Controlled delivery of platelet-rich plasma-derived growth factors for bone formation

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Abstract: Platelet-rich plasma (PRP) represents an autologous source of growth factors essential for bone regeneration. The clinical efficacy of PRP is, however, unpredictable, and this is likely due to the inefficient and inconsistent delivery of PRP-derived growth factors. Previous investigations have shown that current methods of PRP preparation result in a premature release of the relevant bone stimulatory factors. As successful bone regeneration requires multiple factors presented in a physiologic temporal and spatial cascade, the objective of this study is to control the bioavailability of PRP-derived growth factors using a hydrogel carrier system. Specifically, the release of platelet-derived growth factor, transforming growth factor beta-1, and insulin-like growth factor from two types of alginate carriers was compared over time. The effects of the released factors on the growth and alkaline phosphatase (ALP) activity of human osteoblast-like cells were also evaluated. It was found that factor release profiles varied as function of carrier type, and binding of growth factors to the alginate matrix also modulated their release. The bioactivity of released factors was maintained *in vitro* and they promoted cell proliferation and ALP activity. These results demonstrate the potential of this autologous multifactor delivery system for controlling the bioavailability of PRP-derived factors. Future studies will focus on optimizing this system to increase the clinical efficacy of PRP by matching the distribution and temporal sequencing of PRP-derived factors to the bone healing cascade. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 86A: 1128–1136, 2008

Key words: platelet-rich plasma; alginate; growth factor delivery; bone regeneration

INTRODUCTION

Platelet-rich plasma (PRP) is derived from blood plasma[1,2] and serves as an autologous source of growth factors important in vascularization and bone regeneration. Orthopedic and maxillofacial bone defects are frequently reconstructed with either autologous or allogeneic bone grafts,[3] and PRP has been used as an adjunct to the healing bone grafts. The significant clinical interest in PRP stems from its potential as an autologous reservoir of essential growth factors for wound healing and bone regeneration. Furthermore, when combined with biological or synthetic bone grafts, PRP has been reported to improve the aggregation and cohesiveness of particulate-based bone substitutes.[2,4]

A number of growth factors are present in PRP, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-β1), and insulin-like growth factor (IGF).[5,6] In the early stages of wound healing, platelets are activated by thrombin and collagen, and growth factors are released by these activated platelets to facilitate repair and healing. The sequence of events (Fig. 1) leading to bone formation (chemotaxis, cell migration, proliferation, and differentiation) are regulated by growth factors,[7–12] many of which are present in PRP. For example, the recruitment of mesenchymal stem cells and progenitor cells to the site of bone regeneration is mediated by collagen as well as chemotactic

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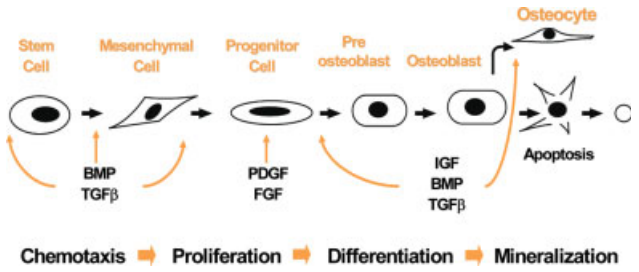

Figure 1. Schematic of bone regeneration cascade, including growth factor sequence necessary for the induction of mesenchymal stem cells for the formation of a mineralized matrix.

factors such as PDGF and TGF-β1. Moreover, PDGF and TGF-β1 stimulate cell proliferation,[7] and TGF-β1 also induces the osteogenic differentiation of mesenchymal stem cells.[8] Osteoblasts differentiation is controlled by IGFs and bone morphogenetic proteins,[9–12] and VEGF is critical in stimulating the angiogenesis necessary for bone formation and remodeling.

The compelling advantages of PRP for bone repair have fueled its increasing popularity in both orthopedic and maxillofacial surgical procedures. Unfortunately, the clinical efficacy of PRP is unpredictable and a significant controversy exists regarding the ability of PRP to augment bone repair.[1,2,13,14] This inconsistent response may be secondary to the rapid wash out of critical growth factors when PRP is used with graft materials as opposed to the temporal release of growth factors observed in nongrafted bone healing. Schmitz and Hollinger emphasized that successful utilization of PRP is hindered by insufficient understanding of the underlying biology.[15] The uncertainty regarding clinical efficacy of PRP is further exacerbated by limited knowledge regarding the bioavailability of PRP-derived growth factors.

