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Effects of platelet-rich and -poor plasma on the reparative response of gingival fibroblasts

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Abstract

Objectives: Although platelet-rich plasma (PRP) has been proposed as a therapeutic tool to enhance wound repair, the cellular and molecular mechanisms stimulated by this agent are still not completely understood. The present study was designed to characterize the effects of PRP and platelet-poor plasma (PPP) supernatants on cell responses involved in gingival tissue repair.

Methods: We studied the response of human gingival fibroblasts (HGF) to PRP and PPP fractions on: matrix contraction, cell migration, myofibroblastic differentiation, production of matrix components and proteolytic enzymes. PRP and PPP were obtained from donors using a commercial kit. Matrix contraction was evaluated by means of collagen lattices in the presence of matrix metalloproteinase (MMP) and actin polymerization inhibitors. The production of matrix molecules and proteinases was assessed through Western-blot. RhoA activity was evaluated through a pull-down assay. Actin distribution and focal adhesions were assessed through immunofluorescence. Transforming growth factor-beta (TGF-β) was quantified through ELISA.

Results: Both PRP and PPP stimulated human gingival fibroblasts-populated collagen gel contraction and Ilomastat and cytochalasin D inhibited this response. PRP and PPP also stimulated MT1-MMP and TIMP-2 production, RhoA activation and actin cytoskeleton remodeling, cell migration/invasion and myofibroblastic differentiation. TGF-β1 was found at significantly higher concentrations in PRP than in PPP.

Conclusions: Both PRP and PPP promote wound tissue remodeling and contraction through the stimulation of actin remodeling, the activity of MMPs, promotion of cell migration, and myofibroblastic differentiation. The similar biological responses induced by PRP and PPP suggest that both platelet-derived fractions may exert a positive effect on gingival repair.

Oral mucosal repair is a key issue in oral implantology since it is critically important to avoid the exposure of membranes or bone-filling materials during reconstructive surgery. Moreover, the reconstitution of a masticatory mucosa is essential for the long-term success of osseointegrated implants (Cairo et al. 2008). Platelet-rich plasma (PRP) contains a high concentration of growth factors and soluble molecular components that have been utilized to enhance wound healing in several tissues including bone (Hakimi et al. 2010), tendons (Kajikawa et al. 2008), skin (Villela & Santos 2010), and the periodontium (Plachokova et al. 2008). Although initial studies were focused on the potential therapeutic effect of PRP on bone repair, recent data have provided interesting results concerning the role of platelet-derived fractions on oral soft tissue healing (Lindeboom et al. 2007; Alissa et al. 2010; Torres et al. 2010). PRP is obtained after the centrifugation of a venous blood sample that leads to three typical layers: red blood cells, a "buffy coat," and an acellular plasma fraction (Anitua 1999). Although some variations are found in the different protocols used to obtain platelet-derived fractions, the buffy coat corresponds to PRP and it is normally used to promote tissue healing (Dohan Ehrenfest et al. 2009). The upper fraction of acellular plasma is known as platelet-poor plasma (PPP). Given that PPP contains smaller quantities of growth factors compared to PRP (Schnabel et al. 2007), few studies have been performed to analyze the role of PPP on gingival repair (Hamdan et al. 2009; Yılmaz et al. 2010).

After tissue injury, a blood clot that contains several growth factors initiates a

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series of coordinated events that stimulate cell recruitment and vascular growth leading to the development of granulation tissue (Martin 1997). A critical event occurring during the late phases of granulation tissue maturation is the reduction of the wound size. This process has been explained by the migration of cells into the damaged tissues (Gross et al. 1995). It has also been proposed that this phenomenon is achieved by the contractile activity of the actin cytoskeleton of fibroblasts populating the wound environment (Arora & McCulloch 1994; Grinnell 2003). Moreover, matrix metalloproteinases (MMPs) have been implicated in the remodeling of skin wounds (Lund et al. 1999) and in the retraction of fibroblasts-populated collagen lattices that resemble granulation tissue contraction (Scott et al. 1998; Berton et al. 2000). Within this group of proteolytic enzymes, MT1-MMP plays a key role in collagen degradation, angiogenesis, and wound healing (Okada et al. 1997; Hiraoka et al. 1998; Holmbeck et al. 1999). In addition, MT1-MMP may promote cell invasion in mesenchymal cells (Sabeh et al. 2004). Therefore, several evidences suggest a role for MT1-MMP in tissue repair.

