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ENHANCED SKIN WOUND HEALING BY A PLASMA RICH IN GROWTH FACTORS OF RABBITS: HISTOPATHOLOGICAL EVALUATION

Sena a Malik Hussein

Department of Animal Production, College of Agriculture, University of Sumer, 64005, Rifai DhiQar, Iraq.

Abstract

The objective of the study was to evaluate the benefits of applying self-plasma rich growth factors (PRGF) in induced lesions in the tongue of rabbits and to study its effects in epithelial and wound inflammation at 7 and 28 days after application. This study was carried out on 20 adult rabbits. Each rabbit had two longitudinal incisions on the midline of the dorsal surface of the nest in each animal, one control, and the other the PRGF. A distance of about 1.5 cm was left between one incision and another. The defects were filled with platelet plasma rich in growth factors, and the last incision served as control and biopsy were taken. Histopathological analyses were performed to evaluate the effect of these materials on acceleration of wound healing. Wound healing was evaluated and compared in the control and treatment groups for four weeks where the tissue was taken in days (7, 28) of the experiment. The histological evaluation of PRGF-treated lesions showed that wound healing was much more ($P<0.05$) than control lesions during the four-week study. The tissue results revealed that PRP-treated wounds enhanced cellular, increased blood vessels, with increased amount of granular tissue accompanied by an increase in the number of skin accessories suggesting improved skin regeneration rather than untreated wounds. Based on clinical findings and pathological anatomy, this study confirms that topical implantation of PRGF accelerates and improves wound healing.

Key words : Platelet-rich growth factors, Soft tissue wound healing, tongue, Experimental animals.

Introduction

Healing is a process that restores the internal and/or external physical integrity of body structures and involves complex interactions between cells and several other factors. It is a dynamic and complex process, consisting of three phases: tissue inflammation, proliferation and remodeling (Sengupta *et al.,* 2015). The healing process comprises the extracellular matrix, cytokines, blood cells, and growth factors. Growth factors are proteins that stimulate and activate cell proliferation through activation of angiogenesis, myelogenesis, and gene transcription, among other reactions, which activate and accelerate the healing process (Sengupta *et al.,* 2015; Hom *et al.,* 2007). Among the growth factors, the most important ones for wound healing include: epithelial growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF-b), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and insulin growth factor (IGF); the latter stimulates cell proliferation, tissue remodeling, and collagen and elastin increase. VEGF acts on angiogenesis and tissue granulation at the early stage of healing. PDGF is crucial for inflammation, granulation, re-epithelialization, and remodeling in the three stages of wound healing (Xie *et al.,* 2013, Kutlu *et al.,* 2013). Due to the pathological and physiological complexity of the healing process, the perfect regeneration of tissues is difficult to achieve (Kutlu *et al.,* 2013); Cervelli *et al.,* 2009). Therefore, the assessment of new treatments is needed, as well as the use of new strategies. The use of growth factors and their combinations have been suggested as promising treatments, because they accelerate the healing process. However, a major obstacle is that the growth factors are degraded by proteinases or removed by exudates before they reach the wound bed (Cervelli *et al.,* 2009). A great number of growth factors and cytokines are present at the wound site. Their dynamic expression manifests temporal and spatial characteristics in the regulation and changes in the pattern of expression of growth factors that are associated with impaired wound healing. Important alterations in the levels of one factor eventually affect the production of other growth factors and cytokines.

Author for correspondence : E-mail : moh07709879860@gmail.com

Thus, it has been shown that pro-inflammatory cytokines and growth factors are released in serum during the early phase of wound healing, and act as potent stimulators of the expression of several other growth factors. One example is the regulation of FGF7, a growth factor produced by fibroblasts at the wound site. Another example is the regulation of VEGF, a major regulator of angiogenesis, which is produced by keratinocytes and macrophages at the wound site. It was found that pro-inflammatory cytokines can induce VEGF expression in both cell types. These examples highlight the complex interactions that occur during wound healing. Such interactions should be considered when interpreting the results obtained by the overexpression or elimination of a single growth factor at the wound site Werner and Grose 2003). The Plasma Rich in Growth factors (PRGF) is a blend mixture of autologous proteins concentrated from a determined volume of Platelet Rich Plasma (PRP) (Sengupta *et al.,* 2015; Hom *et al.,* 2007). In the Alpha granules discharged by the platelets there are a few development factors, including: Platelet Derived Grow Factor (PDGF), Transforming Growth Factor β (TGF-β) and the Endothelial Growth Factor (VEGF), which are involved in the injury mending forms (Xie *et al.,* 2013) . In the logical writing the debate encompassing this method is clear about the genuine impacts of the PRGF (Kutlu *et al.,* 2013; Cervelli *et al.,* 2009; Werner and Grose 2003). The primary inconsistencies are presumably identified with the absence of institutionalization of conventions of the distinctive PRP and PRGF arrangements and with the numbness of the genuine impacts of these proteins focuses on the diverse careful methods in which they have been connected (Anitua *et al.,* 2007; Anitua 1999). The target of this investigation was to think about the impacts of the autologous PRGF in the epithelialization and aggravation of the injuries incited in the tongue of New Zealand pale skinned person rabbits.

