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Two Novel Techniques in the Treatment of Corneal Alkaline Burns: Platelet-Rich Fibrin Membrane and Concentrated Growth Factor Membrane: A Rabbit Model

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ABSTRACT

To investigate the effect of platelet-rich fibrin membrane (PRFM) and concentrated growth factor membrane (CGFM) on the healing of corneal alkaline burns. 24 New Zealand rabbits were divided into four groups equally. Alkaline burn was created in the cornea of each one of the rabbits in each group. Group I: Tarsorrhaphy was applied by fixing PRFM obtained from the whole blood samples of the subjects to the lower and upper conjunctivae to cover the cornea. Group II: Tarsorrhaphy was applied by fixing CGFM obtained from the whole blood samples of the subjects to the lower and upper conjunctivae to cover the cornea. Group III: Tarsorrhaphy was applied after alkaline burn was created in the cornea. Group IV: Alkaline burn was created in the cornea and the eye was left open. Fluorescein staining was applied to the subjects in all groups and their intraocular pressure was measured using a rebound tonometer. At the end of the Day 10, all animals in the groups were killed. Histopathological staining was performed and this was compared between the groups. As a result of clinical examinations, the most healing was seen in Group I and Group II, respectively. When evaluated in terms of epithelial regeneration formation, histopathological examinations were also seen to support clinical examination results.This study revealed that the treatment of corneal alkaline burns with PRFM and CGFM contributes positively to the healing process.

Key words

alkaline burns, blood, concentrated growth factor, cornea, platelet rich fibrin

Introduction

The cornea is a transparent and non-vessel structure that has an important function, such as refracting the light, to realize the visual function (Şaroğlu, 2013). Direct trauma, bacteria, virus, distichiasis, trichiasis, genetic predisposition, entropion, ectropion, ectopic cilia, chemical agents(acidic or alkaline burns) are included in the etiology of corneal lesions (Akın & Samsar, 2005; Şaroğlu, 2013)**.** Alkaline burns cause vascularization, ulceration, perforation and opacification on the corneal surface (Kim et. al., 2012; Yang et al., 2012). Many treatments have been tried in corneal lesions. Some of these methods are as follows: corneal tissue sealants (Gelatt, 2008; Martin, 2010) **,** conjunctival grafts (Cullen & Grahn, 2005; Sandmeyer et al., 2016), third eyelid flap (Gelatt, 2008), tarsorrhaphy (Gelatt, 2008) ,

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keratotomy (Gelatt, 2008), applications of soft contact lens (Grinninger et al., 2015), amniotic membrane application (Fernandes et al., 2005)**,** small intestinal submucosa application (Featherstone et al.,2001; Vanore et al., 2007), renal capsule application (Andrade et al., 1999), pericardial application (Dulaurent et al., 2014), stem cell applications (Ke et al., 2015;Rocca et al., 2015), platelet-rich plasma application (Ronci et al., 2015). It was found that all these methods have certain disadvantages and various complications. Platelets contribute to the healing process through factors such as hemostasis, angiogenesis, cellular proliferation, increased collagen production, stimulation of cell differentiation, and tissue regeneration (Broos et al., 2012). PRF introduced by Choukroun *et al.* (2001) and CGF introduced by Sacco *et al.*(2009), are second-generation platelet concentrates, consisting of many growth factors and cytokines. They play a key role in hemostasis and wound healing (Dohan et al., 2006; Lee et al., 2007; Can et al., 2016). Departing from such information, this study aims to investigate the contributions of PRFM and CGFM techniques in the treatment of experimentally induced corneal alkaline burns in rabbits.

Materials And Methods

Experimental animals: This was a prospective experimental animal study. The material of the study includes 24 New Zealand rabbits aged 12 months weighing 2-4 kg that were taken from The Bingöl University Experimental Research Center. Rabbits were kept in quarantine by Erciyes University, Experimental Research and Application Center for one week. Rabbits were fed *ad libitum* in single housing cages of ERÜ DEKAM. Besides, rabbits were kept on a 12-hour light-dark cycle at an ambient temperature of 21 ± 1°C with 50% humidity rate. At the end of the study, all rabbits were euthanized with sodium pentobarbital.

