The temporally specific application of HBOT during digit regeneration

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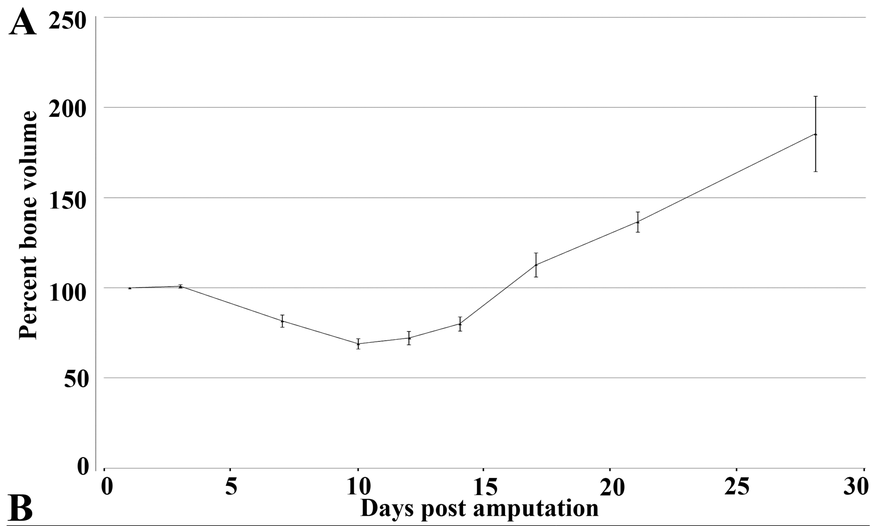
Introduction

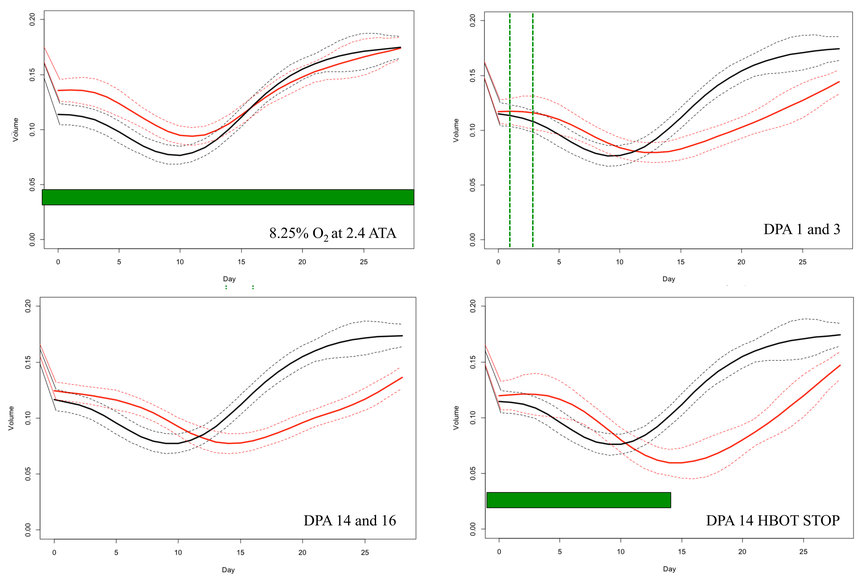
Oxygen has long been known to be a key player in both bone repair and bone development, and we have recently shown that oxygen is critical for optimal bone regeneration [(1)]. While axolotls and salamanders have retained the ability to regenerate whole limbs, mammalian regeneration is restricted to the distal two thirds of the digit tip [(2-6)] in mice [(5, 7)], primates , and humans [(8-11)]. This multi-tissue regenerative model provides a predictable phase progression of regeneration. After amputation of the distal tip of the third phalangeal element there is an initial bone degradation phase, followed by wound closure and blastema formation, and finally redifferentiation of the blastema into bone [(1, 4, 5, 12)]. This regenerative model provides an excellent opportunity to more closely study the influence of oxygen during bone regeneration.

Our previous study revealed the oxygen microenvironment during regeneration is a dynamic event and temporally influential in building and degrading bone [(1)]. These tissue oxygen levels in turn have wide-reaching cellular and molecular effects. This makes the timing, application, and frequency of hyperbaric oxygen three independent influences *in vivo*. Here we show that the application of HBOT during the amputation phase and blastema phase produces a degradation effect on the bone, while the digit appears to insulated from the effects of HBOT during the degradation phase. Our data provide a strong argument for a closer investigation of the timed relationship between HBOT and regenerative events *in vivo*. Here we detail the influence of HBOT on P3 regeneration *in vivo* and investigate the histological and cellular changes effected by HBOT application.

