Original Article

The Possible Role of Platelet Rich Plasma in Treatment of the Induced Surgical Corneal Injury in Rabbits: Histomorphometric and Electron Microscopic Studies

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ABSTRACT

Introduction: The health of the ocular surface that is important for visual acuity is dependent on the corneal epithelial integrity. Platelet-rich plasma is considered a therapeutic approach to corneal wounds because of its content of growth factors. These growth factors help to promote and accelerate wound healing in different tissues including the cornea

Aim of the Work: To evaluate the effect of PRP on corneal wound healing in surgically induced corneal injury in rabbits.

Material and Methods: Eighteen rabbits each weighing 2.5–3 kg were used in this experiment. Blood from the marginal vein of the ear was obtained from Rabbits assigned to be donors for PRP. The center of the cornea was marked, and the epithelium was scrabbed by an ophthalmic blade, then four incisions were made in the center of the corneal stroma. Rabbits were assigned into one of three groups: group I represented the control group that didn't receive any treatment. Group II represented the saline group that received one drop of sodium chloride. Group III received PRP as single subconjunctival injection. Animals of all the groups were then euthanized one week after the surgery. The Cornea of all groups was extracted, processed and subjected to light, and Scanning electron microscopic examination

Results: Platelet rich plasma led to restoration of the regular arrangement of stromal collagen fibers and regular surface epithelium of the corneal wound that showed a good amount of microvilli.

Conclusion: Platelet-Rich plasma led to a more regular pattern of wound healing with better quality in induced corneal injury.

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Key Words: Corneal wounds, corneal wound healing, PRP.

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INTRODUCTION

The cornea is the anterior central part of the outer coat of the eye. It is transparent to permit passage of light and sight. It consists mainly of five layers; the outer epithelial layer; Bowman's layer which is formed of collagen fibers that are arranged randomly. This layer is absent in some species like rabbits. The next layer is the stroma that forms most of the corneal thickness. It is composed of dense, regularly packed collagen fibrils that coalesce to form stacked lamellae; these fibrils are formed by specialized cells called Keratocytes. The fourth layer is Descemet's membrane that lies just above the inner layer that is made up of endothelial cells[1,2]. The health of the ocular surface is dependent on the corneal epithelial integrity, which in turn depends on the balance between a lot of factors including function of limbal stem cells, the quantity of tear film and its quality, the eyelid anatomy in addition to corneal sensitivity[24]. Corneal epithelial wound healing is a complex mechanism and could be sustained by vitamins, glucose, and a lot of growth factors[3].

The process of Wound healing includes recruitment of a lot of inflammatory and immune cells to the wound site. These cells as macrophages, and other mesenchymal stem cells help to eliminate detritus. Then, mesenchymal stem cells together with Platelets secrete factors that enhance the process of wound healing[4]. This complex process requires a lot of elemental materials such as growth factors, glucose, and different types of vitamins. These requirements are provided in the avascular cornea by the limbal blood vessels at the corneal periphery, aqueous humor, and tear film[3,9]. Serum had been found to be similar to natural tears in a lot of properties such as osmolarity, pH in addition to biomechanical properties[3]. In addition, there are similarities in the content of proteins including antimicrobial proteins, enzymes as proteases, lipids, vitamins, cytokines, and growth factors[5]. A major source of the growth factors that play important role in repair and regeneration of damaged tissue is the alpha granules of platelets[6].

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The use of platelets as delivery vehicles of the healing factors had emerged Since the late 1990s. Serum derivatives that have high concentration of platelets are generically called platelet-rich plasmas (PRPs)[7]. It's an autologous bio-adhesive product where a small volume of serum has a very high concentration of platelets that qualifies it to contain a lot of serum components. It could help in the process of healing like the three isomers of Platelet-Derived Growth Factors (PDGF1, PDGF2, and PDGF3), Transforming Growth Factors (TGF1 and TGF2), Vascular Endothelial Growth Factors (VEGF), fibronectin, and epithelial growth factor. These factors contribute to tissue healing[8].

That's why PRP had been used as an accelerator to improve corneal wound healing. It can provide factors required for wound healing from blood circulation. It can be used in the form of eye drop on the surface or clot (as tissue glue) by subconjunctival injection[9].

Moreover, it is used as a tear substitute as an alternative treatment for ocular surface disorders. This is mainly due to its lubricating properties in addition to its huge content of growth factors and anti-inflammatory factors[10,11]. The use of PRP in promotion of wound healing depends on the elaboration and use of autologous PRP which is obtained from patient's own blood. It forms a biodegradable fibrin scaffold in situ after its activation by calcium chloride; this occurs simultaneously with event cascade that leads to release of a pool of biologically active proteins in addition to mix of pro-inflammatory and anti-inflammatory cytokines that initiate group of processes including recruitment of cells, their growth and differentiation. This in turn promote wound healing[12].

The present study aimed at evaluation of the possible therapeutic effect of single subconjunctival injection of PRP on promotion of wound healing in induced corneal injury in rabbits as an experimental animal model.

MATERIAL AND METHODS

The study experiment had been done at the Medical Research Centre, Faculty of Medicine Ain-Shams University at its Animal house. The study plan was performed after review and approval of CARE (Committee of Animal Research Ethics) according to its guidelines. Eighteen rabbits were used in the study each weighing 2.5–3 kg. Rabbits were housed in metallic cages 1 rabbit per cage, exposed to circumstances that simulate their normal life environment like dark/light cycle, 22°-25° C temperature. They were provided with good ventilation, had a free access to water, and standard diet. They were housed for five days before experiment to allow their acclimatation to the environment.

PRP preparation

Rabbits assigned to be donors for PRP were put in a restrainer. 70% alcohol was used to clean the ear. Then, a local anesthetic cream was applied on the collection site before sampling by 10 minutes. A 25 G needle was used to collect blood from the marginal vein of the ear[13]. A blood volume of 8.7 ml was aspirated by 10 ml syringe that was first anticoagulated using 1.3 ml of acid citrate dextrose as anticoagulant[9]. Blood was immediately deposited in a sodium citrate test tube and placed in a centrifuge at 3200 rpm for 15 minutes.

