Partially Demineralized Macroporous (PDM) Allografts for Cranial Tissue Engineering

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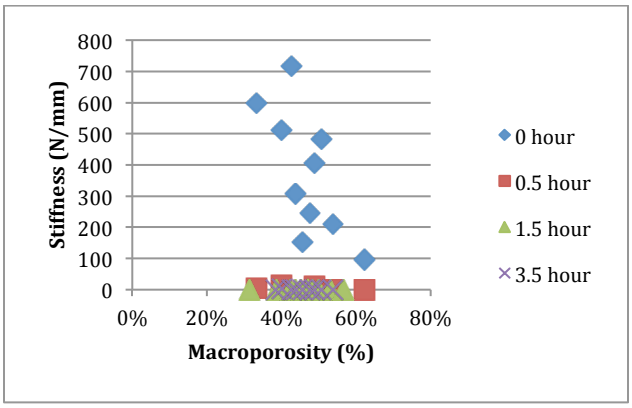
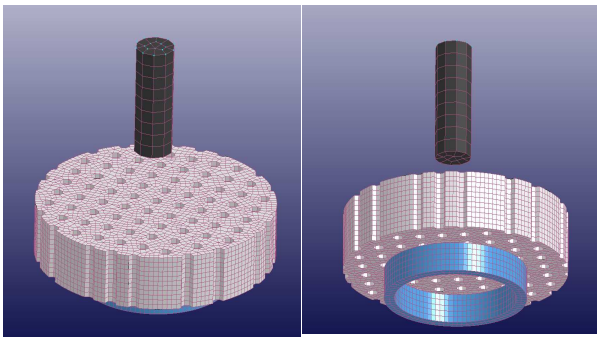
Introduction: Decompressive Craniectomy is a cranial surgery where a large part of the cranial bone is removed in order to mitigate swelling in the brain tissue. Consequently, a scaffold biomaterial is required to substitute the lost bone. Ideal cranioplasty biomaterials should have the following features: fit the cranial defect and achieve complete closure, radiolucency, resistance to infections, no dilation with heat, resistance to biomechanical wear, pliability, and inexpensive[1]. It is possible to create porous, bioactive and biocompatible demineralized bone scaffolds that can potentially be implanted in the area of defect by the surgeon. Partially Demineralized Macroporous (PDM) allografts exhibit such properties to correct these cranial defects. PDM allografts serve as scaffolds that can deliver vital cells to the defective area for maximum integration, optimal mechanical strength and minimal systemic or local cytotoxicity. The main objectives of this study include: (1) examining the effects of demineralization and macroporosity formations on the mechanical and biological properties of allograft bone disks; (2) conducting finite element analysis (FEA) to stimulate the mechanical properties of the PDM allografts; and (3) evaluating the *in vitro* response of the PDM allografts utilizing pre-osteoblast cell lines.

Methods: Tibias were harvested from Ossabaw minipigs and cylindrical cortical bone sections of 2 mm in thickness and 8 mm in diameter were obtained. Macropores of 600 micrometers in diameter were created to generate porosity levels of 30-64% in the bone disks. The bone disks were then demineralized in 1N HCl for 0.5 to 3 hours. The relative stiffness was determined for each class using a material testing machine with a loading rate of 1 mm/min using a piston-on-ring set up. To analyze the deformation characteristics, FEA software LS-DYNA was employed. In order to understand the *in vitro* response, biocompatibility of PDM scaffolds were evaluated by culturing MC3T3-E1 cell lines where XTT and ALP assays were conducted.

Results: It was found that there is an inverse proportionality between the stiffness of the PDM scaffolds and their porosities for each demineralization time. The stiffness values of non-demineralized specimens of 30% and 60% porosity were found to be 300 N/mm and 600 N/mm, respectively, **Figure 1**. The FEA results show the von Mises stress distribution with the characteristic stress concentration in the contact area between the pushing rod and the bone allograft disk, **Figure 2**. The effect of the axial loading through the holes with respect to stress concentration is less prominent. For the *in vitro* studies, the MC3T3-E1 cell lines displayed elevated XTT and ALP behavior when cultured onto PDM allografts compared to the 2D tissue culture plates.





Conclusions: PDM allografts display the suitable stiffness required for cranial defects. The PDM allograft scaffolds aid in osteogenic proliferation and differentiation of pre-osteoblast cell lines *in vitro*. However, there will be further *in vivo* testing regarding the validity of PDM allografts in rat cranial defects.

1. Aydin S, Kucukyuruk B, Abuzayed B, Aydin S, Sanus GZ. Cranioplasty: Review of materials and techniques. J Neurosci Rural Pract 2011;2(2):162-7.