



## Treatment of localized aggressive periodontitis with platelet-rich plasma and bone allograft. Clinical case report

### *Tratamiento de periodontitis agresiva localizada con plasma rico en plaquetas y aloinjerto óseo. Un caso clínico*

Beatriz Raquel Yáñez Ocampo,\* María Guadalupe Marín González\*

#### ABSTRACT

**Introduction:** Platelet-rich plasma (PRP) has emerged as an alternative in periodontal therapy. Presently, there is an evidence-based learning curve showing a first stage when it was used as cementing biomaterial as well as bone tissue regeneration stimulant. In a second current stage based and substantiated on biological evidence, it is applied for soft tissue wounds healing. This has elicited great expectations in several medical specialties, including dentistry. **Method:** The case here presented is of a healthy, non-smoker, 29 year old female patient who attended the Graduate and Research School, National School of Dentistry, National University of Mexico (UNAM). Diagnosis emitted was localized aggressive periodontitis. After phase I, the patient was subjected to flap surgery with PRP and bone allograft. **Results:** The patient was assessed 6 and 12 months after treatment. Based on the six maintenance parameters established by Drs. Lang and Tonetti, she was classified as presenting low risk of periodontal disease recurrence. **Conclusions:** Today's clinical operator increasingly understands the need of making decisions based upon scientific evidence. To the present date we recognize that it is biologically possible for a higher platelet concentration to foster healing.

**Key words:** Periodontitis, platelet-rich plasma, bone allograft.

**Palabras clave:** Periodontitis, plasma rico en plaquetas, aloinjerto óseo.

#### INTRODUCTION

Periodontal diseases are mixed, endogenous infections caused by microorganisms which colonize and cause periodontal pockets, recession or both.<sup>1</sup> These diseases were classified in 1999 at the International Meeting for Classification of Periodontal Conditions and Diseases. At said meeting aggressive periodontitis as such was characterized and named for the first time.

Aggressive periodontitis mainly appears in young, systemically healthy patients. It is characterized by rapid loss of attachment and bone destruction, irrespective of the amount of present microbial deposits. In these cases, flora is specific with high

#### RESUMEN

**Introducción:** El plasma rico en plaquetas ha emergido como una alternativa en la terapia periodontal. Hoy tenemos una curva de aprendizaje basada en la evidencia que nos muestra una primera etapa donde se utilizó como biomaterial cementante y como estimulante de la regeneración de tejido óseo. En una segunda etapa actual se aplica para la curación de heridas en tejidos blandos basado y fundamentado en la evidencia biológica, lo que ha generado grandes expectativas en varias especialidades médicas, entre las que se encuentra la odontología. **Método:** Se presenta un caso clínico de una paciente de 29 años de edad, no fumadora y sin enfermedad sistémica, la cual fue captada en la DEPel de la UNAM. El diagnóstico fue una periodontitis agresiva localizada. Después de la fase I se le realizó una cirugía por colgajo con PRP y aloinjerto óseo. **Resultados:** Se valoró a la paciente a los 6 y 12 meses después del tratamiento y se clasificó como paciente de bajo riesgo a la recurrencia de enfermedad periodontal durante el mantenimiento con base en los seis parámetros del Dr. Lang y Tonetti. **Conclusiones:** El clínico de hoy entiende cada vez más la necesidad de tomar decisiones basadas en la evidencia científica. Hasta este momento sabemos que biológicamente es posible que una concentración más alta de plaquetas pueda ayudar en la cicatrización.

proportions of *Aggregatibacter* as well as *P. gingivalis*. In this situation, phagocytes can be affected. Moreover, other family members might be afflicted with the same condition.<sup>1</sup>

In cases when first molars and central incisors are involved aggressive periodontitis is sub-classified into localized.<sup>1</sup>

Along the time, different techniques have been used to treat this disease. These techniques targeted

\* Professor, Implantology and Periodontics Department, Graduate and Research School, National School of Dentistry, National University of Mexico (UNAM).

