

Study Goals

- Type H-vessels consist of specific endothelial cells that engage in direct interaction with perivascular osteoprogenitor cells, osteoblasts and preosteoclasts required for bone formation and remodeling. This angiogenic-osteogenic coupling leads to synchronous propagation of vasculature and bone regeneration.
- H-vessel response to injury is adequate to facilitate healing in normal healthy tissue but remains inadequate to overcome radiotherapy's devastating effects on bone healing.
- In the setting of radiotherapy (XRT), there is a significant loss of H vessel formation leading to a severe diminution of angiogenic-osteogenic coupling necessary for repair and regeneration of bone.
- The purpose of this study was to evaluate the therapeutic potential of deferoxamine (DFO) to bolster H-vessel formation and restore angiogenic-osteogenic coupling thereby recapitulating the normal pathway of bone healing in irradiated tissues and subsequently act as a marker for adequate bone healing.

Methods

- **Experimental Design:** Male Lewis rats (n=36) were divided into three groups: Distraction Osteogenesis (DO), XRT with DO (xDO), and XRT with DO and Deferoxamine (xDO-DFO).
- **Surgery:** All groups underwent mandibular osteotomy and external fixation. The treatment group received 200 µM DFO injections into the DO gap every other day during the active DO period which began on the evening of post-operative day (POD) 4 and went through the evening of POD 12. Animals were sacrificed on POD 40.
- **XRT:** Experimental groups received a human equivalent dose of 35Gy fractionated at 7Gy for 5 days.
- **Metrics:** Immunofluorescent analysis was utilized to determine the abundance of H-vessels in each specimen. Bone sections were stained with primary antibodies overnight at 4 °C. Nuclei were stained with Hoechst. Images were acquired with a Leica fluorescence microscope. H-vessel quantity was calculated as a percent of total specimen area (Regenerate) for each sample section.

