Controlled Release of PRP-Derived Growth Factors from Alginate Hydrogel Carriers Promotes Osteogenic Differentiation of Mesenchymal Stem Cells

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Statement of Purpose: Platelet-rich plasma (PRP) is derived from blood plasma enriched with platelets, and contains growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGFβ), and insulin-like growth factor (IGF)[1]. PRP has been increasingly utilized in orthopedics and oral and maxillofacial surgery because of its reservoir of autologous growth factors and their potential to enhance bone regeneration. Despite its obvious advantages, a significant controversy exists regarding the clinical efficacy of PRP[2].

The osteogenic and osteoinductive potential of PRP are dependent on growth factor dosage, distribution and temporal sequencing[3,4]. To control the bioavailability of the PRP-derived growth factors, we have designed an alginate hydrogel-based PRP-delivery system based on solid alginate beads and capsules[5]. Previous work has shown that the released factors promoted cellular proliferation and alkaline phosphatase (ALP) activity of human osteoblast-like cells. The objective of the current study is to evaluate the osteoinductive potential of the PRP-derived growth factors released from alginate carriers on human mesenchymal stem cells (hMSCs). It is hypothesized that the released factors will promote proliferation and osteogenic differentiation of hMSCs.

Methods: *PRP Alginate Beads and Capsules*: PRP was prepared following Landesberg *et al.*[6]. The alginate carriers (see Fig. 1) were formed following the methods of Lu *et al.*[5]. Specifically, for alginate beads, the PRP was mixed with 2% alginate (Sigma) and dispensed into a 6% $CaCl_2$ solution. To form capsules, PRP in 6% $CaCl_2$ was dropped into a 1% alginate solution.

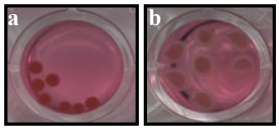

Fig. 1. Alginate Beads (a) and Capsules (b) with PRP.

Cells and Cell Culture: The hMSCs (Cambrex) were pre-seeded (5,700 cells/well) for 24 hrs and then incubated with alginate carriers over the two-week study period. Experimental groups include hMSC cultured with PRP only, beads w/ PRP, or capsules w/ PRP. Control groups were hMSC monolayer, hMSC cultured with beads w/o PRP, and hMSC stimulated with osteogenic media (50µg/ml ascorbic acid, 10mM beta-glycerophosphate and 10^{-7}M Dexamethasone). Cell growth (n=6) was measured by the PicoGreen assay and ALP activity (n=6) was determined via a colorimetric assay as well as histological staining (n=2). Degree of mineralization was assessed using Alizarin Red (ALZ) staining (n=2).

Results: *Cell Proliferation:* The hMSCs proliferated over time (Fig. 2), with a significant increase ($p<0.05$) in cell number in the control monolayer and beads w/ PRP groups at Day 14. Cells cultured in osteogenic media measured a significantly lower proliferation rate compared to all other groups at Day 14, suggesting that these cells may be undergoing active differentiation.

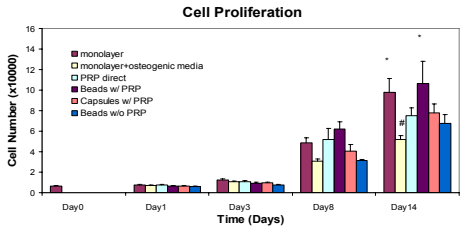

Fig. 2. Cell Proliferation for hMSCs Incubated with Alginate Carriers.

ALP Activity and Mineralization: Cell ALP activity was significantly higher ($p<0.05$) for hMSCs incubated with PRP-alginate capsules beginning at Day 3 (Fig. 3). Interestingly, the capsules w/ PRP group exhibited an ALP activity level similar to those of hMSCs treated with osteogenic media. Histological staining confirmed the quantitative results. ALZ staining revealed that mineralization was evident in the alginate capsule group and hMSCs treated with osteogenic media (Fig. 4).

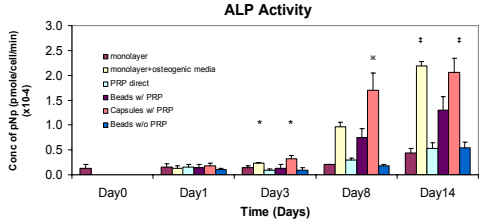

Fig. 3. ALP Activity of hMSCs Incubated with Alginate Carriers.

Discussion/Conclusions: The results of this study demonstrate the feasibility of controlling the bioavailability of PRP-derived growth factors using a hydrogel-based delivery system. More importantly, the released factors maintained bioactivity and had a positive effect on hMSC osteogenic differentiation. The PRP-derived factors released from the hydrogel carriers thus remained osteoinductive and osteoconductive *in vitro*. The role of specific factors in modulating hMSC differentiation in system is not yet known, and will be investigated in future studies, along with *in vivo* testing of the efficacy of the PRP delivery system.

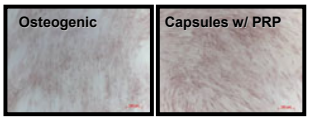

Fig. 4. hMSC Mineralization ALZ red staining, Day 14. (10X)

References: 1. Marx RE *et al*. Oral Surg 85:638, 1998; 2. Freymiller EG and Aghallo TL. J Oral Maxillofac Surg 62:484, 2004; 3. Tsay RC *et al*. J Oral Maxillofac Surg 63:521, 2005; 4. Dimitriou R *et al.* Injury 36:1392, 2005; 5. Lu HH, *et al*. J. Controlled Release, submitted; 6. Landesberg R, et al. J Oral Maxillofac Surg 58:297, 2000.