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*Review Article*

# Platelet concentrates in the replantation of avulsed teeth: a systematic review

Cristina Bucchi[1,2], Alain Arias[1], Ramón Fuentes[1,2]

*[1]Research Center in Dental Sciences (CICO), [2]Department of Integral Adults Dentistry, Faculty of Dentistry, Universidad de La Frontera, Temuco, Chile*

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**Abstract:** It is accepted that replantation is the treatment of choice for avulsed teeth, however resorption and ankylosis are frequent complications in clinical practice. In such situation it is clinically relevant to evaluate the effectiveness of platelet concentrates in reducing these complications and improving the prognosis for the tooth. The object of this review was to analyse the available evidence on the effectiveness of the use of platelet concentrates on replanted teeth (regeneration of the periodontal ligament and lower complication rates). Only two studies carried out in animals were included in this review. PPP and PRP were used in one and PRF in the other. Both used platelet concentrates for replantation of intentionally extracted teeth. According to a qualitative analysis of these studies, it was observed that PRF is superior in terms of neoformation of tissue similar to periodontal ligament. Evidence exists to support the use of these concentrates, however no controlled, randomised clinical studies were found which compared the effectiveness of the various concentrates with one another.

**Keywords:** Tooth avulsion, platelets concentrates, tooth replantation

## Introduction

Avulsion, defined as the complete removal of the tooth from its alveolus, is a severe form of dental trauma which involves the loss of continuity of many tissue compartments, including rupture of the tooth's neurovascular support and damage to the internal layer of the periodontal ligament [1].

Subsequent to treatment of these teeth, the prognosis will depend on various factors such as the time elapsed between trauma and repositioning, the maturity of the root, associated coronary fractures [2] and the preservation medium [3]. Post-operative complications subsequent to tooth avulsion and replantation are loss of vitality of the pulpal organ, ankylosis, replacement or inflammatory resorption and tooth loss [4]. Ankylosis is the most prevalent complication associated with the periodontal ligament (42.9% to 61% according to different studies) [4, 5].

Tooth replantation is accepted as an effective treatment in such cases [6], however subsequent repair of the periodontal ligament is difficult to achieve in clinical practice [1]. Only 25% of avulsed teeth present ligament repair after replantation [5]. Some works have recommended the use of substances or additives for treating the surface of the tooth root immediately prior to replantation [1, 7].

Among the treatment options are autologous platelet concentrates; these are products derived by centrifuging the patient's own blood and include Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF). The platelets contained in the different types of concentrates have been shown to play an important role in tissue repair [7]. Their effectiveness lies in the continuous, local release of a wide range of growth factors which supply the needs of the physiological wound-healing and tissue-repair processes [7]. Growth factors are biological mediators capable of regular key cell events, including cell migration, proliferation and differentiation, as well as synthesis of the extracellular matrix [1, 8, 9].

*In vitro* studies have shown that the high levels of Platelet-Derived Growth Factor (PDGF) and Transforming Growth Factor-β (TGF-β) con-

tained in human PRP modulate the cell-specific proliferation of cultures of gingival fibroblasts, oral epithelial cells, and periodontal ligament cells and osteoblasts [10, 11]; they also appear to be related with an increase in type I collagen production by the cells of the periodontal ligament [12]. PRF, a second-generation platelet concentrate, has shown similar effects. An *in vitro* study of avulsed teeth kept in a medium with PRF and PPP (Platelet-Poor Plasma) showed a positive effect on periodontal cell viability compared with other media [13]. Furthermore, PRF has several advantages over the traditional concentrate, PRP, since the platelets, leukocytes, growth factors and cytokines are contained in a dense matrix of fibrin which allows slow, long-lasting release. It is easy to prepare and the blood requires no biochemical manipulation [7]. Unlike PRP, which is prepared by adding anticoagulants and activators, PRF is strictly autologous [14].

