Biologically Active Blood Plasma-Based Biomaterials as a New Paradigm for Tissue Repair Therapies

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Abstract

Platelets contain an array of growth factors, extracellular matrix molecules, and other signaling molecules that are released into the injury site upon platelet activation, thus providing the cues needed to help initiate and orchestrate tissue repair. In an effort to harness this activity for therapeutic use, autologous concentrated platelet-rich plasma (PRP) has become a popular therapy, particularly in sports medicine, where the desired outcome is to overcome the body’s limitations to tissue repair and accelerate healing. However, the effectiveness of PRP therapies remains controversial due to variable clinical outcomes. Potential sources of variability include the different types of platelet concentrators as well as the inherent variation in patient-to-patient platelet and growth factor quality and quantity. Additionally, PRP, either activated pre- or postinjection, cannot be spatially contained in an injury site and is rapidly resorbed. A further limitation to PRP is that it is not a simple, off-the-shelf solution. We have developed solid, bioactive plasma-based biomaterials (PBMs) that may address these issues. Unlike platelet concentrators, which concentrate the platelets and subsequently discard a significant volume of plasma, we utilize the entire plasma (including the platelets), which contains vital growth factors and other components that are not platelet-derived. Pooled plasma is utilized to reduce PBM lot-to-lot variability. PBMs are inexpensive to manufacture, safe, available as off-the-shelf products, formable into complex 3D shapes, and biodegradable with tunable biomechanical and degradation properties. PBM retention of growth factor activity has been demonstrated with the use of cell proliferation assays. Biocompatibility has been established both in vitro and in vivo. Further, the initial feasibility of PBMs to stimulate the repair of a bone defect was demonstrated in a mouse calvarial defect model. Overall, PBMs represent a platform technology with significant potential to be a disruptive new therapy option in a variety of clinical applications, not only in major clinical markets, but also in third world and developing countries, where the need is high, but where the cost is a barrier to treatment.

Key words: biology; biomaterial; engineering; growth factors; medicine; platelet-rich plasma

Introduction

The therapeutic use of autologous, concentrated platelet-rich plasma (PRP) to enhance healing is an exciting, but somewhat controversial new treatment option for a variety of orthopedic and sports medicine applications. Under normal physiology, platelets circulating in blood are responsible for initiating the healing of tissue injuries. This is due to the array of growth factors and other signaling molecules that are released into the injury site upon platelet activation, thus providing the cues needed to help orchestrate self-repair by the body. Therefore, it seems logical that collecting a patient’s own plasma, concentrating the platelets, and then injecting the PRP back into an injury site should accelerate healing and could overcome the body’s limitations to tissue repair caused by age, disease, or tissues of low vasculature that require long healing times.

Motivated by this logic, PRP injections have become a popular therapy, especially in sports medicine. PRP is also being explored for use in emerging regenerative medicine technologies, including PRP-supplemented media for stem cell expansion cultures and PRP-augmented tissue-engineered scaffolds. The effectiveness of PRP therapies, however, remains controversial due to variability in the clinical outcomes of this treatment. A further limitation to PRP is that it is not a simple, off-the-shelf solution.
We have developed solid, biologically active materials called plasma-based biomaterials (PBMs) that may address these issues. Unlike with platelet concentrators, which concentrate the platelets by discarding a significant volume of the platelet-poor plasma, we fully utilize the blood’s plasma and platelet fractions (which we will refer to as a PRP), which retains the vital plasma-derived growth factors, including the insulin-like growth factor 1 (IGF-1) that the platelet concentrators discard—a primary distinction between PRP therapies and PBMs.

PBMs are inexpensive, safe, available as off-the-shelf products with low lot-to-lot variability, shelf stable at room temperature, formable into complex 3D shapes, and biodegradable with tunable properties (Fig. 1). PBMs are made with pooled plasma units from multiple donors collected in U.S. blood banks and processed into plastic-like materials that remain bioactive. Although U.S. donated and screened blood products are considered the safest in the world, proprietary viral inactivation processes are employed in PBM manufacturing as an added safety precaution against contamination.

