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The Therapeutic Effect of Platelet-Rich Fibrin, Curcumin and Ozone Among Oral Wound Healing Process

Oral wound healing process by Platelet-Rich Fibrin, Curcumin and Ozone

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Abstract

Background: It is difficult to treat the wounds in the mouth for whatever reason, especially in the combination of mucosa and hard palate. In our study, we discuss which have been shown to have positive effects on wound healing in the mouth, whether platelet-rich fibrin, ozone therapy and curcumin treatment are superior to each other. **Aim:** We aimed to demonstrate its effectiveness in oral wounds by evaluating epithelial and mucosal cell regeneration and healing histopathologically.

Methods: We used fifty-one Wistar Hannover rats in our study. The study was designed in 5 groups, including 9 rats in each group. 6 rats were used as donors to obtain PRF. In group 1, only palate defect was created as the control, while PRF was applied locally to the 2nd group, Ozone to the 3rd group, Emu to the 4th group, and the combination of Curcumin and Emu to the 5th group. On the 15th day after the application, all palatine specimens were sent for histopathological examination. The results obtained were made using the ANOVA test for intergroup comparison. For statistical evaluation, the Kruskal-Wallis test and then Mann-Whitney U test were used.

Results: When wound healing is examined in total, epithelial thickness, regeneration, granulation, and collagen formation; While Ozone, PRF and Curcumin were also good, they were the least in the control group. While the most prominent vascularization was observed in the PRF and Emu groups, it was observed the least in the Ozone and Curcumin groups. There was no histopathological difference between the groups in terms of sufficient tissue formation.

Conclusion: Repair of intraoral defects is difficult in every sense. In addition to surgery, a lot of topical things have been tried. In the literature, there are studies on intraoral wound healing, especially cleft palate, showing the topical therapeutic effect of Ozone, Curcumin and PRF. However, in addition to surgical interventions, additional studies are needed for such topical applications to have a positive effect on wound healing. There is still a gap in the literature about which of the Ozone, PRF and Curcumin is more suitable as a topical supplement and at which stage of wound healing it is more effective.

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Introductıon

Although various methods have been determined in the treatment of intraoral wounds, the healing of the wounds that are persistent and with tissue loss and especially after tumor surgeries takes a long time, which causes various complications and significantly decreases the patient's quality of life. Wound healing is a process involving many biochemical and cellular mechanisms. In this process, growth factors modulate the growth and differentiation of cells and the wound healing process. Curcumin treatment prevents fluid passage to the extravascular compartment by regulating capillary permeability caused by hypoxia. Venous lymphatic return is increased. Anti-edema effect is used in cases such as crush, compartment syndrome, reperfusion injury, brain edema, burns (1,2). A number of events such as extracellular matrix accumulation in the wound bed required for all steps of wound healing, is dependent on tissue oxygen level. Proline and lysine hydroxylation required to form the tissue is carried out using molecular oxygen (2). In neovascularization, which starts with budding from the intact capillaries at the wound edge, only if connective tissue is formed, it can progress on it and the wound cavity is filled with granulation tissue. Curcumin's therapeutic capability is limited critically by its poor bioavailability. Since curcumin has poor water solubility and poor envelope permeability, its metabolism is fast and it breaks down rapidly before it enters the cell. In our study, an easy model for topical applications was chosen to create a defect in the rat palate (3).

In studies conducted with ozone, it has been observed that there is an increase in the healing of chronic venous ulcers in diabetic patients, it increases epithelization in patients with burns, and accelerates wound healing in the soft palate (4,5,6). We also thought that ozone would accelerate the healing of intraoral wounds, based on its enhancing effect on wound healing. We aimed to analyze the effect of the regional application of ozone on the healing of intraoral wounds. Platelet-rich fibrin (PRF); act a part in every phase of wound healing with cytokines such as PDGF, TGF-Beta, IGF-1, EGF, which it contains intensively. To observe the clinical and histopathological effect of PRF on wound healing using the model (7,8).