The application of platelet concentrates to periodontal regeneration has been researched using *in vitro* and *in vivo* models, and promising results have been obtained which provide a basis for subsequent clinical studies in humans [15]. However there is very little *in vivo* evidence of the use of these concentrates to treat avulsed teeth or teeth which have been extracted intentionally and replanted.

The object of this systematic review is to analyse the available evidence on the effectiveness of tooth replantation with platelet concentrates in terms of periodontal ligament regeneration and lower complication rates.

## Material and methods

A systematic literature review was carried out using the standard protocol.

*Eligibility criteria*

Studies in human beings and/or animals in which teeth were replanted using platelet concentrates on the tooth root or the alveolus during replantation and/or as a medium for preserving the tooth.

Studies in which the teeth were replanted as a result of intentional extraction or traumatic avulsion.

Studies with comparative designs; for this reason case reports or series were excluded.

Original studies, either prospective or retrospective. Reviews, technical articles and letters to the editor were excluded.

Studies with a clear description of the type of platelet concentrate used, and how it was obtained and applied.

Studies reporting histological, radiographical and/or clinical results related to the effectiveness of platelet concentrates on replanted teeth.

Studies published in Spanish, Portuguese or English.

No limit was set on publication date.

No limit was set on the follow-up time of the studies.

*Search strategy*

An electronic search was carried out in MEDLINE, Scopus, SciELO, Science Direct, EMBASE and Cochrane CENTRAL.

The search terms used were: “platelet concentrates”, “platelet-rich plasma”, “platelet-rich fibrin”, PRP, PRF, L-PRP, L-PRF, PRGF, “platelet gel”, reimplant*, avuls*. The terms were combined with conjunctions OR and AND. The bibliographies of the articles included in this review were also checked to identify relevant articles not found in the electronic search. The last search was carried out on 27 November 2015.

*Selection of articles and data extraction*

Two reviewers studied the titles and abstracts obtained from the electronic search independently. In case of disagreement, they reviewed the article in question again and reached a joint decision. The complete text was analysed independently by the same two reviewers to check for compliance with the inclusion criteria.

The data extracted were: study design, sample size, age, animal species, type of concentrate used and processing method, reason for tooth loss (extraction or avulsion), presence of infection in the surgery zone, type of tooth, follow-up period, and the result variables (regeneration


**Figure 1.** Risk of bias analysis of the articles included in the systematic review.

of periodontal ligament and cement, organization of ligament fibres, and replacement, superficial and inflammatory resorption). The data were extracted by two reviewers independently. In case of disagreement they analysed the article jointly until agreement was reached.

The methodological quality of the animal studies included was evaluated by ARRIVE (*Animal Research: Reporting In Vivo Experiments*) and by the risks of biascriteria laid down for a systematic review [16].

An evaluation was made of the risk of bias in the articles included, considering the following: declaration of approval by an ethics committee, details of the surgical procedures, details of the animals used, randomisation, information on the rate of abandonment/death, calculation of sample size, declaration of conflicts of interest and blinding (**Figure 1**). The risk of bias was evaluated by two researchers independently. Studies were considered to have a low risk of bias when 2/3 of the above parameters were found to be sufficient.

## Results

*Selection of studies*

The result of the electronic search is shown in diagram form in **Figure 2**. The total number of articles found by the searchers was 78, and 15 more were found by manual search. After initial reading of the titles and abstracts, 86 articles were discarded of which 38 were duplications, 43 were unrelated to the topic or studies not carried out on humans or animals and 5 were reviews. The only human studies found were 4 case reports [2, 6, 17, 18], which were excluded. No human studies were found with comparative designs. After evaluation of the complete texts, only two studies, carried out on animals, complied with the eligibility criteria [1, 7].

*Characteristics of the studies included*

Both articles included were approved by an ethics committee and reported in detail the surgical procedure carried out. Both articles described experiments on dogs.

