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**USING OF PLATELET-RICH PLASMA (PRP) IN HEALING OF EXPERIMENTAL
ORAL SOFT TISSUE INJURY IN RABBIT**

ERFANPARAST L¹, TAGHIZADIEH M^{2*} AND AFSHARI F³

1: Department of Pediatric Dentistry, Faculty of Dentistry, Tabriz University of Medical Sciences,
Tabriz, Iran

2: Department of Pathology, Tabriz Medical Sciences branch, Islamic Azad University, Tabriz, Iran

3: Department of Histology, Tabriz Medical Sciences branch, Islamic Azad University, Tabriz, Iran

***Corresponding Author:** mohammadtaghizadieh@yahoo.com; **Tell:** +989143165783

ABSTRACT

Wound healing is a complex and dynamic process. Once a wound begins healing, normally the process resolves with complete wound closure. However, healing of acute and chronic wounds can become impaired by patient factors (ie, comorbidities) and/or wound factors (ie, infection). In present study, we aimed to use third type of study to wit surgical wounds in experimental oral soft tissue injuries in rabbit. In this study, 14 New Zealand female rabbits weighing 1.5 kg were selected. All animals are 3 months old. Animals were allocated into 2 groups by chance. In both groups, for making wound in the rabbits oral soft tissue at the same size, we used sterile biopsy set and biopsy punch with 6 mm in diameter. In group 2, we needed to use PRP so a thin layer of oral soft tissue was incised very smoothly and after application of PRP (at a dose of 0.1ml), the layer was sutured. It must be noted that, in group 1, the same suturing method was used without any application of PRP. Samples from right side of oral soft tissue were approached and were fixed in the formalin (10%) and sent to pathology laboratory. Histopathology studies revealed that healing in the treatment group was obvious than group 1 which indicates PRP activity in healing process.

Keywords: Wound, PRP, Healing, Oral Soft Tissue, Rabbit

INTRODUCTION

Wound healing is a complex and dynamic process [1]. Once a wound begins healing, normally the process resolves with complete wound closure. However, healing of acute and chronic wounds can become impaired by patient factors (ie, comorbidities) and/or wound factors (ie, infection) [2]. Restarting a wound with impaired healing is difficult because good standard wound care does not always provide an improved healing outcome and often more advanced therapies are employed [3, 4].

Platelet-Rich Plasma (PRP) Therapy is a particular lyhot topic, nowadays—in the laboratory and the clinics. It refers to PRP Therapy as a means of delivering a “growth-factor cocktail” to several injuries.

Platelet rich plasma (PRP) gel is considered to be advanced wound therapy for chronic and acute wounds. For more than 20 years, PRP gel has been used to stimulate wound healing. Autologous PRP gel consists of cytokines, growth factors, chemokines, and a fibrin scaffold derived from a patient's blood [5, 6]. The mechanism of action for PRP gel is thought to be the molecular and cellular induction of normal wound healing responses similar to that seen with platelet activation [6].

[7] went a step further and measured the degree of concentration of platelets and growth factors in PRP. They reported platelets as being concentrated up to 8-fold. Various growth factors, including PDGF, TGFbeta, and vascular endothelial growth factor (VEGF), were found to be concentrated from 3- to 6-fold.

[8] report on the delivery of PRP-impregnated, biodegradable, gelatin hydrogel microspheres to a rabbit model of intervertebral disc degeneration. The experimental PRP group showed significant healing of the disc degenerative process.

[9] describe the delivery of thrombin activated PRP to a rat model of myocardial infarction (i.e., coronary heart attack). The thrombin-PRP injection resulted in the improvement of several parameters that demonstrated enhanced myocardial remodeling and accelerated myocardial healing.

