A Comparative Assessment of Implant Site Viability in Humans and Rats

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Abstract

Our long-term objective is to devise methods to improve osteotomy site preparation and, in doing so, facilitate implant osseointegration. As a first step in this process, we developed a standardized oral osteotomy model in ovariectomized rats. There were 2 unique features to this model: first, the rats exhibited an osteopenic phenotype, reminiscent of the bone health that has been reported for the average dental implant patient population. Second, osteotomies were produced in healed tooth extraction sites and therefore represented the placement of most implants in patients. Commercially available drills were then used to produce osteotomies in a patient cohort and in the rat model. Molecular, cellular, and histologic analyses demonstrated a close alignment between the responses of human and rodent alveolar bone to osteotomy site preparation. Most notably in both patients and rats, all drilling tools created a zone of dead and dying osteocytes around the osteotomy. In rat tissues, which could be collected at multiple time points after osteotomy, the fate of the dead alveolar bone was followed. Over the course of a week, osteoclast activity was responsible for resorbing the necrotic bone, which in turn stimulated the deposition of a new bone matrix by osteoblasts. Collectively, these analyses support the use of an ovariectomy surgery rat model to gain insights into the response of human bone to osteotomy site preparation. The data also suggest that reducing the zone of osteocyte death will improve osteotomy site viability, leading to faster new bone formation around implants.

Keywords: drill, bone, ovariectomy, computer model, osteogenesis, osteotomy

Introduction

The cutting of living bone is one of the oldest surgical procedures for which there still exists archaeological evidence (Capasso 2002). Clinicians universally agree that the preservation of cell viability is of utmost importance, but beyond a recognition that high-speed rotary cutting tools generate heat that can damage bone (Moss 1964), there is little guidance on how to limit cell death and optimize new bone formation after osteotomy. Discounting the importance of osteocyte death has clinical consequences: in the early 1970s, it was concluded that “one explanation for the occasional failure of procedures demanding stable fixation . . . [around] bone screws may be the increase in resorption of bone . . . as a result of thermal necrosis caused by preparative drilling” (Matthews and Hirsch 1972).

In the intervening decades, it has become abundantly clear that heat generated during implant site preparation has a negative influence on dental implant osseointegration (reviewed in Mohlhenrich et al. 2015), but the source of this heat is not well understood. Some data have suggested that friction creates the heat responsible for cell death around an osteotomy (Albrektsson 1985); other data call this conclusion into question (Davidson and James 2003). The presence of dead (necrotic) bone stimulates resorption by osteoclasts (Cha et al. 2015; Dolan et al. 2015), and this resorptive phase must be on the wane before new bone formation can initiate, for example, around an implant (Wang et al. 2017). A logical extension of this line of reasoning is that,

if a method could be developed whereby osteocyte cell death was minimized, this could have a positive impact on the rate of new bone formation after osteotomy. Here, our objective was to understand how patients responded to osteotomy site

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preparation and, in doing so, potentially gain insights into how to improve this process to better support implant osseointegration.

Materials and Methods

Animals, Experimental Plan, Ovariectomy, and Tooth Extraction Surgeries

Stanford APLAC approved all procedures (#13146), which conform to ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. In total, 18 female Wistar rats were used in this study (Charles River Laboratories). After anesthesia via intraperitoneal injection of ketamine/xylazine, ovariectomy surgery (OVX) was performed. Simultaneously, both maxillary first molars (e.g., mxM1) were extracted. Bleeding was controlled by local compression. Rats were fed a soft food diet and housed in groups of 2. Weight changes were <10%. No adverse events were encountered. After an 8-wk recovery, rats were randomly assigned to treatment groups outlined in Appendix Table 1.

Human Tissue Collection

The multicenter clinical investigation was performed at a private practice in Beverly Hills, California; a private practice in Newport Beach, California; a private practice in St. Charles, Illinois; and a private practice in Munich, Germany. Eleven male and 14 female healthy volunteers, ranging in age from 31 to 84 y, participated. Informed consent was obtained. All patients were undergoing osteotomies in preparation for implant placement. Patients were informed that a biopsy specimen would be harvested from the osteotomy site prior to implant placement and that this harvest would have no untoward effects on subsequent implant osseointegration. Procedures and materials were approved by local ethics committees and institutional research boards (WIRB#20160967 for US private practices, FECI#016/1399 for Germany private practice), conformed to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines, and followed principles of the Declaration of Helsinki.

