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Helical Rosette Nanotubes as a Potentially More Effective Orthopaedic Implant Material

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ABSTRACT
Organic nanotubes called helical rosette nanotubes (HRN) have been synthesized in this study for bone tissue engineering applications. They possess intriguing properties for various bionanotechnology applications since they can be designed to mimic the nanostructured constituent components in bone such as collagen fibers and hydroxyapatite ($Ca_5(PO_4)_3(OH)$) which bone cells are naturally accustomed to interacting with. This is in contrast to currently used orthopaedic materials such as titanium which do not possess desirable nanometer surface roughness. The objective of this in vitro study was to determine bone-forming cell (osteoblasts) interactions on titanium coated with HRNs. Results of this study showed for the first time increased osteoblast adhesion on titanium coated with HRNs compared to those not coated with HRNs. In this manner, this study provided evidence that HRNs should be further considered for orthopaedic applications.

INTRODUCTION
A class of organic nanotubes called helical rosette nanotubes (HRN) have been synthesized [1-4]. They possess properties suited for various nanotechnology applications such as implant materials, molecular electronic or photonic devices and, drug delivery systems [2, 4]. These HRN are assembled from a single bicyclic block with a Guanine-Cytosine (GC) motif designed to have hydrogen-bond donor-donor-acceptor array on one side and complementary acceptor-donor array on the other side (Fig 1a). These building blocks assemble through H-bonds to form the rosette, which then stack to form a nanotube with a hollow core 11 Å across and up to several micrometers long (Fig 1b) [2]. The tube structure is maintained by electrostatic, hydrophobic and stacking interactions.

A variety of functional groups suited for different applications can be attached to these building blocks. In addition, the entropic nature of the assembly process, similar to entropy-driven processes found in nature (such as assembly of type I collagen fibrils and polymerization of tobacco mosaic virus coat protein), offers opportunities for supramolecular engineering of scaffolds with predefined chemical and physical properties for biomedical applications. With such flexibility and design, it is thought that these HRN are also suited for orthopaedic implants since one can attach growth factors and/or specific bone recognition peptide sequences that will preferentially attract bone cell adhesion.

Moreover, HRN possess biologically-inspired nanometer features that resemble naturally-occurring nanostructured constituent components in bone such as collagen fibers and hydroxyapatite ($Ca_5(PO_4)_3(OH)$) that bone cells are accustomed to interacting with. Previous studies have provided evidence of increased new bone synthesis on nanostructured ceramics,

metals and polymers [5, 6]. Yet, currently used orthopaedic materials such as titanium do not possess desirable nanometer surface roughness. It is believed that this is one reason why titanium sometimes fails clinically as a bone implant material. In addition, reorganization of tissues adjacent to implants depends largely on early protein adsorption onto surfaces, which subsequently affects response of cells to a material [5]. Implant surfaces therefore play an important role in forming the connection and integration between implant and tissue. Much effort has thus been dedicated to developing improved bioactive nanostructured surfaces and nano-materials for biospecificity [5, 6]. The objective of the present in vitro study was therefore to determine osteoblast (bone-forming cells) adhesion on titanium coated with HRN.

