Regeneration of Microcirculation and Alveolar Bone after Application of Platelet-Rich Plasma

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Abstract

In order to model the vascularization stage of periodontal recovery, platelet-rich plasma (PRP) was applied to the sockets of the beagle dog's dentition. Microvascular resin injection was performed 14, 30, and 90 days later and examined by light and scanning electron microscopy to investigate the relationship between bone formation and vascular changes. Bone formation ratios were measured from the scanning electron microscopic images. Fourteen days after the operation, newly formed blood vessels filled the untreated sockets, except for the center portion. These blood vessels had regenerated along the pre-existing bone wall of the socket. In the sockets treated with PRP, however, the sockets were filled with newly formed bone, and regenerated blood vessels were surrounded by new bone. Thirty days after the operation, the insides of the sockets were filled with newly formed porous bone in both groups. In untreated sockets, porous new bone formation was observed along the blood vessels, but in sockets treated with PRP, bone trabecula and blood vessels were arranged in the porous bone. Ninety days after the operation, both treated and untreated sockets contained regenerated normal bone tissue, with bone marrow reproduced along the trabeculae and vascular networks observed. However, the bone formation ratios of the PRP-treated sockets were significantly higher than the untreated sockets after 14 and 30 days. At 90 days, nearly identical bone formation was measured in both groups. Taken together, these observations suggest that PRP application to the extraction sockets advanced bone regeneration and promoted regeneration of the blood vessels. [MVRC 4(1): 12-17, 2011]

Key words: platelet-rich plasma, bone regeneration, microcirculation, resin cast, SEM
**Introduction**

Dental implants are a major treatment option in modern dentistry. However, it is difficult to implant successfully into the narrow width and the low height of the jawbone. Application of platelet-rich plasma (PRP) is extensively used in dental bone regeneration therapy, as it contains many growth factors such as PDGF, VEGF, FGF, EGF and TGF-b. These factors are thought to accelerate regrowth of vasculature and bone, explaining why PRP applications are utilized for skin regeneration in plastic surgery. The growth factors in PRP likely operate at the level of tissue healing, participating in the vascularizations that are the first stage of tissue recovery.

Microvascular resin casts are generated from injection of low-viscosity synthetic resin into blood vessels and dissolving of the peripheral tissue, permitting observation of all blood vessels. Clear, three-dimensional images can be obtained through the complete infusion of a viscous resin up to the capillaries and observation using a scanning electron microscope (SEM) with deep focus. When this method is used, only bone, teeth, and a vascular cast remain for observation.

Here we combine the experimental PRP treatment of extraction sockets with the observational power of the microvascular resin cast method. In this way, we hope to elucidate the characteristics of the regenerative process promoted by PRP treatment following tooth extraction.

**Materials and Methods**

**Surgical procedures:** The six beagle dogs (9 to 10 kg) used in this study were given general anesthesia (Nembutal®, 25 mg intravenously; Abbott, USA) for the performance of all experimental procedures, which adhered to the Animal Care Committee guidelines of our institution. Both mandibular first, second, and third premolars were extracted (Fig. 1a), generating sockets suitable for experimental use. PRP was made (see below) and applied to one side of the dentition (b), while the other side remained untreated as a control (c). The gingival flap was sutured (d), and microvascular resin injection was performed 14, 30, and 90 days later (e).

**Preparation of PRP:** PRP was prepared for each experimental animal. Blood and 2% sodium citrate solution were mixed at a ratio of 1:9, then centrifuged for 10 min at 1000 rpm. The red blood cell layer was removed, and the solution was centrifuged again. The PRP was adjusted by mixing the platelet and the plasma. Platelet was counted in the automated hematology analyzer (KX-21, Sysmex, Japan, 500000-1470000 platelets/μl). Afterward, bovine thrombin was mixed with 10% calcium chloride for the solution to become a gel.

