Synthesis and Characterization of Chondroitin Sulfate Methacrylamide Micelles

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Introduction: Micelles are stable, self-assembling particles that are capable of delivering drugs and growth factors in an aqueous environment. Chondroitin sulfate (CS) is a common glycosaminoglycan found in tissues such as cartilage and is highly negatively charged. Amphiphilic CS-poly(L-lactic acid) and CS-poly(lactic-co-glycolic acid) microspheres have been previously reported for use as drug delivery carriers (Lee CT. Biomacromolecules. 2006;7:1179-86. Lee ES. Biomaterials. 2007;28:2754-62). The negatively charged nature of CS makes it an appropriate material for polyionic complexation with cationic molecules, including growth factors such as basic fibroblast growth factor and transforming growth factor β1. In these studies, we describe the synthesis and characterization of novel chondroitin sulfate methacrylamide (CSMAm) micelles for tissue engineering and bioactive factor delivery.

Methods: N-(3-aminoproyl) methacrylamide (Polysciences, Warrington, PA) was covalently conjugated to chondroitin sulfate A (Sigma Aldrich, St. Louis, MO) using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (Sigma Aldrich) chemistry in distilled water. The reaction mixture was dialyzed for a total of 72 hrs (1,000 Da molecular weight cutoff), and the product was lyophilized for 4 days.

CSMAm micelle formation in distilled water was verified using light microscopy and dynamic light scattering (DLS). A solution of CSMAm in distilled, deionized water (ddH_2O) was mixed with 1 μm diameter latex microspheres (Polysciences), and particles were observed under light microscopy at a total magnification of 100X to determine the morphology and relative size of CSMAm micelles. DLS measurements were taken to determine the hydrodynamic diameter of CSMAm micelles in ddH_2O, and zeta potential measurements were also recorded as a measure of surface charge and colloidal stability. DLS (n=4) and zeta potential (n=3) measurements were recorded at 23°C in ddH_2O (Particle Sizing Systems NICOMP 380ZLS, Santa Barbara, CA), while samples of unmodified CS at the same concentration served as controls.

Cytotoxicity was characterized *in vitro* using bovine marrow stromal cells (MSCs). MSCs were plated at 10,000 cells/cm^2 in a tissue culture-treated 96-well plate, and cultured in media containing CSMAm equivalent to 1, 10, 100, 320 mg per 10^6 cells. After 24 hrs of exposure, cells were stained with LIVE/DEAD solution (Invitrogen, Carlsbad, CA), causing live and dead cells to fluoresce green and red, respectively. Fluorescence was measured in a plate reader (n=3), and relative viability was analyzed in comparison to live controls. Statistical significance was determined using a one-way ANOVA with Tukey's multiple comparison test ($p<0.05$).

Results: Light microscopy images indicate that CSMAm micelles were smaller than 1 μm diameter microspheres, providing a qualitative estimation of the relative size. DLS measurements indicated that a bimodal distribution of the CSMAm micelles were formed, with average diameters of 324.1 ± 8.5 nm and 73.2 ± 4.4 nm (Figure 1). Unmodified CS, however, did not produce sufficient levels of scattering for DLS measurements. Zeta potential measurements showed that the micelles possessed a zeta potential of -38.7 ± 1.1 mV, indicating that CSMAm micelles were negatively charged and stable in solution.

LIVE/DEAD staining of bovine MSCs in the presence of increasing concentrations of CSMAm revealed that cells remained largely viable at the lowest concentrations of 1 and 10 mg/10^6 cells with 1.21 ± 0.14 and 0.77 ± 0.05 viability, respectively, compared to live controls (Figure 2). A significant decrease in viability was observed at 100 mg and 320 mg/10^6 cells concentrations with 0.38 ± 0.06 and 0.18 ± 0.07 viability, respectively.

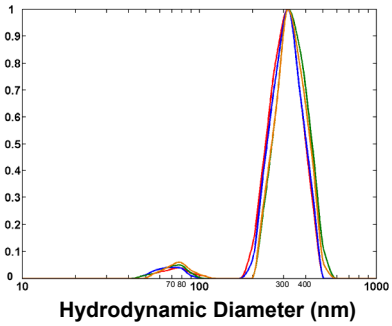

Figure 1: Dynamic light scattering distribution of hydrodynamic diameter. 4 separate samples are shown in red, blue, green, and orange.

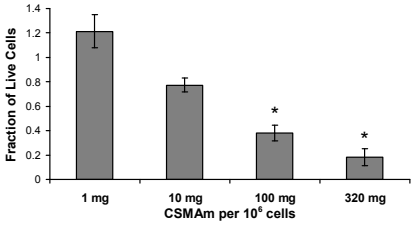

Figure 2: Fraction of live MSCs per well compared to live controls as determined by LIVE/DEAD staining. * indicates statistically different from live controls ($p<0.05$).

Conclusions: These results confirm that CS-based nanoparticles were formed via modification of CS with methacrylamide. These highly negatively-charged CSMAm micelles may have application in delivery and controlled release of cationic growth factors by pH-driven dissociation or enzymatic cleavage by chondroitinase. The presence of CS itself may also directly influence cell behavior, including proliferation, ECM production, and differentiation (van Susante JLC. Biomaterials. 2001;22:2359-69. Varghese S. Matrix Biol. 2008;27:12-21. Wu YN. Biomaterials. 2007;28:4056-67). The concurrent delivery of CS matrix molecules and soluble factors may simultaneously affect cell function, making this an exciting technology for bioactive factor delivery for a variety of regenerative medicine applications.

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