The P3 regenerative response

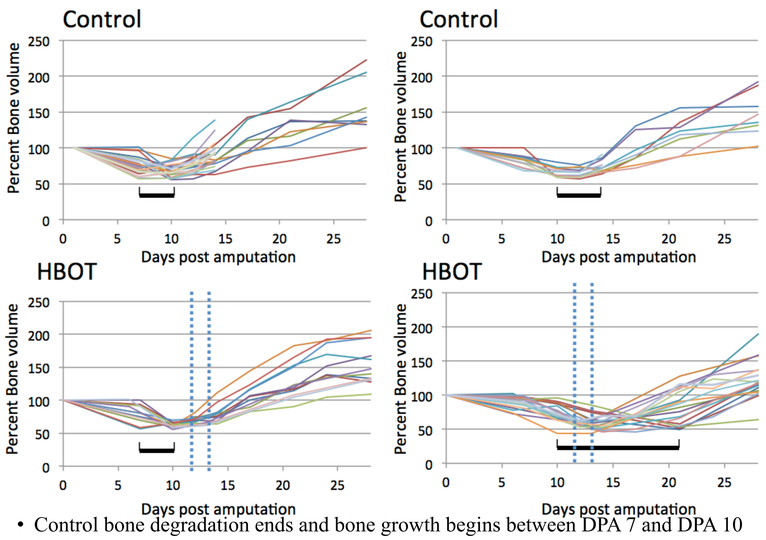


Temporally specific HBOT pulses differentially affects digit regeneration



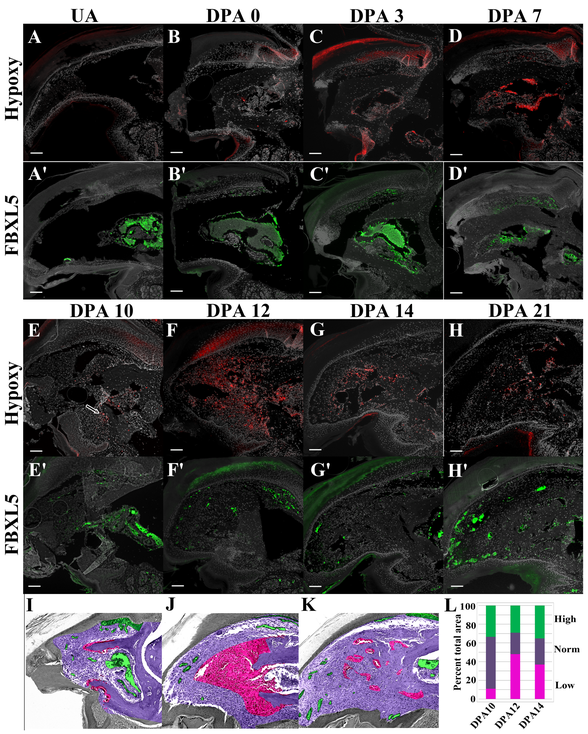
- Regeneration algorithm is a Smoothing Spline ANOVA generated by Dr. Michelle Lacey
- Regeneration algorithm for control digits shown in black and test group in red
- Enhanced and extended bone degradation is due to oxygen concentration and not pressure (n = 16)
- A single HBOT applied once at DPA 1 and 3 enhances and prolongs the degradation phase
- Pulsed HBOT applied once at DPA 14 and 16, after the digit is has begun to grow bone, reduces the rate of bone formation and causes bone volume to plateau slightly
- Continuous HBOT applied twice a day from DPA 0 to DPA 14 has largely the same effect as HBOT applied once at DPA 14 and 16

DPA 11 and 13 HBOT disrupts optimal regeneration[1]



- Control bone degradation ends and bone growth begins between DPA 7 and DPA 10 (n=21 digits).
- Control bone degradation ends and bone regeneration begins between DPA 10 and 14 (n=11 digits). Black bars show inclusive area of bone volume turn-around.
- HBOT at DPA 11 and DPA 13 applied after bone regeneration has already begun (n=13) show little response to hyperbaric treatment and graphs appear similar to control digits.
- HBOT at DPA 11 and DPA 13 prior to the bone growth phase (n=19) show a prolonged period of degradation and a wider range of bone degradation to bone growth turn-around points (black bar).

Regeneration demonstrates a dynamic oxygen environment[1]



- (A-H) Hypoxyprobe-1 (<1.3% oxygen) indicates hypoxic micro-environments (<1.3% oxygen) at DPA 7 (marrow), 12 (blastema), and 14 (trabecular spaces). (E) Arrow indicates hypoxic signal at the distal edge of the bone stump.
- (A'-H') Oxygen-stabilized protein FBXL5 (>5%-6% oxygen) shows signal in the marrow in the unamputated and DPA 0-10.
- (I-K) Hypoxyprobe (pink) and FBXL5 (green) as compared to normoxic (purple) areas at DPA 10, 12, and 14.
- (L) Hypoxyprobe (pink) and FBXL5 (green) stained cells versus DAPI staining. Scale bar represents 100 µm. N=4 with representative sample shown.

Conclusions and Future Research

- HBOT does not inhibit digit regeneration
- The effect of HBOT on digit regeneration is due to oxygen and not pressure
- HBOT cues enhanced and extended degradation when applied during the blastema phase and during the initial wound phase
- HBOT degradation cues are attenuated when pulsed HBOT is applied after bone formation has begun
- Temporally specific single applications of HBOT elicits a parallel bone degradation response when compared to continuous HBOT
- Future studies will concentrate on the applied timing of HBOT during other bone wounding models, such as fracture

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