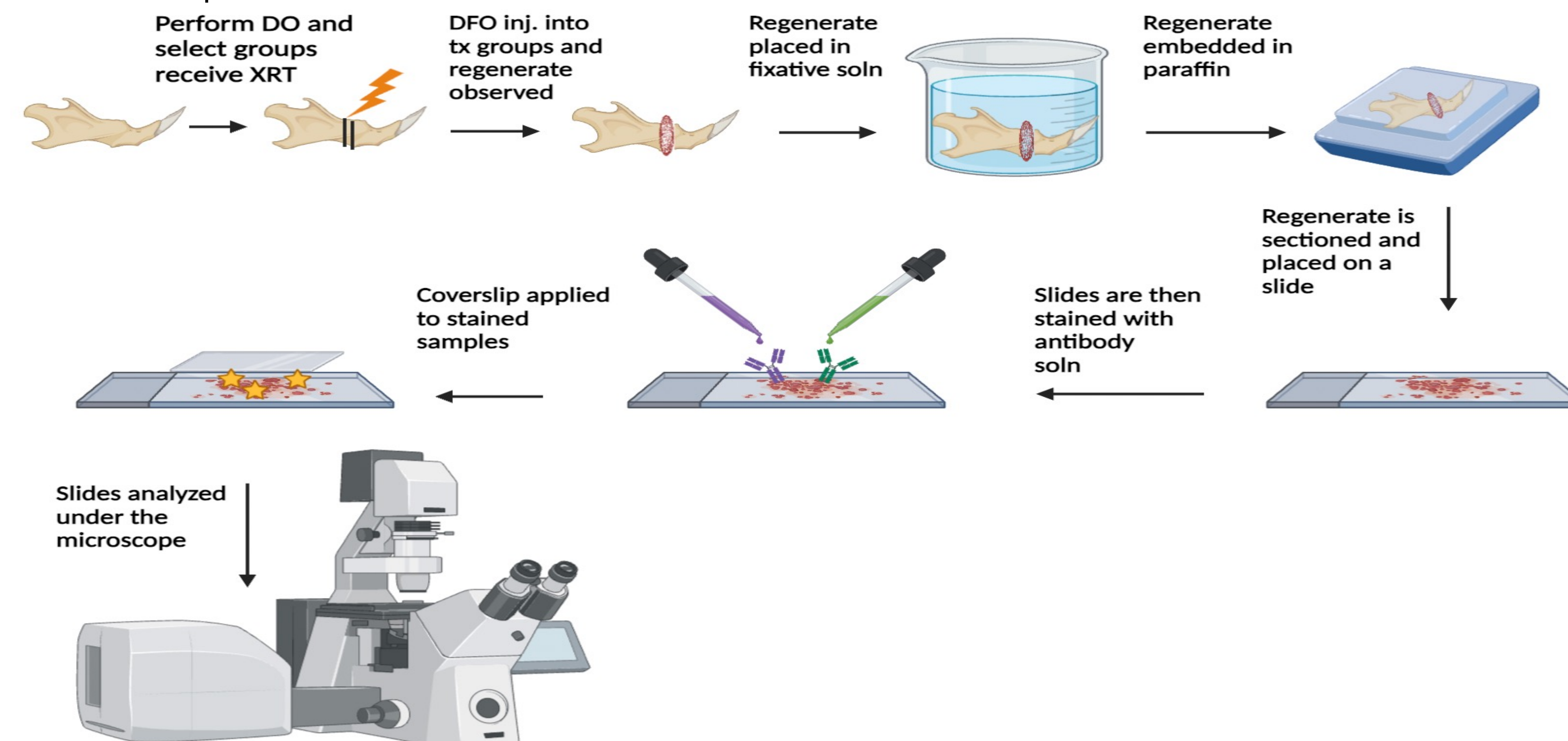


FIG. 1 Protocol of tissue harvesting, fixation, and immunofluorescent staining

Results

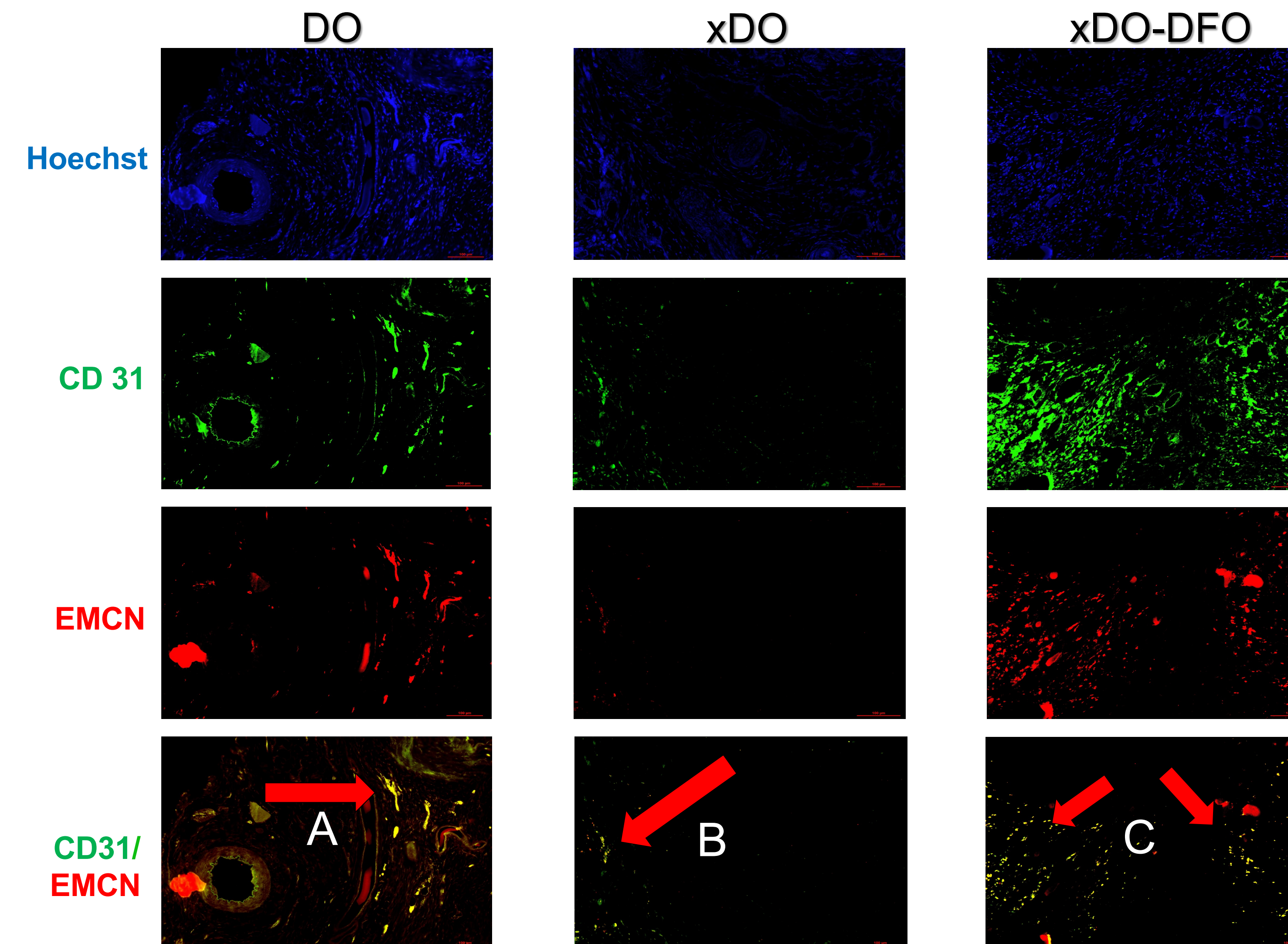


FIG. 2 Visualization of H vessels. In the first column, Hoechst represents nuclear staining, CD 31 and EMCN are markers of vascular endothelial cells and CD31/EMCN is the merged image of the markers to visualize H vessels. H vessels were moderately expressed in the DO column as demonstrated by red arrow A. In the XDO column, H vessels were decreased significantly as depicted by red arrow B. In the last column, DFO rescued and increased the amount of H vessel expression after radiation as seen by red arrows C.

