分科会：生体・医療材料開発分野

Osteogenic differentiation and calcification behaviors of preosteoblast on titanium modified with femtosecond laser

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1. Research Object

The topography of a substrate material surface plays an important role for cellular attachment, proliferation, and differentiation. In our privious studying, it showed that cells spread along with the direction of the submicron gooves on titanium (Ti) surface [1]. This initial cellular adhesion orientation would have effect on the subsequent cell osteogenic differention and calcification behaviors. In this work, to elucidate the effect of submicron topography on cell osteogenic differentiation and calcification of a preosteoblast (MC3T3-E1), the femtosecond laser was used to fibricate the grooves topography on Ti. After 7 d osteogenic differentiation induction, the osteoinduction in MC3T3-E1 on metals were evaluated through genic diagnosis using real time RT-PCR. Then, the visualized formation of calcified extracellular matrix were presented by alizarin red S staining at day 28 after osteogenic differentiation induction. This study is expected to contribute to promote the novel biomaterials-cell interface design for controlling the specific cellular function.

2. Experimental Results

2.1 Materials and Methods

The surface topography was fabricated on mirror polished Ti plate (as named “Mirror Ti”, grade 2, 10 mm × 10 mm) by femtosecond laser scanning. The conditions of laser irradiation were optimized to form periodic submicron structures [1]: sapphire laser system, which had a wavelength, repetition rate, and pulse duration of 775 nm, 1 kHz, and 150 fs, respectively. The laser fluence was 0.35 J cm^{-2}. This surface modified specimen was named “Grooves Ti”. The surface morphology was observed by scanning electron microscopy (SEM) and atomic force microscopy. The results showed that the periodicity and height of the femtosecond laser processed submicron structures are about 590 nm and 270 nm, respectively [1].

A general *in vitro* test process in this work was showed in Figure 1. A mouse preosteoblast (MC3T3-E1) was seeded onto all specimens at an approximate initial density of 6000 cells cm^{-2} and incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air. The tissue-culture-treated polystyrene dishes (TCPS) were used as control. After cells reached to 100% confluence on specimens, the osteogenic differentiation was induced. The cell osteogenic differentiation was evaluated by target genes’ (Fig. 2) expression after 7 d differentiation inducing. In addition, the calcified extracellular matrix were presented by alizarin red S staining after 28 d differentiation inducing.

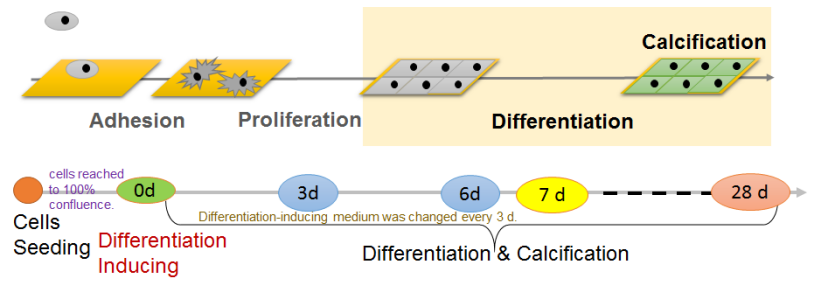
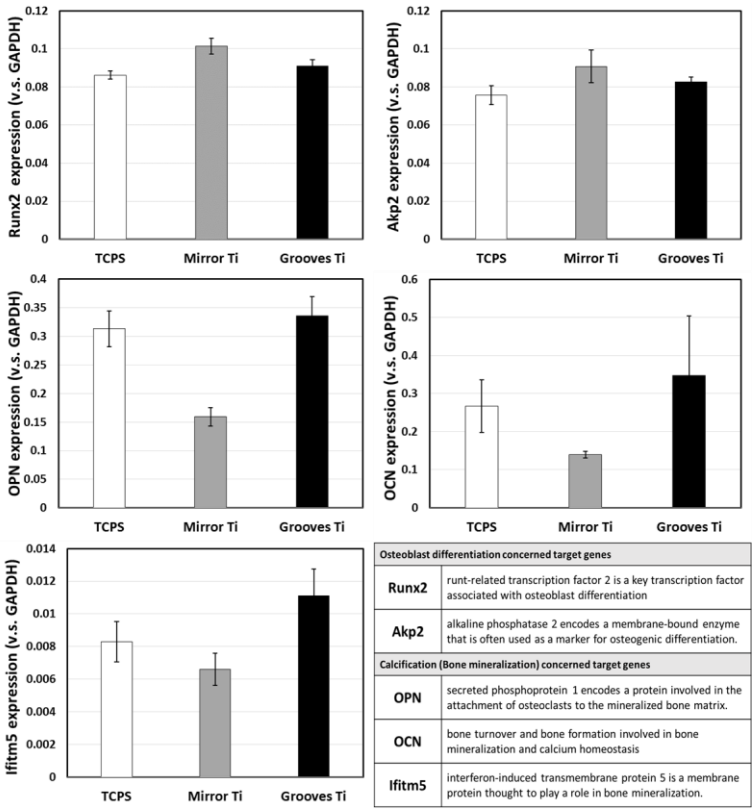


Figure 1. Schematic diagram of the *in vitro* test process in this work.