Upon tissue injury, fibroblastic cells are differentiated into myofibroblasts characterized by the expression of the actin isoform α-smooth muscle actin (α-SMA) (Hinz 2007). An extensive remodeling of the actin cytoskeleton is observed during myofibroblastic differentiation (Hinz 2007). Actin remodeling is regulated by RhoA, a GTPase that promotes the polymerization of actin stress fibers (Ridley 2001). Myofibroblasts are the main cellular source of Type-I collagen during tissue repair and these cells are involved in collagen remodeling as well (Hinz 2007). Periostin corresponds to a matricellular protein that plays a key role in the physiology of the periodontium. Mice deficient in this gene develop a severe defect in the organization of collagen fibers and in the mechanical response to physical forces in the periodontal ligament (Rios et al. 2008). Moreover, periostin expression is highly induced during wound repair (Roy et al. 2007; Jackson-Boeters et al. 2009). Therefore, factors regulating the differentiation of myofibroblasts and the production of Type-I collagen and periostin are important modulators of wound healing in gingival tissues. Several growth factors such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin like growth factor (IGF) have been identified in PRP fractions and may modulate wound repair when locally applied into tissues (Lacci & Dardik 2010). TGF-β proteins constitute a family of growth factors involved in the regulation of the immune response, tissue repair, and scarring (Prime et al. 2004). After tissue injury, TGF-β released by platelets and other cellular sources stimulates wound healing. One of the most prominent cellular effects of TGF-β1 is to promote the differentiation of myofibroblasts by inducing the expression of the actin isoform α-SMA that plays a key role in extracellular matrix (ECM) remodeling and wound contraction (Arora & McCulloch 1994; Hinz 2007). TGF-β1 may also promote cell migration and the synthesis of ECM components such as collagen and fibronectin (Hinz 2007). After binding to the Type-II TGF-β receptor, TGF-β1 phosphorylates the Type-I receptor and activates the phosphorylation of the Smad proteins that modulate the expression of several TGF-β-regulated genes like Type-I collagen and α-SMA (Shi & Massagué 2003; Hinz 2007).

Although several mechanisms have been proposed to explain the remodeling and maturation of granulation tissue during wound healing, the precise effects of PRP and PPP on tissue remodeling are still not well understood. The present study was designed to characterize the role of PRP and PPP on several functions exerted by fibroblasts during wound closure and remodeling including tissue contraction, cell migration, myofibroblastic differentiation, and production of ECM molecules.

Material and methods

Cell culture

Primary cultures of HGF were established by the explant method and were cultured in α-Minimal Essential Medium (α-MEM) (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc., Logan, UT, USA) and antibiotics as previously described (Smith et al. 2006). Tissue explants were obtained from clinically healthy gingivae of nine individuals undergoing extraction of third molars at a private dental practice in Santiago, Chile. Tissue samples were harvested with the informed consent of the patients. The protocol for tissue obtainment was approved by the Ethical Committee, Faculty of Medicine, Pontificia Universidad Católica de Chile. All experiments were performed using cells expanded between passages four and ten.

PRP and PPP

The PRP and PPP were obtained from six healthy male volunteers (age range: 20–30 years) using a commercial kit (GPS®II Platelet Concentration System; Biomet Biologics, Warsaw, IN, USA) after obtaining an informed consent of the patients. PRP was prepared from 50 ml blood samples following the manufacturer's instructions. Blood samples were centrifuged at 3,200 rpm for 15 min. To induce platelet activation, PPP and PRP fractions were incubated 1 h at 37° C with 10% $CaCl_2$ and 1.5 ml of autologous thrombin obtained from each patient. After activation, both PPP and PRP were agitated in a vortex for 1 min and centrifuged for 10 min at 3,200 rpm. Platelet-released supernatants were then frozen and maintained at −80°C until experiments were performed.

Collagen gel contraction assays

For the assays 2×10^5 cells were embedded in 1 mg/ml Type-I collagen gels in 24-well plates for 1 h at 37°C and then cultured in α-MEM in the presence of a series of concentrations of PPP or PRP for 72 h. Quantification of gel contraction was estimated by πR^2. Collagen was prepared from rat tail tendons as previously described (Elsdale & Bard 1972). Since previous studies have determined that collagen gel contraction depends on the activity of MMPs (Scott et al. 1998) and on the polymerization of the actin cytoskeleton (Arora & McCulloch 1994), cells were cultured in the presence of the MMP inhibitor ilomastat (Millipore, Billerica, MA, USA) or the actin-disrupting agent cytochalasin D (Merck, Darmstadt, Germany) 1 h prior to stimulation with PRP or PPP.

Pull-down assay for RhoA

Serum-starved HGF cultures were exposed to 25% PRP or 25% PPP for 30 min. Active RhoA was detected as described previously using a pull-down assay that allows the detection of GTP-bound RhoA (Smith et al. 2006). Whole cell lysates were immunoblotted for RhoA as a loading control.

Cell migration from gingival tissue explants

Isolated explants of gingival tissue were sectioned in 1 mm^3 fragments and embedded in 1 mg/ml Type-I collagen gels in 24-well plates and cultured in α-MEM in presence of PPP or PRP (Chun et al. 2004). Images of migrating cells were captured after 5 days.

Cell migration in nested matrices

Cells were cultured within a neutralized collagen solution (1 mg/ml) and 0.2 ml aliquots

were placed in 24-well culture plates (2×10^5 cells/gel). After 60 min, matrices were gently released from the underlying culture dishes and cultured in α-MEM plus 10% FBS to allow contraction (Miron-Mendoza et al. 2008). Contracted gels were placed on top of 20 μl of collagen solution and covered with the remaining 180 μl. After 1 h at 37°C, α-MEM was supplemented with 25% PPP or 25% PRP for 24 h. Cell migration was evaluated by counting all the periphery of the contracted gels (Grinnell et al. 2006).