Fluorescein staining of the cornea: Fluorescein staining application was performed for all the subjects in the groups on Day 0, 1, and 10. The results obtained were graded as follows (Karabulut, 2018):

0: no staining on the all corneal surface

1: staining 1/8 or less area of the all corneal surface

2: staining 1/4 or less area of the all corneal surface

3: staining 1/2 or less area of the all corneal surface

4: staining all the corneal surface

The Schirmer tear test: The test strips (Schirmer Tear Test Strip) were folded from where they were marked and then they were unpacked and placed in the anteromedial one-third part of the lower eyelid conjunctival sac. A 60-second time was measured with a stopwatch, the test strip was removed from the conjunctival sac and the wetness in the test strip was evaluated in millimeters and recorded. The Schirmer tear test (STT) was performed for all subjects in the groups three times on Day 0, 1, and 10.

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Measurement of intraocular pressure monitoring by rebound tonometry: A Tonovet (Icare Tonovet, Vantaa, Finland) rebound tonometer was used in the study. The device was calibrated according to the manufacturer's instructions for use. The application was performed to all subjects in the groups on Day 0, 1, and 10.

Anesthesia: A combination of 5 mg/kg dose of Xylazine HCL (Rompun 2% Bayer Türk Kimya San. Ltd. Şti., İstanbul, Turkey) and a combination of 35 mg/kg dose of Ketamine-HCL (Ketasol 10% Richter Pharma AG, Wels, Austria) for general anesthesia was administered intramuscularly (IM) for each subject fasting subject for 12 hours prior to anesthesia. Subsequently, the subjects were intubated and connected to the inhalation anesthesia device (SMS 2000 Classic Anesthesia, Sms Medikal, Ankara, Turkey) and anesthesia was maintained with 2-3% sevoflurane (Sevorane Liquid 100% AbbVie, Queenborough, England).

Experimental Design: The subjects were trimmed under anesthesia in periorbital region until os frontal in dorsal and os zygamaticum in ventral; moreover, the trimmed region was made sterile ready by undergoing disinfection procedures. The subjects were placed on the operating table, positioned laterally, left eyes upright. The filter paper (in diameter of 3 mm) was soaked into 1 N sodium hydroxide (NaOH, Sigma-Aldrich, İstanbul, Turkey) and it was fixed in the center of the relevant eye cornea for 1 minute. At the end of the applied time, filter paper soaked in 1N NaOH was removed and the eye was washed for 2 minutes with 0.9% saline. Following that, the fluorescein sodium %2 (Alcon a Novartis Company,İstanbul, Turkey) was performed. All animals were administered butorphanol at 0.3 mg/kg subcutaneously following surgery.

Preparation and Application of PRF Membrane: For each subject in Group I, 5 mL of blood taken from the vena marginalis in the ear periphery was collected in a sterile tube without anticoagulation and it was centrifuged in the centrifuge device (Rotofix 32 A, Hettich Lab Technology, Tuttlingen, Germany) at 400 g force for 12 minutes. At the end of this process, the product in the tube was divided into 3 layers. These layers consisted of erythrocyte on the lowest layer, platelet-rich fibrin in the middle layer, and cell-free plasma on the top layer. The platelet-rich fibrin was placed between the two gauzes and the serum was removed by pressuring slightly with the fingers and thus, a platelet-rich fibrin membrane was obtained. The membrane was placed on the left eye cornea with experimental alkaline burn and it was fixed to the conjunctiva with 6/0 nylon suture (Ethilon, Johnson & Johnson Medical N.V., Belgium) by applying simple separate stitches and eyelids were stitched with a 3/0 nylon suture (Ethilon, Johnson & Johnson Medical N.V., Belgium) and temporary tarsorrhaphy was performed.