Different platelet concentrates were used in the two studies. Reichert da Silva Assunção et al. [1] used PPP and PRP. The latter was used in different presentations: activated with calcium chloride (PRP/Ca); activated with calcium and bovine thrombin (PRP/Thr/Ca); and joint use of bone marrow mononuclear cells and cells activated with calcium (BMMCs/PRP/Ca). Zhao et al. [7] used PRF, periodontal ligament stem cells (PDLSCs) and a construct based on both (PDLSCs in a PRF membrane). The methods for obtaining and using these preparations described in the studies are summarised in **Table 1**.

The two studies used the corresponding preparations for replanting teeth which had been extracted intentionally in the least traumatic way possible; the study material was placed in the alveolus before the tooth was replanted. In both studies the histological results for each group/preparation were compared; teeth replanted with no additive acted as control. In one of the studies [1], the teeth were kept in a dry medium for 30 minutes after extraction and then treated endodontically with a calcium hydroxide-based material (Sealapex®) before being replanted. In the other study [7] the teeth

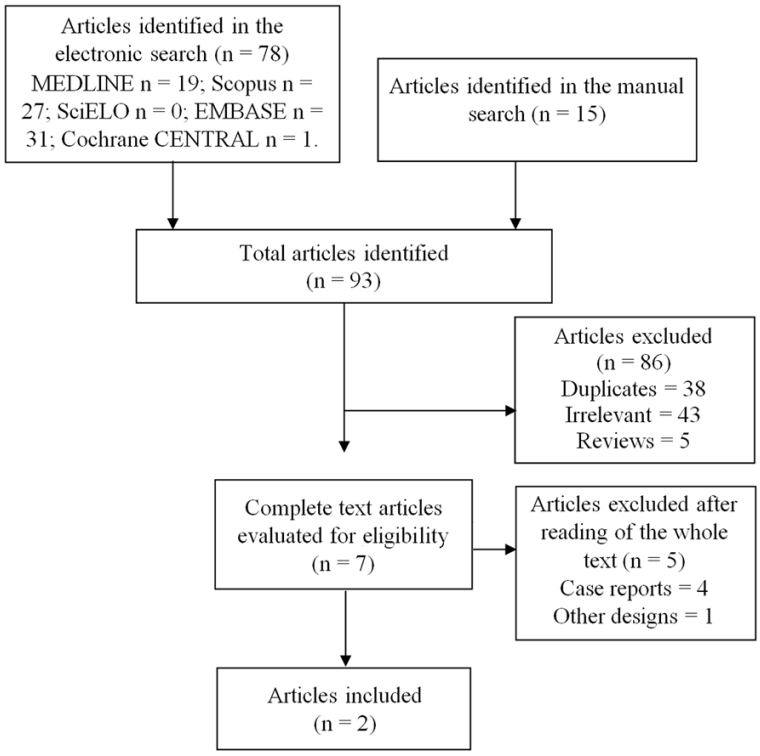


**Figure 2.** Flow chart of the search and selection of articles included in the systematic review.

were also kept in a dry medium after extraction, for 2 hours, and endodoncy was carried out on the teeth two weeks after replantation using a filler paste (AH Plus®) and gutta percha. In both studies the treated teeth were splinted immediately. The follow-up period was variable, as were the post-operatory care procedures until euthanasia. In the first study [1] the animals were controlled for 120 days (approximately 17 weeks) on a soft diet. In the second [7] the follow-up period was 56 days (8 weeks), also on a soft diet. The principal characteristics of these studies are summarised in **Table 2**.

Neither of the articles included evaluated clinical or radiography results. Both articles evaluated histological results.

*Characteristics of the animals*

A total of 10 dogs were treated between the two studies, and 100 teeth were replanted using different preparations to induce tissue regeneration. In the first study [1] 16 teeth per animal were carefully extracted (4 dogs; total 64 teeth) and assigned a type of preparation at random for the treatment of each dog (8 teeth per experimental group). The control group was established as the teeth of the opposing arch (total of 32 teeth in the control group). In the second study [1] 6 teeth per animal were extracted (6 dogs; total 36 teeth) and this sample was divided into four experimental groups of which one was the control (9 teeth per group). The teeth treated were incisors (the majority) and premolars. In the second study only [7], radiography was used to ensure that the teeth presented complete radicular formation and closed apex (the dogs were aged between 1 and 2 years). The first study [7] on the other hand used the animals' age as the only parameter for dental maturity (mixed breed dogs were used, aged between 1 and 1.5 years).

