Autologous Platelet Concentrates for Pulp and Dentin Regeneration: A Literature Review of Animal Studies

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Abstract

Introduction: The purpose of this study was to evaluate the effectiveness of autologous platelet concentrates (APCs) in promoting pulp and dentin regeneration in animal models. Methods: An electronic search was performed on MEDLINE, Embase, Scopus, SciELO, LILACS, and CENTRAL. Animal studies using APC as a root filling material after pulpectomy in mature or immature teeth were included. Articles underwent risk of bias assessment. Histologic evaluation of intracanal neofomed tissue was the primary outcome; root development, root wall thickening, apical closure, and periapical healing in apical periodontitis were the secondary outcomes. Results: Seven articles were included. Platelet-rich plasma (PRP) was used as root filling material during regenerative procedures in the experimental group in either mature or immature teeth. After revascularization with PRP alone or in conjunction with stem cells of a different source, the histologic analyses revealed that, in addition to an odontoblastic cell layer or dentinlike structure, the neofomed intracanal tissues were mainly cementumlike, bonelike, and connective tissues. Conclusions: True regeneration of necrotic pulp may not be achieved with current techniques using PRP, all of which stimulated tissue repair. Benefits of PRP adjunct for pulp tissue regeneration in preclinical studies remain unclear. Further studies with standardized protocols are necessary to assess the actual contribution of PRP in endodontic regenerative therapies. (J Endod 2016;42:250–257)

Key Words

Endodontic regeneration, immature teeth, platelet-rich plasma, pulpectomy

The goal of tissue regeneration is to form a new tissue with the same anatomy and function as the original one (1). Regenerative endodontic procedures rely on tissue engineering and are defined by the American Association of Endodontists as “biologically-based procedures designed to physiologically replace damaged tooth structures including dentin and root structures as well as cells of the pulp-dentin complex” (2). Although several approaches have been used to date, there is still no protocol able to achieve predictable endodontic tissue regeneration (3, 4).

In cases of immature teeth, the necrotic process involving pulp tissue halts further root development and condemns it to a lack of apical closure and reduced thickness of dentinal walls, which compromises the prognosis of the tooth. In the same manner, the pulp necrosis of mature teeth may produce tooth discoloration and infection of the periapical tissues, among other complications. Root canal therapy has been the traditional approach for mature necrotic teeth as well as for immature teeth after an apexification procedure. However, a vital pulp is critical for the maintenance of tooth homeostasis and longevity (4). In cases of absence of a functional pulp tissue and vascular perfusion, the root canal is not able to support the new tissue formation on its own (5–7). Consequently, current revascularization procedures using blood clot or hemocomponents represent an aid for the management of necrotic teeth.

Regeneration of pulp tissue may be enhanced by the combination of the patient’s own growth factors and bioscaffold. Autologous platelet concentrates (APCs) have recently emerged as a possible tool for enhancing regeneration procedures in the medical field; APCs gained popularity among oral and maxillofacial surgeons as well as in other fields such as orthopedics, plastic surgery, and sports medicine, assuming an important role for increasing the predictability of hard and soft tissue regeneration procedures (8–13). APCs are hemocomponents obtained through the centrifugation of a blood sample of the patient. The basic concept of this technology is to collect the most active components of the blood sample (eg, platelets, fibrin, and in certain cases leukocytes). This process produces a very high-concentration gradient of platelets whose granules are rich with many substances fundamental to promote the healing process including adhesive proteins; procoagulant factors; cytokines and chemokines; antimicrobial proteins; and a number of mitogenic growth factors such as platelet-derived growth factors, transforming growth factor-beta, epidermal growth factors, and vascular endothelial growth factors (14–17), which may trigger angiogenesis and improve tissue vascularization. The APCs can be classified based on the fibrin architecture and cellular content as follows: platelet-rich plasma (PRP) with or without leukocytes (L-PRP and P-PRP, respectively) and platelet-rich fibrin (PRF) with or without leukocytes (L-PRF and P-PRF) (18). L-PRF is characterized by the presence of leukocytes and a high platelet concentration (up to 5–8 times the baseline value). It is prepared from anticoagulated blood undergoing a double centrifugation step and requires an activator before use. P-PRF is characterized by the absence of leukocytes and a modest increase in platelet concentration (2–3 times the baseline value). It is prepared from anticoagulated blood undergoing a single centrifugation step and requires an activator before use (14). L-PRF is characterized by the presence of most platelets and leukocytes in a dense fibrin matrix that does not require an activator before use (19). It is prepared from nonanticoagulated blood undergoing a single centrifugation step. The rational basis for the use of APCs for the treatment of pulpectomy roots rests on the assumption that the high concentration of growth factors represents a potent stimulation for tissue healing obtained through the patient’s own...
In addition to granule content release, the polymerization of fibrinogen into a fibrin mesh forms a platelet gel or clot that is delivered to the surgical site (14).