the repair or regeneration of damage caused. They purported the aim of improving periodontal circumstances as well as preserving esthetics and function. This does not mean that one technique might be better than the next, it means that each technique has a precise indication. In the present clinical case, it

**Table I.** Medical specialties which have published PRP use.

Dentistry
<ul style="list-style-type: none"> <li>• Mandibular reconstruction</li> <li>• Bone grafts</li> <li>• Dental implants</li> </ul>
Traumatology
<ul style="list-style-type: none"> <li>• Arthroplasty, prostheses and implants</li> <li>• Bone implants</li> <li>• Spinal fusion</li> <li>• Fractures and bone defects treatment</li> <li>• Intra-articular infiltrations</li> <li>• Chondro-articular regeneration</li> </ul>
Ophthalmology
<ul style="list-style-type: none"> <li>• Incision closure</li> <li>• Reparation of ulcers, abrasions and burns</li> </ul>
Neurosurgery
<ul style="list-style-type: none"> <li>• Duramater reparation</li> <li>• Craniotomy in general</li> <li>• Pituitary tumor surgery</li> </ul>
Cardiovascular surgery
<ul style="list-style-type: none"> <li>• Vascular grafts and prostheses</li> <li>• Aortic, abdominal, thoracic or thoracic-abdominal aneurysms</li> <li>• Carotid surgery</li> <li>• Venous ulcers</li> </ul>
Dermatology
<ul style="list-style-type: none"> <li>• Dermis and epidermis regeneration</li> <li>• Skin grafts</li> <li>• Burns</li> <li>• Bulbar implants and mesotherapy</li> <li>• Body and facial mesotherapy for esthetic and reparation purpose</li> </ul>

was decided to repair damage caused by periodontal disease with platelet rich plasma (PRP) combined with bone allograft.

We would like to mention as precedent that PRP was first reported by Dr. M Ferrari in 1987 as an autologous component used in an open heart surgical event with the aim of decreasing bleeding.<sup>2</sup>

Unfortunately, in recent years PRP could only be obtained through a cell separator or plasmapheresis machine. These machines were large and costly, therefore, use of PRP was limited to the operating theatre and mainly used for major surgical events.<sup>2</sup>

Usage for wound healing showed possible benefits to promote growth when applied to soft tissues; this technique generated great expectations and numerous publications were included in varied medical specialties (*Table I*).<sup>3</sup>

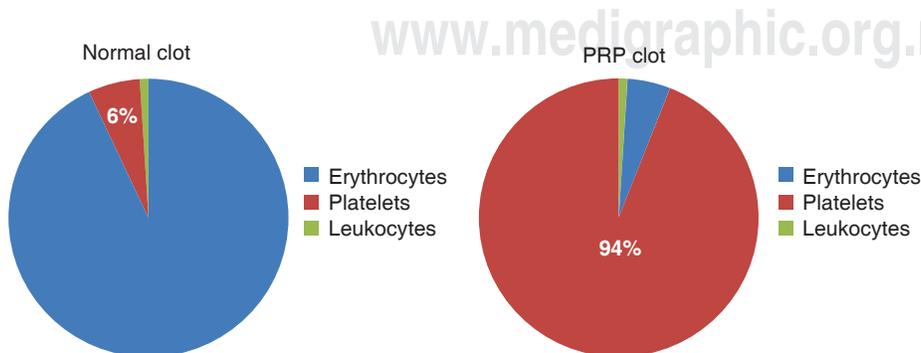
PRP is an autogenous blood clot which contains highly concentrated amounts of platelets. In this clot, the minimum platelet count is one million platelets/microliter. This represents from 4 to 7 times over the normal count, which is 200,000 platelets/microliter.

A normal blood clot contains 94% erythrocytes, 6% platelets and less than 1% leukocytes. In comparison, a PRP blood clot contains 94% platelets, only 5% erythrocytes and 1% leukocytes (*Figure 1*).<sup>2</sup>

Platelets rise from cytoplasmic fragmentation of megakaryocyte in the bone marrow. They enter circulation as anuclear elements; therefore they have a limited 7 to 10 day life.<sup>4</sup>

Platelets possess many pseudopods, invaginations of cell membrane and internal vesicles (storage granules).<sup>5</sup> Vesicles are composed by three granule types: lysosomal, dense and alpha.<sup>4,5</sup> Alpha granules are growth factors storing granules.

