Effect of Platelet Releasate on Bone Cell Migration and Recruitment In Vitro

Wanda E. Oprea, BASc*
Jeffrey M. Karp, BEng*†
Morris M. Hosseini, PhD*
John E. Davies, BDS, DSc*†‡

Toronto, Ontario, Canada

The use of platelets and platelet products has become increasingly popular clinically as a means of accelerating endosseous wound healing. It is likely that growth factors released by activated platelets at the site of injury play a role in bone regeneration by stimulating the migration and proliferation of bone cells. In this study, a novel in vitro assay was developed to study the effects of platelet releasate (PR) collected from activated platelet concentrate on rat bone marrow–derived cells. Cultures of primary rat bone marrow cells were overlaid with a fibrin matrix, and the number of cells migrating within the three-dimensional matrix and the leading front of migration were quantified. The addition of PR to the top of the fibrin gels at different time points caused a 25% increase in the leading front of migration and a 3.5-fold increase in the number of migrating cells. Platelet releasate was also shown to have a mitogenic effect on bone cells in proliferation studies. Comparison between migration and proliferation data indicated that PR stimulates the initial recruitment of bone marrow cells to migration. This assay further allowed the determination that rat bone marrow cells are capable of exerting contractile forces on fibrin matrices and that matrix contraction is directly related to the migratory activity of cells. The results provide a potential mechanism to explain why biologically active platelet-derived factors enhance endosseous wound healing.

From the *Institute of Biomaterials and Biomedical Engineering, †Department of Chemical Engineering and Applied Chemistry, and ‡Faculty of Dentistry, University of Toronto, Toronto, Ontario Canada.

Address correspondence to Dr Davies, Institute of Biomaterials and Biomedical Engineering, University of Toronto, 4 Taddle Creek Road, Toronto, Ontario, Canada M5S 3G9; e-mail: davies@ecf.utoronto.ca

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Endosseous wound healing involves a complex cascade of biological events that ultimately result in the regeneration of bone. As an immediate result of injury, local extravasated blood fills the healing compartment and leads to the formation of a blood clot. The provisional network of fibrin fibers, which forms the structural component of the clot, serves as a matrix for the inward migration of osteogenic cells that originate in the marrow and on endosteal surfaces.[1] The subsequent differentiation of these migratory cells to secretory osteoblasts results in de novo bone formation. In addition to the matrix component, a blood clot contains a rich mixture of growth factors released by activated blood cells. Most notable of these are platelets; on activation, they release a library of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). Conventional wisdom holds that these factors play an important role in the regulation of the wound healing cascade[2,3] based on in vitro and in vivo evidence of their stimulatory effects on the proliferation and migration of various cell types.[4–6]

Such thinking has encouraged the development of new healing therapies that incorporate platelets and their products to enhance and accelerate the wound healing process. These approaches have been applied in a variety of clinical areas, including plastic surgery and orthopedic surgery, with general success. For example, in dermal wound healing studies, an acceleration of granulation tissue formation and healing was observed when PDGF, platelet concentrate, or platelet releasate (PR) was applied at injury sites.[5,7] At endosseous sites, the application of PDGF has been shown to promote bone formation,[8] whereas platelet concentrate has been used successfully to enhance lumbar spinal fusion.[9] Platelet con-

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centrate has also been shown to double the rate of bone formation and improve bone density after bone grafting,[10] which has stimulated the development of several commercially available platelet concentrating machines.[11] Despite such reports, however, clinical success has been inconsistent, and more basic research is required to elucidate thoroughly the underlying mechanisms of platelet growth factor enhancement of endosseous wound healing.

The specific mechanisms of action of platelets during wound healing have been deduced in large part from in vitro studies. Such work has concentrated on the effects of individual platelet growth factors such as PDGF on fibroblasts and inflammatory cells. In the dermal wound literature, PDGF and TGFβ have been shown to be mitogenic for fibroblasts.[12–14] Migration assays designed to investigate the directed migration of cells in response to a soluble growth factor gradient, or chemotaxis, have indicated that PDGF and TGFβ are chemotactic factors for fibroblasts, neutrophils, and smooth muscle cells.[4,12,15,16] Such studies have also been undertaken with osteogenic cells. It has been established that PDGF also stimulates the proliferation of human osteoblasts derived from cancellous bone as well as from osteogenic cell lines.[17,18] In a recent report, Gruber et al[6] have demonstrated that not only platelets but PR, platelet microparticles, and platelet membranes are all capable of stimulating the mitogenic activity of human bone-derived cells. Using two-dimensional migration assays, PDGF and TGFβ have been shown to be chemotactic factors for osteogenic cells.[19–22] Although many increasingly complex migration assays have been developed in the past decade to study stable gradients or three-dimensional migration,[23,24] osteogenic cells have not been used in such assays.

