Long-Term *In Vitro* Cytocompatibility of Hydrogel Nanocomposites as Injectable Bone Substitutes

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Statement of Purpose:

Today various bone diseases and injuries present a common and significant clinical problem all over the world. Traditional autografts and allografts to treat bone defects have many shortcomings including donor site morbidity, extensive inflammation and transmission of diseases, etc., leading to implant failures. Therefore, the necessity of developing novel biomimetic bone substitutes to quickly cure bone defects is reasonably justified.

Osteogenic rosette nanotubes (RNTs) are novel biologically inspired nanotubes obtained through the self-assembly of DNA base pair building blocks in water. Since RNTs can undergo a phase transition from a liquid to a viscous gel when either heated (as shown in Figure 1, after heating the same concentration nanotube solution, the nanotubes formed a densely packed networks), they may provide an exciting therapy as injectable bone substitutes to heal bone defects quickly. Our previous studies have demonstrated that the biomimetic bone substitute based on RNTs with different osteogenic peptide and amino acid side chains and biocompatible hydrogels (poly(2-hydroxyethyl methacrylate) can greatly enhance osteoblast (bone forming cell) short-term functions[1]. In this study, the efficacy of the novel biomimetic and injectable nanostructured bone substitutes for long-term bone regeneration *in vitro* is investigated.

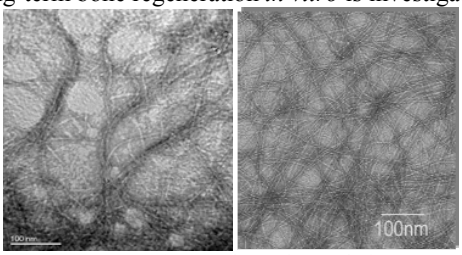

Figure 1. TEM images of (A) unheated 1 mg/ml RNT-K in water, and (B) heated 1 mg/ml RNT-K in water.

Methods: Firstly, 0.01 and 0.001 mg/mL RNTs with lysine (RNT-K) were prepared by dissolving Guanine∧Cytosine motifs with lysine in water. Then these RNT-K embedded in and coated on hydrogels samples were fabricated (as described in [2]) for further long-term osteoblast differentiation study. Hydrogels without RNTs served as controls. Borosilicate glass coverslips were adopted as a reference substrate in this study. All hydrogel samples were sterilized using 70% ethanol solution, air dried overnight and rinsed with phosphate buffered saline (PBS) prior to cell experiments. The human fetal osteoblast cell line (ATCC) was seeded at a density of 100,000 cells/cm^2 for a 21-day study. Then they were cultured in DMEM medium with 10% FBS, 1% P/S, 50μg/mL L-ascorbic acid (Sigma) and 10 m*M* β-glycerophosphate (Sigma) for 7, 14, and 21 days. Medium was changed every other day. At the end of the prescribed time periods, osteoblasts were lysed using three distilled water freeze–thaw cycles. Total protein content, alkaline phosphatase and calcium deposition activity were determined by using commercially available kits following respective manufacturer's instructions.

Results: The three weeks of osteoblast differentiation study demonstrated that nanostructured RNT-K hydrogels even at a very low concentration (such as 0.001 mg/mL) had greater and quicker calcium deposition and alkaline phosphatase activity (not shown here) than hydrogel controls and glass references. Although calcium deposition on all RNT-K hydrogels was similar to hydrogel controls after 21 days, the more and quicker calcium deposition (Figure 2) on RNT-K in hydrogels after 7 days may play a crucial role for further successfully regenerating strong bone at defect sites.

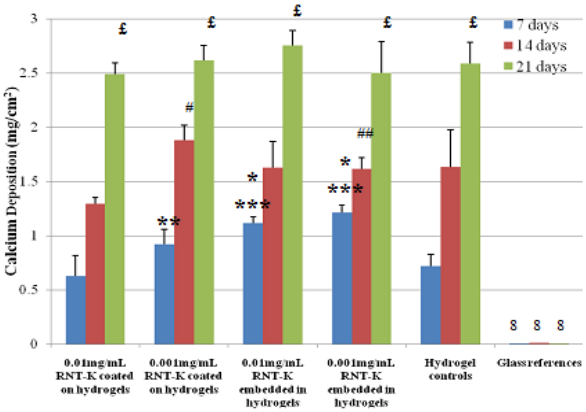

Figure 2. Increased early calcium deposition on RNT-K in hydrogels. Data are mean ± SEM, n=3; *p<0.05 and **p<0.1 compared to hydrogel controls at 7 days. ***p<0.1 compared to 0.01 mg/mL RNT-K coated on hydrogel at 7 days. #p<0.05 and ##p<0.1 compared to 0.01 mg/mL RNT-K coated on hydrogel at 14 days. £p<0.05 compared to respective substrates at 7 days. □p<0.05 compared to all hydrogel substrates at respective 7, 14 and 21 days.

Conclusions: This long-term *in vitro* study showed the excellent cytocompatibility of RNT hydrogel as bone substitutes. The nano bone substitutes are biomimetic, flexible in design and minimally invasive, thus hold potentials for improving current bone defect repair.

References:

1. Zhang L., et al. Biomaterials. 2009;30(7):1309-1320.
2. Zhang L., et al. Tissue engineering, 2008;14(8):1353-1364

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