The majority of *in vitro* and *in vivo* studies using PRP for bone healing have failed to demonstrate efficacy unless PRP is used in conjunction with an osteoconductive biomaterial, mesenchymal stem cells, or embedded in gelatin.[16–18] It is well established that the spatial and temporal gradient of relevant growth factors is critical for bone healing.[10,11,19–21] Reported studies of fracture healing have shown that cell proliferation peaks at 1 week while maturation and ossification proceed during weeks one to three.[1–3] It is, therefore, likely that exogenous factors presented in a physiologically relevant temporal sequence and spatial distribution will be more effective for bone regeneration. Previously, we compared growth factor release from PRP clotted by thrombin with that of PRP clotted with thrombin receptor activated peptide[22] and bone substitutes[4] such as BioOss®, Allogro® and BioGlass®. Over 80–90% of PDGF and TGF-β1 were released within the first 6 h when PRP was activated by thrombin. In contrast, only half of the factors were lost when PRP was mixed with bone substitutes. Therefore, the superior retention ability of the bone substitutes for PRP-derived factors is likely responsible for their enhanced bone healing potential *in vivo*.

Our long term goal is to increase the clinical efficacy of PRP by matching the distribution and temporal sequencing of PRP-derived factors to the bone healing cascade through the use of controlled delivery systems. There are currently no reported studies aimed at the controlled release of PRP-derived factors. The ideal release system should facilitate multifactor release, and more importantly, must be able to match temporal release of PRP-derived factors with those necessary for bone regeneration. Single and multigrowth factor delivery systems based on biological and synthetic polymers have been successfully used to promote bone formation and vascularization in a variety of applications.[23–26] In this study, to control the release of PRP-derived growth factors, we have designed an alginate hydrogel-based delivery system. Alginate is a polysaccharide biopolymer derived from algae, and has been investigated as simulated extracellular matrix,[27–29] scaffolds for tissue engineering[30–33] as well as factor delivery vehicles.[25,34,35] It is a well characterized biopolymer with the advantage of being biocompatible, nonimmunogenic, and biodegradable.[36] The polymer structure is comprised of linear block copolymer and unbranched polysaccharide chains based on D-mannuronic acid and L-guluronic acid.[37] The polysaccharide chains are crosslinked by divalent ion (e.g., Mg^{2+}, Ca^{2+}) binding of carboxylic acid groups on the guluronic acid chain.[38] The ambient gelation preserves the bioactivity of cells and factors, and both polymer matrix (bead) or membrane-based (capsule) hydrogel systems can be readily fabricated.

To exert greater control over the temporal availability of PRP-derived factors, this study examines PRP release from both alginate beads and capsules. Factor delivery is expected to be faster from the beads than the capsules, as an immediate surface is available for diffusion from the bead matrix. Specifically, the release of PRP-derived factors from the alginate carriers was evaluated over a 3-week period, and the bioactivity of the released factors was assessed by determining their effects on the growth and differentiation of human osteoblast-like cells. It is anticipated that the findings of this study will facilitate the design and optimization of an autologous and functional multifactor PRP delivery system. The successful utilization of such a system for bone regeneration would represent a major advance in the repair of orthopedic and maxillofacial bone defects.

MATERIALS AND METHODS

Preparation of PRP

PRP was prepared by a modification of the method of Landesberg et al.[6] Briefly, 60 mL of venous blood from healthy adult volunteers was mixed with Anticoagulant-Citrate-Dextrose (ACD). The ACD solution contained 13.2 g/L trisodium citrate, 4.8 g/L citric acid, and 14.7 g/L dextrose. The mixture was centrifuged at 200 × *g* for 15 min (ACE Surgical Supply Company, Brockton, MA). The plasma and buffy coat layers were collected and spun at 200 × *g* for 10 min. After discarding the platelet-poor plasma, the lower half of the plasma and pellet were resuspended and collected as the PRP. This preparation method yields an average platelet count of 0.5 to 1.0 × 10^6 platelets/μL.

Fabrication of PRP alginate beads and capsules

Carrier fabrication protocol was achieved after optimization with respect to the volume ratio of PRP-to-alginate or PRP-to-$CaCl_2$, needle gauge and drop height. The goal was to form spherical beads and capsules with uniform wall thickness. Specifically, alginate beads containing PRP were fabricated through the internal gelation process by first adding PRP to 2% alginate solution made from alginic acid sodium salt derived from brown algae (Sigma, Medium Viscosity). The mixture was then dispensed via a syringe needle (26½-gauge) into 6% $CaCl_2$ (Sigma). The PRP + alginate mixture was gelled by the diffusion of Ca^{2+} ions into the polymer mixture. After gelation, the beads were incubated in $CaCl_2$ solution for 5 min to complete the gelation process. Alginate capsules with PRP were formed via the external gelation process. Specifically, PRP was first combined with a 6% $CaCl_2$ solution (2:5 volume ratio; Sigma), and the mixture was then dispensed dropwise through a syringe needle (26½ gauge) into a stirring 1% alginate solution. The alginate capsules were formed by an outward diffusion of Ca^{2+} ions from the PRP + $CaCl_2$ mixture. After gelation, the capsules were stirred in the $CaCl_2$ solution for 5 min to ensure the completion of the gelation process. The morphology and dimensions of the alginate-PRP capsules and beads were characterized post fabrication.