This article describes certain, nonproprietary aspects of PBM formulation, in vitro and in vivo feasibility studies, and hurdles for commercialization and broad acceptance of this novel and disruptive technology.

Materials and Methods
Platelet-rich plasma

There is currently confusion regarding what constitutes platelet-rich plasma (PRP). The decade-old and accepted definition by the American Association of Blood Banks (AABB) is simply that PRP is blood soft spun to remove red blood cells. The platelets in this material can be concentrated further to produce platelet concentrates, which are similar to the autologous PRP produced by the platelet concentrators utilized for therapeutic use. To differentiate the AABB-defined PRP from the platelet concentrator-produced PRP, we will call the AABB PRP “aPRP” and leave the platelet concentrator version as “PRP” due to its entrenchment in the vernacular. These aPRP units were prepared by standard methods at a FDA registered and AABB accredited blood center, the Central Blood Bank, part of the Institute for Transfusion Medicine (Pittsburgh, PA). Citrate Phosphate Dextrose (CPD) supplemented whole-blood units were spun to pack the red cells, with the remaining plasma and platelets expressed into a satellit bag and frozen within 8 h of collection. In these preparations, the white blood cells are approximately evenly split between the red blood cell and PRP fractions.

PBM manufacturing

Frozen aPRP is thawed and clotted by adding calcium chloride. The clotted material is freeze-dried and ground into a powder. The powdered material is mixed with glycerol (plasticizer) and other components, such as crosslinking agents and fillers, as needed. The resultant mixture forms a dough-like material that is compression molded at low temperature to form a shelf-stable PBM retaining native plasma biological activity. The typical formulation tested consisted of 65% plasma powder, 35% glycerol, and 0.25% genipin and was molded at 65°C for 25 min at 2,200 lbs unless otherwise noted in the text. For some experiments, beta tricalcium phosphate ($\beta$-TCP) or bone morphogenetic protein 2 (BMP-2) were added as indicated.

Enzyme-linked immunosorbent assays

Levels of IGF-1, platelet-derived growth factor (PDGF)-AB, and transforming growth factor beta 1 (TGF-$\beta$1) were determined using QuantiKine<sup>®</sup> enzyme-linked immunosorbent assays (ELISAs) from R&D Systems (Minneapolis, MN). All assays were performed as described in the kits instructions for plasma/serum samples. ELISAs were performed on components at three different steps along the manufacturing pathway: (1) the initial liquid aPRP; (2) a phosphate-buffered saline (PBS) extract of the freeze-dried, clotted plasma; and (3) a PBS extract of milled PBM (milling was performed to increase extraction efficiency) Detected growth factor levels were corrected for sample dilution and plasma levels.

Mechanical testing

Mechanical testing was performed according to ASTM D638 for Type V samples. Samples were tested on a Mark-10 ESM test stand (Mark-10, Copiague, NY) at a crosshead rate of 10 mm/min. Young’s moduli, ultimate stress, and percent elongation at break were measured as directed per the ASTM guidelines.

In vitro biocompatibility

Genipin-crosslinked PBM, approximately 5×5×1 mm, were placed in 48-well plates. The samples were sterilized in 70% ethanol for 1 min followed by two 10-min washes in calcium- and magnesium-free (CMF) PBS. NIH 3T3 mouse fibroblasts, human MG-63 preosteoblasts, mouse C2C12 stem cells, and human mesenchymal stem cells (all from ATCC, Manassas, VA) were seeded into the wells at 15,000 cells per well in serum media. Three days postseeding, the specimens were washed in PBS and fixed in 2% glutaraldehyde in PBS for 2 h at room temperature followed by overnight at 4°C. After three PBS washes, the specimens were fixed for 1 h in buffered 1% OsO<sub>4</sub>. The OsO<sub>4</sub> was removed with 3 five-minute distilled deionized water (ddH<sub>2</sub>O) washes, followed by dehydration in an ascending series of ethanol.
media containing 50 l Dulbecco’s modified Eagle medium (DMEM) serum-free extracts, the crushed sample powder was incubated in cooled mortar and pestle. To prepare PBM growth factor gen and pulverized into a powder using a liquid nitrogen-cooled mortar and pestle. Retention of bioactivity

