Silk sericin hydrogels as a promising sustainable platform for skin tissue engineering

Anabela Veiga[1,2,3], Sara Baptista-Silva[1], Olivia Foster[4], Raquel Costa[1,5], Viviana Ribeiro[1], Filipa Castro[2,3], Fernando Rocha[2,3], David L. Kaplan[4]* and Ana Leite Oliveira[1]*

[1] CBQF-Centro de Biotecnologia e Química Fina–Laboratório Associado, Universidade Católica Portuguesa, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto
[2] LEPABE-Laboratory for Process Engineering, Environment, Biotechnology & Energy, Department of Chemical Engineering, Faculty of Engineering of the University of Porto, R. Dr. Roberto Frias, 4200-465 Porto, Portugal
[3] ALiCE-Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[4]Tufts University, Department of Biomedical Engineering, 4 Colby St., Medford, MA 02155, USA
[5]Department of Biomedicine, Biochemistry Unit, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

s-anveiga@ucp.pt

Introduction

Silk sericin (SS) as a protein-rich natural material has traditionally been discarded during silk processing, generating high chemical and biological oxygen demand in the environment as well as contamination of waters. In recent years there has been a growing interest in the recovery and utilization of sericin due to its economic, ecological impact and beneficial properties such as antioxidant, moisturizing ability and mitogenic effect on mammalian cells [1].

In particular, SS-hydrogels have been studied for skin-tissue engineering (TE) owing to their hydrophilic nature and gelling properties. However, most systems utilized to generate hydrogels involve harsh and complex chemistries that can compromise the biological behaviour and gelling kinetics. Our team has developed a SS-horseradish peroxidase (HRP) enzymatic crosslinked hydrogel, using a simple and natural approach, for utilization in wound healing (WO 2018/011732 A1) (Figure 1 A), promoting cell viability (Figure 1 A1) and complete skin regeneration after 21 days post-wounding, when applied in an *in vivo* diabetic wound model (Figure 1 A2). Moreover, the hydrogels also promoted collagen deposition which may boost re-epithelialization (Figure 1 A3) [2].

The incorporation of nanoparticles (NPs) in protein matrices is a popular methodology for obtaining systems that are tunable in terms of properties, as well as multifunctional. These systems can also result in enhanced mechanical and biological properties. In this context, the incorporation of NPs in the SS-hydrogels is a promising approach for skin-TE. Recent efforts in the field of particle production are focused on applying scalable methods to improve batch-to-bath reproductivity, homogeneous properties and environmentally-friendly processes