Centrifugation separated the blood into three layers; the platelet poor plasma (superior layer), buffy coat (intermediate layer; rich in WBCs), and the red blood cells (inferior layer). The supernatant plasma fraction just above the buffy coat was obtained with a sterile syringe in a test tube. This fraction was further centrifuged at 3200 rpm for another 15 minutes for the purpose of obtaining two parts: the upper part is the platelet poor plasma (PPP), and the lower part is the platelet rich plasma (PRP) that constitutes 25% of the whole plasma. PRP was aspirated with a sterile plastic syringe to be used[14].

Corneal injury protocol

Each animal initially received a combination of intramuscular ketamine 50 mg/kg and midazolam 1 mg/kg in addition to topical benoxinate 0.4% eyedrops.

An eye speculum was applied for retraction of the right eyelids, then the center of the cornea was marked using a 3mm trephine where the corneal epithelium in the marked area was scrapped by an ophthalmic blade. Afterwards, four incisions were made in the center of the corneal stroma using a 3.2 mm keratome where a crossed pair of parallel vertical and horizontal incisions were formed[15].

Randomization of animals into one of three groups was made as follows

Group I: (Control group) (GI) Consists of six rabbits not subjected to induced corneal injury. They were euthanized at the end of the experiment.

Group II: (experimental group) (GII) Consists of six rabbits. They were subjected to induced corneal injury and received one drop of sodium chloride (9mg/ml). They represent the corneal injured group[15].

Group III: (Platelet rich plasma group) (GIII) Consists of six rabbits. Animals in this group were subjected to induced corneal injury and received a single dose of 0.5 ml of autologous PRP injected subconjunctival under the superior bulbar conjunctiva by 27-gauge half inch needle put on 1ml syringe[9].

Animals of all the groups were then euthanized at one week after the surgery.

Tissue preparation

Animals of groups GI, GII, and GIII were euthanized at one week after surgery. The eyeball was enucleated then was placed in paraformaldehyde 10%. The cornea was extracted from the globe using delicate Colibri forceps and Vannus scissors. Then, five specimens were used for examination under light Microscope and one specimens was used for examination using Scanning Electron Microscopy (SEM).

Histological study

Specimens for light microscopic study were immersed in 10% formol saline for fixation. After fixation, tissues were subjected to dehydration then embedded in paraffin blocks. Sections 5 µm thick were cut from the paraffin blocks of all specimens and stained with Hematoxylin and Eosin (H&E), Masson Modified Trichrome[16]. Stained sections were examined under the Olympus BX50 photomicroscope.

Scanning Electron Microscopy study

Specimens for SEM were cut into cubes (1mm thick) immediately, then subjected to overnight fixation using 2.5% phosphate-buffered glutaraldehyde (pH 7.3) at 4°C. Afterwards, specimens were postfixed in 1% buffered osmium tetroxide for 1–2 hours. Then, subjected to gradual dehydration using ascending grades of ethyl alcohol, and were dried with critical point dryer (Bal-tec cpd030). Specimens were coated with gold pallidum (bal-tec SC005) and were examined by SEM at the regional Centre of Mycology and Biotechnology, Al-Azhar university using the high vacuum mode of JEOL JSM-5500LV Scanning Electron Microscopy.

Morphometric and statistical Analysis

Thickness of collagen fibers was measured in Masson trichrome-stained sections at magnification of x100. The image analyzer (Image J) Program was used for making measurements of the photos of the sections. The measurements were made in five different non overlapping fields chosen randomly in five different sections from the five animals' specimens used for light microscopy examination in each group[17]. The image analyzer was calibrated for automatic conversion of the units measured in pixels produced by the program into actual micrometer units.

Statistical analysis of the measurements of collagen fibers thickness was performed using one-way analysis of variance (ANOVA) to compare between the three groups (GI, GII, and GIII) followed by Tukey's post hoc multiple comparisons test. The results are expressed as mean difference and confidence interval. A *P-value* ≤ 0.05 was used to determine the level of significance where $p \leq 0.05$ was considered significant and $P \leq 0.001$ was considered highly significant.

Data were analyzed using GraphPad Prism Software (V.8.0.2)

RESULTS

GI

Microscopic examination of a corneal section of GI stained with H&E showed the four layers of the cornea; epithelium, stroma, Descemet's membrane, and the endothelium with no Bowman's layer (Figure 1).

The corneal epithelium is formed of 5-7 layers of a stratified squamous non-keratinized epithelium with basal cells being columnar with an almost palisade arrangement resting on the epithelial basal lamina. The middle cell layer appeared as polygonal or wing like cells. The most superficial layer was formed of squamous cells. The stroma was formed of collagen fibers with thin fibroblasts (keratocytes)in between (Figures 2,3).

Masson Trichrome stained sections showed regularly arranged dense collagen fibers occupying the whole stroma between the epithelium and the Descemet's membrane with fibroblasts (keratocytes) in between (Figures 4,5).

Scanning Electron Microscopy

The surface epithelium of the cornea of the GI showed that the cells had a polygonal appearance of large dark cells and smaller light cells. The cells had microvilli on their surface with the dark cells containing more microvilli / square micron than the surrounding light cells. Additionally, there were holes inside the epithelial cells that contained condensed microvilli inside. There were clumps of mucin stuck to the surface of the epithelium (Figures 6,7,8).

The stroma was formed of collagen fibers that were compact and regularly arranged (Figure 9).

GII

Microscopic examination of a corneal section GII stained with H&E showed restoration of the surface epithelium with all its layers: the basal cuboidal to columnar cells, the middle layer wing cells and the superficial layer flat cells along with the basement membrane. However, it showed irregular thickness with areas of hyper cellularity compared with other groups. There are wide spaces in the stroma with disarray of the collagen fibers and minimal infiltration with inflammatory cells (Figures 9,10).

Masson Trichrome stained sections showed widely spaced collagen fibers of the stroma (Figure 11).