Each patient presented with an edentulous space requiring implant placement in the maxilla or in the mandible. A full-thickness periosteal flap was elevated. A slow-speed handpiece and saline irrigation were used; the drilling protocol followed the procedure outlined for rats (see Fig. 1K). Bone biopsies that encompassed the osteotomies were then collected using 3.0/3.5-mm or 2.8/3.3-mm (inner diameter/outer diameter) trephine burs (Ustomed Instrumente; Brasseler USA) that was centered on the osteotomy; therefore, the calculated lateral thickness of the bone sample annulus ranged from 0.65 to 0.75 mm. After collection of bone biopsies, an implant (NobelActive, Nobel Replace Select, or Nobel Tapered Conical Connection implants; Nobel Biocare AB) was placed according to the manufacturer instructions, without incident. Tension-free primary closure of the flap was achieved using sutures. Handling of human tissues is described in the Appendix. In total, 40 samples (1 to 4/patient) were collected, 34 of which were fully intact.

Implant Site Preparation, Cone Beam Computed Tomography, Tissue Preparation, Histology, Histomorphometry, Cellular Activity Assays, Immunostaining, and Mathematical and Finite Element Modeling

Details are in the Appendix.

Statistical Analyses

Results are presented in the form of mean $\pm$ standard deviation, and *t* tests and paired *t* tests were also performed (Appendix). Significance was attained at $P < 0.05$, and all statistical analyses were performed with SPSS software (SPSS, Inc.).

Results

Establishing an Osteopenic Rat Model to Assess Oral Osteotomy Site Viability

The purpose of this study was to characterize the response of patients to osteotomy site preparation and align these data with a relevant animal model. Therefore, our first step was to associate the bone health of a rat with the bone health of the average dental implant patient. Epidemiological surveys place the median age of these patients at >50 y (Grisar et al. 2017; Schimmel et al. 2017), a demographic whose bone health tends toward osteopenia (Looker et al. 2010). To align our rat model with these human epidemiological data, rats underwent OVX (Kalu 1991). Eight weeks later, micro-computed tomography (μ CT) imaging was used to validate the osteopenic phenotype.

This experimental model consisted of 3 cohorts: “young” (19 wk old) rats (Fig. 1A, A’), “young + OVX” (19 wk old, 8 wk post-OVX) rats (Fig. 1B, B’), and “aged” (>12 mo old) rats (Fig. 1C, C’). The “young” and “aged” rats represent the 2 control groups. Compared to the young control group (white bar), both the young + OVX group (blue bar) and the aged control group (red bar) exhibited a significantly lower bone volume/total volume (BV/TV; Fig. 1D). Compared to the young group, both young + OVX and aged groups showed a significantly greater degree of trabecular spacing and endosteal bone loss (not shown). These data, in conjunction with established literature (Kalu 1991; Sophocleous and Idris 2014; Kim et al. 2015), served to validate the osteopenic phenotype of rats in the young + OVX group.

Our next step was to assess alveolar bone density in a cohort of patients who were candidates for dental implants. Although there are inherent limitations in calculating gray values from cone beam computed tomography (CBCT) images (see the Appendix), CBCT image quality was sufficient to measure bone density for 36 of 40 osteotomy sites. Mean bone density varied significantly between anatomic locations (Fig. 1E), in agreement with previous reports (Monje et al. 2015). Nonetheless, statistical analyses demonstrated that bone density at the implant site was similar to pristine alveolar bone in the same anatomic location (Fig. 1E, Appendix Fig. 1). These

Figure 1. A model in ovariectomized rats that mimics a patient population undergoing osteotomy in preparation for dental implant placement. Representative sagittal micro-computed tomography (μ CT) sections of distal femur of (A) young (19 wk old), (B) young + ovariectomy surgery (OVX) (19 wk old; 8 wk post-OVX), and (C) aged (>12 mo old) rats. Representative transverse μ CT sections of distal femur of (A') young (19 wk old), (B') young + OVX (19 wk old; 8 wk post-OVX), and (C') aged (>12 mo old) rats. (D) Quantification of distal femur bone volume/total volume (BV/TV). (E) Scatterdot plot of mean alveolar bone density (HU) in the indicated anatomic regions and proposed osteotomy site. Representative aniline blue-stained (F) transverse and (F') sagittal tissue sections showing the extraction socket in young + OVX rats, harvested on postsurgery day 0 (PSD0). On PSD7, aniline blue staining of representative (G) transverse and (G') sagittal tissue sections from the healing extraction socket of young + OVX rats (arrow indicates new bone formation). On PSD21, aniline blue staining of representative (H) transverse and (H') sagittal tissue sections from the healed extraction site of young + OVX rats. (I) Quantification of aniline blue⁺ pixels/total pixels in the region of interest (ROI) ("—" refers to healed mxM1 extraction site; "+" refers to adjacent pristine bone). (J) The KLS, Twist, and Osteomed drills and (K) their corresponding protocols used for osteotomy site preparation at the healed mxM1 extraction sites. ab, alveolar bone; es, extraction socket; HU, Hounsfield units; pdl, periodontal ligament; RPM, revolutions per minute. Sample sizes (N) as indicated. Scale bars = 300 μ m.

