

## Bone healing of dental alveoli in smokers with platelet-rich plasma obtained using single or double centrifugation.

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**Abstract:** Introduction: the habit of smoking alters the bone healing process, a problem to consider in oral surgery. Objective: To evaluate the bone healing of dental alveoli with PRP obtained using single or double centrifugation in smokers. Methodology: Extraction of mandibular third molars was performed in a study population divided into smoking group (A), which had PRP applied with the protocol using a single centrifugation step (P1C) in the alveolus of tooth 38 and the protocol of double centrifugation (P2C) in alveolus of tooth 48; a smoking group (B), to whom no PRP was applied; and a non-smokers group (C) to whom PRP was applied obtained using P1C and P2C protocol. Radiographic examination was performed at 8, 30 and 60 days post procedure. Results: Thirty patients met the criteria, 57% were female. When evaluating bone healing between the group of smokers and non-smokers, statistically significant differences were observed in the non-smoking group at 30 and 60 days, showing better results with the P2C protocol ( $p < 0.005$ ). Statistically significant differences were found at 30 and 60 days ( $p < 0.005$ ), both with the P1C and P2C when comparing bone healing of group A and B. Conclusions: Bone healing in the alveoli of mandibular third molars that which PRP applied was higher in non-smoking patients, compared with the group of smokers. Bone healing was better in patients smokers to whom PRP was applied than those without PRP treatment. Regarding the method of obtaining PRP, bone healing was better when a double centrifugation protocol (P2C) was applied.

**Keywords:** Bone regeneration; dental alveolus; platelet-rich plasma; smoking.

### INTRODUCTION.

Bone healing of the tooth socket (alveolus) after extraction is an important aspect to consider in oral surgery, as delays in the healing process increase the risk of infections, extend the time of disability, inflammation, pain, loss of the bone tissue and can affect the periodontal status of neighboring teeth;<sup>1,2</sup> One of the risk factors for delays in bone healing is cigarette smoking, which decreases tissue oxygenation; smoking is one of the most common habits in patients who present to dental consultation and its prevalence is increasing worldwide.<sup>3</sup>

To improve healing post dental extraction, different materials may be used, including platelet-rich plasma (PRP),<sup>4</sup> consisting of a platelet concentrate in the form of a supernatant following centrifugation of blood obtained from the patients themselves; it acts as a carrier vehicle for important growth factors for bone, increases cellular mitosis, collagen production, initiates vascular growth and induces cell differentiation.<sup>5</sup>

It presents a low risk of infection or immunological reactions, since platelets play an important role in host defense mechanisms at the site of the wound, due to a release of signal peptide that attracts macrophages.<sup>6</sup>

The PRP can be obtained by different laboratory methods or protocols, the increase in the number of centrifugation steps allows for having a greater concentration of platelets, but this could affect its activity and function, since it may generate more mechanical damage.<sup>7</sup> As such, studies that compare the different methods for obtaining PRP are important.

The present article describes a study evaluating the bone healing of the dental alveoli of mandibular third molars, using PRP as an adjuvant in cigarette smoking patients; we also report which centrifugation technique for obtaining PRP provides the most bone healing.

## **MATERIALS AND METHODS.**

A quasi-experimental intervention study model was used, in which patients underwent an intervention. The study population consisted of young adults between 18 and 30 years old. This age range was selected as extraction of third molars is common among this age range. A sample with an alpha error of 5% and a power of 90% was calculated; data processing yielded a number of 8 patients per group and, accounting for an expected 20% dropout rate, it was increased to 11 patients per group.

Study subjects included non-smokers and smokers with a minimum of one year of smoking history of at least one cigarette a day, with lower third molars erupted in an upright position, with good oral health, without systemic compromise, and in good nutritional conditions, as assessed by a general physical examination, including determination of body mass index, lipoprotein profile and food consumption frequency survey, which were performed by a medical expert on nutrition. We excluded patients who were using drugs that influence the healing process, with alterations in coagulation tests, altered blood glucose levels, were pregnant or breastfeeding, and subjects with decreases number of platelets or immunosuppressed.