Scanning Electron Microscopy

Scanning of the surface epithelium of the cornea at the epithelial cut wound of GII showed more approximation of the wound edges that were advancing to fill the wound gap with the polygonal epithelial cells appearing on the side of the wound edge.

There were few mucin particles on the surface of the epithelium that took the appearance of snowflakes. The advancing epithelial cells showed microvilli which were more in the dark cells present at the edges than in the more peripheral light cells that were away from the wound edge. However, the microvilli were apparently fewer as compared to the control group and the PRP group (Figures 12,13,14).

The cut edge of the corneal wound showed spacing in between the collagen fibers in the middle of the stroma with disarray of the formed collagenous fibrils in addition to remnants of fibrin clots intervening between them (Figures 15,16).

GIII

Microscopic examination of a corneal section GIII stained with H&E showed healing and restoration of regular epithelium in full thickness together with the restoration of the palisade arrangement of the basal epithelial layer cells with apparently less widely spaced and regularly arranged collagen fibers in the stroma (Figures 17,18,19).

Masson Trichrome stained sections showed less widely spaced collagen fibers in the corneal stroma that are regularly arranged with the fibroblasts (keratocytes)in between (Figure 20).

Scanning Electron Microscopy study

Scanning of the surface epithelium of GIII showed approximation of the wound edges that were filled with amorphous material of multiple fibrin clots between the advancing epithelial surface (Figure 21).

The cut edge of the corneal wound of GIII showed regular arrangement of the collagen fiber bundles in the cut edge of the stroma that was crossed at spaces by single collagen fibrils (Figures 22,23).

Statistical analysis

Statistical analysis of the thickness of collagen fibers of the stroma between the control group, the experimental group, and the PRP group is shown in (Table 1, Chart 1) (one-way ANOVA).

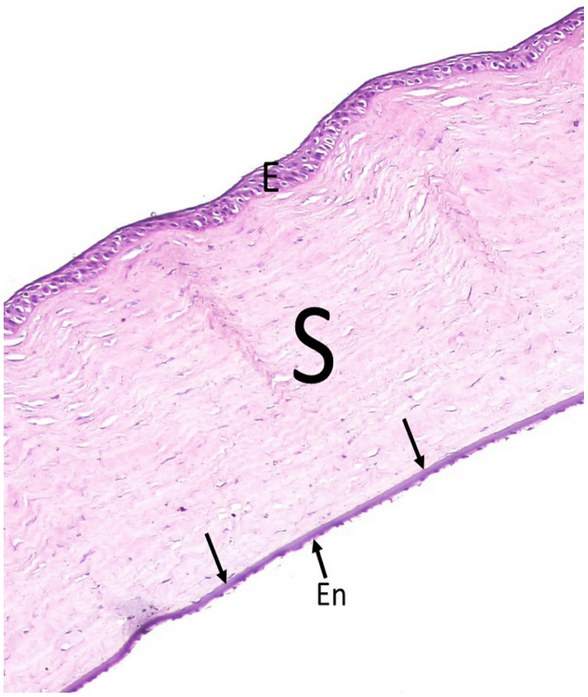

Fig. 1: A Photomicrograph of a corneal section of the GI showing the corneal epithelium (E), the corneal stroma (S), Descemet's membrane (black arrows) and the endothelium (En) (H&E x100)

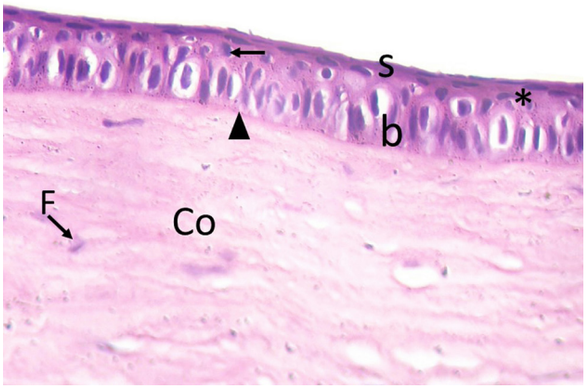

Fig. 2: Photomicrograph of a corneal section of GI showing the corneal epithelium formed of 5-7 layers of cells. The basal cells are columnar (b) and the middle layer cells appeared as polygonal (black arrow) or wing cells (*) and the flattened surface cells (s). The stroma is formed of collagen fibers (Co) with thin fibroblasts (F) in between. Note: The epithelial basal lamina (arrowhead). (H&E x400)

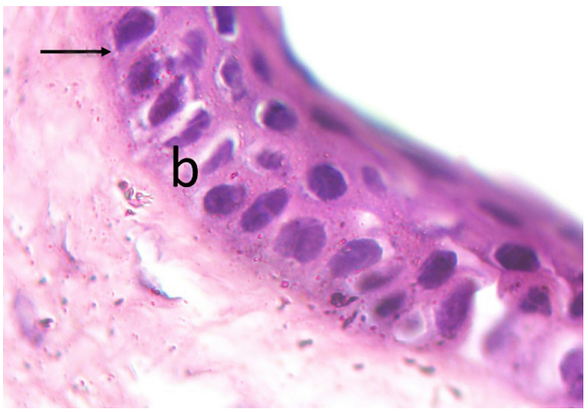

Fig. 3: A Photomicrograph of a corneal section of GI showing the palisade arrangement of the corneal basal cells (b) on the epithelial basement membrane (arrow). (H&E x1000)

