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BASIC RESEARCH

Selective laser melting titanium with nanonet topography inhibits osteoclast differentiation through MAPK signaling pathway.

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Abstract

Selective laser melting (SLM) is a promising technique in manufacturing custom-made titanium implants. In view of the important role of osteoclasts in peri-implant bone resorption, we modified SLM-manufactured titanium surfaces using sandblasting/alkali-heating (SAH), and investigated their effect on osteoclast differentiation as well as their underlying mechanisms. We induced osteoclasts derived from primary mouse bone marrow-derived monocytes (BMMs) on specimens, then osteoclast differentiation was determined by tartrate-resistant acid phosphatase (TRAP) activity assay, calcitonin receptors (CTR) immunofluorescence staining and the expression of osteoclast-related genes. SAH established nanonet topography, thus suppressed BMMs osteoclastogenesis in vitro. It was further revealed that the nanonet topography attenuated the activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinases (JNK). The anti-osteoclastogenesis effect of modified SLM implant might contribute to preventing peri-implant bone loss.

Results

TRAP activity on SAH surfaces was significantly lower than SAN (p<0.001). Multinuclear cells expressing CTR were observed on the SLM surfaces while the SAH samples showed a markedly lower number of multinuclear cells. The mRNA expression of TRAP, CTSK, MMP-9, NFATc1 was significantly lower in SAH group (p <0.001), compared with SLM. A possible underlying mechanism may be that SAH surfaces block the activation of the ERK and JNK.

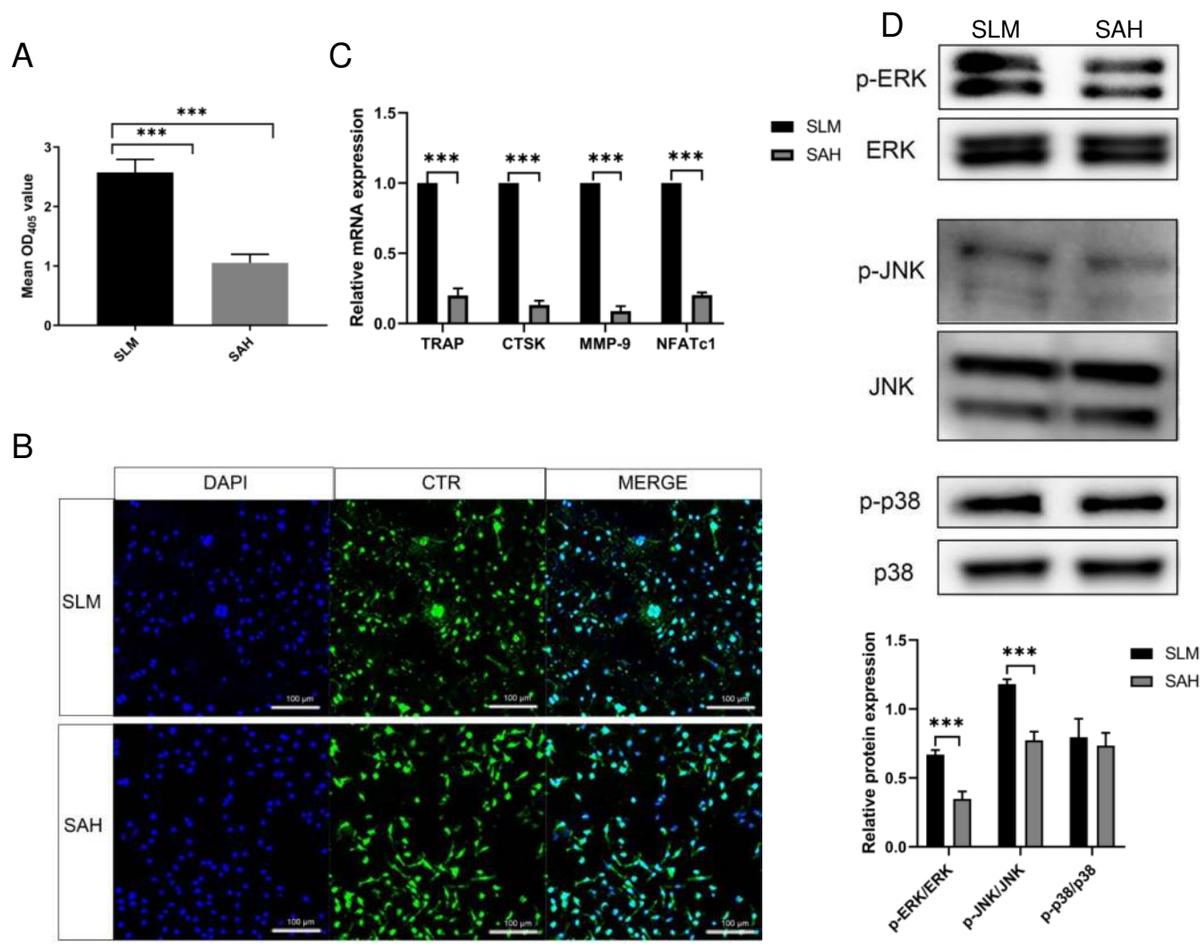

Figure A. TRAP enzyme activity measured at OD_{405} (***p<0.001).

Figure B. Immunostaining for DAPI (nuclei), CTR and merged images of cells cultured on samples.

Figure C. Quantitative PCR for osteoclast-related genes (TRAP, CTSK, MMP-9, NFATc1) of cells on samples (***p<0.001).

Figure D. Western blot analysis for phosphorylation of ERK, JNK and p38 of cells cultured on samples (***p<0.001).

Background and Aim

Selective laser melting (SLM) is widely used in biomedical implants fabrication. It has been demonstrated that SLM titanium with nanonet topography promoted osteogenesis, but its anti-osteoclastic effect is still unknown. The study aimed to define the effect of SLM titanium with nanonet topography on osteoclastogenesis and elucidate the underlying mechanism.

Conclusion

Selective laser melting titanium surfaces with optimized nanonet topography have better anti-osteoclastic effect through MAPK signaling pathway, which may reduce the bone absorption.

Methods and Materials

We used sandblasting to modify native selective laser melting titanium (SLM), followed by alkali-heat treatments (SAH) to create optimized nanonet topography. BMMs were obtained from 5-week-old mice, and they were cultured on the samples with 60 ng/ml nuclear factor- KB ligand (RANKL) and 30 ng/ml monocyte-colony stimulation factor (M-CSF). Osteoclastic differentiation was evaluated by tartrate-resistant acid phosphatase (TRAP) activity assay, calcitonin receptors (CTR) immunofluorescence staining and the mRNA expression of osteoclast marker genes TRAP, CTSK, MMP-9, NFATc1, measured by reverse transcription polymerase chain reaction (RT-PCR). The molecular mechanisms of the effect of SLM titanium surfaces on osteoclastogenesis were investigated by western blotting for osteoclast specific molecules.

References

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