Spider silk-based particles: new drug delivery vesicles for targeted cancer therapy

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Bioengineered spider silk is a biomaterial that combines superb mechanical properties, biocompatibility and biodegradability with a good accessibility and simple purification procedure. Thus, spider silk has extensively been explored as material for numerous biomedical applications. Silk protein can self-assemble in the high phosphate ion concentration and under mixing conditions into spheres of nano- and micrometrical sizes. Moreover, the bioengineered silk protein may be further modified to gain new functions. Particularly, nanoparticles able to target cancer cells could be designed by addition of tumor homing peptides within the silk structural sequence.

Current strategies for cancer treatment are to design nanosystem for a delivery of therapeutic agents specifically to the site of tumor, therefore avoiding potential side effects. The aim of present study was to obtain novel drug delivery carriers based on functionalized spider silk proteins. The specific aim of the study was the evaluation of physical properties, as well as biological activity of bioengineered spider silk-based particles made of two different silk proteins and their blends.

We constructed two bioengineered silk proteins based on dragline silk proteins of *N. clavipes* spider: MaSp1 and MaSp2, named MS1 and MS2, respectively. Moreover, their Her2-directing functionalized variants able to target cancer cells (MS1 and MS2 fused to Her2 receptor-binding peptides: H2.1MS1, H2.2MS1, H2.1MS2, H2.2MS2) were designed. Silk proteins were produced in *E.coli* expression system using bioreactor. Proteins were purified by thermal extraction method. Obtained proteins were processed into spheres in an aqueous process triggered by potassium phosphate. The spheres were based on MS1 and MS2 proteins (and their hybrid variants), as well as on silk fibroin MS1 and MS2 blends at a different weight ratios. Spheres were characterized in terms of morphology, size (confocal and scanning electron microscopy), zeta potential (ZP), and biological activity (flow cytometry and confocal microscopy). The binding potential of silk nanoparticles to cancer cells was determined using cancer cells overexpressing Her2 (SKOV3 and SKBR3) and Her2-free cells (fibroblasts MSU1.1) as control.

Control and functionalized MS1 and MS2 particles demonstrated differences in size, morphology and zeta potential. MS1 formed less spherical, aggregated particles of positive ZP. MS2 formed well-defined spherically shaped particles of negative ZP. Moreover, the influence of blending of MS1 protein with MS2 on sphere properties was analyzed. The increasing MS2 concentration into MS1/MS2 blend improved sphere properties what was observed by different yield and morphology of obtained blend-based spheres. Moreover, the biological activity of silk blends-based spheres (control and functionalized) was further investigated. The spheres made of functionalized spider silk indicated significantly higher binding to the Her2-overexpressing cells comparing with Her2-negative cells and comparing to control spheres without the targeting domains. Functionalized MS1, MS2 and MS1/MS2 blends-based particles revealed differences in cell binding efficiency depending on the amount of MS2 added. Increase of MS2 protein in the blends lowered the cell binding efficiency. However, for blend-based spheres made of proteins at the weight ratio of 80/20% (functionalized MS1/MS2, respectively), the binding was maintained at the level of homogeneous spheres made of functionalized MS1 proteins, with greatly improved physical properties.

The bioengineered spider silk protein can be processed into stable particles. Blending process can be used as a controlling factor of particle properties. The obtained results indicated the potential for the delivery of a therapeutic agent confined in functionalized silk spheres to the specific tumor microenvironment.