Approximately 100 mg of PBM was frozen in liquid nitrogen and pulverized into a powder using a liquid nitrogen-cooled mortar and pestle. To prepare PBM growth factor extracts, the crushed sample powder was incubated in Dulbecco’s modified Eagle medium (DMEM) serum-free media containing 50 l/mL bovine serum albumin, 100 l/mL streptomycin, and 100 U/mL penicillin (1 l/mL of media per 100 mg of bioplastic) for 4 h at room temperature on an inverting mixer. The samples were centrifuged at maximum speed for 5 min and the supernatants isolated. NIH 3T3 cell proliferation in response to the PBM extracts was assayed using a Promega CellTiter (Madison, WI) MTT-based cell proliferation assay. PBM extracts were tested at a 1:10 dilution in DMEM serum-free media. Cells grown in DMEM serum-free and serum media were used as negative and positive controls, respectively. Sample absorbance levels (AU) were reported as a percentage of the positive control.

In vitro degradation

Samples of known size and mass were added to individual wells of a 24-well plate. Next, 1 mL of PBS was added to each well and incubated for 12 h at 37°C. The media was removed, spun down, any solid pellet was added back to the well, and the supernatant stored in microtubes at 4°C. This was designated time zero. PBS containing 100 l/mL trypsin was then added and the wells incubated for ~12 h at 37°C. As before, the media was removed and centrifuged. The supernatant was stored and any pellet transferred back into the well. This process was repeated approximately every 12 h until the samples were fully degraded. The absorbance of the supernatants were determined at 350 nm (general PBMs) and 580 nm (genipin-crosslinked PBMs) for each time point and scaled by the initial sample masses. Total accumulated scaled absorbance values at each time point were determined. The percent-remaining scaled absorbance values were calculated, and then plotted versus time. The maximum slope of the degradation curve was reported as the maximum degradation rate (in% per hour).

Radiolabeled growth factor release

BMP-2 (Peprotech, Rocky Hill, NJ) was used as a model protein to examine release from PBMs. Radioactive iodine (125I) was labeled onto the BMP-2 using a previously published procedure.30 The 125I-BMP-2 was added as a component to form the PBM. Standardized samples (6 mm D x 3 mm H) were measured for their initial radioactivity using a gamma counter. The samples were then placed in glass tubes with 1 mL of serum media containing 0.02% sodium azide. Every 24 h, the media was isolated and replaced and the radioactivity of the isolated media read on the counter. When 125I-BMP-2 release had essentially stopped, the samples were washed with PBS and replaced with PBS containing 100 lg/mL trypsin and 0.02% sodium azide. Every 24 h, the media was isolated and replaced and the isolated media’s radioactivity was counted as well as the media’s absorbance at 570 nm for bioplastic degradation (see method above). All radiolabel data were corrected for radiolabel decay.

Mouse calvarial defect

All surgeries were performed under aseptic conditions according to IACUC-approved protocols. An anterior–posterior skin incision on the midline of the scalp exposed the underlying calvarial bones. The periosteum was incised and carefully dissected from the underlying bones. With a 5-mm OD trephine, full-thickness defects were made into the parietal bones. After washing with saline to remove all debris, a 5-mm circular sample disk of PBM was inserted to fill the defect. Soft tissues were closed in layers with resorbable sutures. Mice were monitored until ambulatory, and then returned to their individual cages and given water and food ad libidum. Each treatment group was implanted into 12 animals.

After 4 and 8 weeks, the mice were sacrificed, and crania were recovered, fixed in 10% neutral-buffered formalin, and radiographed by high spatial resolution µCT (µCT 40 scanner (Scanco Medical)). To obtain accurate radiographic signals from the implants, µCT data were thresholded against the cervical vertebrae. Three-dimensional models were rendered from the scan datasets. Afterward, the cranial explants were decalcified and histologically processed–6-µm serial sections were cut and alternate sections stained with hematoxylin–eosin and a modified Gomori trichrome.

