

Enhancement soft tissue healing by using platelets rich plasma in rabbits

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Abstract

Objective: the study was carried out in an attempt to find out the effects of 0.05 ml of autologous of platelet rich plasma on traumatic skin injury.

Materials & methods: sixteen male rabbits were used & divided into two group according to killing after trauma in to group A after 1 day & B. after 4 days each group subdivided in to control & experimental. The skin wound induced traumatically by blade 1cm length & 2mm depth in the skin of rabbit's back, the experimental wound filled with 0.05ml of autologous PRP, while the control wound left for normal healing. The wound was dressed for one day.

Result: platelet rich plasma (PRP) showed marked angiogenesis, granulation tissue formation, histological examination of biopsies revealed difference in the rate of healing process between the control & experimental groups platelet rich plasma (PRP) showed significant enhancement of healing.

Conclusion: platelet rich plasma (PRP) accelerate the soft tissue healing

keywords: platelet rich plasma (PRP), healing, growth factors

الخلاصة

اهداف البحث:- الدراسة الحالية عملت على ايجاد تاثيرات 0.05 مل من البلازما الغنية بالصفائح الدموية الذاتية في جرح الجلد.

الطرق والمواد:- في هذه الدراسة تم استعمال ستة عشر ارنب ذكور قسموا الى قسمين حسب وقت قتلهم، المجموعة الاولى تم قتلهم بعد يوم واحد بعد احداث الجروح فيهم والمجموعة الثانية تم قتلهم بعد اربعة ايام بعد الجروح. كل مجموعة قسمت الى قسمين (قسم تجريبي وقسم للسيطرة). تم احداث الجرح باستعمال سكينه عمليات (شفرة) (1 سم طول و 2 ملم عمق) على ظهر الارنب. الجرح التجريبي ملئ بـ (0.05 مل) من البلازما الغنية بالصفائح الدموية الذاتية، بينما مجموعة السيطرة تركوا للالتئام الذاتي الطبيعي.

النتائج:- البلازما الغنية بالصفائح الدموية بينت زيادة في تكوين الاوعية الدموية الجديدة في نسيج الالتئام. الدراسة النسيجية للعينات اوضحت اختلاف في سرعة عملية الالتئام بين المجموعتين (التجريبية والسيطرة).

الاستنتاجات:- البلازما الغنية بالصفائح الدموية تسرع من التئام الانسجة الرخوة.

كلمات المفاتيح:- البلازما الغنية بالصفائح الدموية، الالتئام، العوامل الوراثية.

Introduction

Wound is defined as any anatomical or functional interruption in the continuity of a tissue that is accompanied by cellular damage and death⁽¹⁾. In regard to healing process there are two types of wounds, it is regarded as *acute wound* when it proceed through an orderly reparative process and result in a sustained restoration of anatomic & functional

integrity, *chronic wound* occurs when some factors causes the disruption of the normal, controlled phases of the wound healing⁽¹⁾.

Most patients with chronic wounds fail to heal in a reasonable period of time⁽²⁾. Researches were report that the traumatic injuries or wounds can be caused by physical or chemical insults as in table (1) below⁽³⁾.

Table 1. physical & chemical insults

physical	Chemical
Incision	Agent with unphysiological ph
Crushing	Agents with unphysiological tonicity
Overheating	Proteases
Overcooling	Vasoconstrictors
Desiccation	thrombogenic
Irradiation	
Compromise blood flow	

The wounds also can be caused by biological insults (e.g. parasite, infection & autoimmunity)⁽⁴⁾.

The wound healing process is a complex series of that begins at the moment of injury & can continue for years⁽⁵⁾. There are several basic principle must be followed to achieve that goal⁽⁶⁾:

1. The obliteration of the dead space.
2. The even distribution of remaining tension along deep suture line.
3. The maintenance of tensile strength across the wound by the suture until tissue tensile strength is adequate.
4. The approximation & eversion of the epithelial portion of the closure.

