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Albumen Glue, New Material for Conjunctival Graft Surgery, an Animal Experiment

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Abstract

Attach conjunctival graft commonly used are suture technique and fibrin glue. This study was to investigate albumen glue as an alternative to suture technique in attaching conjunctival graft in rabbits. Aim of this study was to compare the conjunctival wound healing between albumen glue and suture technique in rabbit eye as a model. There was an experimental animal study included 32 eyes (16 rabbits) in PT. Bio Farma (Persero) and Histology Laboratory, Faculty of Medicine, Padjadjaran University from March 2014 to July 2014. The study consisted of albumen glue group and suture technique group. The examination included the comparison of conjunctival graft attachment and histologic examination by microscopically was done to obtain the wound gap, then analyze by Mann-Whitney test. The results indicated that the graft attachment was significantly better using albumen glue (grade 4) compared to suture (grade 2-3) on day-1 after surgery ($p=0,000$). The wound gap was smaller using albumen glue (0-0,33 μm versus 5,33-14 μm ; $p=0,0005$) on 10 minutes after surgery and 0 μm versus 0,33-4 μm ; $p=0,0005$ on day-7 after surgery. In conclusion, the graft attachment using albumen glue was better and the wound gap was smaller using albumen glue than suture technique.

Keyword : albumen glue, conjunctival wound healing, suture

1. Introduction

Conjunctival graft has been used in the field ophthalmology. In pterygium surgery, this technique was first promoted by Kenyon et al. in 1985 [1,2]. Pterygium is one of eye problem in Indonesia, as this country is located in the equatorial and has tropical climates. Morbidity of pterygium in Indonesia ranks as second (13.9%) after cataract. Total of pterygium patients visit in Cicendo Eye Hospital in Bandung during 2011 was 2158, and the number of bulbar conjunctival graft using sutures technique was 358 eyes [3]. Bulbar conjunctival graft technique may decrease pterygium grows after the operation, but may cause dissatisfaction for operators and patients due to its sutures [4]. In addition to sutures, methods used today for attaching conjunctival graft can also be using fibrin glue [5].

Bulbar conjunctival graft attachment using a suture technique is micro-surgically complex, requiring a high level of operator learning, long operation time and wound healing, and also can cause complications including retraction tissue grafts, granuloma formation, infection, and death of tissue grafts. Additionally, using a needle, cord, and suture knot will damage the tissue graft, which can cause poor adhesion tissue grafts. Another disadvantage of this technique, it will cause more tissue manipulation so the graft tissue adhesion and healing will be time-consuming [6]. Albumen is a clear liquid contained in an egg [7]. Albumen can repair burns injury due to its natural collagen form. Collagen is the main component of extracellular matrix (ECM) that supports the growth of cells and tissues. Besides the ability to support, ECM strongly affects embryonic development, cell migration flows, signaling, and supply growth factor [4,8].

The main protein consisting in egg albumen is including ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme. Ovalbumin is the highest content in egg albumen, which is 54% and acts as a cell adhesion substrate that serves to support the attachment and cell adhesion [9]. Tissue transglutaminase contained on the surface of conjunctival cells, play a role in cell adhesion processes



and can interact with the ECM. It also plays a role in wound healing [10]. Transglutaminase bond with the matrix protein, such as egg white can enhance cell adhesion.

2. Materials and Methods

This research is an experimental animal study to determine the degree of rabbit conjunctival graft tissue attachment to the essential tissue (sclera) and comparison slit wounds (wound gap) between the albumen glue and suture technique. The inclusion criteria New Zealand White rabbits breeding Bio Farma age 2-3 months, weight 2-2.5 kg, healthy physical (including health both eyes), duck egg albumen healthy local cultures derived from eggs are not older than 3 days. Exclusion criteria were to have two eyes with congenital anatomical abnormalities seen in general, only one eye is healthy, weight loss occurs when quarantined.

The research was conducted after the approval of the Commission and Use of Laboratory Animal Welfare (Institutional Animal Care and Use Committee) PT. Bio Farma (Persero). Place of research at PT Bio Farma Bandung and Histology Section of Faculty of Medicine, University of Padjadjaran, the period from March to July 2014. The subject of the study 16 rabbits (32 eyes) appropriate inclusion criteria and quarantined for one week more, then divided randomly into two groups: group I : albumen glue technique then suture technique; Group II: suture technique then albumen glue technique. The operation includes the manufacture of conjunctival graft, graft attachment in an open sclera same eye using albumen glue were made sterile suture technique using 8-0 vycril thread three pieces. The degree of adhesion conjunctival graft between albumen glue and suture technique assessed on day-1 after surgery by a veterinarian who has received training from an ophthalmologist. After one week, the other eye treated the same as the previous eye. Ten minutes the surgery, the ball left, and right eye of each rabbit was extracted in the anesthetized state. Histological preparations were created by 0.5-1 cm conjunctival tissue of the eye with hematoxylin eosin staining and viewed with a light microscope using a magnification of 100x by an expert doctor histology. The assessment is based on the size of the wound gap the rabbit conjunctival graft between albumen glue and suture technique on 10 minutes after and on day-7 after surgery.