PRP-derived growth factor release from hydrogel carriers

The temporal release of growth factors was examined as a function of alginate carrier type. Specifically, beads + PRP and capsules + PRP were incubated in Dulbecco's Modification of Eagle's Medium (DMEM, Mediatech, Herdon, VA) at 37°C in a humidified environment. The cumulative factor release from beads was measured at earlier time points (0 h, 2 h, 1, 3, 7 days) as preliminary trials showed a rapid release from the alginate beads when compared with capsules. Factor release from capsules was thus determined over a 3-week period. For each sample, both the supernatant and carrier were collected to measure the amount of factor released as well as retained by the carrier.

Quantification of growth factor release (PDGF-AB, TGF-β1, and IGF-1)

The supernatant concentrations of PDGF-AB, TGF-β1, and IGF-1 released from the beads and capsules were determined by Enzyme-Linked Immunosorbent Assay (ELISA, R&D Systems, Minneapolis, MN). The corresponding concentrations of growth factors remaining in the beads or capsules were also measured. When comparing between carrier types, the percentage factor released was calculated based on the ratio of the amount released and the amount preloaded at 0 h. To extract the factors from the hydrogel, the samples were first dissolved in 55 m*M* of sodium citrate, as the competitive binding of sodium citrate for Ca^{2+} leads to dissociation of the matrix.[39]

The amount of PDGF-AB (n = 4) was measured following the manufacturer's protocol. Briefly, the samples were incubated for 3 h at 4°C on a plate coated with monoclonal antibody to PDGF-AA. Following washing, a conjugated secondary antibody to PDGF-BB was added and the samples were incubated at room temperature for another hour. After buffer washes, a substrate solution was added and the reaction was terminated after 20 min. The absorbance was determined at 450 nm (SpectraFluor Plus, Tecan, Maennedorf, Switzerland). A standard curve was generated and used to determine sample PDGF concentrations (pg/mL). The amount of growth factor was calculated based on total sample volume and dilution factor.

Similarly, the concentrations of TGF-β1 (n = 4) and IGF (n = 4) were assayed by ELISA. For TGF-β1, a dilution series of standards was prepared in 100 μL in microtiter plates coated with TGF-β1 receptor type II. The samples were activated with 0.1 mL of 1.0*N* HCl, then neutralized by 0.1 mL of 1.2*N* NaOH/0.5*M* HEPES. After 3 h, an enzyme-conjugated polyclonal antibody to TGF-β1 was added and incubated for 2 h at room temperature. The absorbance was measured at 450 nm. For IGF-1, the samples were incubated with precoated monoclonal antibody specific to IGF-1 for 2 h, followed by 1-h incubation with the secondary antibody at 4°C. The substrate solution was added, and absorbance was determined at 450 nm. Standard curves for TGF-β1 and IGF-1 were generated and the amount of growth factor (pg) was calculated based on total sample volume and dilution factor used.

In Vitro evaluation of bioactivity of released growth factor

A short term *in vitro* study was conducted to evaluate the bioactivity of the PRP-derived growth factors released from the hydrogel carriers by incubating the alginate beads and capsules with human osteoblast-like cells (SaOS-2, ATCC#HTB-85). Briefly, the cells were preseeded at 5 × 10^4 cells/well, and the alginate carriers were added to the well at day 2. The cells were cultured with the carriers in DMEM supplemented with 10% fetal bovine se-

rum, L-glutamine, 1% nonessential amino acids, and 1% antibiotics (Mediatech). Control groups included alginate beads without PRP and monolayer cultures of SaOS-2 cells. Cell growth and alkaline phosphatase (ALP) activity were monitored over 2 weeks. Cell proliferation ($n = 6$) was quantified using the Picogreen dsDNA Quantitation assay (Molecular Probes, Eugene, OR). Fluorescence intensity was measured at 535 nm (Tecan) and correlated to DNA concentration using a standard curve. Cell ALP activity was assessed histologically by Fast Blue staining.[40] The ALP staining solution was prepared by first dissolving the Fast Blue RR Salt (Sigma) in deionized water, and diluting the solution with Naphthol AS-MX Phosphate Alkaline Solution (Sigma). The samples were prefixed with neutral buffered formalin, incubated in the ALP staining solution for 30 min, and then imaged under light microscopy.