Growth factors are signaling proteins which regulate key events in a process of tissue reparation, such as cell differentiation and proliferation as well as their maintenance.<sup>6,7</sup>



**Figure 1.**

Platelet percentages comparison in a normal clot and a PRP clot.

Growth factors contained in these granules are:

1. Growth factor derived from platelet PDGF-aa isomer, PDGF-bb isomer, and PDGF-ab isomer.
2. Beta transforming growth factor 1 and 2 (TGF $\beta$ -1 and TGF $\beta$ -2).
3. Endothelial vascular growth factor (VEGF).
4. Epithelial growth factor (EGF) (Table II).

Studies report that platelets additionally contain abundant cytokines. This term is used to describe a group of molecules which regulate cellular communication, such as interleukins which were grouped as IL1 to IL15, interferons, three tumor necrosis factors, three colony stimulating factors, and a diverse group of cytosines such as osteopontin, oncostatin and lymphotactin among others.<sup>3</sup>

Platelets do not contain insulin growth factor (IGF<sub>1</sub> or IGF<sub>2</sub>) or bone morphogenetic protein (BMP).<sup>2</sup>

The basis for PRP is the existence of growth factors and other cytokines in the alpha granules of these cellular elements.<sup>3</sup>

#### PRP interaction mechanism

Alpha granules contained in platelets, be it in a normal blood clot or in a PRP clot, begin to degranulate in a 10 minute period. They secrete over 90% of their pre-packed growth factors in less than one hour.<sup>2,5</sup>

Secreted growth factors join the external surface of the cellular membrane in the graft, flap or wound by means of the trans-membrane receptors. Studies have reported that adult mesenchymal stem cells,

osteoblasts, fibroblasts, endothelial cells and epidermal cells express receptors for factors in their cellular membrane.<sup>12</sup> These transmembrane receptors in turn induce activation of an internal signaling protein which is directed to the nucleus (Figure 2).

Within the nucleus, transducing protein unblocks a specific genetic sequence for a regulated cellular function, such as mitosis, collagen synthesis, osteoid production etc.

After the initial release of PRP-related growth factors, platelets synthesize and secrete additional growth factors during the remaining 7 days of life. Once the platelet is depleted and dies, the macrophage, upon continuing to secrete growth factors, assumes the function of wound healing regulation.<sup>5,8</sup>

#### PRP involvement in healing process. Intervention of growth factors

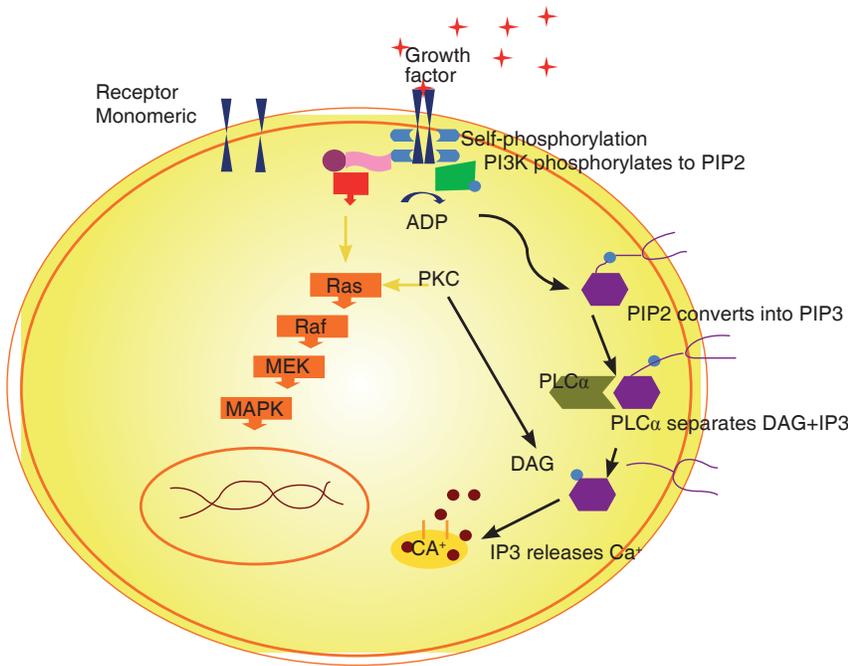
Growth factors are in charge of sorting out tissue reparation and regeneration. This action depends on the release of these active principles at the right moment and place, to thus initiate a sequence of events which has the aim of restoring normal architecture of the tissue where they are found (Figure 3).<sup>3</sup>