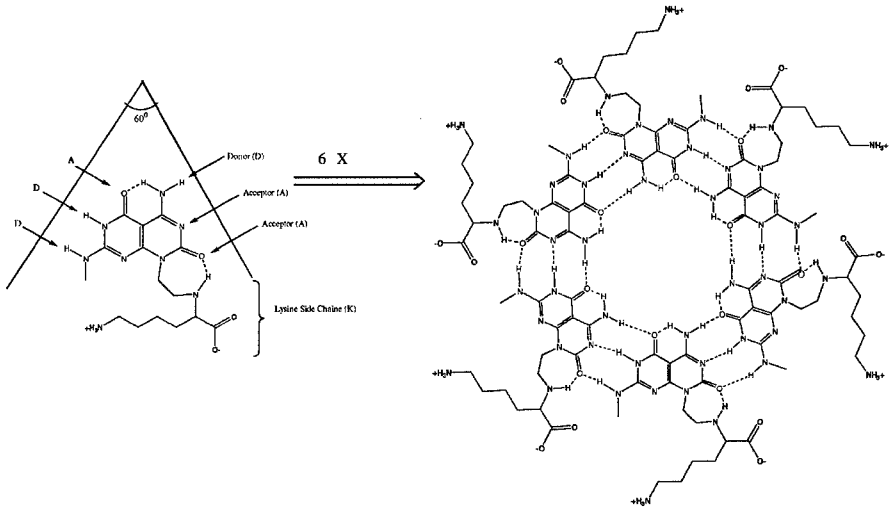
MATERIALS AND METHODS

Osteoblasts

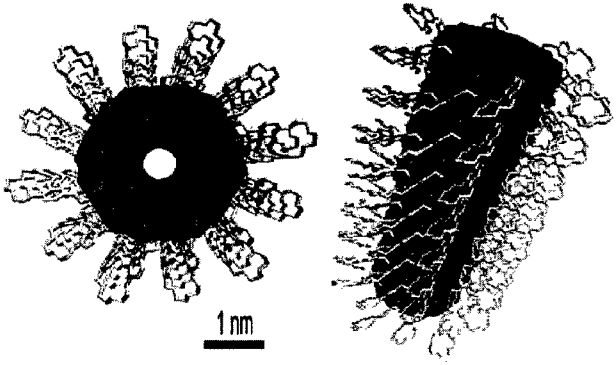
Human fetal osteoblasts (bone-forming cells; American Type Culture Collection: CRL-11372) were cultured in Dulbecco's Modified Eagle Medium/ F-12 Ham (DMEM/F-12 Ham; Hyclone) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (P/S; Hyclone). Cells were cultured under standard culture conditions (37°C and 5% CO_2/95% air humidification) and used as purchased from the vendor without further characterization.

Osteoblast Adhesion on Titanium Coated with Helical Rosette Nanotubes (HRN)

HRN (either containing a lysine residue or an arginine residue) were dissolved in Milipore™-filtered water and filtered once using a 0.2 μm syringe filter. These residues were used model amino acid extensions to the HRN structure. Commercially pure grade 2 titanium (Ti) (1 cm x 1 cm x 2 mm; ASTMB-265-98; SupraAlloys) were coated with HRN by simple adsorption at concentrations of 1 mg/ml, 0.5 mg/ml, 0.125 mg/ml and 0.005 mg/ml for 30 minutes. Substrates were stored for up to 3 weeks at room temperature. Each coated substrate was sterilized with chloroform for 10 seconds immediately prior to experiments with cells. Osteoblasts were then seeded at 3,500 cells/cm^2 and were allowed to adhere in DMEM/F-12 Ham (supplemented with 10% FBS and 1% P/S) for 1 hour under standard cell culture conditions. Controls were uncoated titanium and borosilicate glass coverslips (Fisher) served as a reference. Glass served as a reference due to a firm understanding of how osteoblasts interact with borosilicate glass. After 1 hour, substrates were rinsed with phosphate buffered saline (PBS) to remove non-adherent cells. Cells were then fixed with 10% normal buffered formalin (NBF; Sigma) and stained with 0.3 μg/ml Hoechst 33258 stain (Sigma) for 15 minutes. Five random fields were counted in situ per substrate under a fluorescent microscope. Cell counts were converted to cell densities (cells/cm^2) and one-tail two-sample T-tests were performed. All experiments were run in triplicate and repeated three times.



(a)



(b)

Figure 1: Building blocks of helical rosette nanotubes (HRN). a) The guanine-cytosine (GC) motif possesses the Watson-Crick H-bond donor-donor-acceptor of guanine and acceptor-acceptor-donor of cytosine (*left*). Six GC motifs self-assemble via 18 H-bonds to form the six-membered supermacrocycle (called the rosette) (*right*). b) Second level of organization produces a stack with a hollow core 11 Å across and up to several micrometers long. Top view (*left*). Side view (*right*).