[10] present an “in-vitro” (i.e., laboratory counter-top) model in which porcine chondrocytes (mature pig cartilage cells) were grown in culture media and PRP was introduced into the culture media. While, the cells remained structurally and molecularly unchanged, including their proteoglycan (e.g., glucosamine) and collagen molecular types,

cell proliferation and glucosamine-collagen synthesis were enhanced. [11] investigated PRP's ability to enhance bone repair in a rabbit model. The PRP was incorporated into a gelatin hydrogel. This PRP delivery system was applied topically to rabbit ulna bone defects. They observed that growth factors, such as PDGF and TGFbeta, were released directly from the PRP and more slowly released from the hydrogel as it degraded. Successful bone regeneration and bone defect healing resulted.

[12] present an application of PRP by injection of autologous platelet-leukocyte-rich gel to delayed-union and nonunion fracture patients—a supposed to employing standard orthopedic surgical open grafting procedures. All delayed union cases demonstrated successful union after an average of 9.3 weeks. Then on union group demonstrated 13/20 successful unions after an average of 10.3 weeks.

In present study we aimed to use of platelet-rich plasma (PRP) in healing of experimental oral soft tissue injury in rabbit.

MATERIALS AND METHODS

Study Design

In this study, 14 New Zealand female rabbits weighing 1.5 kg were selected. All animals are 3 months old. All rabbits were developed under laboratory conditions to prevent

common infections, drug interactions, etc. These rabbits were numbered by chance with a waterproof marker in the inner layer of ear and were allocated into 2 groups by chance. Group 1: were made oral soft tissue injury without any treatment. Group 2: were made oral soft tissue injury and received PRP. Conditions, such as temperature, humanity, bed, light and ventilation were provided, same for all animals. Storage temperature was 25°C.

Preparation of PRP

Four milliliter blood was collected from the jugular vein of each rabbit into a sodium citrate tube. The tubes were centrifuged at 1240 rpm for eight minutes. The tubes showed three different density compartments, the lower red blood cells, the middle buffy coat of white blood cells, and the top plasma. The plasma had three distinct layers in ratio of 2:1:1 from the top. The first top layer was platelet poor plasma (PPP), the middle plasma average platelet (PAP) and the lower platelet rich plasma (PRP). The first (PPP) and the second (PAP) were removed by pipette. The third (PRP) layer was carefully separated by pipette and centrifuged again for 5 minutes at the same speed.

Pre-operation Measures

The operation (induction wound in the gingival) required general anesthesia,

analgesia and muscle relaxation. In term, we used ketamine (10%, 35 mg/kg) and Xylazine (2%, 5 mg/kg) for the induction of anesthesia and pre-operation drugs, respectively.

Operation Measures

In both groups, for making wound in the rabbits gingival at the same size, we used sterile biopsy set and biopsy punch with 6 mm in diameter. In group 2, we needed to use PRP so a thin layer of oral soft tissue was incised very smoothly and after application of PRP (at a dose of 0.1ml), the layer was sutured. It must be noted that, in group 1, the same suturing method was used without any application of PRP. Samples from right side of oral soft tissue were approached and were fixed in the formalin (10%) and sent to pathology laboratory.

Pathologic Evaluation

At the 12th day after the surgery and application of PRP, rabbits from each group were euthanized using sodium thiopental overdose. The oral soft tissue tissues were fixed into 10% buffered formalin. In laboratory, slides were provided and were

stained by Hematoxylin and Eosin method (H and E).

RESULTS

In clinical view, healing differences from day 5 were seen among control and treatment group. These differences were significantly increased such that on day 13 (final sampling day), significant difference was seen between the control and treatment group; although the treatment group had good healing than group 1.

Histopathological Findings

Histopathology studies revealed that healing in the treatment group was obvious than group 1. This finding is obviously approved by looking at the epithelial layer. In treatment group, healing occurred in a higher extent than in the group 1 as it detected by checking the epithelial. In treatment group, healing process was very rapid and inexistence of inflammatory cells and hyperemia in healing area was more obvious than group 1. This indicates the rapid healing process and cessation of inflammation (**Figures 1-4**).

