

Lip Reconstruction, Intraoral Barrier and Autologous Platelet Rich Plasma (PRP) Application in a Dog With Lip Necrosis due to Snake Bite

Key words

dog;
flap operation;
large tissue lost;
lip reconstruction;
PRP;
interdental silicone

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Abstract: The study case consisted of a 2-year-old male Jack Russell mixed breed dog with a delayed wound due to snake bite. The necrosis detected on clinical examination in the area starting from the left maxillary and mandibular canine teeth level to the anterior part of the ear base, the upper and lower lips including the left maxillary and mandibular teeth, and the cheek skin, with tissue loss. In the blood sample collected, platelets were recorded at $44 \times 10^9 /L$; however, the analysis of PRP revealed an approximately sixfold rise, with platelet levels reaching $240 \times 10^9 /L$. During the first 15 days, medical treatment was used to prepare the wound for surgery. Caudal Auricular Axial Pattern Flap technique was used in the first operation, and Pedicle Advancement Flap technique was used in the second operation. After both operations, dressings with ointments and systemic treatment continued. At the end of first operation, as a result of the large donor tissue area and the resulting inadequate nutrition and the mechanical irritation created by the patient's tongue, tissue rejection and necrosis occurred at the tip of the flap applied to the lower jaw at the level of the mandibular canine tooth, leading to loss of flap tissue. Therefore, to prevent tissue necrosis, accelerate flap acceptance and ensure flap vitality, platelet-rich plasma (PRP) was applied one week postoperatively. It was concluded that PRP is a useful method that accelerate angiogenesis, epithelialization, acceptance of the flap and to prevent flap necrosis.

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Introduction

A wound is the disruption or loss of cellular or anatomical integrity of tissue. Wound healing is a complex and dynamic process consisting of local and generalized responses such as blood vessel formation, production of mediators such as cytokines from inflammatory cells, and extracellular matrix formation. In order to provide primary healing in wound healing, sutures, grafts, or flaps can be applied, while infective wounds can return to primary healing after the infection is eliminated (delayed primary healing). In recent years, various biomaterials or products have been used to induce or accelerate wound healing. One of these is platelet-rich plasma (PRP). PRP is a non-transfusional hemocomponent that can be used therapeutically as an autologous source of platelet-derived growth factors to promote wound healing and tissue repair for a variety of clinical applications. It can be applied topically in inactive liquid

form via infiltration, injection, or in gel form for the treatment of skin, joint or tendon disorders (1, 2, 3). In this case, platelet-derived growth factors are released at the site of application after platelet activation by endogenous substances such as collagen (4). In addition, they also play a role in mesenchymal cell recruitment and extracellular matrix synthesis, and as they accelerate wound healing, an increase in re-epithelialization, contraction and neovascularization can be observed (5, 6, 7, 8). It is reported to be used in veterinary medicine both in acute wound experimental models (9) and in acute or chronic wound and flap treatments in dogs (1, 2, 10, 11, 12, 13, 14).

The aim of this article is to show that PRP increases flap acceptance and survival in flap operations, one of the reconstructive surgical methods applied to wounds with extensive tissue loss in the facial area.

Case history and Clinical findings

The study case was a 2-year-old, 19 kg, un-neutered male Jack Russell hybrid dog, which presented with a tissue loss wound that was reported to be due to a snake bite. In the patient's first clinical examination, it was determined that there was necrosis in the area starting from the level of the left maxillary and mandibular canine teeth to the front of the ear base, and in the upper and lower lip and cheek skin, including the maxillary and mandibular teeth on the left (Figure 1).



Figure 1: Appearance of the patient before medical and operative treatment

Clidamycin HCl (Clindane 150 mg) PO at a dose of 13 mg/kg for 15 days, Nitrofurazone (Furacin) and 5% Dexpanthenol (Panthenol) were used topically. Although recovery was observed in necrotic bone and soft tissues after 15 days of treatment, necrosis caused bone loss in the mandibular and maxillary alveoli and the mandibular body.