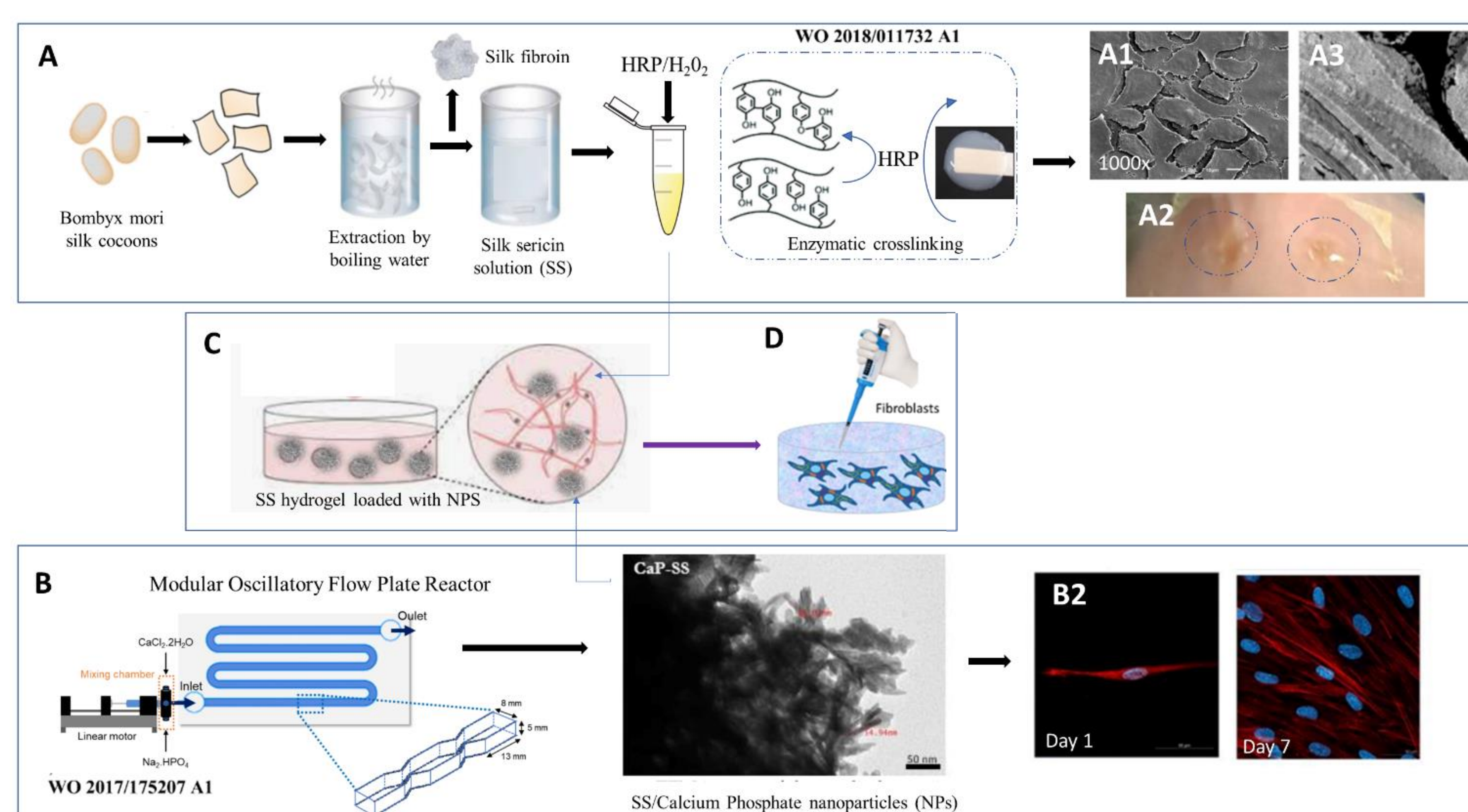


Figure 1. A) Sericin extraction process and crosslinking mechanism with HRP/H_2O_2: A1) L929 fibroblast viability and morphology after 7 days of culture, A2) skin regeneration in wounds induced in diabetic mice 21 days post-wounding, A3) Orientation and distribution of collagen fibrils. B) Production of HAp/SS NPs using a MOFPR: A1) HDFs viability and morphology after 7 days of cell culture. C) combination of A and B to develop a novel SS hydrogel loaded with functional NPs for skin-TE.

Methods and Results

Experimental set-up

The precipitation of NPs was carried out in batch in a modular oscillatory flow plate reactor (MOFPR) (WO 2017/175207), by mixing equal volumes of a solution $CaCl_2.2H_2O$ (Merck, 99.5%) (0,02 M) (pH ≈ 5.84) with and without SS (0.1 g/L) (HAp and HAp/SS) to a Na_2HPO_4 (Sigma-Aldrich, 99.0%) (0.012 M) (pH ≈ 8.52) solution (initial Ca/P molar ratio = 1.67). The reactor is provided with smooth periodic constrictions that are present in two parallels faces of the rectangular cross section tube.

Sericin hydrogel and NPs incorporation

Boiling water extraction was used to obtain a SS solution, followed by concentration and HRP/H_2O_2 crosslinking (Figure 1A). An initial screening with HAp and HAp/SS particles incorporated in the SS-hydrogel at different concentrations (0.1 (1), 0.5 (2) and 0.05 (3) mg/mL) was performed using alamarBlue cell proliferation assay (AB) after seeding with human dermal fibroblasts (HDFs) (Figure 2).