Statistical analysis

Results are presented as mean ± standard deviation, with n equal to the number of samples analyzed. Two-way analysis of variance (JMP IN®, SAS, Cary, NC) was used to determine significant differences between means. The Tukey-Kramer *post hoc* test was performed for all pair-wise comparisons and significance was set at $p < 0.05$.

RESULTS

Characterization of PRP hydrogel carriers

The as-fabricated alginate-PRP beads [Fig. 2(A)] were uniformly shaped, with an average diameter of 1.9 ± 0.06 mm and an average volume of 2.3 μL PRP was incorporated. The alginate-PRP capsules [Fig. 2(B)] were also uniform (4.2 ± 0.4 mm by 3.1 ± 0.2 mm) and measured a wall thickness of 0.6 ± 0.1 mm. The PRP was uniformly distributed in the carriers, with the platelets found throughout the bead matrix, and enclosed within capsule membrane. The intense red coloration of PRP within the carriers was prominent post fabrication and diminished thereafter. Matrix integrity was maintained throughout the 3-week study period.

Release of PDGF, TGF-β1, and IGF from alginate beads and capsules

The temporal release of PDGF from alginate carriers is summarized in Figure 3. The amount of PDGF released is combined with the amount remaining in the carrier, then compared to the total factor preloaded into the carrier. Post fabrication, the total amount of PDGF in the beads was 965 ± 278 pg, approximately double the amount of factors loaded into the capsules (414 ± 68 pg). The release of PDGF from the beads reached a maximum at 2 h [198 ± 20 pg, 21% released, Fig. 3(A)], with no significant release measured after day 1. PDGF release from capsules increased significantly with time [Fig. 3(B)], reaching a maximum after 14 days, with a significantly higher amount of PDGF measured at day 14 compared to day 1.

The release of TGF-β1 from alginate beads and capsules are shown in Figure 4. Post fabrication, the total amount of TGF-β1 in the beads was 625 ± 83 pg [Fig. 4(A)], while the average amount of TGF-β1 preloaded into the capsules was 279 ± 103 pg [Fig. 4(B)]. The amount of TGF-β1 released increased with time as the factor level within the beads decreased. By day 7, over 80% of the factor was released. In contrast, TGF-β1 release from capsules was not detectable until day 7, reaching 15% by day 14 and 20% by day 21.

The release of IGF from the alginate carriers was monitored over time (Fig. 5). A similar amount of IGF-1 was loaded into the capsule and beads (2.3 ± 0.3 pg and 2.8 ± 0.2 pg, respectively). The majority of the IGF was released from the beads [1.9 ± 0.1 pg, 68% released, Fig. 5(A)] and capsules [2.3 ± 0.6 pg, 97% released, Fig. 5(B)] within 24 h. There was a significant decrease in the level of IGF present in the PRP alginate beads after 2 h.

Effects of carrier type on kinetics of growth factor release

The effects of carrier type on PDGF-AB, TGF-β1, and IGF release are summarized in Figure 6. For PDGF [Fig. 6(A)], a significantly higher and continuous release was found in the capsules, with over 60% of the growth factor released by day 7, while PDGF release from the beads plateaued at day 1 at about 20%. In contrast, TGF-β1 release was much greater from the beads compared to the capsules [Fig. 6(B)], with over 75% of the factor released from the beads by day 7, and less than 10% released from the capsules over the same period. For IGF [Fig. 6(C)], over 90% of the factor was released from the capsules by day 1 versus about 70% from the beads. Significantly higher IGF release from the capsule was found at days 3 and 7.

Effects of released PRP-derived growth factors on osteoblast differentiation

The bioactivity of the growth factors released from the hydrogel carriers was evaluated using human osteoblast-like cells. As shown in Figure 7 (A), no significant difference in cell number was found between groups at day 1. Two days later, cell number was significantly higher for the group cultured

