Electrospun silk fibroin/gelatin composite tubular matrices as scaffolds for small diameter blood vessels regeneration

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Statement of Purpose: Cardiovascular diseases are presently a leading cause of death. The great outcomes obtained by synthetic large diameter vascular prostheses have not been replicated for small diameter blood vessels (Ø < 6 mm). Indeed, compliance mismatch between grafts and natural blood vessels and thrombogenicity of synthetic materials led to graft occlusion.

The aim of this work was to develop an innovative small diameter vascular graft (SFgel) composed of an electrospun silk fibroin (SF) tubular structure, coated by a crosslinked gelatin gel (gel) [1]. SF tubular grafts have previously demonstrated adequate properties as vascular grafts [2,3], also with a collagen coating [4]. As collagen is not devoid of some risks of immunogenicity [5], the use of gelatin as a coating material can evade this problem, still promoting cell adhesion. Furthermore, our proprietary method for crosslinking gelatin occurs in mild conditions, giving cytocompatible gels that show good stability (up to 50 days) at 37°C [1].

Methods: SF tubular structures (ID = 6 mm) were obtained by electrospinning on a rotating mandrel, as previously described [2]. Gelatin A (from porcine skin, Sigma) was crosslinked by Michael-type addition reaction with methylenebisacrylamide (MBA) [1].

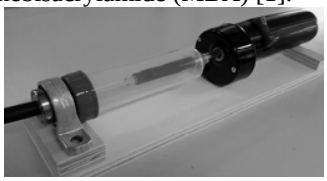

Figure 1: the home-made coating system.

A home-made system for coating SF tubular structures with the crosslinked gelatin was designed and fabricated (Fig. 1). Tubular SF samples were mounted on a rotating glass mandrel, dipped in gelatin/MBA solution and placed in the coating apparatus to complete the crosslinking reaction (50°C, 24h). SEM analyses were performed to investigate the SF nanofibrous structure and coating homogeneity. Swelling and weight loss behavior was investigated at 37°C up to 25 days in water. Mechanical tensile properties of wet SFgel and SF, used as control, were assessed at 37°C by circumferential, cyclic, creep/recovery, and stress relaxation/recovery tests. From the cyclic tests, compliance values (between 80 and 140 mmHg) of SF and SFgel were obtained. *In vitro* tests were carried out with the L929 murine fibroblast cell line. Indirect cytotoxicity test was performed on the eluates of SFgel and SF samples, by dipping them in DMEM for 1, 3, 7 days. Direct cytocompatibility test was performed up to 7 days, by investigating cell viability with MTT assay.

Results: Electrospun matrices had a random nanofibrous structure with an almost uniform fiber size (755 ± 174 nm). SFgel tubular structures were successfully obtained with a homogeneous distribution of crosslinked gelatin coating (Fig.2), which proved stability in distilled water up to 25 days at 37°C.

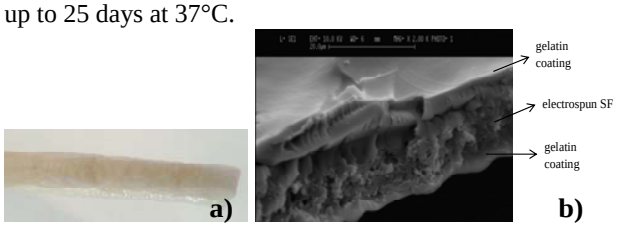

Figure 2: a) macro image of the SFgel composite tubular scaffold; b) SEM image of the cross-section of SFgel

Circumferential tensile tests showed higher stiffness for SF than SFgel structures (1.82 vs 0.58 MPa, respectively). Both SF and SFgel proved compliance values similar to autografts and higher than synthetic grafts commonly used (Fig. 3).

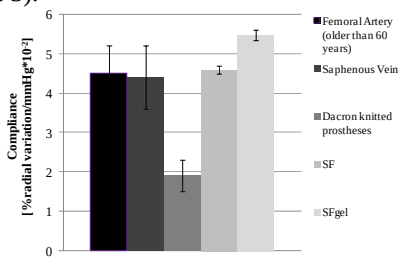

Figure 3: compliance values of autologous and synthetic grafts, SF and SFgel tubular structures.

Creep and stress relaxation tests evidenced a higher contribution of the viscous component for SFgel compared to SF alone.

In vitro cytotoxicity test revealed no release of cytotoxic substances. *In vitro* cytocompatibility test showed, at 7 days, a cell viability significantly higher on SFgel ($p<0.05$) than on SF (Fig. 4).

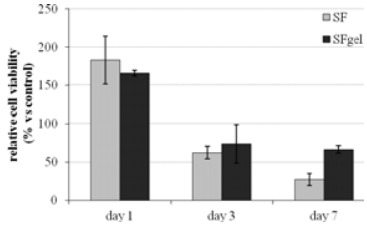

Figure 4: cytocompatibility test: MTT assay results

Conclusions: Novel 6 mm I.D. composite tubular structures were successfully obtained by coating electrospun SF tubes with a homogeneous layer of crosslinked gelatin. Indeed, SFgel mechanical properties, in particular compliance, were similar to clinically used autografts. In addition, the gelatin coating was able to improve the biological characteristics of SF.

References:

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