Cell invasion assay

Cell migration was assayed using Transwell chambers (BD Bioscience, Bedford, MA, USA) with 8 μm pore polycarbonate filters (Collaborative Research, Bedford, MA, USA) coated with 10 μg/ml of Matrigel (Costar, Cambridge, MA, USA). Cells were suspended in serum-free medium and seeded on the upper compartment of the chamber. 10% FBS, 25% PRP or 25% PPP was added to the lower compartment of the chamber. Migration was allowed to occur for 16 h. Staining and cell counting was performed as previously described (Cáceres et al. 2008).

Immunofluorescence of human gingival fibroblasts

To morphologically assess the effects of PRP and PPP on actin polymerization and focal adhesion development, serum-starved HGF were stimulated with 25% PPP or 25% PRP for 24 h in the absence of FBS and fixed with 4% paraformaldehide. Immunofluorescence was performed to identify focal adhesions and actin cytoskeleton using antibodies against Vinculin (Sigma, St. Louis, MO, USA) and F-actin (phalloidin-rhodamine) (Invitrogen Molecular Probes, Carlsbad, CA, USA) as previously described (Smith et al. 2006).

Evaluation TGF-β1 levels in PRP and PPP fractions

A quantity of 1N HCl was added to PRP or PPP fractions (1 : 25) for 60 min at 4°C. Fractions were neutralized by adding 1N NaOH (1 : 25). Total TGF-β1 was quantified using a commercial ELISA kit (BD Bioscience, San Diego, CA, USA). Fractions were tested immediately or stored at −80°C until analysis.

