Spatial control of mES cell differentiation within microsphere based scaffolds.

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Statement of Purpose: Embryonic stem (ES) cells are a useful cell source for tissue engineering and repair due to their pluripotency and infinite capacity for self renewal[1]. Murine ES (mES) cells have been successfully differentiated into osteoblasts for bone tissue engineering using dexamethasone and ascorbic acid supplemented media[1]. PLGA microspheres have been extensively used for the sustained delivery of proteins and small drug molecules[2]. In this study, we aimed to use PLGA microsphere-based scaffolds for the controlled delivery of osteogenic supplements for the *in situ* induction of murine ES cells to form bone.

Methods: 1.0% Dexamethasone (Dex) and 10% ascorbate-2-phosphate (Asc) loaded microspheres were fabricated. Scaffolds were made by sintering microspheres (9:1parts (Asc:Dex)) at 60ºC for 2 hours. The controlled release of Dex and Asc from the scaffolds in PBS was investigated over a 28 day period. The supernatant was harvested at various intervals and concentrations of Dex and Asc determined using HPLC. mES cells were seeded on the scaffolds and incubated in 15% FCS + 10mM β-glycerophosphate supplemented DMEM media for 28 days. Bone nodule formation was investigated through immunocytochemical and histochemical staining of scaffolds. The degree of osteogenesis was quantified using a mouse osteocalcin ELISA kit.

Results: Controlled release experiments showed the sustained release of Asc and Dex from the Dex/Asc loaded scaffolds over a period of 7 & 23 days respectively. The presence of Asc accelerated the release of Dex from the scaffold as indicated by the reduced initial burst (17.34 vs. 50.03%) and the gradual rate of release from Dex only containing scaffolds (Fig 1a).

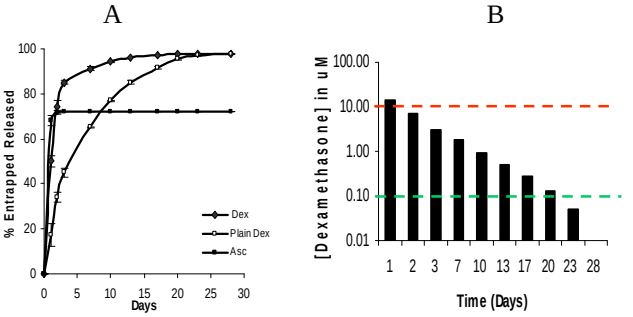

Figure 1: (A) Controlled release of dexamethasone (Dex) and ascorbate (Asc) from Dex + Ascorbate loaded PLGA microparticle-scaffolds and dexamethasone from Dex loaded PLGA microparticle scaffolds (Plain Dex). (B) Chart showing mES cells are exposed to dexamethasone concentrations within the range required for osteogenic induction.

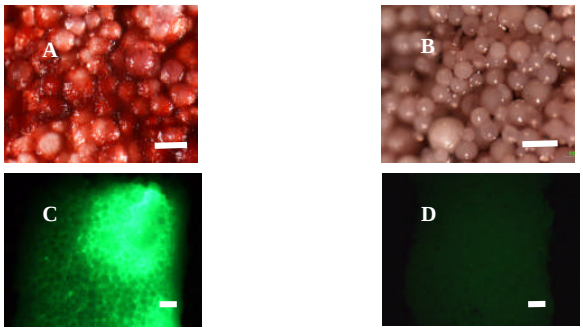

Figure 2: mES cells cultured on Dex /Asc loaded scaffold resulted in increased mineralized matrix deposition and osteocalcin production as detected by (A) Alizarin Red staining for calcium and (C) Immunocytochemical staining for osteocalcin. (B & D) Control Samples. Scale bar is 500µm.

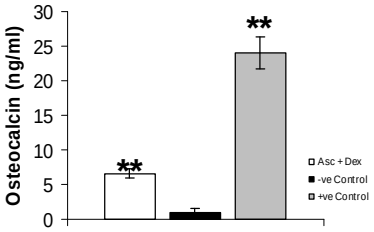

Figure 3: Osteocalcin levels present in media following 28 days of culture were measured to indicate differentiation of mES cells into osteoblasts. Those cultured on Dex + Asc loaded scaffolds, and on non-loaded scaffolds with exogenous addition of Dex + Asc (positive control) secreted significantly higher levels of osteocalcin when compared to mES cells cultured on non loaded scaffolds without exogenous addition of these supplements (negative control). Results are mean ± standard deviation (n = 3; **p<0:01).

Dex released from Asc/Dex scaffolds was observed to be within the range required for osteogenic differentiation of ES cells (fig 1b). Osteocalcin immunostaining, alizarin red staining (fig 2) and osteocalcin quantification (fig 3) indicated the presence of mineralized matrix deposition and mature osteoblast-like cells.

Conclusions: PLGA microsphere based scaffolds were successfully used for the sustained delivery of Asc and Dex to mES cells. Delivery of these factors stimulated differentiation into osteoblast-like cells as indicated by osteocalcin production and mineralized matrix deposition. Microsphere based scaffolds hold great potential to spatially deliver such factors to generate tissue composites[3]; this is the focus of current research.

References: (1) Buttery, L *et al.* Tissue Eng. 2001 **7:** 89 - 99 (2) Wei, G *et al.* J. Controlled Release 2006 **112**: 103–110. (3) Suciati, T, *et al.* J Mater Sci: Mater Med 2006 **17**:1049–1056.