Exposure to Near Infrared Light Alters the Temporal Expression of Bone Morphogenetic Proteins: Implications for the Enhancement of Fracture Healing

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Introduction: Fractures are a major cause of morbidity and mortality in the elderly population and are common diagnoses leading to long term disability and financial burden. For example, hip fractures can result in a 12-20% reduction in survival with 5-20% excess mortality in first year after injury. Therefore, enhancing the rate of fracture healing would produce a concomitant decrease in morbidity, mortality, and the costs associated with long term care.

Recently, investigators have shown that treatment of fractures with near infrared light (NIR) increases bone callus formation and bone mineral density in the early stages of fracture healing. Our lab recently showed that NIR exposure decreases osteoblast apoptosis and promotes cell proliferation.

Bone morphogenetic proteins (BMPs), part of the TGF-beta superfamily that regulates growth, differentiation and apoptosis of various cells, are an important aspect in both initial bone formation *and* fracture healing. Of the many BMP isoforms, BMP-2, BMP-4 (92% amino acid homology) and BMP-7 are most commonly associated with bone formation and fracture healing. It has been suggested that there is a temporal variation between release of these different BMPs**.** BMP-2 and BMP-4 have been seen during the early stages of bone formation, while BMP-7 was seen within osteoblasts surrounding the newly formed osteoids of *in vivo* fracture healing (later stage). Other investigators have shown that BMP receptors (BMPRs) types I & type II are also up-regulated during the fracture healing process. BMPs are released by multiple cell types other than osteoblasts, including endothelial cells that are found within the microvasculature of the bone.

Therefore, we hypothesized that one of the mechanisms by which exposure to NIR light enhances fracture healing is through an alteration in the rate and extent of secretion of BMP's and their corresponding receptors.

Materials & Methods: Murine MC3T-E1 osteoblasts and human umbilical vein endothelial cells (huvec) were grown in tissue culture. The experimental group was exposed to four consecutive days of one minute of NIR (NIR Light Technologies, LLC, Brookfield, WI) per day (8 Joules/cm^2). Light sources consisted of arrays of monochromatic light emitting diodes at a wavelength previously shown to inhibit apoptosis and enhance cell proliferation (830nm). Controls consisted of cells grown in the absence of NIR light exposure. Cells and conditioned media were harvested at 24, 48 and 72 hours following the final treatment of NIR, frozen in -20°C for analysis. The content of the BMP's in the conditioned media were determined by commercially available ELISA, while BMPRs were analyzed by Western blot. Each experimental condition was assessed with at least triplicate determination, and statistical analysis was performed using the analysis of variance with the Bonferroni-Dunn post hoc modification and a p value of ≤ 0.05 as significant.

Results: Exposure to near infrared light increased BMP-4 accumulation in the conditioned media in both osteoblasts and endothelial cells at 72 hours following the last NIR treatment. (Figure 1). Following exposure to NIR light, osteoblasts secreted significantly more BMP-4 than endothelial cells ($p < 0.05$). In contrast endothelial cells secreted significantly more BMP7 than osteoblasts ($p < 0.05$) (Figure 2).

Western blots revealed that exposure to NIR light increased BMPR-II compared to unexposed controls ($p<0.05$). In contrast, NIR light did not alter the immunoreactivity for BMPR-IA.

Figure 1

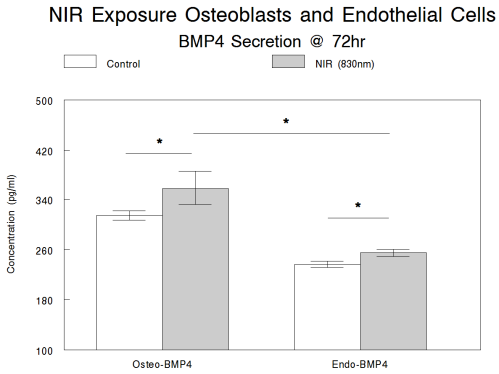
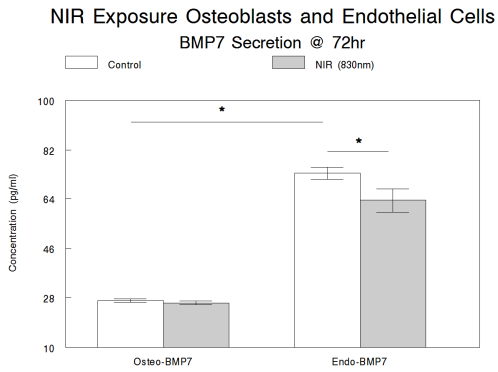


Figure 2



Discussion: These results demonstrate that exposure to near infrared light *increases* BMP-4 production in both osteoblasts and endothelial cells, but does not alter BMP-7 production. This suggests that NIR light may enhance or prolong the early stage of bone formation, resulting in increased release of BMP-4. BMP-2/4 has been shown to be the signal for recruitment of osteoblast/osteocyte progenitor cells, as well as for monocytes. This corresponds to our previous findings that NIR light also increased osteoblast proliferation. In contrast, BMP-7 has been shown to further differentiate progenitor cells into osteocytes. During the early phase of fracture healing, recruitment and proliferation, versus differentiation of osteoblast precursors, would likely be beneficial, leading to increased numbers of cells such as osteoblasts that are capable of bone formation and fracture healing. (i.e. corresponding to low levels of BMP-7 secretion).

This concept is also supported by our results of the up-regulation of BMPR-II, which has been associated with the early stage of fracture healing. These results suggest increased fracture healing by first prolonging or enhancing the early stage of fracture repair. Therefore prolongation or enhancement of the early stage would also enhance and strengthen the end stage of fracture healing because of the increased number of progenitor cells, as well as other factors involved in the early stage of repair.

These findings suggest that exposure to near infrared light may be an important adjunct to stimulate fracture healing, and that one possible mechanism is through alterations in the temporal expression and quantities of proteins that regulate osteoblast maturation and bone formation.

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