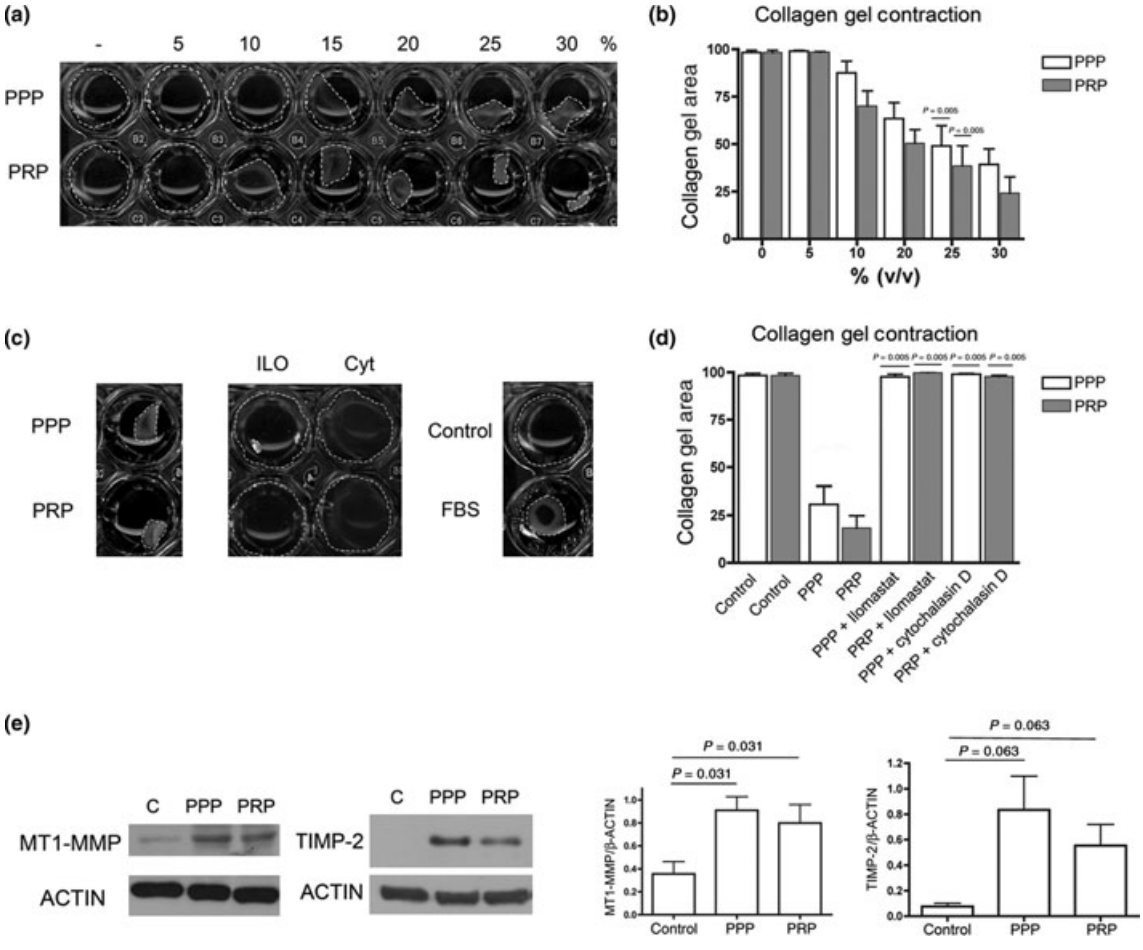

Fig. 1. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) stimulate fibroblast-populated collagen gel contraction through matrix metalloproteinase (MMP) production and actin cytoskeleton remodeling. (a) Human gingival fibroblasts (HGF) (2×10^5 cells) were cultured within collagen gels (1 mg/ml) and exposed to a range of PRP or PPP concentrations for 3 days. Dotted lines indicate the periphery of the contracted gel. (b) Collagen gel areas are represented as average and standard error. Statistical significance between PPP, PRP, and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test. (c) HGF cultured within collagen gels were incubated with 10 μM cytochalasin D (Cyt) or 10 μM ilomastat (Ilo) for 1 h and then stimulated with 25% PRP or 25% PPP for 3 days. 10% FBS was used as a positive control. (d) Collagen gel areas are represented as average and standard error. Statistical analysis was performed between PPP or PRP plus Ilo or Cyt, and PPP or PRP alone using the Wilcoxon test. Exact *P*-values indicate differences between each experimental condition and PPP or PRP alone. (e) Serum-starved HGF were cultured on 60 mm cell culture plates and stimulated with 25% PRP or PPP for 72 days. MT1-MMP and TIMP-2 levels were assessed through Western-blot of the cell lysate. MT1-MMP and TIMP-2 levels, normalized against actin and tubulin are represented as average and standard error. Total actin levels are shown as a loading control. Statistical significance between PPP, PRP and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test.

Statistical analysis

All the assays were performed using cell cultures stimulated with PRP and PPP derived from five different plasma donors in independent experiments. Data were expressed in each graph representing the average and standard error (Cumming et al. 2007). For comparing TGF-β1 levels present in PRP and PPP, the Wilcoxon test was performed. For statistical comparison between control, PPP and PRP, the Friedman test followed by Wilcoxon test was performed. Statistical significance was set at $P < 0.05$. All these analyses were performed using the SPSS software for Windows (version 16.0.2).

Results

PRP and PPP stimulate collagen gel contraction through MMP production and actin cytoskeleton remodeling

To evaluate the role played by PRP and PPP on granulation tissue contraction, we first analyzed the ability of HGF to remodel three-dimensional (3D) collagen gels. HGF were cultured within Type-I collagen gels and stimulated with a range of PRP or PPP concentrations. After 72 h, 25% PRP, or 25% PPP stimulated collagen gel contraction at statistically significant levels (Fig. 1a,b). To assess whether MMPs or actin polymerization were involved in PRP or PPP-induced collagen gel contraction, gels were cultured in the presence of 10 μM ilomastat or 10 μM cytochalasin D. As a positive control, collagen gels were incubated in the presence of 10% FBS. As shown in Fig. 1c,d, both inhibitors induced a statistically significant reduction on collagen gel contraction when compared to PRP or PPP. Considering the dependence of collagen remodeling on the activity of MMPs, we also evaluated whether or not MT1-MMP and its inhibitor TIMP-2 were modulated by PPP or PRP. As demonstrated through Western-blot in Fig. 1e, both PRP and PPP induced a significant stimulation on MT1-MMP and TIMP-2 protein levels.

PRP and PPP stimulate RhoA activation and actin polymerization

Considering that cytochalasin D inhibited PRP and PPP-stimulated collagen gel contraction, we further evaluated the activation of RhoA by both platelet-derived fractions. To this end, serum-starved HGF were stimulated with 25% PRP or 25% PPP. After 30 min, RhoA activity (RhoA-GTP) was evaluated by a pull-down assay. As demonstrated in Fig. 2a, both PRP and PPP stimulated the activation of RhoA. Subsequently, we evaluated the effects of PRP and PPP on actin polymerization and the development of focal adhesions. To this end, serum-starved HGF were stimulated with 25% PRP or 25% PPP for 24 h. Actin stress fibers were stained with phaloidin-rhodamine and vinculin was identified through immunofluorescence. Fig. 2b shows that focal adhesions and actin stress fibers were more clearly developed in PRP or PPP-stimulated cells when compared to control cultures.

PRP and PPP stimulate cell migration and invasion

To evaluate whether PRP or PPP may stimulate cell migration into the wound environment, we first evaluated the migration of fibroblasts from gingival tissue explants embedded in cell-free collagen gels supplemented with 25% PRP, 25% PPP, or 10% FBS. After 5 days, all the agents evaluated stimulated cell migration from the explants into the collagen gels (Fig. 3a). To quantify this phenomenon we used a nested cell migration assay by embedding contracted fibroblasts-populated gels surrounded by a second cell-free collagen matrix supplemented with 25% PRP, 25% PPP, or 10% FBS. As shown in Fig. 3b,c, after 24 h, both PRP and PPP significantly stimulated cell migration. Moreover, to evaluate the ability of cells to traverse a matrix and migrate into a second chamber, we used a Transwell cell migration assay in which fibroblasts were placed on top of a reconstituted extracellular matrix and 25% PRP, 25% PPP, or 10% FBS was added on the lower chamber as a chemotactic stimulus. Fig. 3d,e show that PRP and PPP were able to stimulate cell migration. Although PRP exerted a greater effect on cell migration when compared to PPP, this difference did not reach statistical significance (Fig. 3d).

