Pharmaceutical and Genetic Depletion of Sclerostin and the Effect on Fracture Healing

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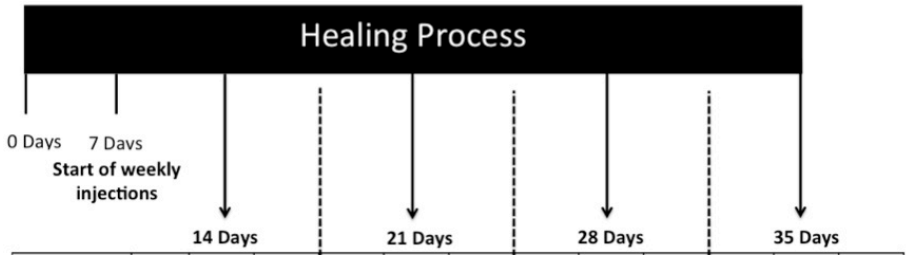
Purpose: Sclerostin is a secreted glycoprotein that interacts with LRP5 (low density lipoprotein receptor-related protein 5) receptor on osteoblasts and inhibits the intracellular Wnt signaling pathway, leading to decreased bone formation. When sclerostin is inactivated, bone formation is therefore stimulated. This stimulation has been proven in fracture studies, which showed that sclerostin-deficient mice have larger and stronger calluses with accelerated fracture healing, both in sclerostin knockout and sclerostin antibody injection models. These observations suggest that sclerostin inhibition and depletion show improved and accelerated fracture healing, but the effect of these two mechanisms have not been compared to asses the accurate effect of the Scl-Ab (sclerostin-neutralizing monoclonal antibody) injections. Therefore we designed a study to compare the effect of sclerostin depletion (sclerostin knockout) and inhibition (Scl-Ab injection).

Methods: 10-week-old male SOST (sclerostin) knockout (KO) (N = 20) and Wild-type (WT) (N = 40) mice underwent insertion of a tibial intramedullary pin after which a midshaft tibial osteotomy was performed. The mice were divided into three groups: SOST KO (N = 20), WT with Scl-Ab injection (N = 20) and WT with saline injection (N = 20). The Scl-Ab group received an intravenous dose of 100 mg/kg weekly starting on day 7. Each group was managed and sacrificed according to the specified protocol (Fig. 1). For data analysis, one-way ANOVA (analysis of variance) was performed followed by Tukey's post hoc test at each time point. P values <0.05 were considered statistically significant.

Results: Both Scl-Ab and KO groups showed significantly increased trabecular BV/TV (bone volume/total volume) at the fracture site (midshaft of the tibia) compared to the saline group at all time points and also showed no significant difference between them at all time points (except at 28 days postoperative) (Fig. 2). On biomechanical testing, the Scl-Ab and KO groups showed significant increased strength in stiffness at days 14, 28, and 35 compared to the saline group (Fig. 3A). Concerning ultimate force and work to failure the KO group showed significant increase in the force required compared to both the Scl-Ab and saline groups at 21, 28, and 35 days. While the Scl-Ab group showed increased force required to fracture the callus compared to the saline group at these time points, this was only significant for work to failure at 28 days (Figs. 3B and D).

Conclusion: Scl-Ab injections showed promising results, which were comparable to the complete depletion of sclerostin, especially at earlier stages of the healing process. In addition, our results indicate that sclerostin antibody exerts its greatest effect in the earlier stages of fracture healing (days 14 and 21), after which the healing process plateaus and thus completing this process at an earlier time point. Further research into accurate dosage and adequate timing of administration is required before these promising results can be implicated as a modality for accelerating fracture healing in humans and management of delayed union/nonunion.

See pages 47 - 108 for financial disclosure information.



Groups	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline	KO	Scl-Ab	Saline
µCT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5
BT	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5	N=5

Figure 1: Fracture model protocol.