After the necrosis stopped, dead tissue was debrided and the soft tissue was prepared for the operation, the patient was taken to the operation in order to eliminate the soft tissue loss in the region. Anaesthesia was induced by IV administration of Propofol (Propofol-lipuro 10 mg/ml, 10 ml ampoule, Braun) at a dose of 6 mg/kg before endotracheal intubation. Maintenance of anaesthesia was provided with Sevoflurane (Sevorane liquid, 250 ml vial, Abbvie) oxygen mixture. As an operation technique, the 5x10 cm area in the mandibular region was closed using the Caudal Auricular Axial Pattern Flap method, and the 2x6 cm area covering the upper lip was closed using the Upper Labial Pull-Down method. (Figure 2) At the same time, all premolars and molars except for mandibular molar 3 were extracted from the teeth in the upper and lower jaws whose roots were exposed. Postoperatively, systemic antibiotic ceftriaxone (Ceforce® 1 g IV,

İstanbul İlaç Sanayi A.Ş.), analgesic tramadol HCl (Contramal® 100 mg ampoule, Grünenthal Pharma), and dexpanthenol (Bepanthen 500 mg 5 vial, Bayer) were administered intravenously twice a day for one week. The dressing was done with pomades containing nitrofurazone (Furacin® 0.2% ointment, Zontiva) and 5% dexpanthenol (Pantenol Pomad®, Saba İlaç San. Ve Tic. A.Ş.). One week after the operation, a 3x4 cm tissue rejection and necrosis were observed at the tip of the flap applied to left mandibular canine tooth level (Figure 3). After the necrotic part was removed and no abnormality was observed in the patient's general condition, the pedicle advancement flap technique was applied (Figure 4 A, B, C) (15). In order to prevent mechanical irritation caused by the tongue, a barrier made of condensation silicone material (Oxasil Soft Putty, Kulzer) was made on a framework made of cerclage wire between mandibular canine (204) and molar (209) teeth (Figure 4 D). After the second operation, it was decided to inject PRP into the flap area to increase flap survival and acceptance. On the first postoperative day, 2 mL of PRP was injected under the flap via the drain and 2 mL PRP was injected around the flap. (Figure 5).

PRP Preparation

The venous blood samples were drawn from the dog using standard venipuncture technique. A total of 24 mL of whole blood was collected into ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer, 4 mL x 6, 13 x 75 mm). One tube of the collected blood was sent for complete blood count (CBC) analysis using an automated hematology analyzer (BC-5000 Vet, Mindray, Shenzhen, China). The CBC provided baseline values for red blood cells (RBCs), white blood cells (WBCs), and platelets.

The method described by Muthuprabakaran et al. (2021) was followed to assess the effects of various centrifugation speeds and the inclusion of the buffy coat in platelet-rich plasma preparation. The collected blood samples were subjected to an initial centrifugation at a relative centrifugal force (RCF) of 100 g for 10 minutes at room temperature (24-25°C) using a non-refrigerated centrifuge (K241, Centurion Scientific, West Sussex, UK). This step resulted in the separation of the blood into three distinct layers: RBCs at the bottom, the buffy coat in the middle, and plasma at the top. Following the first centrifugation, the buffy coat layer, which contains platelets and WBCs, was carefully extracted using a sterile pipette and transferred into a new sterile centrifuge tube. The transferred buffy coat was then subjected to a second centrifugation at an RCF of 400 g for 10 minutes. This centrifugation further separated the components into two layers: platelet-poor plasma (PPP) at the top and platelet-rich plasma (PRP) at the bottom (Figure 6) (3). The PRP was carefully collected from the bottom layer using a sterile pipette. While platelets were measured as $44 \times 10^9 /L$ in the blood sample taken, it was determined that platelets increased approximately 6-fold in the analysis of PRP, reaching $240 \times 10^9 /L$. The PRP was then ready for use in therapeutic administration.