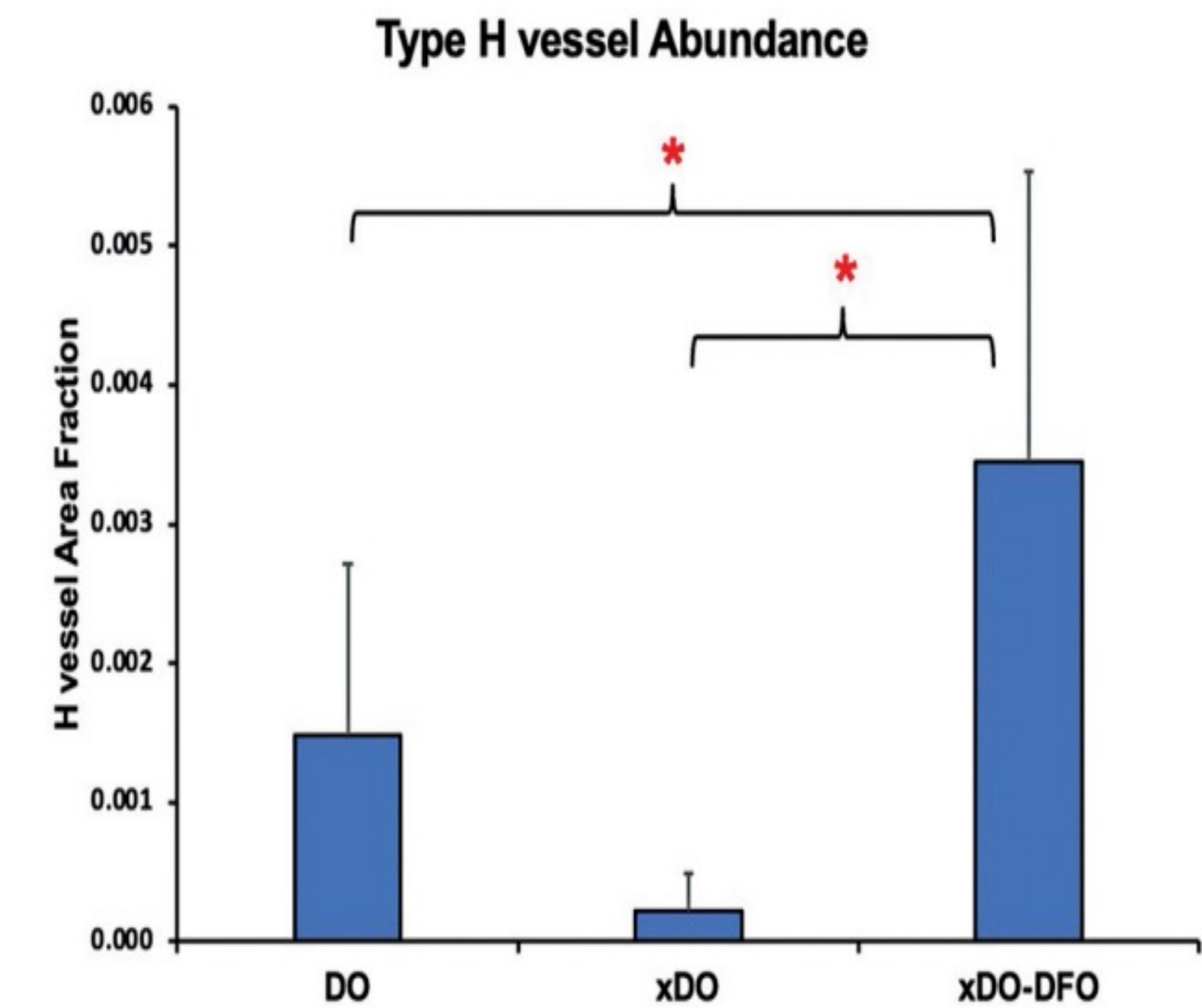


FIG. 3 H vessel abundance within the distraction regenerate was compared. Irradiation decreased H vessel formation by 6.8x compared to DO controls. DFO treatment significantly enhanced and sustained H vessel proliferation by 15.9x compared to xDO levels (p=0.001). We observed a supraphysiologic response with DFO administration to the distraction gap as xDODFO groups demonstrated an impressive 2.3x increase in H vessel quality compared to DO controls (p=0.045).

Conclusions

Use of injectable DFO for bone tissue engineering demonstrates great potential for clinical utilization, including applications in irradiated fracture healing and distraction osteogenesis. In this study, we developed a novel method to visualize and quantify the proliferation of H-vessels in irradiated distraction osteogenesis, demonstrating their crucial role in bone repair and regeneration. Our results indicate that H-vessels are upregulated in irradiated bone treated with DFO and facilitate healing in distraction osteogenesis in the setting of XRT. We believe that DFO restores the angiogenic-osteogenic coupling in the irradiated distraction gap, thereby remediating bone regeneration and potentially highlighting the translational capabilities of DFO.