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Regulation of osteogenesis by micro/nano hierarchical titanium surfaces through a Rock-Wnt5a feedback loop

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ABSTRACT

Titanium substrates with micro/nano hierarchical features could positively mediate the osteogenesis of a titanium implant; nevertheless, the underlying molecular mechanism needs to be further revealed. In this work, we fabricated a micro/nano hierarchically structured Ti (MNT) sample and attempted to evaluate its topography-mediated biological effects and potential molecular mechanisms *in vitro*. The results proved that MNT could not only affect cell morphology and osteogenic differentiation, but also regulate ROCK activity cell biological functions of osteoblasts involved in ROCK activation, β-catenin accumulation, and high-Wnt5a expression in respect to topographical features. Moreover, blockade of ROCK activation resulted in significant inhibition of cell differentiation and Wnt5a expression. Furthermore, the anti-Wnt5a significantly down-regulated ROCK activity. In short, these results indicate the important role of ROCK-Wnt5a feedback loop in regulating cell differentiation by topographies.

1. Introduction

With the rapid progress in biomaterials and regenerative medicine, bone grafts and bone substitutes were widely used [1]. Titanium (Ti) is one of the most commonly used materials for the fabrication of orthopedic implant, due to its good mechanical properties and excellent corrosion resistance [2,3]. But the native titanium implants are highly prone to implant failure due to their inherent surface bio-inertness and poor integration capacity [4,5]. Therefore, the surface modification of titanium is deemed of great importance for strengthening the interaction between cells and titanium. Due to the multi-level (from nanometer to micrometer) structure of natural bone extracellular matrix [6], various manufacturing methods have been used to develop micro- and/or nano-scale surface topologies in recent years, such as polishing [7], grinding [8], sand blasting, etching [9,10] and plasma spraying [11]. Meanwhile, micro/nano hierarchical structures mimicking the natural bone have been fabricated, which are expected to have good biological effects, including osteogenic differentiation and bone formation [12,13].

In regards to the advancement of research, people began to study the mechanism of cell-material interaction after paying attention to the preparation method and biological performance. Previous studies successfully determined the cell-material interaction through integrin and cell fate regulation via cell biochemistry and cell morphology [14–16]. Moreover, cell morphology affected cell biological processes through the ROCK signal pathway [17,18]. Tang et al. proved that hierarchical macropore/nano significantly activated the ROCK signal pathway regulating the osteogenic differentiation of MC3T3 and bone marrow stromal cell (BMSC) [19,20]. Zhang et al. reported that micropitted/nanotubular surface topographies (MNTs) promoted osteoblast differentiation via Wnt/β-catenin pathway in MG63 [21]. The Rho/ROCK, as a common signal pathway for mechanical signal transmission, responded to extracellular physical stimulation, thus altering the cytoskeletal tension. Wnt signaling pathways play a key role in cell proliferation, differentiation and bone formation. To the best of our knowledge, previous reports commonly focus on the importance of independent ROCK or Wnt signal pathways in osteogenic differentiation. However, few studies directly concerned with the relationship between ROCK and Wnt pathways during osteogenic differentiation. Thus, we hypothesized that ROCK-mediated cytoskeleton regulation could influence the interaction of Wnt signaling pathways with osteogenic differentiation of osteoblast grown onto Ti substrates with different surface topographies.

The aim of this study was to confirm the relationship between ROCK and Wnt pathways involved in the promotion of osteogenic differentiation on hierarchical macro/nano structure, revealing the underlying mechanisms associated with surface topography-dependent biological effects.