Statistical test using the Mann-Whitney test with consideration of the small sample size (n = 8).

3. Results and Discussion

The result assessed by comparing the grade of bulbar conjunctiva attachment on day-1 after surgery and wound gap at ten minutes and on day-7 after surgery between albumen glue and suture technique.

3.1 Bulbar Conjunctiva Attachment Grading in Rabbit Eyes

We put the result on conjunctival graft tissue attachment to base tissue (sclera) on the criteria that consists of grade 1, 2, 3, and 4, which conducted on day-1 after surgery.

Table 1. Analysis of Bulbar Conjunctival Graft Attachment Grade in Rabbit Eye Between Albumen Glue Technique and Suture Technique on Day-1 After Surgery

Group	Number of eyes and attachment grade				Mann-Whitney Test	
	1	2	3	4	Z	p
Albumen glue technique (8 eyes)	0	0	0	8	-3,771	0,000
Suture Techniques (8 eyes)	0	1	7	0		

Description: p: the value of significance

Grade 1 of attachment : percentage of attachment $\leq 25\%$

Grade 2 of attachment : percentage of attachment $> 25\% - 50\%$

Grade 3 of attachment : percentage of attachment $> 50\% - 75\%$

Grade 4 of attachment : percentage of attachment $> 75\%$

Table 1 shows the significant differences in the grade of attachment of the bulbar conjunctival graft on day-1 after surgery between albumen technique (grade 4) and suture technique (the value range between grade 2 and 3) with a p-value = 0.000.

3.2 Wound gap result

Histologically, we compared wound gap on the use of albumen glue technique and suture technique by assessing the microscopic picture and measured gap between the donor and recipient tissue.

Table 2. Comparative Analysis of Wound Gap between Albumen Glue Technique and Suture Technique at 10 minutes After Surgery (μm)

Rabbit	Average Albumen (n=8 eyes)	Suture (n=8 eyes)	P
1	0	11,67	0,0005*
2	0	5,33	
3	0	14,00	
4	0	9,67	
5	0	12,33	
6	0,33	12,33	
7	0	11,00	
8	-	10,67	

Description: p: the value of significance (Mann-Whitney)

Sign (-) indicates that wound gap of albumen glue cannot measured, then dropped from the analysis

Table 2 shows there is a significant difference between the average size of the wound gap in albumen glue technique (0 to 0.33 m) and suture technique (5,33-14 μm) at 10 minutes after surgery with p-value = 0.0005.

Table 3. Comparative Analysis of Wound Gap between Albumen Glue Technique and Suture Techniques on day-7 After Surgery (μm)

Rabbit	Average Albumen (n=8 eyes)	Suture (n=8 eyes)	P
1	0	4,00	0,0005*
2	0	0,67	
3	0	2,67	
4	0	0,33	
5	0	-	
6	0	-	
7	0	-	
8	0	-	

Description: p: the value of significance (Mann-Whitney)

Sign (-) indicates that wound gap of albumen glue cannot measured, then dropped from the analysis

Table 3 shows there is a significant difference between the average size of the wound gap between albumen glue technique (0 m) and suture technique (0,33-4 μ m) on day-7 after surgery with a p-value = 0.0005.

Table 4. Analysis of Wound Gap Difference between Luka Gap Between Albumen Glue Technique and Suture Technique at 10 minutes and on day-7 After Surgery

	Wound Gap 10 minutes after surgery	Wound gap On day-7 after surgery	p	Delta
Albumen glue technique	0,05 \pm 0,13 μ m	0,00 \pm 0,00 μ m	0,1425	0,05 μ m
Suture technique	10,88 \pm 2,59 μ m	1,92 \pm 1,73 μ m	0,003	8,96 μ m
P	0,0005	0,0005		
Delta	-10,83 μ m	-1,92 μ m		

Description: p: the value of significance (Mann-Whitney)

Table 4 shows that wound gap in albumen glue technique on day-7 after surgery was not significantly smaller than the 10 minutes after surgery, with p-value = 0.1425. The average difference (delta) wound gap between 10 minutes and on day-7 after surgery for albumen glue technique was 0.05-0.00 = 0.05 μ m. While in suture technique, wound gap on day-7 after surgery was less than on 10 minutes after surgery. The average difference (delta) between 10 minutes and on day-7 after surgery for suture technique was 10.88 to 1.92 = 8.96 μ m.