Current protocols developed in the context of regenerative endodontic therapy aim at meeting the 3 main ingredients of tissue engineering: scaffold, growth factors, and stem cells. Specifically, fibrin within the blood clot or autologous platelet concentrates may act as a natural scaffold through which stem cells from the apical tissues may embed and repopulate the canal space. Growth factors released from an intracanal blood clot or APCs may modulate such cellular recruitment as well as stem cell proliferation and differentiation (20).

Early animal studies in beagle dogs treated using blood clot observed new tissue formation inside the root canal after revascularization (5, 21–24). In particular, Wang et al (21) in 2010 reported that neoformed intracanal tissues, after blood clot induction, in immature teeth consisted of cementoid and osteoid tissues (that were hypothesized to be responsible for root lengthening and thickening) and periodontal ligament–like tissue. Similar results have been shown in revitalization procedures in mature teeth (25). This suggested that the neoformed intracanal tissues may have little similarity to the healthy pulp tissue and raised the question whether the revascularization procedures with blood components might lead to pulp regeneration or just tissue repair.

The growth factors released by platelet concentrates proved to be effective in inducing angiogenesis and regeneration of different tissues and might therefore represent a useful tool for necrotic pulp treatment (26–28).

The aim of the present systematic review of the literature was to evaluate current preclinical evidence about the effectiveness of APCs in restoring the pulp-dentin complex of a necrotic tooth by promoting pulp and dentin tissue regeneration when they are used after pulpectomy.

**Materials and Methods**

**Search Strategy**

A systematic literature search was performed on the following electronic databases (PubMed, SciELO, LILACS, ScienceDirect, Scopus, and Cochrane Central Register of Controlled Trials) using (platelet OR fibrin) AND (endodont* AND regenerat* OR apex*) as the search string. Once the studies were identified, the search was then restricted to only animal studies in which histologic outcomes were reported. An additional hand search of issues from 2000 up to the last issue available on December 15, 2014, including the “early view” (or equivalent) section was undertaken on the following journals: *Australian Endodontic Journal*, *Dental Traumatology*, *International Endodontic Journal*, *Journal of Endodontics*, and *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*. The reference lists of the retrieved reviews and the included studies were also searched for possible additional eligible studies not identified by the electronic search. The last electronic search was performed on January 15, 2015. Only articles published in English were considered, and no restrictions regarding publication date were placed.

**Inclusion Criteria**

Animal studies assessing the effectiveness of APCs for stimulating the regeneration of pulp tissue and/or inducing radicular development...
were included. Studies had to have a comparative design in which APC was used as a root filling material and had to report any type of findings of endodontic regenerative procedures. Studies treating either mature or immature teeth were included. Any type of platelet concentrate was included, and it could be used alone or in combination with other materials or stem cells of a different source. Publications that did not meet these inclusion criteria and those that were not dealing with animal studies (eg, reviews, clinical cases, and in vitro studies) were excluded.

### Selection of the Studies

Titles and abstracts of the initially retrieved articles were screened independently by 2 reviewers (A.L. and M.D.F.) to identify possible eligible studies meeting the inclusion criteria. The concordance between reviewers was assessed by means of the Cohen kappa coefficient. In case of disagreement, a joint decision was reached by discussion with a third reviewer (C.B.M.). When the abstract was not available, the full text was obtained and checked. Publications that did not meet the selection criteria and those that were not dealing with animal studies (eg, reviews, clinical cases, and in vitro studies) were excluded.

