Local Delivery of Deferoxamine Augments Large Osteochondral Allograft Osseointegration in a Preclinical Canine Model

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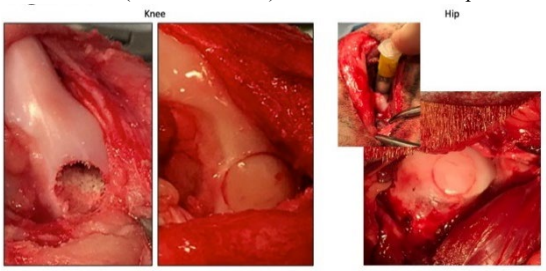
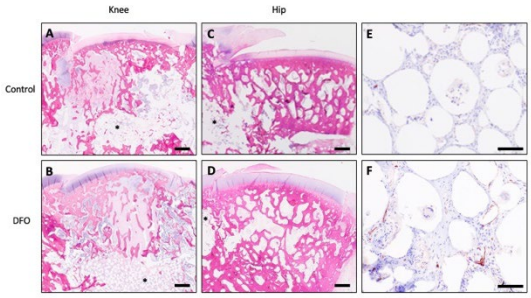
INTRODUCTION: Osteochondral allograft (OCA) transplantation can be a successful treatment option for large articular defects in the knee, hip, ankle, and shoulder, but is still associated with significant revision and failure rates. Patient outcomes and functional graft survival after OCA transplantation are dependent on allograft bone osseointegration via creeping substitution, which is a slow and prolonged process. Insufficient OCA osseointegration remains a major cause of failure. Deferoxamine (DFO) has been reported to be an effective angiogenic and osteo-anabolic iron chelator that consistently promotes bone neovascularization and regeneration. This study was designed to investigate local delivery of DFO for augmenting OCA bone osseointegration using a preclinical canine model for OCA transplantation in the knee and hip. We hypothesized that local delivery of DFO using a controlled microsphere release system may reduce bone resorption and improve revascularization and cellular repopulation to increase new bone ingrowth, potentially expediting OCA osseointegration after transplantation.

METHODS: DFO-encapsulated and DFO-free polycaprolactone microspheres (100-150um) with 10% w/w polyethylene oxide were produced using a simple emulsion method as previously described, and verified for *in vitro* release kinetics. DFO-encapsulated microspheres achieved a controlled release of 0.15mg of DFO over 48 hours for two weeks while DFO-free microspheres were verified to release no measurable DFO.
With IACUC approval (#9957,9961), the femurs of skeletally mature purpose-bred mongrel dogs (n=8) were aseptically recovered after humane euthanasia was performed for reasons unrelated to this study. The distal femurs (n=16) and femoral heads (n=16) were removed by aseptic osteotomy and stored in individual sterile containers for 28 days at room temperature using the Missouri Osteochondral Preservation System (MOPS®) method. Skeletally mature purpose-bred mongrel dogs (n=12; mean weight = 21.58 ± 1.65 kg) were premedicated, anesthetized, and prepared for aseptic surgery of one randomly assigned hindlimb for one of four OCA transplant groups based on joint treated (knee or hip) and use of DFO-encapsulated (DFO) or DFO-free (Control) microspheres: DFO Knee (n=4 defects), Control Knee (n=4 defects), DFO Hip (n=4 defects), Control Hip (n=4 defects). Knee OCAs (n=8) were transplanted in 4 dogs after creating osteochondral defects (recipient sockets) (8mmØ x 7mm depth) on each femoral condyle and implanting cylindrical OCAs (8mmØ x 6mm depth) created from MOPS-preserved donor distal femurs and using press-fit fixation. Hip OCAs (n=8) were transplanted in 8 dogs using recipient sockets (10mmØ x 7mm depth) on the craniodorsal (anteriosuperior) weightbearing surface of the femoral head and implanting cylindrical OCAs (10mmØ x 6mm) created from MOPS-preserved donor femoral heads and using press-fit fixation. Prior to OCA transplantation, microspheres (100mg) were placed into each socket with each dog randomly assigned to DFO or control. (**Figure 1**)
Eight weeks following transplantation, knees and hips were assessed for OCA revascularization, cellular repopulation, and integration on validated functional, radiographic, micro-computed tomography (uCT), histologic, and immunohistochemical outcome measures. For comparisons between treatment cohorts and pre- and post-transplant time points, continuous data were compared for statistically significant differences using t-Tests and categorical data were compared for statistically significant differences using rank sum tests. Statistical significance was set at $P < .05$.





RESULTS: Based on the functional, diagnostic imaging, and uCT assessments performed, local delivery of DFO into OCA transplant recipient sites was associated with maintained or improved joint function, superior radiographic appearance, and significantly greater trabecular thickness, higher bone volume, and new bone ingrowth when compared to DFO-free controls. Histologic and immunohistochemical assessments supported these findings and provided evidence for potential mechanistic effects related to osteoclast-mediated resorption and neovascularization (**Figure 2**), however, lack of statistically significant differences for these outcome measures prevented conclusions regarding the direct effects of DFO in this model.

DISCUSSION: Using this preclinical canine model, local delivery of DFO via elution from polycaprolactone microspheres into OCA transplant recipient sites was associated with maintained or improved joint function, superior radiographic appearance, and significantly greater trabecular thickness, higher bone volume, and new bone ingrowth when compared to DFO-free controls. Importantly, OCA chondrocyte viability was not adversely affected in OCAs exposed to local delivery of DFO and no negative effects on recipient bone or other articular tissues were noted. These results associated with local delivery of DFO for augmenting OCA osseointegration are similar to those previously reported for DFO augmentation of synthetic bone grafts, fracture, and distraction osteogenesis applications, and are encouraging regarding the capabilities for DFO microspheres to promote more rapid and complete allograft bone osseointegration through iron chelator-mediated angiogenesis and osteogenesis.

SIGNIFICANCE/CLINICAL RELEVANCE: Taken together, the results of the present study suggest that local delivery of DFO using a controlled microsphere release system may reduce bone resorption and improve revascularization and cellular repopulation to increase new bone ingrowth, potentially expediting OCA osseointegration after transplantation. As graft survival and function after OCA transplantation are highly dependent on rate and extent of allograft bone incorporation, further development and validation of this clinically relevant method for potentially improving OCA transplantation outcomes are warranted.