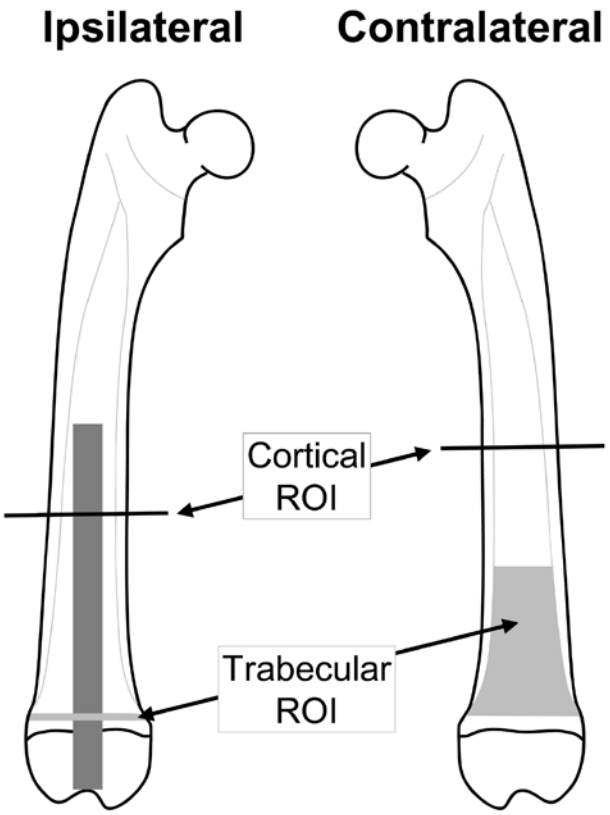

Fig. E-1

Illustration showing the location of the implant and the regions of interest in the femur containing the implant and the contralateral femur. The rationale for the differing placement of the regions of interest (ROIs) is given in the Appendix, and none of the conclusions of the study depend on a direct comparison of the ipsilateral and contralateral bones.

Detailed Methods

Implant Preparation

Implants (1.5 mm diameter, 20 mm length) were made from commercially pure titanium rods (99.6% purity; Goodfellow, Oakdale, Pennsylvania). Commercially pure titanium was used because some porous coatings of orthopaedic implants are made from this material. The surface of the implant was prepared by etching with a dual acid treatment as described previously[48]. Implants were sterilized in 70% ethanol overnight, air dried, and stored in sterile saline solution until surgery.

Surgical Procedure

An adaptation of the marrow ablation method described by Suva et al.[49] was used. The rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine (5 mg/kg), supplemented as necessary. The hindlimb was shaved and scrubbed with ethanol and Betadine (povidone-iodine) solution. An incision (approximately 1 cm) was made along the medial aspect of the patella, and the patella along with the quadriceps tendon and patellar tendon were retracted to expose the distal condyles of the femur. A 1.5-mm hole was drilled through the patellar groove to gain access to the medullary canal. The contents of the medullary canal were disrupted (reamed by hand) with a 1.5-mm drill bit proximally up to the lesser trochanter. The canal was then irrigated with 10 mL of saline solution. The implant was introduced into the medullary canal until the distal part of the implant was slightly countersunk, and the distal opening hole in the bone was filled with bone wax. The patella was repositioned, and the deep fascia and skin were sutured separately. The animals were treated with antibiotics (potassium penicillin G, 100,000 IU in 0.2 mL subcutaneously twice per day) at the time of surgery and for the next three days. Buprenorphine was given subcutaneously at 0.04 mg/kg preoperatively and then twice per day for two days.

Micro-CT

Both the femora containing the implants and the contralateral femora were examined by micro-CT (μCT 40; Scanco, Wayne, Pennsylvania) (Fig. E-1). For the implanted femora, a 1.3-mm-thick transverse region of interest (ROI) approximately 0.5 mm proximal to the growth plate was chosen to characterize the peri-implant trabecular bone, and a 1.3-mm-thick transverse ROI approximately 5 mm distal to the proximal end of the implant was chosen to characterize the peri-implant cortical bone. For the contralateral femora, the trabecular ROI extended from 30% of the bone length as measured from the distal end of the bone to approximately 0.5 mm proximal to the growth plate, and the cortical bone ROI was a 1.3-mm-thick transverse slab at the midshaft. For the trabecular ROIs, the external boundary was the endocortical surface. For femora containing implants, the three voxels closest to the implant and the implant itself were excluded from the analysis.

