Influence of Bone Morphogenetic Protein-7 Encapsulated and Coated Chitosan Microparticles on Osteoblasts Proliferation and Differentiation

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Statement of Purpose: Extensive research in orthopedics has led to immense development in the fabrication and design of implant materials. But there are still several complications like rejection, non-union, infection, delayed healing associated with the use of autografts, allografts, metallic implants. Natural polymers along with growth factors are being considered as an effective alternative for bone substitute materials, as they regulate bone growth by their osteoinductive and osteoconductive characteristics. Thus, the current study focuses on fabricating microparticles using a natural polymer, chitosan. BMP-7 was added to the microparticles by two methods, firstly during the microparticles preparation, thus encapsulating the BMP-7 and secondly after microparticles preparation, thus coating the BMP-7 onto the microparticles. Bioactivity of the BMP-7 released from the microparticles was confirmed by cell study to prove that the presence of the growth factor improved attachment, proliferation and differentiation of osteoblasts.

Methods: i) *Cell attachment* –Cells on the microparticles were fixed in 2.5% glutaraldehyde followed by dehydration in graded ethanol series. Samples were dried by critical point drying and observed by scanning electron microscopy (SEM) ii) *Cell viability* – The viability of cells attached to the chitosan microparticles was confirmed by live/dead cell assay (Invitrogen) iii) *DNA quantification* –DNA was extracted and quantified using DNA kit from Qiagen to quantify cells iv) The real time rt-PCR analysis was carried out to determine the gene expression levels of osteopontin (OPN) and bone sialoprotein (BSP), which were normalized to the expression of housekeeping gene GAPDH. Verso cDNA kit (Thermo Scientific) and SYBR green master mix (Applied Biosystems) were used for this assay v) *Mineralization* – In the Von Kossa staining experiment, 2% silver nitrate was added to determine mineralization. The images were analyzed using bright field microscopy.

Results: i) *Cell attachment* – BMP-7 encapsulated microparticles, BMP-7 coated microparticles and microparticles without any growth factors were all in the size range of 700 µm and appeared to have similar surface morphology. We studied the release kinetics of BMP-7 from the chitosan microparticles. There was a burst release observed till day 1 and from day 2 to day 14, we observed a sustained release. On day 10, BMP-7 coated microparticle showed a significant increase in the number of cells attached its surface in comparison (Fig 1) with BMP-7 encapsulated and microparticles without any growth factors. ii) *Cell viability* – The results indicated that there is a significant difference ($p < 0.05$) in the number of viable cell attached to BMP-7 coated and BMP-7 encapsulated microparticles, thus indicating the influence of higher concentration of BMP-7 on cell attachment and proliferation. There is also a significant difference ($p<0.05$) observed between BMP-7 encapsulated and coated microparticles and microparticles

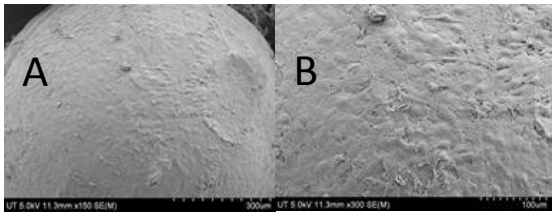

Fig. 1: SEM image of mouse osteoblast cells attached and proliferating on BMP-7 coated chitosan microparticle as observed on day 10 A, B) lower and higher magnification.

without any growth factor, indicating the bioactivity of the released growth factor iii) *DNA quantification* – This assay was in correlation with the above two experiments and proved the influence of BMP-7 on cell growth iv) *Real time rt-PCR* – OPN expression was increased on day 7 in comparison with day 5 for BMP-7 encapsulated (7-fold) and BMP-7 coated microparticles (8-fold). There is a significant increase ($p<0.05$) in mRNA levels of OPN for BMP-7 coated microparticles on day 10 in comparison with day 7, while all the other samples maintained a similar fold change. BSP mRNA levels did not increase much till day 7, however on day 10, there was a significant increase in BSP expression in microparticles only (9-fold), BMP-7 encapsulated (54-fold) and BMP-7 coated (53-fold) microparticles v) *Determining Mineralization* – The results showed that increased mineral deposition was observed by day 10 for both BMP-7 encapsulated and BMP-7 coated microparticles (Fig. 2). Mineral deposits were mainly localized in the areas adjacent to the microparticles.

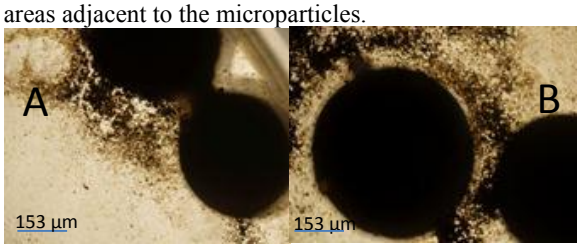

Fig. 2: Voncossa assay images for A) BMP-7 encapsulated and B) BMP-7 coated microparticles on day 10.

Conclusion: Significant increase in cell attachment, proliferation and differentiation of osteoblasts was observed from BMP-7 coated microparticles. Thus, it can be concluded that due to the controlled release of BMP-7 from the chitosan microparticles, there was an improved response from osteoblasts.