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Synergistic interaction of topographic features in the production of bone-like nodules on Ti surfaces by rat osteoblasts

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Abstract

The objective of this study was to study the responses of osteoblast-like cells to rough Titanium (Ti)-coated epoxy surfaces of differing topographic complexity. Four topographies were studied: polished (PO), coarse-blasted (CB), acid-etched (AE) and coarse-blasted + acid-etched (SLA). Rat osteoblasts were cultured on these surfaces and their morphology, thickness as well as the number and size of bone-like nodules measured. To determine cell shape and cell thickness, fluorescein-5-thiosemicarbazide was used to stain the cell components including the cell membrane, the stained cells were optically sectioned using epifluorescent microscopy and the optical sections were computationally reconstructed to obtain three-dimensional images in which cell volume and cell thickness could be determined. Similarly optical sections of bone-like nodules labeled with tetracycline were also reconstructed to determine their size. The different surface topographies were found to alter the thickness and morphology of osteoblasts cultured on these surfaces. Osteoblasts produced significantly more and larger nodules on SLA compared to other surfaces . Nevertheless and perhaps surprisingly, given the evidence in various cell populations that cell shape can affect cell differentiation, cell thickness was not directly correlated with an increase in bone-like nodule formation. Data were analyzed by factorial analysis of variance. In this way the primary effect of each surface treatment (i.e. blasting and acid etching) could be assessed as well as their interaction. Both the acid etching and blasting processes significantly affected the number and size of bone-like nodules cultured on Ti surfaces. Moreover there were significant interaction effects indicating that surface topographic features can act synergistically to enhance bone formation. This result suggests that a useful approach to the optimization of surfaces for bone production could involve systematic investigation of combinations of processes each of which produces distinct surface topographical features.

Keywords: Osteoblast; Bone; Dental implant; Surface roughness; Surface topography; Cell Morphology; Cell culture

1. Introduction

The topography of an implant surface, including surface texture and surface roughness, has a major influence on the evolution and properties of the implant-tissue interface and the long-term success or failure of integration with the body [1,2]. Roughened surfaces achieved through processes such as machining, particle blasting, Titanium (Ti) plasma spraying, chemical/electrochemical etching or particle blasting + chemical etching, have been successfully used to promote bone integration and long-term stability of the implant in experimental in vivo studies [3–8] as well as in patients [9,10]. Another approach to study the effects of surface topography has been the use of microfabricated surfaces which enable precise control of features to be obtained [2].

Cell adhesion is affected by surface topography and varies with the type of cell. More human gingival fibroblasts [11] or periodontal cells [12] attach to smooth surfaces than to rough surfaces. In contrast, osteoblast-like cells demonstrate significantly higher levels of cell attachment on rough surfaces than they do on smooth surfaces [13].

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In vitro studies using osteoblast-like cells indicated that cell differentiation, proliferation, protein synthesis and local factors are affected by surface roughness; alkaline phosphatase specific activity, osteocalcin production, transforming growth factor beta (TGF-β) and prostaglandin E_2 (PGE_2) increased on rough Ti surfaces in comparison to smooth Ti surfaces whereas cell proliferation decreased (reviewed in [14]).

The effects of microfabricated surfaces on cell shape, cytoskeletal organization, and cell orientation have been reviewed recently by authors from laboratories active in this area [2,15–18]. Grooved microfabricated surfaces can also affect tissue organization and epithelial downgrowth on percutaneous devices in vivo [19]. In a recent study we demonstrated that rough surfaces influence the cell thickness but not cell volume of fibroblasts [20]. The first part of this study investigated the effects of surface topography found in commercially available implants on cell morphology, shape and cytoskeletal organization of osteoblasts under conditions of constant surface chemistry. The approach of a two-stage replica technique was used to produce Ti-coated epoxy resin replicas of different Ti surfaces [20]. In view of the importance of topography in obtaining osseointegration, the second part of this study examined the effects of surface topographies on the properties and numbers of bone-like nodules produced in cell culture.

Cell thickness was found to be affected by surface topography, but was not directly related to an increase in bone-like nodule formation. When the data were analyzed using factorial analysis of variance considering the factors of presence or absence of acid etching and/or grit blasting, it was found that both nodule size and nodule number were affected by these surface-modifying processes. Moreover there were significant interaction terms indicating that the surface features produced by blasting and etching can act synergistically to enhance bone formation.

2. Materials and methods

2.1. Surfaces

Ti-coated epoxy-resin replicas of mechanically polished (PO), acid etched (AE), coarsely blasted (CB), and coarsely blasted and acid etched (SLA, Institut Straumann AG, Waldenburg, Switzerland) surfaces were produced as described previously [20]. In brief, impressions of PO, AE, CB, and SLA surfaces were made with vinyl polysiloxane impression material (PROVIL®*novo* Light, Heraeus Kulzer, Dormagen, Germany). Vinyl polysiloxane negative replicas were used to cast epoxy-resin (EPO-TEK 302-3, Epoxy Technology, Bellerica, MA) positive replicas of these surfaces. The positive epoxy replicas were cleaned by ultrasonication in a detergent (7X, ICN Biomedicals Inc., Costa Mesa, CA, USA) and baked at 60°C for 4 days and sputter-coated (Randex 3140 Sputtering System, Palo Alto, CA) with 50 nm of Ti. Samples were sterilized for 3 min. In an argon-gas glow-discharge chamber and placed into six-well culture plates (35 mm diameter each well) prior to plating with the osteoblastic cell suspension. All Ti-coated epoxy-resin replicas were discs, 15 mm in diameter and 1 mm in thickness. Ti-coated PO replicas served as controls.

2.2. Surface characterization

In an earlier study, the chemical composition of the Ti-coated epoxy resin replicas was determined by X-ray photoelectron spectroscopy (XPS) and surface topography was characterized using laser profilometry (LPM), scanning electron microscopy (SEM) and stereo-SEM [20]. In brief, XPS data were obtained on a SAGE 100 (Specs, Berlin, Germany) using unmonochromatized Al K_α radiation at 325 W (13 kV) with an electron detector pass energy of 50 eV for survey spectra and 14 eV for high-resolution spectra. The chemical composition of the surfaces was calculated from the survey spectra whereas binding energy, oxide-layer thickness and oxidation state of the elements were determined from the high-resolution spectra. Surface topographies of the original samples and their replicas were characterized using LPM (UBM, UBM Messtechnik GmbH, Ettlingen, Germany) equipped with a Microfocus sensor based on an autofocusing system, SEM (Cambridge Stereoscan 260, Cambridge, UK) and stereo-SEM (Cambridge Stereoscan 260, Cambridge, UK). Two-dimensional (2-D) profiles of the original surfaces and their replicas were randomly obtained using the LPM. A measurement distance of 4.096 mm with a lateral resolution of 1 μm was chosen. In the case of stereo-SEM, 2-D profiles of SLA and CB surfaces and their replicas were computed from the reconstructed stereo-SEM micrographs. The profile length was 52 μm with a lateral resolution of 0.083 μm.

2.3. Cell culture

Osteogenic cells obtained from newborn-rat calvaria were isolated and cultured as described previously [21–25]. This culture system has been shown to produce mineralized tissues on standard culture dishes reproducibly. In brief, osteoblastic cells from the subcultures 1–3 were cultured on tissue-culture plastic (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) in alpha minimal essential medium (α-MEM, MEM Stemcell, Vancouver, BC, Canada) supplemented with antibiotics (100 μg/ml penicillin G (Sigma, St. Louis, MO, USA); 50 μg/ml gentamicin (Sigma); 3 μg/ml amphoteracin B