data indicated that the elapsed time between tooth extraction and implant site preparation was sufficient to allow for complete extraction site healing.

In patients, around half (18/36) of the osteotomies were slated to be produced in the posterior maxilla. In the rat model, we chose a single site for the osteotomy and selected the posterior maxilla to better align with our patient population. Bilateral maxillary first molar (mxM1) extractions were performed in the young + OVX group. The sequence of healing events was analogous to human extraction socket repair. Immediately after extraction, the socket contained remnants of the periodontal ligament (PDL) and blood (Fig. 1F, F'). Within 7 d of surgery (postsurgery day [PSD] 7), new bone formation was detectable (arrow, Fig. 1G, G'). By PSD21, the site was occupied by a mineralized bone matrix punctuated with vascular sinusoids (Fig. 1H, H'). By postsurgical day 21, histomorphometric analyses confirmed that bone densities in the healed extraction sites were equivalent to bone densities in adjacent sites (Fig. 1I).

Osteotomies were prepared in healed, edentulous alveolar bone of both rats and humans, using the same drilling protocol for each (Fig. 1J, K). In rats, 3 commercially available drills

were evaluated. Drilling speeds aligned with manufacturer's guidelines, and among the drills, a constant cutting speed was used (see Materials and Methods).

Osteotomy Site Preparation Triggers a Programmed Cell Death Program in Osteocytes

In a previous mouse study, we found that micro-damage and programmed cell death were detectable within 24 h of implant site preparation (Wang et al. 2017). In this study, we chose a shorter time point (12 h) to better align with patient data. At postosteotomy day (POD) 0.5, all 3 drills produced a smooth cut edge that was occasionally interrupted by the presence of sharp indentations that appeared to correspond to the cutting flutes (arrows, Fig. 2A–C). Picrosirius red-stained samples, viewed under polarized light, showed the surfaces of cut bone were continuous (Fig. 2D–F). Osteocytes away from the cut edge were viable, indicated by the expression of the osteogenic protein Runx2 (Fig. 2G–I). There were, however, osteocytes near the cut edge that were not Runx2⁺, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining identified these as apoptotic (Fig.

Figure 2. KLS, Twist, and Osteomed drills produce comparable osteotomies. On postosteotomy day 0.5 (POD0.5), aniline blue staining of representative transverse tissue sections from osteotomies produced in young + ovariectomy surgery (OVX) rats using (A) KLS, (B) Twist, and (C) Osteomed drills; arrows indicate damaged bone at interface between the cutting tool and alveolar bone. Adjacent transverse tissue sections stained using picrosirius red, an indicator of collagen organization, then visualized under polarized light from osteotomies produced by (D) KLS, (E) Twist, and (F) Osteomed drills. Near-adjacent tissue sections immunostained with Runx2 when osteotomy sites were prepared using (G) KLS, (H) Twist, and (I) Osteomed drills. Near-adjacent tissue sections stained using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), an indicator of apoptotic osteocyte distribution, in osteotomies produced by (J) KLS, (K) Twist, and (L) Osteomed drills; arrows indicate the approximate extent of the radial zones of cell death. os, osteotomy site. Scale bars = 100 μ m.

2J–L). All drills produced a similar radial zone of apoptotic osteocytes (Fig. 2J–L).

Osteotomy Site Preparation Produces Heat That Creates a Radial Zone of Dead and Dying Osteocytes

We compared the distribution of apoptotic osteocytes produced by the 3 drills. No significant differences in the number or location of TUNEL⁺ osteocytes produced by the KLS, Twist,

or Osteomed drills were identified (Fig. 3A–C). The distribution of apoptotic osteocytes as a function of distance from the cut edge of the osteotomy was mapped. Within 12 h of implant site preparation, dying osteocytes were detectable in a radial zone that extended ~100 μ m from the osteotomy edge (Fig. 3A–C; quantification in 3D). There were 50% fewer apoptotic osteocytes in the radial zone extending 100 to 250 μ m from the osteotomy edge (Fig. 3D). Considerably lower numbers of apoptotic osteocytes were found between 250 and 500 μ m away of the osteotomy edge (Fig. 3D).