Two groups were established: one of smokers and the other a control of non-smokers. All underwent extraction of teeth number 38 and 48, the group of smokers was sub-divided into group A with 11 patients to whom PRP obtained with the P1C protocol was applied to the alveolus of tooth 38, and PRP obtained with the P2C protocol was applied to the alveolus of tooth 48; and group B with eight patients, to whom PRP was not applied. Group C consisted of 11 non-smokers to whom PRP obtained with the P1C protocol was applied to the alveolus of tooth 38 and PRP obtained with the P2C protocol was applied to the alveolus of tooth 48.

Three radiographic controls were performed by an expert evaluator, at 8, 30 and 60 days post-procedure, using a Owandy® brand digital radiology equipment, using the periapical projection. In order to evaluate changes in bone density, the RadiAnt Software DICOM Viewer Version 2.2.9.10728 was used, with the Ellipse tool, which allowed the quantification of the image contrast with the average pixel value or Hounsfield units. The resulting gray scale changes were given a numerical code, with the cervical, middle and apical areas evaluated in the radiographic image of the dental alveolus.

To obtain the PRP, three tubes of venous blood were drawn, with sodium citrate. Two methods were applied for the separation of the PRP: a one-step centrifugation (P1C) and a two-step or double centrifugation (P2C) were used. In the P1C protocol, the sample was centrifuged at 1400rpm for 8min, in a refrigerated centrifuge at a temperature of 22-24°C; then the layer of plasma near the leukocyte layer (0.3ml) where the largest number of platelets is found was removed, at this is one of the methods frequently reported in the literature for application in oral cavity.<sup>8</sup> For the P2C, the sample is was first centrifuged taking into account the aforementioned P1C8; then the extracted PRP was centrifuged at 2,000rpm for 10min, at a temperature of 22 to 24°C; the platelet-poor plasma (0.2cc) was eliminated and the fraction with the highest platelet content was extracted.<sup>9</sup>

The surgical procedure for the extraction of lower third molars was performed applying the mandibular

syndesmotic technique, with dislocation, extraction of the teeth and rinsing the alveolus with 0.9% NaCl. Then the PRP concentrate was applied and 100µl of 10% calcium chloride was added for its activation within the alveolus, and after 10min a clot was obtained. Finally simple suturing was performed.

**Statistical analysis**

The resulting data were tabulated in an Excel 2010 spreadsheet (Microsoft Office 2010®) by a single operator. Next, a second operator exported this data to the statistical software SPSS version 2.0 (IBM Corp. IBM SPSS statistics for Windows version 2.0, Armonk, NY, USA) for statistical analysis.

The statistical analysis consisted in the first instance of a univariate descriptive analysis of the data, showing statistics of central tendency and dispersion (mean±standard deviation) for the case of the quantitative variables. While frequency tables

were obtained for the qualitative variables (absolute frequency and relative frequency). The bivariate analysis consisted in the comparison of the means of the groups by Student t-test for independent samples. For comparing bone healing at the three follow-up time periods, the Student t-test for paired samples was applied. Values for  $p < 0.05$  were considered significant.

The study complied with the ethical aspects and international recommendations of the *Declaration of Helsinki of the World Medical Association and of the health research norms of the Ministry of Health of Colombia*. According to resolution 008430 of 1993 (Ministry of Health of Colombia) the present investigation represented greater than minimal risk for the participants, and the possible risks were clearly expressed in the informed consent. The study was approved by the *Universidad de Cartagena* institutional ethics committee.

**Table 1.** Evaluation of bone healing in smokers.

Smokers treated with PRP (n=11)									
Single centrifugation method				Double centrifugation method				p-value	
Tooth 38	Follow-up	mean	SD	Tooth 48	Follow-up	mean	SD		
Cervical	8 days	5.18	1.08	Cervical	8 days	5.18	1.54	1.000	
Middle		4.91	1.14	Middle		5.27	1.49	0.053	
Apical		4.82	1.17	Apical		5.09	1.76	0.067	
Cervical	30 days	9.00	1.48	Cervical	30 days	9.64	1.43	0.032	
Middle		8.73	1.42	Media		9.55	1.51	0.021	
Apical		8.64	1.43	Apical		9.18	1.47	0.039	
Cervical	60 days	14.82	1.25	Cervical	60 days	15.36	1.50	0.037	
Middle		14.55	1.37	Media		15.27	1.68	0.028	
Apical		14.55	1.29	Apical		15.18	1.66	0.033	
Smokers not treated with PRP (n=8)									
Single centrifugation method				Double centrifugation method				p-value	
Tooth 38	Follow-up	mean	SD	Tooth 48	Follow-up	mean	SD		
Cervical	8 days	3.75	0.89	Cervical	8 days	4.25	0.89	0.028	
Middle		3.75	0.71	Middle		4.25	0.89	0.023	
Apical		3.63	0.74	Apical		4.00	0.93	0.039	
Cervical	30 days	6.25	1.28	Cervical	30 days	7.00	1.51	0.030	
Middle		6.63	1.19	Middle		6.88	1.25	0.069	
Apical		6.63	1.19	Apical		6.75	1.39	0.085	
Cervical	60 days	10.25	2.12	Cervical	60 days	10.25	2.12	1.000	
Middle		10.25	2.12	Middle		10.25	2.12	1.000	
Apical		10.13	2.23	Apical		10.00	2.39	0.092	