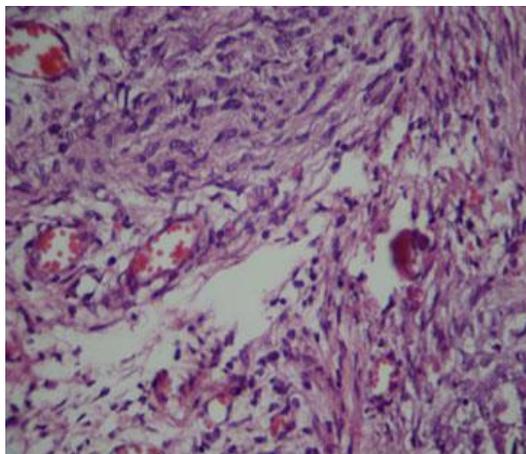


Figure 1: Microscopic view of healing process from one rabbit of the group1. Newly formed epithelial tissue did not cover completely epithelial gap and incomplete areas were covered by growth and development of lining tissue from adjacent areas. Existence of fibrin discharges in the superficial lining tissue indicates incomplete healing. Hyperemia, sever hemorrhage and inflammation under the new formed lining tissue is obvious. Newly formed epithelial cells are not organized and regular. (H and E, 40×)

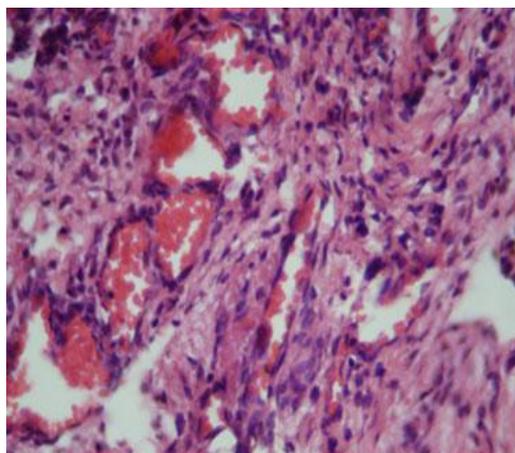


Figure 2: Microscopic view of healing process from one rabbit of the group1. Hyperemia and hemorrhage in healing tissues are obvious. (H and E, 100×).

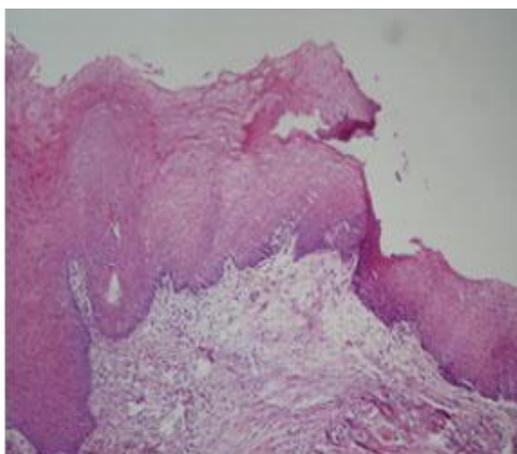


Figure 3: Microscopic view of healing process from one rabbit of treatment group. Epithelial gap because of regeneration of lining tissue has disappeared. Newly formed epithelial is disorganized and irregular, and thicker than the adjacent epithelial tissue. Epithelial cells have degenerative changes as cloddy swelling. New formed granulation tissue is immature and has low density of fibrous strands. Edematous changes in this tissue are obvious and new formed vessels indicate active angiogenesis. (H and E, 40×).

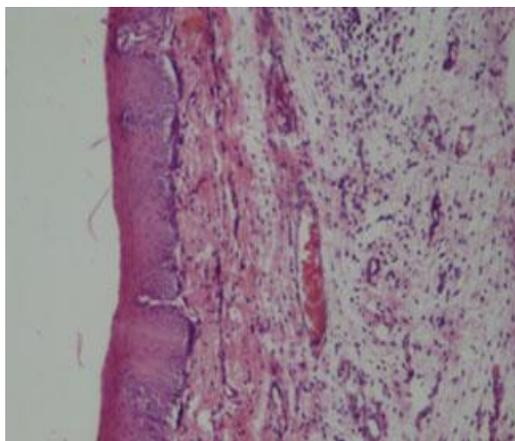


Figure 4: Microscopic view of healing process from one rabbit of the treatment group. Numerous new formed vessels with fibroblasts in the location indicate an active healing. Inflammatory cells are existing in the fibrous filaments. Foreign body type of giant cell is obvious that indicates tendency of healing tissue to making epulis. (H and E, 100×).

