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Cell viability after osteotomy and bone harvesting: comparison of piezoelectric surgery and conventional bur

S. Mouraret[1,2,a], K. S. Houschyar[1,a], D. J. Hunter[1], A. A. Smith[1], O. S. Jew[1], S. Girod[1,3,a], J. A. Helms[1,3,a]

[1]Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford School of Medicine, Stanford, CA 94305, USA; [2]Department of Periodontology, Service of Odontology, Rothschild Hospital, AP-HP, Paris 7 – Denis, Diderot University, U.F.R. of Odontology, Paris, France; [3]Stanford Oral Medicine & Maxillofacial Surgery Service, Stanford School of Medicine, Stanford, CA 94305, USA

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Abstract. The aim of this study was to evaluate and compare the influence of a piezoelectric device versus a conventional bur on osteocyte viability and osteoblast and osteoclast activity using an in vivo mouse model. Osteotomies were created and bone grafts were harvested using either a conventional bur or a piezoelectric device; the resulting injuries and bone grafts were evaluated over an extended time-course using molecular and cellular assays for cell death (TUNEL assay), cell viability (4′,6-diamidino-2-phenylindole (DAPI) staining), the onset of mineralization (alkaline phosphatase activity), and bone remodelling (tartrate-resistant acid phosphatase activity). Osteotomies created with a piezoelectric device showed greater osteocyte viability and reduced cell death. Bone grafts harvested with a piezoelectric device exhibited greater short-term cell viability than those harvested with a bur, and exhibited slightly more new bone deposition and bone remodelling. The difference in response of osteocytes, osteoblasts, and osteoclasts to bone cutting via a bur and via a piezoelectric device is negligible in vivo. Given the improved visibility and the margin of safety afforded by a piezoelectric device, they are the instrument of choice when cutting or harvesting bone to preserve soft tissue.

Key words: bone; ultrasound; cell viability; bone graft; osteoconduction; osteoinduction; in vivo; cutting; harvesting.

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Successful bone regeneration depends on retaining the viability of osteoblasts lining the cut edges of the bone, and of osteocytes within the harvested bone.[1,2] To that end, a wide variety of techniques for bone cutting and bone harvesting have been developed in an attempt to improve cell viability.[3,4] One such technique is the piezoelectric osteotomy.[5] Over time, piezoelectric devices have been optimized to allow effective cutting of mineralized tissue while simultaneously avoiding damage to surrounding soft tissues.[6] The piezoelectric surgery device is an ultrasound machine with modulated frequency and a controlled tip vibration range, which allows a cutting action; the osteotomy site is simultaneously maintained in a relatively blood-free state because of the physical phenomenon of cavitation.[7]

Here, our goal was to understand how piezoelectric devices performed relative to traditional surgical tools in maintaining the cell viability of the cut bone. Most publications on piezoelectric devices are

[a] Contributed equally to the manuscript.

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clinical case reports, or provide an assessment of the cutting qualities of the surgical instrument. There are no in vivo studies reporting the molecular or cellular responses to bone-cutting by a piezoelectric device as compared to a traditional bur. Therefore, in this study we employed two in vivo model systems: one representing an osteotomy in situ, and the other a bone harvesting technique. In both cases we used histology and immunohistochemistry to evaluate how osteoblasts on the surface of the cut bone, and osteocytes in the harvested bone itself, responded to the ultrasonic device as compared to a traditional bur. As this is the first study to appraise the in situ response of osteoblasts, osteocytes, and osteoclasts, we began with the null hypothesis that there would be no discernible difference between the cellular response elicited by bone-cutting with a piezoelectric device versus bone-cutting with a traditional bur.

Materials and methods

Animal care

All procedures followed protocols approved by the Stanford Committee on Animal Research. Animals were housed in a temperature-controlled environment and were given a soft food diet and water ad libitum. There was no evidence of infection or prolonged inflammation at the surgical site, therefore no antibiotics were administered.