$p < 0.005$ : Student t-test.

**Table 2.** Evaluation of bone healing in the non-smokers.

Non-smokers treated with PRP (n=11)									
Single centrifugation method				Double centrifugation method					
Tooth 38	Follow-up	mean	SD	Tooth 48	Follow-up	mean	SD	p-value	
Cervical	8 days	6.11	0.92	Cervical	8 days	7.25	1.53	0.009	
Middle		6.67	1.00	Middle		7.25	1.73	0.169	
Apical		5.89	1.16	Apical		7.00	2.02	0.056	
Cervical	30 days	9.78	1.92	Cervical	30 days	12.56	2.24	<b>0.000</b>	
Middle		10.00	1.93	Middle		12.56	2.40	<b>0.000</b>	
Apical		9.78	1.98	Apical		12.33	2.12	<b>0.000</b>	
Cervical	60 days	14.11	2.14	Cervical	60 days	15.78	2.10	<b>0.000</b>	
Middle		14.22	2.22	Middle		15.89	2.20	<b>0.000</b>	
Apical		14.22	2.43	Apical		15.56	2.12	<b>0.002</b>	

*p*<0.005: Student t-test.

**Table 3.** Comparison of bone healing in smokers in areas of teeth 38 and 48.

Smokers treated with PRP (n=11)									
Smokers (N=8) without PRP				Smokers (N=11) with PRP - Single centrifugation method					
Tooth 38	Follow-up	mean	SD	Tooth 48	Follow-up	mean	SD	p-value	
Cervical	8 days	3.75	0.88	Cervical	8 days	5.18	1.07	0.007	
Middle		3.75	0.70	Middle		4.91	1.13	0.021	
Apical		3.63	0.74	Apical		4.82	1.16	0.022	
Cervical	30 days	6.25	1.28	Cervical	30 days	9.00	1.48	<b>0.001</b>	
Middle		6.63	1.18	Middle		8.73	1.42	<b>0.003</b>	
Apical		6.63	1.18	Apical		8.64	1.43	<b>0.005</b>	
Cervical	60 days	10.25	2.12	Cervical	60 days	14.82	1.25	<b>0.000</b>	
Middle		10.25	2.12	Middle		14.55	1.36	<b>0.000</b>	
Apical		10.13	2.23	Apical		14.55	1.29	<b>0.000</b>	

  

Smokers treated with PRP (n=11)									
Sin PRP				Con PRP					
Tooth 38	Follow-up	mean	SD	Tooth 48	Follow-up	mean	SD	p-value	
Cervical	8 days	4.25	0.88	Cervical	8 days	5.18	1.53	0.144	
Middle		4.25	0.88	Middle		5.27	1.48	0.103	
Apical		4.00	0.92	Apical		5.09	1.75	0.130	
Cervical	30 days	7.00	1.51	Cervical	30 days	9.64	1.43	<b>0.001</b>	
Middle		6.88	1.24	Middle		9.55	1.50	<b>0.001</b>	
Apical		6.75	1.38	Apical		9.18	1.47	<b>0.002</b>	

*p*<0.005: Student t-test.

## RESULTS.

Thirty patients participated in this study, 36.5% were assigned to group A, 27% to B and 36.5% to group C, 57% were female, with an average age of 21.7 years ( $\pm 3.19$ ). All smokers consumed regular cigarettes, had a mean 3.89 years smoking history, and smoked on average 4.10 cigarettes per day.

