Novel Osteogenic Peptide Modified Helical Rosette Nanotubes for Improving Orthopedic Applications

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Statement of Purpose:
Today, bone diseases represent a common and significant problem in the world. However, traditional autografts and allografts to treat bone defects have many shortcomings including donor site morbidity, inflammation and possible transmission of diseases, etc., which can lead to implant failure. Therefore, the objective of this in vitro study was to design novel osteogenic peptide-modified self-assembled helical rosette nanotubes (HRNs) for improving orthopedic applications. HRNs are a series of biomimetic nanomaterials that self-assemble spontaneously in aqueous solutions to mimic the nanostructure of collagen in bone. They are composed of Guanine∧Cytosine (G∧C) DNA motifs with tailorable amino acid and peptide side chains. Previous studies have reported that HRNs with lysine (HRN-K1) have excellent cytocompatibility properties and can remarkably improve osteoblast (bone-forming cell) functions when coated on traditional implant materials (i.e. titanium, Ti) [1] or embedded in hydrogels [2] because of its biologically inspired nanoscale features and rich lysine side chains. In this study, the novel osteogenic HRNs conjugated with cell-adhesive RGD and KRSR (which selectively promotes osteoblast adhesion) peptides were synthesized and investigated for improving orthopedic applications.

Methods: RGDSK and KRSR peptides were grafted onto G∧C motifs and twin based G∧C motifs in order to obtain novel modified nanotubes. The normal G∧C base with lysine side chains was also prepared. 0.01 mg/mL HRN-K1, 5% HRN-RGD-K (RGD to K is 1:20) and HRN-KRSR were prepared by dissolving various motifs with different side chains in water. Then various HRNs were coated on cleaned titanium surface by absorption for 45 min at room temperature and were dried overnight. Uncoated titanium and KRSR peptide coated titanium served as controls. Furthermore, the cytocompatibility of these coatings was tested using human fetal osteoblasts (ATCC). Osteoblasts were seeded at 3500 cells/cm^2 and cultured in DMEM/F-12 Ham supplemented with 10% fetal bovine serum, 1% Penicillin-Streptomycin under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air) for 4 h. After 4 h, adherent cells were fixed by 10% formalin and 0.1% Triton X-100. Then osteoblasts were stained with rhodamine-phalloidin (Molecular Probes) to examine cell spreading and were further stained with DAPI (Invitrogen). Five different fields per substrate were counted under a fluorescence microscope. Experiments were repeated at least three times each with three replicates each time.

Results: The results of this study provided evidence that these biomimetic HRNs greatly improved osteoblast adhesion on titanium compared to uncoated titanium after 4 h (Figure 1). For example, 0.01 mg/mL of 5% HRN-RGD-K and HRN-KRSR coated titanium increased osteoblast adhesion by 124% and 122% compared to uncoated titanium. Moreover, RGD or KRSR modified HRNs stimulated more osteoblast adhesion than unmodified HRN-K1. This indicated that the novel RGD and KRSR modified HRNs contributed to a more cytocompatible coating material for improving orthopedic applications. Figure 2 revealed the relatively flat and well spread osteoblast morphologies on HRN-KRSR coated titanium compared to uncoated controls.

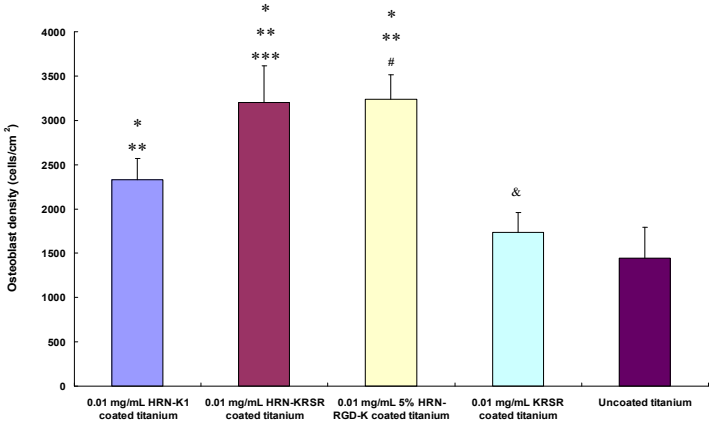

Figure 1. Enhanced osteoblast adhesion on HRNs coated on titanium. Data are mean values ±SEM, N=3. *p<0.01 and &p<0.1 compared to uncoated titanium. **p<0.05 compared to 0.01 mg/ml KRSR coated on titanium. ***p<0.1 and #p<0.05 compared to 0.01 mg/ml HRN-K1 coated titanium.

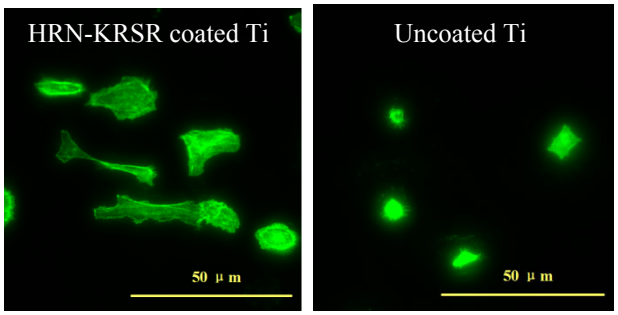

Figure 2. Fluorescence microscopy images of osteoblast adhesion morphologies (rhodamine stained F-actin).

Conclusions: In summary, this study created novel nanostructured and cytocompatible coatings on titanium by modifying HRNs with osteogenic peptides. RGD and KRSR peptide modified HRNs greatly promoted osteoblast adhesion and spreading due to their biomimetic nanoscale dimensions and controllable surface chemistry, which make them promising for orthopedic applications.

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References:

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2. Zhang L., et al. Tissue Eng. 2008;14:1353-1364.