Preparation and Application of CGF Membrane: For each subject in the Group II, 5 mL of blood taken from the vena marginalis in the ear periphery was collected in a sterile tube without anticoagulation and it was centrifuged in the centrifuge device (Medifuge CGF) (in acceleration for 30 seconds) at 408 g for 2 minutes, at 323 g for 4 minutes, at 408 g for 4

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minutes, at 503 g for 3 minutes, and 36 seconds slow down and stop process. At the end of this process, the product in the tube was divided into 4 layers. Serum was formed on the top layer (4th layer), fibrin white blood cells beneath it (buffy coat) (3th layer), growth factors and unipotent stem cells beneath it (2nd layer), and clot on the bottom layer (1st layer). The middle part with concentrated growth factor was placed between the two sterile gauzes and the serum was removed by pressuring slightly with the fingers and thus, a resistant membrane was obtained. The membrane was placed on the left eye cornea with experimental alkaline burn and it was fixed to the conjunctiva with 6/0 nylon suture (Ethilon, Johnson & Johnson Medical N.V., Belgium) by applying simple separate stitches. Eyelids were stitched with a 3/0 nylon suture (Ethilon, Johnson & Johnson Medical N.V., Belgium) and temporary tarsorrhaphy was performed.

Application of Temporary Tarsorrhaphy: Eyelids of the rabbits in Group III of which alkaline burn was created in the left eye cornea were stitched with a 3/0 nylon suture (Ethilon, Johnson & Johnson Medical N.V., Belgium) and temporary tarsorrhaphy was performed.

Process of Enucleation and Tissue Collection: All subjects in four different groups were euthanized at the end of the 10 th day by applying Na-pentobarbital (Ekipental 0.5 gr, Tüm Ekip İlaç, İstanbul, Turkey) at a dose of 100 mg/kg intravenously (IV). The left eyes of the rabbits were enucleated for histopathological evaluation.

The Preparation of Histology Slides and Grading of the Staining Pattern: Samples were taken from the experimentally formed corneal ulcer region in the left eyes of the subjects, including normal corneal tissue. The samples taken were left in a 10% formol solution. After the fixation, tissue samples were trimmed and they were passed through alcohol-xylol series and blocked in paraffin-embedded tissue blocks. 5-6 micron thick sections of tissue were prepared from paraffin blocks and examined under light microscope by staining with Hematoxylin-Eosin (HE, Sigma-Aldrich, İstanbul, Turkey) stain. Mononuclear cells infiltration, collagen bundles, neovessels, and regeneration in the area where the experimental corneal alkaline burn was created were examined in histopathological examination.

Statistical Evaluation: Results were shown as mean ± Standard error mean (SEM) and $p < 0.05$ value was considered statistically significant. One-way analysis of variance (ANOVA) was used to show differences between groups, while Tukey's post-hoc test was chosen for comparisons. The Kruskal-Wallis test was used to determine the statistical difference in the scoring, and the Mann-Whitney U-test was chosen as post hoc for paired comparisons. The Shapiro-Wilk test was performed to determine whether data distributed normally. IBM SPSS 22.0 for Windows (SPSS Inc.) program was used for statistical analysis. GraphPad Prism 5.0 (GraphPad software Inc.) program was preferred for creating graphics.

Results

Clinical findings: For all subjects in the group, epithelial cell losses in the central cornea following the formation of alkaline burns were noted. Since the standard application was performed to create an experimental corneal alkaline burn, it was observed that the corneal

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burn was equal in all groups. Afterwards, fluorescein staining test was applied and the damage caused was detected.

In the examination performed on the first day after the experimental corneal alkaline burn was created, clinical findings of photophobia, corneal opacity, pain, and blepharospasm were observed in all subjects. Group I: In this group where PRFM was applied, no complications were developed pre-, during, and post-operation. The subjects' left eyelids where stitches were applied were followed for 10 days for the continuity of the stitches applied for the infection and tarsorrhaphy procedure. No tear discharge or infection was observed during this process. At the end of the tenth day, the sutures of the subjects whose tarsorrhaphy was opened fixed to their conjunctiva were removed. No discharge, infection, pigmentation, and the edema of cornea was observed in the eyes. When evaluated in terms of corneal opacity, in all subjects in this group, 100% full opacification was observed on the 1st day, whereas the examination of the left eyes on the 10th day revealed that opacity became translucent in all subjects. Group II: In this group where CGFM was applied, no complications were developed pre-, during, and post-operation. The subjects' left eyelids where stitches were applied were followed for 10 days for the continuity of the stitches applied for the infection and tarsorrhaphy procedure. No tear discharge or infection was observed during this process. At the end of the tenth day, the sutures of the subjects whose tarsorrhaphy was opened fixed to their conjunctiva were removed. No discharge, infection, pigmentation, and the edema of cornea was observed in the eyes.

