Effect of Platelet-Rich Plasma on the Peri-implant Bone Response: An Experimental Study

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Platelet-rich plasma (PRP) is used as a source of growth factors to stimulate and accelerate bone formation and soft tissue healing. The use of PRP in bone regeneration, both around dental implants and in periodontic treatments, has become particularly appealing. The aim of this study was to evaluate the effect of PRP in an experimental model of osteogenesis around laminar implants. Fifteen male Wistar rats, weighing 90 ± 10 g, were used in this study. One milliliter of blood was obtained from each animal by intracardiac puncture and transferred into Eppendorf tubes containing 10% sodium citrate. The tubes were centrifuged at 1500 rpm for 15 minutes and PRP was prepared. The laminar test was used to evaluate the bone peri-implant response. PRP and a titanium laminar implant were introduced into the right tibia (Ti/PRP group), whereas the left tibia (control) received only a laminar implant (Ti group). Thirty days postimplantation, the tibiae were resected, radiographed, and processed for embedding in acrylic resin. Ground sections (50 μm) were stained with toluidine blue. The peri-implant bone volume was evaluated histomorphometrically. Statistical analysis of the data was performed. The amount of newly formed bone in the Ti/PRP group (30 ± 7 cm^2) was significantly greater than in the Ti group (16 ± 3 cm^2). A greater volume of peri-implant bone was observed when PRP was used in the laminar implant test model. (Implant Dent 2004;13:73–78)

Key Words: platelet-rich plasma, titanium implants, bone volume, growth factors

Recent advances in biotechnology, the presence of growth factors, and the study of different biomaterials contribute to the knowledge of what is known today as "tissue engineering."[1] One of the most critical elements of tissue engineering is the ability to mimic the extracellular matrix scaffolds (ECM) that normally serve to organize cells into tissues. ECM can modulate growth and morphogenesis through their ability to bind, store, and eventually release soluble regulators (growth factors). It is well known that growth factors are important to initiate and accelerate the normal processes involved in going from injury to repair.[2]

Platelets are considered to be important mediators in the initiation of inflammation and subsequent tissue remodeling and repair. Circulating platelets are invariably associated with wounds and are now known to release factors originally stored within intracellular compartments such as the platelet granules into wounds.[2] The bone-reparative process is the result of a cascade of tissue responses, including remodeling, whose final outcome is the formation of a lamellar bone structure. There are a number of bone reparative processes that require bone formation such as fracture healing, postextraction alveolar wound healing, and orthopedic or dental implantation, among others.

Regarding dental implantation, systemic conditions such as alterations in bone metabolism, hormone balance, corticosteroid therapy, smoking habits, and local conditions such as quantity and quality of bone, the presence of bone fillings, a traumatic surgical technique, radiation therapy, and so on, can affect the peri-implant bone healing response.[3–8]

In 1994, Tayapongsak et al.[9] suggested that the incorporation of autologous fibrin adhesive to bone would increase osteoconduction. Other authors proposed that platelet-rich plasma (PRP) could successfully stimulate bone formation around implants in the mandible.[10] PRP has been shown to enhance the bone response when it is combined with particulate bone allografts or other bone-filling materials such as chitosan/tricalcium phosphate sponge carrier, particulate dentin–plaster of Paris, methylcellulose gel, and bioactive glass.[11–13]

In recent years, PRP has been used as a source of growth factors for

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bone regeneration.[10,14] Clinical studies have shown that PRP accelerates soft tissue wound healing.[15] Platelets have been shown to contain various growth factors that have a significant role in tissue repair processes, ie, platelet-derived growth factor (PDGF), transforming growth factor β1 and β2 (TGF β1 and TGF β2), and insulin growth factor (IGF).[10] The aim of this study was to perform a histomorphometric evaluation of the effect of PRP on the peri-implant bone response in rats.

Materials and Methods

Animals

Fifteen male Wistar rats weighing 90 g ($\pm$ 10 g) were used. The animals were housed in plastic cages and maintained on a 12:12-hour light:dark cycle. They were fed rat chow and water ad libitum. National Institute of Health guidelines for the care and use of laboratory animals (NIH publication No. 85-23, revised 1985) were observed.