After euthanasia, the maxillary bones were fixed and demineralised and the samples were then kept in paraffin. The histological cuts were analysed by staining with Hematoxylin-Eosin (HE) and light [1, 7] and electronic [7] microscopy.

*Evaluation of the risk of bias*

The risk of bias in the two articles included in this review was low according to the parameters evaluated in **Figure 1**.

*Results of the studies included*

In the study by Zhao et al. [7], neoformation of tissue similar to periodontal ligament was significantly greater in the groups with PRF than those without. However the formation of tissue similar to periodontal ligament was significantly greater in the group which contained the construct of PDLSCs and PRF than the group with only PRF. Likewise the groups with PRF presented rates of replacement and inflammatory resorption which were significantly lower than the empty alveoli.

**Table 1.** Summary of methods of obtaining platelet concentrates and use of preparations described in the studies included in the review

| Authors | Type of concentrate | Number of times centrifuged | Anticoagulant | Rpm first centrifuging | Rpm second centrifuging | Activator (number of teeth) | Platelet count |
|---|---|---|---|---|---|---|---|
| Reichert da Silva Assunção et al., 2011 | PPP | 2 | ACD-A | NR | NR | Calcium chloride at 10% (8) | 52.5 × 103 µl |
| | PRP | | ACD-A | NR | NR | Bovine thrombin and calcium chloride at 10% (8) Calcium chloride at 10% (16) | 615 × 103 - 820 × 103 µl |
| Zhao et al., 2013 | PRF | 1 | No anticoagulant | 400 g × 10 min | - | No activator | 213.34 × 109 µl |

PPP: Platelet-Poor Plasma; PRP: Platelet-Rich Plasma; PRF: Platelet Rich Fibrin; ACD-A: anticoagulant citrate dextrose solution; NR: Not recorded.

**Table 2.** Principal characteristics of the studies included in the review

| Author | Animal species | Sample n animals (n teeth) | State of root maturity | Avulsion-replanting time (min) | Extraoral conservation medium | Intervention groups (n) | Concentrate application method | Additional treatment of tooth | Follow-up period (days) | N teeth lost |
|---|---|---|---|---|---|---|---|---|---|---|
| Reichert da Silva Assunção et al., 2011 | Dogs | 4 (64) | Complete | 30 | Dry | PPP (8)<br>PRP activated with calcium (8)<br>PRP activated with calcium and bovine thrombin (8)<br>PRP activated with calcium and bone marrow mononuclear cells (8)<br>No intervention (control) (32) | In the alveolus prior to replantation | Endodoncy prior to replanting and splinting | 120 | NR |
| Zhao et al., 2013 | Dogs | 6 (36) | Complete | 120 | Dry | PRF/PDLSCs (9) | In the alveolus prior to replantation | Splinting and endodoncy 2 weeks after replanting | 56 | 2 |
| | | | | | | PDLSCs (9) | | | | 2 |
| | | | | | | PRF (9) | | | | 1 |
| | | | | | | No intervention (control) (9) | | | | 0 |

PPP: Platelet-Poor Plasma; PRP: Platelet-Rich Plasma; PRF: Platelet Rich Fibrin; PDLSC: Periodontal Ligament Stem Cells; NR: Not recorded.

In the study by Reichert da Silva Assunção et al. [1] on the other hand, the rate of inflammatory resorption was similar in all groups. However replacement resorption was significantly lower in the groups with PPP, PRP activated with bovine thrombin and calcium chloride (replacement resorption was not observed in any case), and PRP activated with calcium chloride, than in the group with PRP activated with calcium chloride and bone marrow mononuclear cells.

