

In Situ Antioxidant Activity of an Enzymatically Crosslinked Sericin Hydrogel for Healing of Chronic Wounds

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Introduction

Chronic wounds are one of the most frequent complications associated to diabetes mellitus, contributing to a high morbidity. Its long healing process is mostly associated with the overproduction of reactive oxygen species (ROS) and may result in ulceration and serious infections [1]. In this context, the choice of the adequate dressing is of great importance and it needs to reflect the requirements of a particular wound according to its stage. Recently, natural-based hydrogel formulations are gaining increasing attention since they can provide control over shape, physical properties and encapsulated cargo, offering the possibility that more advanced products will soon reach the clinic [2].

In this work, a novel *in situ* crosslinked sericin-based hydrogel for wound healing was prepared by a simple methodology using horseradish peroxidase (HRP). The hydrogel was characterized in terms of its thermal stability, fluid interaction and antimicrobial properties. The antioxidant effect was assessed *in vitro* and *in vivo*, after *in situ* application on a diabetic mouse model.

Experimental Methods

Silk sericin extracted from *Bombyx mori* cocoons was used to prepare a fast gelling hydrogel by HRP-mediated crosslinking [3]. The hydrogel was characterized by differential scanning calorimetry (DSC) analysis and its transparency evaluated by colorimetry. The hydration degree and the degradation behavior were also assessed. The antimicrobial activity of sericin solution was determined using an inoculum of 0.5 McFarland (1.5×10^8 CFU/mL). Antioxidant potential was quantified *in vitro* by ORAC assay under physiological protease degradation and investigated *in vivo*. An excisional wound-healing model in genetically-induced diabetic db/db mice was performed to evaluate the effect of sericin-based hydrogel in chronic wounds. Animals were randomly divided into three experimental groups (n=6 per group) as follows: Control (wounds were left untreated); Tegaderm group (wounds covered with Tegaderm); and sericin hydrogel group (sericin hydrogel covered with Tegaderm). Superoxide dismutase (SOD) and catalase activity were quantified to clarify if sericin hydrogel could affect the endogenous protective mechanisms against oxidative stress. Transmission electron microscopy (TEM) was performed to analyse collagen fibres at the wound bed treated with sericin hydrogel.

Results and Discussion

HRP-mediated silk sericin hydrogel was developed with a gelation kinetics of ~2-3 minutes, in agreement to previous rheological study [3]. The hydrogel presented a high degree of transparency. This study evaluates, for the first time, the antioxidant potential by ORAC methodology under physiological protease degradation (3.5 U/mg), up to 24 h at 37 °C. The results showed an increase of antioxidant activity of the

sericin hydrogels. This can be related to the availability of the phenolic groups that were previously bound by the crosslinking and became free to react after degradation.

The sericin solution and sericin-based hydrogel formulations did not present antimicrobial activity against important pathogens of wound infection, such as *S. aureus*, *P. aeruginosa* and *E. coli*, since there was no bacterial growth up to 24 h for all strains as well as there was no inhibition halo for hydrogels.

Sericin hydrogel was applied into diabetic wounds to evaluate their *in vivo* antioxidant behavior. Wounds treated with sericin hydrogel closed at a similar rate when compared to Tegaderm group, although with reduced granulation tissue and decreased wound edge distance and wound thickness, demonstrating that this treatment is more effective in relation to Tegaderm alone. Application of wound dressings of sericin hydrogel could promote a more controlled inflammatory response and deposition of collagen fibers with smaller diameter that could be an advantage to stimulate re-epithelialization, when compared to Tegaderm alone. Oxidative stress and ROS production have been implicated as major contributors in non-healing wounds [4],[5]. The results showed that sericin hydrogel treatment slightly induced two important endogenous antioxidant defenses, SOD and catalase, although without statistical significance. This result was interesting, since the effect of this wound dressing was explored on oxidative damage. It was possible to denote a significant decrease in the content of advanced oxidation proteins products at wound bed, being the sericin hydrogel able to protect wound from oxidative protein damage.

Conclusion

Sericin hydrogel is biocompatible and a promising candidate to be applied in diabetic wound healing to protect against oxidative stress. This is of paramount interest to diabetic patients, due to improvements in some of the comorbidities associated with non-healing wounds.