Platelet-Rich Plasma

The animals were anesthetized intraperitoneally with 1.28 mg xylazine/100 g body weight (Bayer, Argentina) and 8 mg ketamine/100 g body weight (Ketalar; Parke Davis, Morris Plains, NJ). One milliliter of blood was extracted from each animal by cardiac puncture. Samples were taken to prepare blood smears and perform platelet counts. Autologous PRP was then obtained and used to perform smears and platelet counts in a Neubauer chamber to verify adequate platelet concentration. PRP was coagulated with calcium chloride and bovine thrombin before use (Soluplastín; Wiener Lab, Argentina).

Surgical Procedure

A commercially pure (c.p.) titanium laminar implant (5 $\times$ 0.1 mm; Implant Vel, Buenos Aires, Argentina) was placed in the left tibia of each rat (Ti group) and a c.p. titanium laminar implant covered with PRP was placed in the right tibia (Ti/PRP group). The nontraumatic surgical technique described by Cabrini et al.[16] was followed. All the animals were killed by ether overdose on day 30 postimplantation. The tibiae were resected, fixed in 20% formalin solution, and radiographed.

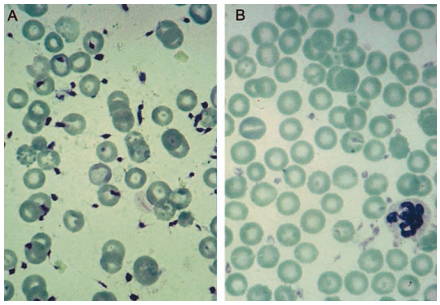

Fig. 1. Smears. (**A**) Platelet-rich plasma smear**.** (**B**) Blood smear. Note that the concentration of platelets is greater in **A**. May-Grünwald-Giemsa stain. Original magnification $\times$400.

Histologic Processing

The tibiae were processed for embedding in methylmethacrylate resin. The samples were sectioned using a saw, and 3 slices were cut perpendicular to the longer axis of the implant. The slices were ground using a grinding machine and finished manually with sandpaper to obtain sections approximately 50 μm in thickness. The sections were then stained with 1% toluidine blue.

Histomorphometric Analysis

Histomorphometric determinations were performed on sections using a light microscope (Zeiss Axioscop 2 MOT; Carl Zeiss, Jena, Germany) online with an image-analysis system (Kontron KS300 version 2; Kontron Elektronik, Munich, Germany). The peri-implant bone volume was evaluated. The results were statistically analyzed by Student *t* test. Data were reported as mean $\pm$ standard deviation at a significance level of $P < 0.05$.

Results

None of the animals showed alterations in body weight, behavior, or general health. The smears of the PRP fraction were richer in platelets than whole blood smears (Fig. 1A, B). This finding was confirmed by counting in a Neubauer chamber (1,188,000 per mm^3 in the PRP fraction and 493,000 platelets per mm^3 in the whole blood fraction).

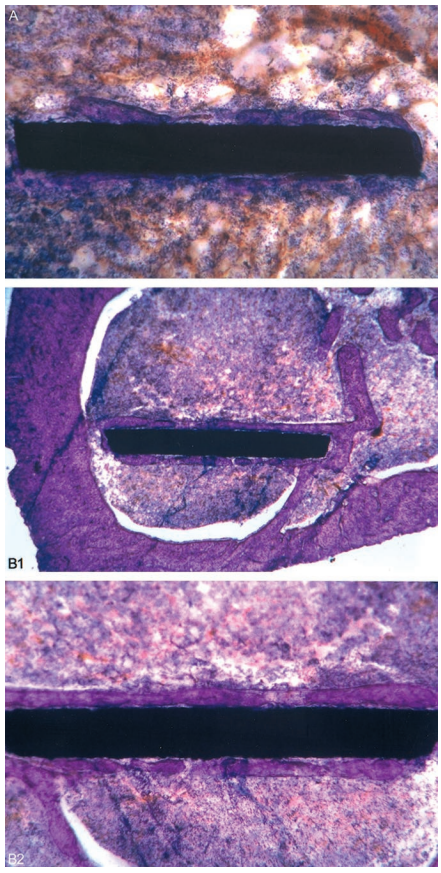

Fig. 2. (**A**) Group Ti. (**B**) Group Ti/PRP. 30 days postimplantation. Note the increase in peri-implant bone volume in the Ti/PRP group (**B**1–2) compared with the Ti group (**A**). Ground sections, toluidine blue stain, **A–B**2 original magnification $\times$100, **B**1 original magnification $\times$50.

