

Histopathology Study of the Platelet Rich Plasma on the Wound Healing in Rabbits

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Abstract

The study aimed to evaluate the beneficial effect of platelets rich plasma (PRP) on wound heal in rabbit. Eighteen healthy male adult rabbit were used they were randomly divided into two equal groups (control group 9, treated group 9). Under aseptic technique 3 cm skin incision was made in the back (dorsal) of animal by scalpel, after that the incision was sutured immediately by silk suture materials 0.2 USP used simple interrupted technique treated group receive immediately post operation PRP (1ml) was injected at the site of the wound, while control group left without treatment. Skin healing process follow up 1,2 and 3 weeks post operation by histopathological examination, the result display in treated group on the first week post operation development of granulation tissue with epithelial regeneration. 2 weeks post operation show intense inflammatory infiltration with desquamation tissue, granulation tissue and new blood vessels formation. 3 weeks post operation showed proliferation of fibroblast with deposition of collagen, while in control group histopathological examination in the first week show intense inflammatory infiltrate with desquamated tissue and hemorrhage, 2 weeks post operation show area of intensive acute inflammatory infiltrate, 3 weeks post operation show area of chronic inflammatory infiltrate with tissue debris and giant cells, the healing of treated group better than control group.

Keywords: Platelet Rich Plasma, Wound Healing, Rabbits.

Introduction

Skin is that the largest and most visible organ of the body, comprising up to 15-20% of the whole weight. It receives around one third of the body's blood offer at a rate of three hundred mls/minute. Traditional skin consists of 2 layers: stratum and stratum. Underneath the stratum lies the connective tissue (or hypodermis), a layer of loose animal tissue. The skin has six major functions. They're protection, thermoregulation, elimination of waste product, synthesis of D, sensation and communication⁽¹⁾.

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Wound may be a consequence of wound fatal if not treated timely and square measure aggravated by secondary microorganism infection. Improper development of animal tissue tissues, angioblasts and fibroblasts additionally the facts of wound complications⁽²⁾. There square measure several factors which will have an effect on wound healing that interfere with one or a lot of phases during this method, therefore inflicting improper or impaired tissue repair. Wounds that exhibit impaired healing, as well as delayed acute wounds and chronic wounds, usually have didn't progress through the conventional stages of healing. Such wounds often enter a state of pathologic inflammation because of a deferred, incomplete, or uncoordinated healing method. Most chronic wounds square measure ulcers that square measure related to ischaemia, diabetes, blood vessel stasis illness, or pressure.

Sometimes the healing of wounds square measure

delayed and therefore the success of the wound healing another extra taxes to the homeowners. Numerous biomaterials, proteins, antibiotics, vitamins and minerals come back to the scene because the angel to accelerate the repair of the wound by stimulating growing, fibroblastosis and epithelialization of wound^(2,3,4,5). Platelets are incontestible to be the natural supply of many growth factors and cytokines that promote blood clotting, tissue repair, and therefore the method of bone mineralization^(6,7,8,9). Platelet-rich plasma (PRP) is that the second price effective supply of protein that effectively hemostasizes and stimulates cellular regeneration^(11,12,13). Application of PRP may be a new approach for tissue regeneration used as gel formulation containing completely different bioactive substances^(10,14). PRP gel exhibits fast animal tissue differentiation and enhance organization of dermal scleroprotein in contemporary wound⁽¹⁵⁾. Degranulation of platelets causes unleash of remodeling growth factor- β 1 (TGF- β 1), platelet-derived protein (PDGF), fibrinogen, epidermic protein (EGF), histamine, and hydrolytic enzymes^(16,17). These square measure concerned within the angiogenic cascade that assists in arduous and soft tissue wound healing^(7,9).

Materials and Method

Experimental Animals: A total of 18 apparently healthy adult local breed male rabbits were recruited for this study. All animals were evaluated clinically by physical examination before initiation of the experiments. The animals were housed in metal cages 30/70/60 cm in an air- conditioned room in the animal house along the period of the experiments. They were received free accesses to water and food. The animals were left two weeks for adaption with experimental condition with using of prophylactic drug, the animals were divided into two equal groups (control and treatment groups).. Control group left without treatment, Specimens from the injured skin were taken three animals per group at 1, 2 and 3 weeks postoperative for histopathological examination to evaluate the progress of wound healing process.

Surgical Procedure: Surgical operations were made under general anesthesia by a mixture of xylazine ketamine given by IM injection (50mg/kg. B.W. ketamine, and 10mg/kg. B.W. xylazine)⁽¹¹⁾. The operation site was prepared aseptically. Skin incision (3cm) on the back (dorsal) was made by use of scalpel, then the incision sutured immediately using silk suture material 0.2 USP by using simple interrupted technique.