The ROIs for the implant-containing femora and the contralateral femora differed in location and, in the case of the trabecular ROIs, size. None of the analyses required a direct comparison, but it might be helpful to clarify the reasons for using different ROIs. For the contralateral limb, we used ROIs that were analogous to those used to characterize bone phenotypes in transgenic mice. For the implant-containing limb, the location of the implant as well as anatomic landmarks were used to define the ROIs. We assumed that the slight difference in cortical bone location would have a minimal effect on the comparison of the two sides. However, the trabecular ROI in the contralateral limb was much larger than that in the implanted femur. To assess how the size and location of the trabecular ROIs might influence the comparison of the ipsilateral and contralateral trabecular bone, we performed a second analysis of sixteen contralateral specimens in which the ROI was made comparable in size and location to that used for the implanted (ipsilateral) femur. We found the following relationship: BV/TV for the global ROI = 0.956 × BV/TV for the restricted ROI + 0.075 ($r = 0.953$, $p < 0.001$), with the 95% confidence interval for the intercept being 0.048 to 0.102 and the 95% confidence interval for the slope being 0.782 to 1.130. Thus, it is not correct to directly compare the BV/TV values of the peri-implant measurement site (which used a restricted ROI) and the contralateral measurement site (which used a global ROI). However, this limitation is not very important because we did not directly compare the values of the ipsilateral and contralateral sites. Rather, we compared the values of the sclerostin antibody-treated and control rats within the ipsilateral site and within the contralateral site.

The implanted femora were scanned perpendicular to the long axis of the bone at 70 kVp and 114 μA or 90 kVp and 66 μA, 0.3 s integration time, isotropic voxel size 16 μm. The two different scan energies were needed because of an unanticipated change in scanner configuration that occurred during analysis of specimens from this study and was related to the need to replace the x-ray tube. As noted below, we went to some effort to ensure that the data were not affected. The scanning medium was either the fixative (10% neutral buffered formalin) or saline solution, depending on whether the specimen was used subsequently for histology or mechanical testing, respectively. All contralateral bones were scanned in fixative perpendicular to the long axis of the bone at 70 kVp and 114 μA, 0.3 s integration time, isotropic voxel size 16 μm. For the trabecular regions of interest, the threshold chosen to segment the bone was 270 on the Scanco grayscale for the 70 kV scans (or its equivalent of 150 for the 90 kV scans). For the contralateral femur, the threshold for trabecular bone was 300. Five trabecular architecture parameters were examined[51]: bone volume per total volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spacing (Tb.Sp), and the structural model index (SMI). The trabecular architecture variables were determined with use of the direct transform method except for SMI, which is a model-based characterization in which plate-like bone has a lower value than rod-like trabecular bone. On the side with the implant, cortical bone was segmented at a threshold of 400 for the 70 kVp scans (or 280

for the 90 kVp scans). For the contralateral bones, the cortex was segmented at 300. The parameters examined included total subperiosteal area (Tt.Ar), cortical bone area (Ct.Ar), medullary area (Ma.Ar), and cortical thickness (Ct.Th). In general, thresholds that appeared to appropriately segment the bone from marrow were chosen. In cases in which different energies were used, we scanned trial samples under both conditions and chose a threshold for each energy such that they gave similar results.

Mechanical Pull-Out Testing

Pull-out tests were performed on thawed, fully hydrated, unfixed specimens[52]. A small portion of the distal end of the femur was removed to expose the distalmost 2 to 3 mm of the implant. The proximal end of the femur was stabilized in dental cement (Lang Dental, Wheeling, Illinois) in a custom-designed fixture. Both ends of the setup were gripped and placed in a jig that centers the line of action of the materials testing system (model 8847; Instron, Canton, Massachusetts) and the long axis of the implant. The force required to pull the implant out of the bone was determined at a displacement rate of 0.25 mm/min. The force-displacement curves were recorded and the ultimate force was normalized to the outer surface area of the implant, taking into account the part of the implant that was exposed and held in the grip, to determine the strength of fixation. The linear part of the load-displacement curve was used to calculate the interface stiffness, and the area under the curve was used to calculate the energy to failure. We did not see evidence of grip slippage.

Histological Evaluation

Histological examination was performed with use of plastic-embedded specimens from which 1-mm slabs were cut with use of a diamond-tipped saw blade (IsoMet 5000; Buehler, Lake Bluff, Illinois). The slabs were attached to slides and ground to a nominal thickness of 50 to 100 μm and then polished (Phoenix 4000; Buehler). These specimens were briefly acid-etched and surface-stained with basic fuchsin/toluidine blue as described previously in detail[9,53-55]. The qualitative nature of the tissue found within the medullary canal and at the bone-implant interface was examined with use of conventional and polarized light microscopy (Nikon Eclipse 80i).