In this study, it was our aim to develop an in vitro model for the investigation of the effects of PR on bone marrow cell migration, recruitment, and proliferation. Our model, which was extended from the soft tissue healing field,[25] consisted of a layer of cultured bone marrow cells overlaid with a 1.5-mm fibrin matrix over which PR was added. Using this model, we were able to observe and quantify cell departure from the culture dish and infiltration into the fibrin matrix. Thus, there were two novel aspects of our assay. First, our model allowed for the investigation of three-dimensional migration of osteogenic cells within a biologically relevant fibrin matrix. Second, we used PR containing all factors released by platelets, which was prepared by the activation of platelet concentrate obtained from a commercially available platelet concentrate system. Thus, not only is the in vivo wound healing scenario simulated more closely but, given the clinical use of PR, our study is directly relevant to current healing therapies.

Materials and Methods

Materials

For this study, Hemaseel HMN human fibrinogen and thrombin were kindly donated by Haemacure Corporation (Haemacure Corporation, Kirkland, Quebec, Canada). Platelet concentrate was obtained using the Platelet Concentrate Collection System generously provided by Implant Innovations (3*i*, Palm Beach Gardens, FL). Bone marrow cells were harvested from the femurs of young Wistar rats (115–130 g) (Charles River, St. Constant, Quebec, Canada) and cultured in fully supplemented media (FSM) consisting of α-minimal essential media (Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada) supplemented with 15% (vol/vol) fetal calf serum (GIBCO BRL Life Technologies, Gaithersburg, MD); 50 μg/ml L-ascorbic acid; 5 mM β-glycerophosphate; 10^{-8} M dexamethasone (Sigma, St. Louis, MO); and a 10% (vol/vol) antibiotic/Fungizone solution consisting of penicillin G (1670 U/ml), gentamicin (500 μg/ml), and amphotericin B (3 μg/ml) (Sigma).

Cell Isolation and Culture

Rat bone marrow cells were obtained according to previously published methods.[26,27] Briefly, femora were excised from young Wistar rats and transferred into 10 ml of the same antibiotic solution as used in the culture media. Epiphyses were removed, and the marrow from each diaphysis was flushed out with 5 ml FSM. Marrow cells of both diaphyses were thus collected in a total of 10 ml of FSM to which was added an additional 20 ml of FSM. Fifteen milliliters of this cell suspension was cultured in Falcon T75 flasks (Becton Dickinson Labware, Franklin Lakes, NJ). Cultures were maintained at 37°C in an incubator with an atmosphere consisting of 95% air, 5% CO_2, and 100% relative humidity. The culture medium was changed every second to third day.

On day 5 or 6 after isolation, depending on when cells reached ~80% confluency, the cells were subcultured by enzymatic release using 0.01% trypsin (GIBCO BRL Life Technologies) for 12 to 15 minutes. Cells were counted using a hemacytometer and seeded at a density of 10,000 cells/cm^2 in six-well plates (Becton Dickinson Labware). This count-

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ing procedure was also used in the proliferation studies.

Fibrin Gel Overlay

When cells reached confluency after the first subculture, usually after 5 to 6 days, the culture medium was removed and each dish was washed in phosphate-buffered saline (PBS) (pH 7.4) (Sigma). Some cultures were treated with the drug cytochalasin-D (Sigma) by incubation for 2 hours at 37°C in 50 mg/ml cytochalasin-D in FSM. These cultures were washed again twice with PBS before the fibrin gel overlay. Reconstituted fibrinogen (60 mg/ml fibrinogen containing 35 IU/ml factor XIII) and thrombin (500 NIH U/ml) were diluted 1:10 and 1:200, respectively, in FSM containing 225 KIU/ml aprotinin protease inhibitor from bovine lung (Sigma). A fibrin gel was cast in each culture dish by the addition of equal amounts of diluted fibrinogen and thrombin, which were mixed by gentle hand swirling for 30 seconds to achieve even clot distribution. The final concentrations obtained were 3 mg/ml fibrinogen and 1.25 NIH U/ml thrombin. The gel was allowed to polymerize for 30 minutes in the incubator, resulting in a fibrin clot overlying the cells. For subsequent culture, 2 ml FSM with 225 KIU/ml aprotinin protease inhibitor was added on top of the gels; this medium was used for all experimental groups and in all subsequent media changes (Fig 1).

