MaSp2 based recombinant spider silk particles: processing of new drug delivery vesicles.

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Statement of Purpose: Bioengineered spider silk (BSS) is a biomaterial that combines superb mechanical properties, biocompatibility and biodegradability with a good accessibility and simple purification procedure(1). Like natural spider silk, BSS has ability to form solid structure polymers(2). BSS protein can self-assemble in the high phosphate ion concentration and under mixing conditions into spheres of nano- and sub-micrometrical size(3). In this study, the bioengineered silk protein MS2.9x based on MaSp2 protein from *N. clavipes* spider was constructed, produced, and then purified using two methods. Obtained proteins were processed into particles in order to develop a new drug delivery system. Spheres were prepared at several process parameters and then characterized in terms of morphology, size, size distribution, zeta potential and loading efficiency. The influence of protein purification method on sphere properties was analyzed.

Methods: MS2.9x BSS was constructed and then produced in *E.coli* using bioreactor. Protein was purified using propionic acid extraction or thermal extraction method(4). Spheres were produced by mixing protein solution, in ratio 1:10, with K_3PO_4 using increasing initial protein concentration, increasing phosphate concentration and potassium phosphate of pH ranging from 6 to 12. Obtained particles were analyzed in terms of morphology, size and size distribution. Morphology and size was observed using scanning electron microscope. Stability in suspension was measured as Zeta Potential (ZP) value. Spheres were loaded with model drug Rhodamine B and their loading efficiency was calculated.

Results: Protein MS2.9x was successfully purified using both methods with similar efficiency. In general, the size and size distribution of produced spheres depended on silk concentration. Higher BSS concentration resulted in bigger spheres of higher size distribution. The phosphate concentration had opposite effect on size and strongly influenced on the morphology and stability of silk particles. Lower K_3PO_4 concentrations were unable to produce fully shaped and dispersed particles. Changing pH of potassium phosphate had no effect on particle properties.

Particles produced of MS2.9x protein purified by different methods and processed at the same conditions demonstrated the difference in size, morphology and ZP. Acid-extracted protein formed bigger particles with porous-like surface whereas spheres made of protein extracted thermally were smaller and smooth on their surface. Particles made of acid extracted MS2.9x presented more negative ZP oscillating around colloidal stability threshold of -30 mV, while spheres produced from thermal extracted BSS had higher ZP, localizing around -15mV. Spheres obtained after both protein purification methods showed equal model drug incorporation properties.

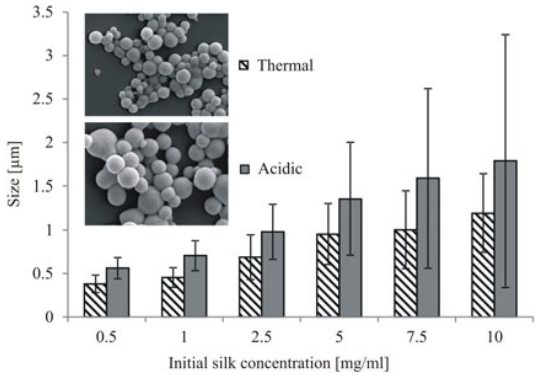

Figure 1. Sphere size and its distribution depends on protein concentration and used protein purification method.

Conclusions: Bioengineered spider silk protein can be processed into stable particles. The processing conditions can be used as a controlling factor for particle size and properties. To obtain the nano-sized, monodispersed and colloidally stable particles, the high concentration of potassium phosphate, low concentration of BSS should be used and the protein should be purified using thermal method.

Obtained results indicated the big potential of silk particles as drug carriers. This non-toxic, non-immunogenic and biodegradable protein polymer is an ideal matrix for drug loading. The next step on BSS-based drug delivery system development will focus on drug incorporation mechanisms, release kinetics as well as silk modification to maximize the drug loading efficiency.

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