Fabrication of Silk/Chitosan-based Hydrogels by Gamma Irradiation

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Statement of Purpose: Biologically derived silk fibroin (SF) protein and chitosan (CS) have gained considerable attention as candidates for tissue engineering due to their excellent mechanical strength, water absorption, and biocompatibility [1-3]. In the fabrication of hydrogels from SF and CS, various techniques including chemical reagents [3] and irradiation [2-5] have been utilized to crosslink the biopolymer chains. Among these techniques, irradiation by gamma ray offers a clean, one-step process to generate free radicals that initiate crosslinking of polymers and sterilize hydrogels at the same time. In this paper, we combined both SF and CS together with poly(vinyl alcohol) (PVA) as starting materials to form hydrogels by gamma irradiation and evaluated their physical properties and biocompatibility to determine their suitability for biomedical applications.

Methods: The cocoons of Thai silkworms *Bombyx mori*, CS (M_w 200,000) and PVA (hot water soluble grade) were used as starting materials. After the degumming process, SF protein was extracted from the cocoons in a ternary solvent of 1:2:8 calcium chloride:ethanol:water by mole, dialized and freeze dried. A 1 % (w/v) chitosan solution was prepared in 1 % acetic acid at pH 6.5. Subsequently, SF powder was added to the CS solution to prepare 2, 5 and 10 % (w/v) mixtures and stirred overnight until completely dissolved. The SF/CS solutions were then mixed with a 15% (w/v) PVA at a ratio (v:v) of 2:1, evaporated to remove 1/3 of the water content before transferred into 12-well tissue culture plates for gamma irradiation by a Co-60 source at doses of 40, 60, 100 and 120 kGy. A solution containing only CS and PVA (0% SF) was used as control. The gel strength was determined by an indentation method with a 4-mm ball probe and the compressive load was recorded at 50% gel height. The swelling of hydrogels was represented by a swelling ratio, which was defined as the weight of hydrogels incubated in distilled water at 37ºC for 72 h divided by the initial dried weight. The gel fraction was determined by a ratio of the dried weight after autoclaving for 1 h to the initial dried weight. As a preliminary test for biocompatibility, dermal fibroblasts were grown in tissue culture media that had been preconditioned with hydrogels (CM), and then cell nuclei were counted and viability was determined by a live-dead assay.

Results: The formation of SF/CS-based hydrogels strongly depended on irradiation dose. At the lowest dose, 40 kGy, all SF/CS combinations resulted in malleable gels, whereas firm and flexible gels were only obtained at doses above 60 kGy. By contrast, in the absence of SF CS/PVA did not form gels until the irradiation dose was raised to 100 kGy. The gel strength (Fig. 1A) and gel fraction (Fig. 1B), both of which correlated to the extent of crosslinking within the gel matrix, increase proportionately to the irradiation dose before reaching saturation at 100 kGy. The presence of SF significantly enabled the gels to withstand a more than 4-fold increase in compressive load compared to control (Fig. 1A, 100 and 120 kGy). This result was observed for the range of SF concentrations from 2-10% tested in this study. The swelling ratio was similar across all irradiation doses and decreased slightly with increasing SF concentration (Fig. 1C), which may be attributed to crosslinking of CS and PVA with SF.

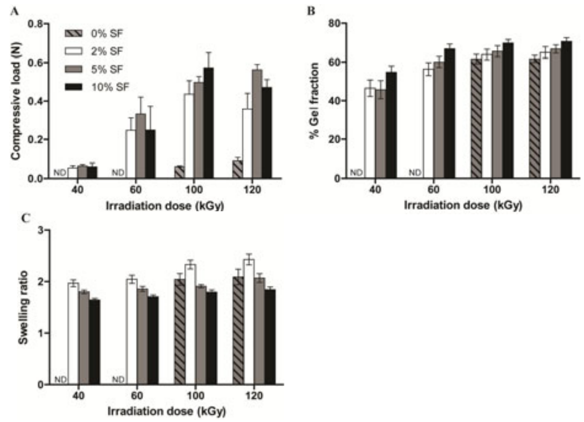

Figure 1. (A) Gel strength, (B) gel fraction and (C) swelling property of SF/CS-based hydrogels. Data are mean ± SEM from 4 independent experiments.

Based on gel strength and gel fraction, hydrogels formed at 100 kGy were selected for the biocompatibility assay. Cells were able to grow in all conditioned media without detectable cell death and eventually reached confluence on day 6 (Fig. 2).

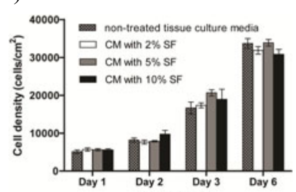

Figure 2. Cell proliferation in conditioned vs. non-treated tissue culture media. Data are mean ± SEM from 3 replicates.

Conclusions: The irradiation dose of 100 kGy was found to be optimal for the formation of SF/CS-based hydrogels. Our data suggest that the minimal concentration of SF used in this study can improve the mechanical properties without affecting the swelling property. The non-toxicity of these hydrogels warrants the future evaluation in animal models.

References:

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