Enhanced bone cell adhesion and proliferation on BMP-7 peptide functionalized self-assembled rosette nanotubes

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Statement of Purpose: Bone morphogenetic 7 (BMP-7) is one of the most important cytokines of the transforming growth factor beta family involved in skeletal development and bone remodeling [1]. However, in conventional applications, it requires several milligrams of BMP-7 for increasing bone growth, and the high dose may induce a burst effect to trigger an inflammation response, but also possibly cause excessive bone formation outside the fracture site. Previous researchers have identified several peptide sequences from the knuckle epitope of the BMP-7 protein and showed that these peptides have the capability to promote bone cell functions [1]. In this study, we further improved the use of BMP-7 by using self-assembled twin based linker molecules (TBL). Specifically, TBL rosette nanotubes (RNTs) were functionalized with three peptides derived from BMP-7 and were tested for osteoblast and fibroblast adhesion and proliferation.

Methods: Synthesis TBL and peptide functionalized TBL. Unfunctionalized TBL and peptide funtionalized TBL building blocks were synthesized according to a previously reported synthetic strategy [2,3].

Characterization of TBL. Transmission electron microscopy (TEM) was utilized to examine the morphology of TBL and peptide functionalized TBL in water. TEM (JEOL, JEM-1010) images were recorded at 50,000× operating at 80 kV acceleration voltages.

Cell adhesion and proliferation studies. To determine the adhesion and proliferation of osteoblasts (Promocell, C-12720) and fibroblasts (ATCC, CCL-110) on the proposed materials, the MTS CellTiter 96 aqueous one solution assay (Promega, G3581) was used. Briefly, for cell adhesion, cells were seeded at 10,000 cells/cm^2 in standard cell culture media and were incubated for 4 hours. For the proliferation study, cells were seeded at 10,000 cells/cm^2 for 1, 3, and 5 days. A plate reader was used to determine cell density. Numerical data were analyzed with a Student's t-test to make pair-wise comparisons. Statistical significance was considered at $p<0.05$. Experiments were completed in triplicate and repeated at least three times.

Results: TEM images showed that the TBL formed a nanotubular structure in water. Peptide A (SNVILKKYRN) and C (KAISVLYFDDS) funtionalized TBL maintained a very long tubular structures, while peptide B (KPSSAPTQLN) funtionalized TBL had shorter segments. In the proliferation study, A-TBL effectively increased osteoblast density after 5-days of culturing compared to formulations without TBL (Figure 1). On the other hand, C-TBL promoted the highest fibroblast density after 5-days of culturing. Moreover, osteoblasts had a higher cell density than that of fibroblasts after 5-day culturing

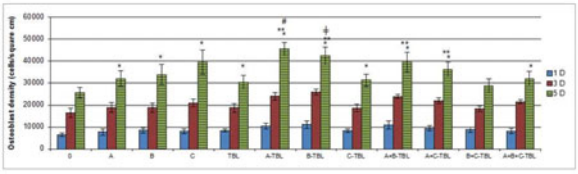

Figure 1. Osteoblast density when culturing with peptide A, B, C, A-TBL, B-TBL, C-TBL, A+B-TBL, A+C-TBL, B+C-TBL, A+B+C-TBL after 1, 3, and 5 days of culturing. Values are mean ± SEM; n=3. (*) $p<0.05$ compared to cells cultured in 96-well plate after 5 days of culturing. (**) $p<0.05$ compared to cells cultured with pure TBL after 5 days. (#) $p<0.05$ compared to cells cultured with peptide A after 5 days. (ǂ) $p<0.05$ compared to cells cultured with peptide B after 5 days.

Conclusions: BMP-7 peptide functionalized TBL was effective to increase the bioactivity of RNTs for novel orthopedic applications.

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