#### Interaction of PRP with bone allograft

Due to its higher platelet concentration, PRP initiates a faster cellular response in the bone graft than that observed with a normal blood clot. Healing of bone graft takes place during the first three weeks and it is characterized by capillary growth, proliferation and cell activity.

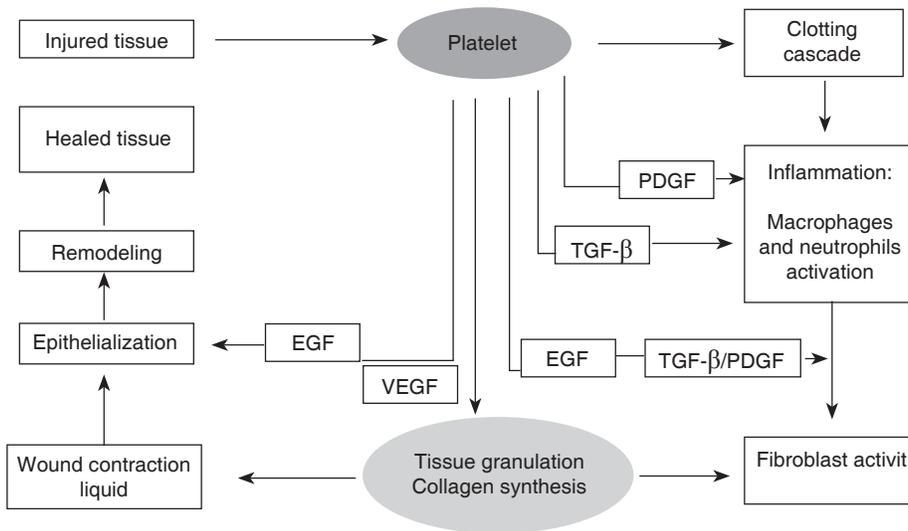
Table II. Function of growth factors.<sup>7</sup>

Growth factor	Action	Stimulated cells
PDGFaa PDGFbb PDGFab	Mitogens	<ol style="list-style-type: none"> <li>1. Mesenchymal stem</li> <li>2. Osteoblasts to create</li> <li>3. Endothelial cells for replication so they secrete basal lamina for new blood vessels.</li> <li>4. Fibroblasts for replication and collagen production.<sup>8,9,10</sup></li> </ol>
TGF $\beta$ 1 TGF $\beta$ 2	Morphogenic mitogens	<ol style="list-style-type: none"> <li>1. Stimulate cell replication</li> <li>2. Stimulate matrix production</li> <li>3. Direct differentiation towards cartilage or bone</li> </ol>
VEGE		<p>Their effect is limited to epithelial cells</p> <ol style="list-style-type: none"> <li>1. They stimulate them so that they synthesize basal lamina.</li> <li>2. Recruitment of pericytes to support development of new blood vessels<sup>11</sup></li> </ol>
EGF		<p>Their effect is limited to mucous membrane and skin basal cells</p> <ol style="list-style-type: none"> <li>1. Induce replication and migration on biological surface.</li> <li>2. Stimulate these cells so they produce specific components of basal membrane<sup>12</sup></li> </ol>



**Figure 2.**

Growth factors' action mechanisms.



**Figure 3.**

Healing process. Intervention of growth factors.

After 3 to 6 weeks, osteoprogenitor cells have proliferated and differentiated enough to produce osteoid.

