Involvement of N-cadherin/β-catenin interaction in the micro/nanotopography induced indirect mechanotransduction

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Statement of Purpose: Topographical modification at micro- and nanoscale is widely applied to enhance the tissue integration properties of biomaterials, but the underlying molecular mechanism is poorly uncovered. We propose that N-cadherin may play a role in the topographically induced indirect mechanotransduction by regulating the β-catenin signaling.

Methods: Four different samples on titanium (Ti) were fabricated including the nanotubular topography (NT), the micropitted topography (MT), the hybrid micro- and nanoscale topography (MNT) and polished sample (PT). The cell functions, gene expression, N-cadherin and β-catenin protein level were compared. After down-regulated the N-cadherin expression by siRNA, the supposed downstream events and the cell function changes are monitored.

Results: The morphology of the fabricated Ti samples was displayed in Fig.1 with the insets showing the nanoscale details.

Fig.1. FE-SEM images of the Ti samples including PT, NT, MT and MNT. The insets with higher magnification show the nanoscale details.

The cytoskeleton of the MC3T3-E1 cells was displayed by actin staining and the cell morphology was inspected by FE-SEM (Fig.2A,B). The cells on PT and NT mainly showed a polygonal shape, while those on MNT and MT mainly assumed a two-polar spindle shape, especially MT. Compared to MT, MNT gave rise to enhanced cell spread due to the addition of nanotubular cue.

Fig. 2. (A) Fluorescence microscopy images of the cells with dual staining with phalloidin for actin filaments (red) and Hochest for nuclei (blue); (B) Osteoblast cell morphology on the Ti samples after 4 days of culture. The insets show represent cells in higher magnification;

The cell differentiation markers including the osteogenesis related gene expression, Alp staining, collagen secretion and ECM mineralization (data not shown) were observed before and after NCADsi (Fig.3). Before down-regulated, Alp staining, collagen secretion and ECM mineralization all showed the similar trend of MT ≥ MNT > PT ≥ NT. NCADsi significantly increased Alp staining, collagen secretion and ECM mineralization. The total and membrane N-cadherin protein levels showed the trend of NT > PT > MNT > MT (Fig.4A,B). While both the cytoplasmic and nuclear (Fig.4D,E) protein levels of β-catenin showed the trend of MT > MNT > PT > NT, reverse to that of N-cadherin expression levels. NCADsi significantly depressed the total and the membrane bound N-cadherin amounts on all the four surfaces (Fig.4A,B).

On PT and NT NCADsi induced significantly higher β-catenin expression, whereas on MT and MNT no statistical significance is observed even though NCADsi led to slightly higher β-catenin expression in number (Fig.4C). Comparatively more obvious enhancing effect of NCADsi on β-catenin protein product was observed on PT and NT (Figs.4D,E).

Fig. 4. Western blot analysis of membrane N-cadherin protein levels (A) and total N-cadherin protein levels (B) in the absence and presence of NCADsi; Western blot analysis of cytoplasmic β-catenin protein levels (C) and nuclear β-catenin protein levels (D) in the absence and presence of NCADsi. * p < 0.05.

Conclusion: The N-cadherin negatively regulates the β-catenin signaling and thus the osteoblast differentiation, which is differentially modulated by the micro- or nanotopography. Down-regulating the N-cadherin expression can significantly increase the β-catenin signaling and the consequent osteoblast differentiation on all the Ti surfaces, which provides a potential new strategy for improving biomaterial performance.