In both groups, histologic sections of the tibiae showed lamellar bone formation in close contact with the metallic surface of the implant (osseointegration). Greater bone formation was observed in the Ti/PRP group (Fig. 2A, B_{1-2}). No macrophages or related inflammatory cells were found. The histomorphometric parameters evidenced greater bone volume around the implant in the Ti/PRP group as compared with the Ti group (30 cm^2 $\pm$ 7 and 16 cm^2 $\pm$ 3, respectively) (Table 1). These differences were statistically significant ($P < 0.05$).

Discussion

The use of PRP has become widespread in traumatology, gastroenterology, and ophthalmology[17] among other fields. It is also used extensively

Table 1. Histomorphometric analysis.

Histomorphometric Parameters	Ti Group (control) n: 15 $\bar{\chi}$	Ti/PRP Group (experimental) n: 15 $\bar{\chi}$	P
Peri-implant bone volume	16 $cm^{2*} \pm 3$	30 $cm^{2*} \pm 7$	<0.05

A lower peri-implant bone volume was observed in control group compared to experimental group ($P < 0.05$).

* Values of projections. Magnification ×160.

$\bar{\chi}$: mean. ±: standard deviation. Ti: titanium. Ti/PRP: titanium-platelet rich plasma.

to favor wound healing in skin.[18,19] Its efficacy in bone tissue when applied in combination with bone fillings has been evaluated clinically and experimentally. Most of the clinical studies involve the use of PRP associated with different types of bone-filling materials, ie, autologous bone,[10,15] allografts,[11,20,21] or synthetic substitutes of bone.[22] These studies reveal an increase in bone formation in the cases treated with PRP. Experimental studies using recombinant growth factors[12,23,24] or autologous PRP[13,21] in combination with different types of bone-filling materials evidenced the efficacy of PRP in peri-implant osteogenesis.

Each platelet is known to have approximately 1200 molecules of PDGF.[25,26] Transforming growth factors β1 and β2 are also found in the platelets and are involved in connective tissue repair and bone regeneration.[27,28] These factors are released by platelet degranulation and are involved in the modulation of long-term bone response and the stimulation of osteoblast precursors.[10]

Experimental studies in dogs[24] at 3, 8, and 12 weeks using recombinant growth factors in methylcellulose sponges placed with implants in dental alveoli suggest that PDGF and IGF do participate in the initial phase of the bone repair process.

Young-Moo Lee et al.[12] showed an osteogenic effect in bone regeneration *in vivo* using chitosan to release the recombinant growth factor PDGF-BB in the experimental model of rat calvaria. The PRP fraction used in this study contains 240% more platelets than whole blood. A higher percentage of platelet concentration exerts a positive effect in osteogenesis, increasing mitogenesis and chemotaxis of bone cells. Thus, the rise in the concentration of growth factors achieved by the use of PRP elicited an increase in the volume of newly formed bone. These findings are in agreement with Marx et al.,[10] who proposed that the amplification of the effect of growth factors achieved by separating and concentrating platelets in PRP would result in a significant effect on osteogenesis and the final volume of newly formed bone. There are no reports in the literature on the use of PRP alone around implants. This study shows that PRP elicits a statistically significant rise in osteogenesis.

Stefani et al.[24] failed to find significant differences in bone area associated with implants. These authors used an experimental model in dogs, placing titanium implants and recombinant growth factors (PDGF and IGF-1) in postextraction alveoli. The model differs from the one used here that showed that at 30 days, the volume of newly formed bone was significantly greater in the Ti/PRP group than in the Ti group. This response could be attributed to the presence of different growth factors and/or a higher amount of them.

Conclusion

Our experimental model was designed to evaluate, histologically and histomorphometrically, the way that PRP might affect the peri-implant bone response. In this experimental model, the use of PRP resulted in a significant increase in peri-implant bone volume.

