INTRODUCTION
Fracture healing is a complex biological process involving inflammation, granulation, callus formation, and bone modeling/remodeling. Optimal fracture healing results in complete restoration of bone structure and function to the pre-fracture levels. However, some fractures are associated with a high risk of delayed union, non-union and other complications. This leads to significant healthcare costs. Therefore, there is a significant unmet medical need for novel, systemic pharmacologic agents that can accelerate fracture repair and reduce the risk for fracture-associated complications.

Recently, it has been reported that beta-catenin signaling plays an important role in fracture healing. Lithium treatment increases beta-catenin signaling and has been shown to improve fracture healing, whereas treatment with Dickkopf 1, an antagonist of the Wnt/beta-catenin pathway, has shown to inhibit the fracture healing process (1). These results suggest that agents that activate the Wnt/beta-catenin signaling may have the potential to improve fracture healing.

Sclerostin, a protein secreted by osteocytes, is a negative regulator of osteoblast differentiation and function and acts as an inhibitor of bone formation (2). Humans with inherited sclerostin-deficiency (sclerosteosis, van Buchem disease) have increased bone mass and are resistant to fracture (3,4). Sclerostin knock-out mice have higher bone mass and bone strength due to increased bone formation (5). Although the mechanism by which sclerostin negatively regulates bone formation is an area of continuing investigation, one body of research supports the hypothesis that sclerostin binds to LRP5/6 and inhibits Wnt/beta-catenin signaling (6), thus impairing osteoblast differentiation and function. Therefore, inhibition of sclerostin may lead to increased Wnt/beta-catenin signaling, thus increasing bone formation and improving the fracture healing process. To test this hypothesis, sclerostin monoclonal antibodies (Scl-Ab) that neutralize sclerostin and reverse the inhibitory effects of sclerostin on Wnt/beta-catenin signaling were developed. It has been previously reported that treatment with a murine Scl-Ab significantly increases bone formation on trabecular, periosteal, endocortical and intracortical bone surfaces, leading to an increase in bone mass and bone strength in rat models of osteoporosis (7-10). Similarly, monoclonal antibody-mediated inhibition of sclerostin resulted in increased bone formation, bone mass and bone strength in intact monkeys (11), and increased biochemical markers of bone formation in healthy postmenopausal women (12).

The objectives of the current studies were to investigate the effects of systemically administrated Scl-Ab on bone healing in a mouse osteotomy model and in a rat closed femur fracture model. These models have been used previously to investigate the effects of therapeutic agents on fracture healing (13-16).

METHODS
The following animal studies have been reviewed and approved by the Institution Animal Care and Use Committee.

Mouse osteotomy study:
Eighty 9-week-old male CD1 mice underwent osteotomy on their right femurs as previously described (13-14). After the surgery, the mice were subcutaneously injected with vehicle or a murine sclerostin neutralizing monoclonal antibody (Scl-AbII) at 25 mg/kg, twice per week for 4 weeks. There were 9 to 12 mice per group. The treatment effects were monitored by X-ray imaging and mechanical testing. Weekly digital radiographs were performed with standardized positioning and were analyzed for callus density changes. A standard, three-point bending test was used to obtain bone strength data on the fracture sites of femurs at the end of the study.

Rat closed femur fracture study:
Nine week old male Sprague Dawley rats underwent standard closed diaphyseal femoral fracture (15-16). After surgery, the rats were subcutaneously injected with vehicle or ratized Scl-AbII at 25 mg/kg, twice per week. A group of vehicle treated rats and a group of Scl-AbII treated rats were necropsied at the end of week 2, while another group of vehicle treated rats and a group of Scl-AbII treated rats were necropsied at the end of week 4. In addition, there was one group of rats treated with Scl-AbII for the first two weeks, and then with vehicle for the next 2 weeks (on/off treatment group) before necropsy. There were 9 to 12 rats per group. Effects were monitored by weekly X-ray analysis. Fractured femurs were excised for mechanical testing.

RESULTS

Mouse osteotomy study:
Mice treated with Scl-AbII had significantly higher callus density in the fracture area compared with the vehicle controls. Mechanical testing of the fractured femurs revealed increases in strength parameters with Scl-AbII treatment. Compared with vehicle controls, maximum load and stiffness were increased by 117% and 195% (p<0.05) respectively in the Scl-AbII treated group. In addition, energy to failure was increased non-significantly by 66% in the Scl-AbII treatment group compared with vehicle controls. These results indicate that sclerostin inhibition by Scl-Ab improved fracture healing in the mouse osteotomy model.

Rat closed femur fracture study:
Analysis of X-ray images showed that the 4 week treatment group and the on/off treatment group had improved callus density than vehicle treatment controls by the end of study.

At week 2, maximum load and stiffness were increased by 34% and 39%, respectively, in the Scl-AbII treatment group compared with the vehicle group. At week 4, maximum load and stiffness of fractured femurs in the on/off treatment group were significantly increased by 105% and 110%, respectively, compared with the vehicle group. Similarly, maximum load and stiffness of fractured femurs were increased by 54% and 70% respectively in the 4-week treatment group compared with vehicle.

These results indicate that Scl-Ab treatment improved fracture healing in the closed femoral fracture rat model.

DISCUSSION
These studies are the first to show that inhibition of sclerostin via systemic treatment with Scl-Ab can enhance fracture healing in animal models. In both the mouse and rat fracture healing models, Scl-Ab increased callus density and bone strength at the fracture sites. The potential mechanism for these beneficial effects of Scl-Ab could be increased Wnt/beta-catenin signaling, since Scl-Ab neutralizes sclerostin and reverses the inhibitory effects of sclerostin on Wnt/beta-catenin signaling. These results are consistent with other reports that show Scl-Ab acts as a potent bone anabolic