Chemically Modified Light-curable Chitosans with Enhanced Potential in Bone Tissue Engineering

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Statement of Purpose: Chitosan, a natural abundant polymer, holds great promise attributing to its biocompatibility and osteoconductivity. However, the potential of chitosan in bone repair has been compromised largely due to its poor solubility in organic solvents, which hinders its utility for most scaffold fabrication processes [1]. In addition, raw chitosan are not instantly curable, therefore, does not qualify for in situ fabrication of scaffold that reproduces the size and morphology of the defect. This is particularly important for bone repair since most bone defects are in irregular shapes. In this study, we have chemically modified chitosan with light curability and enhanced solubility by replacing the hydroxyl side groups with benzoyl and methacryloyl groups at appropriate ratios. Alterations in the chemistry of chitosan after modification were confirmed by FTIR and NMR. The modified chitosan was fabricated into 3-D scaffolds with interconnected pores, which promoted osteoblast ingrowth and bone-like tissue formation in vivo.

Methods: Chemical modification and characterization: The mixture of benzoyl chloride and methacryloyl chloride at different ratio was added dropwise to the methane sulfonic acid solution of chitosan and allowed to react for 3 hrs before transferred to –20 °C overnight. The solution was then poured into icy water, filtered, and stirred in 4% ammonia solution overnight. The final products were collected by filtering, and vacuum-dried. The structure and chemistry of the modified chitosan were examined using FTIR and HNMR. Scaffold fabrication: The modified chitosan was dissolved into DMSO to form a precursor solution. Light cured crosslinked chitosan was obtained by UV light exposure of the precursor solution in the presence of photoinitiator, Iragure 2979. Porous chitosan discs were fabricated using a salt leaching technique. Cytocompatibility and osteoconductivity: Cytocompatibility of the light-cured chitosan was evaluated by co-culturing with 3T3 fibroblasts up to 1 week and determining the cell viability. To determine the osteoconductivity, bovine osteoblasts were seeded into the chitosan discs and subcutaneously implanted into the back of nude mice. The implants were retrieved at 6 weeks and the histology was examined with H&E staining.

Results/Discussion: The scheme of chitosan modification is shown in Fig.1. The hydroxyl side groups in the raw chitosan were replaced by benzoyl and methacryloyl groups (two types of R2) at appropriate ratios, as determined by the feeding ratio of benzoyl chloride to methacryloyl chloride. Introduction of methacryloyl groups confers light curability to the material, i.e., upon UV light exposure in the presence of photoinitiators, the precursor solution undergoes polymerization to form crosslinking networks. The concentration of methacryloyl groups in the light curable chitosan therefore determines the crosslinking density of the chitosan networks. Characterization of the light curable chitosan using FTIR (Fig.2 A) indicates the presences of benzoyl (down-arrow) and methacryloyl groups (up-arrow), with the intensities change as a function of the benzoyl chloride to methacryloyl chloride feeding ratios (decrease in a descending order in Fig.2 A). NMR analysis also demonstrated that double bonds are incorporated into the backbone of chitosan (Fig. 2B). Solubility tests indicate that the modified chitosan can be dissolved in organic solvents such as DMSO, DMF, and acetone before light-cure. SEM images of the porous scaffolds fabricated from the light curable chitosan display highly interconnected pores throughout the scaffolds with pore sizes ranging from 150 to 210 um in diameter (Fig. 3). The light cured chitosan is cytocompatible, as evidenced by the lack of cell death in the in vitro co-culture with 3T3 fibroblasts. Histology examinations of the subcutaneous implants of bovine osteoblast-seeded chitosan discs (Fig.4) display the growth of a large number of osteoblasts into the pores of the discs and the formation of bone tissue-like structures at 6 weeks, suggesting that the light curable chitosan scaffold is highly osteoconductive.

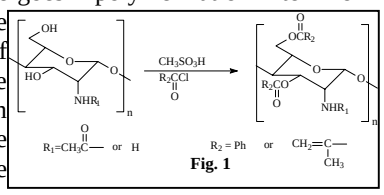

Fig. 1

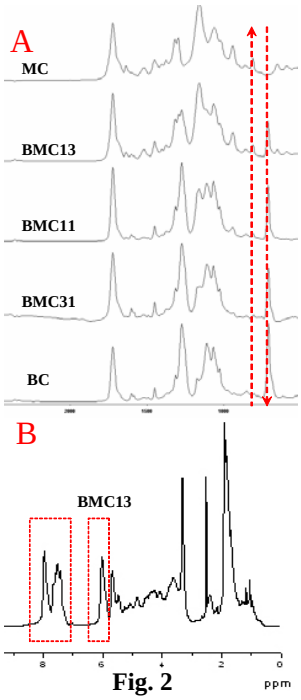

Fig. 2

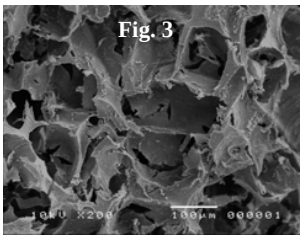
Fig. 3

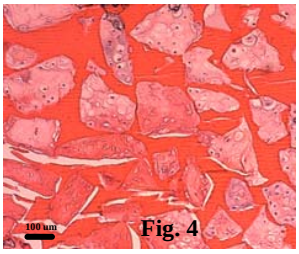

Fig. 4

Conclusions: Light curable chitosan with enhanced processibility for scaffold fabrication can be obtained through chemical modification of the side groups in the raw chitosan. 3-D scaffolds with interconnected pores that are fabricated from the light curable chitosan are cytocompatible, and osteoconductive, therefore, represent ideal substrates for bone tissue engineering purposes. Studies underway are focused on optimizing the synthesis process for light curable chitosan to achieve the maximal potential in promoting bone repair in vivo.

References: Kumar MNV, etc, Chem. Rev. 2004, 104, 6017

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