

### **Δ The Effect of Iron Chelators on Bioceramic Bone-Graft Remodeling**

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**Purpose:** The clinical success of bone-graft substitutes for posttraumatic reconstructive procedures relies on the rapid vascularization of the construct while temporizing graft resorption to maintain structure and strength during bone ingrowth. Local delivery of the widely available iron chelator, deferoxamine (DFO) has recently been shown to augment both angiogenesis and osteogenesis in fracture models through activation of the hypoxia-inducible factor (HIF) signaling pathway. Hypoxic conditions are also known to induce osteoclast differentiation and resorptive function through HIF activation: however, mimicking this effect with iron chelators has interestingly shown contradicting in vitro results. We aimed to determine the effect of the local delivery of DFO on new bone growth in a rabbit ulnar defect bridged by anatomical 3-dimensional (3D)-printed monetite (CaHPO<sub>4</sub>) bone-graft substitutes. Secondly we aimed to accurately quantify the effect of iron chelator delivery on osteoclast mediated graft resorption using a monetite graft cranial onlay model.

**Methods:** Microporous 10-mm monetite grafts were 3D-printed to anatomically match a rabbit midshaft ulna. Cylindrical grafts (9-mm diameter, 4-mm thick) were prepared for the cranial model. In six rabbits, grafts were inserted into bilateral 10-mm middiaphyseal ulna defects. Starting on day 4 postoperatively, 600 μL of DFO (200 μM) was injected into one graft of each rabbit every 48 hours for 6 doses. The contralateral limb received saline injections. In 10 rabbits, two circular grafts were fixed subperiosteally onto the cranium. Four of these rabbits had DFO injected in a similar fashion into both grafts and four rabbits were given saline. To verify if the results could be replicated using another chelator, two rabbits were injected with 1,10-phenanthroline. At 8 weeks, micro computed tomography (CT) and histology were used to assess new bone growth and graft resorption. Additionally, histologic sections of the cranial grafts were TRAP (tartrate-resistant acid phosphatase)-stained to assess for osteoclast density at the bone-graft interface.

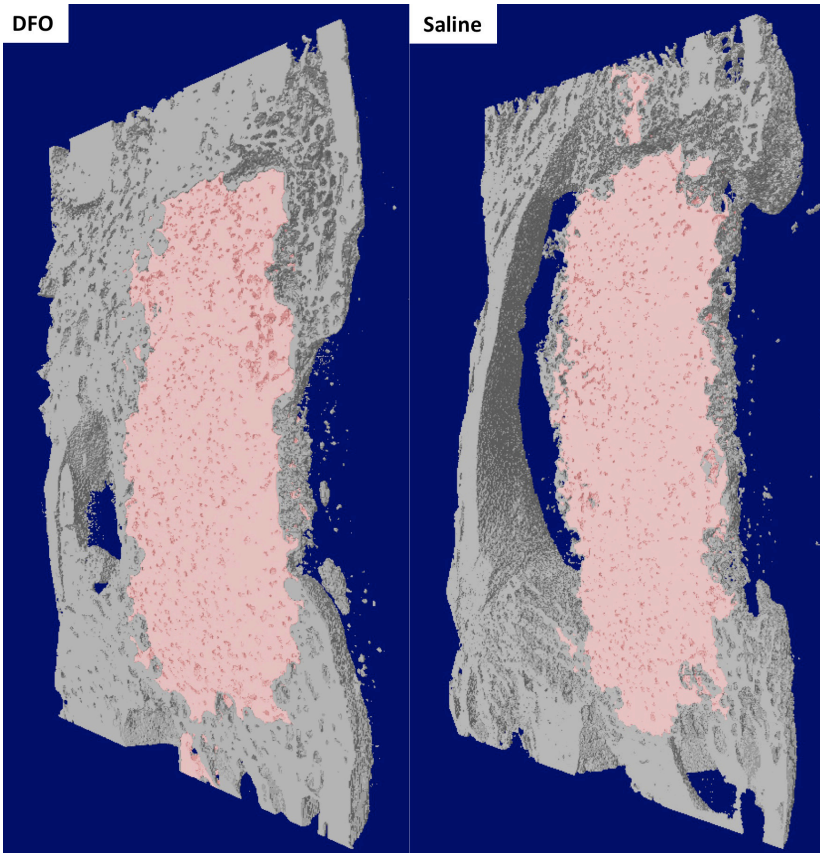
**Results:** Ulnar model: At 8 weeks postimplantation, micro CT analysis demonstrated a significant increase in new bone growth in the DFO group compared to the saline group (bone volume/tissue volume 19.50% vs 13.65% [P = 0.042], Fig. 1). Histologic analysis of coronal sections showed increased bone within the osteotomy gap, more bone integrated at the graft surface, as well a more matured soft-tissue callus in the DFO group. Cranial model: Micro CT and histologic analysis showed a markedly decreased resorptive front in the DFO and PHT group as compared to saline controls. TRAP stain quantification showed a 3-fold significant decrease in osteoclast density in the chelation groups compared to controls.

**Conclusion:** DFO significantly increased bone formation in a long bone defect bridged by a bioresorbable bone graft substitute. The cranial onlay model exposes the grafts to a more static environment whereby cell mediated resorption can be tracked from a single front. Local delivery of chelators reduced graft resorption and osteoclast numbers at the bone graft interface in this model. This study proposes a second mechanism by which iron chelators

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The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device he or she wishes to use in clinical practice.

may function as bone anabolic agents; in addition to HIF activators, they may also reduce osteoclast mediated resorption by additional mechanisms.



**Figure 1:** Representative 3D coronal cuts of the osteotomy site. Grey represents new bone and pink represents the remaining graft material.