Platelet Releasate

Some experimental groups contained PR added to the media on top of the fibrin gel. To obtain PR, platelet concentrate was initially obtained using the 3*i* Platelet Concentrate Collection System. Briefly, 54 ml whole blood was obtained from a healthy, consenting volunteer (two donors with 1–2 donations each were used for this study) and mixed with 6 ml sodium citrate anticoagulant (Cytosol Laboratories, Braintree, MA). The blood was transferred to the collection system and subjected to two centrifugation cycles. Red blood cells were removed after the first centrifugation, and a platelet pellet was obtained after the second, which was resuspended in 6 to 8 ml overlying plasma. We have demonstrated an average sevenfold increase in platelet count relative to whole blood using the Platelet Concentrate Collection System (not shown), which is consistent with published results.[11] For subsequent use, platelet concentrate was stored at room temperature with gentle agitation for up to 3 days.

Platelets were allowed to rest for 30 minutes and were then activated by incubation with 2 IU human thrombin per milliliter (Hemaseel HMN; Haemacure Corporation) for 15 minutes at room temperature. An equal amount of FSM was added, and the mixture was centrifuged at 2000*g* for 10 minutes. The supernatant containing the released platelet factors was removed and filtered with a 0.22-μm filter (Millipore, Bedford, MA). Platelet supernatant was added in dilutions of 1:4 (vol/vol) in FSM with aprotinin to different experimental groups at various time points (Table 1). Some groups received human recombinant PDGF-BB (Sigma) added to the media on top of the gel at a concentration of 20 ng/ml FSM. Medium changes were performed every other day.

Five days after the fibrin gel overlay, cell cultures were fixed in a solution of 2% glutaraldehyde and 2% formaldehyde in PBS at 4°C for a minimum of 1.5 hours. Samples were washed three times in PBS after fixation and prepared for confocal microscopy or transmission electron microscopy (TEM).

Confocal Laser Light Microscopy

For confocal microscopy, fixed cultures were first permeablized with 0.1% Triton X-100 in PBS for 90 seconds (Sigma) and labeled with a solution of 5 μl/ml fluorescein isothiocyanate phalloidin (Alexa Fluor 488; Molecular Probes, Eugene, OR), an F-actin stain, and 0.7 μl/ml ethidium homodimer (Molecular Probes), a nuclear stain, in PBS for 20 minutes. The microscopy was performed using the Zeiss LSM 510 confocal microscope (Zeiss, Thornwood, NY) equipped with a ×40 Axiovert water lens. Sectional images were taken along the Z axis. Cross-sectional images were computed from the Z stack data by LSM 3.80 software (Zeiss). To quantify cell migration, cultures were analyzed at 15 random fields on the culture dish for three dishes per experimental group. The thickness of fibrin gels was measured using the stage height function on the LSM 510 microscope.

Table 1. Experimental Groups, Abbreviations and Respective Culturing Conditions

Experimental Group	*Culturing Media on Days after Subculture*	
	Day 5	*Day 7*
Negative control	FSM	FSM
PR - 5	FSM supplemented with PR	FSM
PR - 5, 7	FSM supplemented with PR	FSM supplemented with PR
PR - 5	FSM	FSM supplemented with PR

FSM, fully supplemented media; PR, platelet releasate.

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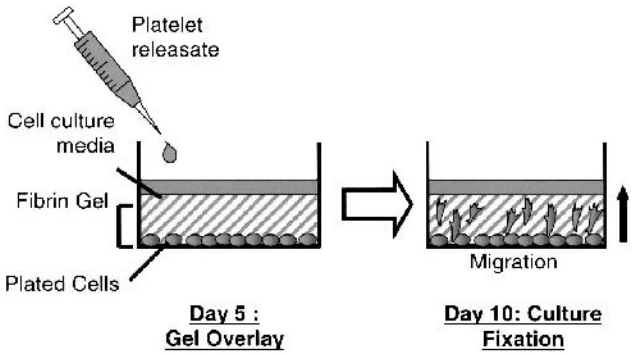

Fig 1 Experimental method. Rat bone marrow cells were subcultured in 35-mm tissue culture dishes. After cells reached ~80% confluency, usually on day 5 of subculture, a fibrin gel was overlaid. Medium was added on top of the gel, and platelet releasate was added to the medium in some culture groups. The culture medium was refreshed on day 7, and cultures were fixed on day 10, allowing cell migration to proceed for a total of 5 days.