PRP and PPP stimulate myofibroblastic differentiation and production of ECM molecules

To evaluate whether PRP or PPP may differentially modulate myofibroblastic differentiation, serum-starved HGF were stimulated

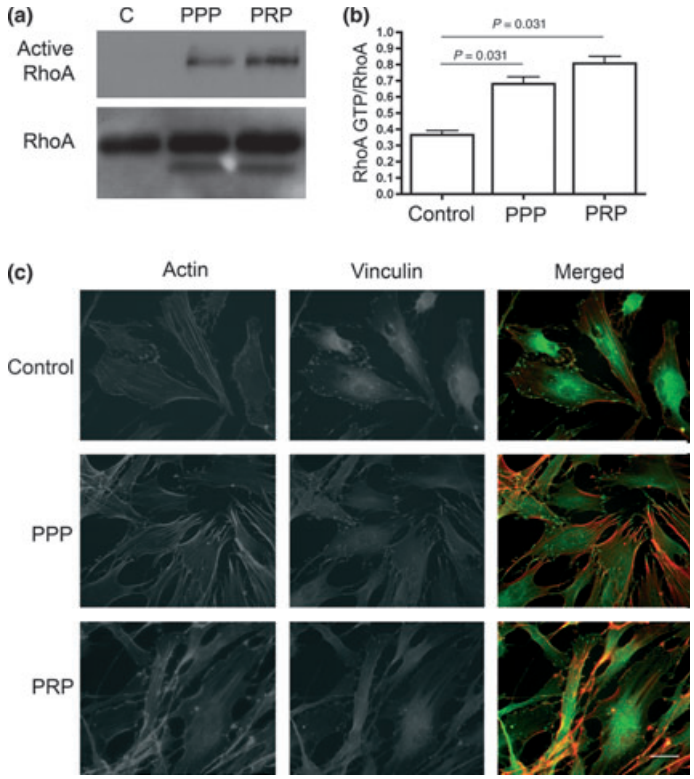

Fig. 2. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) stimulate RhoA activation and actin cytoskeleton remodeling. (a) Serum-starved human gingival fibroblasts (HGF) were stimulated with 25% PRP or 25% PPP. After 30 min, cells were lyzed and RhoA-GTP levels (active RhoA) was evaluated through a pull-down assay. Total RhoA levels are shown as a loading control. (b) RhoA-GTP levels, normalized against total RhoA are represented as average and standard error. Statistical significance between PPP, PRP and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test. (c) HGF were stimulated with 25% PRP or PPP for 24 h and the distribution of the actin cytoskeleton and vinculin were evaluated through immunofluorescence. Bar = 10 μm.