3.3 Hypothesis testing

Hypothesis 1: The grade of conjunctival graft attachment in the rabbit with albumen glue technique is better than suture technique. Testing : based on Mann-Whitney test, grade of bulbar conjunctival graft attachment on day-1 after surgery in albumen glue technique is significantly better than suture technique (Table 1). Mann-Whitney test results obtained statistical value of $U = 0.000$ and $Z = -3.771$ with a chance of error or p-value = 0.000. It appears that ($Z = -3.771$) < (Z tabel = -1.645) or (p-value = 0.000) < ($\alpha = 0.05$): significant. Z table values obtained from normal Z table, in error rate of 5% of 1-side type test. This finding shows that using albumen glue technique to transplant the bulbar conjunctival graft proven to be proficient in producing better attachment grade than suture technique on day-1 after surgery.

Conclusion: Based on the above test, the first hypothesis is accepted (tested).

Hypothesis 2: Wound gap of conjunctival graft in the rabbit with albumen glue technique is smaller than suture technique.

Testing:

- Results of Mann-Whitney U statistical test = 0.000 and $Z = -3.350$ with a chance of error or p-value = 0.0005. It appears that ($Z = -3.350$) < ($-Z$ tabel = -1.645) or (p-value = 0.001) < ($\alpha = 0,05$): significant. Z table values obtained from normal Z table, in error rate of 5% of 1-side type test. This finding shows that albumen glue technique in bulbar conjunctival graft was proven to have 10-minute postoperative wound gap smaller than suture techniques.
- Results of Mann-Whitney U statistical test = 0.000 and $Z = -3.233$ with a chance of error or p-value = 0.0005. It appears that ($Z = -3.233$) < ($-Z$ tabel = -1.645) or (p-value = 0.0005) < ($\alpha = 0,05$): significant. Z table values obtained from normal Z table, in error rate of 5% of 1-side type test. This finding shows that albumen glue technique in bulbar conjunctival graft was proven to have wound gap smaller than suture techniques on day-7 after surgery.

Conclusion: based on test results above, the second hypothesis is accepted (tested).

3.4 Tissue Attachment

The attainment of the bulbar conjunctival graft is tempted by the attachment of graft tissue to the base tissue, so vascularization occurs optimally and tissue grafts can survive and be healthy. Treatment of bulbar conjunctival graft using suture technique should be carefully done because the tissue graft is very thin and easily torn by the withdrawal of sutures, and the knot will damage the conjunctival graft of donor and recipient. Thus, the more manipulation conducted in this technique, will lead to failure of tissue graft attachment and slow healing process. In contrast to the use of albumen glue as an alternative to suture technique, bulbar conjunctival will stick to base tissue adequately because ovalbumin, which is the most numerous protein in albumen acts as a substrate adhesion functioning in support cell attachment [9].

In this study, albumen was taken from the duck egg. The duck egg is known to have more albumen than chicken eggs by 60%. Based on these explanations, the researchers decided to use duck egg albumen. Duck eggs were used in this study were less than three days of age, as according to Q Huang et al. study, stated that ovalbumin would gradually turn into S-ovalbumin, which has a lower viscosity than ovalbumin and may affect adhesion process. The formation of S-ovalbumin affected by pH, temperature, duration or life-length of eggs. S-ovalbumin increased to 91.24% at the age of 12 days, with a 37°C of temperature [11].

In this study, the grade of attachment of rabbit conjunctival graft with albumen glue technique obtained to be better than suture on day-1 after surgery, p-value = 0.000. There were no similar studies reported, but in medicine research, there have been several studies reported to using ovalbumin of albumen glue tissues such as research conducted by Gabrielle et al. that used ovalbumin to reconstructed bone tissue. It was reported that ovalbumin could improve bone healing because of this protein biopolymer, which consisting of 380 amino acid, is equivalent to 10% amino acid content in human serum albumin. Gabrielle et al. study showed that ovalbumin as an adhesion tissue substrate that affects cell growth, proliferation, and differentiation of preosteoblast cells for bone tissue regeneration [12]. Kaiparettu et al. showed a growth of mammary epithelial cells in humans and mice using albumen as a three-dimensional cell culture media. It described that albumen has a function as an ECM that support growth and cell development. Sergey et al. reported that there were transglutaminase and ECM protein bond that play a role in cell adhesion. Transglutaminase is a soluble enzyme in cells and plays a role in cell adhesion. Transglutaminase activity increases when injuries occur, so we can say that the transglutaminase and albumen bond as ECM proteins increases cell adhesion and will encircle wounds that help to protect cells from further damage so that wound healing can occur [9,10].

In this study, tissue attachment on albumen glue technique is denser than suture technique, so conjunctival tissue donor was possible to adequately attached to the base tissue without tissue retraction.