### Data Extraction

Relevant data from included articles were extracted and analyzed by 2 independent reviewers (A.L. and C.B.M.). Cases of disagreement were subject to joint evaluation until an agreement was reached. The primary outcome was histologic evaluation of intracanal tissues. The secondary outcomes were radiologic evaluation of root development, root wall thickening, apical closure in case of immature teeth, and periapical healing in case of periapical periodontitis.

### Risk of Bias Analysis

Methodologic quality of the selected studies was also evaluated as part of the data extraction process. The ARRIVE (Animals in Research: Reporting In Vivo Experiments) guidelines for reporting animal experiments in periodontology and implantology (29) and a systematic review on instruments for assessing risk of bias and other methodologic criteria of animal studies (30) were used to identify 10 items suitable for the evaluation of the risk of bias of the studies included in this review.

Quality criteria taken in consideration were as follows:

1. Ethical statement (nature of ethical review permissions and national or institutional guidelines for the care and use of animals)
2. Experimental procedures (precise details of all procedures performed)
3. Experimental animals (details of animal used including species, developmental stage or mean age, type of teeth, and diagnosis)
4. Randomization
5. Allocation concealment
6. Sample size calculation
7. Completeness of information on dropouts
8. Blinding of the evaluator
9. Financial conflict of interest

The evaluation of the methodologic quality of the selected studies was performed by 2 reviewers independently and in duplicate according to the previously described parameters. All the criteria were assessed as adequate, unclear, or inadequate. The authors of the included studies

### TABLE 1. Main Characteristics of the Included Studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal species, n</th>
<th>Tooth type, test/control</th>
<th>Pretreatment state of the root</th>
<th>Treatment of the root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu et al, 2012, 2014 (33, 38)</td>
<td>Beagles, 4</td>
<td>Upper premolars/ lower premolars</td>
<td>Mature permanent teeth</td>
<td>cDPSCs, 8^A PRP, 8 PRP + cDPSCs, 8^B BC + PRP, 8 BC + BMA gel, 8 BC + PRP + BMA, 8</td>
</tr>
<tr>
<td>Gomes-Filho et al, 2013 (25)</td>
<td>Beagles, 2</td>
<td>Upper and lower premolars</td>
<td>Mature permanent teeth</td>
<td>PRP + HA, 8^*</td>
</tr>
<tr>
<td>Petrović et al, 2013 (34)</td>
<td>Monkeys</td>
<td>Lower canines and lower central incisors/ Lower premolars</td>
<td>Immature permanent teeth</td>
<td>PRP + HA, 8*</td>
</tr>
<tr>
<td>Zhu et al, 2013 (35)</td>
<td>Beagles, 4</td>
<td>Immature permanent teeth</td>
<td>DPCs, 10 PRP, 10 PRP + DPCs, 10</td>
<td></td>
</tr>
<tr>
<td>Torabinejad et al, 2014 (36)</td>
<td>Ferrets, 7</td>
<td>Upper and lower canines</td>
<td>Immature permanent teeth</td>
<td>PRP, 9^*</td>
</tr>
<tr>
<td>Zhang et al, 2014 (37)</td>
<td>Beagles, 3</td>
<td>Premolars</td>
<td>Immature permanent teeth</td>
<td>PRP, 12^C</td>
</tr>
</tbody>
</table>

BC, blood clot; BMA, bone marrow aspirate; cDPSCs, canine dental pulp stem cells; CH, calcium hydroxide; DPCs, dental pulp cells; HA, hydroxyapatite; MTA, mineral trioxide aggregate; NR, not reported; NaOCl, sodium hypochlorite; PRP, platelet rich plasma; TAP, triple antibiotic paste (metronidazole, ciprofloxacin, minocycline).

Reasons of exclusion from histologic analysis: A1 root cracked during the histologic sectioning procedure, B1 root cracked during the histologic sectioning procedure, C6 roots damaged during sectioning, and D1 root was excluded because it was damaged during sectioning.*

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were contacted for providing clarifications or missing information as needed. Studies were considered at low risk of bias if more than two thirds of the parameters were judged as adequate.