**Measurement of bone formation ratio:** The bone formation area was measured from the SEM images taken of 6 specimens for each group. Edges were detected with the image analysis application Image-J (NIH), and the area of bone addition was measured. The ratio of the bone addition areas to the area of each socket was determined, and statistically significant differences were detected with Student’s t-test.

**Results**

Fourteen days after the extraction, light microscopy of the resin cast models from untreated sockets revealed the regeneration of blood vessels along the pre-existing bone of the socket (Fig. 2a). Blood clots filled the center of the socket, which became a gel.

**Fig. 1. Experimental design.** The mandibular premolars were extracted from six beagle dogs (a). Platelet-rich plasma (PRP) was applied to sockets on one side of the dentition (b), while the other side remained untreated as a control (c). The gingival flap was sutured (d), and microvascular resin injection was performed 14, 30, and 90 days later (e).

**Fig. 2. Light microscopy of resin cast models from untreated sockets 14 days after extraction.** Blood vessels (BV) were regenerated along the pre-existing alveolar bone (AB). Few blood vessels were seen in the center of the socket (marked with an asterisk). NFB, newly formed bone.
and few blood vessels were detectable in this area (Fig. 2b). In the PRP-treated group (Fig. 3), blood vessels were regenerated not only in the vicinity of pre-existing bone, but also in the center of the socket.

High-magnification SEM images of untreated sockets (Fig. 4a) indicated that the blood vessels that were connected to the bone marrow vessels of pre-existing alveolar bone were regenerated in the socket, and several new bone formation was observed along the bone wall. Following PRP treatment (Fig. 4b), the socket was filled with regenerated blood vessels, and new bone formation had taken place near blood vessels. The density and amount of blood vessels and new bone was larger in the PRP-treated group than in the control group.

The bone formation ratio of the PRP group (56.95±1.30%) was significantly higher (p<0.05) than in the control (43.64±3.53%) group.

When resin cast models made thirty days after the extraction were examined under the light microscope, sockets from both untreated (Fig. 5a) and treated groups (Fig. 6a) were filled with porous new bone. In the untreated group, remarkable amounts of porous bone formation had taken place in the vicinity of the blood vessels (Fig. 5b). Porous bone formation was also observed near blood vessels in PRP-treated sockets (Fig. 6b).

Osteoneogenesis advanced and bone trabeculae spaces began to form, phenomena that were observed even more clearly under high-magnification SEM (Fig. 7). Blood vessels were observed in sockets untreated with PRP, which were filled with porous new bone (Fig. 7a). Thick bone trabeculae appeared in PRP-treated sockets, and blood vessels were arranged in networks in the newly formed bone marrow (Fig. 7b).

The bone formation ratio of the PRP-treated group...
(66.46±2.69%) was significantly higher (p<0.05) than the ratio of the untreated (54.25±2.22%) group thirty days after extraction.

Matured bone structure was observed in both groups ninety days after extraction (Figs. 8 and 9). Compact bone and spongy bone was reconstructed from the porous new bone in 30 days. Trabeculae was regenerated and blood vessels consists of 50- to 100µm venous plexus. arranged in networks in the bone marrow.

Divergence of thick trabeculae was revealed by high-magnification SEM of both untreated (Fig. 10a) and PRP-treated (Fig. 10b) sockets. Blood vessels were arranged in networks in the newly formed bone marrow.

Ninety days after extraction, the bone formation ratios of the PRP-treated (72.21±4.99%) and untreated (71.09±5.36%) sockets were nearly indistinguishable.

**Fig. 6.** Light microscopy of resin cast models from sockets 30 days after extraction and treatment with platelet-rich plasma (PRP). Remarkable amounts of porous newly formed bone (NFB) were seen in the sockets after PRP treatment (a). Bone trabeculae (BT) and bone marrow space began to form (b). AB, alveolar bone; BV, blood vessels.

**Fig. 7.** Scanning electron microscopy of resin cast models made 30 days after extraction. Blood vessels (BV) were observed in untreated sockets, which were filled with porous newly formed bone (NFB; panel a). Sockets treated with platelet-rich plasma (PRP) displayed thick bone trabecula (BT), and blood vessels (BV) were arranged in networks in the bone marrow space (b). AB, alveolar bone.