Figure 3. Computational modeling and biological analyses demonstrate alignment in the response of human and rodent tissues to implant site preparation. On postosteotomy day 0.5 (POD0.5), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-stained representative transverse tissue sections across osteotomies produced by (A) KLS, (B) Twist, and (C) Osteomed drills in ovariectomy surgery (OVX)/mxM1 rats. (D) Quantification of TUNEL⁺ apoptotic osteocytes, expressed as a function of distance from cut edge of osteotomy. (E) Computational model maps the radial heat distribution from the osteotomy edge. (F) Bone temperature expressed as a function of radial distance from the drill; shaded bars correspond temperature ranges to the distribution of dying osteocytes within specified distances from osteotomy edge. Bone biopsies from 2 patients (G, H) costained with TUNEL and 4',6-diamidino-2-phenylindole (DAPI) to identify dying and viable osteocytes at the periphery of osteotomy sites; merged images show the distribution of TUNEL⁺ and DAPI⁺ cells at the osteotomy edge. (I) Quantification of TUNEL⁺ apoptotic osteocytes, expressed as a function of distance from osteotomy edge. ab, alveolar bone; os, osteotomy site. Scale bar = 100 μm.

To characterize the relationship between apoptotic osteocyte distribution and temperature elevation produced by drilling (Fig. 3E), a computational model was developed. The computational parameters were derived from the *in vivo* experimental parameters (see the Appendix). This model demonstrated that temperatures theoretically decreased as a function of distance from the drill tip: in a radial zone that extended 50 μm from the osteotomy

edge, calculated temperatures ranged from a maximum of ~74°C to a minimum of 55°C (orange bar, Fig. 3F). In a radial zone extending 50 to 100 μm from the osteotomy edge, calculated temperatures were 55°C to 48°C (green bar, Fig. 3F). In a radial zone that extended from 100 to 250 μm, calculated temperatures were between 48°C and 42°C (light blue bar, Fig. 3F). Outside this zone, calculated temperatures were near-physiologic (e.g.,

42°C to 37°C, dark blue bar, Fig. 3F). When considered in conjunction with the TUNEL data, this computational model suggested that temperatures above 42°C were associated with osteocyte apoptosis.

Implant Site Preparation Triggers an Apoptotic Program in Patient Tissues

We next evaluated the response of patient tissues to osteotomy site preparation. Since our analyses in rats indicated no detectable differences in cutting performance with different drills (Fig. 3), osteotomies in patients were produced using Osteomed drills only. The same drilling protocol was used in both patients and rats, and tissues were harvested within hours after osteotomy.

As observed in the rat model, patient samples exhibited extensive osteocyte apoptosis around the osteotomy edge (Fig. 3G, H). 4',6-Diamidino-2-phenylindole (DAPI) staining identified viable cells at a distance from the osteotomy edge and in the vascular sinusoids (Fig. 3G, H), while TUNEL costaining exhibited the same distribution of dying osteocytes as was observed in rat tissues (Fig. 3G, H). The distribution of TUNEL⁺ apoptotic osteocytes was calculated as a function of distance from the cut edge of the osteotomy, and once again, most dying osteocytes were located in a radial zone that extended 100 μ m from the osteotomy edge (red and green bars, Fig. 3I). There were ~50% less apoptotic osteocytes in the radial zone extending 100 to 200 μ m from the osteotomy edge (blue bars), and a fraction of apoptotic osteocytes was found between 200 and 400 μ m away of the osteotomy edge (light blue bar, Fig. 3I). Collectively, these analyses indicated that in humans and OVX rats, osteotomy site preparation produces a zone of cell death.

