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Topographic scale-range synergy at the functional bone/implant interface

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ABSTRACT

We sought to explore the biological mechanisms by which endosseous implant surface topography contributes to bone anchorage. To address this experimentally, we implanted five groups of custom-made commercially pure titanium implants of varying surface topographical complexity in rat femora for 9 days; subjected them to mechanical testing; and then examined the interfacial bone matrix by electron microscopy. The five implant surfaces were prepared by combinations of dual acid etching and grit blasting the titanium substrates and, in some cases, modifying the created surfaces with the deposition of nanocrystals of calcium phosphate, which resulted in 10 samples per group. In parallel, we cultured rat bone marrow cells on surrogate implants constructed from polymer resin coated with the same calcium phosphate nanocrystals, and monitored the deposition of bone sialoprotein by transmission electron immunohisto-micrography. We found that implant samples modified with sub-micron scale crystals were bone-bonding, as described by the interdigitation of a mineralized cement line matrix with the underlying implant surface. The in vitro assay showed that bone sialoprotein could be deposited in the interstices between, and undercuts below, the nanocrystals. In addition, when mineralized, the cement line matrix globules occupied micron-sized pits in the implant surfaces, and in part obliterated them, creating an additional form of anchorage. Our results also showed that collagen, elaborated by the osteogenic cells, wrapped around the coarse-micron features, and became mineralized in the normal course of bone formation. This provided a mechanism by which coarse-micron implant features contributed to a functional interface, which we have previously described, that is capable of resisting the mechanical loading that increases as peri-implant bone matures. Thus, our findings provide mechanistic explanations for the biologically-relevant criteria that can be employed to assess the importance of implant surface topography at different scale-ranges.

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1. Introduction

Endosseous implant surface topography has been defined at three scale-ranges: nano (or sub-micron), micron and coarse micron (or macro) [1,2]; and we have recently demonstrated that these scale-ranges mimic those found at natural remodeling sites in bone [3].

In bone remodeling, the initial matrix elaborated by differentiating osteogenic cells is the cement line, which interdigitates with nano-scale features in the underlying bone that has been resorbed by osteoclasts [4]. We referred to this as the “true” interface, since it represents the junction of new and old bone. However, the Howship’s lacunae [5] created by individual osteoclasts provide a micron-scale topography that can be extended over a further order of magnitude by the synergistic resorptive activity of migratory osteoclasts [6]. Thus the bone/bone interface extends over three distinct scale-ranges [3].

In the case of the bone/implant interface we have argued that such biologically-relevant criteria could be employed to demonstrate the importance of known differences in scale-ranges in implant surface designs. Thus, even the smoothest surfaces, when modified to present a sub-micron scale surface with undercuts, were rendered bone-bonding [3]. This bonding phenomenon can be demonstrated by some form of mechanical disruption assay, where failure occurs within either the implant or bone, but the interface remains intact [4]. Bone bonding was previously thought to be limited to calcium

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phosphate-based materials [7,8]. However, it is also a characteristic of metal oxide surfaces with a reticulate surface nano-topography [1,9–11], which suggests that physical interlocking at the nanometer scale (also referred to as micro-mechanical interdigitation), rather than interfacial chemistry, is driving the bone-bonding mechanism. However as locomotor load, through bone to the implant, increased with time of healing such interfaces failed, while surfaces with sub-micron features superimposed on surfaces of greater microtopographical complexity, remained intact, demonstrating that micron and higher-order topography is a requirement for longer-term interfacial stability. Thus, the "true" interface could be both topographically and functionally distinguished from the "functional" interface [3].

This previous work demonstrated the functional significance of the sub-micron, micron and coarse-micron topographic scale-ranges, but failed to demonstrate the distinct biological mechanisms relevant to each scale range. Specifically, we did not provide either direct evidence of critical initial stages of cement line matrix deposition into nano-scale undercuts on candidate implant surfaces, or any morphological evidence of the importance of the bony relationship with implant surface features in the coarse-micron scale-range.

Therefore, in this work, we sought to reconcile the differing scales of implant topography at the sub-micron and micron scale-ranges with the biologic mechanisms involved in the creation of the interface at each of these scale-ranges. To address this experimentally we undertook both in vitro and in vivo assays. The former were designed to specifically mimic the earliest deposition of cement line proteins within the undercuts created by a nano-topographically complex surface. In the in vivo experiments, we placed custom-made commercially pure titanium (cpTi) implants, with surfaces of five different topographies in rat femora and subjected the model to a mechanical disruption test, and examined the interface at both sub-micron and micron scale-ranges with scanning electron microscopy.

2. Materials and methods

2.1. In vivo model

Fifty custom designed rectangular plates (1.3 mm × 2.5 mm × 4 mm) were fabricated from commercially pure titanium (cpTi, grade IV) by Biomet 3i (Palm Beach Gardens FL, USA). A central hole was milled down the long axis of each plate to enable suture fixation at surgery and facilitate mechanical testing. This model was inspired by Nakamura et al. (1985) [12].

Different groups of implant surface topographical design and complexity were generated by the following methods, listed by increasing complexity of surface features: Grit blasting (GB; using calcium phosphate particulates of 180–300 μm in size as the blasting medium; $n = 10$), dual acid etching (DAE; with H_2SO_4/HCl; $n = 20$), and grit blasting followed by acid etching with H_2SO_4/HCl (GB/AE; $n = 20$). Half of the DAE and GB/AE implants were further modified by discrete crystalline deposition (DCD) of calcium phosphate (CaP) nanocrystals on the implant surfaces. For this, the samples were dipped in a solution containing a suspension of nano-structured CaP particles (nominally 10–100 nm in size; ≥95% crystalline; no amorphous content detected; suspended in an alcohol based solution) at room temperature and, following withdrawal from solution, were dried in an oven at 100 °C. Thus, the final implant samples consisted of 5 groups containing 10 implants each.