PRFM and CGFM were found to be both ideals for the reconstruction of the ocular surface in terms of micro and macro architecture, and they can be easily stitched due to their solid structure. In the group where PRFM was applied, it was found that membranes disappeared when the temporary tarsorrhaphy was opened at the end of the tenth day; in the group where CGFM was applied, at the end of the tenth day, membrane residues were found around the 6/0 nylon suture, which was fixed only on the conjunctiva. Group III: In this group, where only tarsorrhaphy treatment was applied as treatment, no complications were observed during the operation. In the postoperative period, it was observed that there was discharge in the eyes of the subjects numbered 1, 3, and 4. Ofloxacin 0.3% (Exocin, Allergan Pharmaceuticals, Ireland) was used topically in the relevant eye for medical purposes (2 drops morning and evening for 5 days). Evaluating in terms of corneal opacity, in all subjects in this group, while 100% complete opacification was observed on the first day as corneal opacification, as a result of the examination of the left eyes on the tenth day, opacification was found to be translucent in the peripherals of corneal burns in all subjects, and there was still opacification in the center. Group IV: 0.9% saline was dropped in the cornea burns in the left eyes of the subjects in this group and tarsorrhaphy was not applied and their eyes were left open. Ocular discharge, photophobia, corneal opacity, blepharospasm were observed in all subjects in this group. To control the infection, Ofloxacin 0.3% (Exocin, Allergan Pharmaceuticals, Ireland) was used daily in the relevant eye topically (2 drops morning and evening for 5 days). Evaluating in terms of corneal opacity, in all subjects in this group, while 100% complete

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opacification was observed on the first day as corneal opacification, as a result of the examination of the left eyes on the tenth day, opacification was found to be translucent in the peripherals of corneal burns in all subjects, and complete opacification was identified in many areas, including the central cornea.

As the eyes of all subjects in the groups were healthy on Day 0, since the corneal epithelium did not adhere stain when fluorescein staining was applied, this value was determined as 0 according to the grading scale. On Day 1, as the standard procedure to the eyes of all subjects in the groups, the filter paper (in diameter of 3 mm) was soaked into 1 N NaOH and it was kept in the center of the relevant eye cornea for 1 minute. At the end of the applied time, filter paper soaked in 1N NaOH was removed and the eye was washed for 2 minutes with 0.9% saline. The degree of corneal burns that occurs in this way was found as 4 according to the grading scale when fluorescein staining was performed. On Day 10, fluorescein staining was applied to all subjects in the groups (Figure 1). It was observed that the most staining was in the subjects in Group IV, while subsequently in Group III and Group II respectively, and the least capacity of fluorescein staining adherence was in the subjects in Group I. A substantial increase was found in IOP values of all subjects in the groups on Day 1, when experimental corneal alkaline burn was created. After the measurement using the tonometer, the IOP values in Group I and Group II were found within normal limits on Day 10. In the measurement using a tonometer, the IOP values in Group III and Group IV were found to increase on Day 10 compared to Day 0, and it was seen to exceed the normal limits. On the 1st day of experimental corneal alkaline burn, an increase in tear values was observed in all groups. According to the Schirmer test repeated on the 10th day, normal tear values and significant improvement were found in Group I and Group II according to the STT-1 result, while tear values decreased significantly and dry eye syndrome occurred in Group III and Group IV.