In addition, emu oil has low levels of saturated fatty acids that are rich in omega fatty acids. It contains quite a lot of natural antioxidants such as polyphenols, flavones, carotenoids, vitamins D and E, and phospholipids. As known, the basic problem with curcumin administered orally is the high first pass metabolism it has. Therefore, this study was conducted to prove the mucosal permeability property of emu oil, apart from comparing curcumin, PRF and ozone (9,10).

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Materıal And Methods

Animals

Approved by the Bagcilar Education and Training Hospital Animal Ethics and Research Committee (consent number 2019-144), this project was completed in our experimental animal laboratory. Fifty-one male Wistar Hannover rats weighing between 250 and 350 g were used in this study. Rats were kept in a room with limited access where temperature and light were controlled. They were also fed standard rat food and allowed unlimited access to tap water. Rats were placed individually in standard rat cages.

Surgical operation

All animals fasted overnight before surgery. The rats were anesthetized with 1.5-2 MAC isoflurane in a mixture of half oxygen and air. In order to adapt to rat physiology, rats were ventilated with a respiratory rate of 40 /min and 15 cm H2O and their expiratory / inspiratory ratio of 2: 1. After the anesthesia was applied, a circular incision with a diameter of 3 mm was made on the soft palate to create the wound in the mouth (Figure 1), and the tissues up to the periosteum were dissected and removed (Figure 2).

Figure 1: To reveal the hard and soft palate by holding the rat to create a defect in the palate

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Figure 2: Removing the rat's palate by creating a full-thickness defect up to the periosteum

The PRF Preparation

The whole blood of 6 rats in the PRF preparation group was taken intracardially to prepare platelet-rich fibrin (PRF), and the rats were sacrificed with a high-dose anesthetic agent. During sacrification of rats, 6 ml amount of blood samples were collected from each rat, and a total of 36 ml of blood was taken. The collected blood was centrifuged and an average of 3.6 ml PRF was obtained (Figure 3).

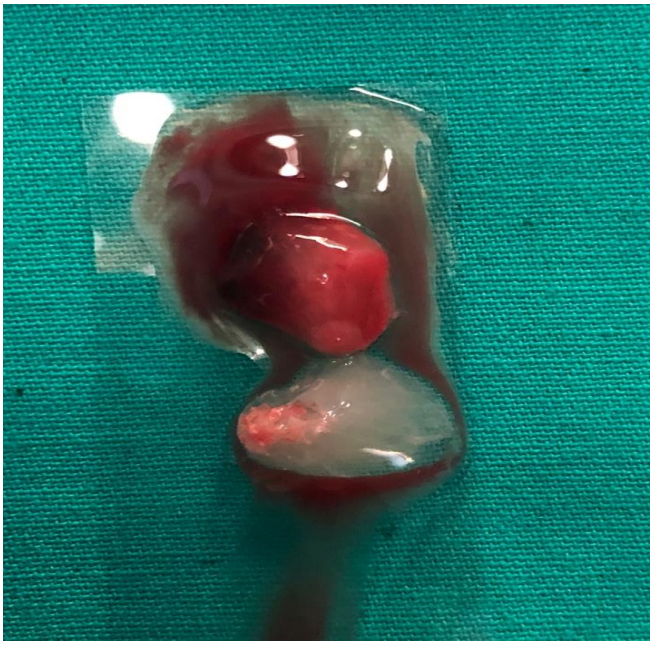

Figure 3: Macroscopic view of the PRF

Experimental Groups

Group 1: The control group (9 rats); A wound was opened on the soft palate of each rat with a skin biopsy device up to 3mm in diameter. No additional intervention was performed.

Group 2: The PRF group (9 rats); The soft palate of each rat was wounded with a skin biopsy device up to 3mm in diameter. Under inhaler isoflurane anesthesia, 0.1 ml thrombocyte rich fibrin (PRF) injection was performed on days 1,3 and 7 over the wounded area.