Results

Mechanical characteristics

PBM mechanical properties can be controlled by a variety of methods. One method is to manipulate the formulation components, such as by varying the plasma content (Table 1). Through the addition of fillers, Young’s Moduli as high as 250 MPa have been achieved to date. Processing conditions can also be utilized to control physical properties. For example, higher molding temperatures increased the Young’s Modulus and ultimate strength to 180 MPa and 6 MPa, respectively.

In vitro degradation rate

PBM degradation in vivo is mediated by proteolysis. To manipulate the PBM degradation rate, genipin crosslinker is

| Table 1. Mechanical Property Data for Three PBM Formulations with Varying Plasma Content |
|---------------------------------|------------------------------|----------------|----------------|----------------|
| PBM, % plasma powder (w/w)     | Young’s modulus (MPa)        | Ultimate stress (MPa) | % elongation at break |
| 60                             | 21.4 ± 2.0                   | 1.60 ± 0.15      | 140 ± 2        |
| 65                             | 43.2 ± 1.7                   | 2.47 ± 0.13      | 96 ± 17        |
| 70                             | 93.1 ± 3.7                   | 3.78 ± 0.83      | 38 ± 14        |

All values are reported as the average± the standard deviation. PBM, plasma-based biomaterials.
Table 2. In Vitro Degradation and In Vitro Bioactivity Data for all Formulations

<table>
<thead>
<tr>
<th>PBM, % crosslinker (a/a)</th>
<th>Max. in vitro degradation rate (%/h)</th>
<th>Bioactivity (% of pos. control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.37 ± 0.17</td>
<td>167.2 ± 6.4</td>
</tr>
<tr>
<td>0.25</td>
<td>2.17 ± 0.01</td>
<td>102.5 ± 3.0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.46 ± 0.01</td>
<td>77.8 ± 3.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.96 ± 0.03</td>
<td>75.9 ± 3.8</td>
</tr>
<tr>
<td>2.0</td>
<td>0.61 ± 0.02</td>
<td>38.5 ± 1.8</td>
</tr>
<tr>
<td>Negative control</td>
<td>3.9 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>100 ± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

All values are reported as the average ± the standard deviation.

employed (Table 2). Genipin is a low toxicity31–33 natural crosslinker that is finding wide application in biomaterials research34–39 and we employ it in some PBM formulations. By using approximate correlation to observations in completed animal studies, it is estimated that PBM can be designed to reside from approximately 1 week to 6 months depending on the formulation and anatomical location. Processing conditions, such as molding temperature and time, can also modulate PBM crosslinking and the resulting degradation rate (data not shown).

Growth factor retention, elution, and activity

Retention of the native plasma’s biological factors is a significant innovation with the PBM technology. To study this, levels of IGF-1, PDGF-AB, and TGF-β1 were measured for ten individual plasma units in the starting liquid plasma as well as in the recoverable extracts of the powdered plasma and final PBM (Table 3). Retention of growth factor in the powdered plasma (i.e., lowest recoverable levels in the extract) was highest for TGF-β1 followed by PDGF-AB and IGF-1, most likely due to their differential binding to the fibrin clot. This characteristic is paralleled in the PBM, but overall retention is improved within the PBM matrix, as expected.

Growth factor binding to the PBM matrix can be manipulated via formulation components and processing conditions, which also affect growth factor elution rates. As a demonstration, radiolabeled [125I]-BMP-2 (a low-abundance platelet protein60 and protein of interest for bone repair) was formulated into the PBM. BMP-2 possesses a heparin-binding site,61 which allows it to potentially bind to the fibrin component of the PBM. Free release and protease-mediated release were monitored by radioactive and spectroscopic measurements (Fig. 2). Approximately 50% of the [125I]-BMP-2 freely released (over 3 weeks) for the base PBM formulation, but was reduced to ~30% free release (over 10 days) when the formulation contained 10% β-TCP. When free release of [125I]-BMP-2 had ended, trypsin was added and both [125I]-BMP-2 release and PBM degradation were monitored. Both formulations were shown to release [125I]-BMP-2 in concert with PBM degradation, indicating protease-mediated [125I]-BMP-2 release.

