Editorial

Silk fibroin in ocular surface reconstruction – what is its potential as a biomaterial in ophthalmics?

Damien G Harkin
School of Biomedical Sciences and Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland 4000, Australia, and Queensland Eye Institute, 41 Annerley Road, South Brisbane, Queensland 4101, Australia.
Tel: +61 7 3138 2552
Fax: +61 7 3138 6030
E-mail: d.harkin@qut.edu.au

Traian V Chirila
Queensland Eye Institute, 41 Annerley Road, South Brisbane, Queensland 4101 and Faculty of Health Sciences, University of Queensland, Herston, Queensland 4006, and Faculty of Science and Engineering, Queensland University of Technology, Brisbane, Queensland 4001, Australia.
Tel.: +61 7 3010 3368
Fax: +61 7 3010 3390
E-mail: traian.chirila@qei.org.au
Corresponding author

KEYWORDS: Silk fibroin, tissue engineering, regenerative medicine, biomaterials, eye disease, corneal transplantation.

"...in the second decade of the Asian Century it is perhaps befitting that a material of so much importance to the continent's cultural and economic history, should become the focus of cutting-edge biomedical research."

Hardly a month goes by within the scientific literature without some new material "*X*" being reported as a suitable material on which to grow cell type "*Y*", for the potential purpose of treating disease "*Z*". Thus when fibroin, a protein found in silk, was first proposed as a biomaterial for cell growth [1] it joined a long list of other materials of both natural as well as synthetic origin. Nevertheless, in the second decade of the Asian Century it is perhaps befitting that a material of so much importance to the continent's cultural and economic history, should become the focus of cutting-edge biomedical research. Sentiments aside, however, silk fibroin possesses quite a unique combination of properties which make it a promising candidate for repairing the eye and especially for treating damage to the cornea, the transparent window at the front of the eye.

Silks belong to the group of fibrous proteins, known also as 'structural' or 'fibrillar' proteins, which also includes collagens, elastins, keratins, and myosins. Specifically, the silk proteins contain highly repetitive sequences of amino acids and are stored in the organism as liquids before being configured into fibres when sheared/spun upon extrusion from the organism for the purpose of generating webs or cocoons. In nature, the silks are produced

solely by species of the phylum *Arthropoda*, and only by organisms in classes *Arachnida* and *Insecta*, and subphylum *Myriapoda*. Although variable in composition and structure, the silks serve the same purposes for all these organisms, i.e. protection, structural support, assistance in reproduction and foraging. By far, the most investigated silk is that produced by the domesticated silkworm, the larva of the *Bombyx mori* silkmoth. As part of the reproductive cycle, the larvae generate cocoons that are made of silk threads and constitute the predominant source for both the textile industry and research community. A silk thread is organized hierarchically starting from macromolecules of a protein called 'fibroin' to nanofibrils, then microfibrils and ultimately to brins. The fibroin monofilaments in brins are coated and glued together with another protein called 'sericin' to generate silk threads. Chemically, *Bombyx mori* silk fibroin (henceforth BMSF) is a composite of evolutionary, naturally designed block copolymers. As a protein, its primary structure is dominated by three amino acids: glycine, serine and alanine. The BMSF protein composite consists of a heavy-chain fibroin (~ 390 kDa) linked through covalent disulfide bonds to a light-chain fibroin (~ 25 kDa), to which a glycoprotein known as protein P25 is associated non-covalently. In the insect silk fibroins, a disulfide bond connects 6 molecules of heavy fibroin to 6 molecules of light fibroin, while a single molecule of P25 is associated by physical interactions to the complex [2,3]. The secondary structure of insect silks involves a mixture of conformations including α-helix, parallel and anti-parallel β-pleated sheets, random coil and cross β-pleated sheets, but not all of them have been identified in BMSF [4]. The distribution of these conformations determines the existence of at least two types of BMSF (known as types I and II), and ultimately dictates the evolutionary value of the silk for the organism that generated it. BMSF may also posses a tertiary structure due to a combination of extensive hydrogen bonding, high crystallinity and predominantly hydrophobic characteristics.