Wound healing is a tissue remodeling process in which, the injured tissue is removed & replaced by normal tissue⁽⁷⁾. There are four basic responses that can occur following an injury⁽⁸⁾. The response of the body to tissue destroyed by an insult can lead to complete restoration of tissue architecture & function is called regeneration⁽⁹⁾. Or it may lead to restoration of function & of tissue continuity but with distortion of the normal architecture is called repair⁽¹⁰⁾. Normal repair is the response where there is a reestablished equilibrium between scar formation & scar remodeling. In excessive healing there is too much deposition of C.T. that results in altered structure & thus, loss of function, fibrosis & contractures are examples of excessive healing. Deficient healing is the opposite of fibrosis, chronic ulcers are examples of

deficient healing⁽⁸⁾. Shortly time exposure of the fibroblasts cells to hypoxic condition enhanced their proliferation but persistence of this condition may actually impair wound repair & tissue integrity⁽¹¹⁾

There are three categories of wound closure: primary healing, which involve closure of a wound within hours of it's creation, secondary healing, involves no formal wound closure, the wound closes spontaneously by contraction & reepithelialization, & the tertiary wound closure, also known as delayed primary closure, involve initial debridement of wound for an extended period & then formal closure with suturing or by other mechanism⁽¹²⁾. The wound healing process involve a complex interplay of cells, mediators, growth factors & cytokines. The cascade of events begins with clotting, & aggregation of inflammatory cells & then proceed to a highly proliferative state, in which the fibroblasts cells involve in the synthesis of collagen matrix, keratinocytes form the new epithelial layer & angiogenesis for neovascularization⁽¹³⁾. Skin wound repair in most mammals follows a similar orderly sequence of events⁽¹⁴⁾.

Platelets are small discoid blood cells made in bone marrow with life span of 7-10 days. Inside the platelets are many intracellular structure containing glycogen, lysosomes. The platelets are activate by thrombin, then they change into different shape & developed branches called pseudo-

pod cells that spread over injury site, this process called aggregation.⁽¹⁵⁾ Several angiogenic regulating proteins that are carried by platelets are present at different times during wound healing & are stored in separated in alpha granules in the platelet's cytoplasm⁽¹⁶⁾

Platelet rich plasma is defined as a volume of the plasma fraction of autologous blood having a platelet concentration above baseline. Normal platelet concentration is 200,000 platelets/ul. Studies have shown that clinical efficacy can be expected with a minimum increase of 4* this base line (1 million platelets/ul)⁽¹⁷⁾. It contains many growth factors, such as PDGF (Platelet-Derived Growth Factors), TGF-B (Transforming Growth Factor-B) & VEGF (Vascular Endothelial Growth Factor). It has been reported a significant enhancement of bone & soft tissue healing when PRP is used in cardiac surgery, bone, & in oral & maxillofacial surgery where it enhanced the healing of the fresh extraction sites was observed⁽¹⁸⁾. Clinical trials indicate that the combination of bone graft substitutes & growth factors such as cytokines contained in platelet rich plasma may be suitable to enhance bone density (19). Clinicians prepare & applied of PRP increase the early wound strength & regulate key cellular processes⁽²⁰⁾.

Materials & Methods

Sixteen adult male healthy rabbits of the same age weighed 1-1.5kg were used in this study. Divided in to two group (8 rabbits in each group) according to the skin specimens taken dividing them in to group A killed 24 hours after the trauma & group B which were killed 4 days after the trauma. Each group subdivided in to

subgroup [control & experimental]. The animals were purchased from the animal center at the local animal market. All animals received care in compliance with the Islamic Religion guidelines on the care & handling of laboratory animals. The rabbits were kept in an open ground area, where they received food & water at ad libitum. The animals were fed on conventional diets.