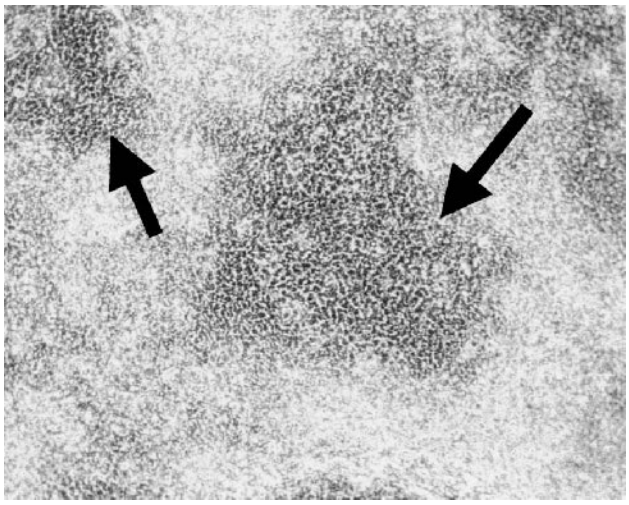

Fig 2 Bone nodule formation, as indicated by the arrows, observed by light microscopy in cultures at time of fixation. The field width is 1,125 μm.

Measured fibrin gel heights in groups with cells were compared with fibrin gels without cells that were cast on the same day and maintained under the same conditions.

Transmission Electron Microscopy Analysis

For TEM analysis, samples were fixed using Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M Na-cacodylate at pH 7.3) at 4°C for 4 hours. After fixation, cell cultures were postfixed in 2% osmium tetroxide in 0.1 M Na-cacodylate for 90 minutes at room temperature to preserve the cell membranes. Subsequently, samples were dehydrated in graded ethanol and thoroughly washed before en bloc staining with uranyl acetate in 50% ethanol for 2 hours at room temperature. Specimens were again dehydrated in graded alcohols and subsequently infiltrated with a series of alcohol-Epon mixtures before two final changes with pure Epon. After Epon polymerization at 40°C overnight and afterward at 60°C for 3 days, selected areas of the tissue were re-embedded in beam capsules. Silver to pale gold, thin, transverse sections were cut on an ultra microtome and mounted on copper grids. Sections were stained with 3% uranyl acetate in 30% ethanol and afterward with Reynold lead citrate. Samples were examined with a Phillips 400T transmission electron microscope and, in the case of electron diffraction, with a Phillips EM 430 transmission electron microscope. Electron diffraction patterns were projected directly onto a photographic plate.

Statistical Analysis

Experiments were performed in duplicate for $3 \leq n \leq 5$. For each experiment, the error in all results was expressed as the SD for the indicated number of experimental groups. Statistical significance of the results was determined by the paired, one-tailed, Student *t* test. *P* values less than 0.05 were considered significant.

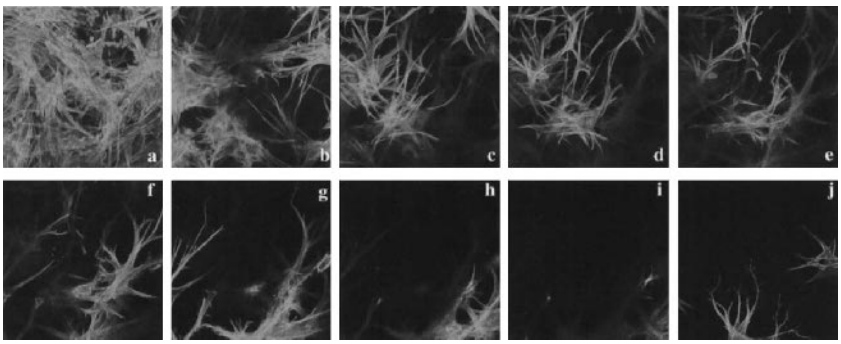

Fig 3 Series of horizontal images obtained by confocal microscopy. The image series (a–j) begins at the bottom of the culture dish (a), where a confluent cell layer is visible, and proceeds up through the unstained fibrin gel. Cells are stained green for F-actin, rendering the cell body and extended cell processes clearly visible. The vertical distance between images is 15 μm, and the field width is 265 μm.

