INTRODUCTION

Avascularity and hypoxia play a role in skeletal repair, particularly in challenging healing situations such as impaired fracture healing or avascular necrosis (AVN) of the femoral head. The Hypoxia Inducible Factor (HIF) and the Vascular Endothelial Growth Factor (VEGF) pathways are activated in human fracture healing and AVN models. Small molecules have been identified that activate the HIF pathway and have been shown to increase VEGF and vascular response in bone healing environments with local application. One of these, desferoxamine (DFO), is the focus of the present study. DFO exerts its effects by chelating iron, a co-factor for the prolyl hydroxylase enzyme that degrades HIF. In the absence of the cofactor, the enzyme does not degrade HIF and it accumulates, translocates to the nucleus, and activates gene programs such as VEGF.

DFO was shown to be effective in bone healing and distraction osteogenesis when applied by repeated local injection. This delivery method would be impractical in a clinical setting, as repeated injections are poorly tolerated and liquid application may result in delivery to unintended sites. Therefore, a method for sustained, controlled local delivery is desirable and is the object of this study.

A mouse metatarsal model was used to examine angiogenesis in response to DFO administered through controlled-release carrying agents. The controlled-release systems that were explored are all materials which are readily available to orthopaedic surgeons in the operating room and include CaSO₄ pellets, collagen sponges, and demineralized cortical bone matrix. The ultimate goal of this study was to find a carrier agent suitable for delivery of desferoxamine in order to more rapidly re-vascularize necrotic bone in AVN, as well as in stimulation of fracture healing, all while hopefully minimizing heterotopic effects and systemic toxicity.

METHODS

Embryos were harvested from timed pregnant C57BL/6 mice on day 17.5 and metatarsals were dissected. Each isolated metatarsal was immediately placed into its own well of a tissue culture plate with alpha-minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. Explants were then incubated at 37°C in 5% CO₂ for a period of 10 days with replacement of media every 3 days. After incubation, metatarsals were exposed to either calcium sulfate pellets containing DFO (CaDFO), collagen sponges soaked in DFO (ColDFO), demineralized bone matrix soaked in DFO (DBMDFO), VEGF (VEGF) as a positive control, DFO (DFO) in media as a second positive control, or media alone as a negative control for 24 hours.

We felt it important to be sure that desferoxamine did not immediately diffuse away from the carrier when exposed to fluid during surgical procedures. Therefore, the experiment was repeated with the calcium sulfate pellets and collagen sponges, though not with the demineralized bone matrix, but this time the sponges and pellets were placed in separate Petri dishes with media for 30 minutes before being introduced to the metatarsals (CaMed and ColMed).

After the 24 hour exposure to the experimental delivery agents and controls, the metatarsals were fixed in zine formalin for 15 minutes at room temperature and subsequently stained for the endothelial marker Cluster of Differentiation 31 (CD31).

Bright field images were obtained at 2x magnification. Other investigators have used pixel counting methods to objectively assess the vascularity after treatment but we were unable to do so because debris in the microscopic field (from our carrier agents) caused spurious pixel counting results. Therefore, three novel methods were used to evaluate vascularity resulting from treatment.

First, a numerical scale ranging from 1- avascular to 5- extremely hypervascular was developed to grade vascularity of the metatarsals. Images were graded by 3 blinded observers to compare “vascularity grading” by group. Second, the lengths of the 5 longest vessels sprouting from each metatarsal were measured by a blinded observer to determine the “mean longest vessel length” by group. Third, an ellipse was laid over each metatarsal with a length 110% the length of the metatarsal and a width 55% the length of the metatarsal. A blinded observer then counted the number of vessels which crossed over the edge of the ellipse to objectively evaluate the “mean number of vessels” by group.

RESULTS

**Vascularity Grade**

![Vascularity Grade Diagram](image)

**Mean Length of Vessels**

![Mean Length of Vessels Diagram](image)

**Mean Number of Vessels**

![Mean Number of Vessels Diagram](image)

DISCUSSION

This study confirms that DFO significantly increases capillary sprouting from bone, supporting the concept that it might be a useful agent in the treatment of orthopaedic disease. It also confirms that it can be released from several orthopaedic materials that might be used as carriers for implantation of DFO into desired locations. Although both collagen sponges and demineralized bone matrix loaded with DFO increased angiogenesis when compared to control, calcium sulfate pellets appeared to be the most effective controlled release system that we tested. Not only did the calcium sulfate pellets significantly increase overall vascularity, increase the mean length of the longest vessels, and increase the mean number of vessels compared to the negative control, but they were shown to still be efficacious in inducing angiogenesis after being submerged in fluid for 30 minutes prior to being introduced to the fetal metatarsals. Additional avenues of exploration include evaluations of the in vivo effectiveness of this calcium sulfate pellet controlled release system for DFO.

REFERENCES