Angiogenic and Osteoinductive Hydrogel Scaffolds for Bone Regeneration

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Statement of Purpose: The mechanical and functional properties of bone are related to its microstructural organization. In order to direct the formation of mature bone, scaffolds for regeneration are likely to require delivery of angiogenic as well as osteoinductive signals. Hydrogel scaffolds made from the naturally occurring polymer, hyaluronic acid (HA), can be designed to have variable degradation rates due to hydrolysis and the enzymatic activity of hyaluronidase. These scaffolds can also be designed to exhibit controlled protein release rates due to electrostatic interactions of positively charged proteins with the negatively charged HA. Vascular endothelial growth factor (VEGF) has been shown by others to have a positive effect on bone growth[1,2] and thus was selected for delivery from these HA scaffolds in a critical size defect model.

Methods: *Scaffold Formation*: HA (110kDa or 220kDa, Lifecore, Chaska, MN) was modified with glycidyl methacrylate (Sigma-Aldrich, St. Louis, MO) to attach reactive vinyl groups. The modified HA was cross-linked to form hydrogels using UV irradiation (365nm) and the photoinitiator Irgacure® 2959 (Ciba, Tarrytown, NY).

In Vitro Degradation: Hydrogels were incubated at 37°C in buffers at various pH to show hydrolytic degradation and in buffers containing hyaluronidase (Sigma) to show enzymatic degradation. Buffers were sampled at selected time points, and HA content was assayed using a modified carbazole assay[3].

In Vitro Protein Release: Hydrogels were loaded with protein (VEGF – Genentech, South San Francisco, CA; bone morphogenetic protein (BMP)-2 – Genetics Institute, Cambridge, MA; or bovine serum albumin (BSA) – Sigma) and placed in a release buffer at 37°C. The release buffer was sampled at selected time points, and protein release was measured by ELISA (R&D Systems, Minneapolis, MN) for VEGF and BMP-2 or by a Bradford assay (Pierce, Rockford, IL) for BSA.

In Vivo Bone Regeneration: Scaffolds were implanted in a 5mm circular critical size defect in the parietal bones of adult male Sprague Dawley rats (Harlan, Indianapolis, IN). At sacrifice, the parietal bones were harvested and fixed. Extent of mineralization was measured by X-ray and/or micro-computed tomography (microCT). The tissues were then decalcified and embedded in paraffin prior to histology. Immunohistochemistry was also performed for bone and endothelial cell markers.

Results / Discussion: *Hydrogel Formation and Characterization*: Decreasing the molecular weight and increasing the concentration of HA resulted in hydrogels that were less susceptible to hydrolysis but could still be degraded by hyaluronidase. Release of positively charged proteins (VEGF, BMP-2) was slower than controls (BSA), and release of nanogram quantities of protein could be maintained for several weeks *in vitro* using the slow degrading 110kDa HA hydrogels (data not shown).

Rat Calvarial Critical Size Defect Model: At a three-week time point, the faster degrading 220kDa hydrogel scaffolds with no protein resulted in negligible mineralization compared to an unfilled defect (figure 1). Hydrogels loaded with 5µg of BMP-2 resulted in significant mineralization compared to the negative controls. Hydrogels loaded with VEGF showed a dose response with significant mineralization (similar to the BMP-2 positive control) at a dose of 25µg.

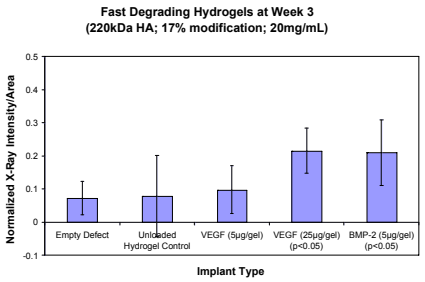

Figure 1: *Extent of mineralization as measured by X-ray; shown as mean ± standard deviation*

Histological analysis showed that the 25µg VEGF dose resulted in a greater volume of tissue filling the defect compared to lower VEGF doses or the negative controls (figure 2). Immunohistochemistry for vonWillebrand Factor (vWF) also showed increased angiogenic blood vessels with the higher dose of VEGF (representative image shown in figure 2).

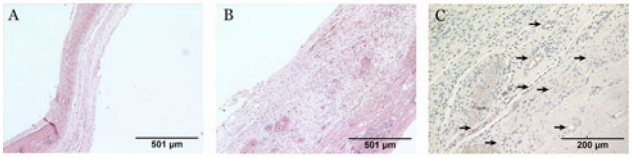

Figure 2: *(A) 5µg VEGF dose, H&E stain; (B) 25µg VEGF dose, H&E stain; (C) 25µg VEGF dose, IHC for vWF (red stain, arrows), hematoxylin counterstain*

At the three-week time point, the 110kDa hydrogel scaffolds had not degraded. However, X-rays showed that hydrogels loaded with 5µg BMP-2 were still able to induce significant bone formation (data not shown).

Conclusions: We were successfully able to create HA hydrogel scaffolds with controlled degradation and protein release properties. These scaffolds induced significant bone regeneration when loaded with BMP-2 and showed a dose response with VEGF. VEGF delivery also resulted in tissue that was well-vascularized, as seen with immunohistochemistry for endothelial cell markers. These results show that cytokine-loaded HA hydrogel scaffolds can be used to rapidly regenerate bone as well as that increasing the vascularization of the defect region by delivery of angiogenic molecules is sufficient stimulus to initiate bone regeneration.

References:

[1]Murphy DL *et al*. J Dent Res. 2004;83:204-210.
[2]Street J *et al*. PNAS. 2002;99:9656-9661.
[3]Cesaretti *et al*. Carbohydrate Polymers. 2003;54:59-61.