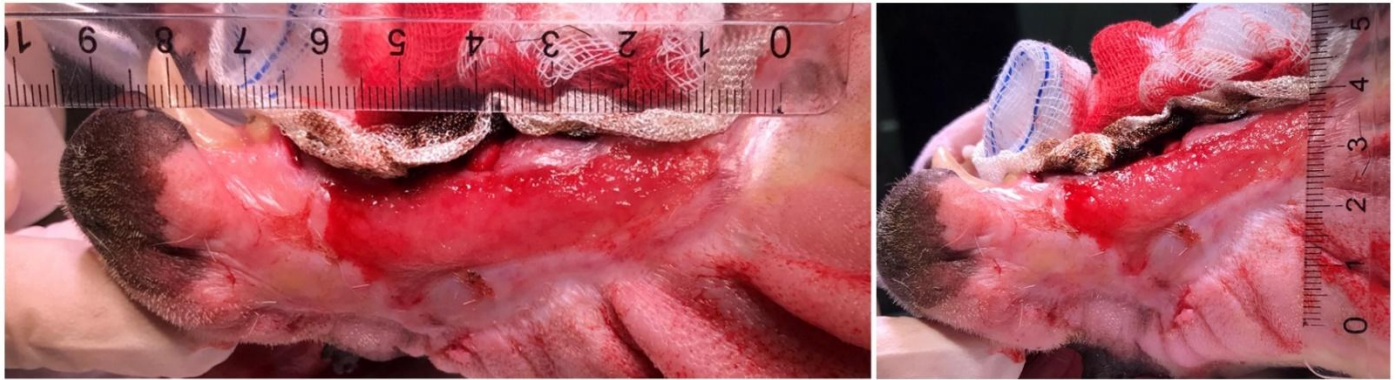


Figure 2: Mandibular lip wound measurements after use of ointment dressing



Figure 3: Lateral view of the wound on the 3rd day after the first operation

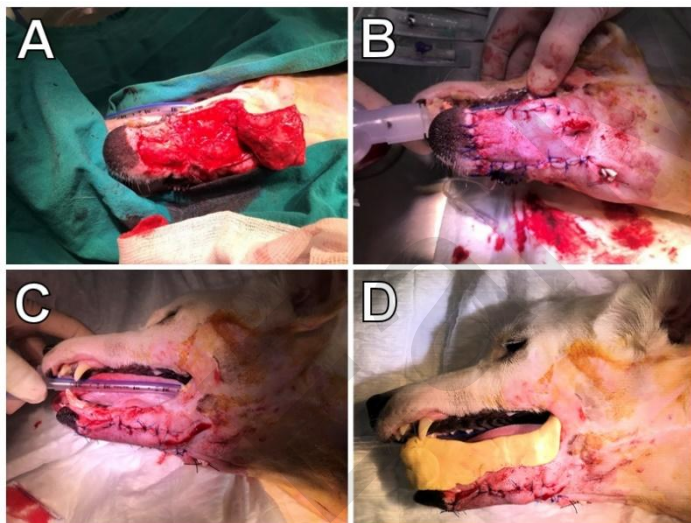


Figure 4: The second operation. (A) the Full-Thickness labial advancement flap preparation (B) Ventral and (C) Lateral view of the flap application. (D) A barrier made of condensation silicone material

Outcome

The drain was removed on the 2nd postoperative day and no discharge was observed in the area (Figure 7 A). Although the application was later attempted to be continued as an injection, it was continued only topically due to strong tissue acceptance

and it was observed that the tissue acceptance was achieved in 3th day (Figure 7 B). It was determined that epitelization achieved and the flap area was hairy and healthy on the 8th postoperative day (Figure 7 C). The sutures were removed on the 10th postoperative day.



