant proteins are reviewed and discussed in a biomedical context.

1. Introduction

Silk drawn from spiders has successfully been employed in the regeneration of peripheral nerves and has performed well in biocompatibility studies [1–3]. This, combined with its renowned mechanical properties, makes spider silk a highly interesting material for use in regenerative medicine, but difficulties in producing the material have prevented industrial applications [4]. Since spiders are predators, cannibalistic and produce small amounts of silks, they are difficult to farm and therefore heterologous production of silk proteins is necessary. The nature of the spidroins, i.e. their large size, tendency to aggregate and repetitive sequence, has been a major obstacle for recombinant production [5,6]. Recent progress has resulted in a number of different approaches to obtain recombinant spidroins. These include the use of different hosts as well as different strategies for purification and assembly [4,7]. When purified, most procedures include dissolving the recombinant spidroins in denaturing agents before they are spun into fibers, while a few processes render spidroins that are able to self-assemble in aqueous solutions into fibers, films and/or foams [4]. Some of the recombinant silk produced has been used for cell culture and biocompatibility studies with encouraging results [8]. However, for commercial applications more defined and reproducible properties of the polymerized material are needed. Also, if we could find a way to mimic the spider's spinning procedure and employ that on recombinant spidroins, we could most likely enhance the properties of the material produced.

2. Spider silk in regenerative medicine

Cell culture studies using recombinant spider silk as matrices are so far relatively few but the results overall are encouraging, even though the silk produced varies a lot in amino acid sequence, mode of production and format [4,7]. Fibroblasts have successfully been cultured on three-dimensioinal (3-D) scaffolds made from recombinant spider silk [9–11] and human mesenchymal stem cells can be differentiated on recombinant spider silk in the presence of osteogenic medium [12]. Recently, recombinant spider silk films and foams were shown to support attachment and proliferation of rat neural stem cells (NSCs) as well as subsequent differentiation into neurons, astrocytes and oligodendrocytes [13] (Fig. 1). Apart from spider silk matrices, nanofiber assembled from peptides is the only defined 3-D material that has been shown to support NSC culture [14,15]. In contrast to the mechanical robustness and versatility (e.g. fibers, foams and films) of recombinant spider silk matrices [10,16], self-assembling peptides form hydrogels of entangled nanofibers of about a few micrometers in length [15], and may therefore be less useful for providing guidance for regenerating cells and support to damaged organs and tissues. Studies of the local tolerance of spider silk and spider silk replicas have also

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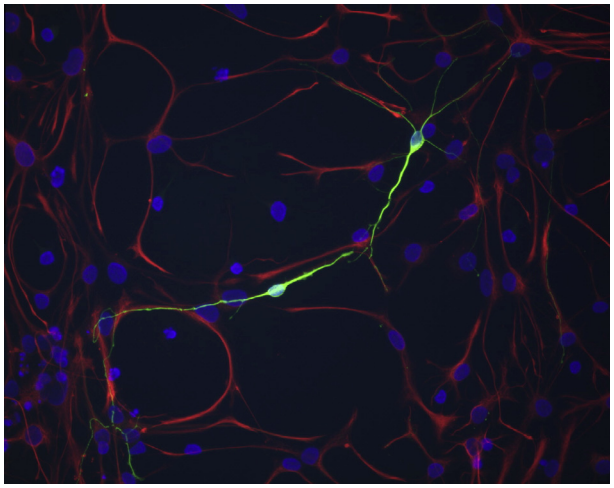

Fig. 1. Neural stem cells from day E15.5 rat embryos grown on recombinant spider silk (4RepCT) film in medium supplemented with bone morphogenetic protein (BMP) 4 for 7 days (168 h) and stained with anti-Neuronal Class III B-Tubulin (TuJ1; green), anti-glia fibrillary acidic protein (GFAP; red) and nuclei are visualized by DAPI-staining (blue) [13]. Photo: Michalina Lewicka.

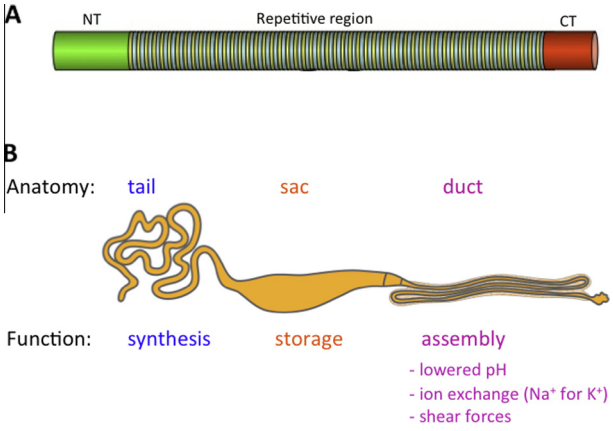

Fig. 2. (A) Schematic illustration of a typical spidroin composed of three distinct parts: a non repetitive N-terminal domain (~130 amino acid residues long), an extensive repetitive region (~3000 residues) and a C-terminal non-repetitive domain (~110 residues). (B) Spiders' spinning apparatus consists of a long tail where the spidroins are mainly produced, a sac where they are stored and an S-shaped duct where the spidroins are assembled into a solid fiber.

shown promising results [1–3,17]. The silk is degraded with time and does not evoke strong immunological reactions, perhaps as a consequence of the repetitive nature of the spidroins (see below).

