Synthesis and Characterization of Chitosan Based Composite Scaffolds for Bone Tissue Engineering

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Statement of Purpose:: Bone is a living tissue and it constantly remodels and adapts the stresses imposed upon it. Bone disorders are of significant concern due to increase in the median age of our population. Synthetic scaffolds have been heavily researched as bone graft substitutes to restore damaged bone. The role of traditional scaffolds is to provide mechanical support to grow cells on it. These days scaffolds are further engineered to be bioactive and bioresorbable to enhance bone formation and vascularization. The scaffolds are often porous, made of natural and synthetic materials that harbor growth factors, drugs, genes, or stem cells. The objectives of this research are to synthesize a chitosan/carobxymethyl chitosan based composite scaffolds for bone regeneration and measure their physio-chemical properties. We hypothesize that the positively charged chitosan, a bioactive natural polymer combined with negatively charged polysaccharides will result complex cosorbate that can result a scaffolds with superior mechanical properties and appropriate microstructures suitable for bone regeneration.

Methods: The chitosan and CMC solutions were mixed in a container with the weight ratio of 1:1. The total weight of the container was observed to set the counter balance in a thinky mixture (Planetary Centrifugal Mixture (ARM-310)). The solutions were mixed in the planetary centrifugal thinky mixture which uses a mechanism where the container holding the material revolves clockwise and the container itself rotates counter clockwise. The centrifugal force exerts pressure within the material inside the container and generates vertical spiral convection continuously. The mixing process was carried out for 30 minutes at 2000 rpm. After the solutions were thoroughly mixed, they were casted in 48 well cell culture dishes. The cast was kept at 4°C for about 4 hours and then transferred to -20°C for 4 hours and then finally to -80°C for 12 hours. The scaffolds were allowed to lyophilize for about 36 hours in the freeze. Scanning Electron Microscope (Hitachi SU8000, Japan), Instron 5525 and Mcro/Nano CT-X ray mictotomography (GE Phoenix Nanotom- M^{TM}, GE sensing and inspection technologies GmbH) were used to measure the porosity of the scaffolds.

Result/Discussion: Micro CT images were used for quantization of porosity of the scaffolds. Histogram analysis showed the porosity percentage in the range of 80 to 90%. Morphology of as synthesized porous 3D scaffolds was assessed by using Micro CT and SEM images. Both analyses showed that the scaffolds did have uniform porosity with pore size in the range of 100-150 µm. This size range is very beneficial for bone cell to grow

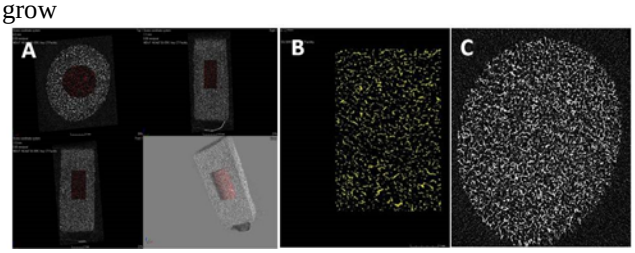

Figure 1: Micro CT image of scaffold for porosity analysis. (A) CT-Images show the projection of image of the scaffolds in three different planes, (B) Micro CT image showing corresponding voxel of scaffold's struts in yellow and void pores in dark background, and (C) top/plane view of inner slice of scaffold.

Conclusion: Biofunctional and biodegradable chitosan based composite scaffolds were successfully synthesized. Scaffold's physical properties such as microporosisty, mechanical strengths and morphology were analyzed. Porosity of the scaffolds was in the range of 80 to 90 percent where as mechanical strength under compression was in the range of 0.08 to 0.12 MPa with compressive modulus of 2.5 MPa. The prepared scaffolds were also found intact with original three-dimensional frameworks and ordered porous structures maintaining sufficient mechanical strength in cell culture medium. These observations provide a new effective approach for preparing bone tissue engineering scaffold

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