Novel injectable *in-situ* forming hydrogel for the treatment of diabetic ulcers

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Introduction/Resume

Lower extremity diseases are twice common in diabetic compared with nondiabetic patients, which is known to be related to impaired production of insulin (1). Ulcers frequently become infected, causing great morbidity, and ultimately lower extremity amputation. This work suggests a simple and practical strategy to rebuild the function of tissues in diabetic ulcers using an *in situ* formed sericin hydrogel, for healing and tissue regeneration, enabling cell recruitment and proliferation. This enzyme-mediated crosslinking hydrogel is capable of gelling *in situ* by crosslinking polymer hydroxyphenyl groups using horseradish peroxidase (HRP). This system is able to induce fast gelation (~2 min) at physiological temperature (Patent Application (2)). The prepared hydrogels are mainly of amorphous conformation and transparent appearance, stable in PBS (pH 7.4) for 17 days, up to 7 days in protease XIV biological concentration (3.5 U/mg), and 4 days under acute and chronic physiological pH values. These hydrogels will incorporate insulin which is aimed to be released at the diabetic wound site in an attempt to accelerate tissue healing and regeneration. With this, new insights might be found to help explain the connection between diabetes and poor healing. Besides tackling one of today's major public health issues, this work promotes the re-use and valorization of a textile industrial by-product, sericin, by its integration in the biomedical arena.

Methods

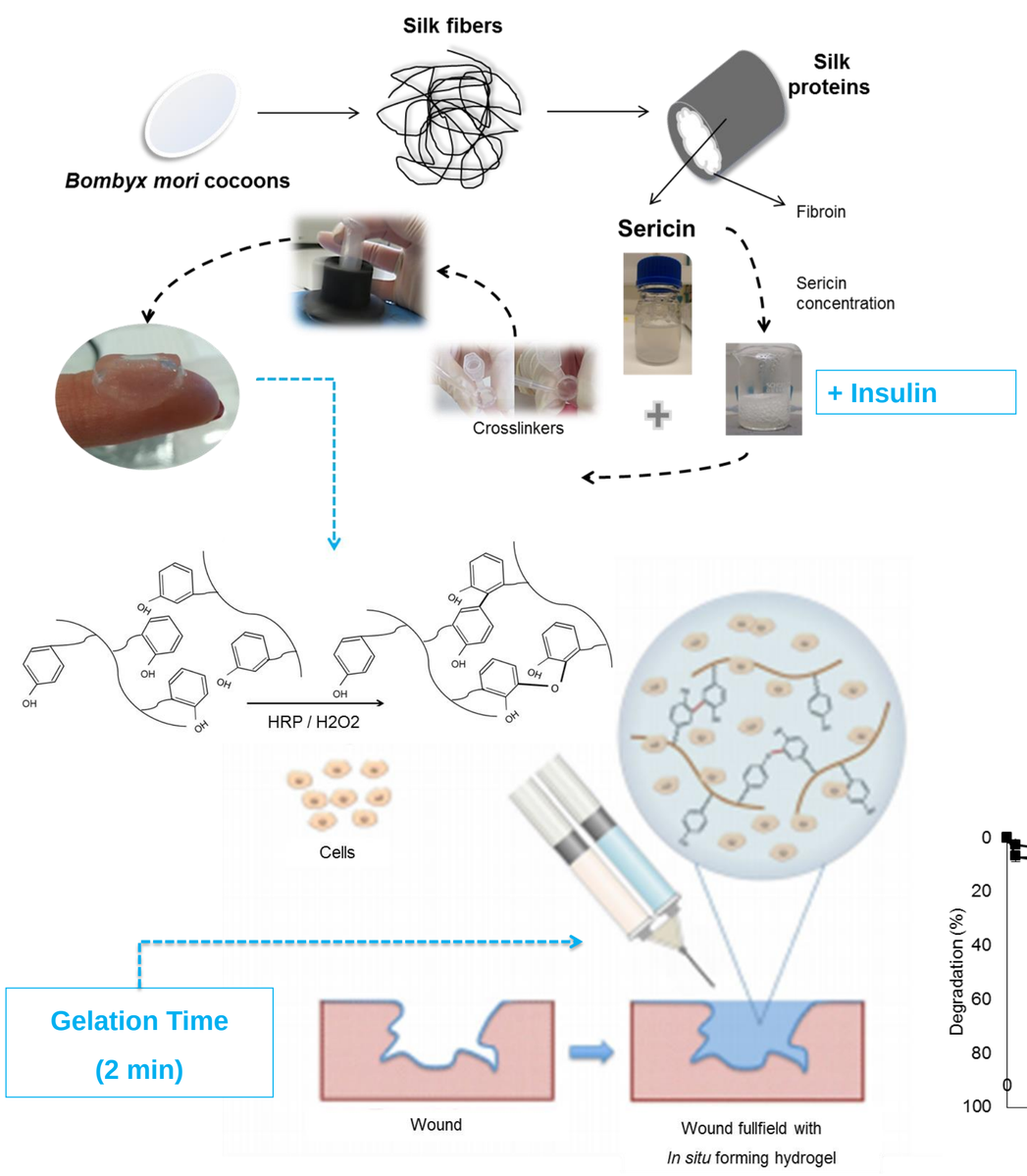

Figure 1. Schematic illustration of *in siu* enzimatic-mediated crosslinking silk sericin hydrogels.