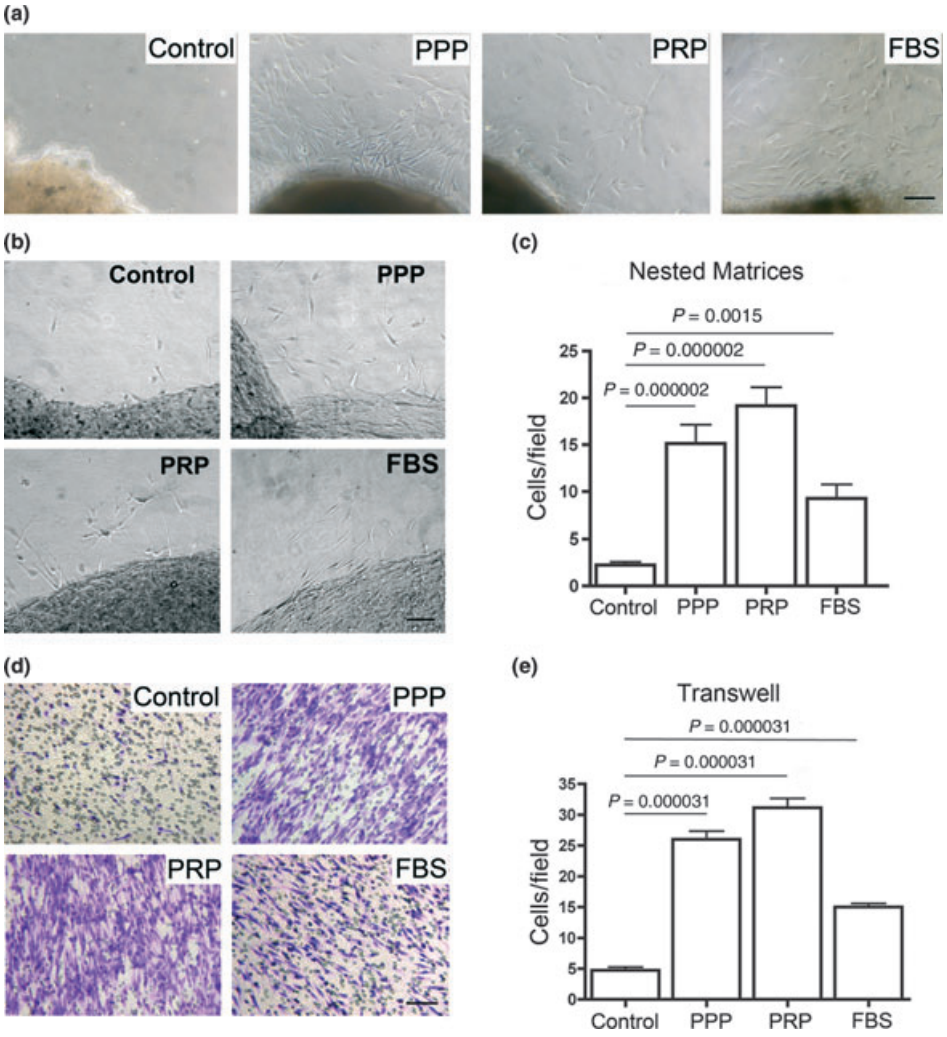

Fig. 3. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) stimulate cell migration and invasion. (a) Human gingival tissue explants were cultured within cell-free collagen gels and stimulated with 25% PRP, 25% PPP, or 10% fetal bovine serum (FBS). Images were captured after 5 days of cell migration. Bar = 30 μm. (b) Human gingival fibroblasts (HGF) migration was evaluated using a nested cell migration assay in which contracted collagen gels were immersed within cell-free collagen gels supplemented with 25% PRP, 25% PPP, or 10% FBS. Images of migrating cells were obtained after 24 h. Bar = 30 μm. (c) The number of migrating cells is represented as the average and standard error. Statistical significance between PPP, PRP, FBS and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test. (d) Cell invasion was evaluated through a Transwell assay in which cells were cultured on top of a reconstituted extracellular matrix (Matrigel). On the lower chamber of the Transwell device, 25% PRP, 25% PPP, or 10% FBS were added as a chemotactic stimulus. After 16 h, migrating cells were stained with crystal violet on the lower side of the filter. Bar = 30 μm. (e) The number of migrating cells is represented as the average and standard error. Statistical significance between PPP, PRP, FBS and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test.

with 25% PRP, 25% PPP, or 5 ng/ml TGF-β1 for 72 h. As shown in Fig. 4a, both PRP and PPP stimulated the production of α-SMA, an actin isoform considered as a hallmark of the myofibroblastic phenotype (Hinz 2007). We also evaluated the expression of EDA-Fibronectin, a spliced variant of this glycoprotein involved in myofibroblastic differentiation (Hinz 2007). As shown in Fig. 4b, cells stimulated with PRP and PPP demonstrated higher EDA-fibronectin protein levels. We also compared the production of the matricellular protein periostin. It was interesting to identify that fibroblasts stimulated with PRP and PPP demonstrated significant increases in periostin levels when compared to control cultures (Fig. 4c). We finally evaluated the protein levels of Type-I collagen under the stimulus of PRP, PPP, and TGF-β1. We observed that Type-I collagen levels were stimulated by PRP and PPP when compared to control cells (Fig. 4d). To assess the presence/activity of TGF-β ligands in platelet-derived fractions we tested the activation of the Smad signaling, the TGF-β canonical activation pathway. As shown in Fig. 4e, both 25% PRP and 25% PPP potently stimulated the phosphorylation of Smad 2/3. To assess whether or not other Smad activators such as activin were present in platelet-derived supernatants, we added the activin inhibitor follistatin to the PRP and PPP stimulus and did not find any change in Smad 2/3 activation (data not shown). Finally, we quantified TGF-β1 levels in PRP and PPP by means of an ELISA assay. The average of TGF-β1 concentrations in PRP and PPP was 18.3 and 4.1 ng/ml, respectively.

Discussion

Platelet-rich plasma has been proposed as a therapeutic tool to promote tissue repair (Dohan Ehrenfest et al. 2009). However, the biological response of cells exposed to these formulations is not well understood. Wound closure involves the maturation of granulation tissue, a process that includes several cell responses such as migration, myofibroblastic differentiation, ECM production, and the remodeling of this matrix. In the present study we provide novel findings that contribute to understand the role of PRP and PPP in wound repair. Our results show that these agents promote the contraction of three-dimensional collagen matrices through a mechanism that involves the proteolytic activity of MMPs and the remodeling of the actin cytoskeleton. In addition, both PRP and PPP stimulate the differentiation of myofibroblasts, the migration of cells, and the production of ECM components. It is interesting to note that recent studies have reported that PRP may improve oral mucosa and gingival wound healing (Lindeboom et al. 2007; Alissa et al. 2010; Torres et al. 2010). Our results reinforce the possibility that PRP, and even PPP, may exert a positive effect on gingival wound repair through the above-described mechanisms.