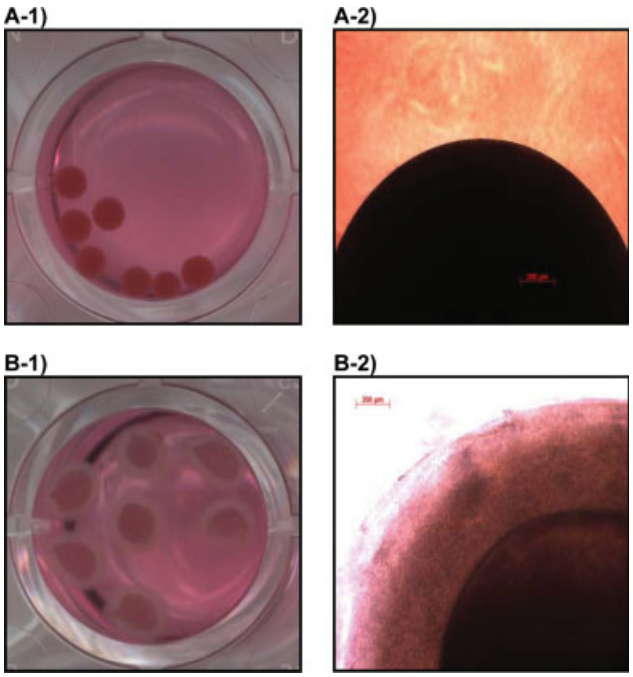

Figure 2. Alginate carriers for PRP delivery. (A-1) Alginate beads with PRP; (A-2) alginate beads with PRP (×20); (B-1) alginate capsules with PRP; (B-2) PRP encapsulated within alginate capsule membrane (×20). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

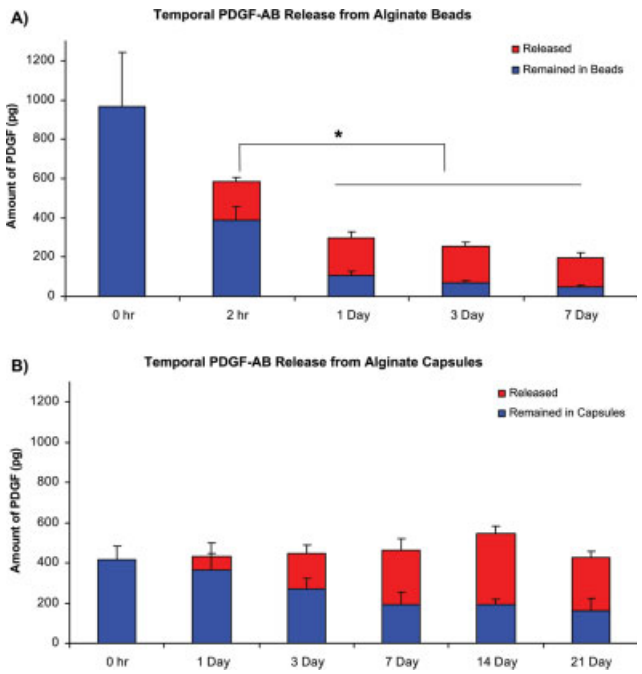

Figure 3. Temporal release of PDGF-AB from the alginate carriers. The amount of growth factor released is combined with the amount of factor remained in the carrier and compared to the total amount of factor preloaded into the carrier at 0 h. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with alginate beads ($p < 0.05$), while cell number increased significantly at day 14 for the group cultured with the capsules. The ALP activity of the SaOS-2 cells [Fig. 7(B)] was higher at day 1 for cells cultured with capsules, with the greatest staining intensity found in the capsule group when compared with the bead group and controls.

DISCUSSION

We have described here an alginate-based delivery system for regulating the release of PRP-derived growth factors. The temporal release profiles of PDGF-AB, TGF-β1 and IGF-1 were monitored as a function of carrier type, and the potential of the released factors to modulate osteoblast growth and differentiation was evaluated. It was found that factor release was dependent on carrier type and were also factor-specific. Interaction of the growth factors with the alginate matrix also modulated their release. Moreover, bioactivity of the released factors was maintained, and these factors promoted cell proliferation and ALP activity in human osteoblast-like cells.

In this study, the release of PRP-derived growth factors from the hydrogel carrier varied as a function of carrier type. We had anticipated that factor release would be more rapid from beads, given the greater diffusion distance associated with release from cap-

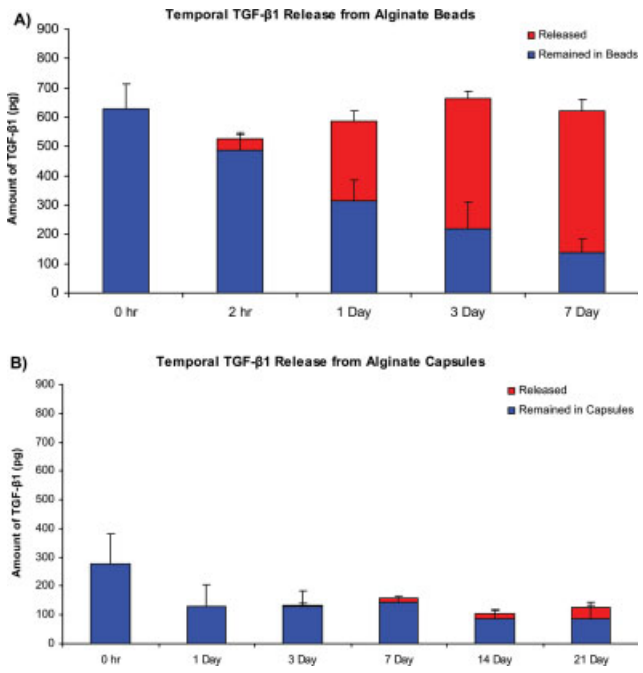

Figure 4. Temporal release of TGF-β1 from alginate carriers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