Osteotomy Site Preparation Creates a Zone of Osteocyte Apoptosis and Necrotic Bone That Is Remodeled and Replaced by New Bone

We used additional assays to characterize the response of patient tissues to implant site preparation. Patient osteotomies (Fig. 4A), like rat osteotomies (Fig. 4B), exhibited uniformly smooth cut edges that were occasionally interrupted by discontinuities, which either corresponded to cutting flutes or micro-damage (arrows, Fig. 4A', B'). Collagen organization was visualized by picrosirius red staining, and when viewed under polarized light, a characteristic basket weave pattern was detectable in both patient (Fig. 4C) and rat (Fig. 4D) osteotomies. Tartrate-resistant acid phosphatase-positive (TRAP⁺) cells were identified in vascular sinusoids of the alveolar bone of patient (Fig. 4E) and rat (Fig. 4F) samples, in keeping with the monocyte origin of osteoclasts.

In patients, harvesting of the osteotomy site had to be performed soon after creation of the osteotomy; in rats, osteotomies could be harvested at multiple time points throughout the entire healing period. This ability to analyze tissues at multiple

time points permitted a more detailed understanding of how implant site preparation affected subsequent new bone formation. For example, we had noted that osteotomies produced in both species were accompanied by significant programmed cell death in the surrounding alveolar bone (Fig. 3); in rats, we could couple DAPI/TUNEL staining at POD0.5 to other analyses, conducted at subsequent time points, to understand the fate of this necrotic bone (Fig. 4G, brackets). In analyses of rat tissues, we found that the necrotic bone underwent extensive remodeling, as shown by cathepsin K immunostaining and TRAP activity (Fig. 4H, I). Cells occupying the osteotomy site were mitotically active and, by POD7, were immunopositive for the osteoprogenitor marker Osterix (Fig. 4J, K). When viewed under polarized light, picrosirius red staining demonstrated that these cells were actively secreting a collagen-rich, ALP⁺ mineralized matrix that had begun to fill the osteotomy site (Fig. 4L, M, N). Thus, within 7 d of osteotomy site preparation, the onset of new bone formation had initiated.

Discussion

There are 2 practical applications of the data shown here. First, the clinical use of dental implants is predicated on preclinical safety and efficacy testing, which until recently has been primarily carried out using large animal models (Pearce et al. 2007). Such studies typically have very small sample sizes and few time points for analysis, which represent significant limitations when studying a process as complex as implant site preparation. If smaller animal models can be validated for such purposes, it would reduce the time, resources, and number of animals that must be sacrificed to gain regulatory approval of a device or drug.

A second practical application of this study is that it can serve as a baseline for understanding how a common practice, osteotomy site preparation, affects bone. It is inescapable: cutting bone kills osteocytes, and this zone of death triggers bone resorption. With this knowledge in hand, we can begin to develop and critically evaluate new tools and technologies that potentially reduce the trauma of osteotomy site preparation, which can translate directly into faster bone formation around implants.

Osteopenia Slows the Rate of Bone Healing

From the orthopedic literature, it is clear that a patient's underlying bone health has a profound impact on his or her rate of bone repair (Giannoudis et al. 2007), but whether this translates to bone healing around dental implants is not known (Guobis et al. 2016). Most dental implant patients are older than 50 y (Wood et al. 2004; Bural et al. 2013; Austin et al. 2015), which coincides with the onset of age-related osteopenia. Even though some clinical imaging studies do not reveal an age-related decline in alveolar bone density (Moriya et al. 1998), others do (Shaw et al. 2011), and there is an accompanying extensive clinical literature indicating that patients with osteopenic/osteoporotic skeletons exhibit slower bone repair (Namkung-Matthai

Figure 4. Implant site preparation creates a radial zone of dead and dying osteocytes, the fate of which can be followed in a rodent model but not in patients. Aniline blue staining of tissue sections from representative osteotomies produced in (A) a patient and (B) an ovariectomy surgery (OVX) rat, collected on postosteotomy day (POD) 0.5. Higher magnification images show areas of micro-damage in (A') patient and (B') rat samples. When visualized under polarized light, picrosirius red-stained tissues from (C) a patient and (D) rat illustrate a similar organization to the collagenous-rich mineralized matrix at the osteotomy edge. Tartrate-resistant acid phosphatase (TRAP) staining for osteoclasts is similar between (E) a 67-year-old patient and (F) an OVX rat. (G) In rat tissues collected on POD7, analyses for osteocyte viability using 4',6-diamidino-2-phenylindole (DAPI); (H) osteoclast activity using cathepsin K immunohistochemistry and (I) TRAP activity; (J) mitotic activity using proliferating cell nuclear antigen (PCNA) immunohistochemistry; and (K) osteogenesis using Osterix immunohistochemistry, (L) picrosirius red staining, (M) alkaline phosphatase (ALP) activity, and (N) aniline blue staining. Abbreviations as noted previously. Scale bars in A, B = 200 μ m; E–N = 100 μ m.

et al. 2001; Schneider et al. 2005; Giannoudis et al. 2007). Both factors were considered in our decision to use an OVX model to more closely match the probable osteopenic phenotype of the average dental implant patient.

