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

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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

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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