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Group 3: The Ozone group (9 rats); Under inhaler isoflurane anesthesia, a wound was opened on the soft palate of each rat extended up to 3mm in diameter with a skin biopsy device. Ozone (Ozonsan Photonics 1014, Hans GmbH Nordring & Iffezheim, Germany), gas 2.3-3.0 mL (60µg / mL) was intraperitoneally performed on day 1,3 and 7 with O2-O3 mixture.
Group 4: The Emu oil group (9 rats); Under inhaler isoflurane anesthesia, the soft palate of each rat was wounded with a skin biopsy device up to 3mm in diameter. A single dose of Emu oil (3 ml/kg) was applied on the wounded area of rats, on days 1,3 and 7.
Group 5: The Curcumin-Emu oil group: (9 rats); Under inhaler isoflurane anesthesia, the soft palate of each rat was wounded with a skin biopsy device up to 3mm in diameter. The rats were treated with a single dose of curcumin (1 mg/kg), (Sigma Chemical Co., St. Louis, USA) which was dissolved in Emu oil (3 ml/kg) on days 1,3 and 7 over the wounded area.
On the postoperative 15th day, all rats were killed by intracardiac puncture while under and xylazine (10 mg/kg Ronpum, Bayer AG) and ketamine (50 mg/kg Ketalar, Pfizer Inc.) anesthesia. After sacrification, a sample of the palatine segment was taken for histopathological examination (Figure 4).

Figure 4: The palate removed and sent for histopathological examination

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Histopathological analysis

All tissues were fixed in 20mL 10% formalin for 24 hours and then placed in the cassettes macroscopically. Then, the tissues were dehydrated with alcohol. And then acetone, xylene, and paraffin steps are completed respectively. After the tissues were embedded in paraffin blocks, 4 mm thick sections were taken. Sections were stained with hematoxylin and eosin (H&E). A blind examination was performed by a histopathologist under a light microscope (Olympus BX51). Histopathological changes such as angiogenesis, epidermal and dermal regeneration, granulation tissue thickening, collagen distribution and inflammatory cells were taken into account in the grading. These parameters were evaluated by selecting 2 areas in each sample, categorizing them as low (+), medium (++), high (+++) and very high (++++) in a total of 200 areas.

Statistical analysis

Calculations were performed based on the use of two-sample t-tests. Power calculations were performed using G-Power version 3.1. The results were shown as median (minimum-maximum). Overall comparison between the groups was performed using 1-way analysis of variance (ANOVA). The Kruskal-Wallis test followed by the Mann-Whitney U test was used for statistical evaluation. SPSS 23.0 software (Chicago, IL, USA) was used for the analysis. The level of significance was set to $p<0.05$.

Results

Histopathological comparison of the groups was shown in Table 1. The cellular layers of the skin were distinguished by their morphological features. Increased angiogenesis and vascularization together with epidermal and dermal regeneration and the wound tissue thickness according to formation of granulation tissue were observed. It was seen that re-epithelization increased within edema areas in the upper epidermal and dermal layers where the inflammatory cell infiltrates increased around the vessels, and the cell clusters (Figure 5).

