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Bone healing response in cyclically loaded implants: Comparing zero, one, and two loading sessions per day

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ABSTRACT

When bone implants are loaded, they are inevitably subjected to displacement relative to bone. Such micromotion generates stress/strain states at the interface that can cause beneficial or detrimental sequels. The objective of this study is to better understand the mechanobiology of bone healing at the tissue-implant interface during repeated loading. Machined screw shaped Ti implants were placed in rat tibiae in a hole slightly bigger than the implant diameter. Implants were held stable by a specially-designed bone plate that permits controlled loading. Three loading regimens were applied, (a) zero loading, (b) one daily loading session of 60 cycles with an axial force of 1.5 N/cycle for 7 days, and (c) two such daily sessions with the same axial force also for 7 days. Finite element analysis was used to characterize the mechanobiological conditions produced by the loading sessions. After 7 days, the implants with surrounding interfacial tissue were harvested and processed for histological, histomorphometric and DNA microarray analyses. Histomorphometric analyses revealed that the group subjected to repeated loading sessions exhibited a significant decrease in bone-implant contact and increase in bone-implant distance, as compared to unloaded implants and those subjected to only one loading session. Gene expression profiles differed during osseointegration between all groups mainly with respect to inflammatory and unidentified gene categories. The results indicate that increasing the daily cyclic loading of implants induces deleterious changes in the bone healing response, most likely due to the accumulation of tissue damage and associated inflammatory reaction at the bone-implant interface.

1. Introduction

Since bone implants unquestionably will remain a mainstream treatment modality for years to come, a better understanding and control of the healing events at the bone-implant interface – where cell fate decisions are made – is mandatory to meet these challenges, especially in the cases where implants are immediately loaded after placement. When implants are loaded, they are subjected to some degree of micromotion; the displacement of the implant relative to bone generates stress and strain that will result in the local deformation of supporting interfacial tissues (Brunski, 1999; Haiat et al., 2014). Micromotion and the ensuing local tissue deformation can affect bone healing, cause fibrous encapsulation, induce bone resorption, and lead to implant loosening (discussed in Wazen et al., 2013a), all of which generate morbidity and ultimately require implant replacement. However, it has been suggested that some degree of micromotion can also positively influence bone formation (Birkhold et al., 2014; Duyck et al., 2007, 2006; Geris et al., 2008; Leucht et al., 2007; Vandamme et al., 2007a, 2007b; Willie et al., 2010; Yang et al., 2013; Zhang et al., 2014).

Most loading studies have examined the healing process around implants using an experimental system in which constant micromotion of the implant is applied, which can then require an increase in loading force throughout the healing period as the interface attempts to heal (Leucht et al., 2007; Wazen et al., 2013a). Clinically, subjects typically use overall similar forces during mastication but the period during which force is exerted varies. In this context, few studies have evaluated the interfacial bone healing response with respect to the number of sessions per day of implant loading. Finally, since virtually all implants have an initial interface with at least some gaps between the cut bone and implant surface, it remains important to study the influence of loading on induction of new bone in such gaps. This latter point has motivated our study of events in a Bone Implant Gap Interface (BIGI) and follows up on our prior work in a murine tibia model where constant displacement was applied (Leucht et al., 2007; Wazen et al.,

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2013a).

The focus of our work is not to replicate any particular clinical situation but rather to investigate the basic bone healing response that occurs near the bone-implant interface where tissue deformation takes place during loading. A rat tibia model was used for correlation of multiple analytical approaches, including DNA microarray, histological, and biomechanical analyses. We hypothesized that the cumulative number of cycles of force (and related interfacial strain) per day can affect events in this gap interface that will fill, or not, with bone. Our data shows that despite no increase in peak applied force, simply doubling the number of loading sessions (and cumulative cycles) per day has a significant influence on healing at the bone implant interface, a point that should be taken into consideration when evaluating clinical loading regimens.

2. Materials and methods

2.1. Ti implants and surface analysis

Machined screw-shaped implants made of cp Titanium Grade 2 (Medical Micro Machining Inc, Colfax, WA, USA) were used. The surface quality of the screws was checked using a JEOL JSM-7400F field emission scanning electron microscope (SEM) operated at 1–2 kV. The screws were 7 mm length, 0.45 mm pitch and 1.7 mm diameter. Before surgery, samples were washed with 70% ethanol and air-dried.