In the third phase, the osteoid, weak and elastic, undergoes resorption by osteoclasts, which liberate BMPs, IL<sub>1</sub> and IL<sub>2</sub> (interleukin one and two), and these in turn induce adjacent osteoclasts and mesenchymal stem cells to differentiate and produce a more mature substitution bone, with lamellar architecture and non present Haversian systems in the osteoid.

This bone regeneration continues during the whole life of the graft, with a normal rhythm of remodeling-resorption replacement similar to the rest of the skeleton (approximately 0.7% per day).<sup>2</sup>

Platelets and PRP act in the initial biochemical first stage of a three phase bone regeneration sequence.

Recent PRP studies have revealed its efficiency when combined with bone-substitute materials.<sup>8</sup>

Bone substitutes produce new bone by way of osteoconduction of adjacent osteoprogenitor cells,

whilst autogenous graft produces new bone by way of the transplant of osteoprogenitor cells from a distant location.<sup>2</sup>

The purpose of presenting this clinical case was to provide an alternative treatment for localized aggressive periodontitis with PRP and bone allograft, and showing obtained results at 6 and 12 months.

Assessment of periodontitis recurrence risk in this patient was determined based upon the individualized risk assessment model. To this effect the diagram proposed in 2003 by Drs. Lang and Tonetti was used. Said diagram included the following aspects:

1. Percentage of bleeding upon probing.
2. Prevalence of residual pockets larger than 4 mm.
3. Tooth loss.
4. Periodontal support loss with respect to patient's age.
5. General state of patient and genetic circumstances.
6. Environmental factors such as smoking.

## METHODS

The present article presents the case of a 29 year old female patient who sought treatment at the Graduate and Research School of the National School of Dentistry, National University of Mexico (UNAM).

The patient reported as dental history having initiated two years before orthodontic treatment, nevertheless, no diagnosis of periodontal disease was established at that time.

At the Graduate School's Periodontics Clinic diagnosis of localized aggressive periodontitis was established. Treatment was undertaken after the patient signed informed consent form (*Figure 4*).

Initially, periodontal phase I was undertaken.

### PRESURGICAL PROCEDURE

PRP was obtained before surgery at a blood bank. Official Norm 003 of the Secretaria de Salud (Mexican Health Ministry) protocol was adhered to.<sup>9,10</sup>

The procedure consisted on taking a 10 mL sample, which was then subjected to preliminary exams of hepatitis and HIV among others.<sup>9,10</sup>

Once the aforementioned studies were completed, a 450 mL blood sample was extracted from the patient in order to obtain PRP. Bearing in mind the fact that blood clots immediately, the bag should contain an anti-clotting agent (sodium citrate) in order to prevent clotting.

Two centrifugations are required to separate and concentrate platelets. In the first centrifugation,

plasma and formed elements are separated, at 1,800 rpm and temperature of 18-22 Celsius for 10 minutes. Once the first centrifugation is completed, two bags are obtained: one with formed elements and the other with platelet-poor plasma. This last bag is taken to a machine called Optipress in order to separate, by concentration gradients, plasma and cellular elements.

The second centrifugation is conducted at 3,500 rpm, 18-22 degrees Celsius for 10 minutes. Once this is completed, approximately 60 mL of platelet-rich plasma is obtained.

PRP will remain in liquid form until activation during surgery.

### Surgical procedure

A flap debridement procedure was undertaken. An incision through the groove was performed and was extended beyond the defect site so as to allow lifting the flap and viewing the whole defect. While debridement procedure was being performed, the bone allograft was hydrated with PRP; root surfaces were equally scraped and planed (*Figure 5*).

Bovine thrombin mixed with calcium gluconate at 1:6 proportion was incorporated into PRP in order to initiate activation. This allowed the cellular adhesion molecules to bond bone allograft and provide a working component which would be easier to manipulate.

Once PRP is combined with bone allograft, the surgeon can use the material in several ways. In the present clinical case, it was additionally used as a membrane by placing on the site several layers of clotted PRP (this membrane has a duration of 5 to 7 days). This allows filtration of growth factors outside of the clot and penetration into the graft.