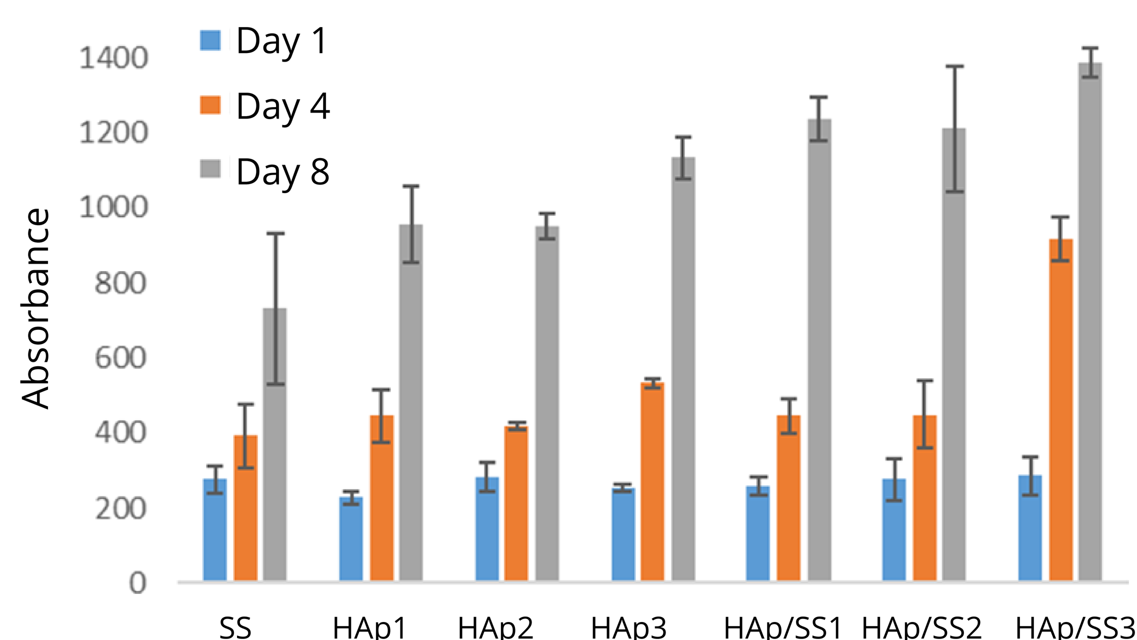
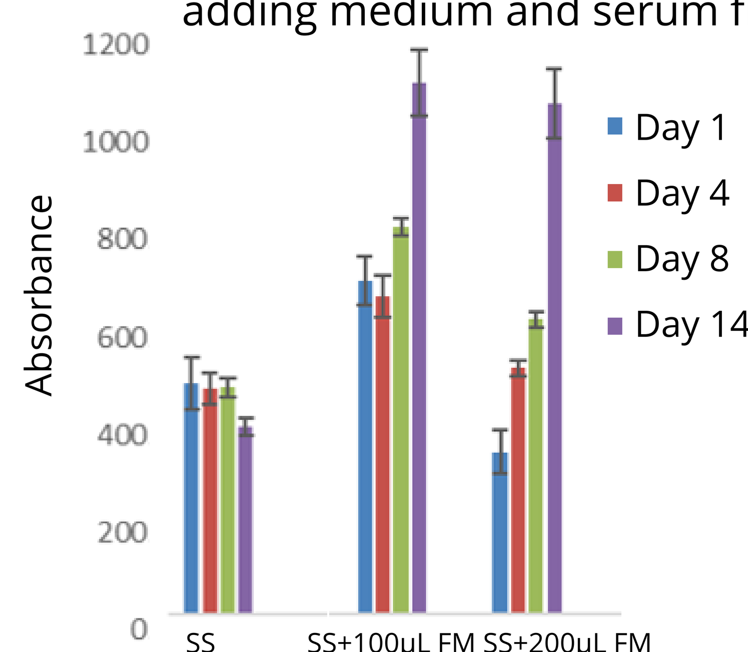


Figure 2. Metabolic activity of HDFs cultured in SS-loaded hydrogels.

HDFs embedding

Cell incorporation in the hydrogels was achieved by adding 6×10^5 HDFs per gel. The effect of adding medium and serum free medium (FM) was assessed using AB and Immunostaining.



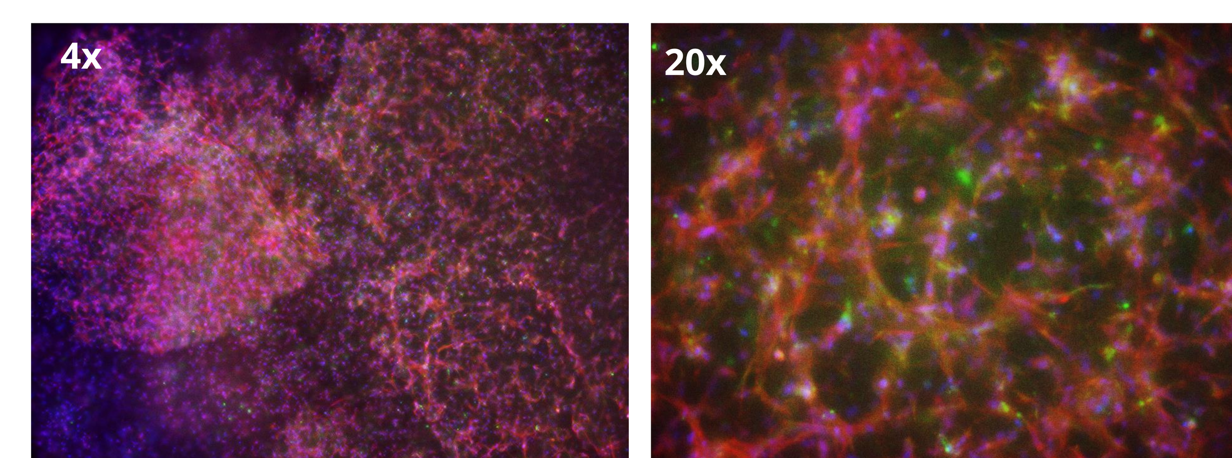


Figure 3. Cell viability and proliferation after cell embedding in the SS hydrogels (red: phalloidin, green: vimentin, blue: DAPI).

Conclusions

In vitro tests using human dermal fibroblasts (HDFs) showed that the SS presence in the particles improved cell viability and proliferation for all concentrations studied and that the lowest concentration was associated with the best performance (0.05 mg/mL). Moreover, the developed hydrogels can be successfully used to incorporate cells, using FM to improve the viability over time. Current work is focused on the optimization of the SS-loaded hydrogel for the development of a full-thickness in vitro skin model.

References

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