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Bone formation and bacterial early adhesion in micro and nanostructured surfaces for biomedical implants

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Background: Titanium (Ti) surface treatments do alter significantly the surface properties of Ti and the cell responses at the bony interface. Nanotechnology seems to generate surfaces presenting faster and effective bone response by creating nanoscale contacts with extracellular matrices. Also, surface morphology, roughness, purity and wettability may directly influence the superficial contacts between cells and biomaterial.

Aim/Hypothesis: This study aims to investigate the influence of nano and micro alterations of the Ti surface in terms of its physicochemical properties, and verify the possible influences on early bone cell responses and bacterial colonization.

Materials and Methods: For the present investigation, 90 pure Ti disks were prepared and later divided into five groups (G1-machined, G2-etched surface, G3- double etched surface, G4- etched surface; anodization and G5- double-etched surface; anodization). The samples were first analyzed by electron microscopy and atomic force (SEM and AFM), and also wettability test (Goniometer). A statistical test was applied to investigate the fidelity of two-dimensional (2D-Ra) and three-dimensional (3D-Sq) roughness measurements. Biocompatibility tests for osteoblastic differentiation, bone matrix formation, osteoblastic adhesion (7 days) and adhesion of *S. epidermidis* bacteria at two periods (1 h and 24 h) were performed to verify the biological responses. Data were analyzed using ANOVA followed by Tukey test.

Results: The applied physicochemical tests characterized five different Ti surfaces in terms of roughness and morphology. The G3 group presented the highest roughness values with Ra and Sq (5000 nanometers) followed by the G5 group with TiO₂ nanotubes (Ra and Sq- 2000 nanometers). The wettability tests presented similar contact angles among all the treatments, which provided a direct analysis of surface roughness and morphology. All surfaces showed biocompatibility for osteoblastic adhesion and proliferation. The observed osteoblastic adhesion suggested better results in the groups with nanotexturization (G5 and G4), while the higher bacterial adhesion showed statistical significance in the G3 group only after 24 h of culture, showing a better performance of nanotexturization in terms of antibacterial properties.

Conclusion and Clinical Implications: In conclusion, three-dimensional analyses of roughness should be prioritized in comparison to two-dimensional for their higher fidelity. Nanotexturing suggests better early performance in terms of osteoblastic adhesion and tissue formation. Considering bacterial adhesion, the nanotexturization presents lower bacterial adhesion compared to surfaces with microtexturization and higher roughness.