BIOMECHANICAL CHARACTERIZATION OF A SILK FIBROIN-BASED BIOINK FOR 3D PRINTING IN TISSUE REGENERATION

Giulia Maria Di Gravina (1), Elia Bari (2), Franca Scocozza (1), Sara Perteghella (1), Benedetta Frongia (1), Sara Tengattini (1), Lorena Segale (2), Maria Luisa Torre (2) Michele Conti (1)

1. University of Pavia, Italy; 2. University of Piemonte Orientale, Novara, Italy

Introduction

Thanks to its excellent mechanical and biological properties, silk fibroin (SF) is a protein used in many applications of tissue engineering (TE), for example the ones involving controlled drug release. Despite this, the literature reports limited examples of SF in bioprinting as many problems still need to be addressed, including its time stability, low viscosity when in the form of solution, and, more importantly, the difficulty of 3D printing this material because of β-sheet formation in its structure during the printing process, leading to nozzle clogging [1]. In this work, we developed and optimized a blend of silk fibroin and sodium alginate (SA) for the release of lyosecretome, a freeze-dried formulation of mesenchymal stem cell secretome characterized by bioactive properties able to improve biological response [2].

Materials and Methods

Preparation SF solution was obtained starting from Bombyx mori cocoons. Three different degumming times in 0.02 M Na_2CO_3 were investigated: 30 min (standard protocol), 1, 2, and 4h. SF (5% w/v) degummed for 1,2, and 4h was blended with SA (10% w/v) obtaining three different formulations of SA-SF hydrogels. A hydrogel composed only by SA (10% w/v) was used as control.

Printing characterization Each formulation was evaluated in terms of printability and shape fidelity at two different time-points (7 and 14 days after SF preparation) to assess the time-dependent behaviour of SF. An extrusion-based 3D bioprinter was used for printing 3D structures, whose strand size, inter-filament distance and printability index were calculated to evaluate quantitively the printing performance.

Mechanical characterization Each formulation was evaluated with tensile and compressive mechanical tests at 7 and 14 days. Before testing, the samples were chemically crosslinked with solution formed by 2% w/v $CaCl_2$ + 20% w/v KCl + 5% w/v protamine. Moreover, to understand the impact of the crosslinking method on the material structure, a comparison of this crosslinking solution with one composed only by 2% w/v $CaCl_2$ was performed on SA hydrogel samples in terms of tensile test.

Release study: lyosecretome was included in each formulation and its release over time was measured.

Results and Discussion

SF solution extraction was successfully optimized by increasing the standard degumming time from 30 min to 1, 2, or 4 h: reduced SF molecular weight allowed to achieve a printable protein solution, that remained capable of the conformational change from Silk I (random coil) to Silk II (β-sheet). This transition is fundamental to improve the scaffold's mechanical properties and drug release. From a printing point of view, SA-SF hydrogels with a degumming time of 2 h and 4 h resulted to have a better performance in terms of shape fidelity and to be printable at both time-points. From a mechanical point of view, adding SF to SA hydrogel increased the compressive response, especially when degummed for 2 and 4 h, while it did not influence tensile performance (Figure 1b). However, the crosslinking method in the material was demonstrated to strongly influence the mechanical response. Indeed, the tensile modulus of SA hydrogel resulted to be three times higher when crosslinked with $CaCl_2$ compared when treated with $CaCl_2$+protamine+KCl solution (Figure 1c). Finally, degumming of SF for 2 and 4 h dramatically slowed the lyosecretome release and modified the kinetics and mechanism of release with respect to the SA hydrogel baseline.

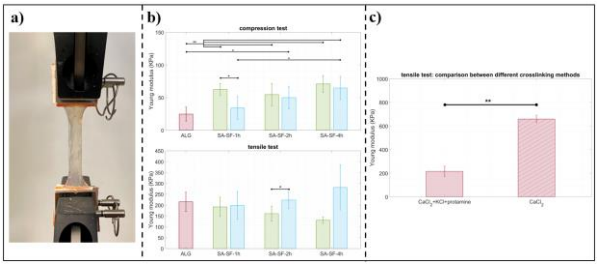

Figure 1: Mechanical characterization: a) example of tensile sample and test; b) tensile and compressive modulus results; c) influence of crosslinking methods on tensile response

Conclusion

A 3D printable SA-SF hydrogel for TE applications was achieved. The results obtained from the different characterizations lay the foundation for future development of SA-SF bioinks with modulable mechanical and release properties, and their use in scaffold 3D printing.

References

1. Wang et al, Materials, 12.3: 504, 2019.
2. Bari et al, Pharmaceutics 15.2: 383, 2023.

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