Angiogenesis in Hyaluronan Treated Bone Repair

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Statement of Purpose: A common objective when using biomedical implants in musculoskeletal tissues is rapid apposition of host tissue to the implant surface. Restoration of the vasculature is critical because it provides nutrients to newly forming tissues and routes for migration of osteoblast progenitor cells. Hyaluronic acid (NaHY) has been used as a carrier for demineralized bone matrix (DBM) to improve its handling characteristics for surgical procedures. While DBM promotes bone formation, it has been observed that NaHY by itself accelerates bone healing. The mechanism by which NaHY promotes bone healing is unknown, however it has been postulated that NaHY promotes neovascularization. The aim of the present study is to compare the angiogenesis that occurs during normal healing with different combinations of NaHY alone and NaHY+DBM using an immunocompromised rat tibial marrow ablation model.

Methods: Angiogenesis was assessed using a rat tibial marrow ablation model. An initial time course study was performed to determine when neovascularization within the marrow cavity was at its peak. At 3, 6, 14, 21, and 28 days post-ablation animals were euthanized and the vasculature was perfused using a radio-opaque contrast agent (Microfil, Carver, MA). To examine the effect of NaHY on vasculogenesis, marrow was ablated and test materials were injected into the empty marrow space as follows: (I) low MW NaHY (35kDa)+DBM, (II) high MW NaHY (700-800kDa)+DBM, (III) I:II 50:50, (IV) low MW NaHY, (V) high MW NaHY, and (VI) IV:V 50:50. (VII) DBM in PBS, (VIII) high MW NaHY + heat inactive DBM, and (IX) an empty defect were used as controls.

μCT images were taken of ablated tibias. Samples were subsequently decalcified, imaged a second time, and analyzed for blood vessel volume fraction, connectivity, number, thickness, and spacing. Samples were then processed for histology using H&E staining.

Results: Peak vessel volume within the marrow cavity was observed on day 14 post-ablation (Fig 1). Neovascularization within the marrow cavity at 14 days post-ablation varied with the implant formulation. Active DBM increased blood vessel volume and number. Addition of low molecular weight NaHY to the DBM further increased blood vessel volume and blood vessel branching but did not have an additive effect on the number of vessels within the marrow cavity. High molecular weight NaHY, low molecular weight NaHY and high+low molecular weight NaHY were all angiogenic by themselves to the same degree as DBM alone (Fig 2). Histomorphometry of sagittal sections showed that all three formulations of NaHY increased blood vessel number within the marrow cavity.

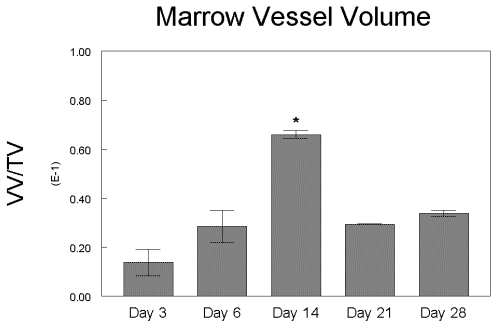

Fig 1. Neovascularization following tibial marrow ablation. Blood vessel volume fraction was assessed after tibial marrow ablation at 3, 6, 14, 21, and 28 days. *p<0.05 vs. all other time points.

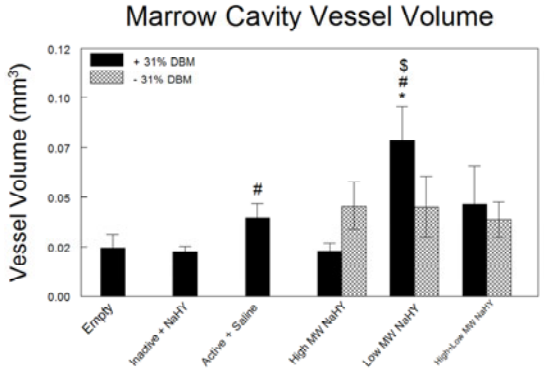

Fig 2. Effect of NaHY on neovascularization following bone marrow ablation. *p<0.05 vs. empty defect; #p<0.05 vs. inactive + NaHY, $p<0.05 vs. active + saline.

Conclusions: This study confirms that hyaluronic acid is vasculogenic and suggests that it is also osteogenic. Overall, addition of NaHY resulted in an increase in total vessel volume. Both high and low MW NaHY increased the total vessel number compared to empty defects while low MW NaHY also significantly increased the marrow cavity blood vessel volume. Histomorphometry of mid-sagittal sections supported the μCT observations. It is important to note that this model assesses effects over those caused by the ablation itself and at the time the measurements of neovascularization were done, remodeling of the marrow was already underway. The contribution of the vascularization to remodeling isn't known. In an orthotopic site, high molecular weight NaHY might contribute to bone formation both supporting MSC and progenitor cell migration in early phases of healing and vascular ingrowth as the NaHY is degraded to smaller fragments.

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