ABSTRACT

Hydrogels from the *Bombyx mori* silk fibroin (SF) are of great interest for drug delivery and tissue engineering applications because of their biocompatibility, water absorbability, controllable biodegradation rate and processability into different formats. Despite these, unmodified SF samples normally display poor mechanical strength with prolonged gelation kinetics thus, limiting their practical use in biomedical applications. To overcome these drawbacks, the new all-aqueous crosslinking methods to form the three-dimensional (3D) porous SF hydrogel network were developed. The physically crosslinked SF hydrogels were prepared by mixing different aqueous SF solutions (2-10% w/v) with poly(ethylene glycol)diacrylate (PEGDA). All formulations were then, incubated at room temperature until there is a pronounced increase in the apparent viscosity. Results indicated that PEGDA (30% w/v) could successfully shorten the silk gelation time and stabilize the silk hydrogels in aqueous media. This was associated with a formation of the interconnected sheet-like structure, as observed by Scanning Electron Microscopy (SEM). Data interpretation of Fourier Transform Infrared (FTIR) spectra suggested that the PEGDA-induced gelation was driven by a conformational transition of SF from α-helix into the β-sheet structure. Furthermore, the process was affected by the type of extraction solvent and the utilization of sonication. Surprisingly, the physically crosslinked hydrogels exhibited moderate antibacterial activity against gram positive *Bacillus cereus*, MRSA-SK1 and *Staphylococcus aureus*, as evidenced by the diameters of the inhibition zone between 9.2-10.9, 5.7-6.1 and 5.7-6.2 mm, respectively. This finding highlighted another unexploited property of soft SF gels in biomedical fields.

Apart from a physical method, a new chemical modification by using a protein crosslinker, *O'O*-bis[2-(*N*-succinimidyl succinylamino)ethyl]polyethylene glycol (NHSP), was also employed to fabricate the more stable SF hydrogels. For this, appropriate amounts of NHSP was mixed with an aqueous SF solution at room temperature in the presence of poly(*L*-lysine) (PLL) as a gel enhancer. A network formation was accelerated by the using of ultrasonication and noticed within 24 hours, depending on the silk concentration. A crosslink reaction was convinced by the

appearance of a new ether linkage observed in the Fourier-transform Infrared (FTIR) spectra, the presence of a new thermal decomposition temperature (T_d) at higher temperature region and a shift of the electrophoretic bands from 35-130 KDa to 37-150 KDa. Such crosslinking contributed to the improved hydrogel properties, such as a structural integrity, thermal stability and water-resistance. Also, this produced an interconnected porous structure with higher β-pleated sheet content, comparing to the physically crosslinked ones. In terms of release behavior, both physical and chemical hydrogels displayed initial burst release, followed by zero-order release kinetic. The release amounts of the model drugs; rhodamine B dye and α-mangostin, are affected by their hydrophobicity, molecular weight, affinity for SF adsorption as well as hydrogel network morphology and the feed composition. As the silk content was increased, the hydrogels could facilitate much slower delivery profile due to the hindered diffusion effect facilitated by the silk hydrophobic β-sheets. These two new methods thus, proven to be capable of improving both silk gelation kinetics and its physicochemical properties, making it more suitable for both biomedical and tissue engineering applications.

As predicted, an aqueous solution of α-mangostin exhibited strong antibacterial activity against Gram-positive *B. cereus*, *MRSA-SK1* and *S. aureus* with the minimum inhibitory concentration (MIC) of around 0.25 μg/mL. Such property became less observable upon encapsulation, as evidenced by the appearance of the diameters of inhibition zone against *B. cereus, MRSA-SK1* and *S. aureus* between 15.2 ± 0.3, 9.2 ± 0.3 and 11.0 ± 1.0 mm, respectively. Furthermore, the released aqueous solution of α-mangostin exhibited weaker antibacterial activity against Gram-positive bacteria with the MIC value of 64±0.0 μg/ml. This could be explained in terms of a diffusion controlled mechanism facilitated by hydrogel matrices and the limited water solubility of the active compound in an aqueous solution. Thus, a direct application of the recent SF hydrogels is likely to be more suitable for hydrophilic or amphipathic active compounds.