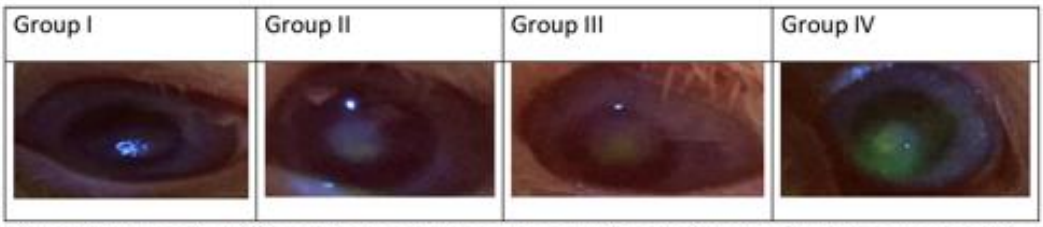

Figure 1: Fluorescein staining application on the corneas, on which experimental alkali burns were created, on Day 10 (under cobalt blue light)

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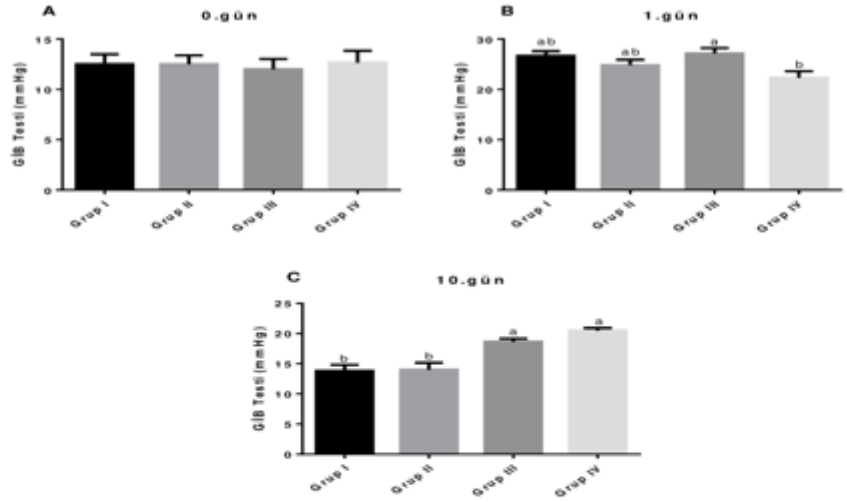

Figure 2: The bar graph shows the Intraocular Pressure values in the rabbit on Day 0 (A), Day 1 (B), and Day 10 (C), respectively. Error bars give the standard error mean. Lowercase letters on each bar show statistically significant difference between groups; p<0,05, Turkey's Post hoc test.

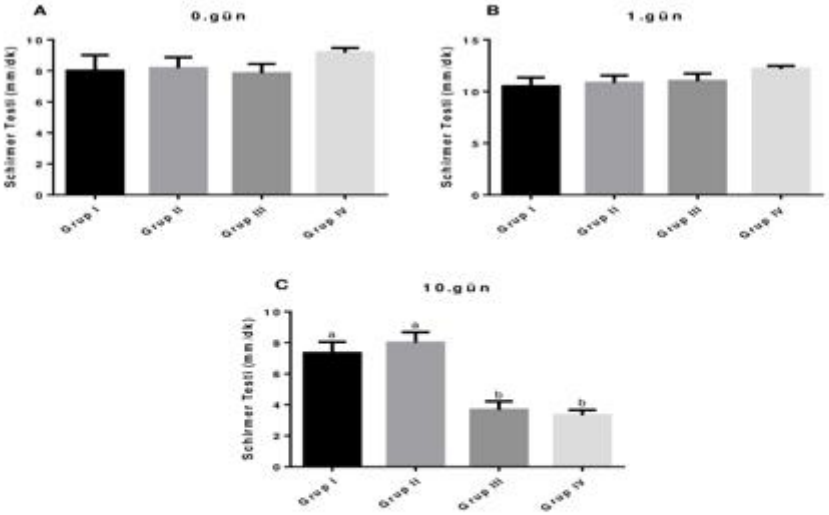

Figure 3: The bar graph shows the Schirmer level in the rabbit on Day 0 (A), Day 1 (B), and Day 10 (C), respectively. Error bars give the standard error mean. Lowercase letters on each bar show statistically significant difference between groups; p<0,05, Turkey's Post hoc test.