The rabbits were anesthetized by intramuscular injection of 0.15ml/kg of body weight of xylocaine in the thigh before 1ml of the blood withdrawal from the marginal ear vein of the rabbits using 1ml volume syringe (21) as shown in figure(1), with 1/10v/v sodium citrate as anticoagulant (16) then the citrated blood centrifuged for 10 minutes, then the plasma which carry the platelets separated & frozen until used(22).

Surgical procedure:

The surgical procedures were done under general anesthetic drugs, that were injected into the rear limb-thigh muscle of the rabbits.

Xylazin, 1.5 mg/kg body weight (B.W.), was given intramuscularly for sedation, analgesia, anesthesia & muscle relaxation. This is followed after 10 minutes with an intramuscular injection of 1 ml/kg (B. W.) Ketamine Hydrochloride to obtain dissociate anesthesia, & this was repeated in every occasion there was a reflex (11). The fur at the site of operation was depilated by the use of depilatory cream, then the operative field was properly draped by sterilized towels, no.15 blade putted on a scalped handle, 1cm length incision was made at the back of the rabbits, the depth of the incision was 2mm (height of the blade)



Figure 1. the wound filled with 0.05 ml of autologous PRP

The experimental wound filled with 0.05 ml of autologous PRP figure(3), while the control one left for normal healing. The wounds covered with dress for one day.

Table 2. Criteria to evaluate histological scores of wound healing (23)

Score	Epidermal and dermal regeneration	Granulation tissue thickness	Angiogenesis
1	Little epidermal and dermal organization	Thin granulation layer	Altered angiogenesis (one to two vessels per site) characterized by a high degree of edema, hemorrhage, occasional congestion, and thrombosis
2	Moderate epidermal and dermal organization	Moderate granulation layer	Few newly formed capillary vessels (three to four per site), moderate degree of edema and hemorrhage, occasional congestion and intravascular fibrin deposition, absence of thrombosis
3	Complete remodeling of epidermis and dermis	Thick granulation layer	Newly formed capillary vessels (five to six per site), moderate degree of perivascular and interstitial edema and congestion, absence of thrombosis and hemorrhage
4	—	Very thick granulation layer	Newly formed and well-structured capillary vessels (more than seven per site) vertically disposed toward the epithelium and at the wound margins, slight degree of degree of perivascular edema

Results

At 1st day postoperatively:

1-Histopathological examination

Control group

Large number of acute inflammatory cells with hemorrhage along the borders of the incision. Few amount of collagen fibers formed, &

little epidermal & dermal regeneration so the granulation tissue thickness& epidermal & dermal regeneration scores were 1 figure (4&5).

Experimental group

Less amount of inflammatory cells were presented with the space of the fluid appeared covered by newly

formed reepithelized layer of the epidermis, more collagen fibers formed in the area of incision than that of the control group, with newly formed

blood vessels, so the granulation tissue thickness & epidermal & dermal regeneration scores were 2, figure (6 &7).

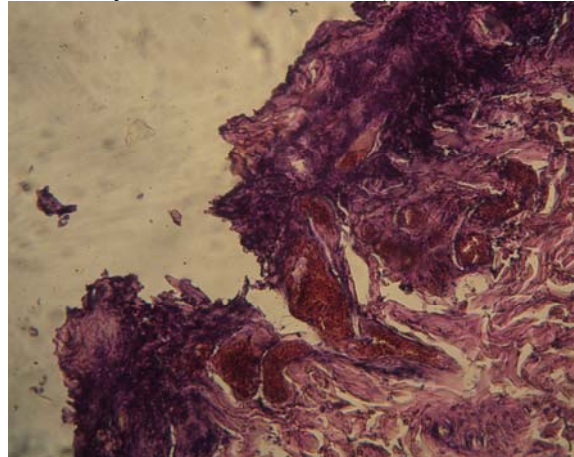


Figure 4. hemorrhage & large amount of inflammatory cells in the incision 10x power