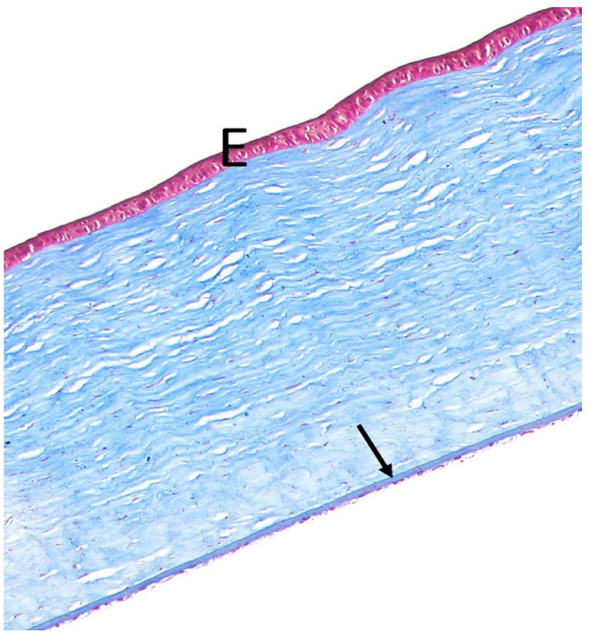

Fig. 4: Photomicrograph of a corneal section of GI rabbits stained with Masson Trichrome showing dense regular collagen fibers occupying the whole stroma between the epithelium(E) and the Descemet's membrane (arrow). (Masson Trichrome X100)

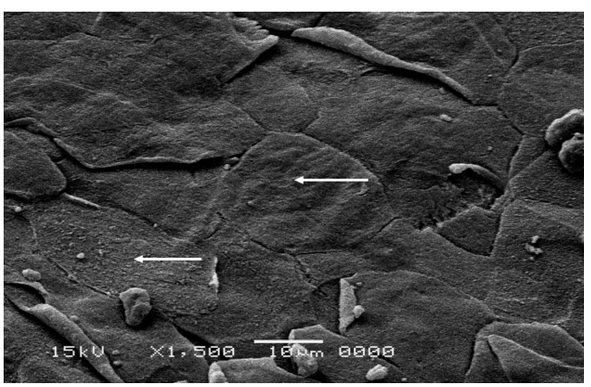

Fig. 5: SEM of the surface epithelium of the cornea of GI showing that the superficial layer s formed of polygonal cellular appearances (arrows). (Scale bar 10µ (X1500))

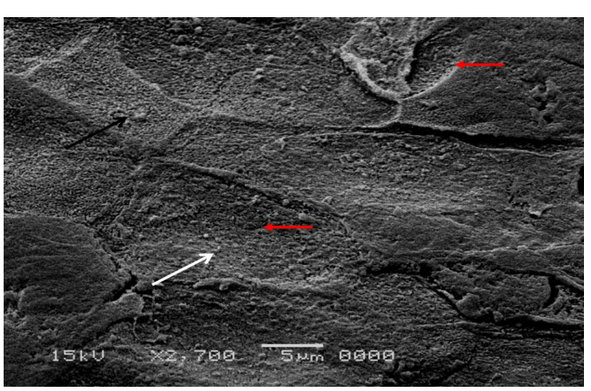

Fig. 6: SEM of the surface epithelium of the cornea of GI showing the large dark cells (white arrow) and the smaller size light cells (black arrow). Note: The Holes in the epithelial cells (Red arrows). (Scale bar 5µ (X2700))

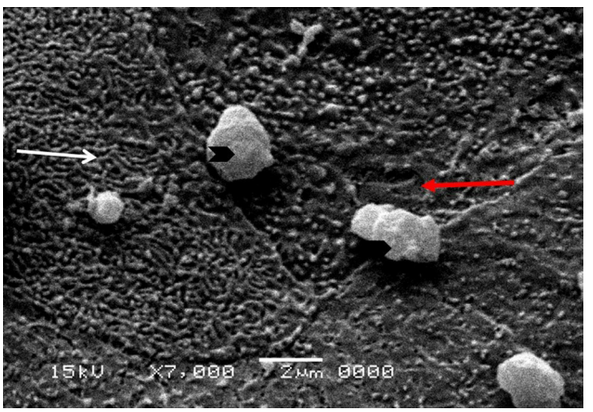

Fig. 7: SEM of the surface epithelium of the cornea of GI showing the microvilli on the surface of the epithelium with the dark cell (white arrow) containing more microvilli /square micron than the surrounding light cells. Globular structures mostly Clumps of mucin (arrow heads) are sticked to the surface epithelium. Crater like holes appears in the cells (Red arrow). (Scale bar:2µ (X7000))

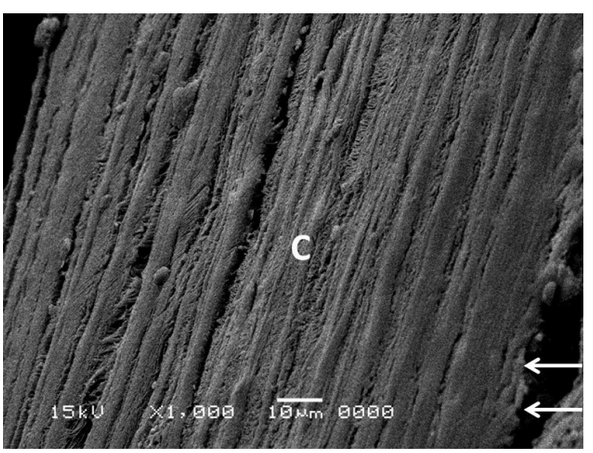

Fig. 8: SEM photograph of the regular compact arrangement of collagen fibers (C) in the stroma with the endothelial layer; Descemet's membrane at the margin of the photo (white arrows) (Scale bar:10µ (X1000))

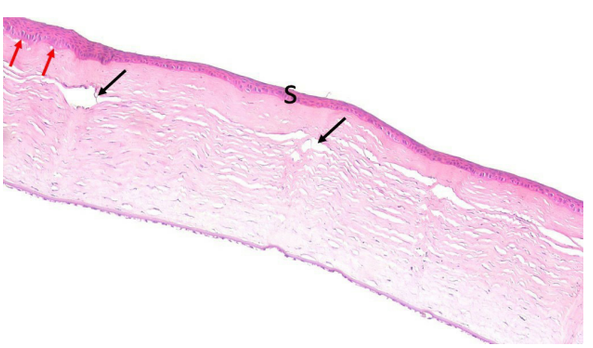

Fig. 9: A photo micrograph of a corneal section of GII showing restoration of the surface epithelium (s) with its basement membrane, However, it showed apparent irregular thickness with areas of hyper cellularity (red arrows). Notice the wide spaces in the stroma (black arrows). (H&E x100)