Materials and Methods

Animals

A sum of 20 grown-up male New Zealand pale skinned person rabbits, weighing 2500–4700 g, with averagely. The animals were kept two by two in individual propylene cages under standard laboratory conditions by the dimensions of 45× 60×90 cm^3. Rabbits were maintained on a 12 h light/dark cycle at 22 ± 1°C and 50 ± 10 % humidity, and fed with standard laboratory diet and water ad libitum.

Surgical procedure

The creatures were anesthetized with a blend of ketamine (60%) and xylazine (40%) managing 1 mL/kg of body weight by intramuscular infusion. To acquire PRGF at least 10 mL of blood by creature are required. The blood was acquired by means of heart aspiration. Quickly after accumulation the blood was put in two sterile extraction tubes with sodium citrate at 3.8% as anticoagulant. At that point, put in an axis (1800 rpm) for eight minutes, along these lines isolating the distinctive periods of the blood. The Plasma Poor in Growth Factors (the most astounding 500 µl of each tube) and the Plasma with Platelet (the accompanying 500 µl of each tube) were disposed of. At long last, we acquired the last 500 µl of PRP that compare to the PRGF and were enacted utilizing calcium chloride at 10%. Two injuries were made on the midline in the center third of the dorsal surface of the tongue in every creature, one as a control and another which PRGF was applied (Fig. 1). The injuries were made

Fig. 1 : Shows, the positions of wounds on the midline in the middle third of the dorsal surface of the tongue regions.

utilizing a 6 mm distance across biopsy punch. At long last, all injuries were sutured with two basic 4/0 polypropylene fastens.

We therefore have a total of 40 wounds (20 control and 20 with PRGF). For the biopsy we used an 8 mm diameter biopsy punch, after the sacrifice of the animals We therefore have a total of 40 wounds (20 control and 20 with PRGF). For the biopsy we used an 8 mm diameter biopsy punch, after the sacrifice of the animals (10 rabbits at 7 days and 10 at 28 días) by CO_2 inhalation.

Histopathologic examination

The samples were promptly presented in a wide-mouthed holder and settled in rich 10% formalin-cradled saline. The examples were at long last inserted in paraffin and were cut into 4 µm segments and recolored with hematoxylin and eosin. All examples were contemplated by the same experienced pathologist.

To quantify the review of epithelialization, the criteria

set up by Eppley *et al.,* (2004) were utilized; review 0: epithelialization at the edge of the injury, review 1: epithelialization covering not as much as half of the injury, review 2: epithelialization covering the greater part of the injury, review 3: epithelialization covering the whole twisted with unpredictable thickness, review 4: epithelialization covering the whole twisted with typical thickness.

The review of irritation was considered utilizing the resolution periods of incendiary procedures depicted by Lindeboom *et al.,* (2007) and connected to the investigation of the injury recuperating in experimentation creatures by different creators (Kimura *et al.,* 2005; Marx 2004); review 1: intense aggravation (pyogenic membrane is framed), review 2: power of diffuse intense irritation (transcendence of granulation tis-sue), review 3: prevalence of interminable aggravation (fibroblasts starting to multiply), review 4: resolution and mending (diminishment or vanishing of perpetual irritation, albeit infrequent round cells may persist).

Table 1: Shows the second intension healing (effect of treatment and days) in epithelialization (%).

		Histopathologic scale to evaluate epithelialization*						
Day	**Groups**	**Total**	**Grade 0**	**Grade 1**	**Grade 2**	**Grade3**	**Grade 4**	***p-value***
7	Wound+PRGF	9	0	0	0	6	2	0.155
	Control	9	0	0	2	7	0	
28	Wound+PRGF	9	0	0	0	0	9	0.030
	Control	9	0	0	1	3	3	

Table 2: Shows the second intension healing (effect of treatment and days) in inflammation (%).

		Histopathologic scale to evaluate epithelialization*						
Day	**Groups**	**Total**	**Grade 0**	**Grade 1**	**Grade 2**	**Grade3**	**Grade 4**	***p-value***
7	Wound+PRGF	9	0	0	1	4	1	0.632
	Control	9	0	1	2	2	1	
28	Wound+PRGF	9	0	0	0	0	9	0.020
	Control	9	0	0	0	3	4	

Statistical analysis

The data were processed using the SPSS.