Growth factor activity is paramount to the function of PBM. It is difficult to isolate individual growth factors from PBM for analysis as proteinolytic degradation of the PBM matrix can also potentially destroy the growth factors. Therefore, an aggregate extraction assay was employed to measure retention of overall growth factor activity. PBM extracts were added to NIH 3T3 fibroblasts and cellular proliferation measured using a standard MTT assay. Cells grown in standard serum media served as a positive control and were used to normalize the data. The PBM extract dilutions were designed to achieve a 1:10 dilution of the original plasma component, comparable to the 1:10 serum dilution in the positive controls. PBM biological activities higher than the serum controls were achieved (Table 2). Besides altering the blood plasma content, biological activity is most directly altered by increasing PBM crosslinking (Table 2) or by changing compression molding temperature (Fig. 3). Biological activity is reduced via crosslinking by sequestration within the PBM changing the elution profile, whereas temperature reduces activity primarily by deactivation, but to some degree by heat-induced crosslinking. In addition to NIH 3T3 fibroblasts, biological activity has also been demonstrated with human MG-63 preosteoblasts, mouse C2C12 stem cells, and human mesenchymal stem cells (data not shown).

In vitro biocompatibility

PBM biocompatibility has been extensively examined. PBM formulations have demonstrated biocompatibility with cell types in vitro, such as human MG63 preosteoblasts, mouse C2C12 stem cells, human mesenchymal stem cells, and mouse NIH 3T3 fibroblasts (Fig. 4). Cellular affinity for PBM surfaces can be affected by changes in PBM formulation. For example, MG63 preosteoblasts cells preferred formulations with increased β-TCP levels where NIH 3T3 fibroblasts did not (data not shown). It is possible that these kind of interactions are tunable for such applications where adhesion barriers are desired.

Table 3. Growth Factor Levels Determined by ELISAs for the Starting Liquid Plasma as Well as Recoverable Growth Factor Levels from Powdered Plasma and PBM Extracts

<table>
<thead>
<tr>
<th>Source</th>
<th>IGF-1 (ng/mL)</th>
<th>PDGF-AB (ng/mL)</th>
<th>TGF-β1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting plasma</td>
<td>111 ± 52</td>
<td>33 ± 10</td>
<td>62 ± 14</td>
</tr>
<tr>
<td>Powdered plasma</td>
<td>107 ± 47</td>
<td>23 ± 7</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>PBM</td>
<td>73 ± 30</td>
<td>7 ± 2</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

Data are reported as the average and standard deviation from ten individual units of plasma.

ELISA, enzyme-linked immunosorbent assays; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

Mouse calvarial defect feasibility study

The efficacy of PBM in vivo has been demonstrated in a variety of animal studies involving a range of animal types and anatomical models. Human blood plasma-derived PBMs and species-specific PBMs have been employed to gain a better understanding of the immunological response to PBMs. In one example, an initial feasibility study was performed using human-derived PBMs in a mouse calvarial defect model. Figure 5 exhibits the calvarial defect response to a PBM formulation containing β-TCP, 8 weeks after implantation. Although in many cases the implant had migrated within the defect site, new bone was clearly formed in response to the PBM implant. Histologically, new bone and...
cellular infiltrate were seen throughout the PBM as the PBM was degraded in concert with tissue healing. Significant new vasculature was also seen in the repair site. No undue inflammation was observed. Inflammation was primarily seen around the periphery of the PBM and was less in magnitude than that typically seen around a suture (data not shown). The low immunological profile has been confirmed in later animal studies involving both species-specific and human plasma-derived PBMs.