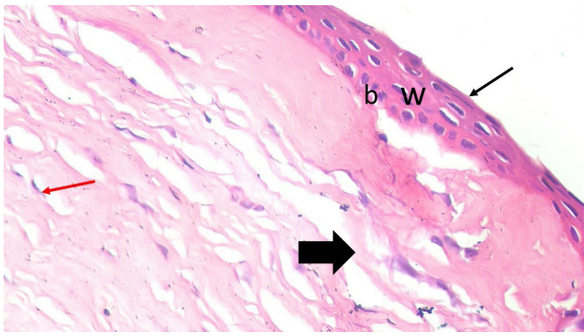

Fig. 10: A photo micrograph of a corneal section of GII showing restoration of the epithelial layers with the basal cuboidal to columnar cells (b), the middle layer polygonal cells (w) and the superficial layer flat cells (arrow). There are wide spaces in the stroma with retraction of the epithelium from with disarray of the collagen fibers (broad arrow) with keratocytes in between (red arrow). (H&E x400)

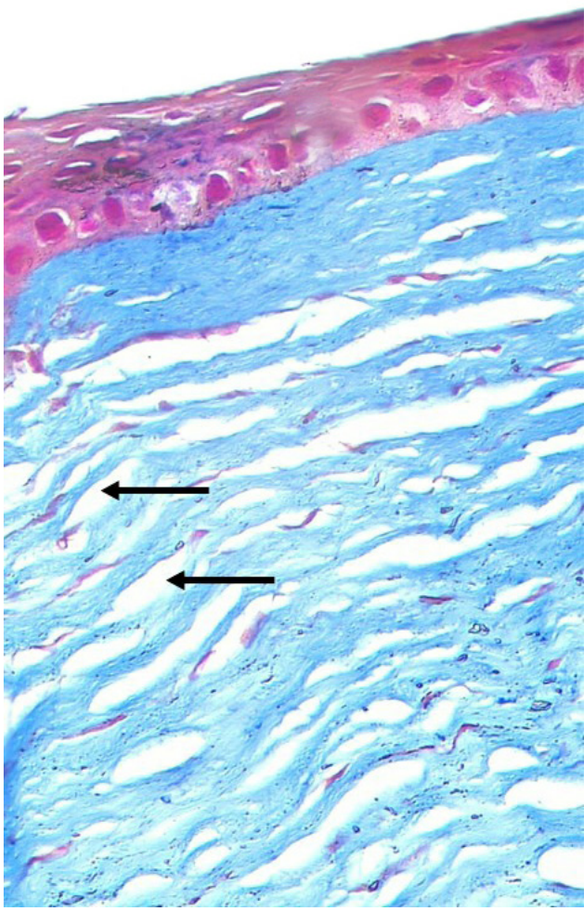

Fig. 11: A photo micrograph of a corneal section of GII stained with Masson trichrome showing widely spaced collagen fibers of the stroma (arrows). (Masson Trichrome x400)

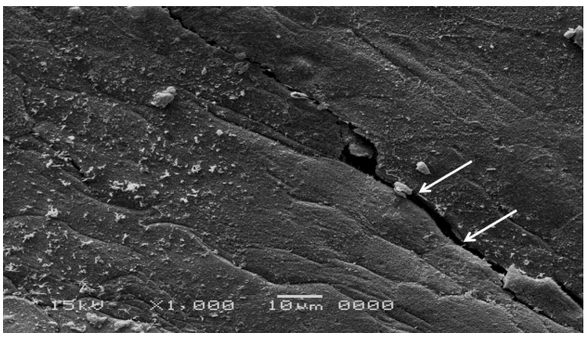

Fig. 12: SEM of surface epithelium of the cornea of GII rabbits showing approximation of the epithelial cut wound in the middle of the specimen (white arrows). Few mucin flakes are present on the surface. Microvilli are apparently decreased in comparison to group I. (Scale bar:10µ (X1000))

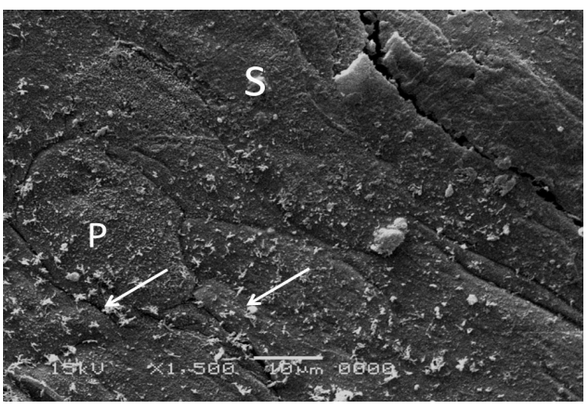

Fig. 13: SEM of surface epithelium of the cornea of GII showing the surface epithelial cells acquiring spindle elongated shape at the wound (S) with few polygonal epithelial cells away from the wound edge (P) with decreased Mucin content (the snowflake appearance) (arrows). (Scale bar:10µ (X1500))

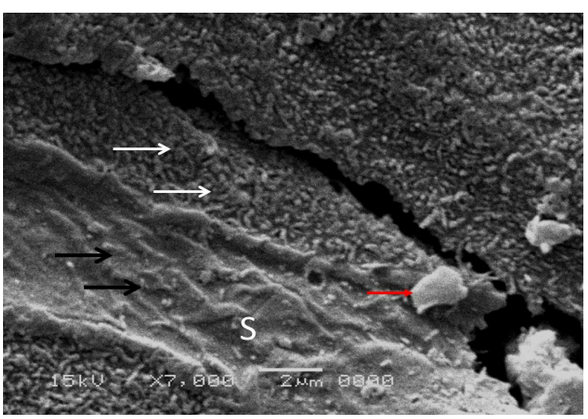

Fig. 14: SEM of surface epithelium of the cornea of GII showing the microvilli of the advancing epithelial cells which are more in the dark cells (white arrows) present at the edges than the more peripheral light cells (black arrows). The epithelial cells acquiring elongated spindle shape (S) with fewer microvilli as compared to group I. (Scale bar:2µ (X7000))