It has been postulated that wound closure is modulated by the contraction of granulation tissue fibroblasts through the remodeling of actin cytoskeleton (Welch et al. 1990; Arora et al. 1999; Tomasek et al. 2002). Actin stress fibers are connected to ECM components through integrin receptors and forces generated at the intracellular level are able to guide tissue remodeling (Tomasek et al. 2002). Fibroblasts-populated collagen gels have been used as a model to study gran-

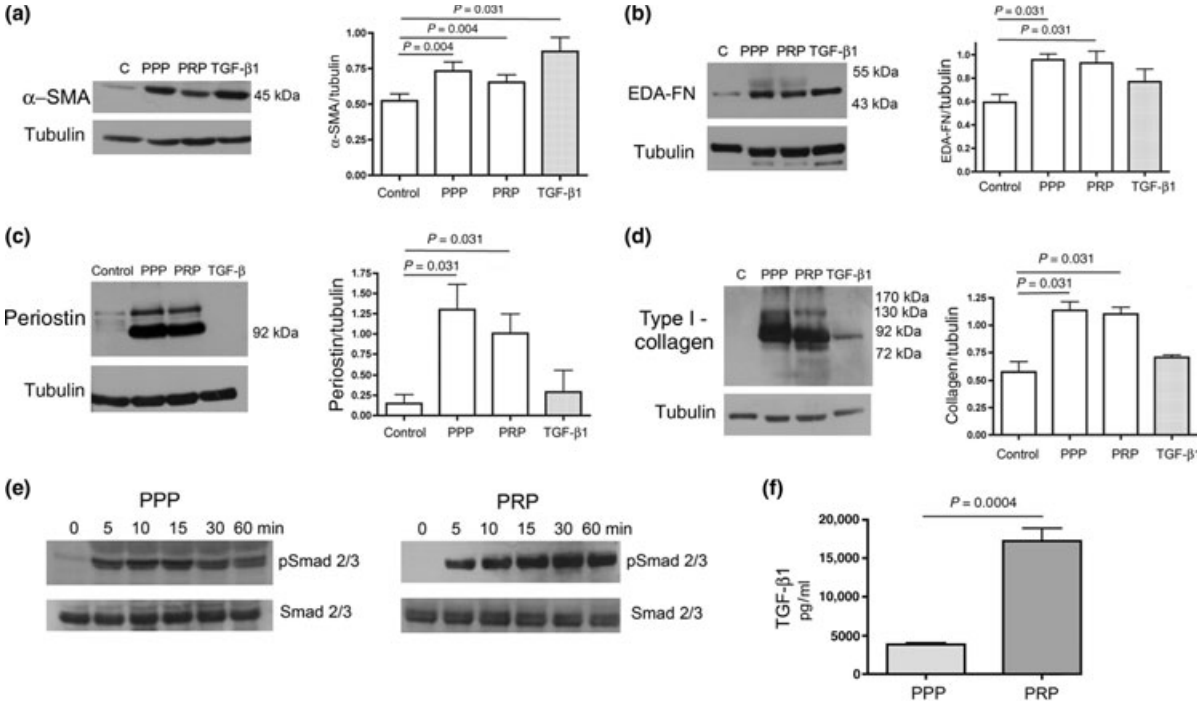

Fig. 4. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) stimulate myofibroblastic differentiation. Serum-starved human gingival fibroblasts (HGF) were stimulated with 25% PRP, 25% PPP, or 5 ng/ml transforming growth factor-β1 (TGF-β1) for 72 h. α-Smooth muscle actin (α-SMA), EDA-FN, Periostin and Type-I collagen levels were analyzed through Western-blot of the cell lysates. α-SMA (a), EDA-FN (b), Periostin (c) and Type-I collagen (d) levels were digitalized and normalized against Tubulin levels (loading control) and expressed as mean and standard error. Statistical significance between PPP, PRP, TGF-β1 and control-stimulated cells was calculated using the Freidman test followed by the Wilcoxon test. Exact *P*-values indicate differences between each stimulated condition and its control using the Wilcoxon test. (e) Serum-starved HGF were stimulated with 25% PRP or 25% PPP from 0 to 60 min. Phosphorylated Smad2/3 and total Smad2/3 levels were analyzed through Western-blot of the cell lysates. (f) TGF-β1 levels were determined in the PRP or PPP fractions derived from six donors through an ELISA assay. TGF-β1 protein levels are shown as average and standard deviation. Statistical significance between PPP and PRP was calculated using the Wilcoxon test. Exact *P*-values indicate differences in TGF-β1 concentrations between PPP and PRP.

ulation tissue remodeling during wound repair (Tomasek et al. 2002). In our study, collagen gel contraction was stimulated by PRP and PPP and this response was highly dependent on the polymerization of the actin cytoskeleton. Moreover, both PRP and PPP stimulated the activation of the GTPase RhoA that is a critical component of the signaling pathways involved in the development of actin stress fibers and focal adhesions (Ridley 2001). In addition, PRP and PPP stimulated the polymerization of actin stress fibers and the formation of focal adhesion contacts, reinforcing the mechanical connection between cells and the ECM allowing collagen contraction. These findings provide an original mechanism of action for PRP and PPP on the modulation of the actin cytoskeleton and the remodeling of granulation tissue.