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Cases	Day 0	Day 1	Day 10
Group I	0 ± 0	4 ± 0	0.5 ± 0.5
Group II	0 ± 0	4 ± 0	2 ± 0
Group III	0 ± 0	4 ± 0	3 ± 0[a]
Group IV	0 ± 0	4 ± 0	4 ± 0[a,b]
p* value	>0.05	>0.05	<0.0001

Table 1. Grading of groups according to adherence capacity of Fluorescein staining
Data were given as mean ± Standard error.
* The Kruskal-Wallis test was used to determine the statistical difference and the Mann-Whitney U-test was selected as post hoc for paired comparisons.
[a] Indicates a significant difference against Group I in the same column.
[b] indicates a significant difference against Group II in the same column.

Histopathological Results

Corneal damage and regeneration: Group I; In the subjects in this group, it was found that collagen bundles were well-formed by intense fibroblast proliferation in the corneal stroma and capillary vessel proliferations were observed in the stroma in the corneal limbus. The anterior limiting membrane of the cornea was observed to be well-formed in all but one subject and epithelial regeneration was observed to occur better than other groups (Figure 4.A). In the subjects in this group, a small number of mononuclear cell infiltrations with mild inflammatory reaction were observed in the application areas but no epithelial cells were observed. Group II: Regeneration was observed in the corneal epithelium where the application was performed microscopically and the epithelial cell cytoplasm was found with vacuolar appearance. The anterior limiting membrane of the cornea and epithelial regeneration was found to be more irregular than Group I. It was observed that collagen fibers were evident in corneal stroma (Figure 4.B). Collagen bundles were well-formed in the corneal stroma in this group, and they were with the appearance of loose connective tissue. Capillary vessel proliferations were observed in the connective tissue in the corneal limbus. In connective tissue, mild inflammatory reaction and mononuclear cell infiltrations were observed. Group III: In terms of microscopic examination, fibroblast proliferation and collagen bundles were less and irregularly formed in the corneal stroma compared to subjects in the other two groups and capillary vessel proliferation in the stroma in the corneal limbus were found to be less. Inflammatory reaction and mononuclear cell infiltration were observed in stromal connective tissue. No necrotic cells were found in the epithelial layer. The anterior limiting membrane of the cornea and epithelial regeneration were more irregularly formed than the other two groups (Figure 4.C). Group IV: It was observed that epithelial regeneration was not fully formed and the single-layer epithelial cells were formed irregularly. It was noted that a loose connective tissue was formed, with mild collagen increase in the stroma and

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mononuclear cell infiltrations (Figure 4.D). When all groups were compared, the best epithelial regeneration, capillary vessel proliferation, and the formation of collagen bundles were observed in Group I, and it was followed by Group II. Healing in Group III was observed to be less than Group I and Group II, while healing in Group IV was at the least level than other groups.

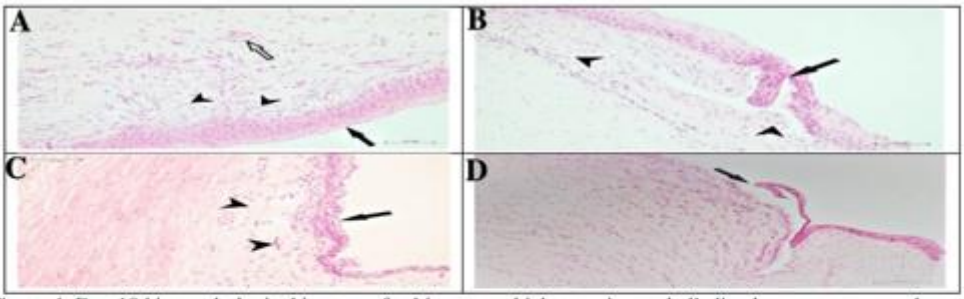

Figure 4: Day 10 histopathological images of subjects on which experimental alkaline burns were created

Figure 4. A: Group I, Regeneration in corneal epithelium (dark-colored arrow), collagen bundles in stroma (arrowheads) and capillary vascular proliferations (light-colored arrow), HEx20µ

