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The effects of multiple high-resolution peripheral quantitative computed tomography scans on bone healing in a rabbit radial bone defect model

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ABSTRACT

The use of *in vivo* high-resolution computed tomography (CT) scanners provides the unique opportunity for evaluating temporal progression in healing of bone defects. However, these *in vivo* scanners impose ionizing radiation that could affect the healing and morphology of the bone. The primary objective of this study was to determine the effects of *in vivo* scanning at 2-week intervals on bone healing of a critical sized radial defect in rabbits and to investigate the effect of this radiation protocol on bone marrow cell viability using clinically applicable radiation doses. Thirty male rabbits were randomized into three groups: two groups received a 15 mm defect in the left radius that was filled with an autologous bone graft (DEF-CT and DEF-SHAM), and one group acted as an intact control (INT-CT). The duration of the study was 6 weeks. DEF-CT and INT-CT had high-resolution CT scans performed at 2-week intervals. The total cumulative radiation dose was 81.6 mGy per animal. DEF-SHAM received sham CT scans at the same time points. In group DEF-CT, the bone volume (BV) in the defect increased significantly over time ($p \leq 0.002$, for all comparisons); the bone mineral density (BMD) in the defect decreased over time and was significantly lower at weeks 4 and 6 than at weeks 0 and 2 ($p < 0.001$, for all comparisons). In group INT-CT, BV and BMD did not change over time ($p = 1$, for all comparison). The BV ($p = 0.50$) and the BMD ($p = 0.37$) in the defect as measured by microCT scan during *ex vivo* analysis was not significantly different between DEF-CT and DEF-SHAM. Similarly, histomorphometry showed no significant difference in the total bone area ($p = 0.22$) and percentage bone within the defect ($p = 0.24$) between these groups.

Bone marrow analysis of the left (radiated) and right (non-radiated) radius of the INT-CT group *via* a Colony Forming Units (CFU) assay demonstrated an average of 25.3 and 28.5 colonies for radiated and non-radiated radii, respectively ($p = 0.72$).

In conclusion, there was no significant difference in bone healing between radiated and non-radiated radius defects in rabbits. This is an important finding as it demonstrates that serial *in vivo* high resolution-CT imaging can not only provide accurate tissue regeneration data, but it can also be used to reduce the number of temporal cohorts within an experimental design.

Introduction

Micro-computed tomography (μCT) imaging has been proven useful for the evaluation of bone micro-architecture in *ex vivo* [1–3] and *in vivo* studies [4–7]. This technique offers the possibility for non-destructive qualitative and quantitative analysis, by the visual inspection of three dimensional reconstructions and calculation of bone morphometric parameters, respectively [8]. These properties have rendered micro-CT extremely useful for evaluation of fracture healing [9] and healing of bone defects [10,11] in pre-clinical research models. *In vivo* micro-CT imaging provides the unique opportunity for evaluating temporal progression in healing of bone defects, thereby reducing the number of animals needed per experiment by a factor as high as the number of measurements performed [12] and thus adhering to the concept of the three Rs (Replacement, Reduction and Refinement) in animal research [13]. Each animal can act as its own control, thereby accommodating the variability present in a cohort of animals and thus facilitating repeated measures comparisons [6]. Additionally, when assessing bone substitute materials which may have similar densities

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to bone tissue and hence cannot be distinguished from bone through thresholding, immediate postoperative data can be used as reference values to normalize subsequent data. Thus, information on changes in bone volume and bone mineral density can be determined in a relative manner.

However, these *in vivo* scans impose ionizing radiation that could potentially affect the healing and morphology of the bone and therefore confound the biological outcome of an experiment. Radiation-induced changes in bone are commonly encountered in patients undergoing radiation-therapy for neoplastic diseases and include alterations in bone growth, osteoradionecrosis, pathologic fractures, and radiation-induced neoplasms [14]. The effects on bone regeneration depend on the radiation dose and the timing of the exposure relative to defect creation [15–17]. *In vitro* experiments with osteoblast-like cells have shown that radiation exposure inhibited cell proliferation and enhanced cell differentiation in a dose-dependent fashion [18–20]. Radiation doses greater than 5 Gy caused cell-death, however low-dose exposure (<1 Gy) induced no changes [20]. These detrimental properties of radiation to cells have led to its use in treating neoplastic diseases *via* radiation therapy. Radiation therapy requires extremely high doses to be effective and a number of preclinical studies have investigated the effect of a single high dose exposure on bone healing in rats [15,16,21] and rabbits [17]. The lowest radiation dose used in these studies is 10 Gy for rats [15,16] and 6.5 Gy for rabbits [17]. However, the radiation doses associated with imaging are substantially lower (<1.3 Gy per scan) [5–7,12,22–25], though investigation of temporal changes requires multiple successive micro-CT scans and therefore results in an accumulation of radiation exposure. Several investigators have examined the effect of repeat *in vivo* micro-CT radiation on morphological changes in long bones from mice and rats with conflicting results [5–7,12,22–25]. Differences in study design, radiation dose, scanning intervals, species sensitivity to radiation, age and maturity of the animals, may account for these discrepancies.

Recently, a high-resolution peripheral quantitative computed tomography system (pQCT) was introduced (XtremeCT, Scanco Medical, Switzerland) for *in vivo* clinical assessment of bone structure [26]. The investigators started using this system regularly for the longitudinal evaluation of healing in segmental bone defects in small animals, but the impact of this repeat radiation from sequential pQCT scans on bone healing and possibly more importantly on cell-based therapies utilized in the treatment of bone defects is currently unknown.