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RESULTS

Harvested rat bone marrow cells were cultured in vitro as described previously. Bone nodule formation was observed by light microscopy (Fig 2). Bone nodules were first detected on day 4 or 5 after subculture and continued to appear and expand until fixation in both control and fibrin overlaid groups.

Confocal microscopy revealed extensive penetration of the cells into the unlabeled fibrin matrix (Fig 3) after 5 days. The cells observed within the fibrin matrix had the characteristic elongated morphology of migrating cells[28] with many cell processes projecting into the fibrin gel.

To confirm the viability of the model, it was important to determine if on fibrin gel overlay, cells became trapped within the fibrin gel. To investigate this possibility, some experimental groups were fixed an hour after gel overlay. Analysis by confocal microscopy showed that no cells were present within the fibrin matrix in these groups.

The leading front of migration, or the furthest vertical distance migrated by any one cell into the fibrin gel, as well as the total number of migrating cells in the volume above the analyzed points was determined. Statistically significant differences in terms of these two parameters were observed between groups treated with growth factors in the form of PR and those without. In the experimental groups that received PR at the early stage of culture (PR 5 and PR 5,7) the average leading front was 20% to 25% greater than in control groups (Fig 4). In these groups, the average leading front ranged from 255 to 275 μm (equivalent to 50–55 μm/d), which corresponds well with published in vivo data.[29] An even more striking difference between groups was observed by counting the total number of migrating cells. Close to a 3-fold increase in the number of migrating cells was observed in PR 5 and PR 5,7 groups as compared with controls (Fig 5).

In groups treated with PDGF instead of PR at parallel time points, a 1.5-fold increase in the number of migrating cells was measured in PDGF 5 and PDGF 5,7 groups as compared with untreated controls (data not shown).

Transmission electron microscopy analysis was performed to examine the presence or absence of signs of de novo bone formation within the fibrin gel, thus confirming that a percentage of the migrating cells were indeed osteogenic. Given that the cellular infiltrate was characterized by stellate-shaped cells, the TEM cross section often only showed cell processes rather than cell bodies (Fig 6A). In proximity to the cellular infiltrate, spherical electron-dense bodies approximately 0.5 to 1 μm in diameter could be observed within the fibrin matrix. At higher magnification, the electron-dense bodies within the fibrin matrices appeared to contain needle-shaped crystallites (see Fig 6B). The presence of mineral within the electron-dense bodies was supported by TEM electron diffraction analysis. Diffraction patterns obtained from areas with electron-dense bodies revealed circular banding patterns (see Fig 6C) characteristic for hydroxyapatite.[30]

To examine further the mechanisms of action of

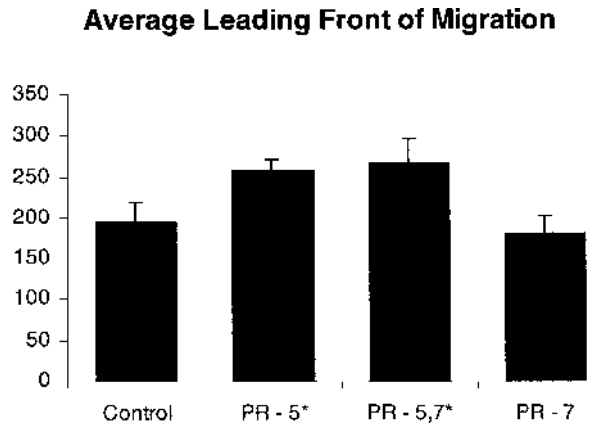

Fig 4 Average leading front of migration (furthest distance traveled by any one cell at analyzed point) in micrometers as determined by confocal microscope analysis at 15 points per well for three wells. *Statistically significant results compared with control ($P < 0.05$).

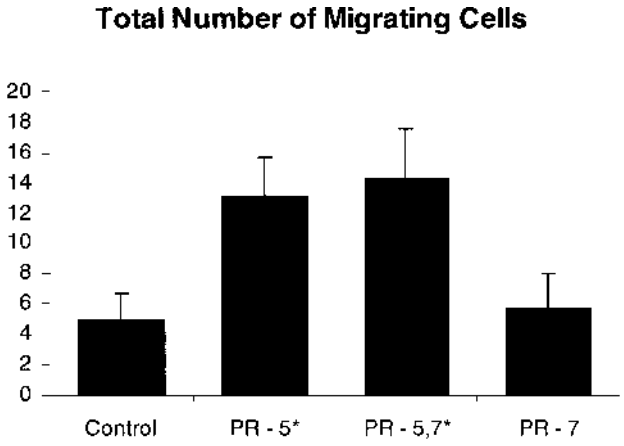

Fig 5 Total number of migrating cells as counted in the volume above the confluent cell layer at specific analyzed points given as average of 15 points per well for three wells. *Statistically significant results compared with control ($P < 0.05$).

