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Controlled Release of PRP-Derived Growth Factors Promotes Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Abstract*—Platelet-rich plasma (PRP) has been gaining increasing popularity in orthopedics and oral and maxillofacial surgery because of its potential efficacy in enhancing bone regeneration. To maximally augment bone healing using PRP and to control the bioavailability of the relevant growth factors, we have designed an alginate hydrogel-based PRP-delivery system. The bioactivity of the growth factors released from PRP carriers was evaluated by determining the ability of these factors to induce osteogenic differentiation of human mesenchymal stem cells (hMSCs). Specifically, monolayers of hMSCs were incubated with the PRP-containing hydrogel carriers over a two-week culture period. Osteoblast-like cells treated with the hydrogel carriers served as controls. The growth and osteogenic differentiation (alkaline phosphatase activity and mineralization) of hMSCs was determined. The results showed that PRP-derived growth factors released from hydrogel carriers stimulated the osteogenic differentiation of hMSCs and most significantly, the cellular response was carrier type-dependent. Future studies will focus on *in vitro* and *in vivo* testing of the efficacy of hydrogel-based PRP release systems.

I. Introduction

PLATELET-rich plasma (PRP) is derived from blood plasma enriched with platelets. Activated platelets release granules that contain growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor (IGF), which have the potential to augment bone regeneration in orthopedic and oral and maxillofacial surgery applications [1,2].

These growth factors are critically important in the natural bone regeneration process. It has been reported that PDGF (27-31kDa) stimulates mitogenesis of osteoblastic precursors and is a major stimulant in wound healing [6]. It has been shown that TGF-β (a dimer of 25kDa) induces proliferation and differentiation of mesenchymal cells into osteoblasts, stimulates collagen synthesis and inhibits degradation of newly-formed collagen [7]. The smallest of the three factors, IGF (7.6kDa), promotes bone formation by stimulating proliferation and protein synthesis in bone forming cells [3-8].

Regardless of the compelling theory behind the use of PRP in enhancing bone regeneration and the increasing popularity of its use in surgical procedures, there has been significant controversy regarding its effectiveness [9]. We postulate that the inconclusive reports regarding the clinical efficacy of PRP arose from a lack of information regarding the temporal bioavailability, dosage and spatial distribution of PRP-derived growth factors. To be effective, the release kinetics of these factors must match the cascade of events leading to bone regeneration as outlined in Figure 1 [10]. Previous work from our laboratory demonstrated that the level of growth factor release from PRP is a function of the preparation method. Current methods of PRP preparation using thrombin resulted in the *in vitro* release of over 90% of the PRP-derived growth factors (*e.g.* PDGF, TGF-β) within 24 hours [11].

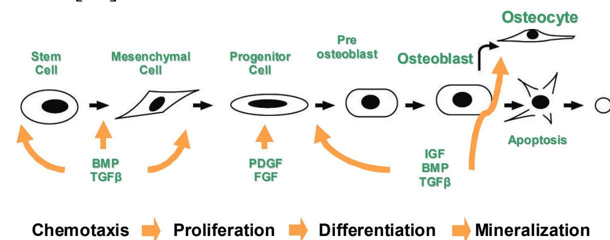

Fig. 1. The temporal sequence of growth factors required during bone regeneration.

To regulate growth factor bioavailability and increase the clinical efficacy of PRP, we propose that a controlled delivery system which temporally coordinates the molecular events during bone regeneration with the delivery of PRP-derived growth factors will maximally augment bone healing. In this study, we have designed a hydrogel delivery system of PRP-derived factors based on alginate biopolymer.

Alginate is extracted from brown algae, and contains linear unbranched polysaccharides consisting of 1-4 linked D-mannuronic acid (M) and L-gluronic acid (G) residues [12]. Gelation occurs in the presence of divalent cations, such as Ca^{2+} from $CaCl_2$ used in this study, and can be achieved by external or internal gelation. The resulting hydrogel contains pore sizes in the range of 5-200 nm. The alginate hydrogel is biocompatible, non-immunogenic and biodegradable. The use of divalent ions as a crosslinking agent preserves the bioactivity of cells, growth factors, and pharmacological agents. With its low cost and ease of use, alginate can also be optimized at room temperature to manipulate properties such as porosity.