**Fig. 8.** Light microscopy of resin cast models from untreated sockets 90 days after extraction. Normal bone structure was achieved 90 days after extraction, even without the addition of PRP. AB, alveolar bone; BV, blood vessels; BT, bone trabeculae.

**Fig. 9.** Light microscopy of resin cast models from sockets 90 days after extraction and treatment with platelet-rich plasma (PRP). Regenerated bone trabeculae (BT) and networks of blood vessels (BV) in the bone marrow appeared after 90 days. AB, alveolar bone.
Discussion

We established a model of periodontal tissue regeneration in beagle dogs, which enabled us to compare the healing process in untreated sockets with sockets treated with PRP. Specifically, we employed the method of resin cast formation to monitor the progression of angiogenesis, the process of vascular formation. In a previous study using the resin cast method, vascularization and bone formation were observed to occur along existing bone in an extraction socket in the initial healing stage (7-14 days); bone remodeling and vascularization was observed in the sockets in the middle healing stage (after 30-60 days), and maturation of bone marrow and formation of bone trabeculae were reported in the final healing stage (90-120 days). Similar results were obtained in the untreated group without PRP application in this experiment. Fourteen days after extraction, the tooth socket was filled with blood vessels. The density and amount of blood vessels and new bone was larger in the PRP-treated group than in the control group. In the thirtieth days after, the socket was filled with porous newly formed bone, trabecula-like bone was formed in the newly formed bone and a vascular network was forming in the bone marrow. Thick bone trabeculae appeared in PRP-treated sockets, and blood vessels were arranged in networks in the newly formed bone marrow. Ninety days after, the bone and vascular structure were recovered as normal in both groups.

Alveolar bone regeneration therapy is divided into major three methods: guided bone regeneration, bone grafting, and growth factor application. Guided bone regeneration, widely used in the clinical setting, is the method of covering the upper side of the bone defect with an artificial membrane. Vascularization and bone addition are promoted under the membrane, which also protects against the downgrowth of gingival tissue from upper regions. Resin casting has revealed rich vascular and bone regeneration under the membrane, compared with control tissues. Although alveolar bone height and width are achieved, the speed of vascularization and bone remodeling is not accelerated.

Bone grafts are subdivided into autografts and synthetic bone grafts. For reasons of safety, allografts and xenografts are not suitable for bone regeneration therapy in human patients. Autografts use bone obtained from the patients; the cancellous bone that contains many growth factors and blood vessels is presumed to promote vascularization and bone formation, while cortical bone acts as a scaffold. This method is the preferred clinical technique. While there is less risk of graft rejection because the graft originates from the patient, it is difficult to gather a sufficient amount of bone. Synthetic bone grafts employ biologically active ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP), and infection and graft rejection are also low-risk with this technique. Non-resorbable HA shows the recovery process of newly formed blood vessels reproducing near the HA particles, and forming a new bone. On the other hand, the regenerative process is similar to resorbable tricalcium phosphate. However, as the HA particles persist in the tissue in non-resorbable material, neither regeneration of bone trabecula nor the reproduction of bone marrow vessels.

The application of various growth factors, including BMP and bFGF, is being tested for bone regeneration therapy. Compared with other extracted growth factors, PRP is one of the safest materials from own blood, a very important consideration in bone regeneration. For example, the use of PRP carries low risk of HIV or herpesvirus infection. PRP also contains large amounts of growth factors that are suitable for clinical application and promote vascularization. Previously, PRP was applied to the sockets of impacted lower wisdom teeth, and bone formation was promoted, suggesting that priming the vascularization with PRP can lead to bone addition. PRP promotes vascularization in the first stage of healing, and is presumed to work through the regeneration of bone structure. VEGF in particular promotes vascular formation.

Taken together with our observations, these results suggest that the most prominent benefits of PRP use are safety and acceleration of vascularization and bone formation.
References


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