Figure 5: First day of the PRP application



Figure 6: Platelet rich plasma obtained from the patient's blood

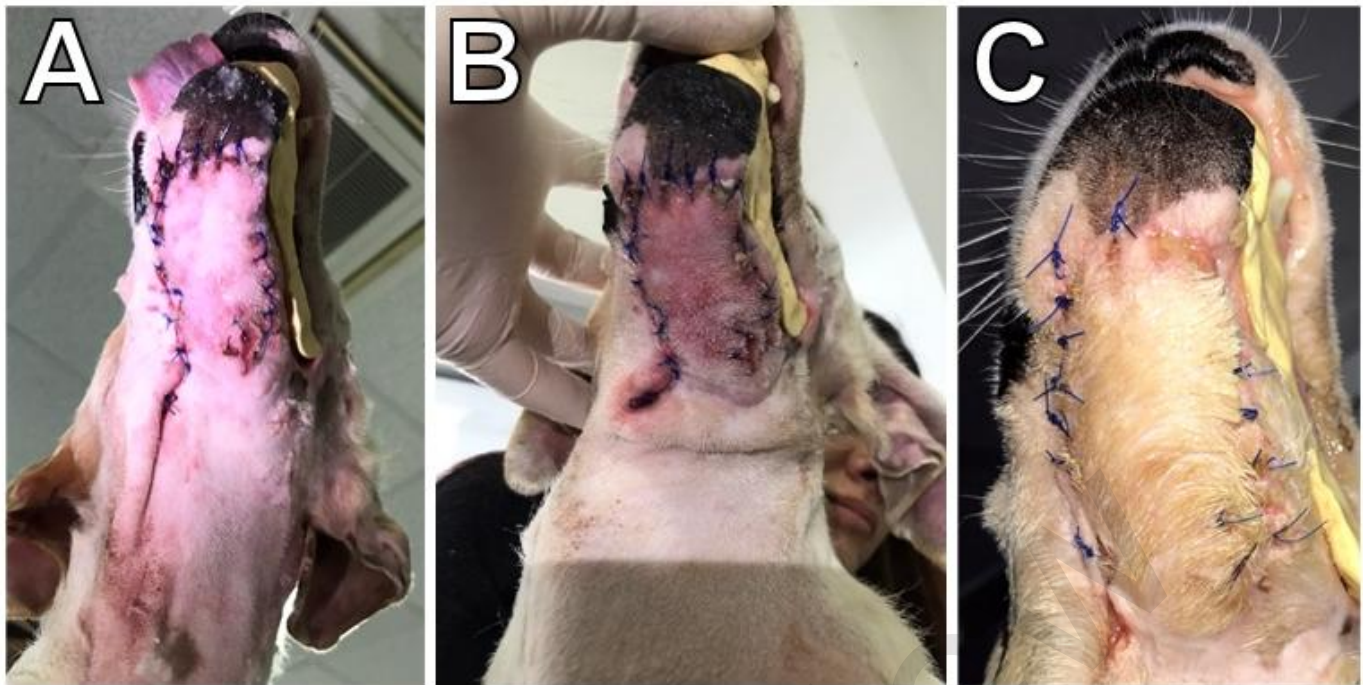


Figure 7: (A) 2nd postoperative day, (B) 3th day, (C) 8th postoperative day PRP application

Discussion

In recent years, alternative applications in veterinary reconstructive surgery, especially for wound healing, have developed and increased. The effectiveness of some of these methods has been demonstrated through experimental studies and has found a place in clinical applications in both human and veterinary medicine (16, 17). One of the most commonly used of these applications is PRP. PRP, which has been shown to contribute to wound healing, especially with the factors it contains, has been reported in different tissues and different application methods (1, 11, 18, 19, 20). In this case, we aimed to report the effects of PRP injection into the flap area after the flap operation we performed on the lip area where there is extensive tissue loss.

Many methods have been described that can be used in reconstructive surgery in the lip region. In general, these are local rotational flaps and axial flaps. Some of the most common complications after flap applications are distal flap necrosis, edema, infection, bruising and seroma formation (21, 22). In the case, the tissue loss in the lip region included a widespread area starting from the level of the left mandibular frenulum, covering the entire left mandibular ventral skin and 2/3 of the right mandibular ventral skin, the entire left lower lip caudally, the angularis oris up to the cranial aspect of the left ear root, and 1/3 of the upper lip turning dorsally up to the maxillary canine tooth. Due to the width of the scar tissue and the lack of sufficient tissue for local displacement flaps, the caudal auricular axial flap method was deemed appropriate for closure of the left mandibular region. The angularis oris region was treated with secondary healing. The healing of the upper lip region was done

by pulling the remaining 2/3 of the upper lip tissue downwards. It was observed that the upper lip healed without any postoperative problems after the application. No seroma formation was observed due to the application of drains in the neck region and under the chin. However, necrosis occurred at the end of the 4 cm flap in the caudal and ventral directions from the mandibular canine tooth level sutured to the gingiva. It was thought that the necrosis in the flap could have been caused by licking and salivation due to the flap being too long or the mechanical obstacle created by the extracted teeth being removed.

In studies conducted with PRP, it has been stated that when PRP is applied to the flap and graft area, it stimulates angiogenesis, increases local tissue perfusion and reduces edema, and accelerates wound healing by providing organization and re-epithelialization of collagen fibers (11, 23). It has also been reported that PRP increases flap survival by providing increased tissue perfusion in preventing distal flap tip necrosis, especially seen in skin flaps (24, 25, 26). In the case, after the necrotic part of the flap was removed, medical treatment was continued to keep the flap bed ready for the second operation. In the second operation, the exposed part of the caudal auricular flap, which was partially accepted in the first operation, was closed with the the pedicle advancement flap method and a drain was applied. In order to prevent mechanical irritation of the tongue after the flap operation, a bridge was established between the mandibular canine and the 3rd molar teeth using cerclage wire, and a barrier was made using silicone toothpaste. Before waking up from anesthesia, PRP prepared from the patient's blood was injected into the area, and the area was dressed. The stitches were removed on the 10th postoperative day. In the postoperative control, it was observed that the barrier established between the teeth stopped the mechanical effect created by licking, but salivation

continued. At the end of the treatment, no serious complications were observed in the mandibular soft tissue and tongue, although slight redness was observed in the fragile commissure, which was left for secondary healing, due to the bridge. Although salivation continued and macerated the area, it was observed that the flap did not become necrotic and no edema or infection occurred with the PRP application on the first and second postoperative days compared to the previous operation. Again, it was observed that the flap was accepted on the second day and granulation tissue formed in the tissue under the flap on the fifth day, and almost complete healing was achieved in the wound edges.

In conclusion, in the flap techniques used in the treatment of the wound with extensive tissue loss of the lower and upper lips in the case, normal healing processes were not sufficient due to mechanical effects and the effects of saliva. In addition to attempts to minimize these effects, autologous PRP was used to benefit from its effects reported in the literature, and thus accelerated flap acceptance and survival and positively affected healing.

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