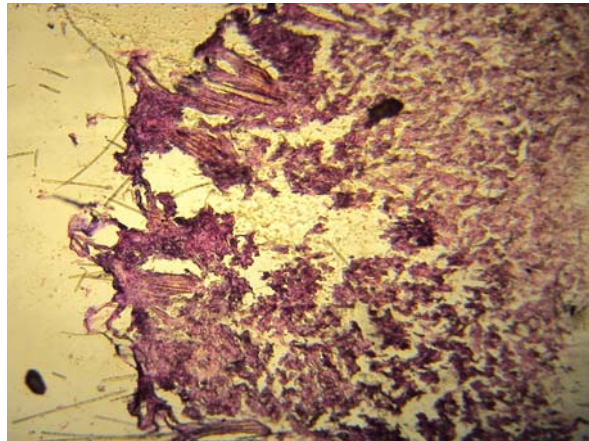


Figure 5. the space of the fluid appeared covered by newly formed reepithelized layer of the epidermis 4x power.

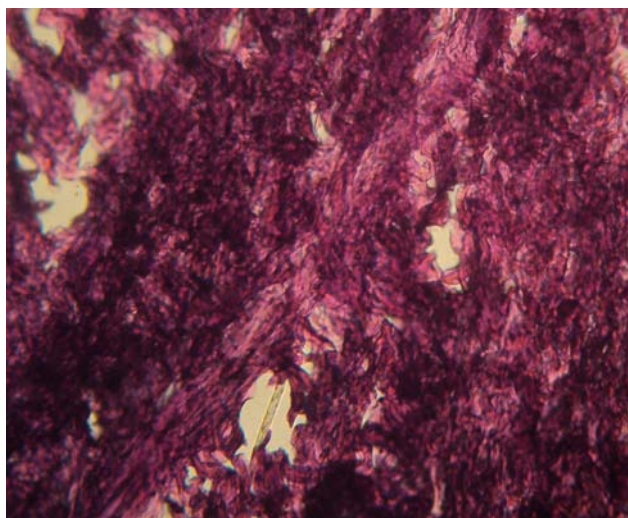


Figure 6. more collagen fibers formed in the area of incision,40x power

2- Statistic analysis

T- Test show there was highly significant increased in scores of the experimental (platelet rich plasma) group (P value< 0.01) in granulation tissue thickness score & there was significant increased in scores of

experimental group (P value< 0.05) in epidermal & dermal regeneration scores. The statistic analysis for this interval shown in table (3), the average granulation tissue thickness & epidermal & dermal regeneration scores shown in figure (8).

Table 3. Statistic analysis at 1st days postoperatively

Scores	groups	Descriptive statistic					Cal. F	P-value
		Mean	SD	SE	95% C.I.			
Granulation tissue thickness Score	Control	1.0000	.0000	.0000	-	-	6.00	.002*
	Platelet rich plasma	2.2500	.5774	.2887	2.2064	-.7936		
Epidermal & dermal regeneration score	Control	1.0000	.0000	.0000	-	-	9.000	.024**
	Platelet rich plasma	1.7500	.5000	.2500	1.3617	-.1383		