Regarding the results from the radiographic evaluations, taking in consideration the three controls performed on

all three groups (A, b and C), of the cervical, middle and apical area of the alveolus of tooth 38 and 48, and both P1C and P2C protocols, statistically significant differences were found in group C at 30 and 60 days. This group at these time points showed better results with P2C (*p*<0.005), (Table 1 and Table 2). No statistical differences were observed when associating bone density with gender.

Comparing the results from the changes found in the radiographic evaluations between group A and B, in the

cervical, middle and apical area of the alveolus of tooth 38, statistically significant values were found at 30 and 60 days with the P1C ( $p < 0.005$ ). Likewise when comparing changes in the alveolus of tooth 48 between group A and B, statistically significant values were also found at 30 and 60 days with P2C ( $p < 0.005$ ), with the application of PRP with either P1C or P2C being more favorable than without the use of PRP (Table3).

## DISCUSSION.

Smoking, of the biggest problems for public health, is an utmost important risk factor because of the local and systemic effects it causes in the oral cavity.<sup>10</sup> Regarding its vascular effects, smoking causes a delay in the revascularization of both soft and hard tissues, and reduces chemotaxis, phagocytosis and adherence to tissue surfaces in neutrophils.<sup>3,11</sup> Patel *et al.*,<sup>12</sup> stated that smoking has a negative effect on bone regeneration after periodontal treatment, Saldanha *et al.*,<sup>13</sup> assessed the dimensions of the alveolus and radiographic bone density after dental extraction in smoking patients and concluded that smoking can lead to a significant reduction in the size of the alveolar ridge and the healing of alveolus after tooth extraction, compared to non-smokers. Rojas *et al.*,<sup>3</sup> reported that both the duration of smoking history and the daily consumption are important factors in the severity of the changes smoking can induce in humans.

Platelet-rich plasma contains not only a high level of platelets, but also of growth factors that are actively secreted by platelets. Although it is difficult to determine the magnitude of the stimulating effect on bone healing, what remains clear are the positive results obtained, such as shortening the healing time, due to its mitogenic and chemotactic properties. This suggests that using PRP<sup>14</sup> is better than not using it Yilmaz *et al.*,<sup>15</sup> reported on the use of PRP in the regenerative treatment in periodontal surgery, combined with a xenograft in smokers and non-smokers. The method of obtaining the PRP was a single centrifugation.

The researchers state that the combination of treatments provides benefits for both smokers and non-smokers, and the results of the radiographic evaluation was better with regard to bone regeneration in non-smoking patients. This is in agreement with the present

study, where we evaluated the bone healing of dental alveoli with PRP in both smokers and non-smokers and bone healing was most favorable non-smokers, and even though the surgical procedure was different in the study of Yilmaz *et al.*,<sup>15</sup> it confirms that the habit of smoking alters the process of bone regeneration.

Regarding the double centrifugation technique for obtaining PRP Valadez *et al.*,<sup>16</sup> conducted a study the optimal method to obtain PRP was evaluated by comparing three different protocols regarding the centrifugation speed, length and number of cycles.

A double centrifugation yielded the highest yield and number of basal platelets ( $p < 0.001$ ); however they clarify that one must work very carefully, since the too much manipulation increases the risk of product contamination. In the current study, the single- and double-centrifugation methods were used to obtain PRP, in order to to evaluate bone healing.

In the analysis and interpretation of the results, non-smoking patients presented significant statistical differences at 30 and 60 days follow-up periods with the double centrifugation method, which indicates that this is a good option as an adjuvant in the bone regeneration of dental alveoli. Meanwhile there were no statistically significant differences between the two methods of obtaining PRP in smokers, but there was a slight difference in the radiographic contrast with the double centrifugation method, due to cigarette smoking, a risk factor in bone regeneration.

## CONCLUSION.

The alveoli of mandibular third molars of non-smoking patients to which PRP was applied display more extensive bone healing compared to smokers. Bone healing was better in smokers with PRP compared to those without PRP, favoring its use nonetheless as an assistant in bone regeneration with any of tested protocols in these patients. However P2C was the best method for obtaining PRP, resulting in better results regarding bone healing.

As recommendations for future studies, we suggest to expand the number of radiographic follow-up controls up to 120 days, to include a group of non-smoking patients to whom no PRP centrifugation protocol is applied, and to increase the sample size.