The MMPs play a significant role in wound tissue remodeling through the degradation of structural components of the ECM and the proteolytic modification of cytokines, growth factors, and chemokines involved in cell signaling (Mirastschijski et al. 2004; Gill & Parks 2008). Using fibroblast-populated collagen gels we observed that both PRP and PPP were able to stimulate tissue contraction through a mechanism efficiently attenuated by the MMP inhibitor GM6001. Our results also demonstrate that PRP and PPP stimulate the production of MT1-MMP and its inhibitor TIMP-2, suggesting that soluble factors present in platelet-derived fractions may exert a balanced activation of the proteolytic cascades involved in granulation tissue remodeling. This report represents a novel description of this mechanism of action for platelet-derived soluble factors that involves the activity of MMPs in the remodeling of granulation tissue. MT1-MMP plays a key role in collagen degradation (Holmbeck et al. 1999) and its expression has been identified in wounds (Okada et al. 1997). MT1-MMP not only degrade Type-I collagen but may also process connective tissue growth factor (CTGF), thereby modulating its biological activity (Tam et al. 2004). Since CTGF is a significant mediator of fibrogenesis and wound repair (Cicha & Goppelt-Struebe 2009), it is tempting to speculate whether PRP or PPP might affect the biological activity of this growth factor.

Cell migration is an important phenomenon that is critical for increasing the cellular content of the wound clot (Martin 1997). Moreover, it has been proposed that forces induced by fibroblasts migrating into granulation tissue are important for wound closure (Ehrlich & Rajaratnam 1990). Our study evaluated cell migration using three *in vitro* models (migration from tissue explants, nested cell migration, and bi-cameral cell invasion assay). We observed that cell migration was similarly stimulated by both fractions. Several growth factors involved in cell migration including PDGF-BB, TGF-β1 and IGF have been detected in PRP (Postlethwaite et al. 1987; Nishimura & Terranova 1996; Lacci & Dardik 2010). Although our effort was restricted to the evaluation of TGF-β1 in PRP and PPP, previous studies have not been able to identify PDGF-BB and IGF in PPP (Schnabel et al. 2007). Therefore, TGF-β1 may be one of the molecular factors present in PPP that stimulate cell migration.

Myofibroblasts represent key players in the reconstruction of damaged connective tissues after injury (Hinz 2007). These cells are characterized by the expression of α-SMA, an actin isoform that enable fibroblasts to induce high contractile forces that permit wound contraction. Our observations revealed that PRP and PPP equally stimu-

lated α-SMA and ED-A fibronectin, suggesting that both platelet-derived fractions stimulate myofibroblastic differentiation. It is interesting to mention that PRP and PPP were also similarly effective in stimulating Type-I collagen and periostin production. Type-I collagen represents the structural framework of the gingival and periodontal connective tissues and periostin plays a key role in the organization of collagen fibers in the periodontium (Rios et al. 2008). Therefore, our results suggest that PRP and PPP may exert a similar stimulatory effect on the reconstruction of damaged gingival tissues through the differentiation of myofibroblasts and the production of structural molecules of the ECM.

Even though several studies have analyzed the responses of different cell types upon PRP or PPP stimulation, there is still no agreement concerning the appropriate concentration of growth factors necessary to promote tissue repair (Cenni et al. 2005; Slapnicka et al. 2008; Dohan Ehrenfest et al. 2009). In our study, PRP and PPP induced a similar effect on RhoA activation and on the production of MT1-MMP, TIMP-2, α-SMA, FN-EDA, Type-I collagen, and periostin. PRP induced a more potent effect on cell migration when compared to PPP but these differences were not significant. PRP also induced a stronger effect on collagen gel contraction when compared to PPP, however, only one of these different responses reached statistical significance at 20% of the platelet-derived fractions. Although our results showed that TGF-β1 was ~4.4 times more concentrated on PRP than on PPP, the cell assays described in the present study showed a similar biological response upon stimulation with both platelet-derived fractions. Two possibilities arise to explain these findings: on one hand, it is possible that the wide range of cellular activities observed in HGF cultured in the presence of PPP or PRP represent the response to factors other than TGF-β1, PDGF-BB, or IGF. On the other hand, it is also possible that these responses are induced by very low concentrations of TGF-β1 (below the concentration identified in PPP). These results suggest that important cell responses involved in tissue repair, such as those analyzed in this study, are appropriately stimulated with relatively low concentrations of growth factors as those identified in PPP. These findings coincide with those described by Graziani et al. (2006), which observed that the best cell proliferation and differentiation responses were not necessarily obtained with the higher concentration of PRP.

Taken together, these results show that gingival fibroblasts may respond to both PRP and PPP with similar biological traits that may be associated with gingival tissue repair. Given the complex composition of platelet-derived fractions, more studies are needed to disclose the role of these agents on the promotion of wound healing.

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