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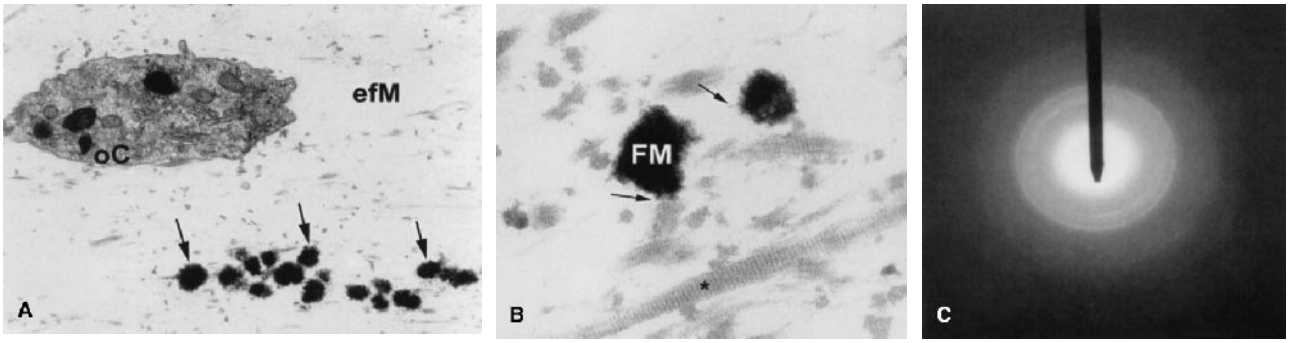

Fig 6 Transmission electron microscopy photomicrographs (A and B) and electron diffraction pattern (C) indicating the presence of de novo bone formation within fibrin gels infiltrated by rat bone marrow cells. (A) An anuclear branch of an osteogenic cell (oC) containing several catabolic organellae can be seen embedded within the extracellular fibrinous matrix (efM). In proximity to the cell branch, several spherical electron-dense bodies on the order of 0.5 μm (arrows) are visible. The field width is 10 μm. (B) At higher magnification, individual needle-shaped particles (arrows) are visible within the electron-dense spherical bodies. The field width is 1.7 μm. (C) The presence of hydroxyapatite in the electron-dense bodies is confirmed by the circular electron diffraction pattern.[30]

PR during wound healing, the effect of PR on cell proliferation was also studied. The proliferation studies were conducted in the absence of fibrin but using the same culture conditions as described previously. Platelet releasate had a significant effect on cell proliferation, causing a 1.5 to 2-fold increase in cell numbers as observed at the time of fixation (Fig 7). Platelet-derived growth factor alone caused a maximum 1.5-fold increase in cell numbers in the PDGF 5,7 group. Gruber et al[6] have recently shown that PR is able to elicit a mitogenic response in human trabecular bone–derived cells but did not generate a growth curve or confirm the osteogenic potential of the harvested cells.

To investigate whether the presence and migration of cells within the three-dimensional fibrin matrix had any effect on the gel, contraction of the fibrin gel was characterized after the culture period. No statistically significant vertical or horizontal contraction of the gel was observed in the absence of cells (not shown). Furthermore, all gels remained firmly attached to the sides of the culture wells, indicating the absence of horizontal contraction. Microscopically aided measurement of the thickness of the fibrin gel revealed significant vertical contraction after the 5-day culture period, however. To investigate further the cause of such contraction, cells were treated with cytochalasin-D, which blocks actin-dependent cell processes such as cell migration by capping actin filaments.[31] As shown in Figure 8, these cells exhibited only minimal migration as compared with untreated cells. Examination of the vertical thickness of these gels showed that significant contraction of the fibrin matrix occurred in the presence of untreated cells after the 5-day culture period. This contraction was further enhanced in the presence of PR. When migration was inhibited by cytochalasin-D, however, gel contraction was also strikingly reduced (Fig 9).