2.2 Results and Discussion

Figure 2 shows the target genes' expression levels by cells cultured on specimens at day 7 after induction of differentiation. Similar expression levels of *Runx2 and Akp2, which* concerning the cell osteoblast differentiation, were obtained by cells cultured on all samples. This result may be explained by the long term osteogenic differentiation inducing. After 7 d incubation, most of preosteoblast already transformed into osteoblast.

The calcification concerned target genes, *OPN*, *OCN* and *Ifitm5*, showed a higher expression levels by cells cultured on Grooves Ti, compared with cells cultured on Mirror Ti or TCPS. These results indicated that the grooves topography of Ti have a calcification promotion on preosteoblast, which may be explained by the alinged cellular adhesion and stronger attachment with the substract.



Osteoblast differentiation concerned target genes	
Runx2	runt-related transcription factor 2 is a key transcription factor associated with osteoblast differentiation
Akp2	alkaline phosphatase 2 encodes a membrane-bound enzyme that is often used as a marker for osteogenic differentiation.
Calcification (Bone mineralization) concerned target genes	
OPN	secreted phosphoprotein 1 encodes a protein involved in the attachment of osteoclasts to the mineralized bone matrix.
OCN	bone turnover and bone formation involved in bone mineralization and calcium homeostasis
Ifitm5	interferon-induced transmembrane protein 5 is a membrane protein thought to play a role in bone mineralization.

Figure 2. Histograms of target mRNA expression levels in MC3T3-E1 cultured on the specimens at 7 days after the induction of osteogenic differentiation.

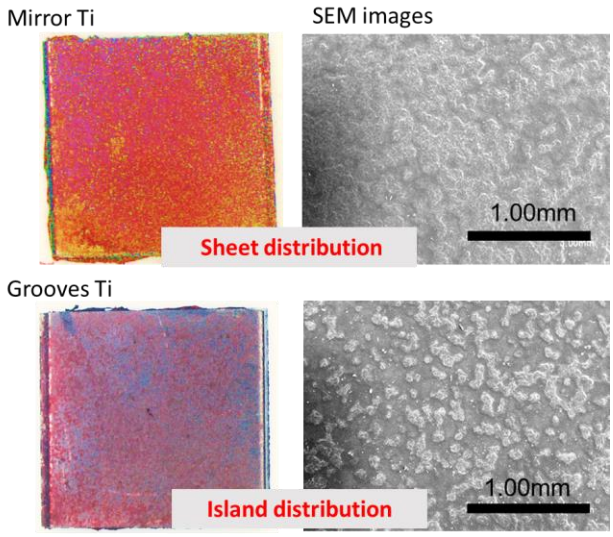


Figure 3. Calcified deposits by MC3T3-E1 cultured on Mirror Ti and Grooves, stained with alizarin red S solution

Figure 3 shows calcified extracellular matrix in MC3T3-E1 cells cultured on specimens at day 28 after induction of differentiation. A calcified deposition with sheet distribution was obtained by cells cultured on Mirror Ti, while, a calcified deposition with island distribution was obtained by cells cultured on Grooves Ti. This phenomenon could be explained by the lack of good cell-cell communication of the well-aligned cells cultured on Grooves Ti (Fig. 4).

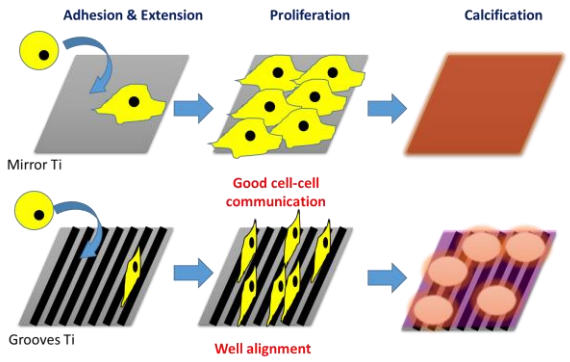


Figure 4. Model of the role of grooves surface topography in cell calcification behavior after the induction of osteogenic differentiation.

In this study, grooves Ti surface was created using a femtosecond laser. This grooves surface promotes osteogenic differentiation after 7 d differentiation inducing and affects the distribution of calcified deposition at day 28 after the induction of differentiation. Our findings provide a basis for designing novel biomaterial-cell interfaces to control specific cellular functions.

3. References intersurface

(1) Shinonaga T, Tsukamoto M, Kawa T, Chen P, Nagai A, Hanawa T. Formation of periodic nanostructures using a femtosecond laser to control cell spreading on titanium. Appl. Phys. B 2015;119:493-496.