Osteotomy

Twelve adult wild-type mice (males, between 3 and 5 months old) were anaesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (16 mg/kg). The mouth was rinsed using a povidone–iodine solution and then a sulcular incision was made that extended from the maxillary first molar to the mid-point on the alveolar crest. A groove was made on the crest, in front of the first maxillary molar towards the incisor, using a piezoelectric device (SATELEC Piezoelectric System, Synthes Inc.) and the 1.2 mm × 0.5 mm insert (Synthes 03.000.407S). The piezoelectric device was always set to program mode D3 and fine tuning level 1; in this condition the frequency modulation was constant at 60 Hz. During its use, surgeons applied a repeated, short pulling movement, with slight pressure, never exerting force. On the other side, the same injury was created with a 0.5-mm diameter fissure carbide bur (H349.104.005; Komet USA, Rock Hill, SC, USA) fit on a low-speed dental engine (800 rpm). Surgeons used new cutting tips and a new bur for every surgery. In both cases, to avoid any risk of burns or overheating, cold irrigation (60 ml/min) was always switched on and active when the hand pieces were in use. The surgical site was rinsed and the flap was closed using non-absorbable single interrupted sutures. Following surgery, clinical examinations were performed and mice received subcutaneous injections of buprenorphine (0.05–0.1 mg/kg) for analgesia once a day for 3 days. Mice were sacrificed at 5, 11, and 14 days post-surgery.

Bone harvest

Twelve mice were anaesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (16 mg/kg). The dorsum was shaved and decontaminated using a povidone–iodine solution for 1 min. A skin incision was made, followed by a muscle incision to access the femur. Bone grafts were harvested from the central part of the femur (8 mm of length to 3 mm of width, through the cortical bone until the bone marrow) with the piezoelectric device (Synthes) and the 1.2 mm × 0.5 mm insert (03.000.407S; Synthes). The piezoelectric device was set to the same settings as those of the osteotomy: program mode D3 and fine tuning level 1 for a constant frequency modulation of 60 Hz. The piezo incision was always performed in the midline of the femur; the cut length was 0.8 mm. Surgeons utilized the same cutting techniques as in the osteotomy, applying a repeated, short pulling movement, with slight pressure and no force. On the other femur, the same graft was harvested with a 0.5-mm diameter fissure carbide bur (H349.104.005; Komet USA) fit on a low-speed engine (800 rpm). Surgeons used new cutting tips and a new bur for every surgery. In both cases, to avoid any risk of burns or overheating, cold irrigation (60 ml/min) was always switched on and active when the hand piece was in use. The surgical site was rinsed and the muscle was closed using synthetic absorbable sterile surgical sutures (coated Vicryl 6–0; Johnson & Johnson Medical, USA) and the skin with non-absorbable single interrupted sutures (Ethilon monofilament 7–0; Johnson & Johnson Medical, USA).

Bone grafts were placed in the dorsum after a small skin, muscle, and fat incision, and fixed with one suture (Ethilon monofilament 7–0). Following surgery, clinical examinations were performed and mice received subcutaneous injections of buprenorphine (0.05–0.1 mg/kg) for analgesia once a day for 3 days. Mice were sacrificed at 3 and 7 days post-surgery.

Sample preparation, processing, histology, and immunohistochemistry

Maxillae and femurs were harvested and prepared as described elsewhere.[8] Alkaline phosphatase (ALP) activity was detected by incubation in nitro blue tetrazolium (NBT; Roche), 5-bromo-4-chloro-3-indolyl phosphate (BCIP; Roche), and NTM buffer (100 mM NaCl, 100 mM Tris pH 9.5, 5 mM $MgCl_2$). Tartrate-resistant acid phosphatase (TRAP) activity was observed using a kit (Sigma). For TUNEL staining, sections were incubated in proteinase K buffer (20 μg/ml in 10 mM Tris pH 7.5) and applied to a TUNEL reaction mixture (In Situ Cell Death Detection Kit, Roche). For immunostaining, endogenous peroxidase activity was quenched by 3% hydrogen peroxide for 5 min and the sections then washed in phosphate buffered saline (PBS). Slides were blocked with 5% goat serum (Vector S-1000) for 1 h at room temperature. Antibodies used included anti-osteocalcin (Abcam ab93876) and anti-macrophages/monocytes (Millipore MAB 1852). Details are described elsewhere.[8]

Histomorphometry

Representative tissue sections were stained for 4′,6-diamidino-2-phenylindole (DAPI) and TUNEL and were imaged in differential interference contrast (DIC) and UV light. For maxilla wounds ($n = 3$ in triplicate), TUNEL-positive and DAPI-positive cells were quantified as an indication of cell death. For the bone grafts ($n = 3$ in triplicate), lacunae were quantified in the region of bone injury; cell nuclei were quantified in DAPI images as an indication of cell viability.

