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A Novel Injectable Succinyl Chitosan/Oxidized Alginate Hydrogel Encapsulating Selenium-Folic Acid Nanoparticles for Spinal Cord Injury Regeneration

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ABSTRACT

Introduction: Spinal cord injury remains a significant clinical challenge due to its limited capacity for self-repair. Advances in biomaterials offer promising solutions for enhancing neural regeneration. Herein, we developed a novel composite hydrogel from succinyl chitosan (S-CS) and oxidized alginate (O-Alg).

Methods and Materials: In this experimental in vitro study, selenium-folic acid (Se-FA) nanoparticles (NPs) were synthesized by mixing FA, silica, and ascorbic acid. The NPs were characterized using field emission scanning electron microscopy (SEM), dynamic light scattering, Fourier-transform infrared spectroscopy, thermogravimetric analysis, and ultraviolet spectrophotometry. Cross-linked gelatin (GE)-tannic acid (TA) nanofibers were created by electrospinning a solution of GE, TA, and Se-FA NPs at concentrations of 0%, 5%, and 10%. The solubility, wettability, and morphology of the mats were evaluated through solubility tests, water contact angle (WCA) measurements, and SEM. Injectable hydrogels were formed by combining S-CS (10 mg/ml) and O-Alg (50 mg/ml) with NP-loaded nanofibers at concentrations of 0%, 1%, 2%, and 5%. Characterizations of the hydrogels included assessments of gelation time, injectability, morphology, mechanical properties, and antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa* using the disk diffusion method. Antioxidant capacity was measured using DPPH assay. Cytotoxicity and cell proliferation were assessed through indirect and direct assays with MTT and DAPI staining at 48 h and 72 h with L929 and PC12 cell lines. Wound healing was evaluated using an L2929 fibroblast scratch assay, and hemocompatibility was also evaluated. All tests were performed in quintuplicate (n = 5). Statistical analyses were conducted using Graph Pad Prism 7.0, employing one-way ANOVA followed by Tukey's post hoc test.

Results: The characterization techniques showed the NPs exhibited a spherical shape, a size of less than 100 nm, a negative zeta potential, and a low polydispersity index of 0.24. Additionally, typical carboxyl bands were observed at 1693 cm^{-1} and 1607 cm^{-1}, with a loading efficacy of 15.38% ± 1.03. A bathochromic peak shift confirmed the successful synthesis of NPs and FA loading. The GE-TA nanofiber exhibited a weight loss of 8.32% ± 0.87, a water contact angle of 80.45°, and a non-bead morphology. The hydrogels exhibited gelatin times ranging from 90.56 to 70.58 seconds, depending on the amount of GE-TA acid nanofibers, along with injectability, and moduli comparable to soft tissue (1-10 kPa). The antioxidant capacity was significant, with values of 59.09% and a peak at 0.443 nm, highlighting the effectiveness of hydrogels in scavenging free radicals. in the disk diffusion test, the hydrogels produced an inhibition zone of 32 ± 1.03 mm ($p < 0.01$ compared to the control). Biocompatibility cellular tests indicated low cytotoxicity and high cell proliferation rates, with viability of 93.54% ± 1.37 ($p < 0.05$). The scratch assay demonstrated accelerated scratch closure, achieving 100% closure after 48 h ($p < 0.001$), and the red blood cell lysis was less than 5% ± 0.35 in all groups.

Conclusion and Discussion: In vitro tests demonstrate the biocompatibility and mechanical properties of this hydrogel, which enable injectability and conformability at the injury site. It exhibits minimal cytotoxicity, operates effectively, and supports neuronal cell proliferation and neurite outgrowth. Future pre-clinical and clinical studies must evaluate its therapeutic efficacy, safety, and relevance.

Keywords: Folic acid, Hydrogels, Nanoparticles, Spinal cord injuries

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