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To control the bioavailability of PRP, we have designed an alginate hydrogel-based PRP-delivery system. The objectives of this study were 1) to determine the growth factor release profile of two hydrogel carriers (beads vs. capsules) and 2) to evaluate the bioactivity of growth factors released from alginate carriers on the osteogenic potential of both human mesenchymal stem cells (hMSCs) and osteoblast-like cells (SaOS-2).

We hypothesize that hydrogel delivery of PRP-derived growth factors will control bioavailability, preserve the bioactivity of the released factors and promote osteogenic differentiation of hMSCs and osteoblast-like cells.

II. Materials and Methods

A. PRP Alginate Beads and Capsules

PRP was prepared following Landesberg *et al* [13]. For alginate beads, PRP was re-suspended in 2% alginate (Sigma) and dispensed via a 26½-gauge needle into a 6% $CaCl_2$ solution. To form capsules, PRP in 6% $CaCl_2$ was similarly dispensed drop-wise into a stirring 1% alginate solution. Beads without PRP served as a control. The effect of substrate on the release of growth factors was also examined by comparing PDGF release from the different alginate preparation to that of various bone substitutes and PRP prepared with only thrombin.

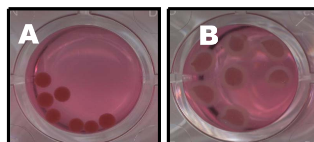

Fig. 2. (A) Alginate beads with PRP. PRP was uniformly distributed within the beads. Alginate beads were spherical and uniform in size (1.862 ± 0.0604 mm). (B) Alginate capsules with PRP in the center. Capsules had elliptical morphologies and were uniform in size (4.22 ± 0.37 mm x 3.09 ± 0.21 mm) with a wall thickness of 0.61 ± 0.05 mm. Morphology and membrane integrity of both carriers was maintained over time.

B. Growth Factor Release from Carriers

Beads and capsules were incubated in Dulbecco's Modification of Eagle's Medium (DMEM) at 37°C. The PRP capsules and supernatant were collected at 1, 3 and 7 days, and the PRP beads and supernatants were collected at 1, 3, 7, 14 and 21 days. Factor release for PDGF, TGF-β1, and IGF-1 from alginate carriers was measured by Enzyme-Linked Immunosorbent Assay (ELISA, R&D Systems).

C. Evaluation of Bioactivity of Alginate PRP carriers

Mesenchymal Stem Cells

Human mesenchymal stem cells (hMSCs, Cambrex) were pre-seeded (5,700 cells/well) and incubated with alginate carriers. Experimental groups were hMSC+PRP, hMSC+alginate beads with PRP, and hMSC+alginate capsules with PRP. Control groups were hMSC monolayer, hMSC+alginate beads without PRP, hMSC+osteogenic media (50μg/ml ascorbic acid, 10mM beta-glycerophosphate (β-GP) and 10^{-7}M Dexamethasone). Cell growth (n=6) was quantified using a PicoGreen assay. Alkaline phosphatase (ALP) activity (n=6) was measured using a colorimetric assay as well as histological staining. All experimental groups were cultured in fully supplemented DMEM with the addition of 3mM β-GP after day 7.

Osteoblasts

Human osteoblast-like cells (SaOS-2, ATCC#HTB-85) were pre-seeded ($5x10^4$ cells/well) and incubated with alginate carriers. Experimental groups were similar to that detailed above with hMSCs, and the cells were cultured in supplemented DMEM with the addition of 3mM β-GP after day 7. Beads without PRP and a monolayer of SaOS-2 served as controls. Cell growth and ALP activity were determined as above.

III. Results and Discussion

A. Growth Factor Release from PRP-Alginate Beads and Capsules

PDGF-AB

Release from the beads reached a maximum at 2 hours and plateaued thereafter. In contrast, PDGF-AB release from the capsules was continuous and increased significantly over time reaching a maximum at Day 14.