Results

The article selection process is presented in Figure 1. The electronic search retrieved 271 articles, whereas the manual search identified 10 additional articles. After the first screening consisting of title and abstract evaluation, 272 articles were excluded: 16 were duplicates, 228 were nonrelated to the topic of this review, and 28 were not dealing with an animal study (15 clinical studies, 8 reviews, 1 letter to the editor, and 4 in vitro studies). Ten full-text articles were assessed for eligibility. After full-text evaluation, 2 articles were excluded: the first one because the endodontic regenerative treatment actually did not involve the use of APC (31) and the second one because it was not possible to obtain translation from Serbian language and the English abstract provided poor information (32). Finally, 7 articles met the inclusion criteria and were included in this review (25, 33–38). The assessed Cohen kappa coefficient value was equal to 0.88, meaning an almost perfect agreement between reviews on the selection of the studies according to the scale of Landis and Koch.

In 2 of the included articles, the same pool of teeth was treated, but different outcomes (radiologic and histologic in 1 study and histochemical and immunohistochemical in the other one) were reported (33, 38). All articles used PRP as a root filling material after pulpectomy, 3 of them in mature teeth and 4 in immature teeth (25, 33–38) (Table 1).

The main characteristics of the animals, teeth, and treatment applied to teeth of the included studies are summarized in Table 1.

### Table 2. Description of the Process for Producing the Platelet-rich Plasma (PRP) Used in Included Animal Studies

<table>
<thead>
<tr>
<th>Authors, Date</th>
<th>Type of concentrate</th>
<th>Manual procedure</th>
<th>PRP Concentration</th>
<th>Anticoagulant</th>
<th>Activator</th>
</tr>
</thead>
</table>
| Petrović et al, 2013 (34) | PRP | 1. 1200 rpm × 20 min  
2. 2000 rpm × 15 min | NR | Na-citrate | NR |
| Zhu et al, 2012, 2014 (33, 38) | PRP | 1. 200 g × 10 min  
2. 360 g × 15 min | 1,200 × 10^9/L | Citrate solution | Bovine thrombin + 10% calcium chloride |
| Gomes-Filho et al, 2013 (25) | PRP | 1. 300 g × 10 min  
2. 640 g × 10 min | NR | Citratephosphate-dextrose-adenine-1 | Autologous thrombin |
| Zhu et al, 2013 (35) | PRP | 1. 200 g × 10 min  
2. 360 g × 15 min | 1,200 × 10^9/L | Citrate solution | Bovine thrombin + 10% calcium chloride |
| Torabinejad et al, 2014 (36) | PRP | 2 steps | NR | NR | Thrombin + 10% calcium chloride |
| Zhang et al, 2014 (37) | PRP | 1. 100 g × 15 min  
2. 3200 rpm × 30 min | 800 × 10^9/L | EDTA-k2 | NR |

NR, not reported; PRP, platelet-rich plasma.
The details of the process for obtaining the production of platelet concentrates for any included studies are reported in Table 2. The histologic outcomes of the included animal studies are summarized in Table 3. In addition, radiographic outcomes are detailed in Table 4. Finally, risk of bias assessment of the included studies is reported in Figure 2.

**Characteristics of the Included Studies**

In all studies included in this review, an ethical statement was reported regarding the type of ethical permissions received for conducting animal experiments and the institutional guidelines followed for the care and use of the animals. Precise details of all experimental animal procedures performed were also reported (Table 1). A total of 28 animals were treated: 13 beagle dogs (25, 33, 35, 37), 8 monkeys (34), and 7 ferrets (36).

All animal studies used PRP in the experimental group as root canal filling material. It was used alone (36, 37), in conjunction with canine dental pulp stem cells (33) or dental pulp cells (35), or with bone marrow aspirate gel and blood clot (25) (Table 1). In almost all studies, blood clot was the standard comparative treatment, except in 1 article in which hydroxyapatite (HA) was used as the control (34). The duration of follow-up was 3 months in 6 studies (25, 33, 35–38) and 6 months in 1 study (34).

**Characteristics of the Experimental Units**

In 4 articles in which teeth were depulped (25, 33, 35, 37), for the histologic evaluation, each root canal was considered as an independent unit, in particular 70 experimental root canals received PRP, whereas 44 root canals were treated with blood clot. On the contrary, in the remaining articles (34, 36), the tooth was used as the histologic unit, with a total of 17 teeth treated with PRP, 12 blood clot–treated teeth, and 8 treated with HA. Incisors, canines, and premolars were used. They were permanent teeth with complete or incomplete root formation, and some of them had experimentally induced apical periodontitis (Table 1).