2.2. Field emission scanning electron microscopy (FE-SEM)

High-resolution photomicrographs of one additional implant in each group were taken, without sputter coating, using a Hitachi S-5200 SEM at the Centre for Nanostructure Imaging, University of Toronto. Implants retrieved after mechanical testing were carbon sputter coated and the remaining peri-implant bone following the disruption test was imaged using the same microscope.

2.3. Surgical procedure

Twenty five male Wistar rats (200–250 g, Charles River Laboratories, Canada) were used in the experiment. The surgical protocol was approved by the Ethics Committee of Animal Research at the University of Toronto. Inhalation anesthesia (Isofluorane in nitrous oxide and oxygen, 900 ml total flow rate-5% induction and 2–2.5% maintenance) was used to sedate the animals. Pre- and post-operative analgesics were provided by subcutaneous administration of 0.01–0.15 mg/kg Buprenorphine. Implants were placed bilaterally in the distal metaphyses of the rat femora and were assigned by partial randomization. The implants placed in the same animal were chosen from different treatment groups.

The surgical procedure was performed as described previously [13]. Briefly, the antero-lateral aspect of each femur was shaved, a skin incision was made on the lateral thigh to access the underlying femur, and the widest aspect of the femur on the anterior surface close to the knee capsule was then exposed for insertion of the implants. To create the defect for implant placement, a bicortical hole was drilled in the bone perpendicular to the long axis of the femur, proximal to the growth plate, using a 1.3 mm twist drill (Brasseler, GA, USA) attached to a dental handpiece (ImplantMED DU 900 and WS-75, W&H, Dentalwerk, Austria). A second bicortical hole was then drilled with the same drill, 2.5 mm proximal to the first one, and the two holes were joined in a proximal–distal direction using a 1.2 mm cylindrical side-cutting bur (Biomet 3i, FL, USA). Irrigation with sterile saline was used throughout the preparation.

An implant was press-fitted into each defect, in an antero-posterior orientation with its long axis perpendicular to the long axis of the femur (Fig. 1A). Threading a suture (4-0 Polysorb™, Syneture, USA) through the central hole of the implant and tying a knot around the lateral femoral margin ensured stability of the implants during the post-operative period. Muscle tissue was sutured with the same biodegradable sutures and the cutaneous tissues were re-apposed using wound clips (9 mm, Becton Dickinson, MD, USA).

Animals were maintained in the animal care facility of the Faculty of Dentistry, University of Toronto, and were observed daily during recovery and checked for signs of compromised ambulatory ability. Rat chow was provided ad libitum and all animals were allowed free access to water. Implant sites were observed and inspected for signs of infection.

2.4. Euthanasia, sample harvesting and preparation

Euthanasia was achieved at 9 days post-operatively ($n = 10$ implants/group) by cervical dislocation after CO_2 exposure. Femora were harvested and stored in a 15% sucrose buffer solution for about 3 h, during preparation for the mechanical testing. A cylindrical diamond bur (Brasseler, GA, USA) connected to a high speed system (Handpiece: KaVo Dental Corporation, IL, USA; Handpiece control: DCI International, OR, USA) was used to trim the bone to the width of the implants and all bone attached to the sides of the implant were removed carefully. Samples prepared for mechanical testing consisted of two arches of bone attached to each side of the implants, except one GB and one GB/AE implant, the arches of which fell off during preparation.

2.5. Mechanical testing

Fifty samples (100 arches) comprising 20 arches of bone of each group were assigned for mechanical testing. The mechanical test, as described previously [13], consisted of a disruption test performed in an Instron™ testing machine (model 8501) at the Mechanical Testing Laboratory, Faculty of Dentistry, University of Toronto. Immediately before the test, a nylon line (Red Wolf fishing line, 4.5 kg (10 lb) IA, USA) was passed through the marrow space of each arch of bone (Fig. 1B and C). The implant was secured in a vice attached to the Instron™ machine, and each nylon line was attached, in turn, to the moving crosshead of the machine. The disruption force to rupture the sample was recorded at a crosshead speed of 30 mm/min and force/displacement results were generated for each implant. Both the lateral and medial sides of the femur were subjected to the test, with the lateral arch tested first for all samples. Samples were collected after the disruption test and fixed in 10% formalin at room temperature. Fixed samples were then immersed in a solution of 3% sodium hypochlorite for 20 min, washed in running tap water, dehydrated in serial concentrations of ethanol (50, 70, 95, 100, 100% v/v) for 1 h in each and critical point dried in preparation for FE-SEM.

2.6. Statistical analysis

Repeated measure analysis of variance was employed to compare the means of the disruption force between groups. The square root transformation was used to transform the distribution of the disruption force values to normal distribution. The Kolmogorov–Smirnov test confirmed the normality of the distribution of the square transformed disruption force values. The Generalized Estimation Equations (GEE) method was used to account for correlations among correlated measurements. A box plot of disruption force values for all groups was presented for visual assessment and comparison of distributions. Data was analyzed by the statistical software SAS 9.1 (SAS Institute Inc., NC, USA) and p values less than 0.05, obtained using the Wald test, were considered significant.

2.7. In vitro model

Thermanox® coverslips (NUNC™ Brand Products, Rochester, NY USA) of 2.2 cm in diameter and 0.02 cm in thickness were used as substrates and further treated with DCD nanocrystals with the same method as described for implants (see above). To remove any contaminants from the DCD process, the DCD-treated substrates

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