Silver/fibroin/alginate microspheres for wound and ulcer therapy

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INTRODUCTION

Silver has been used for centuries for the treatment of wounds and cutaneous ulcers: it is a potent antimicrobial agent, acting by blocking the respiratory enzyme pathways and altering the microbial DNA and cell wall (Tian J. 2007). Several forms of silver have been used to treat difficult wounds, such as colloidal silver solutions, silver proteins, silver salts and silver sulfadiazine. The most common silver dosage forms are dermatological powders and dressings, which present some pitfalls: the powders excessively dehydrates wounds, and dressings have occlusive properties which could halt exudates drain and retard the cicatrization process (Eaglstein W.H. 1993).
Alginate microspheres containing silver have been advantageously prepared to combine alginate absorbent and silver antimicrobial properties (Qin Y. 2005; Shanmugasundaram N. 2006). This approach can be followed and improved by adding silk fibroin in microsphere formulation. Fibroin is a protein derived from cocoons, widely employed in biomedical applications, due to unique properties such as processability, strength, biocompatibility and long-term degradability (Wenk E. 2008). Moreover, silk fibroin presents optimal filming properties which promote epidermal regeneration in skin wounds (Sugihara A. 2000); fibroin films can be thinned until nanoscale dimensions (Wang X. 2005) and tailored to the therapeutic needs.
Fibroin is characterized by a repetitive unit peptide [Gly-Ala-Gly-Ala-Gly-Ser] responsible for protein conformations and cristallinity. This protein shows different molecular conformations: silk II self-assembles to form an anti-parallel β-sheet structure, stable and insoluble in water, dominant in fibers; silk I is characterized by random coil conformation, metastable and water soluble, dominant in fibroin solutions and films. β-sheet content reflects the cristallinity degree of fibroin. Organic solvents, ionic strength, changes in temperature, shear forces and blending with several polymers can modify the molecular conformations, directing towards one of these possible forms. The manufacture of fibrin-based microspheres needs the control of the structural features in terms of β-sheet content and cristallinity degree: the form I (random coil) is mandatory during the preparation process, whereas the form II (β-sheet) as stable form, is useful in the design of controlled release microspheres. The aim of this work is to prepare microsphere of alginate and silk fibroin for topical silver spray administration: the stabilization of the β-sheet form was promoted using PEG 1500 as osmolyte.

MATERIAL AND METHODS

Preparation of silk fibroin solution. Cocoons of *Bombyx mori*, obtained from the Zoological Institute of Padua (Italy), were treated in autoclave in pure water at 120°C (pressure 2 Bar) for 30' and then rinsed with water at 60°C. Degummed cocoons were dried at 60°C and cut in small pieces. The resulting material was stirred in an aqueous solution of $CaCl_2$ and ethanol ($CaCl_2$/ET-OH/Water, 1:2:8 mole ratio) for 1h at 60°C.

The solution was dialyzed with distilled water using cellulose membranes (MW 12.000, Sigma Aldrich). The final concentration of silk fibroin aqueous solution was 2% w/w. Coomassie brilliant blue staining (1% in water/Et-OH solution, Merck) was used to check the sericin absence.
Microencapsulation. Microspheres were obtained by spray drying (Büchi Mini Spray Dryer) of aqueous solutions. Colloidal silver aqueous solution was electrochemically obtained at the concentration of 80 ppm and diluted in silk fibroin solution, 1:1 volume ratio; polyethylenglycol (PEG 1500, Sigma Aldrich) was added at the concentration of 0.05% (Micro B and D) and 0.1% (Micro C); sodium alginate solution was added at the concentration of 1% (Micro D). All concentrations are expressed as weight/volume. The percent composition of microspheres is reported in table 1.
Granulometric analysis. Granulometric analysis of microspheres was performed in water by a laser light scattering granulometer (Beckman Coulter LS230), equipped with a small volume cell, 120 mL volume with refractive index set at 1.330 for water, obscuration 5%. Ethanol suspensions of microspheres were put into the measurement cell and ran in 5 replicates of 90 seconds each.
Scanning electron microscopy (SEM). Microspheres were placed on small aluminum cylinders, point-dried and sputter coated with gold (Sputter coater with Cressington Sputter 108 auto). Morphological analysis was performed by a JEOL JSM-6380LV electron scanning microscope.
Fourier Transform Infrared Spectroscopy (FTIR). Samples were analyzed by FTIR on a Bruker Alpha-E spectrometer equipped with a MIRacle™ attenuated total reflection (ATR) Diamond crystall cell in reflection mode. Background measurements were taken twice with an empty cell and subtracted from the sample readings. The FTIR spectra in the absorbance mode were obtained in the spectral regions of 1000–1800 cm^{-1}. Each spectrum of the samples was acquired by accumulation of 32 scans with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSION

Micro B microspheres (Figure 1) show a size distribution with volume-weighted diameter lower than the other types of microspheres (Micro A=13.03 μm; Micro B=3.36 μm; Micro C=5.99 μm; Micro D=5.15 μm); the presence of alginate increases slightly the particle size with a reduced dispersion in diameter.

Micro	Alginate	Fibroin	PEG
A	0	100	0
B	0	95	5
C	0	90	10
D	48.75	48.75	2.5

Table 1 : percentage composition of microspheres.