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Disclosure

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

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Abstract Translations [German, Spanish, Portugese, Japanese]

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Thrombozytenreiches Blutplasma und dessen Auswirkung auf das das Implantat umlagernde Knochengewebe: ein Laborversuch

ZUSAMMENFASSUNG: **Zielsetzung:** Thrombozytenreiches Blutplasma wirkt unterstützend auf die Stimulation und Beschleunigung des Knochengewebswachstums und der Weichgewebsheilung. Als besonders erfolgversprechend hat sich der Einsatz von thrombozytenreichem Plasma bei der Knochenwiederherstellung sowohl im ein Zahnimplantat umlagernden Gewebe wie auch im parodentalen Gewebe herausgestellt. Die vorliegende Studie hatte sich zum Ziel gesetzt, die Wirkweise von thrombozytenreichem Blutplasma auf die Osteogenese im Schichtimplantate umlagernden Gewebe zu bewerten. **Methoden und Materialien:** Für die Versuchsreihe wurden 50 männliche Wistar-Ratten mit einem Gewicht von 90 g $\pm$ 10 g herangezogen. Durch endokardiale Punktion wurde jedem der Versuchstiere ein Milliliter Blut entnommen. Die Blutproben wurden in mit 10%igem Natriumcitrat gefüllte Eppendorf-Röhren gegeben. Die Röhren wurden für 15 Minuten bei 1500 UpM in der Zentrifuge gehalten und das thrombozytenreiche Blutplasma vorbereitet. Im Schichttest sollte die Knochengewebsreaktion am das Implantat umlagernden Gewebe beurteilt werden. Am rechten Schienbein wurden sowohl thrombozytenreiches Plasma als auch ein Titan-Schichtenimplantat implantiert (Versuchsgruppe), während am linken Schienbein nur ein Schichtimplantat eingesetzt wurde (Kontrollgruppe). 30 Tage nach erfolgter Implantation wurden die Schienbeine operativ entfernt, röntgentechnisch untersucht und zur Einbettung in Acrylharz vorbereitet. Bodenabschnitte (50 μm) wurden mit Toluidinblau eingefärbt. **Ergebnisse:** Es erfolgte eine histomorphologische Messung des Knochenvolumens im das Implantat umlagernden Gewebe. Die Daten wurden einer statistischen Analyse unterzogen. Hierbei wies die Versuchsgruppe einen wesentlich höheren Anteil an Knochengewebsneubildungen auf (30 $\pm$ 7 cm^2) auf als die zum Vergleich herangezogene Kontrollgruppe (16 $\pm$ 3 cm^2). **Schlussfolgerung:** Bei Einsatz von Schichtimplantaten wie im Modellversuch weist das das Implantat direkt umlagernde Gewebe bei Verwendung von thrombozytenreichem Blutplasma wesentlich bessere Volumenwerte auf.

SCHLÜSSELWÖRTER: Thrombozytenreiches Blutplasma, Titanimplantate, Knochengewebsvolumen, Wachstumsfaktoren

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Efecto del plasma rico en plaquetas en la respuesta del hueso periimplante: Un estudio experimental

ABSTRACTO: **Propósito:** El plasma rico en plaquetas (PRP por sus siglas en inglés) se usa como fuente de factores de crecimiento para estimular y acelerar la formación del hueso y la curación del tejido suave. El uso de PRP en la regeneración del hueso alrededor de implantes dentales y los tratamientos periodónticos se ha convertido en un tratamiento interesante. El objetivo de este estudio fue evaluar el efecto del PRP en un modelo experimental de osteogénesis alrededor de implantes laminares. **Métodos Y Materiales:** En este estudio, se usaron cincuenta ratas Wistar macho, con un peso de 90 g ± 10 g. Se obtuvo 1 mililitro de sangre de cada animal a través de un pinchazo intracardíaco y transferido a tubos Eppendorf que contenían un 10% de citrato de sodio. Los tubos se centrifugaron a 1500 RPM durante 15 minutos y se preparó el PRP. Se usó la prueba laminar para evaluar la respuesta periimplante del hueso. Se introdujeron el PRP y un implante laminar de titanio en la tibia derecha (grupo Ti/PRP); mientras que la tibia izquierda (control), solamente recibió un implante laminar (grupo Ti). Treinta días luego de la implantación, se completó una resección de la tibia, con radiografía y procesada para determinar la penetración en resina acrílica. Las secciones molidas (50 μm) se tiñeron con azul de toluidina. **Resultados:** Se evaluó el volumen del hueso periimplante histomorfométricamente. Se completó el análisis estadístico de los datos. La cantidad de nuevo hueso formado en el grupo Ti/PRP (30 ± 7 cm^2) fue significativamente mayor que en el grupo Ti (16 ± 3 cm^2). **Conclusión**: Se observó un volumen mayor de hueso periimplante cuando se usó PRP en el modelo de colocación del implante laminar.