In summary, spider silk can act as support for different clinically relevant cell types in vitro and has the potential to be used as an implantable material in regenerative medicine, but any clinical application of spider silk would require the generation of defined matrices with reproducible properties.

3. Spider silk production apparatus

Spider silk is produced in glands in the opisthoma (abdomen) of the spider. The most studied spider silk, the dragline silk, is produced in paired major ampullate glands and is composed of at least two different proteins, the major ampullate spidroin 1 (MaSp1) and MaSp2 [18,19]. There are several MaSp1 and MaSp2 genes in the spiders' genome, possibly as a result of the high demand of templates for spidroin synthesis [20–22]. The genes are in general large, repetitive and rich in guanine or cytosine and are as such difficult to sequence. The few described full-length genes have revealed a variable intron/exon organization between paralogues as well as orthologues [23]. However, they all encode spidroins in which relatively short non-repetitive terminal domains are separated by an extensive repetitive region [6,23–26] (Fig. 2A).

The major ampullate gland has three distinct parts: the tail where the spidroins mainly are produced, the sac where they can be stored in a highly concentrated soluble form (called the dope) and the duct where the spidroins are transformed into a solid fiber [18] (Fig. 2B). Some have suggested that lipids and/or glycoproteins are added to the spidroins in the gland [27,28], but it is not clear which cells produce these components. In the gland, the spidroins are believed to be stored in micelles where the terminal domains form the hydrophilic outer shell and the repetitive region is shielded in the center [29] or as a liquid crystalline feedstock [30]. The duct is lined by a cuticular intima, the function of which has not been determined, but it has been suggested to act as a hollow fiber dialysis membrane, enabling removal of water from the dope [31]. Along the gland and perhaps also the duct, pH decreases; according to one study, from 7.2 in the tail to 6.3 at the beginning of the duct [32], and according to another, from 6.9 in the sac to 6.3 in the third limb of the duct [33]. The ion concentration probably also changes; the potassium content in the silk gland increases fourfold along the spinning duct while the sodium content decreases to about one tenth in the fiber compared to the gland [34]. In the third limb of the duct, the solid fiber is formed as the dope suddenly narrows and pulls away from the walls of the duct [35,36].

4. Spidroin molecular events in the production apparatus

The amino acid sequences of the protein motifs in the repetitive region of the respective spidroins differ, and can be linked to differences in mechanical properties between different silks [37,38]. Thus, the repeats from, for example, an MaSp can easily be distinguished from a flagelliform spidroin repeat. In contrast, the sequence and structure of the terminal domains are conserved, and they are probably important for fiber formation rather than for the mechanical properties of the fiber. Both terminal domains lack known homologues and form homodimers of five α-helices but they are structurally unrelated to each other and to any other known protein [39]. The N-terminal domain (NT) is the most conserved part of the spidroin and confers solubility to recombinant spidroins at pH 7 and rapid fiber formation when pH is lowered to 6 [39]. It is mainly monomeric at pH > 7 and in the presence of salt [40–43] but dimerizes when pH is lowered below ~6.4, depending on the salt concentration [40,43]. The dimerization is accompanied with significant structural rearrangements, mainly by the medial movement of helix 3 that is a prerequisite for the dimer interface formation [41]. In vitro studies of the C-terminal domain (CT) have revealed that it is a constitutive homodimer of five α-helices [44,45], thus linking the spidroins together already at the synthesis. The CT also plays important roles in fiber formation. It is believed to confer solubility to quarternary structures (micells) during storage, while shear and altered salt concentrations in the duct induce fiber formation by the exposure of hydrophobic regions that could direct the β-sheet formation in the resulting fibers [44]. In contrast to what has been shown for the NT, the proton concentration seems to have little effect on the CT in the interval pH 12–6 as determined by circular dichroism [44].

In summary, in the spinning duct the CT exposes hydrophobic regions that may direct ß-sheet assembly in the repetitive region of the spidroins [44], and simultaneously, the NT dimerizes and interconnects the spidroins into endless protein chains [40,43] (Fig. 3). When the fiber is pulled the forces will be transplanted