Silk became a biomaterial long before the latter term was introduced in the scientific idiom. Indeed, the silk produced by *Bombyx mori* has been extensively used to make surgical sutures. Owing to sensitizing reactions which are attributed to the presence of sericin, currently the latter is removed from the silk sutures. As such, BMSF can be easily isolated from sericin and has been proposed as a biomaterial on its own about two decades ago [1]. There has been an increasing interest ever since in the use of silks, mainly in the form of BMSF, for biomedical applications [5,6]. As a biomaterial, BMSF displays indeed an impressive array of favourable properties including high biocompatibility, good mechanical properties, and permeability to oxygen and to large molecules. In the living tissues, BMSF biodegrades slowly leading to non-toxic residues. It can be processed into a large variety of material types (films, membranes, fibres, nets, sponges), at low cost and without the involvement of toxic solvents. Finally, and importantly, BMSF can be made into transparent materials.

"The remarkable transparency of silk fibroin membrane makes this material an obvious candidate for use as a biomaterial in corneal reconstruction."

Fibroin membranes are highly transparent sheets of protein formed by allowing thin layers of fibroin solution to dry in contact with a flat surface, followed by stabilisation by treatment in polar solvents or using water vapour annealing techniques, which generally enhance the content of β-sheet conformations. This process can be performed simply in standard Petri dishes, but ultra-thin membranes measuring a few microns in thickness can be reliably produced to a high degree of accuracy using tape-casting techniques. The remarkable transparency of the silk fibroin membranes makes this material an obvious candidate for use

as a biomaterial in corneal reconstruction. Moreover, the membranes are sufficiently permeable to allow the translocation of nutrients and other factors deemed important for establishing and maintaining normal tissue structure and function. Further increases in permeability can be achieved by preparation of membranes in the presence of other materials such as poly(ethylene) oxide which promote the formation of pores within the membrane. These procedures had been well established by others at the time of commencing our studies [7], but surprisingly had never been applied for the purpose of growing corneal tissue.

The feasibility of growing corneal cells on BMSF membranes was first reported by our group using primary cultures of corneal epithelial cells grown from human donor eye tissue [8, 9]). Subsequent studies by us and others have confirmed that fibroin membranes support the growth of cells isolated from all three cellular layers of the cornea including stromal cells [10, 11] and corneal endothelial cells [12]. Thus it is conceivable that a full-thickness, corneal tissue substitute might be generated by combining multiple layers of fibroin membrane with appropriate cell types grown on each according to their respective location within the cornea (i.e. epithelial layer on top, endothelial layer on the bottom and several stromal layers in between). Importantly, primary cultures of corneal epithelial cells grown on BMSF retain a similar number of progenitor cells to those observed in cultures grown on donor amniotic membrane, the current standard substrate for transplantation of cultured corneal epithelial cells [13]. Likewise, the phenotype of corneal stromal cells has been found to be unaffected by cultivation on this foreign material [11]. Moreover, preliminary studies of biocompatibility in a pre-clinical model have demonstrated that fibroin membranes appear to be well tolerated when implanted within the corneal stroma [14]. It must be said, however, that similar results to those outlined above have been achieved using other materials including modified formulations of collagen [15]. So what benefits, if any, does silk fibroin afford above those displayed by collagen and especially more complex and highly utilized materials such as amniotic membrane? Ironically, the ultimate benefits of fibroin membrane may be that *Bombyx mori* silk fibroin inherently lacks recognisable cell-binding motifs that are normally required for cells to interact with collagen and other extracellular matrix components.

"In other words, BMSF can be considered to be akin to a blank canvas onto which any required combination of materials can be applied in order to elicit the desired response in cultured cells."

The absence of apparent cell-binding motifs is an intriguing aspect of cellular interactions with fibroin. In the absence of exogenous cell attachment factors such as those normally supplied by inclusion of serum in the culture medium, corneal epithelial cells generally adhere poorly to BMSF membranes and indeed four-fold fewer cells bind to this material than to amniotic membrane under serum-free conditions [13]. BMSF, like culture plastic, therefore appears to be relatively inert, with the type and level of cell attachment being dependent upon the presence of exogenous materials. For example, improved attachment of corneal endothelial cells to silk fibroin has been achieved through coating with collagen IV [12]. In other words, BMSF can be considered to be akin to a blank canvas onto which any required combination of materials can be applied in order to elicit the appropriate cellular response. Such an arrangement is particularly attractive in the case of growing cultures from stem cells since their developmental fate is often highly dependent upon the surrounding milieu of extracellular materials. By comparison, proteins such as collagens with their inherent cell-recognition motifs, present instructions to cells that may or may not be

appropriate to a particular cell type. Alternatively, synthetic biomaterials such as those based for example on polylactides, are often less amenable to decoration with required exogenous cell attachment factors.