PALABRAS CLAVES: Plasma rico en plaquetas, implantes de titanio, volumen de hueso, factores de crecimiento.

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Efeito do Plasma Rico em Plaquetas sobre a Resposta do Osso Periimplantário: um Estudo Experimental

RESUMO: **Objetivo:** O Plasma Rico em Plaquetas (PRP) é usado como fonte de fatores de crescimento para estimular e acelerar a formação de osso e a cura do tecido mole. O uso de PRP na regeneração óssea, tanto em torno de implantes dentários quanto em tratamentos periodônticos, tornou-se particularmente atraente. O objetivo deste estudo foi avaliar o efeito do PRP num modelo experimental de osteogênese em torno de implantes laminares.**Métodos & Materiais:** Cinqüenta ratos Wistar machos, pesando 90 g – 10 g, foram usados neste estudo. Um mililitro de sangue foi obtido de cada animal através de punctura intracardíaca e transferido para tubos Eppendorf contendo 10% de citrato de sódio. Os tubos foram centrifugados a 1500 RPM durante 15 minutos e o PRP foi preparado. O teste laminar foi usado para avaliar a resposta periimplantária do osso. O PRP e um implante laminar de titânio foram introduzidos na tíbia direita (grupo Ti/PRP); ao passo que a tíbia esquerda (controle) recebeu apenas um implante laminar (grupo Ti). Trinta dias após a implantação, as tíbias foram seccionadas, radiografadas e processadas para incrustação na resina acrílica. Seções da superfície (50 um) foram manchadas com azul de toluidina.

Resultados: O volume do osso periimplantário foi avaliado histomorfometricamente. Foi realizada análise estatística dos dados. A quantidade de osso recém-formado no grupo Ti/PRP (30-7 cm2) foi significativamente maior do que no grupo Ti (16-3 cm2). **Conclusão:** Um volume maior de osso periimplantário foi observado quando o PRP foi empregado no modelo de teste de implante laminar.

PALAVRAS-CHAVE: Plasma rico em plaquetas, implantes de titânio, volume do osso, fatores de crescimento.

インプラント周辺の骨反応へのPlatelet Rich Plasmaの影響：実験的研究

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要約：

目的：Platelet Rich Plasma（PRP）は、骨形成と柔組織治癒を促進させる成長要因の素材として使われる。デンタル・インプラント周辺と歯周病治療のための骨再生におけるPRPの使用は、特に好まれる方法である。本研究の目的は、laminarインプラント周囲の骨形成の実験モデルへのPRPの影響を評価することにあった。

素材と方法：実験には体重90g±10gの雄ウイスター・ラット50匹が使用された。各ラットからそれぞれ1mlの血液が心内穿針によって採取され、クエン酸ナトリウム10％を含むエッペンドルフ・チューブに挿入された。エッペンドルフ・チューブは1500RPMで15分間遠心分離されPRPが準備された。Laminar testが、骨インプラント周辺の反応を評価するために使われた。PRPとチタンlaminarインプラントは右脛骨に施され（Ti/PRP群）、左脛骨にはlaminarインプラントのみが設置された（対照実験群）。インプラント設置30日後に脛骨は切除され、X線撮影の後にアクリルレジン埋設用に処理された。切砕断面（50μm）はトルイジンブルーで染色された。

結果：インプラント周辺の骨体積が組織形態計測学的に評価された。データの統計分析が行われた。Ti/PRP群（30±7㎣）における新骨形成は、対照実験群（16±3㎣）より有意に大きかった。

結論：PRPがlaminarインプラントの試験モデルに使われた場合に、インプラント周辺骨の体積がより大きくなることが観察された。

キーワード：Platelet Rich Plasma、チタン・インプラント、骨体積、成長要因

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