The objective of this study was therefore to determine whether *in vivo* pQCT scanning at 2-week intervals negatively impacts healing of a critical sized radial defect treated with an autologous iliac crest bone graft in New Zealand white rabbits and whether this radiation dose affects bone marrow cell viability.

Materials and methods

Animals and surgery procedure

Thirty skeletally mature male New Zealand white rabbits between 28 and 32 weeks of age and a mean body weight of 4.1 ± 0.4 kg were included in this study under the approval of the cantonal ethics committee of Grisons/Switzerland. All animals were screened and assessed to be free of disease and in good physical condition and allowed to acclimatize to their surroundings for two weeks prior to the start of the study. Animals were randomly assigned to one of three treatment groups: DEF-CT (n = 10), DEF-SHAM (n = 10), and INT-CT (n = 10). Rabbits in the DEF-CT and DEF-SHAM groups received a 15 mm critical sized diaphyseal defect in the left radius following the procedures adapted from Wittbjer et al. [27,28]. These defects were augmented with 0.3 cc of morselized autologous iliac crest bone graft. Rabbits in the INT-CT group did not undergo any surgical intervention.

All operated animals were sedated prior to surgery using a combination of 0.2 mg/kg Medetomidine (Domitor®, Pfizer AG, Switzerland), 1 mg/kg Climazolam (Climasol®, Dr. E. Graeub AG, Switzerland) and 1 mg/kg Morphine (Morphin-HCl Sintetica®, Sintetica SA, Switzerland) intramuscularly. Anesthesia was induced with Propofol (Propofol® 1% MCT Fresenius, Fresenius Kabi AG, Switzerland) intravenously to effect (2–6 mg/kg). The rabbits were intubated and anesthesia was maintained with 2% Isoflurane (Isofluran Baxter®, Baxter AG, Switzerland) in oxygen. After induction the animals were placed in left lateral recumbency and anesthesia of the left brachial plexus was performed using 0.1 ml/kg Lidocain 2% (Lidocain 2% Streuli®, Streuli Pharma AG, Switzerland) and 0.1 ml/kg Bupivacaine 0.5% (Carbostesin®, AstraZeneca AG, Switzerland) under guidance of a nerve stimulator (TOF-Watch®, Organon, Swords Co. Dublin, Ireland).

A 20 mm skin incision was created over the dorsal aspect of the right iliac crest and the underlying subcutaneous tissues and fascia were incised to expose the iliac crest. The gluteus medius muscle was partially detached from the iliac crest and approximately 0.3 cc of cortico-cancellous bone was obtained from the dorso-cranial aspect of the iliac crest using a bone rongeur. The incision was closed routinely in three layers using a subcuticular suture pattern and cyanoacrylate tissue adhesive (Epiglu®, Meyer-Haake, Germany) to close the skin.

The diaphysis of the left radius was approached through a 30 mm skin incision. A 15 mm bone defect was created in the diaphysis of the radius using an oscillating saw so that the distal edge of the defect was located 25 mm proximal to the radio-carpal joint (Fig. 1). The defect was treated with 0.3 cc of the morselized autologous bone graft. A titanium pin was placed 8 mm proximal to the proximal edge of the defect to serve as a reference marker for the CT scans (Fig. 1). Muscles and other soft tissues overlying the defect were sutured in three layers with 5–0 non-resorbable polypropylene suture material (Prolene; Ethicon, Norderstedt, Germany) and the skin was closed with cyanoacrylate tissue adhesive (Epiglu®, Meyer-Haake, Germany).

Post-operative analgesia consisted of Carprofen (Rimadyl®, Pfizer AG, Switzerland) (4 mg/kg) subcutaneously once daily for 3 days and Buprenorphine (Temgesic®, Reckitt Benckiser AG, Switzerland) (0.05 mg/kg) intramuscularly three times daily for 2 days. Rabbits were maintained in individual cages and allowed to ambulate normally for the entire study and were assessed 4 times daily for the first two post-operative weeks, followed by twice daily assessments for the remainder of the study. All animals were humanely euthanized by means of captive bolt and exsanguination following the last scan.

Imaging

High-resolution computed tomography

Bone morphometric parameters were assessed *in vivo* and *ex vivo* using a high-resolution peripheral quantitative computed tomography system (HR-pQCT) system (XtremeCT, Scanco Medical, Switzerland) and a high-resolution micro CT (μCT) system (microCT 40, Scanco Medical, Switzerland), respectively.

In vivo high-resolution peripheral quantitative Computed Tomography (pQCT). Immediately after surgery (week 0) the rabbits were placed in the pQCT (DEF-CT and DEF-SHAM) or after inclusion in the study (INT-CT) and at week 2, 4 and 6. Rabbits were anesthetized and secured into a custom-made carbon holder designed to obtain reproducible positioning of the left front leg.

The pQCT was operated at a setting of 60 kVp and 1 mA, with an isotropic voxel size of 82 μm. To determine the radiation dose, the CT Dose Index (CTDI) was calculated from the scanning protocol and resulted in a CTDI value of 20.4 mGy per scan (Appendix A). The total cumulative dose was therefore 81.6 mGy per animal for DEF-CT and INT-CT animals.

For DEF-CT and DEF-SHAM, the start point of the scan was located 25.5 mm distal to the reference marker (Fig. 1). This point corresponded with the same location for INT-CT animals, where the start point was identified as the slice situated 22.5 mm proximal to the radio-carpal