## Nanotubular composites decorated on titanium to regulate cell responses

Kapil D Patel<sup>1,2</sup>, Chinmaya Mahapatra<sup>1,2</sup>, Jung-Ju Kim<sup>1,2</sup>, Tae-Hyun Kim<sup>1,2</sup>, Hae-Won Kim<sup>1,2,3\*</sup>

<sup>1</sup>Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, South Korea.

<sup>2</sup>Department of Nanobiomedical Science & BK21 PLUS NBM Global Research Center for Regenerative Medicine, Dankook University, Cheonan 330-714, South Korea.

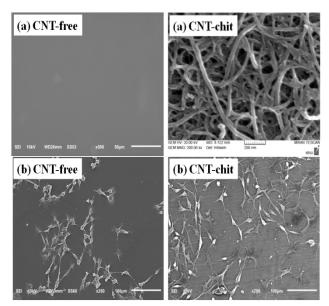
<sup>3</sup>Department of Biomaterials Science, School of Dentistry, Dankook University, Cheonan, 330–714, South Korea

\*E-mail: kimhw@dku.edu

**Statement of Purpose:** Nanotopologies have shown great influences in regulating a series of cell responses, including cell adhesion, migration and differentiation. The manipulation of nanostructures onto metallic surfaces can thus provide a promising platform for cellular control over the implant materials. Here we utilized carbon nanotubes (CNTs) to decorate titanium surface with nanotubular structure via electrophoretic deposition (EPD). As the priori index for the cellular control of the nanotubular surface, the initial adhesion events of cells were examined.

**Methods:** CNTs were dissolved (0.05 wt %) in 30 % ethanol in water. As the binder of CNTs, a range of chitosan solutions (0.012~0.1wt % prepared at pH 3.5) were also used. The CNT-chitosan complexes were decorated onto Ti substrate, under the EPD process adjusted. The coating surface morphology was observed by field-emission scanning electron microscopy (FE-SEM). The degradation of the coatings was investigated in PBS, and the apatite forming ability was examined in simulated body fluid (SBF). To investigate cellular adhesion events, pre-osteoblastic (MC3T3-E1) cells were cultured. The cell adhesion level, spreading area and the expressions of adhesive proteins were analyzed and then compared between the different compositions.

Results: The EPD process was shown to effectively form CNT-chitosan composites on the titanium surface, as observed in different voltages and times. The chitosan molecules were shown to complex with CNT due to charge-charge interactions. The high resolution SEM images of CNT-chitosan decorated surface revealed the nanotubular-structured morphology, which was contrasted by the CNT-free surface morphology (figure 1). The nanotubular morphology was preserved well with chitosan contents up to 0.1wt%. The CNT-chitosan surfaces showed excellent apatite formation in SBF within 1 week. The degradation of the surfaces increased with increasing chitosan content. The adhesion level of cells was significantly enhanced by the CNT-chitosan decoration. Furthermore, the adhered cells spread actively with time on the nanotubular surface, presenting a spreading area being highly improved. The expression of proteins related with adhesion events, including p-FAK, p-paxillin, and vinculin, was significantly up-regulated on the nanotubular surface.



**Figure.** (a) Surface morphologies (CNT-free, CNT-chit) and (b) cell adhesion morphology at 18 h (CNT-free, CNT-chit).

**Conclusions:** The nanostructured CNT-chitosan composite was evenly decorated on titanium surface through the simple application of EPD process. The nanotubular-structured surface significantly stimulated the initial cellular adhesion events, including cell anchorage, spreading, and the expressions of a series of adhesion molecules. The currently-developed nanotubular surface is considered to find extended uses to regulate cellular behaviors, which might not being limited to the initial adhesion events.

**References:** (1) Patel KD et al. J Mater Chem. 2012; 22:24945-24956. (2) Patel KD et al. ACS Appl Mater Interfaces 2.14; 6:20214-20224.