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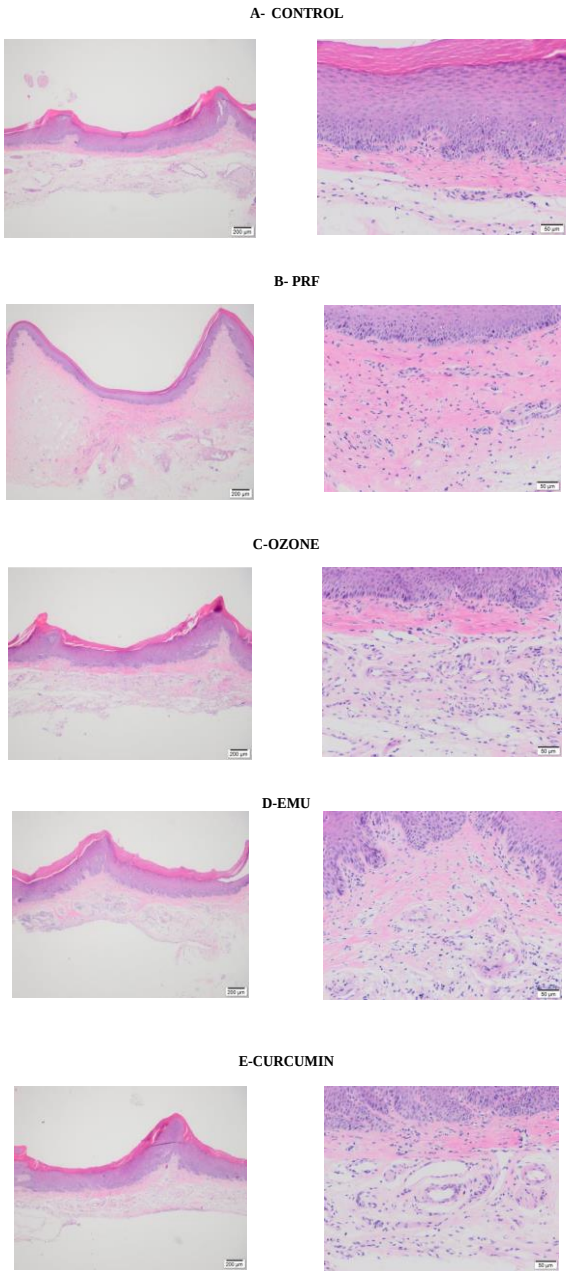

Figure 5: Histological images of wounded area from (A) Control, (B) PRF, (C) Ozone, (D) Emu oil, and (E) Curcumin groups. H&E, X40

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Wound healing process progressed more slowly in the control group compared to the other groups. However, wound healing was more likely improved in Ozone, PRF and Curcumin groups compared to the Emu group. The epithelial thickness, epithelial regeneration, granulation and collagen formation were seen mostly with PRF, Ozone, and Curcumin contrary to the control group ($p < 0.001$). The group of Curcumin showed more likely better epithelial thickness, epithelial regeneration, granulation and collagen formation than the Emu oil group ($p < 0.05$). However, there was clinically not enough tissue formation of the healing process between the groups for histological findings. The vascularization was seen mostly within PRF and Emu application, followed by Ozone and Curcumin compared to the control ($p < 0.001$). The PRF treatment showed more likely vascularization than Emu oil ($p < 0.01$) (Table 1).

Table 1: Statistical comparison of histopathological data

Groups	Epitelial thickness	Epitelial regeneration	Granulation	Vascularization	Collagen
Control	2 (1–2) [a]	2 (1–2) [a]	2 (1–3) [a]	2 (1–3) [a]	2 (1–3) [a]
PRF	3 (1–3)	3 (1–4)	2 (1–4)	3 (1–4) [b]	3 (1–4)
Ozone	3 (1–3)	2 (1–3)	3 (1–3)	3 (1–3)	2 (1–3)
Emu oil	2 (1–4) [c]	2 (1–3) [c]	2 (1–4) [b]	2 (1–4)	3 (1–3) [b]
Curcumin	3 (1–3)	2 (1–4)	3 (1–4) [c]	2 (1–4)	3 (1–4) [c]
Overall p	*p<0.05*	*p<0.05*	*p<0.05*	*p<0.05*	*p<0.05*

All values are given as median(min-max).
[a]$p<0.001$ vs control group.
[b]$p<0.01$ vs curcumin group.
[c]$p<0.05$ vs curcumin group.

