Bone marrow stromal cell function on hybrid microparticles

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Statement of Purpose:

There has not been much study in the application of polymer microparticles (MPs) in bone tissue engineering. Chitosan (CS) scaffold has been used in bone regeneration applications because of its osteoconductive and antimicrobial properties [1]. In our study we look at the application of hybrid CS MPs synthesized by emulsification technique, in bone marrow stromal cell (BMSC) function. Since calcium phosphate ($CaHPO_4$), calcium carbonate ($CaCO_3$) and coral are known to support bone regeneration [2], we have incorporated them in different formulations, to improve the osteoconductive properties of the MPs.

Methods:

We have scaled up the emulsification technique described for 1X batches [3] to prepare 4X batches of CS MPs. We have used 1.5 % (w/v) CS solution, prepared by dissolving 0.75 gm of CS in 50 ml of 1% (v/v) acetic acid. We have also incorporated 10% (w/w) $CaHPO_4$, 20% $CaHPO_4$, 10% $CaCO_3$ or 10% coral, to study the effect of these formulations on cell attachment.

The surface morphology and size of the MPs was examined using a scanning electron microscope (SEM) Hitachi S3200N. The samples were sputter coated with gold prior to analysis and examined using 400 X magnifications. The MPs were also characterized using Fourier transformed infrared spectroscopy (FTIR).

BMSCs were isolated from the femur and tibia of C-57B46 mice [4], and plated on T-75 flasks and allowed to grow until two passages, before using for attachment studies.

12 mg of MPs from each formulation were weighed into 96 well plates. The MPs were sterilized using UV, for 10 min. Four replicates were used for each formulation. Prior to cell seeding 50 µl of cell culture medium was added into each well, to ensure the MPs settle down at the bottom of the plate. Aliquots of 50 µl cell suspension (cell density: 400,000 cells/ml) were seeded on the MPs in the 96 well plates. Cells seeded in wells without MPs served as controls.

Cell quantification was performed on time points 4, 8, 25 and 48 hrs. At each time point the supernatant was removed and the MPs were washed with PBS. The cells were detached from the particles by adding 50 µl trypsin into each well. This process was repeated twice to ensure that all cells were detached. The cells were then counted using a hemocytometer. Statistical analysis was performed using Minitab software-version 15.

Results:

We have successfully scaled up the various formulations from 1X to 4X batches. SEM analysis showed that the MPs from 4X batches had a spherical shape, and most of the MPs were in a diameter range of 20-40 µm (Fig. 1).

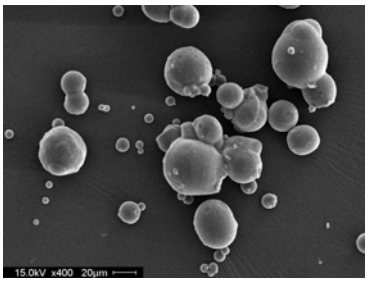

Fig.1. SEM image of 1.5% (w/v) CS+ 10% (w/w) $CaHPO_4$ formulation.

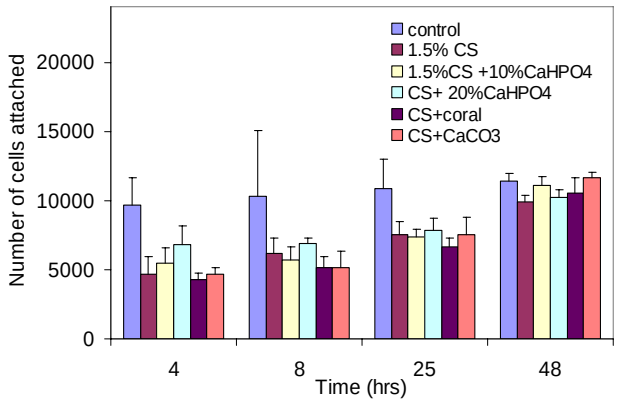

Fig. 2. Attachment of BMSCs on MPs from different formulations.

Fig. 2 shows the attachment of BMSCs on CS MPs from the various formulations. We noticed that after 4, 8, and 25 hrs of incubation there was a significant difference between the number of cells attached to the control wells and each of the different formulations ($p<0.05$). However, after 48 hrs of incubation this difference was insignificant ($p>0.05$). No significant difference was observed in the number of cells attached to the MPs, from the various formulations, at 4 and 8 hrs of incubation. However, the cell attachment to the MPs significantly varied between 8, 25 and 48 hrs of incubation ($p<0.05$).

Conclusions:

Our results show that more than 50% of the cells that were initially seeded on the MPs, attached to the MPs in 2 days. We are currently looking at the proliferation and differentiation of BMSCs on hybrid MPs, to see how incorporation of $CaHPO_4$, coral or $CaCO_3$ helps in bone regeneration. This study also offers one of the most promising approaches to injecting biodegradable MPs along with cells, to repair bone defects of different sizes.

References:

1. Zhang Y et. al, J. Mat. Sci., 2004, 15, 255-260.
2. Wang X et. al, Biomaterials., 2001, 21, 2247-2255.
3. Lopez C et. al; Int. J. Pharm., 2006, 312,166-173.
4. Jayasuriya AC et. al. JTERM 2:1 (In press)

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