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Synthesis and physicochemical characterization of Spider silk / Chitosan based hydrogels for biomedical applications

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The capability of new hydrogel material has achieved various innovative scopes in the biomedical industry. Hydrogel and biomaterial industries have been increasingly developing among recent research aspects among the diverse polymer-based hydrogels, protein hydrogels have garnered considerable attention due to their fruitful features such as biocompatibility, biodegradability, and low immunogenicity [2,3] . Such hydrogels are usually assembled via chemical and physical cross-linking of soluble protein polymers producing water-insoluble 3D-networks. Owing to the genetic basis of sequence, molecular weight, folded structure, and stereochemistry, protein polymers thus offer substantial opportunities for the design of protein hydrogels [4]. Recently, modular biopolymer designs that combine the unique properties of native spider silk proteins - spidroins [1] , chitosan, and other structural or biologically active domains have emerged as an intriguing approach for fabricating advanced biomaterials and attained spotlight in recent research.

Here we report the fabrication of hydrogels based on biopolymers and characterization of their physical and chemical properties leading to involve them in biomedical applications. For the synthesis, polyacrylic acid (PAA), chitosan (CHI), nano-spidroin (nS) and micro-spidroin (μS) were used as main biopolymers. Chitosan (CHI) scaffolded polyacrylic acid / nano-spidroin (PAA / CHI / nS) and Chitosan (CHI) scaffolded polyacrylic acid / micro-spidroin (PAA / CHI / μS) preparations were done separately by 1-ethyl-3- (3 -dimethyl aminopropyl) carbodiimide (EDC) / N-hydroxysuccinimide (NHS) mediated chemical crosslinking.Structural and physical characterization was conducted using scanning electron microscopy (SEM), Fourier transform infrared spectrophotometry, Nitrogen sorption and surface area measurements and contact angle measurements (KRUSS).

Further characterization of hydrogels as functional biomaterials included measurements of their swelling degree, degradation, conductivity, and shrinkage. The swelling abilities of the hydrogels in water suggested that these hydrogels can absorb water several times higher than its original dry weight. According to enzymatic degradation studies, which were done using enzyme Trypsin and Chymosine separately, a declining trend in degradability with increasing spidroin content in the hydrogel samples was observed. In parallel lines, shrinkage tests of the hydrogels, which were conducted in normal environmental conditions (25 $^{\circ}$C and 1 atm), showed a decrease of hydrogels shrinkage rate with the increasing of nano-spidroin and micro-spidroin content in the samples. Viscosity of the hydrogels confirm the extraordinary applicability as a delivery material.

Antimicrobial abilities of the hydrogel samples were tested on gram-negative (Escherichia coli Nissle 1917) and gram-positive (Staphylococcus aureus) bacterial cultures, respectively. Based on the antimicrobial tests, enhanced antimicrobial properties against both gram-negative and gram-positive bacteria were measured in nano-spidroin based materials with a higher nano-spidroin content, compared to micro-spidroin based ones. This fact agrees well with

the literature data suggesting that spidroins possess the slight antimicrobial effect due to the presence of specific inorganic salts, Sulfur containing compounds, ionic forms of amines that are also secreted from the spider follicles [5] .
As the main target of the characterization, drug absorption and drug delivery studies were conducted using an anti-inflammatory drug (ibuprofen). Drug absorption rate and the entrapment efficiency of the hydrogels showed the capability of absorbing ibuprofen is optimum to be used in external anti inflammatory drug delivery systems. The hydrogels with nano-spidroins were approximately less time consuming than the hydrogels with micro-spidroins in drug loading. The hydrogels containing nano-spidroins showed the possibility of short term drug delivery and the hydrogels containing micro-spidroin showed the possibility of applying in long term drug delivery.The cytotoxicity of the nano spidroin and micro spidroin containing samples were observed by surface proliferation of human fibroblast cells. The human fibroblasts were overgrown on the hydrogels with in 72 hours of incubation period which suggest the hydrogels are non toxic for the human fibroblasts.
Applications of these advance materials can be introduced in skin drug delivery and cosmetic products. Furthermore, the rheological properties of the hydrogels will direct the applicability on physiological applications leading to obtain injectable hydrogels in bone joints to treat arthritis and bone joint deformities. In emergency care, the hydrogels can be used as compressive material for control bleeding in deep wounds.

References

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Illustrations

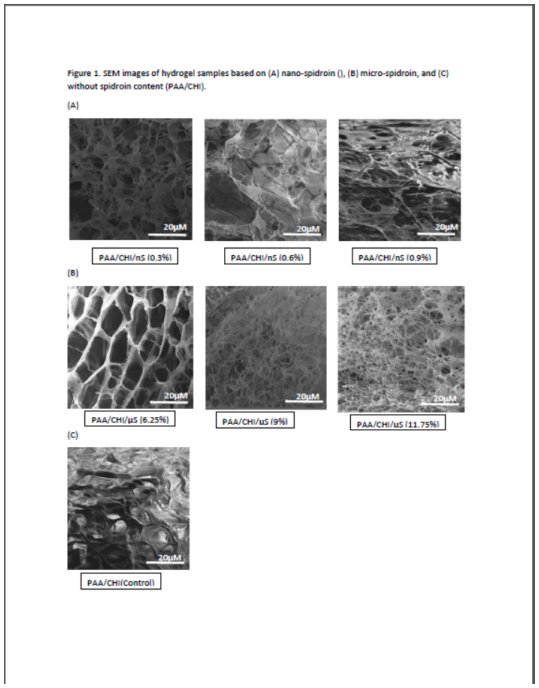

Рис. 1. Figure 1. SEM images of hydrogel samples based on (A) nano-spidroin (PAA/CHI/nS), (B) micro-spidroin, and (C) without spidroin content (PAA/CHI).