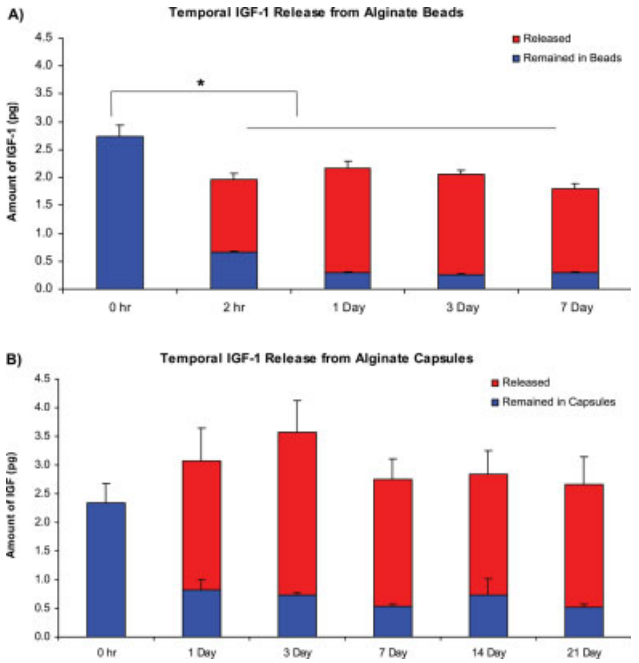

Figure 5. Temporal release of IGF-1 from alginate carriers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sules. This was indeed valid for TGF-β1, as a significantly higher factor concentration was consistently measured for the alginate beads compared to the capsules. In contrast, both PDGF and IGF were released at a significantly higher rate from the alginate capsules compared to the beads, and this difference between carriers was more pronounced for PDGF when compared with IGF.

A closer examination of the data revealed that for alginate beads, the mass balance profile for both PDGF and IGF was significantly lower compared to the preload value of each factor, while no such disparity was found when these factors were encapsulated in the alginate capsules. These observations suggest that unlike the capsules, the loaded factors may be interacting with the alginate matrix instead of being released from the beads. In the present study, to determine the amount of factors remaining in the carriers, the alginate matrix was dissociated by chelation of Ca^{2+} with sodium citrate. Therefore, to test whether all of the growth factors have been extracted, the bead samples were reprecipitated and incubated for an hour with sodium citrate. This second extraction yielded another 5% recovery of PDGF, which was not sufficient to account for the over 60% difference in total PDGF at day 1. Thus, interaction between PDGF and alginate is likely responsible for the significantly lower release measured in the bead group. Alginate is routinely used for bioseparation of proteins,[41] and gentamicin sulfate has been reported to selectively interact with the mannuronic residues of the alginate molecule.[25] In addition, the oligosaccharide domains of the alginate molecules have been reported to be cofactors for VEGF function and endothelial cell proliferation.[42] It is likely that PDGF is retained in the alginate matrix by binding or interacting with the structural domains of the polymer chain.

As the capsules and beads were both composed of alginate, the observed differences in PDGF-matrix interactions may be further attributed to the gelation process. Alginate capsules are fabricated by the outward diffusion of Ca^{2+} through the alginate matrix, while the beads are formed by the inward diffusion of Ca^{2+} into the alginate gel. These two distinct processes led to markedly different exposure times of PRP to alginate. To form beads, the PRP is first mixed with alginate and then dropped into calcium