Statistical analyses

Results are presented as the mean ± standstandard error of the mean. The Student's *t*-test was used to quantify differences described in this article; $P \leq 0.05$ was considered to be significant. For the study we used over 24 mice, which generated reproducible results while respecting the rules of clinical research.[9]

Results

Bur-cut and piezo-cut osteotomies stimulate equivalent levels of new bone formation and bone remodelling

We assessed the molecular and cellular responses observed the at maxillary

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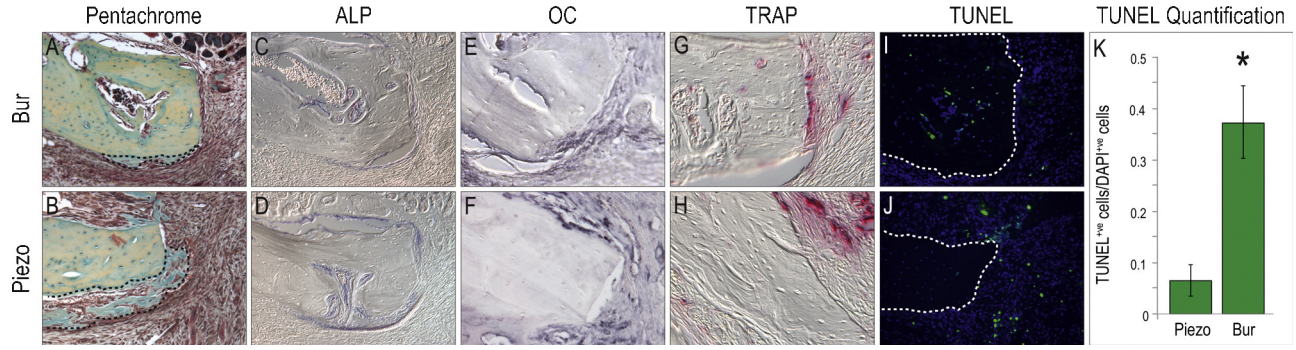

Fig. 1. Osteogenic cell responses to a conventional bur versus a piezoelectric device on post-surgery day 7. Representative tissue sections through a bony defect created by (A) a bur, and (B) a piezoelectric device, stained with pentachrome. Alkaline phosphatase activity indicates sites of new mineralization in (C) bur-cut bones, and in (D) piezo-cut bones. Osteocalcin (OC) is expressed in (E) bur-cut bones, and in (F) piezo-cut bones. TRAP staining to identify sites of osteoclast resorption in (G) bones cut with a bur, and (H) bones cut with the piezoelectric device; TRAP staining is minimal along the edges of bones cut with the piezoelectric device. DAPI staining was used to visualize viable cell nuclei and TUNEL staining was used to identify cells undergoing apoptosis in (I) bur-cut bones, and (J) piezo-cut bones. (K) Histomorphometric analyses revealed a greater ratio of apoptotic (TUNEL-positive) cells compared to viable (DAPI-positive) cells in the bur injury compared to the piezo injury site. Scale bars: A–J 100 μm.

osteotomy sites created with a piezoelectric device ($n = 12$) and with a 0.5-mm diameter fissure bur ($n = 12$). We conducted our first analyses on post-surgical day 7.

Representative sections through each osteotomy site were analyzed, and images shown in the figures represent the average findings observed from all the samples.

The most obvious histological difference noted in the bur-cut (Fig. 1A) versus the piezo-cut bones (Fig. 1B) was the presence of new osteoid matrix surrounding the piezo-cut bone edges (dotted line, Fig. 1B). New osteoid matrix stains a blue–yellow colour versus mature bone matrix, which stains yellow,[10] suggesting that this new bone deposition occurred in response to the osteotomy.