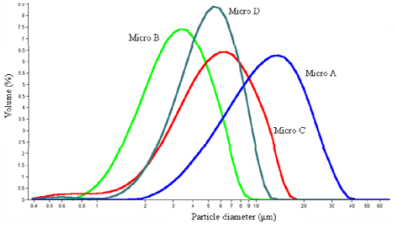

Figure 1: Granulometric analysis of Micro A, B, C and D.

The conformational transition of microspheres containing silk fibroin and silk fibroin/PEG was carried out by means of infrared spectroscopy. The spectrum of β-sheet structure shows peaks at 1629 cm^{-1} and at 1685 cm^{-1} (for amide I) while the spectrum of random coil conformation shows peaks at 1650 cm^{-1} for amide I and 1540 cm^{-1} for amide II. The degummed cocoons have absorption bands at 1620cm^{-1} and 1510cm^{-1} for amide I, both attributed to β-sheet conformation, while Micro A at 1640 cm^{-1} for amide I and 1515 cm^{-1} for amide II, both attributed to random coil; Micro B has

a similar behavior to degummed cocoons (Figure 2). The second derivative spectra suggest that degummed cocoons and Micro B have the same behaviour (Figures 3 and 5), whilst the Micro A present the second derivative of random coil conformation (Figure 4): the transition occurs from the random coil to β-sheet when microspheres contain PEG. FTIR spectra show that small quantities of PEG promote a conformational transition in silk fibroin.

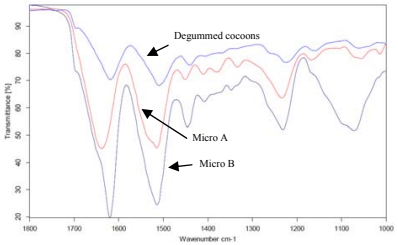

Figure 2: FTIR spectra of degummed cocoons, Micro A and Micro B.

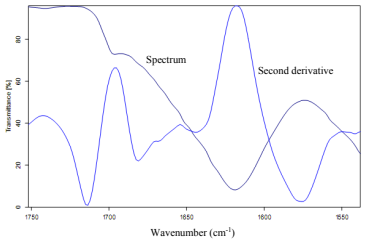

Figure 3: FTIR spectra of degummed cocoons (spectrum and second derivative).

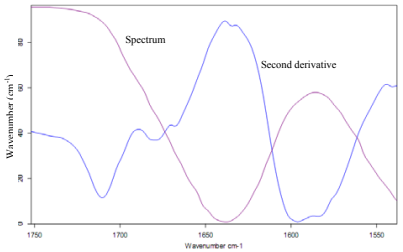

Figure 4: FTIR spectra of Micro A (spectrum and second derivative).

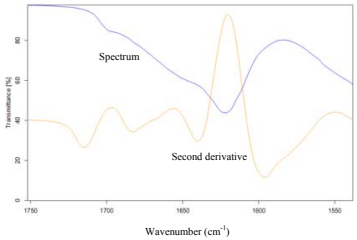

Figure 5: FTIR spectra of Micro B (spectrum and second derivative).

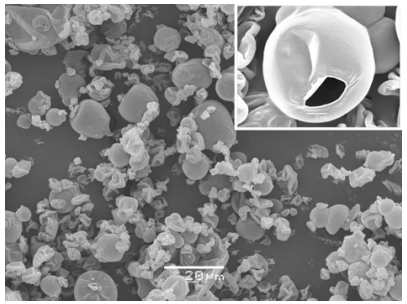

Figure 6: SEM image of Micro A. Inset: magnified view of a hollow microsphere.

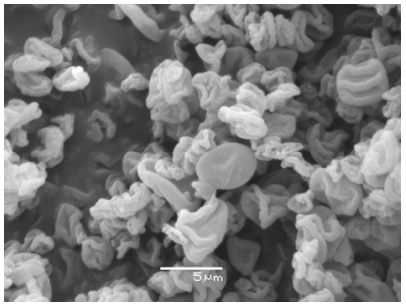

Figure 7: SEM image of Micro B

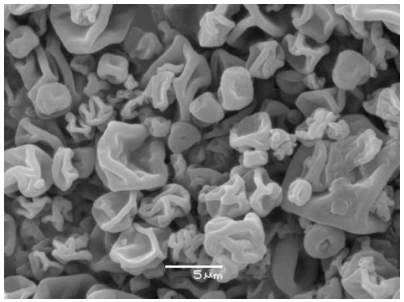

Figure 8: SEM image of Micro C.

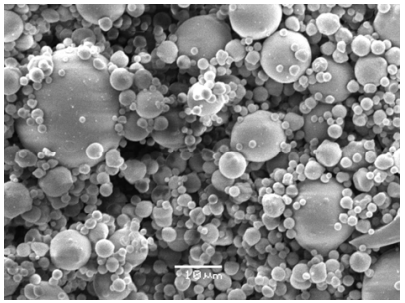

Figure 9: SEM image of Micro D.

Micro A, Micro B, and Micro C containing silk fibroin are generally collapsed and vacuolated (Figures 6-8), while Micro D, containing alginate and fibroin, appears smooth and spherical shaped (Figure 9).

CONCLUSIONS

These data suggest that colloidal silver can be loaded in microspheres containing alginate and silk fibroin. Polyetylenglicol may be added in small quantities to guarantee the silk fibroin β-sheet structure and alginate improves the microsphere quality and morphology. Microspheres containing alginate, silk fibroin and colloidal silver are promising devices for the therapy of wounds and cutaneous ulcers by topical spray administration.

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