**Discussion**

PBMs were designed to take advantage of the regenerative potential of PRP, but reduce its drawbacks and clinical variability. Two primary sources of variability involve PRP placement and residence time. Spatial control of delivery is highly problematic and the material is rapidly resorbed by the body. Other sources of clinical variability include the variation in patient-to-patient platelet and growth factor quality and quantity.62,63 There are also numerous platelet concentrators being marketed, which produce different concentrations and volumes of platelets.64 Additionally, there is debate over whether the presence of leukocytes enhances or diminishes effectiveness and whether PRP should be preactivated before injection or allowed to activate in situ.65 That PRP is not an off-the-shelf therapy is also a drawback to the technology. We believe PBMs reduce these drawbacks.

Although PBMs represent an innovative and disruptive platform technology, the concept of making biological plastics from blood components is not a new idea. Fibrin-based plastic scaffolds were developed in the 1940’s and successfully used during WWII as part of a US defense research program to develop medical strategies for treating wounded soldiers.66,67 Fibrin was refined from pooled, donated human plasma using methods developed by Edwin Cohn at Harvard. John Ferry, then at Woods Hole, led the effort to develop methods to form fibrin into 3D plastics. However, the high-temperature molding processes used to make some forms of these plastics destroyed any bioactivity, and the formaldehyde-crosslinking agent they used was relatively toxic and required multiple steps to deactivate any remaining crosslinker. Nevertheless, these plastics and elastomeric sheet forms of fibrin were successfully used clinically as implants for bone resurfacing, neurosurgical applications, burn treatments, and peripheral nerve regeneration.67,68 Bovine-derived fibrin-based bioplastics were also developed in the 1950s through the early 1970s in research sponsored by the
Hungarian government. The Hungarian IP was sold to Ethicon in the early mid 1970s, and the technology was shelved due to the advent of modern plastics as well as new concerns over the bovine-derived products. Cost was also an issue due to the high cost of isolating the large volumes of fibrinogen needed to make such plastics. Ultimately, even if the fibrin plastics could be made in a fashion that maintained bioactivity, the main purpose of such materials was to create scaffolds to support tissue growth, but not to induce cellular growth and differentiation, which require bioactive materials.

Motivated by the idea that bioplastics could be made from blood components and by the potential for PRP-based therapies, we set out to combine these ideas and to overcome the limitations of the WWII era fibrin plastics and current PRP therapies. We developed a blood plasma-derived biomaterial, which we refer to as a PBM, which is bioactive, inexpensive, safe, off-the-shelf, and has low lot-to-lot variability (Figs. 1 and 6).

The PBM manufacturing process is exhibited in Figure 6. The process utilizes aPRP because it contains the whole-blood plasma fraction that includes important plasma-derived growth and regenerative factors, unlike the platelet concentrators that discard a large amount of this platelet-poor plasma. The aPRP is clotted, freeze-dried, ground, mixed with other components, and compression molded into PBMs. These PBMs can take a variety of forms and physical properties, including flexible sheets, putties, and complex shapes, such as screws (Fig. 1).

With any tissue-derived material lot-to-lot variability is an issue. For PBM products, lot-to-lot variability is reduced through the pooling of multiple aPRP units. This is in contrast to current autologous PRP injections, where patient-to-patient variability in the number of platelets and growth factors make it difficult to predict which patients may respond well to autologous PRP therapy. Additionally, certain growth factors (e.g., IGF-1) are known to decrease with age, making it more likely that older patients will not respond well to PRP therapy; it is these older patients, however, who could benefit the most from a biologic acceleration of healing. By contrast, PBM materials are manufactured from pooled plasma (average donor age ~ 35 years old), reducing the variability.

FIG. 4. Biocompatibility of PBMs was demonstrated with mouse C2C12 stem cells (A), human mesenchymal stem cells (B), human MG63 preosteoblasts (C), and NIH 3T3 fibroblasts (D). Cells are indicated by asterisks. Samples were fixed 3 days postseeding and analyzed by SEM.

