Cellular and Gene Expression Responses in Osteoblast-like Cells to Metals

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Introduction: Titanium (Ti), zirconium (Zr), and gold (Au) are highly corrosion-resistant materials. Ti and Zr are covered by surface passive oxide films, while, Au is not. However, the bioactivity of metals is caused not only by good corrosion resistance but also by other properties. For example, Ti shows the best hard tissue compatibility among them. Thus, the aim of this study was to deep investigation of this difference in cellular and gene expression responses to those metals, which makes it possible to deeper understanding the mechanism of bone formation on metallic biomaterials. In this work, a mouse osteoblast-like cell (MC3T3-E1) was employed. After osteogenic differentiation induction, visualized formation processes of calcified extracellular matrix were presented by alizarin red S staining. Then, the osteoinduction in MC3T3-E1 to metals were investigated through genic diagnosis using real time RT-PCR. Our study is important for understanding of the cellular osteoinduction behaviors at metal-cell interface.

Methods: In order to have an ultra-pure metal surface, Ti, Zr and Au specimens were prepared by sputter-deposition [1] onto cover glasses. All specimens were sterilized by 70% ethanol before using. A MC3T3-E1 (RIKEN cell bank, Japan) were cultured in a culture medium. As a control, cells were incubated on tissue culture treated plastic dishes (TCPS). After cells reach to 100% confluent in culture medium, the medium was changed to differentiation-inducing medium. This medium was changed every 3 d. The calcification of MC3T3-E1 cells differentiation-induced for 5, 7, 10, 14, 21, and 28 d on each specimen was evaluated by alizarin red S staining. Meanwhile, gene expression analysis was carried out as follows; total RNA was isolated from differentiation-induced cells of 0, 3, 5, 7, 10, 14, and 21 d using RNeasy Kit (Qiagen). Extracted total RNA was reverse-transcribed into cDNA with random hexamer primers using the PrimeScript II 1st strand cDNA Synthesis System (Takara, Japan). Gene expression in cells was determined by miRNA TaqMan® qRT-PCR assays (Applied Biosystems) on a LightCycler® 480 (Roche) according to suppliers protocol. Primers for real time RT-PCR were Col1, ALP, BSP, OPN, OCN, and Ifitm5. Housekeeping gene of GAPDH was used as internal control in reactions.

Results: Figure 1 shows calcified deposits in MC3T3-E1 cells on the different metals. A quicker calcification (at 5-day incubation) can be obtained by TCPS comparing with metals. Comparing with Au, MC3T3-E1 cultured on Ti and Zr surfaces showed a better bioactivity at 7 d, 10 d, and 14 d. With a long term (21 d and 28 d) incubation, similar calcification images of cells on different metal surfaces were obtained. On the other hand, gene expression analysis results showed that, TCPS had a higher quicker differentiation rate than metallic surfaces. However, different metals presented different expression levels of genes involved in bone formation during the incubation. In the initial stage (1 to 7 days after inducing the differentiation), Au showed a higher gene expression in BSP, which encodes a major structural protein of the bone matrix, and OPN, which encodes a protein, involved in the attachment of osteoclasts. After 7-day incubation, Ti reached to the highest gene expression level in BSP, OPN, and Runx2, a key transcription factor associated with osteoblast differentiation. Ti also showed an advanced gene expression level in OCN, a biochemical marker of bone turnover and bone formation involved in bone mineralization and calcium homeostasis. At the same time, comparing of Ti with Au, the gene expression responses inMC3T3-E1 to Zr were not prominent. Moreover, at 5-day differentiation incubation, a premier calcification phenomenon was obtained by alizarin red S staining, which was also confirmed by genic diagnosis with target genes of ALP, which encodes a membrane bound enzyme that is often used as a marker for osteogenic differentiation and Ifitm5, which encodes a membrane protein thought to play a role in bone mineralization. Based on this finding, the initial osteoblast-like cells may form a premier calcification during the junior cellular proliferation and differentiation, which would be important for promoting the secondary cellular adhesion and differentiation. The conformation of proteins adsorbed on the metallic surfaces was supposed to be one of important factors for the cellular osteoinduction and calcification at metal-cell interface.

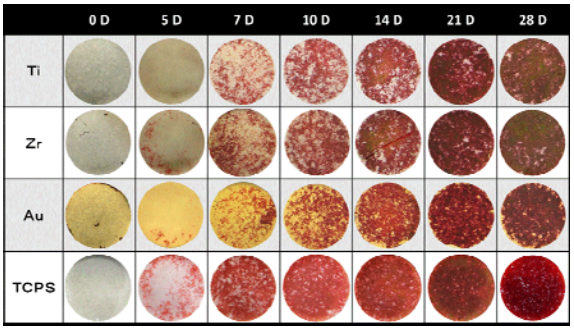

Fig. 1 Pictures of calcified deposits in MC3T3-E1 cells stained with a 1% alizarin red S solution after fixation.

Conclusions: A similar biocompatibility of Ti and Zr was obtained. However, the genic diagnosis showed that the osteoinduction and calcification behaviors of cells were different to metals. This work may contribute to promote the novel metallic biomaterials design and use of those biomaterials for orthopedic/dental applications.

References:

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