## REFERENCES.

1. Prataap N, Sunil PM, Sudeep CB, Ninan VS, Tom A, Arjun MR. Platelet-rich Plasma and Incidence of Alveolar Osteitis in High-risk Patients Undergoing Extractions of Mandibular Molars: A Case-control Study. *J Pharm Bioallied Sci.* 2017;9(Suppl 1):S173–9.
2. Doiphode AM, Hegde P, Mahindra U, Santhosh Kumar SM, Tenglikar PD, Tripathi V. Evaluation of the efficacy of platelet-rich plasma and platelet-rich fibrin in alveolar defects after removal of impacted bilateral mandibular third molars. *J Int Soc Prev Community Dent.* 2016;6(Suppl 1):S47–52.
3. Rojas J, Rojas LA, Hidalgo R. Tabaquismo y su efecto en los tejidos periodontales. *Rev Clin Periodoncia Implantol Rehabil Oral.* 2014;7(2):108–13.
4. Nathani DB, Sequeira J, Rao BH. Comparison of platelet rich plasma and synthetic graft material for bone regeneration after third molar extraction. *Ann Maxillofac Surg.* 2015;5(2):213–8.
5. Dutta SR, Singh P, Passi D, Patter P. Mandibular Third Molar Extraction Wound Healing With and Without Platelet Rich Plasma: A Comparative Prospective Study. *J Maxillofac Oral Surg.* 2015;14(3):808–15.
6. Barona-Dorado C, González-Regueiro I, Martín-Ares M, Arias-Irimia O, Martínez-González JM. Efficacy of platelet-rich plasma applied to post-extraction retained lower third molar alveoli. A systematic review. *Med Oral Patol Oral Cir Bucal.* 2014;19(2):e142–8.
7. Moreno R, Carreño G, Torres J, Herreros A, Villimar A, Sánchez L. Técnicas de obtención del plasma rico en plaquetas y su empleo en terapéutica osteoinductora. *Farm Hosp.* 2015;39(3):130–6.
8. Dugrillon A, Eichler H, Kern S, Klüter H. Autologous concentrated platelet-rich plasma (cPRP) for local application in bone regeneration. *Int J Oral Maxillofac Surg.* 2002;31(6):615–9.
9. Moreno R, Carreño MG, Torres JJ, Herreros MJA, Villimar A, Sánchez PL. Técnicas de obtención del plasma rico en plaquetas y su empleo en terapéutica osteoinductora. *Farm Hosp.* 2015;39(3):130–6.
10. González MC, Hernández MC, Boch MM, Castellanos CMM, Méndez CM, Garrido LC, Fernández CC. Efectos fisiopatológicos del tabaquismo como factor de riesgo en la enfermedad periodontal. *Rev Finlay.* 2016;6(2):134–49.
11. Wyganowska-Swiatkowska M, Nohawica MM. Effect of tobacco smoking on human gingival and periodontal fibroblasts. A systematic review of literature. *Przegl Lek.* 2015;72(3):158–60.
12. Patel RA, Wilson RF, Palmer RM. The effect of smoking on periodontal bone regeneration: a systematic review and meta-analysis. *J Periodontol.* 2012;83(2):143–55.
13. Saldanha JB, Casati MZ, Neto FH, Sallum EA, Nociti FH Jr. Smoking may affect the alveolar process dimensions and radiographic bone density in maxillary extraction sites: a prospective study in humans. *J Oral Maxillofac Surg.* 2006;64(9):1359–65.
14. Floresa JR, Gallego MAP, García-Dencheb JT. Plasma rico en plaquetas: fundamentos biológicos y aplicaciones en cirugía maxilofacial y estética facial. *Rev Esp Cirug Oral y Maxilofac.* 2012;34(1)
15. Yilmaz S, Cakar G, Ipci SD, Kuru B, Yildirim B. Regenerative treatment with platelet-rich plasma combined with a bovine-derived xenograft in smokers and non-smokers: 12-month clinical and radiographic results. *J Clin Periodontol.* 2010;37(1):80–7.
16. Valadez Báez XL, Hernández Santos JR, Torres Huerta JC, Tenopala Villegas S, Canseco Aguilar CP. Método óptimo para la obtención de plasma rico en plaquetas en el Servicio de Clínica del Dolor del CMN 20 de noviembre ISSSTE. *Rev Soc Esp Dolor.* 2016;23(4):175–80.