RESULTS

Increased Osteoblast Adhesion on Titanium Coated with HRN-K1

Compared to uncoated Ti substrates, there was statistical difference in the number of osteoblasts that adhered on Ti substrates coated with HRN containing a lysine residue (HRN-K1) after 1 hour ($p < 0.01$, Fig. 2). There was no statistical difference between the different HRN-K1 concentrations studied. Cell adhesion was approximately 1.5 times more on coated substrates than on uncoated ones.

Increased Osteoblast Adhesion on Titanium Coated with HRN-Arg

There was statistical difference in the number of osteoblasts that adhered on Ti substrates coated with HRN containing an arginine residue (HRN-Arg) after 1 hour ($p < 0.1$, Fig. 3) when compared to uncoated Ti substrates.

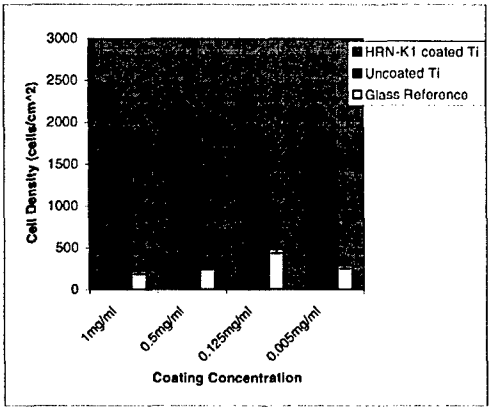


Figure 2: Human osteoblast adhesion. Substrates utilized were borosilicate glass coverslips, commercially pure grade 2 titanium (Ti) and Ti coated with HRN-K1 at 1mg/ml, 0.5mg/ml, 0.125mg/ml and 0.005mg/ml. Data are mean ± SEM; n=3; $*p < 0.01$, $**p < 0.10$ (T-test) compared to uncoated Ti.

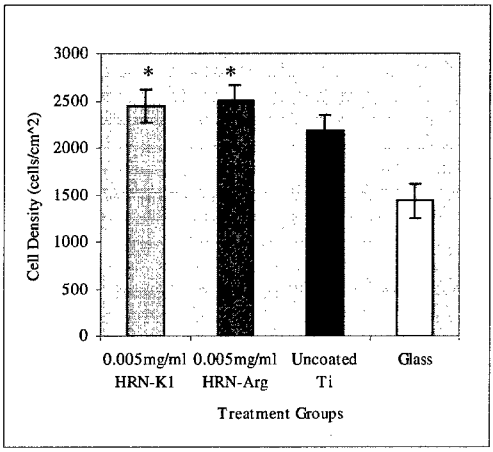


Figure 3: Comparing osteoblast adhesion of HRN-K1 coated-, HRN-Arg coated-, and uncoated Ti substrates. Borosilicate glass coverslips were used as a reference. At 0.005mg/ml both HRN-K1 and HRN-Arg coated substrates showed higher cell densities than uncoated Ti. Data are mean ± SEM; n=3; $*p < 0.1$ (T-test) compared to uncoated Ti.

DISCUSSION AND CONCLUSIONS

Results of the present study show that HRN is a potentially promising orthopaedic implant material. Preferential cell adherence suggests the possibility that both HRN-K1 and HRN-Arg are cytocompatible with osteoblasts. In addition, concentrations as low as 0.005mg/ml still showed enhanced cell adhesion. This is very encouraging since less material is needed to achieve the same cell adhesion properties. Since adhesion of anchorage-dependent cells (like osteoblasts) is imperative for subsequent cell functions such as deposition of calcium-containing mineral, these results encourage us to further explore the possibility of fabricating this material into either a stand alone orthopaedic implant, a composite or as a coating material. These results indicate the potential of these self-assembling HRN (regardless of functionality) for bone tissue engineering applications. It is hoped that through the use of HRN, the optimal material to promote efficient new bone growth will be obtained; such a criterion is critical for orthopaedic implant success.

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