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Topography Influences Adherent Cell Regulation of Osteoclastogenesis

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Appendix

Preparation of Titanium Disks

Commercially available pure-grade IV titanium disks (20.0 × 1.0 mm) were prepared and the surfaces modified as 3 kinds of topography. Briefly, 1 group of disks was polished with sand paper of 600 grits, sonicated 3 times in water for 15 min each to clean, and soaked in 30% HNO_3 for 5 min. These disks served as the smooth surface group. The other 2 groups of disks were polished with sand papers from grade 320 to 400 and 600 grits, subsequently grit-blasted with 100-μm aluminum oxide particles, and sonicated 3 times in water for 15 min to clean. The second group of disks were treated with 5N HCl overnight and sonicated 3 times in water for 15 min and then passive 30% HNO_3 for 5 min. These disks served as the micron surface group. The third group of disks were immersed in 50% (v/v) solution of 30% H_2O_2 and 2N H_2SO_4 for 2 h (Nanci et al. 1998; de Oliveira and Nanci 2004; Vetrone et al. 2009). These disks are referred to as the nano surface group. Before cells were seeded on the disks, all disks were soaked in 70% ethanol for 24 h, then dried, and exposed to ultraviolet light in a steric tissue culture hood for 24 h.

Animal Surgery

Sprague-Dawley rats (250-300 g; Harlan, Indianapolis, IN, USA) were anesthetized by an intraperitoneal injection of a mixture of ketamine (80 to 100 mg/kg) and xylazine (10 mg/kg), and supplemental local anesthesia was obtained with lidocaine 2% with epinephrine (1:100,000). A incision site of the tibia was shaved gently, and a 2-cm incision was made through the skin and periosteum, muscle, and neurovascular bundle, which was retracted to reveal the medial and dorsal aspect of the tibial bone. With sterilized stainless steel burs, osteotomies were created by 1.7-mm diameter holes through the cortex. Sterile implants (1.8 × 2.0 mm; JMR Inc., Niigata, Japan) with smooth, micro, and nano surfaces were placed into the osteotomy, and the skin was closed. Ketoprofen (5 mg/kg) was provided subcutaneously following surgery. The animals were monitored continuously until they ambulated on their limbs and took fluids. After 1-d implantation, the tibiae were harvested to flush out bone marrow for bone marrow–derived macrophage culture.

Appendix References

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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