FIG. 5. Healing in a cranial defect implanted with a human plasma-derived PBM after 8 weeks. Left image: MicroCT 3D reconstruction. Right image: Decalcified paraffin section through the center of the defect with Gomori trichrome staining, 20× magnification. New bone (arrows) surrounds new blood vessels (asterisks) and is on the periphery of the residual plastic (arrow heads).
It is important to note that due to the manufacturing process, final growth and regenerative factor concentrations are approximately 5-10 \( \times \) higher than in the starting aPRP, considerably higher than what the platelet concentrators are currently delivering. However, it should be stressed that these factors are slowly released from the PBM over time (as determined by the PBM formulation and processing conditions) versus the bolus and short-lived delivery from the currently used autologous PRP liquid-based therapies. This bolus growth factor release calls into question the time frame of effectiveness for current PRP treatment and has led some to utilize multiple injections over several weeks. In contrast, PBM growth factor release rates can be substantially controlled. However, since every growth factor interacts with the PBM matrix differently, retention and release strategies are not universal and the large number of growth factors would make optimization for multiple growth factors quite complex. As a result, retention and release strategies of growth factors of interest are application-specific, possibly requiring trade-offs in consideration of other performance requirements, such as desired mechanical properties and degradation rates. For instance, higher molding temperatures typically increase mechanical strength, but they also decrease the degradation rate (data not shown) and biological activity (Fig. 3).

In addition to growth factor retention and release, growth factor fate is also a significant focus of our ongoing research. Each factor may be uniquely susceptible to the various processing conditions employed in manufacturing PBMs. Determining individual growth factor fate requires not only the isolation and total recovery of each individual factor, but also determining the total individual growth factor activity. Unfortunately, recovering individual growth factors bound by the PBM matrix is nontrivial and the paucity of growth factor-specific activity assays impedes this work. As a result, aggregate \textit{in vitro} biological activity is used to evaluate PBM formulations, but because this only measures the freely released aggregate growth factor activity and not the potential activity retained in the PBM matrix, it is an incomplete measure of total PBM activity.

**Disruptive Potential**

There is a great need for effective, consistent, and cost-effective products that can biologically enhance tissue healing. Injuries to connective tissue (tendons, ligaments, cartilage) that are largely avascular are difficult to heal, requiring months of recuperation and often result in poor clinical outcomes. For example, the number of meniscal procedures (including resection and repair) is expected to exceed 1.05 million in 2012, making it one of the most commonly performed orthopedic procedures. Of the 430,000 annual surgical repairs, there is a disappointing 40%–70% re-tear rate. Additionally, there are 400,000 patients with injuries to their knee cartilage who are surgically treated every year in the U.S. but with generally poor prognosis. Cartilage defects are currently considered to be irreversible, and the rationale of surgical intervention is to delay total knee replacement. Restoring articular cartilage represents a massive unmet need in orthopedics, with numerous companies attempting to solve the problem. Finally, bone repair is another area in need of biologic augmentation. Biologics are used to accelerate the healing in only 10.5% of the more than 1.2 million fractures to the extremities (humerus,
The prevalence of nonunion of closed tibia fractures are the most common long bone fractures encountered. The prevalence of nonunion of closed tibia fractures is 2.5% with a five-to-sevenfold increase for open fractures.

Because of a lack of effective products, physicians have begun to look for ways to improve the healing of soft tissue and bone and are rapidly embracing the use of autologous PRP manufactured in the operating room, as it contains a concentration of natural growth factors and other proteins that appear to accelerate healing. Normal platelet counts in blood, average approximately 200,000/\mu l; concentrated PRP for therapeutic use has a concentration 3–5 times larger, with a threshold of 1,000,000/\mu l desired.

PRP is being highly investigated (Fig. 7) and is being rapidly adopted for numerous clinical applications, including the repair of tendons, ligaments, cartilage, bone, skin wounds, and other applications. However, as mentioned above, there are a number of questions and problems surrounding the use and effectiveness of PRP. Clearly, the concept of using a concentration of the body’s own regenerative factors to heal itself is very attractive, but the high number of uncontrolled variables makes the true role of autologous PRP in soft and hard tissue repair difficult to determine. If PBMs can truly mitigate the issues surrounding traditional PRP, they have the potential to be the basis for platform technology providing highly effective treatment options for tissue repair and regeneration.