Flaps were repositioned; a triple zero black silk, with suspensory stitches was used to suture. The patient's brackets were used to avoid further tissue contraction. It is worth mentioning that during this phase, orthodontic treatment was maintained inactive.

### Postoperative care

Postoperative care consisted on use of 12% chlorhexidine oral rinse twice a day avoiding mechanical cleansing of the surgical areas.

Sutures were removed 8 days after surgery. This healing phase was completed with personal plaque control every two weeks during the first two months.

After that period, the patient was included in a bi-yearly maintenance program.



**Figure 4.** Initial circumstances.

### RESULTS

Clinical assessment results after 6 months showed health-compatible circumstances with respect to color, shape consistency, texture and bleeding upon probing. These results were maintained along 12 months.

During maintenance stage, the patient's risk status was assessed, taking into account 6 parameters after a year:<sup>11,12</sup>

1. Bleeding upon probing the distal aspect of tooth number 15.

2. 5 mm deep periodontal pocket at the distal aspect of tooth number 15.

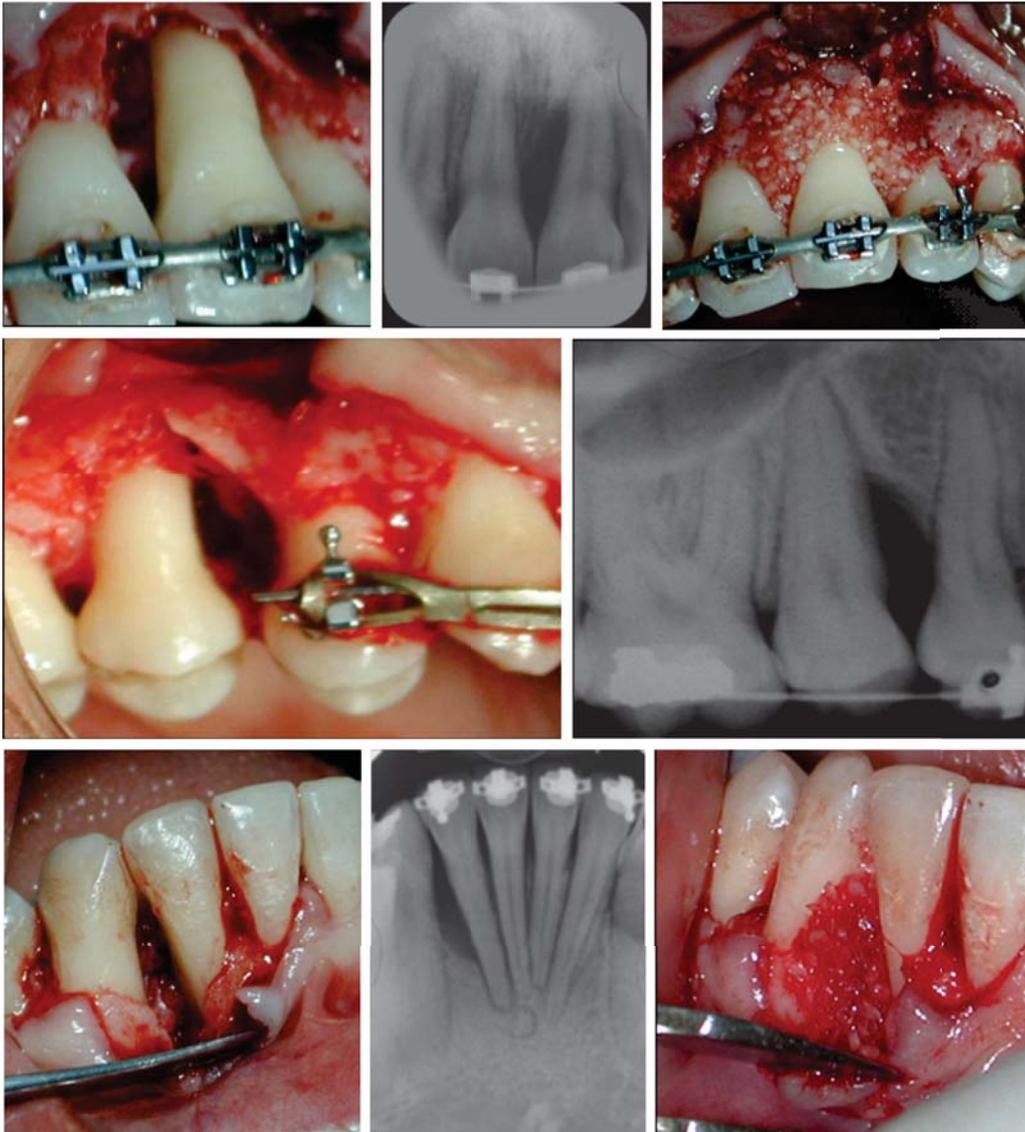
3. Absence of tooth loss during this period.