In conclusion, BMSF displays significant potential as a biomaterial for repairing the cornea. The transparency of BMSF membranes and their amenability to treatment with cell-attachment factors of choice are particularly positive attributes. Favourable responses to BMSF have been observed *in vitro* using all three corneal cell types and preliminary studies of biocompatibility have also yielded positive results. Based upon this success to date, pre-clinical studies of safety and efficacy for corneal cells transplanted on BMSF membranes are therefore expected in the near future.

Acknowledgements

The authors wish to thank Professors I Schwab and D Kaplan for initially encouraging our interest in silk fibroin. We also wish to acknowledge several members of our past and present corneal research teams: Dr Z Barnard, Dr L Bray, Prof L Hirst, T Hogerheyde, Dr K George, T Giovenco, J Lai, Dr F Li, Dr P Madden, C Rayner, Dr N Richardson, Dr S Stephenson, Dr S Suzuki, A Shadforth, J Young and Dr Zainuddin.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Minoura N, Tsukada M, Nagura M. Physico-chemical properties of silk fibroin membrane as a biomaterial. *Biomaterials* 11, 430-434 (1990).

2 Inoue S, Tanaka K, Arisaka F, Kimura S, Ohtomo K, Mizuno S. Silk fibroin of Bombyx mori is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain and P25, with a 6:6:1 molar ratio. *J. Biol. Chem.* 275, 40517-40528 (2000).

3 Sehnal F, Žurovec M. Construction of silk fiber core in Lepidoptera. *Biomacromolecules* 5, 666-674 (2004).

4 Craig CL. Evolution of arthropod silks. *Annu. Rev. Entomol.* 42, 231-267 (1997).

5 Altman GH, Diaz F, Jakuba C *et al*. Silk-based biomaterials. *Biomaterials* 24, 401-416 (2003).

6 Vepari C, Kaplan DL. Silk as a biomaterial. *Prog. Polym. Sci.* 32, 991-1007 (2007).

7 Rockwood DN, Preda RC, Yücel T, Wang X, Lovett ML, Kaplan DL. Materials fabrication from Bombyx mori silk fibroin. *Nat. Protoc.* 6, 1612-1630 (2011).

8 Chirila T, Barnard Z, Zainuddin, Harkin D. Silk as substratum for cell attachment and proliferation. *Mater. Sci. Forum* 561-565, 1549-1542 (2007).

9 Chirila TV, Barnard Z, Zainuddin, Harkin DG, Schwab IR, Hirst L. Bombyx mori silk fibroin membranes as potential substrata for epithelial constructs used in the management of ocular surface disorders. *Tissue Eng. Part A* 14, 1203-1211 (2008).
10 Lawrence BD, Marchant JK, Pindrus MA *et al.* Silk film biomaterials for cornea tissue engineering. *Biomaterials* 30, 1299-1308 (2009).
11 Bray LJ, George KA, Hutmacher DW, Chirila TV, Harkin DG. A dual-layer silk fibroin scaffold for reconstructing the human corneal limbus. *Biomaterials* 33, 3529-3538 (2012).
12 Madden PW, Lai JN, George KA, Giovenco T, Harkin DG, Chirila TV. Human corneal endothelial cell growth on a silk fibroin membrane. *Biomaterials* 32, 4076-4084 (2011).
13 Bray LJ, George KA, Ainscough SL, Hutmacher DW, Chirila TV, Harkin DG. Human corneal epithelial equivalents constructed on Bombyx mori silk fibroin membranes. *Biomaterials* 32, 5086-5091 (2011).
14 Higa K, Takeshima N, Moro F *et al.* Porous silk fibroin film as a transparent carrier for cultivated corneal epithelial sheets. *J. Biomater. Sci.* 22, 2261-2276 (2011).
15 Bentley E, Murphy CJ, Li F, Carlsson DJ, Griffith M. Biosynthetic corneal substitute implantation in dogs. *Cornea* 29, 910-916 (2010).