Acknowledgement

This work was supported by national funds from *Fundação para a Ciência e a Tecnologia* (FCT), through project UID/Multi/50016/2019. Sara Baptista-Silva gratefully acknowledges FCT for the research grant (ref. SFRH/BPD/116024/2016). The authors also acknowledge the support of the i3S Scientific Platform HEMS, member of the national infrastructure PPBI - Portuguese Platform of Bioimaging (PPBI-POCI-01-0145-FEDER-022122).

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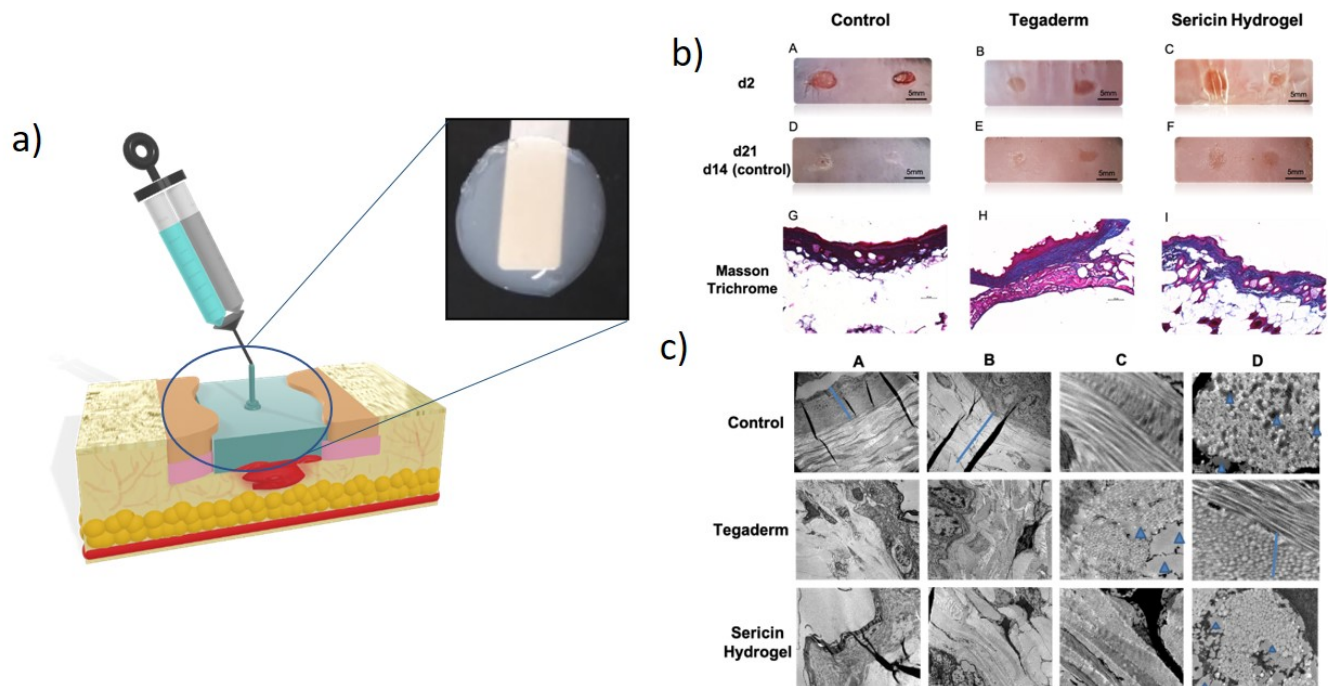


Figure 1

a) Schematic illustration of *in situ* application of the sericin hydrogel in a chronic wound; b) Representative images of wound closure (A-F) and histopathological analysis (G-I) in a mouse model of skin wound healing assay; c) TEM images to analyze collagen fibers at wound bed on diabetic animals treated with sericin hydrogel and covered with Tegaderm, with just Tegaderm or untreated wound (control): A) Dermis organization at low magnification; B) The network of collagen fibres; C) Orientation and distribution of collagen fibrils; and D) Cross-section of collagen fibres.