Treated group receive immediately post operation PRP (1ml) was injected at the site of the wound, while control group left without treatment.

PRP Preparation: 3ml of Blood were collected from each rabbits using a 3ml disposable syringe. The samples were transferred into anticoagulant tubes containing 0.35ml of 10% sodium citrate. The blood was initially centrifuged at 160 rpm, for ten minutes at room temperature. After the first centrifugation, two layers were observed in each sample. A red lower layer that consists of packed red blood cells and an upper straw-yellow layer that contains plasma component. The upper surface of packed red blood cells called Buffy coat is rich in platelets and leukocytes. Plasma and buffy coat were transferred to new sterile tubes. The retained component of blood samples was centrifuged again at 160 rpm for two minutes to obtain more concentrated platelets. Then, the plasma and Buffy coat was centrifuged for the second round at 400 rpm, for 15 minutes. Two layers eventually appeared: the upper two thirds of the sample was designated as platelet poor plasma (PPP) and was discarded, on the other hand, the lower third was PRP (Fig5). Moreover, the platelets were activated by 0.05 ml of 10% calcium chloride solution to each 1 ml of PRP (Maghsoudi *et al.*,2015).



Figure (1): PRP

Results

- Control group:** Shows section of skin of control positive animals reveals the site of surgical incision after one week containing intense inflammatory infiltrate (INF), with desquamated tissue (DE) and

hemorrhage (H). Skin of control positive group after two weeks shows area of intensive acute inflammatory infiltration (INF) at the site of skin wound. Skin of control positive group after three weeks shows area of intensive chronic inflammation (CIN) at the site of skin wound with tissue debris (TD). And in another section of Skin of control positive group after three weeks shows area of intensive chronic inflammation (CIN) at the site of skin wound with giant cells (GS).

- 2. PRP treated group:** Section of Skin of PRP treated group after one week shows development of granulation tissue at the site of skin wound figure

(1) and another section of Skin of PRP treated group after one week shows development of granulation tissue (GT) at the site of skin wound with epithelial regeneration (ER) figure (2). the section of skin of PRP treated animals reveals the site of surgical incision after 2 week containing intense inflammatory infiltrate (INF), with desquamated tissue (DE) and granulation tissue (Gr) new generated blood vessels figure (3). Skin of PRP treated group after three weeks shows proliferation of fibroblast (FB) with collagen deposition (CD) at the site of skin wound figure (4) and another slide shows proliferation of fibroblast (FB) with collagen deposition (CD) at the site of skin wound figure (5)

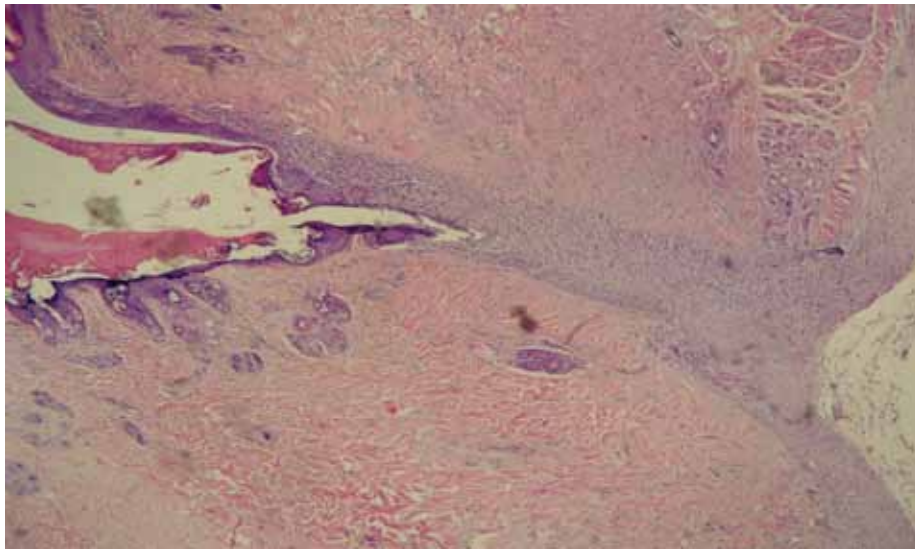


Figure (1) Skin of PRP treated group after one weak shows development of granulation tissue at the site of skin wound (GT) H & E 50X