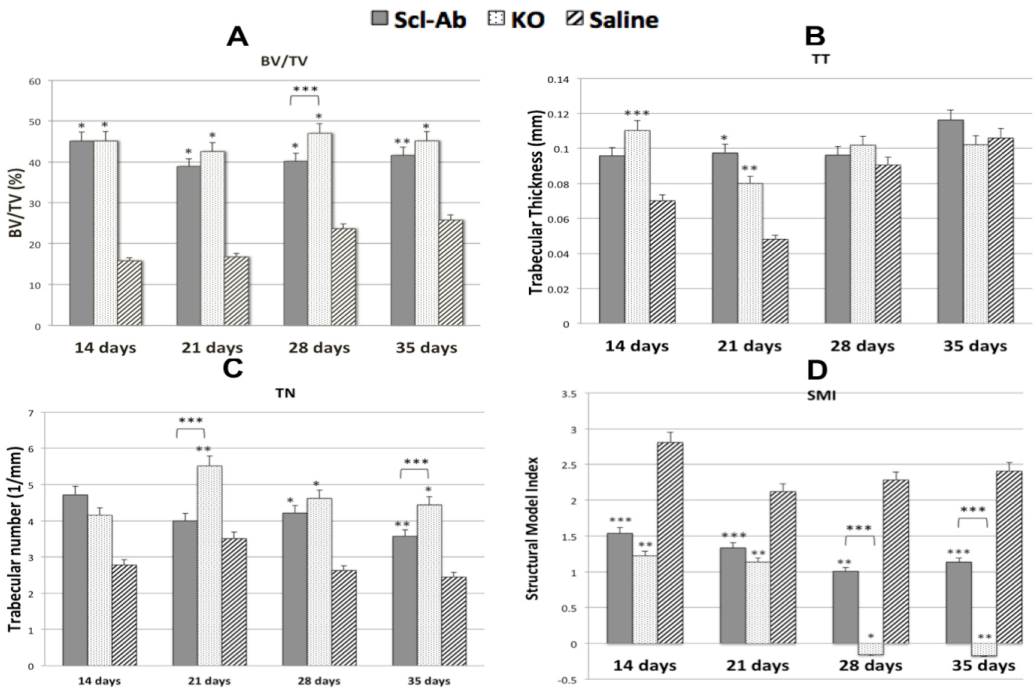


Figure 2: MicroCT results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: BV/TV; bone volume/total volume, Tb.Th; trabecular thickness, Tb.N; trabecular number, SMI; structural model index).

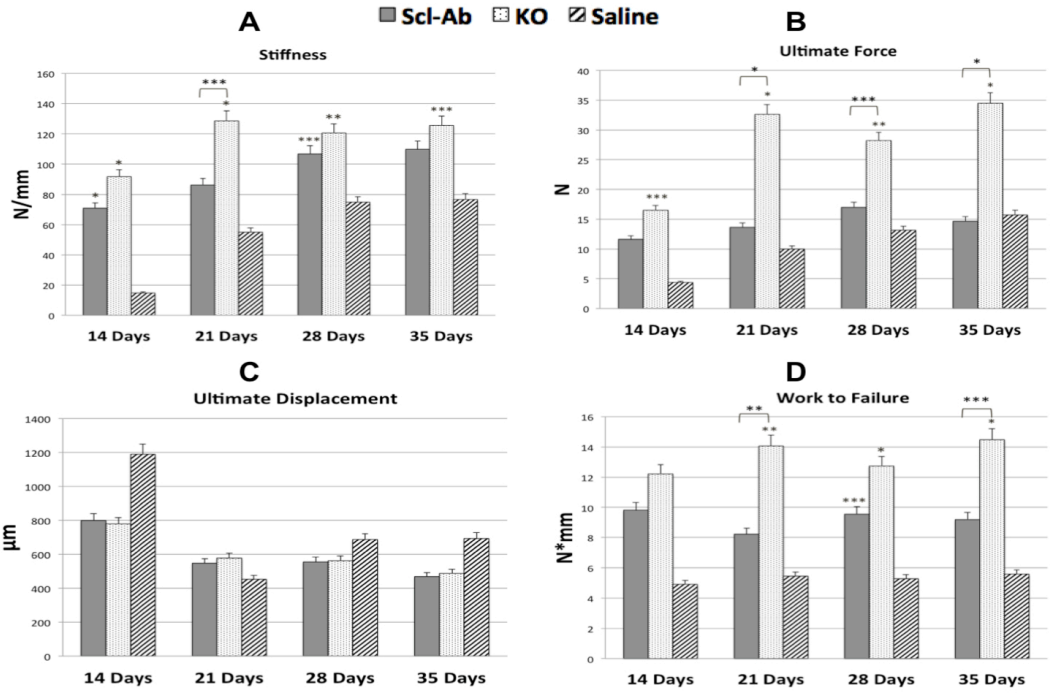


Figure 3: Biomechanical testing results across all time points. Data presented as mean and standard error of the mean (*P<0.001, **P<0.01, ***p<0.05).
Abrev: N; newton

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