Both bone-cut (Fig. 1C) and piezo-cut (Fig. 1D) surfaces showed evidence of ALP activity, with ALP activity slightly more predominant in the piezo-cut samples. Using expression of the osteogenic gene osteocalcin,[11–13] we found a similar response: whether the bones were cut with a bur (Fig. 1E) or with a piezoelectric device (Fig. 1F), all surfaces showed evidence of osteocalcin expression. Thus, the act of cutting bone stimulates new osteoid mineralization within the periosteum.

Bone formation is tightly linked to the process of bone resorption.[14] Therefore, we evaluated the activity of osteoclasts on the cut bone surfaces using TRAP staining.[15] We found evidence of osteoclast activity along both the bur-cut (Fig. 1G) and the piezo-cut (Fig. 1H) bones. Thus, the remodelling response was similar whether the bones were cut with a bur or a piezoelectric device.

Cell death is reduced in piezo-cut bones

Our impression thus far was that cutting a bone with a piezoelectric device resulted in greater cell viability. Within the bone itself, we found that there were more positive cells in the piezo-cut samples (Fig. 1I, J). We used TUNEL staining to detect DNA fragmentation associated with apoptotic cell death[16] and found more positive cells in the bur-cut bones than in the piezo-cut bones (Fig. 1I, J). We used histomorphometric analyses to quantify the number of TUNEL-positive osteocytes relative to the number of DAPI-positive osteocytes in both types of samples and found a greater percentage of apoptotic cells in the bur-cut bones (Fig. 1K). Taken together, these results demonstrate that when the bone is cut with a piezoelectric device, more osteocytes survive within the bone itself. More dying cells, however, were found in the surrounding soft tissues of the piezo-cut samples than of the bur-cut samples (Fig. 1I, J).

Bur- and piezo-cutting elicit a minor inflammatory response

An inflammatory response has to regress before cell proliferation ensues,[17,18] and this can delay bone healing. We evaluated whether the piezoelectric device produced a stronger inflammatory response, and found that it did not. At post-surgery days 7 and 11, the inflammatory response was the same between bones cut with a bur (Fig. 2A, C) and those cut with a piezoelectric device (Fig. 2B, D). Thus, neither method of bone cutting elicited an inflammatory response that curtailed new bone formation.

Osteotomies created with a piezoelectric device show enhanced remodelling

On post-surgery day 11 we began to observe differences between bur-cut bones and piezo-cut bones: first, the bur-cut bones showed less new bone deposition on the cut edges (Fig. 3A) compared to bones cut with the piezoelectric device (dotted yellow line, Fig. 3B). ALP activity was evident along the bur-cut bone surface (arrows, Fig. 3C). In comparison, ALP activity was overall more robust in the piezo-cut bones (arrows, Fig. 3D).

We evaluated TRAP activity on post-surgery day 11 as well. TRAP activity was evident on bur-cut bones (Fig. 3E) but only minimally evident in piezo-cut bones (Fig. 3F). Collectively, these data suggest that osteoblasts and osteoclasts actively remodel bone surfaces whether they are cut with a traditional bur or a piezoelectric device, and that any early differences in cell viability were apparently compensated at later time points.

Bone grafts harvested with a piezoelectric device are superior to those harvested with a bur

In addition to creating osteotomies, piezoelectric devices are also used for harvesting bone grafts. We developed an autologous femur graft harvest model (see Methods for details) to test its function for this invasive application. Once harvested, the bone graft was placed in the dorsal muscle pouch, and 3 and 7 days later, tissues were collected for analysis.

In bone grafts harvested by a bur from a femur ($n = 12$), the cut edge was rough and uneven on post-surgery day 3 (dotted line,

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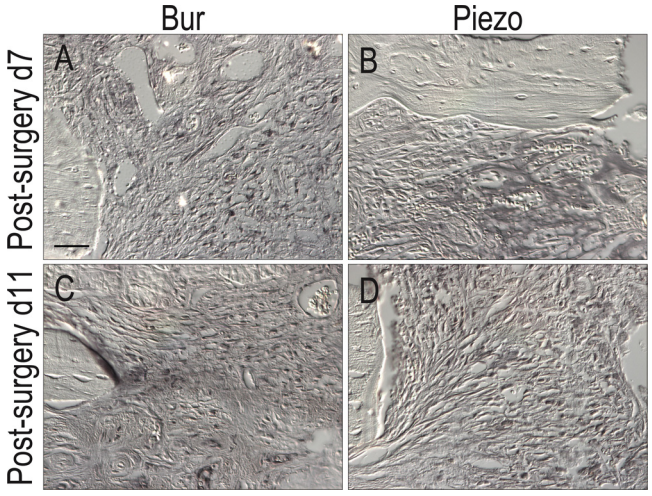