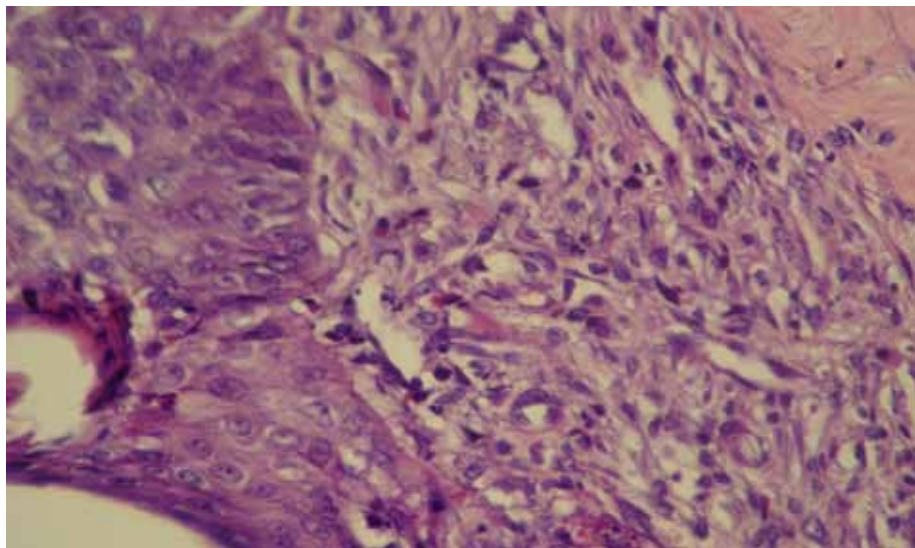


Figure (2) Skin of PRP treated group after one weak shows development of granulation tissue (GT) at the site of skin wound with epithelial regeneration (ER) H & E 500X

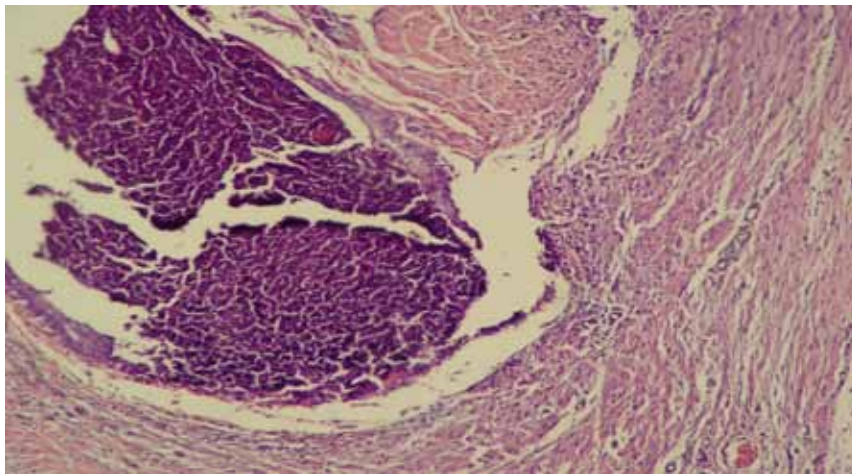


Figure (3): Section of skin of PRP treated animals reveals the site of surgical incision after 2 week containing intense inflammatory infiltrate (INF), with desquamated tissue (DE) and granulation tissue (Gr) new generated blood vessels A) 50 H & E

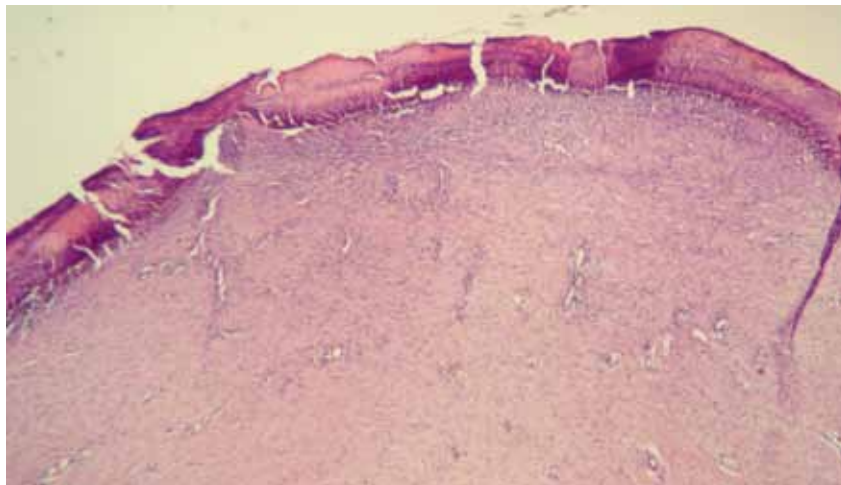


Figure (4) Skin of PRP treated group after three weeks shows proliferation of fibroblast (FB) with collagen deposition (CD) at the site of skin wound H & E 50X