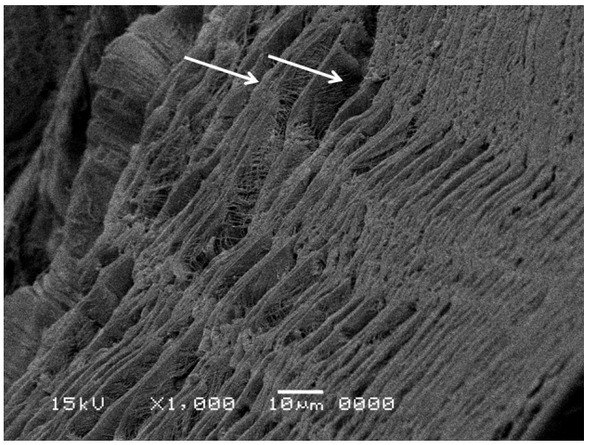

Fig. 15: SEM of the cut edge of the corneal wound of GII showing spacing between collagen fibers in the middle of the stroma (arrows) (Scale bar:10µ (X1000))

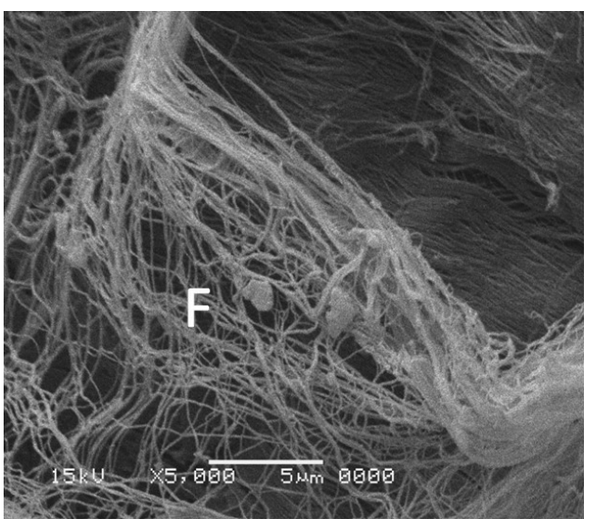

Fig. 16: SEM of the cut edge of the corneal wound of GII showing disarray of the collagenous fibrils (F). (Scale bar:5µ (X4000) & (X5000))

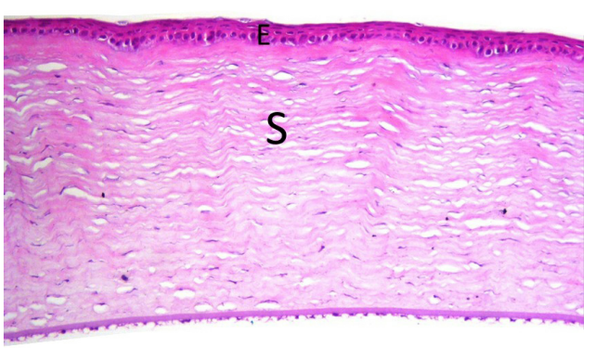

Fig. 17: A photo micrograph of a corneal section of GIII showing restoration of epithelial full thickness (E) with apparently less widely spaced and regularly arranged collagen fibers in the stroma (S). (H&E x100)

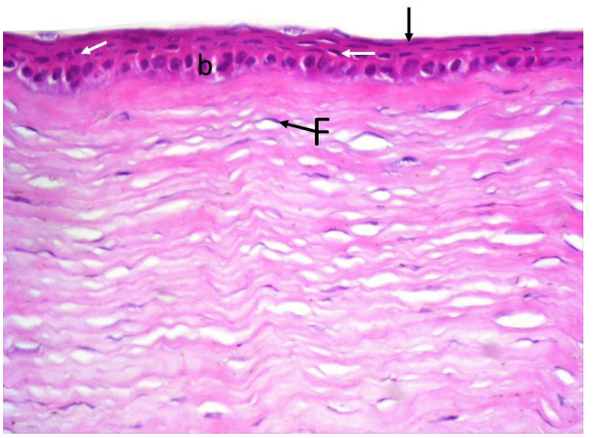

Fig. 18: A photo micrograph of a corneal section of GIII showing the basal cuboidal to columnar cells (b), the middle layer polygonal and wing cells (white arrows), and the superficial layer flat cells layers of the epithelium (black arrow). Notice the less widely spaced collagen fibers in the stroma as compared to group II with fibroblasts in between (F). (H&E x400)

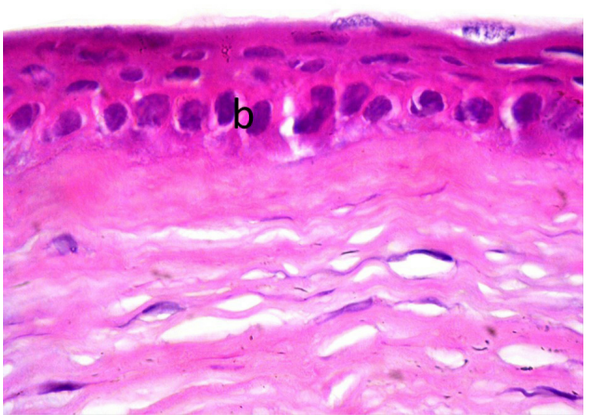

Fig. 19: A photo micrograph of a corneal section of GIII showing the full restoration of the epithelial full thickness with the palisade arrangement of the basal epithelial layer cells (b). (H&E x1000)

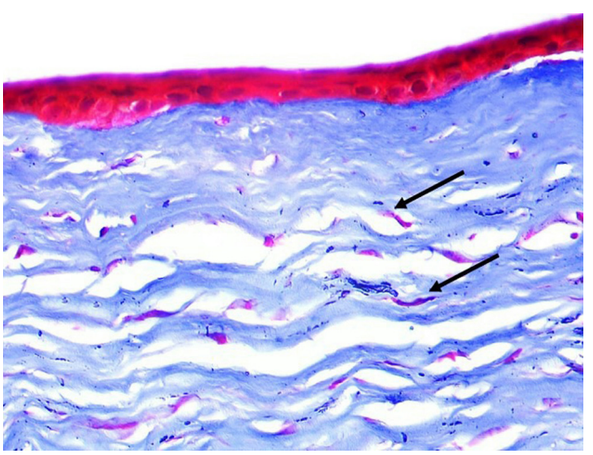

Fig. 20: A photo micrograph of a corneal section of GIII showing the spaced collagen fibers in the corneal stroma. They are regularly arranged with the fibroblasts in between (arrows) (Masson Trichrome x400)

