The assessment of Bone Formation in Vitreous Carbon Foam Using the Rat Calvarial Defect Model

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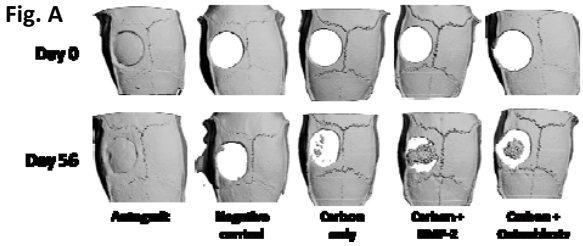
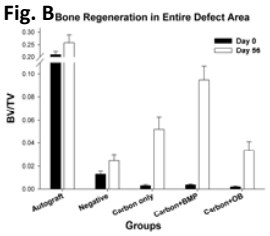
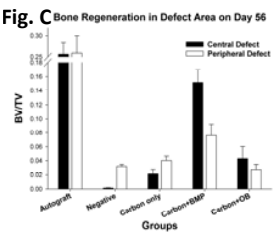
Statement of Purpose: Carbon foam-based composites represent a novel tissue engineering scaffold for repairing bone defects. Carbon foam has an exceptionally low relative density and high surface area, which makes it potentially suitable for osteoconduction and osteoinduction after loading with bioactive materials such as BMP-2 and mesenchymal stem cells (MSCs). A competent carrier is required to retain and activate the osteogenic protein and cells at the site of the bone defect. Thus it is hypothesized that carbon foam can effectively deliver osteogenic proteins and cells, establish various bone formation patterns (endogenously and exogenously), and enhance the bony healing in calvarial defects. We examined carbon foam as an osteoconductive and osteoinductive scaffold for orthopaedic applications.

Methods: Vitreous carbon foam (605±86.38μm at pore size, and 225±67.45μm at pore interconnections) was sculpted as a disk with a diameter of 6mm and a height of 2mm. 35 Lewis rats were assigned to five groups according to bone defect treatment: (1) autograft, receiving excised autogenous calvarial fraction; (2) negative control, receiving no graft; (3) carbon foam alone; (4) carbon foam+BMP-2; (5) carbon foam+osteoblasts (OB). Carbon foam+BMP-2 was prepared by immersing carbon foam in 10μg/ml BMP-2. Carbon foam+OB was prepared using Lewis rat bone marrow MSCs-derived osteoblasts seeded on the center of the carbon disks at 0.2×10^5 cells/scaffold 1 week before implantation. A rat model of a full-thickness calvarial defect 6mm in diameter was surgically established, and graft repaired accordingly. Microcomputed tomography (μCT) was employed to evaluate the bone regeneration in the defect at day 0 and day 56 postoperatively. The extent of new bone formation in the defect site was presented as the ratio of bone volume to total defect volume (BV/TV). The new bone formation was distinguished as central and peripheral bone formation. The central area was defined as the concentric cylinder with a diameter of 3mm in the middle of the defect. The peripheral area was determined as the outer-ring (1.5mm in width) of the defect, which was in continuity with the host calvarias.

Results: On the 8th week after implantation, all groups showed varying bone formation in the defect sites compared with the BV/TV of surgery day (Fig. A). Although all other groups did not repair the defect to the same extent as autograft implantation, bony restoration was significantly greater in BMP-2 treated groups than in the groups of negative control, carbon foam alone and carbon foam+OB ($p<0.05$) (Fig. B). The remarkably radiographical bone bridging was noticed BMP-2 treated groups, revealing the tendency of the defect closure over time (Fig. A). There was no significant differences in bone formation among negative control, carbon foam alone and carbon foam+OB ($p>0.05$). In the groups of negative control and carbon foam alone, only sporadic bone formations could be observed at the defect margin at 8 weeks of implantation (Figure C), possibly originated from the host osteoblasts migration. In contrast, central bone formations were detected in both the BMP-2 and the OB treated groups. The BMP-2 and OB treated groups exhibited statistically higher BV/TV ratios than those of the negative control and carbon foam alone groups at the center. In central regions, the BV/TV of BMP-2 treated groups achieved more than one half of the BV/TV of the autograft group. However, the peripheral BV/TV of the groups of negative control, carbon foam alone and carbon foam+OB were not significantly different.







Conclusions: In this study, the rat calvarial defect model was used to evaluate the osteogenic potential of a carbon foam biomaterial. μCT technology allows the temporal and geographical analysis of bone regeneration at the defects. Carbon foam appears an effective delivery vehicle to accommodate biological regulating agents and osteogenic cells. Combining BMP-2 or osteoblasts on carbon foam resulted in increased bone regeneration in comparison with the negative control and carbon foam alone. Although the defects in the BMP-2 and osteoblasts treated groups did not reach complete repair, it is possibly due to the short-term implantation period. Introduction of BMP-2 in the scaffold apparently accelerated local osteoinductivity and promoted bone regeneration from host calvaria. Pre-seeding osteoblasts on the carbon form scaffold gave rise to bone formation in the center of the defects, generating a second or exogenous ossification center which advanced the osteogenic potential of the carbon foam scaffold. The porosity and pore size of the scaffold are above the critical values required for cell migration. The bone formation was extended beyond the contour of the cell-seeding area, although it was confined to the center during the 8 weeks of repair. The implantation of carbon foam alone did not result in any massive bony repair, supporting the concept to use carbon foam as an effective delivery vehicle for osteogenic cells and osteoinductive proteins.