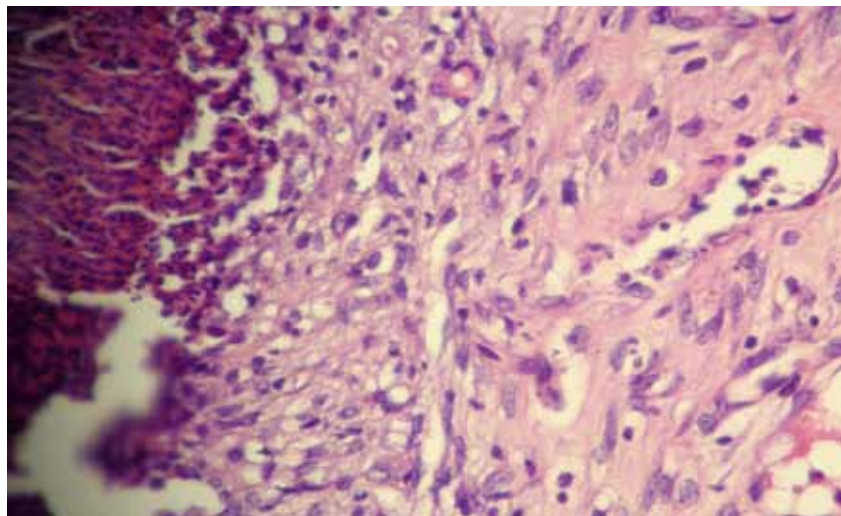


Figure (5) Skin of PRP treated group after three weeks shows proliferation of fibroblast (FB) with collagen deposition (CD) at the site of skin wound H & E 500X

Discussion

The wound healing growth factors which are activated by platelets is greater to continue this process more quickly and smoothly. When these factors are at the wound site, recruitment and differentiation of cells in tissue repair increase and so tissue repair occurs more quickly and well⁽²⁰⁾. is a process which consists of hemostasis, inflammation, proliferation, maturation and remodeling. The effect of growth factors which are activated by platelets is greater to continue this process more quickly and smoothly. When these factors are at the wound site, recruitment and differentiation of cells in tissue repair increase and so tissue repair occurs more quickly and well (20). (Platelet-rich plasma is defined as a platelet concentration of at least 10,00,000 platelets/ μ L in 5 ml of plasma. It contains a 3-5 fold increase in the concentration of growth factors. 10 Proteomic studies have shown that platelets contain over 800 proteins with numerous post-translational modifications, resulting in over 1,500 proteinbased bioactive factors⁽²¹⁾. In our study the treated group at 7 days post operation showed development of granulation tissue at site of incision with epithelial regeneration this agreement with⁽²²⁾ who explained that wounds treated with PRP gel has more rapid epithelial differentiation and enhanced organization of dermal collagen compared to control groups in horses. in present study in treated group 2 weeks post operation showed intense inflammatory cells and granulations tissue this agreement with⁽²³⁾. In a study on dogs performed. it revealed of PRP which was injected into the wound site was investigated on wound healing and then it was reported to have positive effects on granulation formation, collagen deposition, and re-epithelialization. The recent study achieved to show the effect of PRP on the healing of acute wounds in rabbits displayed that the PRP treated group enhanced angiogenesis at the wound beds as compared to control group⁽²⁴⁾ and this has agreement with the present study in an experimental study on rabbits conducted by Ostvar et al.⁽²⁴⁾, PRP was topically applied to the lesions created on the backs of animals and then it was reported that re-epithelialization, angiogenesis, and collagen deposition were statistically significantly increased compared to the control group, this agreement with the our study. It has been reported in different studies that PRP accelerated the inflammatory process⁽²⁵⁾. This agreement with our study the treated group at 2weeks post operation showed high inflammatory process by intense inflammatory infiltrate at site of injury. While⁽²⁶⁾ Application of PRP in cutaneous regeneration and wound healing in dogs,

who revealed that there were no significant differences between median of epithelialization, inflammatory cell infiltration, presence of dermal granulation tissue, fibroblast arrangement, fibroblast proliferation, collagen deposition in the both of treated and non-treated wounds and this disagreed with our study because we found wide differentiation between treated and control group in treated group appear better wound healing by high inflammatory cells, collagen deposition, angiogenesis, a granulation tissue, re-epithelialization and high proliferation of fibroblast.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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