Results

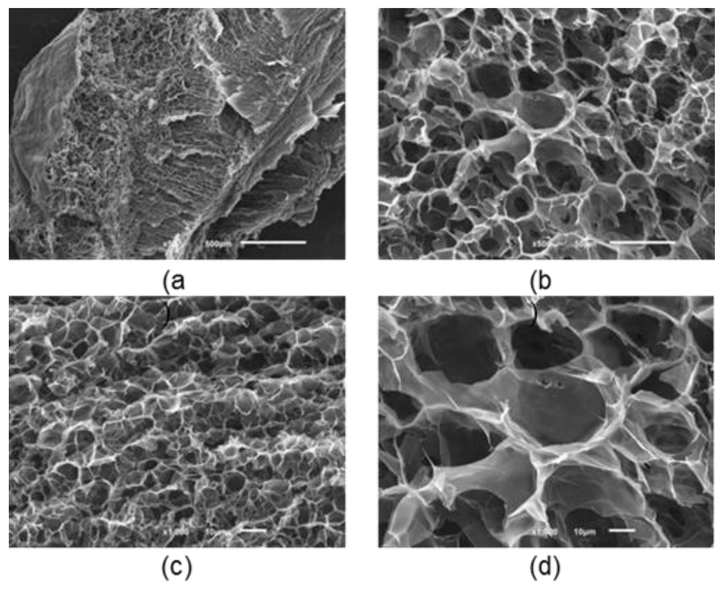

Figure 2. SEM micrographs of lyophilized sericin-based hydrogels at different resolutions (a) and (b) at 500x; (c) and (d) at 1000x.

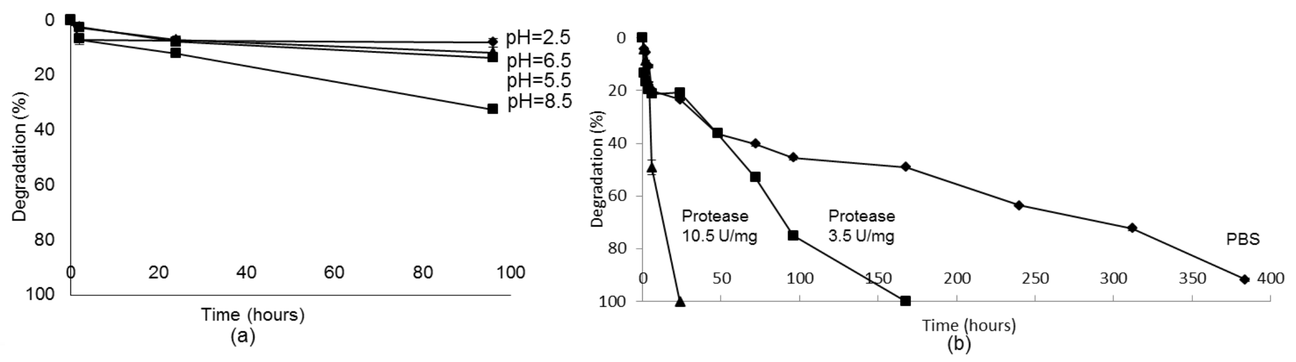

Figure 3. Degradation profile of sericin-based hydrogels (a) pH degradation during 96 hours and (b) enzymatic degradation with protease XVI during 400 hours, both at 37 ºC.

Discussion/Conclusions

In this study, silk sericin from *Bombyx mori* cocoons was extracted, purified and concentrated by a procedure previously developed and optimized in the group (Figure 1). The enzyme mediated crosslinked sericin hydrogels was developed by the addition HRP and hydrogen peroxide at different concentrations of until achieving the ideal gelation time of 2 min. The formulations in this gelation time range and exhibiting better mechanical properties were selected for subsequent studies. The morphology and microstructure of the sericin-based hydrogel were examined by SEM (Figure 2). From the obtained images, sericin hydrogel presented an homogeneous and highly porous structure, with pore size around 50 µm. The pores' diameter suggest that sericin hydrogel may be broadly applicable for repair of different types of tissues (3). The enzymatic cross-linked sericin hydrogels were stable in PBS (pH 7.4) for 17 days, up to 7 days with Protease XIV from *Streptomyces griseus* at physiological concentration of 3.2 U/mg, and 4 days under acute and chronic physiological pH values (Figure 3). The degradation profile can be modulated by the amount of crosslinker in the structure, which indicates that this system can constitute an adequate carrier for the delivery of biologically relevant molecules such as insulin or other therapeutic agents.

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