For a new technology to be truly disruptive, it must not only be functionally unique, addressing unmet technical challenges, but also be cost effective to be available to the masses. The rising cost of healthcare has become a central problem for hospitals, and represents a major barrier for new and expensive technologies, regardless of their effectiveness. Biologic products manufactured from recombinant proteins, such as Medtronic’s Infuse® BMP-2 product, cost hospitals approximately $9,000 for the spinal fusion of a single patient. Tissue-engineered approaches, such as Genzyme’s Carticel® ACI procedure, for treating cartilage defects average $26,000 per procedure. By contrast, 3 cc of bone putty incorporating PBM materials cost under $10 to produce. Plasma is readily available as a starting material—only 22% of plasma is used clinically with the remainder either discarded or sold to fractionators. Thus, PBM materials can be offered at relatively low cost allowing this important new technology to benefit a wide population of patients in healthcare systems, where cost containment limits the use of more expensive therapies. Not only is this an important consideration for First World countries, but also low-cost PBMs could be practical options for Second and Third World nations.

### Barriers to Be Overcome

#### Concerns regarding PRP efficacy

Over the last few years, PRP has been used in a wide variety of applications. The highly publicized use of PRP therapies by professional athletes has created significant hype. Unfortunately, with few controlled studies, conducted evidence of effectiveness in many instances has been anecdotal, and a number of published studies have called into question PRP’s effectiveness. One widely reported study examined the effectiveness of PRP to heal chronic Achilles tendon injuries and found that PRP treatment was no more effective than the control treatment of saline. These mixed reports have created a skepticism regarding the use of PRP and any new PRP treatment, such as PBMs, has to be able to distinguish itself from PRP and clearly demonstrate its effectiveness using well-documented and designed research. Because PBM materials are made from pooled plasma, variables that are difficult to control with the use of autologous PRP can be measured and controlled.

#### Safety of pooled products

Safety is a primary concern when dealing with human tissue. This is one reason why autologous PRP is such an attractive treatment option. In manufacturing PBMs, we incorporate pooled allogenic plasma, which carries with it not only the burden of demonstrating safety quantitatively, but also overcoming perceptions or even fears of nonsafety—this may be, especially, an issue with orthopedic physicians who have been taught that human tissues, such as allograft bone, can never be pooled. The blood plasma received from the Blood Bank is prescreened for known blood-borne pathogens. The pool is then rescreened for known viral contaminants by using sensitive nuclear amplification technology assays. Additionally, multiple proprietary viral inactivation steps are employed that have been validated in their ability to reduce potentially unknown enveloped and nonenveloped RNA and DNA viruses to sterility assurance levels of greater than $10^{-6}$.

#### Regulatory concerns

There are numerous human blood products being marketed, and FDA and EMEA have adopted stringent guidelines to control the safety of these pooled blood products through viral testing and inactivation. However, due to their novel nature as well as their blood derivation, we expect that PBM materials will be carefully reviewed and scrutinized by FDA (U.S.) and EMA (Europe) before clearance to
market PBM products will be granted. Depending on the product’s primary mode of action, either a biologic or device regulatory pathway will be required. We have had discussions with both FDA and EMA representatives to clarify the requirements for marketing, and both agencies have been accommodating and interested in helping to define the data needed to ensure safety and effectiveness of these novel products. While the regulatory pathway always represents an expensive and time-consuming barrier, it is an important and necessary one.

**Conclusions**

Manufacturing novel biomaterials from human platelet-rich blood plasma represents a unique, but simple value proposition with a material that encourages the healing of damaged tissue naturally. PBM products have the potential to accelerate healing and reduce pain and complications, enabling patients to return to work and their daily lives more quickly. The PBM technology enables the manufacture of products incorporating a concentration of natural growth factors at a relatively low cost—unique for any product containing a biologic and truly disruptive.

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