Figure 4. B: Group II Irregular regeneration in corneal epithelium (arrow), collagen increase in corneal stroma (arrowhead). HExµ

Figure 4. C: Group III, Irregular epithelial regeneration (arrow), collagen bundles in stroma and mononuclear cell infiltrations (arrowheads), HEx20µ
Figure 4. D: Group IV Irregular epithelial regeneration (arrow), HEx20µ

Discussion

Corneal alkaline burns are one of the eye diseases with high potential to cause serious visual impairment for both humans and animals. The healing process of the corneal surface depends on a complex pool of growth factors that, as with other tissues, interact and coordinate with the main biological events of tissue regeneration. The presence of corneal receptors was revealed for a large number of growth factors found in platelet granules (Schultz et al.,1994). Platelet-rich fibrin, introduced by Choukroun et al. (2001) and concentrated growth factor, introduced by Sacco et al. (2009), are platelet concentrates, consisting of many growth factors and cytokines, which play a key role in hemostasis and wound healing (Taylor et al., 2011; Albanese et al.,2013; Can et al., 2016). Growth factors such as Epidermal growth factor (EGF), transforming growth factor/beta (TEBF-β1), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) bind to transmembrane receptors in damaged tissue, and thus, cell differentiation, proliferation, migration, angiogenesis begins (Şaroğlu, 2013; Nurden, 2018). In a study where the platelet-rich plasma was applied, in the treatment of ocular burns, it was stated that there was a significant statistical decrease in corneal healing time and conjunctival cicatrization (Márquez-de-Aracena et al., 2007). Sanchez-Avilla et al. (2018) administered a treatment of plasma rich in growth factors (PRGF) membrane to a group of patients who had various corneal lesions and previously failed medical treatments such as therapeutic contact lenses (TCL), corticosteroids, artificial tears, nonsteroidal anti-inflammatory drugs and

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operative treatments such as amniotic membrane transplantation (AMT) and keratoplasty. It was reported that there were no side effects in these patients and 87% of them had a full recovery. The common point of these studies was the fact that they contain more growth factors in their structure than normal blood. In this way, successful results were obtained in various corneal lesions. In our study, we have clearly demonstrated that the effect of PRFM and CGFM on the healing of corneal alkaline burns was supportive due to its biological and physical properties. However, the fact that PRFM and CGFM that do not require sodium citrate use such as PRP and PRGF are obtained only from the patient's own blood also provides convenience. In addition, Marquez et al. (2007) stated that the use of fibrin skeletal structure of platelet concentrates by converting into membrane results in reduced repair and epithelialization time of the cornea and conjunctiva and provides better corneal clarity and visual acuity (Dohan et al., 2006) . In our study, in parallel with this information; we found that epithelialization and corneal clarity levels in subjects in Group I (where we applied PRFM, one of platelet concentrates), and in Group II (where we applied CGFM) were satisfactory compared to Group III with tarsorrhaphy and Group IV with saline 0.9%. In addition, when Fluorescein staining was performed, significant reductions in corneal staining were observed in Group I and Group II. When these results were evaluated, it was seen that re-epithelialization in the cornea was satisfactory in a short period of 10 days. When we compare PRFM and CGFM, which are among the platelet concentrates that we used in our study and where the most clinically satisfactory improvement was observed, in terms of epithelialization and corneal clarity, subjects in Group I where PRFM was applied were found to be superior than the subjects in Group II where CGFM was applied. Our clinical results were found to be compatible with histopathological results. Another method used for the reconstruction of the ocular surface is AMT (Alemañy Gonzalez & Camacho Ruaigip, 2006; Can et al.,2016). The amniotic membrane supports epithelialization by releasing growth factors such as epithelial growth factor and keratocyte growth factor, which are important for healing. However, some eye studies have revealed that AMT was inadequate to treat tissue adhesives and grafts taken from animals (Feng et al., 2014; Can et al.,2016). The amniotic membrane contains both anti-inflammatory and pro-inflammatory cytokines and other molecules that have adverse effects. However, the amniotic membrane is an irregular biological tissue due to variations in its quality (Rahman et al., 2009). It has several disadvantages: the risk of exposure to biological contamination, non-standard production protocols, its complicated and expensive preparation process, risk of transmission of viral infection from the donor and the need for storage (Liu et al., 2010). Additionally, since the amniotic membrane is a natural but allogenic graft, the immunological response is a major problem. However, PRFM and CGFM are autologous and are obtained from the patient's own blood and therefore, there is no risk of immunological rejection in the tissue (Can et al., 2016). Preparation of PRFM and CGFM is practical and cost effective. No tissue bank is needed for its production. Since it is prepared and applied in the same place during the operation, no additional hardware is required for storage. One of the methods frequently used for ocular surface reconstruction is conjunctival grafts. However, they are reported to leave