**Root Canal Treatment Protocol**

The protocol for the radicular canal treatment in the included articles is described as one of the following:

1. The disruption of the pulp and induction of experimental periapical periodontitis (25, 33, 37)
2. Pulp removal from immature or mature teeth (33, 36) followed by root canal irrigation with different solutions as described in Table 1

Canal disinfection using triple antibiotic paste was performed in only 3 studies (25, 35, 37). At the subsequent visit, PRP was applied in the root canals, and root canal access was sealed using different composites. In one study (34) the treatment provided to one group of teeth was defined by the authors as high pulpotomy, meaning the removal of the pulp close to the radiographically visible end of the immature root. Hence, in this review such so-called high pulpotomy was considered as pulpectomy. To these teeth gutta-percha was applied along with selected material (PRP + HA or HA alone) and sealing with glass ionomer cement and amalgam (Table 1).

Protocols for preparing PRP are summarized in Table 2; consistent differences among studies were observed. Different authors followed diverse manual procedures (time and speed of the 2 centrifugation steps) and used a different anticoagulant and activator. The final concentration of platelets obtained in PRP was reported in only 3 articles (35, 37, 38).

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**Table 2: Histologic Outcomes of the Included Articles**

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of teeth (group)</th>
<th>Follow-up (months)</th>
<th>Deformities</th>
<th>Inflammatory reaction of pulp/periapical tissue</th>
<th>Fibrous connective tissue in the canal</th>
<th>Bonelike tissue</th>
<th>Cementumlike tissue</th>
<th>Authors</th>
<th>No. of teeth (group)</th>
<th>Follow-up (months)</th>
<th>Deformities</th>
<th>Inflammatory reaction of pulp/periapical tissue</th>
<th>Fibrous connective tissue in the canal</th>
<th>Bonelike tissue</th>
<th>Cementumlike tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhu et al, 2012, 2014 (33, 38)</td>
<td>4 (cDPSCs)</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>Zhu et al, 2013 (35)</td>
<td>10* (DPCs)</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zhu et al, 2013 (34)</td>
<td>8 (PRP + HA)</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>Zhu et al, 2013 (36)</td>
<td>18* (PRP)</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Petrović et al, 2013 (34)</td>
<td>8 (BC + PRP + BMA)</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>Zhang et al, 2014 (37)</td>
<td>18* (BC)</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A. absence; BC, blood clot; BMA, bone marrow aspirate; cDPSCs, canine dental pulp stem cells; DPCs, dental pulp cells; HA, hydroxyapatite; NR, not reported; P, presence; PRP, platelet-rich plasma.
Histologic and Radiographic Results

In all studies, a histologic evaluation of intracanal and periapical tissues was performed (25, 33–37). One study also reported immunohistochemical and histochemical analyses of intracanal tissues (38). Four of the previously described studies also performed radiographic assessment (33, 34, 35, 37). An analytic description of the histologic findings is provided in Table 3, and detailed radiographic outcomes are reported in Table 4.

Risk of Bias Assessment

Only 2 studies (35, 37) were classified as having a low risk of bias, whereas all other included studies presented a high risk of bias based on the parameters evaluated (Fig. 2).

Discussion

In a healthy condition, the root canal is occupied by dental pulp tissue, which is constituted by a well-organized architecture of blood vessels, nerves, and cells, with the main function of producing dentin and maintaining the vitality of the pulp-dentin complex. In particular, among the pulp cells, odontoblasts are specialized to form dentin, the immune cells ensure a quick response against possible microbial contamination and undifferentiated mesenchymal cells, dental pulp stem cells replace the primary odontoblasts depositing the tertiary dentin, and stem cells from the apical papilla (SCAPs) of immature permanent teeth have dentinogenic potential for the production of primary and secondary dentin (39). When the pulp is no longer vital, tooth becomes vulnerable, and the deposition of the dentin stops. Particularly, the treatment of immature necrotic teeth is a great challenge for endodontists. The main difficulty with young teeth having nonvital pulp is adequately cleaning the root canal, paying attention to the fragile and thin dentinal walls, and sealing the open apex properly. Traditional treatments had failed to predictably manage these teeth because there were no adequate therapeutic strategies (40). Root canal obturation using bioactive cements (41) may ensure a clinical success in terms of healing of the periapical lesion (42–44), but the continued root development and strengthening of the root structure may not be warranted (20).

