

## Zonal release of proteins within tissue engineered scaffolds

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**INTRODUCTION:** The concept of releasing growth factors (GFs) over extended and controlled periods of time using microparticles is widely pursued within the field of tissue engineering<sup>1</sup>. Our approach uses growth factor loaded microparticles that may be built layer-by-layer into a 3D structure. The loose aggregate of microparticles is then sintered to form an interconnected porous matrix. These scaffolds could provide GF gradients to direct cell movement or to provide zonation of cell response to engineer multiple tissues in a single device that may be applied in multiphase tissue repairs (i.e. osteochondral defects). The aim of this study was to establish the methodology of producing such scaffolds with zonal protein release for tissue engineering.

**METHODS:** Horseradish peroxidase (HRP) or recombinant human bone morphogenetic protein-2 (rhBMP-2) was loaded into P<sub>DL</sub>LA microparticles using a Solid-in-Oil-in-Water (S/O/W) emulsion method<sup>2</sup>. The microparticles were then heat sintered to form scaffolds. The release profiles of HRP from both microparticles and scaffolds were performed by incubation in PBS and analysis of the protein concentration and activity using the micro-bicinchoninic acid (BCA) assay and 3,5,3',5'-tetramethylbenzidine (TMB) substrate, respectively. C2C12 mouse myoblasts were cultured on scaffolds consisting of rhBMP-2 loaded and rhBMP-2 free microparticles and the level of osteoblast induced differentiation measured using alkaline phosphatase (ALP). Zonal release of a tri-layered scaffold consisting of a layer of protein-free microparticles sandwiched between two layers of HRP loaded microparticles and a bi-layered scaffold consisting of a rhBMP-2 loaded zone and a rhBMP-2 free zone was analyzed by recording the sequential colour changes of TMB substrate and observing the staining of ALP-induced expression on C2C12 cells, respectively. The C2C12 cells cultured scaffolds were also stained for cell distribution using Toluidine Blue.

**RESULTS:** HRP was released in a controlled manner from microparticles and scaffolds over a 30-day period and the activity of released protein

was maintained throughout this period. The analysis of C2C12 cell response on scaffolds consisting of various portions of rhBMP-2 loaded microparticles showed a linear relationship between increasing ALP induced expression and the ratio of these particles. An intense TMB yellow product was formed almost exclusively in the HRP loaded zones of the triple layer HRP scaffold which confirmed the zonation of protein release within this scaffold. Fig 1 shows the zonal cell response within the bi-layer rhBMP-2 scaffold; a significant increase of ALP activity was observed in the rhBMP-2 loaded zone.

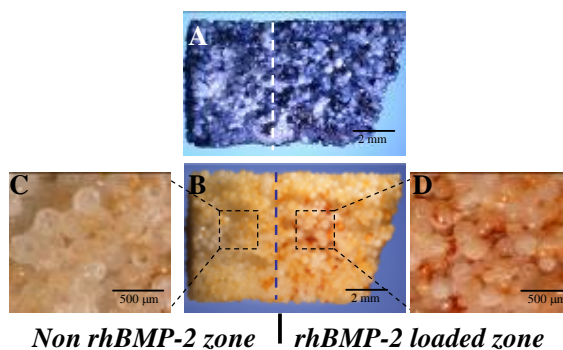


Fig. 1: Homogenous cell distribution (A) and location of ALP staining (B) demonstrating the lack of osteoblast induced differentiation of C2C12 cells in the non-rhBMP2 loaded zone (C) and positive ALP staining (red) in the rhBMP-2 loaded zone (D).

**DISCUSSION & CONCLUSIONS:** This study has demonstrated that zonal release of proteins to induce a location specific cell response can be achieved by organizing protein loaded microparticles into layered scaffolds. The doses of proteins within these scaffolds can be tuned by varying the ratio of protein-loaded and protein-free microparticles.

**REFERENCES:** <sup>1</sup>P.Q. Ruhe, et al, (2005) *J. Contr. Rel.* **106**:162. <sup>2</sup>T. Morita, et al. (2000) *J. Cont. Rel* **69**: 435.

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