## Discussion

The object of this systematic review was to establish the effectiveness of platelet concentrates in the treatment of replanted teeth. Despite the large number of articles which sustain the effectiveness of these concentrates in various fields of dentistry, such as bone regeneration and soft tissue regeneration [19], for this review only two articles were found which analyse the effectiveness of concentrates in avulsed teeth. Furthermore, both these studies used animal models, and no controlled clinical studies were found. The treatment of avulsed teeth with platelet concentrates is a practically unexplored field of research.

The studies included in this review used different types of platelet concentrates on the experimental groups: Zhao et al. [7] used PRF and PDLSC, while Reichert da Silva Assunção et al. [1] used PPP, PRP/Thr/Ca, PRP/Ca and BMMCs/PRP/Ca. There are important differences in the biological properties of the different platelet concentrates, which may affect their effectiveness. The platelet concentration in PRP is 5-8 times the base concentration and it is prepared with an anticoagulant and an activator. Studies have shown that PRP activated with bovine thrombin causes release of 81% of the growth factors during the first day, with a significant decrease at days 3, 7 and 14 [20]. This causes a massive, rapid but short-lasting effect. There is also a risk associated with its use since bovine thrombin can generate anti-bodies to coagulation factors V and XI and to thrombin, possibly leading to life-threatening coagulopathies [21]. PRF on the other hand does not need either an anticoagulant or an activator, since polymerisation of the fibrinogen occurs naturally during centrifuging. Slow polymerisation means that there is an increase in the incorporation of cytokines into the fibrin network [22] and greater concentrations of growth factors are released at 7 and 14 days [22]. *In vitro* and *in vivo* studies have shown promising results, with no findings which would contradict the use of PRF alone or in combination with other biomaterials; it has been shown to have more advantages than disadvantages, principally because it is minimally invasive in technical terms, low risk, and gives satisfactory clinical results [23]. All of the above suggests that platelet-rich fibrin concentrate would be more effective than platelet-rich plasma in treating avulsed teeth. However comparative studies need to be done to demonstrate the superiority of PRF over the other preparations.

The rational basis for the use of platelet concentrates in tissue regeneration is the high number of growth factors which stimulate cell migration, proliferation and differentiation, improving wound-healing. The prognosis of an avulsed tooth depends directly on the viability of the cells of the periodontal ligament and the cells surrounding the damaged zone, and their capability of proliferating and differentiating. Previous studies have indicated that root resorption is due to necrosis of the cells of the periodontal ligament [5], which explains why their vitality must be maintained.

Various studies have shown that platelet concentrates increase the proliferation and viability of periodontal ligament cells in avulsed teeth [7, 13]. This makes it an interesting treatment option, both as a medium for preserving the avulsed tooth and for placing in the alveolus prior to replantation, where it reduces post-plantation complications such as replacement and inflammatory resorption [7].

The studies included presented differences in their reporting of results, preventing quantitative analysis in this review. However the qualitative analysis concludes that teeth replanted with platelet concentrates present more effective periodontal repair, greater ligament regeneration and reduced ankylosis and inflammation [1, 7]. Although one of the studies included in this review [7] shows that the combination of PRF with stem cells from the periodontal ligament has a positive effect seen in greater fibre formation and lower resorption rates, the use of stem cells is still difficult to apply in clinical practice. Furthermore, teeth treated with PRF alone showed formation of tissue similar to

periodontal ligament, which may be explained by the presence of stem cells in the periodontal tissue remaining in the alveolus which are able to differentiate and form tissue similar to periodontal ligament [7].

The treatment of teeth replanted with platelet concentrates is an apparently effective technique, autologous, easy to apply in clinical practice and low-cost. However more evidence needs to be assembled through controlled, randomised clinical studies. Comparative studies between the different types of concentrate (PRP, PRF) are also needed.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Cristina Bucchi, Research Center in Dental Sciences (CICO), Faculty of Dentistry, Universidad de La Frontera, Manuel Montt 112, Casilla 54-D, Temuco, Chile. E-mail: cristina.bucchi@ufrontera.cl

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