Rosette nanotube composites for cartilage applications

Linlin Sun,[1] Usha D. Hemraz,[2] Hicham Fenniri,[2*] and Thomas J. Webster[1*]
[1]Bioengineering Program and Department of Chemical Engineering, College of Engineering, Northeastern University, 360 Huntington Avenue, Boston, MA 02115 USA
[2] National Institute for Nanotechnology and Departments of Chemistry and Biomedical Engineering, University of Alberta, 11421 Saskatchewan Drive, Edmonton, AB T6G 2M9, Canada.
*Corresponding author: E-mail: th.webster@neu.edu, hicham.fenniri@ualberta.ca

Statement of Purpose: Composed of an extracellular matrix and a small percentage of chondrocytes, articular cartilage has limited regenerating capabilities [1]. Rosette nanotubes (RNTs) [2,3] composed of guanine^cytosine blocks are one kind of self-assembled supermolecules. With the similar dimensions as collagen molecules, RNTs have demonstrated increased vitronectin and fibronection adsorption and subsequent cell adhesion and functions [4]. In this study, the composites of twin based linker molecules (TBL) assembled RNTs and poly(2-hydroxyethyl methacrylate) (pHEMA) were tested for cartilage applications.

Methods: Synthesis of TBLs. TBL building blocks were synthesized according to our reported synthetic strategy [2,3], then was dissolved in dH_2O to a final concentration of 4 mg/mL. This solution was sterilized by filtration through a 0.22 μm syringe filter.

Preparation of TBL/pHEMA/H_2O composites. TBLs (0.01 mg/mL), initiator 2,2'-azobisisobutyronitrile (AIBN, 3 mg/mL, Sigma-Aldrich), 2-hydroxyethyl methacrylate (HEMA) monomer (5 mL, Polysciences, PA), and dH_2O were mixed to give 0, 10, 20 and 30 wt% HA/pHEMA solutions. Finally, the composites were heated in an oven at 60ºC until the samples solidified completely.

Cell adhesion and proliferation studies. To determine the adhesion and proliferation of chondrocytes, the cell proliferation assay (CellTiter 96, Promega) was used. Briefly, for cell adhesion, cells were seeded at 3,500 cells/cm^2 in standard cell culture media and were incubated for 4 hours. For the proliferation study, cells were seeded at 1500 cells/cm^2 for 1 day and 3 days. The dye solution was added to the cells after the end of the prescribed period for 4 h, then the stop solution was added and incubated overnight. A plate reader was used to test cell density.

Total protein synthesis. Chondrocytes were seeded at a seeding density of 10,000 cells/cm^2 onto the substrates. Cells were cultured for 3 and 5 days under standard cell culture conditions with chondrogenic medium. Total protein content in the cell lysates was measured using a commercial BCA™ Protein Assay Reagent Kit (Pierce Biotechnology) and following the manufacturer's instructions.

GAG synthesis. For chondrocyte differentiation studies, chondrocytes were seeded at a seeding density of 10,000 cells/cm^2 onto the substrates. Cells were cultured for 3 and 5 days under standard cell culture conditions with chondrogenic medium. Glycosaminoglycan (GAG) concentration was measured spectrophotometrically by a 1- 9- dimethylmethylene blue (DMMB) dye assay.

Statistical analysis. Numerical data were analyzed with a Student's t-test to make pair-wise comparisons. Statistical significance was considered at $p<0.05$.

Results: In the cell adhesion study, chondrocytes adhered more to TBL conjugated pHEMA composites than that on samples without TBL. The cell number on 0.01 mg/mL TBL/pHEMA with 20% H_2O composites was significantly greater than that on non-TBL composites. In the proliferation study, TBLs effectively increased chondrocyte density after 3-days of culturing compared to formulations without TBL (Figure 1). Moreover, TBL/pHEMA composites stimulated chondrocytes to synthesize more protein and GAG.

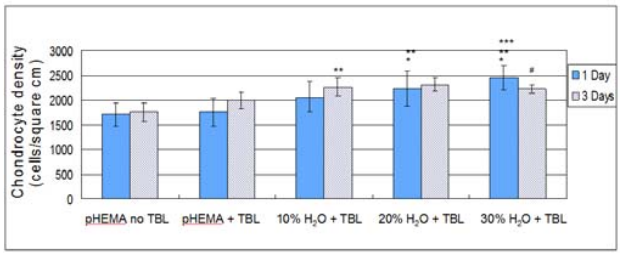

Figure 1. Chondrocyte density on pHEMA composites containing no TBLs, TBLs with 10%, 20%, or 30% H_2O after 1 and 3 day of culturing. All the composites contained TBLs (0.01 mg/ml). Values are mean ± SEM; n=3. (*) $p<0.05$ compared to pHEMA without TBL composites after 1 day of culturing. (**) $p<0.05$ compared to pHEMA with TBL composites after 1 day. (***) $p<0.05$ compared to pHEMA with TBL and 10% H_2O composites after 1 day. (#) $p<0.05$ compared to pHEMA without TBL composites after 3 days.

Conclusions: All composites containing TBLs had higher chondrocyte density than composites without TBLs. The addition of TBLs increased chondrocyte differentiation including total protein and GAG synthesis. Therefore, TBL was effective to increase the bioactivity of the composites, and TBL/pHEMA composites are promising as cartilage implant materials.

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