Dıscussıon

Oral wounds have been treated in various methods for decades. However, the healing of the wounds that are persistent especially after tumor surgeries, causes various complications and significantly reduces the life quality of the patients. The stages of wound healing beginning following the tissue damage are inflammation, proliferation and maturation. The intra-oral tissue regeneration is altered owing to the temperature, moisture and wide-ranging bacterial vegetation (11).In the current study, we investigated the effectiveness of the healing process for oral wounds following surgery by histopathological evaluation with the therapeutics of platelet-rich fibrin, ozone and curcumin.Therefore, PRF has growth factors which was released by α-granular especially good for tissue healing process such as vascular endothelial growth factor (VEGF),

platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF) (12-15).

We aimed to initiate the tissue healing process by applying high concentrate PRF as a growth factor inside the wounds of the PRF group. In some studies, it was reported that cellular healing with different cells has been stimulated by the tissue regeneration of PRF as an initiator which is consistent with our study findings (16). Consistently, PRF is sufficient for typical wound renewal, too (11,17). Especially, the PRF treatment showed more likely vascularization which is consistent with the literature regarding the VEGF activation indeed.

There are a lot of dilemmas and unmet needs to identify the therapeutical mechanism of ozone in tissues which demonstrates a flow of effects, commanding to the combination of various cytokines and growth factors that increases the perfusion and oxygenation, regulation of the immune reaction, and antioxidants enzymes, which triggers the faster and effective wound healing process (18,19).

Though, the amount of fibroblasts was produced more likely by the ozone therapy studies which showed the consequence of ozone following the migration and activation of fibroblasts, as an example of collagen I and TGF-β genes, and proliferation of growth factors as PDGF and TGF-β regarding fibroblasts proliferation, and syntheses of collagen extracellular matrix (18-23). Consistently, our findings demonstrated that Ozone has a positive impact on tissue regeneration following surgery regarding the literature such as treatment by ozone for the ulcerous lesions of intra-oral mucosa demonstrating a healing progression. Following the post-operative results, we reported that Ozone had the potential as an essential initiator for re-epithelization and collagen formation like PRF and Curcumin contrary to the control group ($p<0.05$).

Curcumin has widespread therapeutic approaches in the medical field which exhibits antioxidant functions through free radical-scavenging activity, anti-inflammatory, hepatoprotective, anti-microbial, anti-mutagenic, anti-angiogenic, apoptotic and anti-platelet aggregation features (22). Hence, it is implicated in many therapies to improve wound healing processes. Consistently, in the current study, curcumin showed more likely better epithelial thickness, epithelial regeneration, granulation and collagen formation in the oral wound healing process. In terms of its effect on antimicrobial activity, we can say that curcumin is effective in reducing the total microbial and bacterial population (24,25). Curcumin can cause permanent damage to bacteria by triggering the permeability of the bacteria's cell membrane. It has also been reported that curcumin exerts an antibacterial effect in the mouth by inhibiting the growth of mature plaque formed by periodontal pathogenic bacteria indicating its preventive effect on periodontitis (24-26).

Following the literature as our strength, demonstrating that topical therapeutic effects of PRF, Ozone and curcumin partially improved the wound healing process in combined vascularization, epithelization, collagen formation of rats who underwent intraoral surgery. Regarding the limitations of our study that there is not enough immunohistochemical analysis for the

inflammatory changes and the preferences to place a worth to the alterations of standard principles in detail. In addition, additional donor animals were used instead of autologous tissues, since the amount of blood required to obtain PRF reached levels incompatible with the life of the rat. Indeed, different surgical approaches could be another phase of our research to set an assessment to compete these therapies.

Conclusıon

Subsequently, all therapeutic approaches showed more effective tissue regeneration in the wound healing process, and especially PRF improved the vascularization as a potential initiator. This study also labels the further need for research into the regenerative potential of PRF, enduring the merit of a clinical trial designed to weigh the competence of PRF and curcumin in oral wound healing.

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