* highly significant
 ** significant

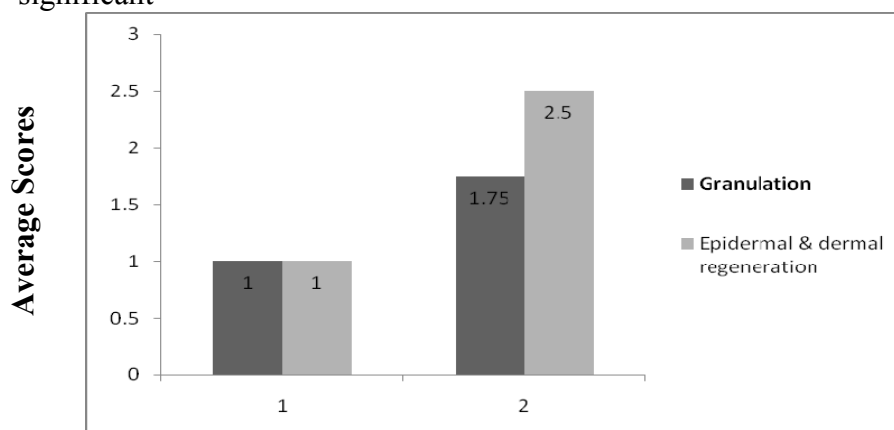


Figure 7. Average granulation tissue thickness and epidermal & dermal regeneration scores at 1st day postoperatively

At 4th days postoperatively:

1-Histopathological examination

Control group

The area of incision filled by thick granulation layer with little epidermal & dermal organization as in figure (9), large number of collagen fibers associated to fibroblasts cells with newly formed blood vessels & chronic inflammatory cells infiltration as in figure (10). The granulation tissue thickness score was 2 while the

epidermal & dermal organization score was 1.

Experimental group

The area of incision characterized by complete reepithelizaion so the epidermal & dermal organization take the score 3 due to complete epidermal & dermal regeneration figure(11), the granulation tissue thickness take the score 1 because the granulation tissue changed by fibrous tissue more collagen fibers bundles less blood vessels as in figure (12)

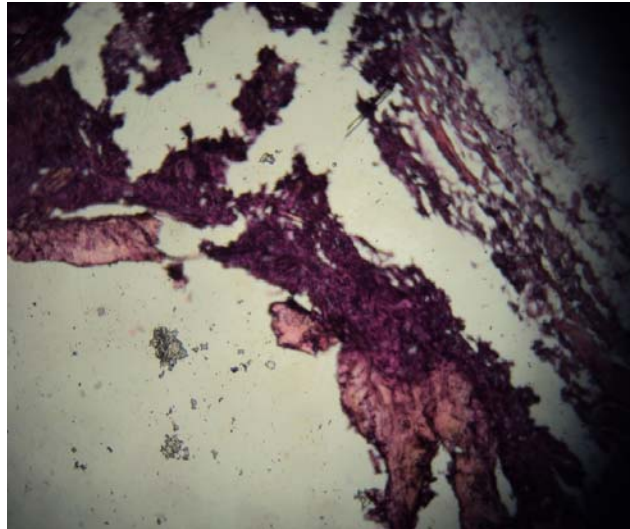


Figure 8. thick granulation layer with little epidermal & dermal organization in the area of incision at 4x power.

