Bone Regenerative Capacity of rhBMP-2 Loaded Carboxymethylchitosan Microspheres in a Rat Calvarial Defect

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Statement of Purpose: Our laboratories have developed chitosan-based microspheres and scaffolds for use as a bone graft substitute.[1] EDC/NHS-crosslinked carboxymethylchitosan (X-CMCS) microspheres demonstrated cytocompatibility, degradability, and a sustained rhBMP-2 elution profile in vitro.[2] The purpose of this study was to determine the bone regenerative capacity of rhBMP-2 loaded X-CMCS microspheres in vivo. We hypothesized that rhBMP-2 loaded X-CMCS microspheres would promote increased bone regeneration in a critical-sized rat calvarial defect compared to treatment with demineralized bone matrix.

Methods: Chitosan microspheres were prepared using a precipitation method as previously described.[1] The chitosan microspheres were carboxymethylated using monochloroacetic acid to yield carboxymethylchitosan (CMCS) beads.[3] The CMCS beads were crosslinked using carbodiimide chemistry. The CMCS beads were added to a solution containing 100mM 2-(N-morphilino) ethane-sulfonic acid, 20mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, and 50mM N-hydroxysuccinimide in 300mL of DI H_2O. The beads were crosslinked for 90 minutes at a pH of 6.5 and then washed with DI H_2O and 70% ethanol, followed by drying in a convection oven at 35°C. These beads are referred to as X-CMCS.

X-CMCS samples were prepared for surgery by placing 100mg of low dosage gamma-sterilized beads in 1mL of a 5µg/mL rhBMP-2 solution overnight. Demineralized bone matrix (DBM) samples were prepared immediately before implantation by hydrating 0.1mL of rat DBM with 100µL of 1x PBS. A critical-sized 10mm diameter defect was created in the calvaria of male Wistar rats using a dental drill and trephine. X-CMCS or DBM samples (n=5) were placed in the defect (Figure 1), and the surgical site was sutured.

The animals were euthanized after one month. The calvaria and surrounding tissue were recovered and stored in 10% neutral buffered formalin. Micro-CT imaging and analysis (9µm resolution) was conducted. The volume of bone, bone density, and bone surface/volume ratio were determined. Undecalcified histological processing and analysis is currently being performed.

Results: One X-CMCS animal was lost due to post-surgical complications. The X-CMCS group promoted more bone formation than the DBM implants (Table 1 and Figure 2). The region of interest contained 30.0 ± 5.2% bone tissue in the X-CMCS samples compared to 18.9 ± 3.9% for the DBM group. In Figure 2, it can be seen that bone nodules are forming throughout the X-CMCS samples; whereas, new bone appears to be forming only near the defect margins of the DBM group. In addition, the quality of the bone found in the X-CMCS group was slightly better than that of the DBM samples (Table 1). In this analysis, 100% bone mineral density corresponds to the quality of calvarial bone outside the defect area. It is very promising that after only one month, the newly deposited bone has a mineral density approaching that of native calvarial tissue. The bone surface/volume ratio is an indicator of active remodeling, and the two groups displayed similar values. The histology results are expected to provide more information on the cellular response to the materials and extent of bone formation and remodeling.

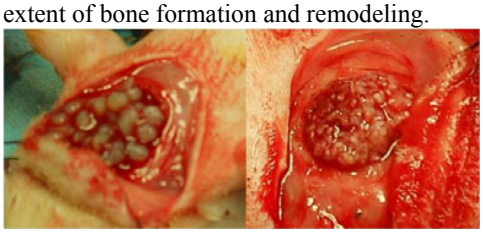

Figure 1. Implants in calvarial defect. L- X-CMCS; R- DBM.

	Bone volume/tissue volume (%)	Bone Mineral Density (%)	Bone surface/volume ratio (mm^{-1})
X-CMCS	30.0 ± 5.2	92.1 ± 3.7	72.1 ± 7.1
DBM	18.9 ± 3.9	87.6 ± 3.8	74.2 ± 13.4

Table 1. Results of the micro-CT analysis.

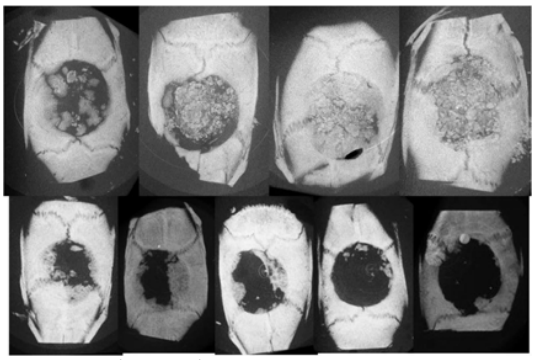

Figure 2. Micro-CT images. Top- X-CMCS; Bottom- DBM.

Conclusions: The ability of rhBMP-2 loaded X-CMCS microspheres to promote bone regeneration in a critical-sized rat calvarial defect model was demonstrated. DBM contains BMP-2 and is commonly used clinically as a bone graft substitute. The X-CMCS beads displayed increased bone filling compared to the DBM implants, and our hypothesis was confirmed. This model will be expanded in the future to include additional timepoints and comparison to an autograft group.

References:

1. Reves BT. JBMR Part B. 2009; 90:1-10.
2. Reves BT. SFB 2012 Fall Symposium. Abstract #140.
3. Shi ZL. Biomacromolecules. 2009; 10(6): 1603-11.

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