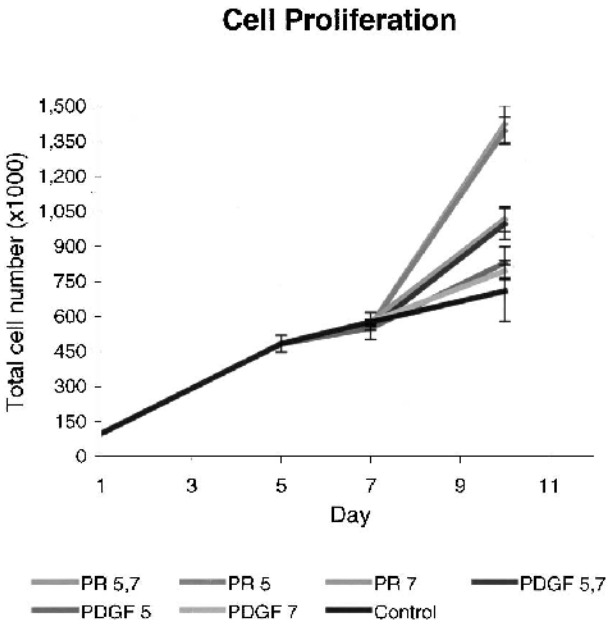

Fig 7 Cell proliferation in the absence of fibrin under standard culture conditions (control) or with added growth factors at days after subculture. Groups listed in order of appearance from top to bottom. Values given as average of three samples counted per group.

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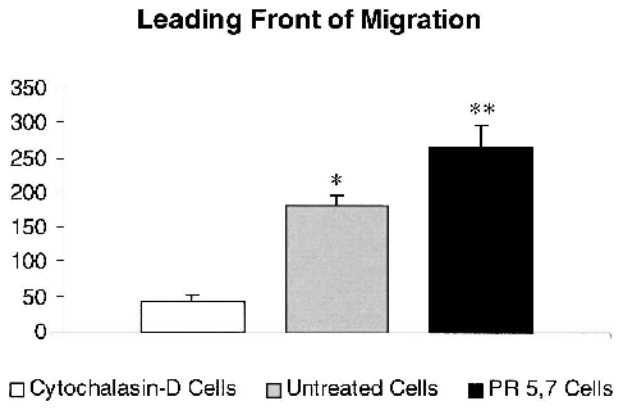

Fig 8 Leading front of migration as recorded from confocal microscopy given as an average of 15 points for three wells per experimental group. *Statistically significant groups as compared with cytochalasin-D–treated cells. **Statistically significant group as compared with cytochalasin-D and untreated cells.

Discussion

In the current study, we present a new in vitro assay to elucidate the effects of PR on osteogenic cell recruitment, migration, and proliferation. Our model consisted of a layer of rat bone marrow cells cultured under osteogenic conditions overlaid by a fibrin gel in the presence or absence of PR. Using this model, we were able to observe and quantify the effects of PR on osteogenic cell migration and recruitment in a biologically relevant fibrin matrix.

Using confocal microscopy, it was observed that rat bone marrow cells were capable of extensive infiltration of fibrin matrices in vitro (see Fig 3). Furthermore, TEM analysis indicated the presence of globular foci of mineralization in the proximity of the cells infiltrating the fibrin gels (see Fig 6A), which were morphologically similar to globules we have observed both in vitro during de novo bone formation at solid surfaces and in vivo in advance of the bone front in the closing sagittal suture of rats.[1,32] The presence of such mineralization foci throughout the fibrin gel indicates that among the multiple bone marrow cell populations, osteogenic cells were indeed infiltrating the fibrin matrices. It can further be concluded that the observed cell migration was an active cellular process because it proceeded counter to gravity and it was confirmed that cells were not trapped within the fibrin gel during the overlay procedure. This was also supported by the observed cell morphology, with the elongated cell body and extended cell processes that are characteristic features of cell migration in three dimensions.[28]

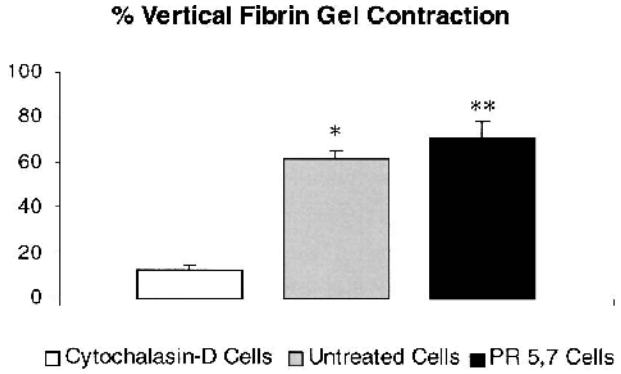

Fig 9 Gel contraction expressed as percentage of contraction of vertical fibrin gel height at the time of fixation as compared with height of gel at the time of casting. *Statistically significant groups as compared with cytochalasin-D–treated cells. **Statistically significant group as compared with cytochalasin-D and untreated cells.