4. Absence of bone loss. Contrary to this, radiographic assessment revealed bony filling in all sites with vertical defects (*Figure 6*).

5. General health status of patient was suitable.

6. Environmental factors such as tobacco use and restorations were assessed in order to avoid compromising the achieved periodontal stability.

Based on the aforementioned six parameters, we could conclude that the patient was under low risk of



**Figure 5.**

Surgical procedure.

periodontal disease recurrence one year after initial treatment (*Figure 7*).

#### DISCUSSION

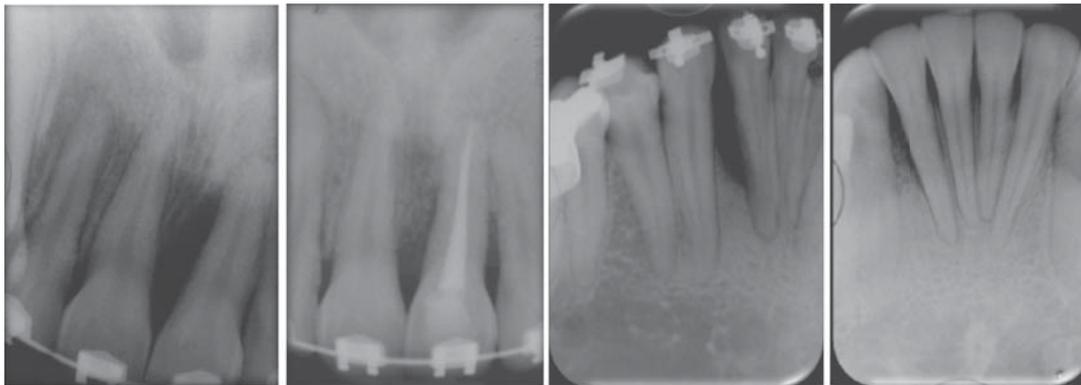
In 1998, Dr. Marx et al. were the first to conduct a formal study and transmit to the scientific dental community the potential presented by platelet rich plasma.<sup>13</sup> From that moment onwards, many clinicians and researchers started to publish countless articles related to PRP.

Unfortunately, along this way, a terminology confusion was established with respect to the systems used to obtain this platelet rich plasma, and many studies were conducted with platelet poor plasma (PPP).

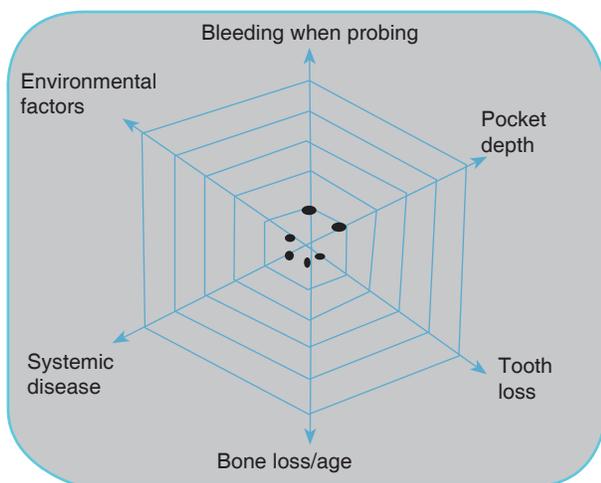
Techniques used to obtain PRP in several studies might influence their success, since some isolation techniques might contribute to early and premature platelet degranulation.

