Increased cell functions using BMP-7 functionalized rosette nanotubes

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Statement of Purpose: Bone has a limited capability to heal following large defects caused by traumatic bone loss, bone tumors or infections. Bone morphogenetic 7 (BMP-7), approved by the FDA for orthopedic applications in 2001, is one of the most important cytokines of the transforming growth factor beta family involved in skeletal development and bone remodeling. However, the amount of BMP-7 needed for inducing bone growth is on the order of milligrams due to a short half-life, which is extremely high and might cause a variety of adverse effects ranging from inflammatory responses to excessive bone growth. As a result, a novel delivery system has to be developed to prolong BMP-7 retention and therefore reduce such a high dose. In addition, due to the high cost of the production and instabilities of all BMP proteins, previous researchers have identified several peptide sequences from the knuckle epitope of BMP proteins and showed that these peptides also have the capability to promote bone cell functions. According to a previous study, short peptides derived from the BMP-7 knuckle epitope (peptide A (SNVILKKYRN), B (KPSSAPTQLN), and C (KAISVLYFDDS)) were able to promote osteoblast functions in vitro[1]. In this study, the use of the BMP-7 short peptides mentioned above were further improved for orthopedic applications by covalently connecting them with twin G∧C base-derived rosette nanotubes[2,3]. The mechanism of increased bone cell functions when using such materials were examined by qPCR.

Methods:

A: The synthesis of BMP-7 peptidomimetic-functionalized RNTs (A-RNT, B-RNT, C-RNT) will be published elsewhere. Unfunctionalized RNTs (U-RNT) were synthesized according to a previously reported synthetic strategy.[2,3] The peptide-functionalized twin G∧C bases were mixed with unfunctionalized twin G∧C base at a ratio of 1:9 in water. The resulting RNT stock solution concentration was 700 μM. Pure U-RNTs in water solution at a concentration of 630 μM was set as a control. All of the solutions were sterilized by filtration through a 0.22 μm syringe filter.

B: Characterization of U-RNT: Transmission electron microscopy (TEM) was utilized to examine the morphology of U-RNT and peptide-functionalized RNTs in water. For this purpose, a carbon-coated 400-mesh copper grid was floated on the stock solution at 700 μM for 30 s. Then, the grid was placed on a droplet of 1.5% aqueous uranyl acetate for 5 s, blotted and dried with filter paper. The staining process was then repeated for another three times. TEM images were taken at 50,000×.

C: 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, MTS assays (Promega, G3581) were used. Briefly, for cell adhesion, cells were seeded at 10,000 cells/cm^2 and were incubated for 4 h. For the proliferation study, cells were seeded at 10,000 cells/cm^2 for 1, 3 and 5 days. The MTS dye solution was added to cells at the end of the prescribed period. A plate reader was used to determine cell density.

D: Mechanisms examined by qPCR: To understand the mechanisms of increased cell functions, the relative expression of COL1A2, RUNX2, BGLAP and IBSP in osteoblasts was determined using real-time PCR. GAPDH was chosen as the endogenous control in this experiment.

E: Statistical Analysis: Numerical data were analyzed with student's t-test to make pair-wise comparisons. Statistically significance was considered at $p<0.05$.

Results: In the cell proliferation studies, A-RNT and B-RNT increased osteoblast density by 177% and 165% (Figure 1), respectively, compared to the control groups over 5 days. For the fibroblast experiments, B-RNT, C-RNT and A/C-RNT promoted the highest fibroblast density after 5 days of culturing. Moreover, it is worth mentioning that osteoblasts did achieve higher densities than fibroblasts over the course of 5 days, which clearly provides some advantages for bone applications.

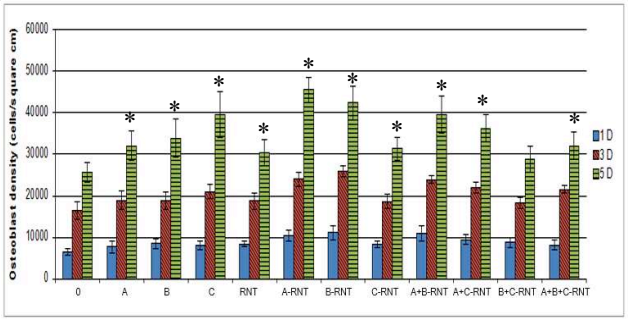

Figure 1. Osteoblast density after 1, 3, 5 day of culturing with samples, date are shown as mean ± SD. (*$p<0.05$ compared to control group)

Abbreviations: "0" (control group), A, B, C (short peptides selected), A, B, C-RNT (functionalized RNT), A/B-RNT (mixtures of A-RNT and B-RNT).

Conclusions: Both short BMP-7 peptides and the functionalized RNTs promoted osteoblast adhesion and proliferation. Among which, B-RNT, C-RNT and A/C-RNT promoted the highest fibroblast density, while A-RNT, B-RNT induced the highest osteoblast density. It is obvious that B-RNT was most effective for both cell types and therefore could be further studied to promote long-term bone functions. Finally, it was shown that BMP-7 peptide functionalized RNT had the ability to increase bioactivity of the U-RNT and the potential in developing novel injectable bone implants.

References: [1] Chen Y.P. et al. J. Biomed. Mater. Res. Part A. 2009, 91A(1): 296–304. [2] Fenniri H. et al. J.Am.Chem. Soc. 2001, 25,123(15): 3854-5. [3] Fenniri H. et al. J.Am.Chem. Soc. 2002, 18,124(37): 11064-72.