Regenerative endodontics uses the concept of tissue engineering to turn the pathological pulp into a vital functional one by promoting the growth of new vital intracanal tissue, resulting in root development (dentin thickening and root apex closure) (40, 45). Although current regenerative endodontic protocols have reported successful clinical and radiographic outcomes in the treatment of immature necrotic teeth, the biological outcomes are still unpredictable, and a true pulp regeneration may not be achieved (20, 46). Moreover, pulp regeneration of mature teeth has been traditionally considered more challenging than the pulp regeneration of immature teeth, mainly because of the lower amount of stem cells and a narrower apical foramen that may allow revascularization (25).

When attempting to regenerate functional pulp/dentin tissue in a root canal, some issues have to be considered. Regenerated pulp must do the following:

<table>
<thead>
<tr>
<th>Authors</th>
<th>No. of teeth (group)</th>
<th>Follow-up (months)</th>
<th>Periapical healing</th>
<th>Root growth retardation</th>
<th>Root wall thickening</th>
<th>Apical closure</th>
</tr>
</thead>
</table>
| Zhu et al, 2012, 2014 (33, 38) | 4 (cDPSCs) | 3 | 2 | 2 | NR | NR | NR | NR | NR | NR | NR
| | 4 (PRP) | 3 | 1 | 1 | NR | NR | NR | NR | NR | NR | NR
| | 4 (PRP + cDPSCs) | 3 | 1 | 1 | NR | NR | NR | NR | NR | NR | NR
| | 4 (BC)† | 0 | 4 | NR | NR | NR | NR | NR | NR | NR | NR
| Petrović et al, 2013 (34) | 8 (PRP + HA) | 6 | 8 | 0 | 3‡ | 4‡ | NR | NR | NR | NR
| Zhu et al, 2013 (35) | 10* (DPCs) | 3 | 8 | 2 | NR | NR | 10 | 0 | NR | NR | NR
| | 10* (PRP) | 10 | 0 | NR | NR | 3 | 7 | NR | NR | NR | NR
| | 10* (DPCs + PRP) | 9 | 1 | 1 | NR | NR | 9 | 1 | NR | NR | NR
| | 10* (BC)† | 9 | 1 | 1 | NR | NR | 6 | 4 | NR | NR | NR
| Zhang et al, 2014 (37) | 18* (PRP) | 3 | 18 | 0 | NR | NR | 13 | 5 | 11 | 7 | 2
| | 18* (BC)† | 18 | 0 | NR | NR | 12 | 6 | 16 | 2 | 1 |

A, absence; BC, blood clot; cDPSCs, canine dental pulp stem cells; DPCs, dental pulp cells; HA, hydroxyapatite; NR, not reported; P, presence; PRP, platelet-rich plasma.

*Number of roots.
†Control.
‡One root failed.
1. Be vascularized and innervated
2. Have cellular density and extracellular matrix architecture similar to the natural one
3. Produce odontoblasts that must line on the existing dentin layer and produce new dentin upon the existing one (47)

However, pulp regeneration is made difficult by the typical anatomic structure of the tooth. Indeed, pulp tissue is encased by the dentin and has the only blood supply, which is essential for the healing and regenerative processes, coming through the root apical foramen. Because of the highly organized pulp structure and composition, the most challenging problem is the ex novo pulp regeneration from a pulpless tooth and restoration of the ordinary function.

The objective of this review was to assess the effectiveness of APCs in achieving pulp and dentin regeneration. The presence of PRP in the root canal space might be beneficial to the stem cells from the apical papilla or other pulp cells seeded as part of the treatment in order to repopulate the root canal. In fact, stem cell proliferation and differentiation are mainly induced by the growth factors secreted from platelets, whose greater content in APCs may promote a stronger biological activity compared with the blood clot. However, a common feature highlighted among the animal studies included in this review was that the odontoblastic cell layer, dentinlike structure, or new pulplike tissue was rarely detected after revascularization using either PRP alone or in conjunction with pulp stem cells or pulp cells. Indeed, the new intracanal tissue formed was cementumlike tissue, bone-like tissue, and fibrous connective tissue. Only in 1 of the included studies in the present review (37) the regenerated tissue in the canal space was described as pulplike tissue with no difference between the PRP and blood clot groups. However, this tissue could not be considered as true pulp because of the absence of an odontoblast layer. Similar findings emerged from other preclinical studies in which odontoblasts were not identified, even if many pulp tissue elements were histologically detected as fibroblasts, blood vessels, and collagen. Besides unexpected cells or tissues, osteoblasts and cementum were sometimes observed in the root canal (5, 21–24).