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residual tags on the cornea that may prevent vision (Bussieres et al., 2004). In our study, we did not find any corneal leucoma in PRFM and CGFM. In addition, in cases with both corneal damage and extensive conjunctival damage, conjunctival graft application to the cornea was insufficient. The fact that PRFM or CGFM obtained from his own blood of a patient with this condition can be easily applied to both the conjunctiva and the cornea is another important advantage of our study. STT-1 was applied to all subjects in our study to detect eye dryness that may occur after alkaline burn. Therefore, the relationship between dry eye and use of PRFM and CGFM was also examined. Looking at the results, an increase in tear values was seen in all groups on Day 1, when experimental corneal alkaline burn was created. According to the results of the Schirmer test repeated on Day 10, normal tear values and significant improvement were found in Group I and Group II compared to the STT-1 result while tear values significantly decreased in Group III and Group IV and dry eye syndrome occurred. Based on these results, it was concluded that PRF and CGF, which are among blood products, may be used as a therapeutic agent for dry eye syndrome in the future. Corneal alkaline burns can lead to increased intraocular pressure (Şaroğlu, 2013) . Therefore, the relationship between IOP and PRFM and CGFM was also examined in our study. Accordingly, all subjects in the groups had high IOP on day 0, while Group I and Group II were found to be within normal values on day 10. In vitro and in vivo studies have shown that blood products can modulate TEBF-β activity, reduce tissue fibrosis, and promote c ell regeneration (Riestra et al., 2016). These mechanisms are thought to be able to explain the decrease in intraocular pressure (Sanchez-Avila et al., 2018) . In light of this information, we concluded that membranes obtained from blood products, PRF and CGF, can be used in the treatment of glaucoma.

Conclusins

Our study, it was concluded that the membranes obtained from the blood products such as PRF and CGF did not cause an immunological reaction since they were obtained from the subject's own blood, and thus, they can be used safely. It was seen that obtaining membranes is extremely easy, and they are very practical methods since they are obtained shortly before the operation and used without waiting. In our study, we found that PRFM and CGFM applications were not only practical but also contributed positively to healing of the cornea. Since platelets secrete antibacterial proteins with antibiotic effects, it was found that there were no symptoms of infection and inflammation in the eye of the subjects in Groups of PRFM and CGFM. This was seen to be able to provide healing in the postoperative period without the need for use of antibiotic preparations topically. Thanks to this feature, it was concluded that it can be easily used in organic animal production. It was clinically and histopathologically proven that the most satisfactory results were obtained in Group I with PRFM and then in Group II with CGFM. In line with these data, it was concluded that PRFM and CGFM are safe methods without any side effects in the treatment of corneal alkaline burns. According to the literature review, our study is the first study to use PRFM and CGFM in experimental corneal alkaline burn treatment. Therefore, it is thought that more clinical

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studies are needed. However, we think that treatment with PRFM and CGFM will contribute to clinical practice in ophthalmology for human and veterinary in healing clinical findings and associated pathology symptoms in patients with corneal alkaline burns. Although there is no study on PRFM and CGFM in patients with dry eye syndrome and glaucoma progressing with keratitis due to alkaline burns, we believe that PRFM and CGFM will make positive contributions when used in the treatment of diseases mentioned.

Ethics approval. The study was approved by the Ethics Committee of the Erciyes University (protocol number 17/013).

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Declaration of interest. The authors declare that they have no competing interest.

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