2.2. Type of interface and surgical procedure

The screw shaped titanium implants were placed in 2.0 mm holes in rat tibiae to create a model of gap-healing at an interface (BIGI, (Wazen et al., 2013a)). Twenty-seven male Wistar rats weighting 200–225 g (Charles Rivers Canada; St-Constant, QC, Canada) were anesthetized with an intraperitoneal injection of a mixture of Ketalean (0.05 mg/g body weight; ketamine hydrochloride; Biomeda-MTC, Cambridge, ON, Canada), Rompun (0.005 mg/g body weight; xylazine; Bayer Inc., Toronto, ON, Canada) and Acevet (0.001 mg/g body weight; acepromazine maleate; Vetoquinol Inc., Lavaltrie, QC, Canada). The antero-medial side of each hind limb was shaved and cleaned with Baxedin® (chlorhexidine gluconate; Omega Laboratories, Montreal, QC, Canada). A 1 cm incision was made through the skin using a 15 C blade (Almedic, Montreal, QC, Canada). The skin and muscle were gently pried apart to expose the periosteum. Using holes near the extremities of the bone plate as guide, two unicortical holes were drilled in bone at low speed using a 0.5 mm drill bit (Drill Bit City, Prospect Heights, IL, USA) and titanium alloy retopins (0.62 mm in diameter) were placed through the holes in the bone plate and into the cortices of the bone, thereby fixing the bone plate to the bone. With the center column of the bone plate as a guide, a main transcortical hole was drilled, at the superior level of the antero-medial tibial metadiaphysis, at low speed using a 2.00 mm diameter drill bit (Drill Bit City). Titanium implants were then inserted into the hole with a silicone rubber O-ring (Apple Rubber Products, Lancaster, NY, USA) situated between the head of the implant and the center column of the bone plate. The cap was screwed onto the center column of the bone plate (Fig. 1). The skin incision was closed around the central column of the Delrin plate using 4-0 Vicryl sutures (Ethicon, Inc, Somerville, NJ, USA) and surgical staples (Becton Dickinson, Franklin Lakes, NJ, EUA) The surgical site was again cleaned and disinfected with Baxedin® (Omega Laboratories). The animals received an injection of Temgesic® (0.2 ml Buprenorphine hydrochloride, Reckitt and Colman, Hull, UK) after surgery, and were fed with soft food containing Temgesic® (Reckitt and Colman).

2.3. Micromotion system and loading regimen

The micromotion system used was sized for use in the rat tibia, but was otherwise identical to the system that we previously used in mice (Leucht et al., 2007; Wazen et al., 2013b, Fig. 1A and C–F). A hand-held Force Gauge Series 5, model M7-2 loading device (Fig. 1B, Mark-10, Copiague, NY, USA) was used to apply controlled force to the implant through a small opening in the top of the protective cap. The loading device – − 2 lb = 8.896 N capacity – can be used in tension or compression and is factory-calibrated, with a maximum error in full scale reading of 0.03%. We checked its performance in our own calibration trials where we recorded the force gauge's output in response to application of known weights. Three loading regimens were applied for 7 days, ***(a)*** zero loading (Unloaded group) (***b***) one daily loading session of 60 cycles with an axial force of 1.5 N/cycle (Micromotion 1× group), and (***c***) two daily sessions of 60 cycles each session with the same force per cycle (Micromotion 2× group). Loading frequency was controlled manually and the approximate rate of loading was 1 cycle per second. For the one and two daily loading sessions groups, the animals were anesthetized and kept under AErrane anesthesia (isoflurane USP, Baxter, Mississauga, ON, Canada) maintained at 1–2% during application of force. Hence, these loaded animals were anesthetized one and two times per day, respectively. The surgical site was cleaned once a day with Baxedin® (Omega Laboratories) before the loading and, the unloaded group was also similarly anesthetized once a day for routine wound cleaning. Each isoflurane anesthesia including the induction never exceeded 5 min. The experimental groups and loading protocols are described in Table 1.

2.4. Ethical approval and animal supervision

All animal procedures and experimental protocols were approved by the *Comité de déontologie de l'expérimentation sur les animaux of Université de Montréal.* Animals were under regular observation at the University animal facilities throughout the period of experimentation. They were given food and water ad libitum and left to move around freely in the cages. The animals' appearance, weight and healing were checked on a daily basis. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research.

2.5. Finite element analysis

To clarify the biomechanical environment over time around unloaded and loaded implants, 3-D finite element (FE) models were formulated (Fig. 2A and B). The geometry of the implant site in the rat tibia was modeled as a 4 mm-diameter composite cylinder made up of a 0.6 mm layer of cortical bone with a 1.6 mm-thick layer of trabecular bone beneath it in the marrow, plus a drill hole (2 mm in diameter, 2.2 mm deep) containing the 1.7 mm-diameter implant. The drill hole was filled with fibrin or healing tissue, depending upon time after implantation. The outer boundaries of the model (except for the top of the bone cylinder) were constrained. The properties of the cortical bone, trabecular bone that forms in the marrow cavity, interfacial region, and implant were as described in Table 2. Note that in simulating the situation immediately after implantation – when the gap interface is filled with a fibrin clot – we assigned the properties of fibrin to the gap (Munster et al., 2013).

In loading the implant, we accounted for the fact that a 1.5 N axial force on the implant in the bone plate system is balanced by a force from the O-ring (beneath the head of the screw) plus a force from the interface on the screw threads, i.e., not all of the force applied to the screw head is transferred to the implant's interface. The amount of force taken by the O-ring vs. interface – and, in turn, the displacement of the implant – depends on the properties of the interface, e.g., with no tissue in the interfacial gap, our experiments showed that the implant moved 93.7 μm when 1.5 N was applied to the screw. (The axial stiffness of the O-ring was 0.016 N/micron.)

The FE model also allowed us to vary the properties of the gap tissue to estimate how healing (or lack thereof) in the gap interface would cause the interface's stiffness to change over time after implantation.