To determine the effects of PR on cell migration and recruitment, two parameters were investigated, namely, the leading front of migration and the number of migrating cells. The addition of PR caused a significant increase in the leading front of migration when added early during culturing (Groups PR 5 and PR 5,7), but no effect was observed when it was added later as in the PR 7 group. Thus, PR has a stimulatory effect on cell migration but seems to be associated with a lag period. It is also reasonable to assume that a gradient was initially present across the fibrin gel and that cells migrated vertically upward through the gel in response to this gradient. No conclusive distinction between chemotaxis and chemokinesis can be made based on these data, however, and additional work is necessary to confirm the extent to which the gradient was maintained over the duration of the experiment.

The most striking effect of PR was on the number of migrating cells within the fibrin matrix. The early addition of PR in the PR 5 and PR 5,7 groups caused a 3- to 3.5-fold increase in the number of migrating cells (see Fig 5). To distinguish whether this increase was caused by the recruitment of a greater number of cells to migrate or by enhanced proliferation of the same initial number of migrating cells in the presence of the PR, we can compare the migration data with data obtained in the proliferation studies (see Fig 7). If we suppose that the number of migrating cells doubled as a result of proliferation as indicated by the proliferation studies, a greater number of cells still had to be initially stimulated to migrate to account for the greater than 3-fold increase in the number of migrating cells in the PR 5 and PR

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5,7 groups. A further examination of the PR 7 group shows that although PR stimulated a 1.5-fold increase in cell proliferation, an increase in the number of migrating cells was not observed in this group as compared with controls. Thus, it appears that increased recruitment stimulated by PR is the more significant factor contributing to an increase in the number of migrating cells within the fibrin gel. The addition of PR therefore stimulates an increase in the migration of osteogenic cells through three mechanisms: first, by recruiting more cells to detach from the culture dish or neighboring cells and to begin to migrate within the fibrin gel; second, by stimulating an increase their migratory activity; and, third, by stimulating cell proliferation.

One parameter that must be taken into consideration, however, is that the proliferation data were obtained from adherent cells on a two-dimensional surface. Proliferation can be significantly affected by the extracellular environment.[33] Thus, the rate of division of cells on a two-dimensional surface can be significantly different than in a three-dimensional biological matrix. Studies that have addressed this issue have not offered consistent trends, however.[33] Fibroblast studies suggest that proliferation in three dimensions compared with two dimensions is either unchanged,[34] inhibited,[35] or stimulated.[36] Such data are currently not available for bone marrow–derived cells. Our data from the PR 7 group seems to indicate that cell proliferation is in fact reduced within the fibrin gels, given that the 1.5-fold increase in cell number that was seen when cells were cultured in two dimensions did not translate to cells within the three-dimensional fibrin gel.

Using this assay, we were further able to investigate the effects of cell migration on fibrin gel contraction. Measurement of fibrin gel height at the time of fixation indicated a substantial vertical contraction of the gels in the presence of cells compared with acellular controls. When cells were treated with cytochalasin-D, a chemical used to block active cell migration,[31] migration was strongly inhibited (see Fig 8), as was gel contraction (see Fig 9). Cytochalasin-D–treated groups exhibited only 20% of the contraction observed in untreated groups. These results indicate that matrix contraction is directly related to the migratory activity of cells, because inhibition of cell migratory activity resulted in reduced gel contraction. The contractile forces generated during migration are well known[37] and have been previously postulated to be responsible for matrix contraction in soft tissue wound healing.[33]

Furthermore, gel contraction was enhanced by the presence of added growth factors. Experimental groups that contained added PR showed statistically more gel contraction than cell controls without added factors (see Fig 9). This supports our previous observation that a greater number of cells were found migrating further in these groups. It follows that an increase in gel contraction is expected.

In this study, a novel in vitro model for the study of the recruitment and migration of osteogenic cells was developed. Using this model, it was determined that PR stimulates the recruitment, migration, and proliferation of bone marrow–derived cells. The model also allowed us to determine that fibrin matrix contraction is directly related to cell migration.

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