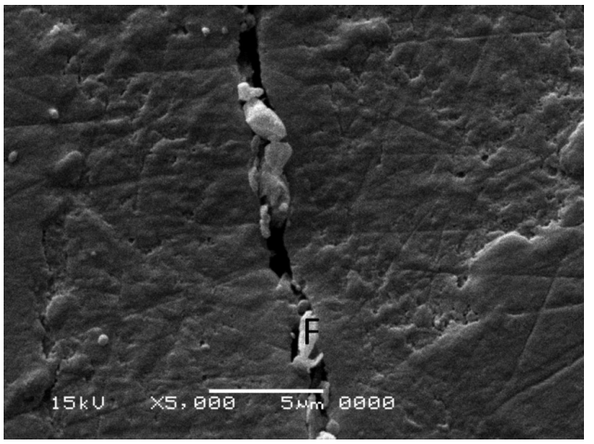

Fig. 21: SEM photomicrograph of the surface epithelium of GIII showing approximation of the wound edges that are filled with amorphous material of multiple fibrin clots (F) filling the slit between the advancing epithelial surface. (Scale bar: 5µ (X5000))

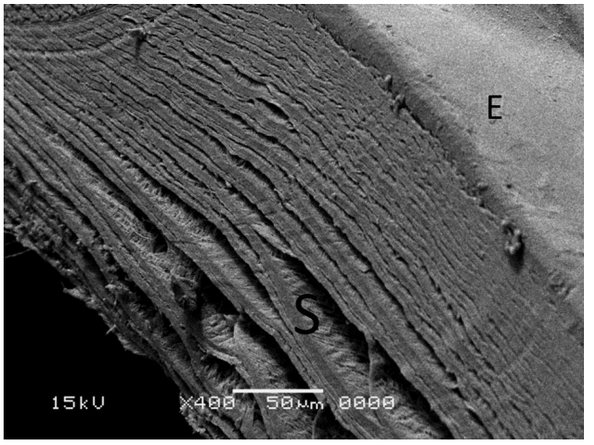

Fig. 22: SEM photomicrograph of the cut edge of the corneal wound showing the regular arrangement of the collagen fibers (arrows) Scale bar: 10µ (X1000)

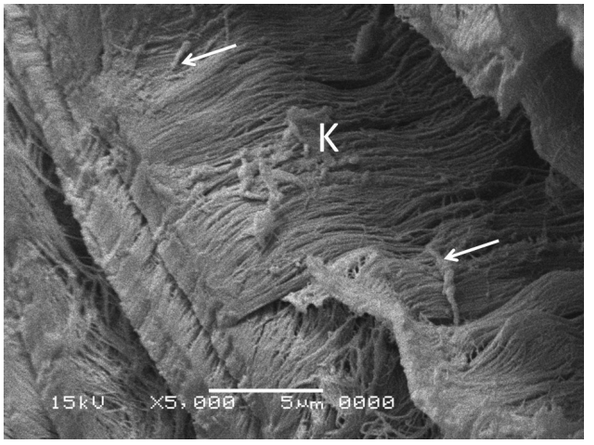

Fig. 23: SEM photomicrograph of the cut edge of the corneal wound showing regular arrangement of collagen fiber bundles that are crossed at spaces by single collagen fibrils (Arrows). Keratocyte (fibroblast) is seen in between the fibers as spindle shaped cell with multiple fibrin strands arising from it (K). (Scale bar: 5µ (X5000))

Table 1: Thickness of collagen fibers between the three groups (one-way ANOVA)

	Mean Diff.	95.00% CI of diff.		Sig.
		Lower bound	Upper bound	
Control vs. experimental	267.5	207.0	328.0	*P*1<0.0001
Control vs. PRP	72.17	11.68	132.7	*P*2=0.0182
experimental vs. PRP	-195.3	-255.8	-134.8	*P*3<0.0001

P1= There was a significant difference in the thickness of collagen fibers between the control and the experimental group.
P2=There was also significant difference in the thickness of collagen fibers between the PRP and the control group.
P3= there is a significant difference in the thickness of the collagen fibers in the stroma between the experimental group and the PRP group.

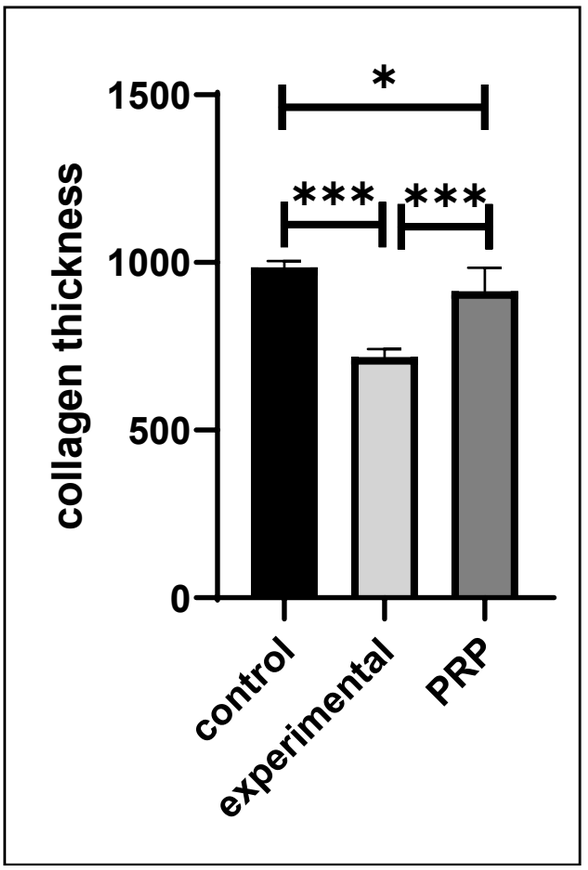

Chart 1: collagen thickness between the three groups

DISCUSSION

Corneal wounds are very common due to its position as the most anterior part of the eye[18]. The integrity of the cornea is crucial for visual acuity.