Avoiding Osteocyte Death to Improve Implant Site Preparation

The current practices of implant site preparation leave behind a zone of dead and dying osteocytes, and our computational model provided some key insights into the basis for this cell death. Drilling raises the calculated temperature in bone but not because of friction; rather, the temperature rises because of the energy required to remove bone (Fig. 3 and see Davidson

and James 2003). If the BV/TV is high, then there will be considerably more heat generated during the bone removal process than if the BV/TV is low (Fig. 3). Our model also shows that even a modestly elevated ($\sim 42^{\circ}\text{C}$) temperature can kill osteocytes. Drilling at lower speeds can reduce temperatures (Fig. 3 and see Wang et al. 2017), but most drills are not designed to be used at very low revolutions per minute (RPM). Copious irrigation is also of limited use in these cases because again, most of the heat generated is related to the energy required to remove the bone, rather than friction between the cutting tool and the bone.

Osteotomy site preparation kills osteocytes (Fig. 3, and see Wang et al. 2017); the extent of apoptosis is further increased if an implant is placed in the osteotomy using high insertion

torque (Cha et al. 2015). This additional osteocyte death is the result of high strains created in the peri-implant bone in response to lateral compression by the implant (Cha et al. 2015). If low insertion torque is used, then the extent of osteocyte necrosis is less, nearly equivalent to that produced by osteotomy drilling alone (Cha et al. 2015).

Combining our biological and computational data, a strategy emerges for improving implant site preparation. Because the extent of osteocyte death is directly related to the amount of bone removed by cutting (Fig. 3E), a tool that minimizes cutting and instead displaces bone to create an implant bed may be preferable. Overcondensing should be avoided, however, because the force used to displace the bone can also cause extensive micro-damage (Wang et al. 2017). Using a very slow cutting speed, with a tool specifically designed to cut at low RPM, may also prove to be advantageous. Existing data support these ideas: for example, in locations where there is minimal osteocyte apoptosis, new alveolar bone forms quickly (Pei et al. 2017), whereas increasing the zone of apoptotic osteocytes results in more bone resorption and slower bone formation (Cha et al. 2015).

Aligning Animal Models with Patient Data: A Continuing Challenge

We demonstrate here a close alignment between the initial responses of patient and rat tissues to implant site preparation, but we could not evaluate healing responses in patients over time. Were it possible to evaluate patient tissues at multiple time points postosteotomy, we could begin to understand both the immediate as well as long-term consequences of a specific drilling protocol or cutting tool. Until there is ample justification, for harvesting multiple bone biopsies from patients over a protracted course of the bone-healing period, we must rely on validated animal models to assess the complex process of tissue healing.

We also sought to align the rat model with the average dental implant patients' underlying bone health, and herein lies a second limitation to our study. The OVX surgery reliably produces osteopenia/osteoporosis in rats (Fig. 1), and while most dental implant patients aged >50 y will have an osteopenic bone phenotype, a subset of those patients is likely to be taking medications to treat this low bone mass disease. Therefore, it is probable that the significant delays in bone healing we demonstrate in the OVX/mxM1 rats likely reflects the delayed healing that would be observed in aged individuals or in osteopenic and osteoporotic patients with uncontrolled disease.

Conclusion

In aggregate, these data demonstrate that the onset of new bone formation, either in an osteotomy or around an implant placed in such an osteotomy, could be significantly enhanced if cutting tools being employed minimized the zone of osteocyte

death. Most of this osteocyte death is a consequence of the energy required to cut the bone and not because of friction between the bone and the cutting tool. Therefore, methods to limit osteocyte death that take advantage of this information will likely have a significant, positive impact on implant site preparation and osseointegration.

Author Contributions

C.-H. Chen, contributed to data acquisition, analysis, and interpretation, drafted the manuscript; X. Pei, U.S. Tulu, M. Aghvami, C.-T. Chen, D. Gaudillière, M. Arioka, M.M. Moghim, O. Bahat, M. Kolinski, T.R. Crosby, A. Felderhoff, J.B. Brunski, contributed to data acquisition, analysis, and interpretation, critically revised the manuscript; J.A. Helms, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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