OSTEOBLASTS ALIGNMENT ON NANOPHSE MATERIALS

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INTRODUCTION

Previous studies have shown increased responses of osteoblasts (bone-forming cells) on materials [1] with nanoscale surface features and enhanced biocompatibility for osteoblast-like cells on hydrophobic coating surfaces [2]. Also, carbon nanofibers (CNF) are considered a promising biomaterial as they are not toxic with osteoblasts and promote their function compared to larger dimensioned fibers [3, 4]. However, besides the importance for superior cytocompatibility with nanophase materials, the next generation biomaterials require detailed knowledge of the interactions of cells with individual nanometer structures. Also, from an orthopedic point of view, understanding the interactions of osteoblasts with ordered nanoscale surfaces (like aligned patterns) is intriguing since bone itself is a highly aligned nanophase material. In this work, it was found that actin filaments were strongly correlated with hydrophobic aligned nanometer surface features.

EXPERIMENTAL

Substrate preparation

In this experiment, square glass chambers (Lab-Tek™ II, Nalge Nunc International) were used as a base substrate. RS (wash step was formulated to improve the consistency of the glass surface after the hydrophobic border had been applied) glass possessed hydrophobic nano-lines. Nanophase composite materials were composed of carbon nanotubes (10nm diameter, Multi-Wall Carbon Nanotubes (MWCNT), Catalytic Materials LLC, Holliston, MA) which were suspended in polymer solutions by using an ultra-sonicator. An FDA-approved polycarbonate urethane carbothane (PCU, catalogue no PC-3575A, Thermedics) was used as the matrix element. Chloroform was used to dissolve polymer in the composites (80:20, CNT: PCU wt % were fabricated).

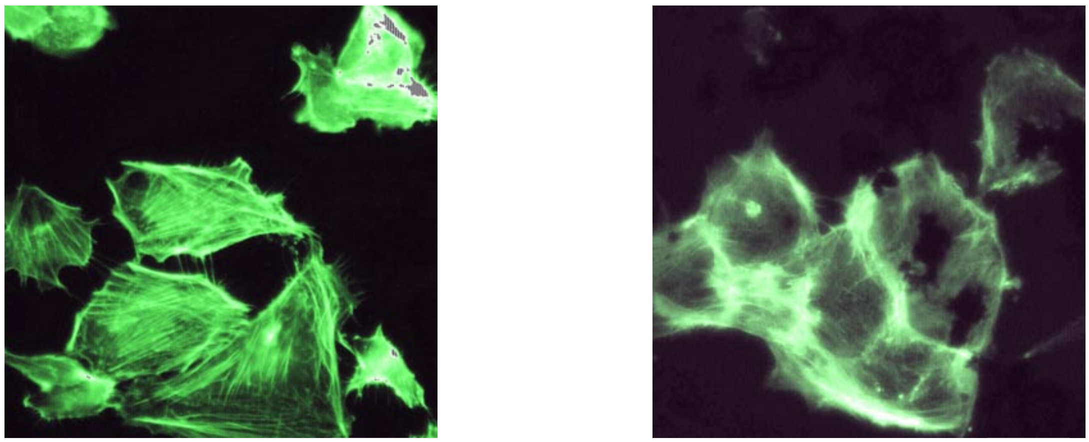
Cytocompatibility

Human osteoblasts (CRL-11372) purchased from ATCC were cultured on the different substrates under standard cell culture conditions(*i.e.*, a 37 °C, humidified, 5% CO_2/95% air environment). Osteoblasts were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S), under standard cell culture conditions. For cytocompatibility experiments, human osteoblasts were seeded at a density of 2,500 cells/cm^2 onto each substrate and were incubated under standard cell culture conditions in osteoblast growth media for 7 days. Osteoblast growth media was replaced every other day. After 7 days, the cells were fixed with 4% formaldehyde (fisher) and stained with Rhodamine Phalloidin (R415, Molecular Probes) to see F-actin filaments and Hoechst 33258(Sigma) to see the nucleus.