Healing of corneal wounds and exploring new interventions to accelerate corneal wound healing

represents an important aspect in clinical and experimental eye research.

Platelet Rich Plasma is one of the most explored therapeutic interventions due to its containment of several growth factors that induce, accelerate and improve wound healing quality. This experimental study was performed to evaluate if injection of PRP in the superior bulbar conjunctiva would accelerate and improve healing of the corneal wound in comparison to just maintaining corneal hydration through application of saline drops.

The present study results confirmed that PRP accelerated corneal wound healing on histopathological, SEM and statistical levels. Platelet Rich Plasma significantly increased thickness of collagen fibers in the stroma with restoration of their regular arrangement. The epithelial thickness had been restored with regular and palisade arrangement of the basal epithelial cells.

Platelet Rich Plasma was investigated before in the literature using different preparation techniques that lead to different concentration of platelets and in turn their growth factors content[3,7,8,9]. Likewise, the current study results came in line with the previous experimental studies[8,9]. Freire *et al*., (2014), and Etxebarria *et al*., (2017) found that blood derivatives that had higher concentration of platelets like serum rich in growth factors derived from plasma (s-PRGF) enhanced and accelerated wound healing more than PRP[3,7].

The ability of PRP to accelerate wound healing is mainly attributed to be vehicles that deliver growth and healing factors contained in their alpha granules to the site of injury. Platelet rich plasma is a blood plasma that has an enriched platelet content that provide supraphysiological concentration of signaling growth factors and cytokines that enhance the wound repair by various mechanisms as angiogenesis and inflammation regulation, as well as synthesis of new tissue and enhancement of its remodeling[7].

Platelets can release various growth factors present in their alpha granules as VEGF, TGF-β1 and-β2, PDGFs aa, bb, and ab, and epithelial growth factor[19].

These growth factors in turn lead to different sequences that helps to accelerate the different stages of the wound healing. For instance, the platelet derived growth factor (PDGF) was stated to be the first factor that appears in the wound and to which the PRP owes most of its efficacy. It stimulates collagen synthesis and regeneration in addition to revascularization through angiogenesis and new blood vessels formation.

Other factors that promote the process of angiogenesis include fibroblast growth factor (FGF) and Vascular Endothelial Growth Factor (VEGF). This process of angiogenesis helps to deliver nutrients and progenitor cells to the wound.

Endothelial growth factor (EGF) is another growth factor that promote and fasten epithelial proliferation and oppose apoptosis[20].

The PRP didn't only accelerated wound healing on the histopathological or ultra-structural level but also on the clinical level; either with subconjunctival injection of PRP or PRP drops[21,22,23].

CONCLUSIONS

The current study could add to the clinical benefit of PRP use in corneal wound healing in different circumstances including traumatic corneal injury and corneal ulcers.

This study proved that PRP initiated normal restoration of the corneal epithelium and stroma through histological and SEM studies in addition to statistical analysis. This might be due to its higher content of different growth factors.

Other advantages include ease of preparation, its autologous origin, absence of intolerance. Further studies may be needed for the longer follow up period and for identifying the most proper preparation method that leads to faster and more optimum physiological healing.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور المحتمل للبلازما الغنية بالصفائح في علاج إصابة القرنية المستحدثة جراحيا في الأرانب

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مقدمة : تعتمد صحة سطح العين والتي تعتبر ضرورية من أجل حدة الإبصار على استواء وسلامة النسيج الطلائي للقرنية .أيضا تعتبر البلازما الغنية بالصفائح إحدي الطرق العلاجية لجروح القرنية لما تحتوية من عوامل النمو المختلفة.
الهدف من الدراسة : تقييم البلازما الغنية بالصفائح كعلاج محتمل لتحسين و تعجيل التئام جروح القرنية المستحدثة جراحيا في الأرانب.
مواد وطريقة الدراسة : تم استخدام ثمانية عشر أرنبًا في هذه الدراسة. تم تحضير البلازما الغنية بالصفائح من الأرانب المعينة لذلك عن طريق أخذ عينة دم من الوريد الطرفي في الأذن. تم إحداث جرح في القرنية للمجموعتين الثانية والثالثة يشمل كلا من النسيج الطلائي من خلال تقشيره بواسطة مبضع القرنية واللحمة عن طريق إحداث أربع جروح قطعية متقاطعة بها. تم تقسييم الأرانب إلى ثلاث مجموعات: المجموعة الضابطة وهي المجموعة الأولى والتي لم تتلق أي علاج كما لم يتم إحداث جرح بها، ومجموعة المحلول الملحي وهي المجموعة الثانية،والتي تلقت قطرة من المحلول الملحي تركيز ٠,٩ ٪ والمجموعة الثالثة والتي تلقت البلازما الغنية بالصفائح من خلال الحقن تحت الجفن العلوي. تم التضحية بجميع الأرانب للمجموعات الثلاث بعد سبع أيام من التجربة ، ثم تم استخراج القرنية و معالجتها من أجل الفحص تحت المجهر الضوئي والمجهر الإلكتروني الماسح.
النتائج : أوضحت الدراسة أن العلاج بالبلازما الغنية بالصفائح قد أدى إلى التئام جرح القرنية بشكل أفضل مع استعادة التركيب المنتظم لنسيجها الطلائي واستعادة ألياف الكولاجين المنتظمة الخاصة باللحمة مقارنة بمجموعة المحلول الملحي.
الخاتمة : أدي استخدام البلازما الغنية بالصفائح إلى التئام جرح القرنية بفاعلية أفضل وأقرب إلى التشريح الفسيولوجي الأكثر انتظاماً في الأنسجة.