Sustained Release of IGF-1 from Chitosan Microparticles to apply in Bone Regeneration

V. Mantripragada, A. Champa Jayasuriya
Department of Orthopaedic Surgery, University of Toledo Health Science Campus, Toledo, Ohio 43614, USA

Statement of Purpose:

Regeneration of diseased or fractured bone by tissue engineering is a much novel and promising approach in comparison to autologous or allogenous bone transplantations. A scaffold based tissue engineering approach will require a bone substituting material fulfilling requirements such as biocompatibility, osteogenecity, malleability, biodegradability. Chitosan, a linear polysaccharide derived by partial de-acetylation of chitin, was found to possess these characteristics. It also has antibacterial activity, mucoadhesivity and wound healing capability. But many studies suggest that substitution with chitosan alone is not sufficient to induce rapid bone regeneration due to its low mitotic potential. Thus a combination of growth factor and chitosan based scaffold material would be an effective approach as the growth factors function as modulators of chemotaxis, proliferation and differentiation of pluripotent cells concerning bone regeneration. Insulin like Growth Factor (IGF), specifically IGF-1 was found to be playing an important role in the longitudinal growth of the bone. Thus, the objectives of this study was to characterize the microparticles (MPs) and to evaluate the controlled release of IGF-1 from the chitosan MPs.

Method:

Medium molecular weight Chitosan, cotton seed oil, Tripolyphosphate (TPP), span85 (purchased from Sigma), acetone, acetic acid, hexane (purchased from Fisher) is used to prepare MPs by emulsification technique. MPs were prepared by method described previously [1]. After preparing the chitosan/acetic acid/acetone mixture, 0.15% IGF-1 (w/w) was added to it and then the mixture was added to cotton seed oil. Scanning electron microscope (SEM) image was taken to characterize the MPs. Release study of IGF-1from chitosan MPs was performed by suspending 20mg of MPs in 3mls of phosphate buffer saline (PBS). The vial was placed in incubator at 37^{o}C and shaken at a frequency of 15 rpm. At predetermined time interval over a period of 2 weeks, samples were withdrawn from vials and replenished with fresh PBS. Loading efficiency was studied by dissolving MPs in 0.1N HCl for 24h and then sonicating for 30mins. The concentration of released IGF-1 was assayed using ELISA. All the data was collected in triplicates and the result was expressed as mean $\pm$ standard deviation.

Results:

The prepared chitosan MPs were in spherical shape with 30-80 µm in diameter as analyzed by SEM. The loading efficiency was calculated based on the total amount of IGF-1 encapsulated in the MPs. Fig. 1 demonstrates the release of IGF-1 from MPs. Initially a very slow release was observed, but by the end of second week, there was a sudden release observed. The IGF-1 release from MPs may be due to the diffusion through the small pores present in the MPs. The range of release of IGF-1 is anticipated to stimulate regeneration of bony defects during implantation. Bioactivity is now being initially studied on the Mesenchymal Stem Cells isolated from mice bone marrow.

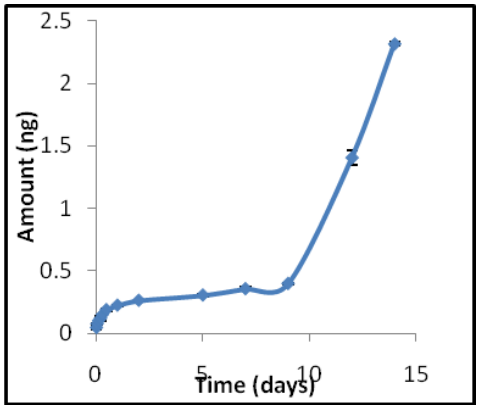

Fig. 1. Release profile of IGF-1 from chitosan MPs

Conclusion:

The release profile of IGF-1 is being controlled by chitosan. Thus, chitosan in combination with IGF-1may be a valuable tool in bone regenerative therapy. Future work would include the testing of different concentration of IGF-1 encapsulation to study the release profile and also the effect of the released IGF-1 on the bioactivity of the cells.

References:

1. Jayasuriya AC, Bhat A. Biomed Mater 4(5):55006, 2009.

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