Fig. 2. Bones cut with a bur or a piezoelectric device demonstrated similar inflammatory responses. Representative tissue sections through the cut edge of bone grafts harvested with (A) a bur, and (B) a piezoelectric device, and evaluated on post-surgery day 7, immunostained for monocyte-macrophage marker MOMA-2 to identify inflammatory cells. Immunostaining for monocyte-macrophage marker MOMA-2 of the cut edge of bone grafts harvested with (C) a bur, and (D) a piezoelectric device and evaluated on post-surgery day 11. Scale bars: A–D 25 μm.

Fig. 4A). In comparison, bone grafts harvested using a piezoelectric device ($n = 12$) had a smooth cut edge (dotted line, Fig. 4B). We evaluated the organization and packing of the collagen at the cut bone edges using picrosirius red staining.[19] Picrosirius red staining clearly demarcated the uneven bur-cut bone edges (dotted line, Fig. 4C). In comparison, picrosirius red staining of the piezo-cut bone edge was uniform (dotted line, Fig. 4D).

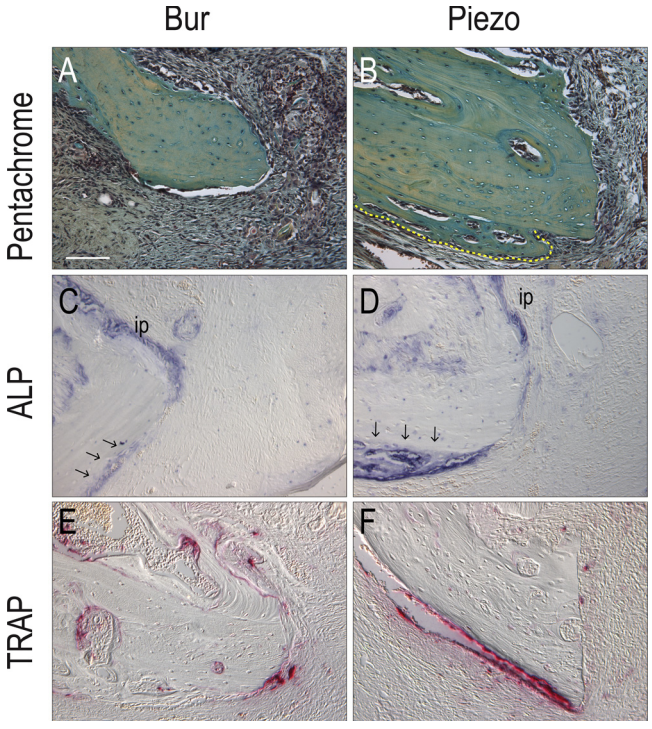

Fig. 3. Osteocyte responses to a conventional bur versus a piezoelectric device on day 11. Representative frontal tissue sections through a bony defect created by (A) a bur, and (B) a piezoelectric device, stained with pentachrome. New bone deposition is denoted by the dotted yellow line. Alkaline phosphatase (ALP) activity is detectable in the newly mineralized bone matrix in (C) bur-cut bones, and in (D) piezo-cut bones (arrows). TRAP staining was evident along the bones cut with (E) a bur, but only minimally evident along the edges of bones cut with (F) the piezoelectric device. Scale bars: A–F 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Representative tissue sections were visualized under fluorescent light and a dramatic difference in cell density was obvious: relative to cell density at the bur-cut bone edge (dotted line, Fig. 4E), cell density was higher at the piezo-cut bone edge (dotted line, Fig. 4F). We used histomorphometric analyses to quantify the number of viable cells relative to lacunae at the bone graft site and found a greater percentage of osteocytes attached to lacunae in the piezo-cut bone graft compared to the bur-cut bone graft (Fig. 4G). To determine if the improvement in cell viability persisted, we evaluated the bone grafts at post-surgery day 7. Pentachrome staining at this later time point showed dramatically increased cell density in the piezo-harvested bone grafts compared to the bur-harvested grafts (Fig. 4H, I). None of the bone grafts showed evidence of ALP activity on the cut surfaces (data not shown). However, ALP activity in the intact periosteum of the bone grafts was equivalent between the bur-harvested (Fig. 4J) and the piezo-harvested samples (Fig. 4K), indicating that the graft had been handled in a manner that preserved cell viability.

