Assessing tissue oxygenation at the fracture site by EPR oximetry

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Oxygen plays an important role during fracture repair. Chronic hypoxia leads to delayed fracture healing in dogs1, and hyperbaric oxygen can accelerate bone repair2. Accumulating evidence also suggests that oxygen levels may affect the function and differentiation of chondrocytes and osteoblasts3,4,5. We hypothesize that tissue oxygenation at the fracture site directs cell differentiation during fracture healing. As a first step to test this hypothesis, we evaluated the feasibility of using EPR (electron paramagnetic resonance) oximetry to assess tissue oxygenation at the fracture site. EPR oximetry is an accurate, quantitative, and minimally-invasive technique to assess in vivo pO26,7. It employs particulate probes of paramagnetic materials, such as Lithium Phthalocyanine (LiPc) and wood chars, which have characteristic changes in their EPR spectra that are proportional to pO2. Subsequent non-invasive measurements can be made for periods of more than one year, depending on the tissue.

Materials and methods

Animals and creation of tibia fractures: With IACUC (Institutional Animal Care and Use Committee) approval, we measured bone/fraction pO2 in male c57B6 mice (8 weeks old). Animals were anesthetized with isofluorane in 100% oxygen. A transverse closed fracture (n=7) was created by three-point bending at the mid-shaft of right tibia. To create tibia fractures with femoral artery ligation (n=4), the right femoral artery branches were ligated and removed immediately prior to fracture.

Implantation of LiPc and EPR oximetry: Throughout the experiment, animals were maintained under general anesthesia (1.8% inspired isofluorane) and kept euthermic (rectal temperature, 37.5°C). LiPc crystals (<50µl) were implanted into the fracture site in the fractured legs or adjacent to periosteum in the contralateral legs using a 25 gauge needle. We first determined the times needed to obtain a state of tissue oxygen equilibrium following fracture and inspired gas change by sequentially measuring pO2 after LiPc implantation every 5 – 15 minutes. We found that oxygen equilibrium was usually achieved in 60-90 minutes after fracture on either 21% or 100% oxygen and it took about 20 minutes for the pO2 to equilibrate after the inspired oxygen change (data not shown). Mice with non-ischemic fractures were then randomly assigned to breathe either 21% or 100% oxygen for 90 minutes. We then measured the pO2 level at the ischemic fracture sites at 90 minutes after the induction of femoral artery resection in animals breathing 100% oxygen (Fig. B). Femoral artery resection significantly reduced oxygen at the fracture sites (p<0.01). The ischemic fractures had virtually no detectable oxygen during the early period after surgery even on 100% inspired oxygen.

Conclusion

In this study we found that fracture significantly reduces tissue oxygenation and femoral artery resection further decreases the pO2 to near 0 at the fracture sites. These data demonstrate that EPR oximetry is a feasible technique to assess tissue oxygenation after fracture.

Reference:

Fig. A. P2O2 at the fracture sites of non-ischemic fractures with intact femoral artery or periosteum of the contralateral limbs. The animals are breathing either 21% (white) or 100% (black) oxygen. Data are shown as mean ± SD. ∩ p<0.01.

Fig. B. P2O2 at the fracture sites of ischemic (with femoral artery resection) and non-ischemic (with intact femoral artery) fractures. The animals are breathing 100% oxygen. Data are shown as mean ± SD. □ p<0.01.