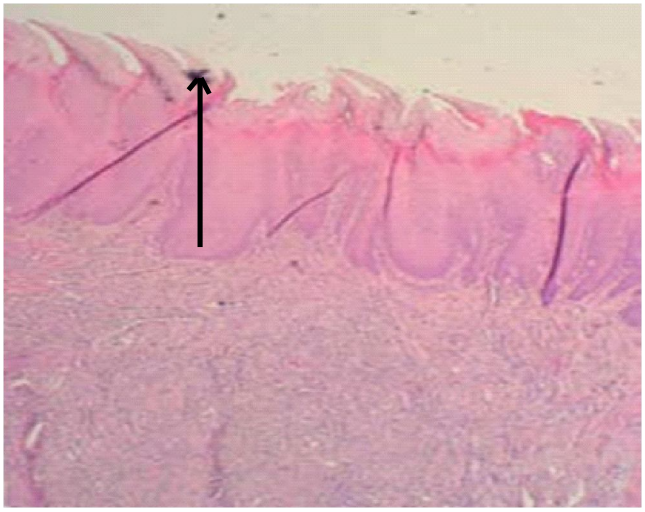

Fig. 2: Histopathologic section of wound related to treatment group, at 28 days post-wounding, show's epithelialization covering the entire wound (white arrow), with normal thickness (H&E; X40).

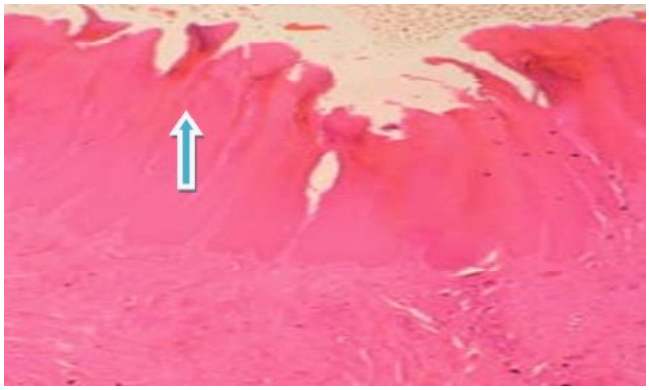

Fig. 3: Histopathologic section of wound related to control group, 28 days post-wounding, show's epithelialization (blue arrow) covering the entire wound with irregular thickness. (H&E; X20).

Results

Seven days after provoking the wounds in the tongue, the majority of the samples presented a grade 3 of epithelialization, irrespective of the application or not of PRGF. Nevertheless, at 28 days, where PRGF had been applied, all the wounds had completed the epithelialization process, with statistically significant differences to the control (p=0.031) (Table 1) (Fig. 2 and 3).

With respect to the resolution of the inflammatory process, at 7 days we found no significant differences between the two groups. Nevertheless, at 28 days, all samples in which PRGF had been applied, demonstrated complete resolution of the inflammatory process, fin- ding statistically significant differences with respect to the controls (p=0.023) (Table 2).

Discussion

Development factors collect in the β granules of platelets and it is by and large acknowledged that they assume a basic part in the injury recuperating. Development factors connected to wounds can quicken recuperating by animating angiogenesis, tissue development and epithelialisation (Sengupta *et al.,* 2015; Hom *et al.,* 2007; Werner and Grose 2003; Martin *et al.,* 1992). PRGF is an autologous item, and therefore keeps away from the danger of transmitting illness. In our investigation, in the oral mucosa the epithelialization and aggravation was not totally settled until 28 days after medical procedure; this might be clarified by the way that the mouth is a wet zone, where the salivation and maceration of the tissue (due partially to rumination) may at first meddle with the mending procedure.

To get PRGF, we have taken after the convention de-scribed by Anitua in 1999 (Hom *et al.,* 2007); in this convention the air conditioner tivator is calcium chloride at 10%, this takes out the danger of immunological responses and the transmission of sicknesses related with the utilization of exogenous cow-like thrombin. Moreover, in this convention the PRGF can be gotten in a solitary centrifuging at 460g (1800 rpm) for eight minutes; conversely with different conventions that utilization twofold axis procedure to acquire PRP and re-quires a higher blood volume (least 50 mL), which is unfeasible in rabbits (National Research Council, 1995).

Taking everything into account, our outcomes propose that the use of PRGF (got by methods for this convention) quickens epithelialization and decreases aggravation at 28 long stretches of inciting wounds in the tongue. Be that as it may, the regenerative impacts of PRGF in delicate tissue are vague, and in this regard we should keep researching.

Conclusions

Wounds treated with PRGF exhibit more rapid epithelial differentiation and enhanced organization of dermal collagen compared to controls in the rabbit.

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