TRAP-positive osteoclasts were more abundant on the bur-cut surfaces of the bone grafts (dotted line, Fig. 4L); no TRAP staining was detectable on piezo-cut surfaces of the bone grafts (dotted line, Fig. 4M). Thus, bone grafts harvested using a piezoelectric device showed slightly lower bone resorption on the cut surfaces.

Discussion

In our study we compared the effects of a conventional bur with a piezoelectric device, and specifically focused on the in vivo response to each of these cutting tools. To our knowledge, this represents the first study to directly compare the in vivo effects of these two widely used methods of bone cutting. Because the study was conducted in vivo, quantifying the molecular and cellular response is necessarily more complicated. In keeping with this inherent constraint, we limited our interpretations of the data.

Nonetheless, a descriptive study such as this provides some essential information regarding the spatial and temporal responses of cells and tissues that cannot be gained through in vitro analyses.

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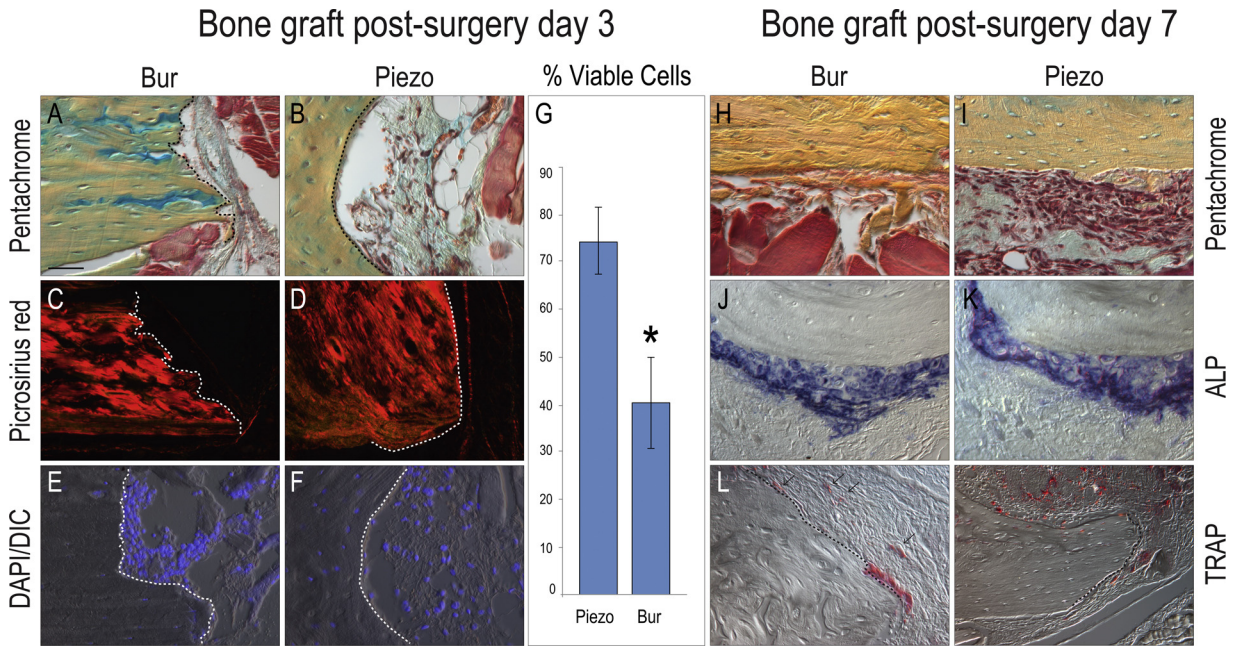