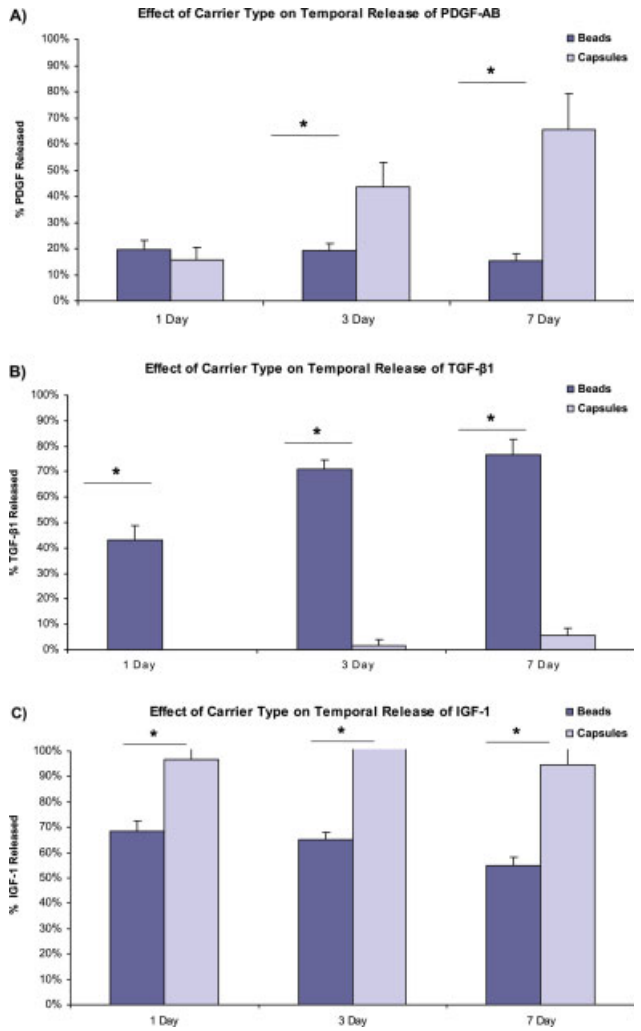

Figure 6. Effects of carrier type on the release of PRP-derived growth factors. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

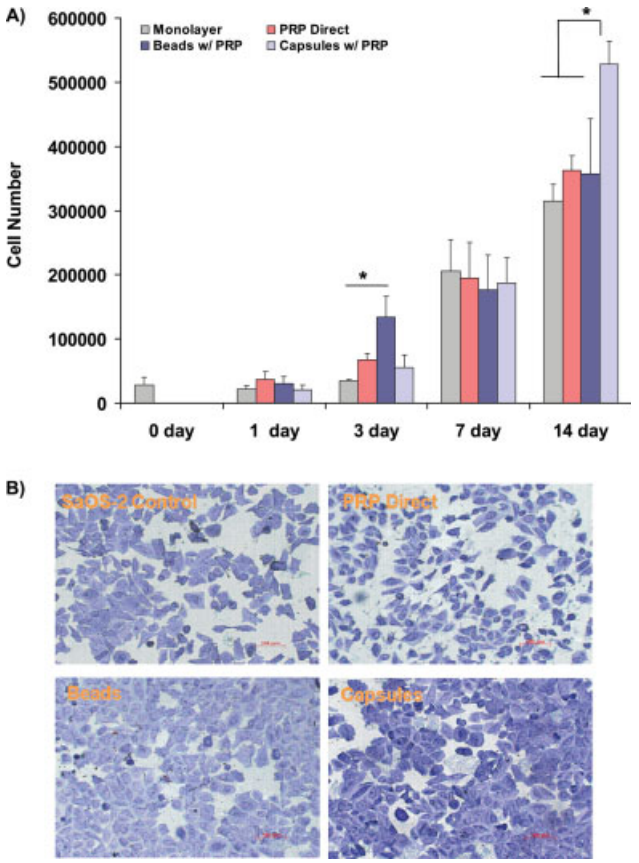

Figure 7. Bioactivity of factors released from alginate carriers. The PRP-derived growth factors released from the carriers remained bioactive and promoted carrier-dependent increases in the (A) proliferation and (B) alkaline phosphatase (ALP) activity of osteoblast-like cells. (A) Factors released from alginate beads increased cell proliferation on day 3 while those from the capsules promoted significant increase on day 14. (∗: $p < 0.05$). (B) The PRP + capsule group promoted the ALP activity of human osteoblast-like cell (day 1, ×10, clockwise from top left: SaOS-2 control, PRP direct, capsules, beads).

chloride solution. In contrast, the PRP is mixed with calcium chloride and then dropped into the alginate solution to form capsules. The prolonged exposure of PRP to alginate during bead fabrication permits the PRP-derived factors to readily bind to the polymer matrix, while rapid crosslinking of the polymer chains by Ca^{2+} during capsule gelation may decrease the availability of PDGF binding domains in alginate.