TGF-β1

Release from the beads increased over time within the 21 day incubation period, peaking at day 14. TGF-β1 release from the capsules was delayed until after day 7 and increased thereafter.

IGF

The majority of the factor was released from both carriers (beads and capsules) within 24 hours. A significantly higher amount of IGF was released from the capsules compared to the beads.

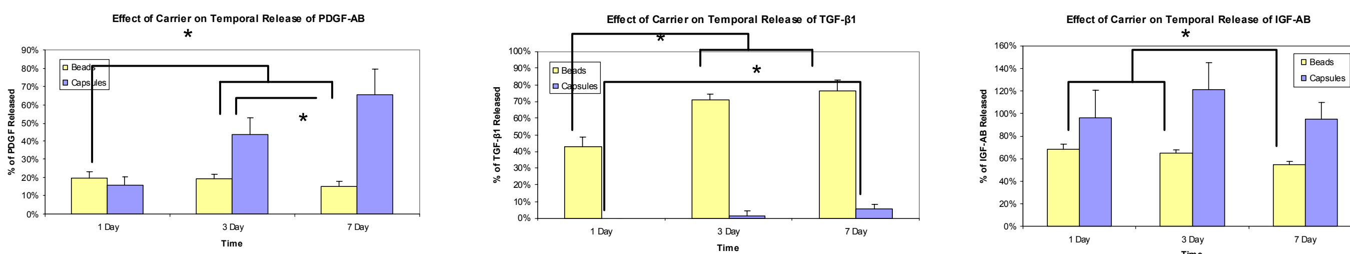

Fig. 3. Growth factor release profile from the two alginate carriers. Alginate beads and capsules demonstrated very different release profiles for PDGF-AB, TGF- β1 and IGF. The results showed that bioavailability and temporal sequence of growth factor release can be changed by manipulating the carriers.

Effect of Substrate on PDGF Release

PDGF release was examined, and it was found that alginate capsules were more effective in retaining the growth factors compared to bone substitutes and thrombin alone [11].

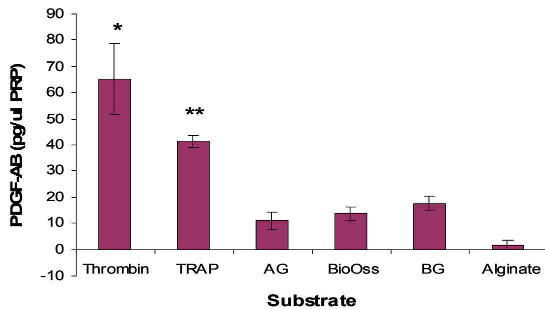

Fig. 4. Comparison of PRP-derived PDGF release from alginate capsules and other substrates.

B. Effect of Released Growth Factors on hMSCs

hMSCs Cell Proliferation

The hMSCs proliferated in all groups examined, with a significant increase in cell number at Day 14 in the control monolayer and PRP-alginate bead groups. Cells cultured in osteogenic media showed a significantly lower proliferation rate compared to the other groups at day 14, suggesting that these cells were undergoing active differentiation.

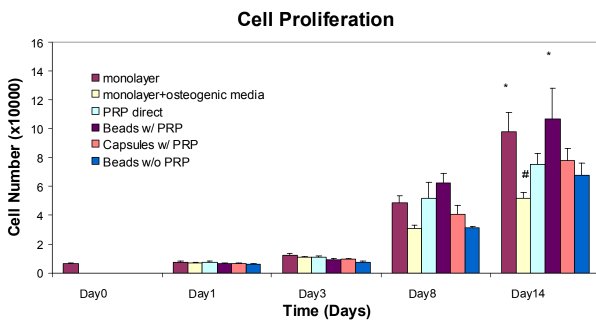

Fig. 5. Cell Proliferation for hMSCs

hMSCs ALP Activity

The ALP activity was significantly higher for the hMSCs with osteogenic media and PRP-alginate capsules beginning at Day 3. Factors released from the alginate capsules consistently induced ALP activity levels similar to those of hMSC culture treated with osteogenic media. The significantly higher ALP activity began on day 3 for the alginate capsule group. This corresponded to an increase in PDGF-AB and IGF release from the capsules during the same period.