Fig. 4. Cellular responses in a bone graft harvested with a conventional bur versus a piezoelectric device. Representative tissue sections through the cut edge of bone grafts harvested with (A) a bur, and (B) a piezoelectric device, and evaluated on post-surgery day 3, stained with pentachrome. The dotted lines mark the edges of the bone grafts. In picrosirius red staining the red colour denotes the orientation of collagen on bone grafts harvested with (C) a bur, and (D) a piezoelectric device on post-surgery day 3. DAPI staining was used to identify the nuclei of viable cells, in (E) bur-harvested bone grafts compared to (F) piezo-harvested bone grafts on post-surgery day 3. (G) On post-surgery day 3, histomorphometric analysis revealed a greater ratio of viable (DAPI-positive) cells to lacunae in the bur-cut bone graft compared to the piezo-cut bone graft. Representative tissue sections through the bone grafts harvested with (H) a bur, and (I) a piezoelectric device and evaluated on post-surgery day 7, stained with pentachrome. Higher magnification images showed alkaline phosphatase activity in (J) bur-harvested bone grafts, and in (K) piezo-harvested bone grafts. Higher magnification images showed TRAP activity in (L) bur-harvested bone grafts and in (M) piezo-harvested bone grafts. Scale bars: A–L 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

We focused on how osteoblast, osteoclast, and osteocyte behaviours were affected by cutting with a bur and cutting with a piezoelectric device. We first tested how the piezoelectric device and a bur affected cell viability at the site of an osteotomy. Although there were differences in the extent of cell viability and cell death (Fig. 1), we did not detect any significant alterations in osteogenesis when bone was cut with a bur or with a piezoelectric device. These results are in agreement with those of other groups.[20–22] In bone grafts harvested with a piezoelectric device we found the same trend, namely that there were more viable cells found in grafts harvested with a piezoelectric device (Fig. 4). The reason(s) for differences in cell viability are not clear. Some in vitro studies indicate that piezoelectric devices generate less heat than burs,[23,24] and there is ample evidence that techniques that generate minimal heat are associated with higher cell viability.[25] There may be factors other than thermal damage, however. For example, ultrasound energy[26] and copious water irrigation[21] can contribute to cell viability in an osteotomy or at a bone-harvesting site. Clearly, a better understanding of the conditions that optimize cell viability will result in improved healing and bone regeneration.

The most critical period of bone healing is the first 2 weeks, during which inflammation and revascularization occur. We evaluated the inflammatory response following bone cutting with a bur versus a piezoelectric device, and found no obvious differences in the extent or duration of inflammation (Fig. 2). There are caveats to this conclusion: our analyses were limited to the detection of macrophages and neutrophils within the wound environment. Nonetheless, we failed to detect any notable differences using these two robust indicators of an in vivo inflammatory response.

The application of ultrasound is emerging as a potential therapy for the treatment of complex bone fractures and tissue damage. Ultrasonic stimuli may improve bone healing by promoting cell proliferation, migration, and matrix synthesis,[27–30] but in none of the piezo-cut bones did we observe an increase in ALP activity, osteocalcin expression, or overt bone formation (Figs 1 and 3). Thus, claims of a pro-osteogenic effect from a piezoelectric device appear to be unwarranted.

We also evaluated whether bone grafts harvested with a bur fared better than those harvested with a piezoelectric device (Fig. 4). At post-surgery day 3, we found improved osteocyte viability in the piezo-cut samples compared to the bur-cut samples, and at post-surgery day 7, we observed slightly more new bone on the piezo-cut surface (Fig. 4). This osteogenic response may prove to be valuable, as the best scaffold materials are those containing living cells that are capable of osteogenic differentiation. Future studies will focus on the osteogenic potential of bone grafts harvested with a piezoelectric device, as any biological improvement in bone grafting procedures will have a profound effect on a large number of patients.

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Competing interests

No conflicts of interest.

Ethical approval

All procedures followed protocols approved by the Stanford Committee on Animal Research.

Patient consent

Not required.

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Address:
Jill A. Helms
Division of Plastic and Reconstructive Surgery
Department of Surgery
Stanford School of Medicine
Stanford
CA 94305
USA
Tel: +1 650 736 0919;
Fax: +1 650 736 4374
E-mail: jhelms@stanford.edu

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