RESULTS

Osteoblast interaction with non aligned substrates

On samples with non-aligned nanoscale features, F-actin filaments in osteoblasts were random.



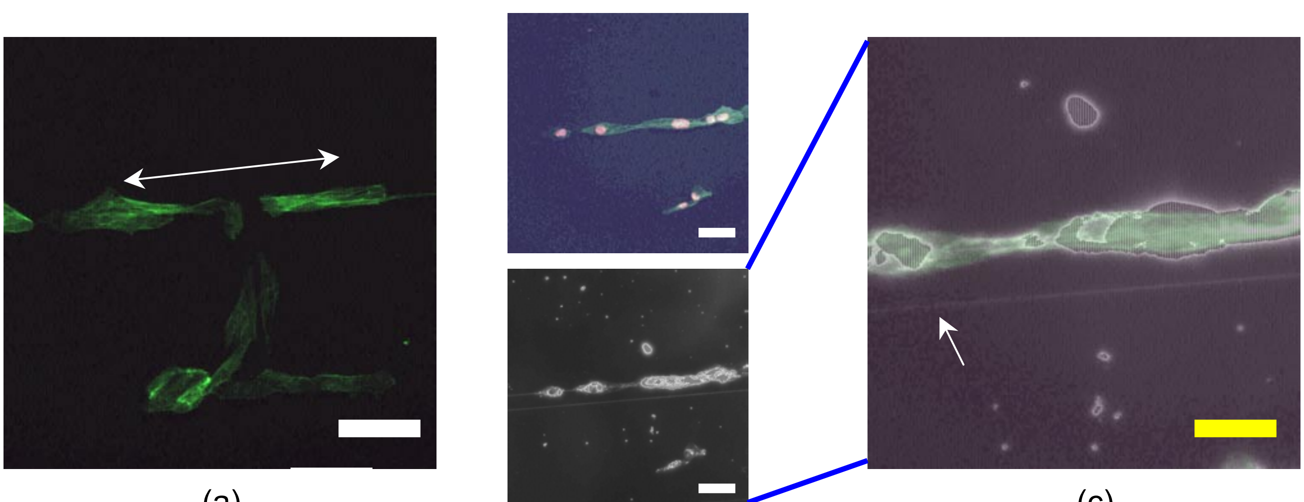
(a) (b)

Figure 1. Osteoblasts on non-aligned substrates

(a) and (b) (bar is 20μm) are the reference glass and the CNT/PCU composite. Actin filaments in osteoblasts on each substrate showed different shapes and no preferred alignment.

Osteoblast interaction with aligned substrates

In contrast, for substrates in which nanoscale features were aligned, f-actin filaments in osteoblasts showed a preferred corresponding alignment.



(a) (b) (c)

Figure 2. Osteoblasts aligned on aligned nano featured glass

(a) Aligned osteoblasts (white arrow) provided evidence of a preferred orientation on substrates with aligned nanometer features (bar is 40μm). (b) Adjacent nuclei and aligned cell bodies match the aligned nano pattern (bar is 40μm). (c) Image (bar is 20μm, arrow shows hydrophobic nano aligned features parallel to the one above it) displays alignment of actin filaments in osteoblasts.

DISCUSSION

In this study, evidence is provided of the ability to control osteoblast alignment through the use of aligned nanoscale surface features. At hydrophobic surfaces, specific proteins that mediate osteoblast attachment may influence the surface tension between the cell and the nano-lined surface. Therefore, highly aligned CNT composite materials with functionalized coatings could be used as promising biomaterials. Result from the present study is coupled with previous reports that demonstrated enhanced calcium-deposition by osteoblasts cultured on non aligned CNF in PCU composites; thus, this study continues to show promise for the use of these materials in orthopedic applications.

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