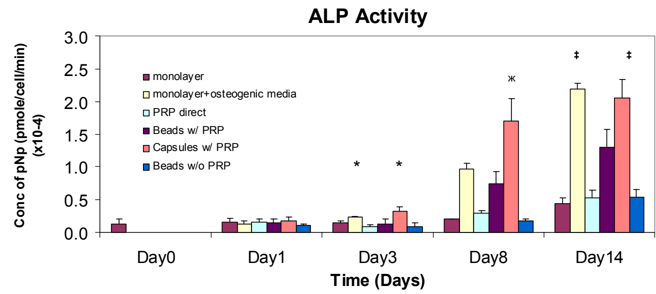

Fig. 6. ALP Activity for hMSCs

ALP Activity Staining

ALP staining on day 14 for monolayer control, hMSC+osteogenic media, hMSC+PRP, hMSC+beads with PRP and hMSC+capsules with PRP (10X). The highest ALP activity was seen in hMSCs treated with PRP-alginate capsules and with osteogenic media.

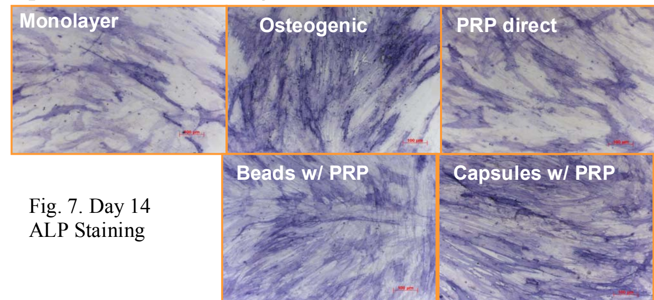

Fig. 7. Day 14 ALP Staining

C. Effect of Released Growth Factors on SaOS-2

SaOS-2 Cell Proliferation

Similar to the hMSCs, the SaOS-2 cells demonstrated a significant increase in cell number at day 3 in cells cultured with PRP-alginate beads and at day 14 for cells cultured with PRP-alginate capsules (data not shown).

SaOS-2 ALP Activity

Cell ALP activity was significantly lower for the PRP direct group at day 1, while it peaked for both the PRP-bead and PRP-capsule groups during the same period. In addition, a significantly higher ALP activity was measured for the capsule group than the bead group at day 1 (data not shown).

D. Correlation between Cell Response and Growth Factor Release

PDGF-AB

Release from the capsules was faster than that from the beads. As only 20% of incorporated PDGF was released from the beads over the 14 day period; these results suggest that PDGF-AB binds to the alginate bead matrix. Further release of PDGF-AB from the carriers is expected to occur after alginate degradation, which may then increase ALP activity in hMSCs.

TGF-β1

A significantly greater release was found from the beads than the capsules with 90% of the growth factor released from the beads by day 7. A continued release from the capsules with a corresponding increase in cell proliferation was demonstrated.

IGF

Release from the capsules was faster than that from the beads. Over 60-80% of the growth factor was released from both carriers after 24 hours, which could be explained by the small molecular weight of IGF (7.6 kDa). The release of IGF may have induced an increase in the ALP activity of hMSCs.

IV. Conclusions

The results of this study demonstrated the feasibility of controlling bioavailability of PRP-derived growth factors using hydrogel carriers with different geometries and surface areas. The released factors maintained bioactivity and had a positive effect on the osteogenic differentiation of hMSCs and osteoblasts. The PRP-derived factors released from the hydrogel carriers were osteoinductive and osteoconductive *in vitro*. How this specificity of cellular response relate to the release of growth factors was not yet clear, however, this will be investigated in future studies. Future studies will focus on *in vitro* and *in vivo* testing of the efficacy of hydrogel-based PRP release systems.

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