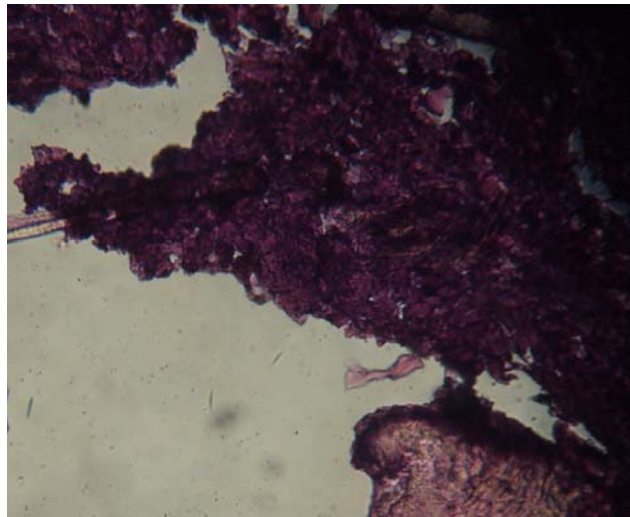


Figure 9. showed thick granulation tissue at 10 x power

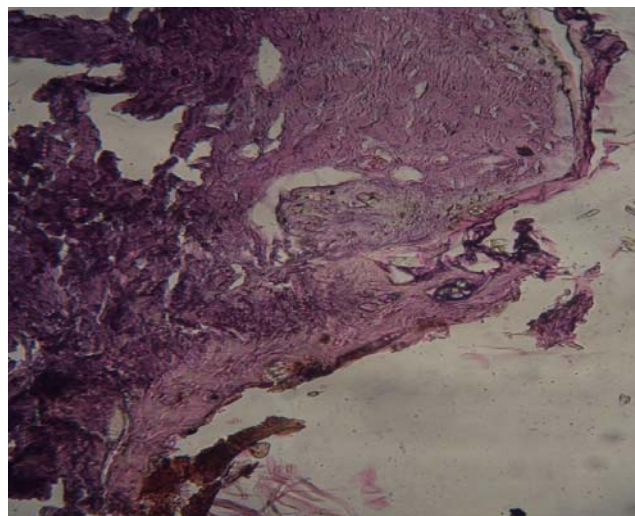


Figure 10. complete epidermal & dermal regeneration at 10x power.

2- Statistic analysis

T- Test show there was no significant difference between the experimental (platelet rich plasma) group & the control group (P value> 0.05) in granulation tissue thickness score & there was significantly increased in scores of experimental

group (P value< 0.05) in epidermal & dermal regeneration scores. The statistic analysis for this interval shown in table (4), the average granulation tissue thickness & epidermal & dermal regeneration scores shown in figure (13).

Table 4. Statistic analysis at 4th days postoperatively

Scores	groups	Descriptive statistic					Cal. F	P-value
		Mean	SD	SE	95% C.I.			
Granulation tissue thickness Score	Control	1.7500	.5000	.2500	-.6844	1.1844	6.000	.356*
	Platelet rich plasma	1.5000	.5774	.2887	-.6891	1.1891		
Epidermal & dermal regeneration score	Control	1.2500	.5000	.2500	-2.1844	-.3156	6.000	.017**
	Platelet rich plasma	2.5000	.5774	.2887	-2.1891	-.3109		

* non significant

** significant

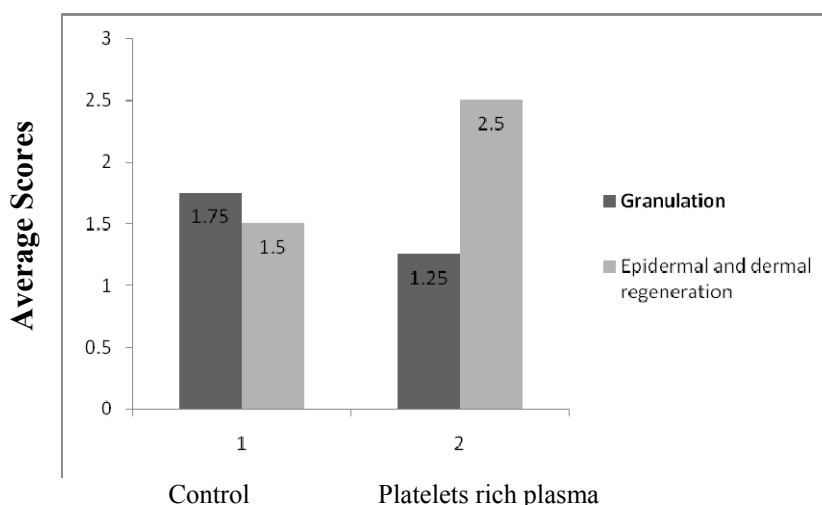


Figure 11. Average granulation tissue thickness and epidermal & dermal regeneration scores at 4th day postoperatively

Discussion

Cell migration, inflammation, provisional matrix synthesis, collagen deposition, & reepithelization play an integral role in skin repair⁽¹³⁾.