When comparing PRP-growth factor release from the alginate carriers to PRP clotted with thrombin and bone substitutes, the percentage of factor released from the hydrogel carriers is significantly lower than that seen with the bone substitutes (Fig. 8). The carriers also serves as a multifactor release system, and if used in conjunction with clinically available bone filler materials, will likely extend the bioavailability of the growth factors and improve the outcome of bone reconstruction surgeries. Another advantage of the hydrogel carrier system is its versatility in design and the inherent potential to control both the temporal sequence and spatial distribution of the released factors. For example, PRP loading efficiency can be increased by using higher volume of PRP, and total factor release may be increased by varying carrier surface area to volume ratio or the number of carriers utilized. The number of beads and capsules and combination thereof, can be controlled to provide sustained delivery of each factor, in addition to tailoring the growth factor release profiles to match that of the temporal gradient necessary for bone repair. Identifying the optimal combination of beads and capsules will be critical for achieving a consistent release of physiologically relevant growth factors corresponding to the bone regeneration cascade, and will be investigated in future studies. The observed interaction between alginate matrix and growth factors could also be advantageous, as bound factors such as IGF-1 or PDGF would become available as the hydrogel degrades, thus extending their bioavailability. Furthermore, the spatial gradient of these factors may be controlled by embedding the hydrogel carrier in a PRP clot, and bone morphogenetic proteins, which are not found in PRP, can also

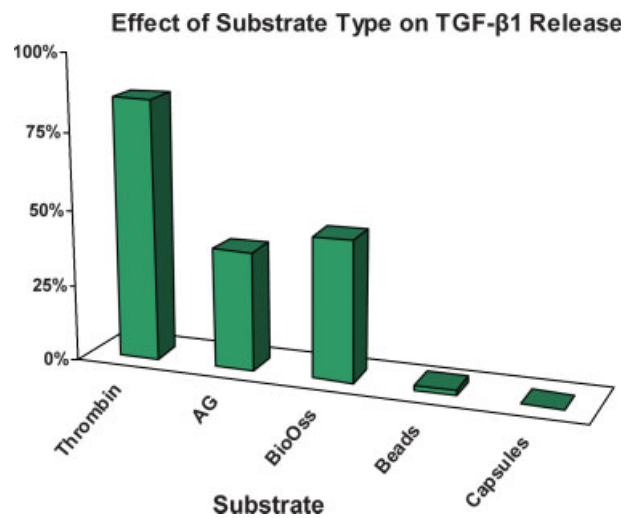

Figure 8. Release of TGF-β1 from alginate carriers compared to current methods of PRP preparation. When PRP is clotted with thrombin, over 90% of TGF-β1 was released within 2 h. The addition of bone substitutes such as Bio-Oss or allograft to PRP significantly reduced growth factor release by 50%. However, when TGF-β1 was delivered from alginate carriers, less than 10% of the factor was released from the beads and capsules. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

be incorporated into the hydrogel matrix to provide the full range of relevant factors necessary for bone regeneration.

From a functional perspective, the growth factors released from the alginate beads and capsules retained their bioactivity and promoted the growth and differentiation of human osteoblast-like cells. Most interestingly, as factor release differed as a function of carrier type, cell response also varied between the bead and capsule groups. A significantly higher cell number was measured at day 3 in osteoblast-like cells cultured with alginate beads, while a similar response was found in the capsule group at day 14. These results suggest that the responsible mitogenic factor was released first from the beads then later from the capsules. This finding is most likely due to PRP-derived TGF-β1, that showed over 40% release from the beads when compared with the capsules (<1%) at day 1. The majority of TGF-β1 (>90%) was released from the beads after 1 week, while TGF-β1 release from the capsules peaked at about 30% on day 14. This coincided with the increase in cellular proliferation seen in the capsule group. Similarly, higher amounts of PDGF and IGF were released from the alginate capsules compared to the beads at day 1, and the IGF may be responsible for the increase in ALP activity observed in the osteoblast-like cells cultured with capsules. It is noted here that the *in vitro* study was conducted in the presence of 10% serum, the specific effects of the released factors will be investigated in the future under serum-free conditions or through blocking assays.

Future studies will also focus on the optimization of the alginate-based carrier system for multifactor release. The objective is to tailor the release cascades to match those required for bone regeneration, enabling essential factors to be available at physiologically effective concentrations and during critical periods for osteogenesis. Carrier degradation kinetics as well as the osteoconductive and osteoinductive potential of the released growth factors will be determined by evaluating the *in vivo* efficacy of the hydrogel delivery system.

CONCLUSIONS

This study describes the design and testing of a hydrogel-based delivery system of PRP-derived growth factors. The temporal bioavailability of these factors can be controlled by altering carrier type between alginate beads or capsules. Furthermore, the bioactivity of the released growth factors was maintained and these factors promoted the proliferation and differentiation of human osteoblast-like cells.

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