We presently have an evidence-based learning curve which overcomes aforementioned problems. Within PRP's clinical use evolution there is a first stage where it was used as cementing biomaterial and as bone tissue regeneration stimulant.

In a second and more recent stage, based on and supported by biological evidence, it was applied to heal soft tissue wounds. This fact has given rise to great expectations in several medical specialties, the field of dentistry among them.<sup>3</sup>



**Figure 6.**  
Pre-surgical and post-surgical X-rays one year after treatment.



**Figure 7.** Maintenance diagram after one year.

**CONCLUSIONS**

Today’s clinician is every day more aware of the need to make decisions based on scientific evidence and not based on recommendations and treatment alternatives presented by the dental commercial industry.

PRP seems to increase the healing process speed, since it is biologically possible that a higher platelet concentration might help wound healing. This would be due to the greater platelet concentration and faster onset of cellular response when compared to normal blood clots.<sup>14,15</sup>

**REFERENCES**

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol.* 1999; 4: 1-6.
2. Marx RE, Garg AK. *Dental and craniofacial applications of platelet-rich plasma.* Chicago: Ed Quintessence; 2005.
3. Arcuri A. *Plasma rico en plaquetas. Recuperación de tejidos con factores estimulantes de crecimiento autólogos.* Argentina: Editorial AMOLCA; 2013.

4. Steven R, Thomas M. “Platelets”. *Encyclopedia of life sciences/* 2001 Nature Publishing Group/www.els.net
5. Guyton H. *Tratado de fisiología médica.* 12a edición. México: editorial Elsevier; 2011.
6. Anitua E. *Un nuevo enfoque en la regeneración periodontal. Plasma rico en factores de crecimiento.* Vitoria-España: Editorial Puesta al Día Publicaciones; 2000.
7. The potential role of growth and differentiation factors in periodontal regeneration. *J Periodontol.* 1996; 67 (5): 545-553.
8. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg.* 2004; 62: 489-496.
9. Norma Oficial Mexicana NOM-253-SSA1-2012. *Para la disposición de sangre humana y sus componentes con fines terapéuticos.*
10. Asociación Argentina de Hemoterapia. *Manual técnico de la Asociación de Banco de Sangre.* 15 edición. Argentina: 2008.
11. Lang NP, Tonetti MS. Periodontal risk assessment for patients in supportive periodontal therapy. *Oral Health Prev Dent.* 2003; 1 (1): 7-16.
12. Lindhe J. *Clinical periodontology and implant dentistry.* Cap. 32. 4ta ed. Editorial Médica Panamericana; 2003; pp. 816-838.
13. Marx RE, Carlson ER, Eichstaedt RM, Strauss JE, Georgeff KR. Platelet-rich plasma, growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998; 85: 638-646.
14. Freymiller EG, Aghaloo TL. Platelet-rich plasma: ready or not? *J Oral Maxillofac Surg.* 2004; 62 (4): 484-488.
15. Carlson NE, Roach RB Jr. Platelet-rich plasma clinical applications in dentistry. *J Am Assoc.* 2002; 133 (10): 1383-1386.

**RECOMMENDED READINGS**

- Linch SE, Genco RJ. *Tissue engineering applications in maxillofacial surgery and periodontics.* Chicago (USA): Quintessence Publishing Co. Inc; 1999; pp. 18-25, 71-82.
- Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor modulated guided tissue regenerative therapy. *J Periodontol.* 1995; 66 (6): 522-530.
- Tolga F, Burak D. Platelet-rich plasma: a promising innovation in dentistry. *J of the Canadian Dental Association.* 2003; 69: 10.
- Sánchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. *Int J Oral Maxillofac Implants.* 2003; 18 (1): 93-103.

Mailing address:  
**Beatriz Raquel Yáñez Ocampo**  
E-mail: raquely0@yahoo.com.mx