Pietramaggiore *et al.* (2008) reported that the early in the wound healing angiogenesis is essential to initiate & support the repair process &

a timely switch between pro -& anti-angiogenesis is necessary to initiate & terminate the healing process, platelet can likely be considered to partake in controlling this angiogenesis switch as pro- & anti -angiogenic proteins. In our study, a similar consequence was evident in our study; angiogenesis was stimulated in the first day post

operatively & reduced in the 4th day postoperatively.

Platelet derived growth factors, main functions are to stimulate cell replication (mitogenesis) of healing capable stem cells. It also stimulates cell replication of endothelial cells, this will cause budding of new capillaries in to the wound (angiogenesis). In addition, platelets derived growth factors seems to promote the migration of perivascular healing capable cells into a wound & to modulate the effects of other growth factors ⁽²²⁾ & the our study was to increase the growth factors to enhancement of healing.

Growth factors are essential for regulation the cellular events involving in wound healing by attracting cells to the wound, stimulating proliferation & significantly influencing matrix deposition ⁽²²⁾.

Growth factors responsible for increasing cell mitosis, increasing collagen production, recruiting other cells to the site of injury, initiating vascular in-growth & inducing cell differentiation ⁽¹⁸⁾. Platelets are release multiple wound healing growth factors & cytokines, including platelets-derived growth factor (PDGF), transforming growth factor beta 1&2, vascular endothelial growth factor (VEGF), platelet derived endothelial cell growth factor (PDEGF), basic fibroblast growth factor (bFGF) (19 &20).

The main sources of transforming growth factor- beta1 (TGF-B1) are the platelets, inflammatory cells, osteoblasts, & chondrocytes ⁽²⁴⁾. The (TGF-B1) one of the most important factors in the stimulation of the fibroblast to proliferation & synthesis of extracellular matrix protein ⁽⁵⁾.

All these studies agreement with our results in this study, where there were highly significantly increased in

granulation tissue thickness in 1st day postoperatively in experimental group & changed in to non significant difference between the experimental & control groups. While the epidermal & dermal regeneration significantly increased in 4th day postoperatively in experimental group.

Our results agree with Lopez-Vidriero *et al* (2010) who reported that, the use of PRP technologies has opened another door in the treatment of soft-tissue injuries. An understanding of the principles of tissue healing and the pathophysiology behind PRP, as well as a basic knowledge of the differences in commercial systems involved in the preparation of these products, is essential to the successful application of this modality in both the conservative and operative management of soft-tissue injury ⁽²⁶⁾.

Conclusion

PRP showed significant enhancement the healing of the skin. Although the Autologous blood injections and the use of autologous blood products continue to be an experimental but potentially exciting therapeutic intervention.

There is no standardization for PRP preparation technique. Various preparation techniques of PRP will produce different platelet counts, depending on the system used, or whether a double- or single-centrifugation technique is utilized. The duration of centrifugation may also provide different platelet counts. In addition, it is not yet well understood what the optimal concentration of platelet is that will provide the best outcome. The effects of too high or too low of platelet concentration are still unknown. Moreover, potential complications or

side effects caused by PRP have not been studied extensively.

Any concerns of immunogenic reactions or disease transfer are eliminated because PRP is prepared from autologous blood. No studies have documented that PRP promotes hyperplasia, carcinogenesis, or tumor growth. Growth factors act on cell membranes rather than on the cell nucleus and activate normal gene expression. Growth Factors are not mutagenic and naturally act through gene regulation and normal wound healing feed-back control mechanisms.

Relative contraindications include the presence of a tumor, metastatic disease, active infections, or platelet count < 10⁵/ul Hgb < 10 g/dl. Pregnancy or active breastfeeding are contraindications. The patients should be informed of the possibility of temporary worsening symptoms after the injection. This is likely due to the stimulation of the body's natural response to inflammatory mediators. Although adverse effects are uncommon, as with any injection there is a possibility of infection, no relief of symptoms, and neurovascular injury. Scar tissue formation and calcification at the injection site are also remote risks.

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