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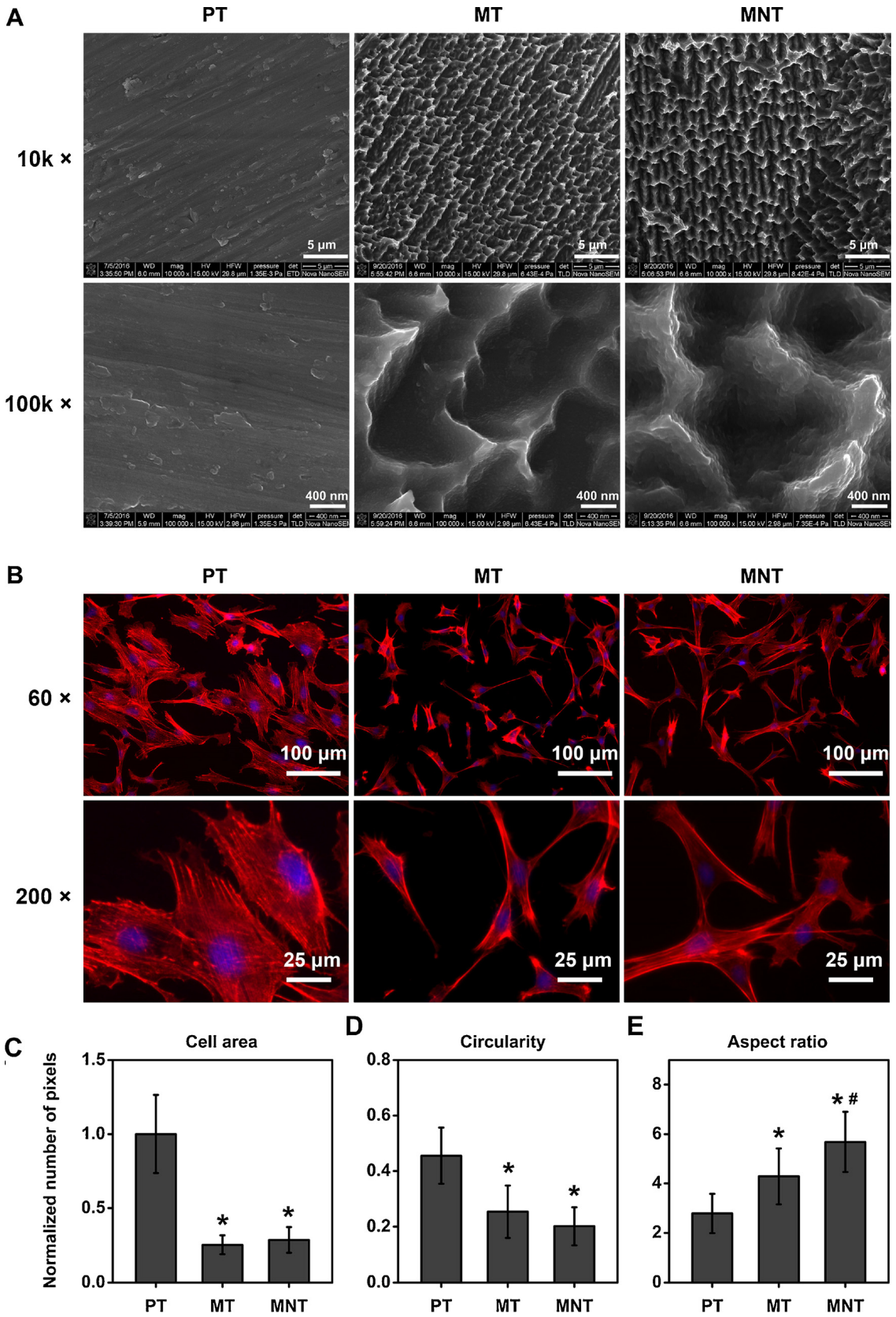

Fig. 1. Characterization: (A) surface morphology of polished smooth titanium (PT), micro-structured titanium (MT) and micro/nano-structured titanium (MNT) detected by SEM; (B) fluorescence images of cells grown on different Ti substrates for 3 days; (C) cll area, (D) circularity and (E) aspect ratio were analyzed using Image J software based on above images (B), $^{*}p < 0.05$ *vs.* PT, $^{\#}p < 0.05$ *vs.* MT.