Intracanal mineralized tissues were detected both adherent to the dentin wall (dentin-associated mineralized tissue [DAMT]) and forming bone islands in the central region (23). DAMT differs from dentin and bone and although resembling cementum tissue in terms of lack of vasculature and the immunostaining pattern, the organization and maturation of collagen matrix are different from the cementum as well. The pattern of mineralization in DAMT is less uniform than other mineralized tissues such as cementum. Bony islands resemble bone matrix in terms of morphology, collagen organization, and immunoreactivity (24). The cellular source responsible for DAMT and bony island formation is poorly understood. It has been suggested that the SCAPs might differentiate into odontoblasts forming new roots. By contrast, the detection of odontoblast-like cells was reported in vitro models when stem cells were seeded into scaffolds of a different type or in the presence of growth factors (48–53).

To date, few clinical studies on immature human teeth with apical periodontitis performed histologic analysis to investigate the nature of the neoformed intracanal tissues after revascularization/revitalization (54–56). Interestingly, in only 1 such study was the presence of intracanal pulplike connective tissue detected. In this clinical study, PRP alone was injected in the root canal as a regenerative endodontic treatment (54). In another study, loose connective tissue resembling immature pulp and containing fibroblasts and mesenchymal cells was observed in the root canal space of an immature human maxillary central incisor (55). Conversely, in the third histologic study, cementoid/osteoid tissue, vital connective tissue, and blood vessels formed in immature mandibular molars with apical periodontitis irrespective of the type of scaffold used (PRP or blood clot) (56).

Pulp tissue and apical papilla may have been destroyed by the overinstrumentation performed in some articles to contrast the root canal infection, thus impairing ex novo pulp regeneration and root maturation. This could be 1 explanation for the missing detection of the odontoblastic cell layer, dentinlike structure, or new pulplike tissue after revascularization procedures. Another explanation is that when the survival of stem cells located in the apical papilla is compromised because of apical periodontitis, no further odontoblast differentiation occurs, and, therefore, root dentin is no longer deposited. To accomplish regenerative endodontic therapy, it is necessary to stimulate DPSCs or SCAPs to adhere to the inner surface of the root canal dentin although it was reported that the use of some disinfection protocols may prevent the repopulation of stem cells of the dentinal surface, impairing the treatment outcome (57, 58). We have to consider that in the absence of a proper cell population, adjunctive treatments like platelet concentrates may become ineffective because growth factors released by platelets need to interact with target cells to affect their biologic activity.

Nevertheless, after the completion of the healing process, root development (consisting of root wall thickening, root lengthening, and root apex narrowing) occurred presumably through the growth of cementumlike and bone-like tissue. Therefore, clinical success may not correspond to histologic pulp regeneration.

In conclusion, from the histologic results of the studies included in the present review, it seems that there is no current protocol using APCs able to achieve a true regeneration of the necrotic pulp tissue either in immature or mature teeth. In fact, tissue repair seems apparently stimulated. Thus, the root canal may become repopulated with a living tissue that only marginally resembles the original pulp but may not have the same functional activity. Despite this, root maturation may be achieved, and teeth function is not compromised, which represents a success from a clinical standpoint. Thus, the results of clinical studies evaluating pulp regeneration procedures should be considered and compared with preclinical ones. In fact, the latter may not fully reflect the clinical environment, at least for the present topic. The benefit of the additional use of PRP for predictable regeneration of the pulp tissue in preclinical studies is unclear. Further studies with a higher evidence level and a standardized protocol are required to shed light on the actual role of PRP in such clinical applications.

Acknowledgments
The authors deny any conflicts of interest related to this study.

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