Osteogenesis in Encapsulated Cultures of Mesenchymal Stem Cells: A Modular Platform for Improved Bone Regeneration

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Statement of Purpose: Current stem cell+scaffold bone regeneration strategies fail to completely regenerate bone *in vivo*. A modular tissue engineering strategy based on forming scaffolds *in situ* from stem cell loaded microcapsules could ameliorate this problem. In this study, rat bone marrow mesenchymal stem cells (MSCs) and calcium hydroxyapatite (HAP) microcarriers were encapsulated via ionic complexation of chondroitin 4-sulfate (C4S) with chitosan, for use as a modular bone regeneration platform. C4S has been shown to facilitate HAP nucleation *in vitro* [1], and HAP has been shown to stimulate MSC osteogenesis, even in the absence of osteogenic supplements [2]. MSC proliferation and osteogenesis in this modular system was studied *in vitro*.

Methods: Microencapsulation of HAP microcarriers and MSCs was performed using an established protocol [3]. Breifly, a C4S solution containing suspended HAP microcarriers and MSCs was extruded as 500 μm droplets into rapidly stirring chitosan. Reaction between C4S and chitosan produced a polyelectrolyte complex membrane, encapsulating the suspended cells and microcarriers. After buffer washes and surface stabilization, the microcapsules were transferred to static dish culture for eight weeks. Four capsule culture conditions were evaluated: 1) capsules formed without HAP microcarriers (EXP) cultured in non-osteogenic medium, 2) capsules formed with HAP microcarriers and cultured in non-osteogenic medium (EXP+HAP), 3) capsules without HAP but cultured in medium with ostoegenic supplements (OSTEO), 4) capsules with HAP and cultured in osteogenic medium (OSTEO+HAP). Intra- and extra-cellular alkaline phosphatase activity (ALP), along with osteocalcin (OC) and osteopontin (OP) secretion, were quantified to assess the extent of MSC osteogenesis over 8 weeks of culture. Results were normalized to sample dsDNA content. Deposited calcium mineral was quantified using the colorimetric *o*-cresolphthalein complexone method. The extent of new mineralization inside the capsule was visualized with tetracycline fluorescence, and MSC location in the capsule with Calcein Red Orange (Calcein RO) fluorescence.

Results: After two weeks of culture, both osteo-induced cultures showed rapid mineralization of the interior of the capsule and capsule walls. By week four, the mineralization was extensive, with a stark contrast between the EXP control and OSTEO+HAP conditions (Fig. 1A-1D). Intracellular ALP activity peaked between weeks two and three, and declined slightly as mineralization proceeded through week 4 (Fig. 1E). OC deposition in capsule cultures increased from negligible levels to over 2 and 4 ug OC/ ug dsDNA in OSTEO and OSTEO+HAP cultures respectively), thus indicating osteogenic differentiation of the MSCs. A similar trend was observed for OP deposition. Surprisingly, intracellular ALP activity and OC deposition of EXP+HAP capsules was similar to the OSTEO capsules, suggesting the occurrence of HAP-induced osteogenic differentiation.

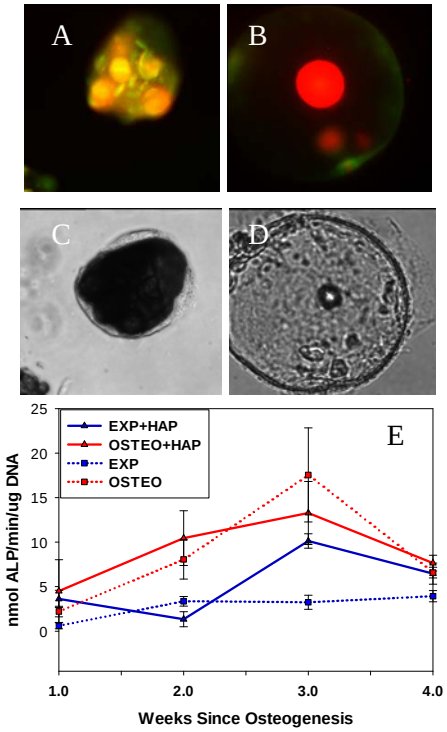

Figure 1: Tetracycline fluorescence (green) indicating new mineralization and Calcein RO (red) fluorescence indicating viability in (A) OSTEO+HAP culture at week 4, and (B) EXP culture at week 4. (C, D) Corresponding phase contrast images (E) Intracellular ALP activity over 4 weeks of culture.

Conclusion: Results to date show that the encapsulated culture system described here facilitates MSC differentiation to an osteogenic lineage. Moreover, it is noteworthy that the ALP activity and OC deposition of MSCs cultured in the EXP+HAP condition (without osteogenic media) were comparable to OSTEO capsules (capsules cultured in osteogenic conditions). Overall, these results, coupled with our ability to endothelialize and fuse capsules into 3D constructs [4], suggest that this encapsulation procedure is a materials foundation suitable for developing modular tissue engineered constructs for bone regeneration that can be readily vascularized *in vivo*.

References:

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