DISCUSSION AND CONCLUSION

When a wound occurs and is exposed to external environment, it is more prone to attack by microbes, which invade through the skin and delay the natural wound healing process. Reactive Oxygen Species (ROS), are vital part of healing and serve as cellular messengers that drive numerous aspects of molecular and cell biology.

ROS can trigger the various beneficial pathways of wound healing, for example, at micromolar concentrations of hydrogen peroxide can promote Vascular Endothelial Growth Factor (VEGF) expression in keratinocytes [13, 14, 15]. During the inflammation phase of healing neutrophils and macrophages are attracted into the injured tissue by various chemotactic factors. They locate, identify, phagocytize, kill and digest microorganisms and eliminate wound debris through their characteristic “respiratory burst”

activity and phagocytosis [16]. At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation, which further impede the healing process by causing damage to cellular membranes, DNA, proteins and lipids as well [17]. Hence, if a compound or a plant extract having antioxidant potentials and antimicrobial activity additionally, it can be a good therapeutic agent for accelerating the wound-healing process. Our results showed that PRP-treated rabbits represent rapid healing and improvements in oral soft tissue which can be concluded that it is because of several factors exist in PRP.

[18] mentioned that PRP contains various growth factors that play important roles in cell proliferation, chemotaxis, cell differentiation, and angiogenesis concentrations. The basic cytokines identified in PRP include platelet-derived growth factor, transforming growth

factor β , vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, epidermal growth factor, and endothelial cell growth factor.

[19] said that platelets and thrombin activated platelet products induced mitogenic activity of cultured human trabecular bone-derived cells and that platelet concentrates also enhance the proliferation of human osteo blast like cells.

[20] reported that thrombin activation would yield inflammatory cells that were not seen in the non-activated group.

[21] reported that the platelet-activating factor (PAF) produces several biochemical responses associated with inflammation and wound healing.

[22] mentioned that it also activates mitogen-activated protein kinases and stimulates both calcium influx into cells and the expression of the cyclooxygenase (COX-2) enzyme, an inducible isoform responsible for synthesis of various prostaglandins associated with inflammation.

In a research by [23, 24, 25] the PAF activates the gene expression of selective metallo proteinases (MMPs) involved in tissue remodeling, such as MMP-1 and MMP-9, urokinase plasminogen activator (uPA), and their inhibitors.

[26] reported that the Platelet rich plasma (PRP) is an autologous blood-derived product that has an increased concentration of platelets that are rich in growth factors, and has the potential to enhance the healing of tissue at the cellular level via the recruitment, proliferation, and differentiation of cells involved in tissue regeneration.

[27] said the platelet-rich plasma (PRP) acts as a rich source of autologous growth factors.

Thus [28] mentioned that, PRP is a very good clinical source for osteochondral regeneration.

[29] proved that PRP was effective in normal tissue regeneration. But there is no clue that platelet rich plasma would normalize the diabetic wound healing pathway.

In a study [30] observed an extracellular matrix-regulating effect of PRP. By considering above mentioned literatures it becomes that PRP is rich from growth factors which accelerate healing process.

[31] carried out a review study on the effect of PRP in oral cavity wound healing and concluded that PRP to be effective at improving surgical results in a variety of procedures in the field of oral and maxillofacial surgery. There was no experimental study regarding PRP use in oral soft tissue so our study is unique and in this study we showed the beneficial effects of PRP in the oral soft tissue in laboratory scale.

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