In tissue graft, cell and surrounding tissue injury will occur, the surgical trauma that occurs will depend on the performed technique and will result in tissue healing. Acceleration of tissue healing is strongly induced by the quality of the tissue attachment. The phase of wound healing on the first day that stands out is the inflammatory phase. Injury due to surgical incision in the conjunctiva is the extracellular matrix, cells, and blood vessels. This will lead to the formation of excess cytokines, causing irregularities in wound healing [4,14]. Chronic wounds will form necrotic tissue or contained foreign material that cannot be phagocytosis during acute inflammation. Lymphocytes, monocytes, and macrophages will stay on the sites of inflammation. The macrophages attract fibroblasts and in a long time will produce amount of collagen, form mass slowly from the fibrous tissue called granuloma [15]. Suture technique is considered as additional trauma to the conjunctival graft due to needle cut and is also considered as trauma for the cell, ECM, and blood vessels while the cord is a foreign body and knot can cause irritation and inflammation on the tissue. It can also occur granuloma formation and prevent settlement of tissue grafts and slow healing process [4,6]. Angiogenesis, growth, and proliferation of new blood vessels is one of the special marks on the histological tissue remodeling of

inflammation which caused by allergies. Clinically, there are symptoms such as itching, redness, photophobia, and watery [16]. However, this study did not assess the symptoms mentioned above.

3.5 Wound Gap

The healing process of the conjunctiva is faster than healing process of the skin. Kenyon et al. observed that conjunctival graft has been attached to the sclera and got vascularization within 3-5 days postoperatively [17]. Conjunctival epithelial is permeable epithelium because its close attachment between epithelial cells and goblet cells, so the protein that is administered topically will seep easily into the lumen of the conjunctiva capillaries [18]. In this study, 10 minutes and seven days postoperatively wound gap for albumen glue technique were obtained, and it was significantly smaller than the suture technique.

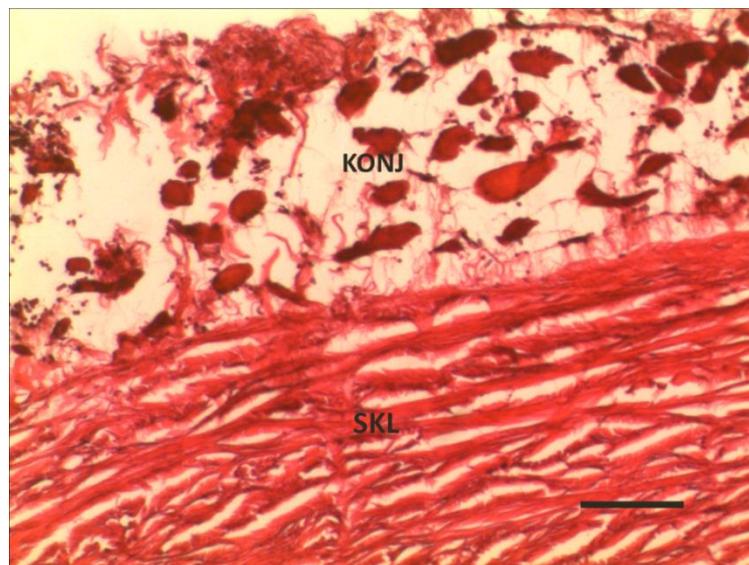


Figure 1. Histological figure of wound gap 10 minutes after surgery in albumen glue technique
Description: Konj = conjunctiva; skl = sclera; Bar = 10 μ m

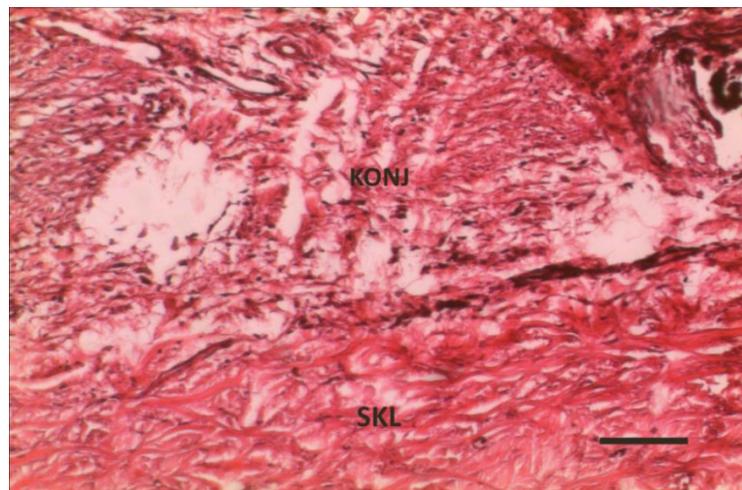


Figure 2. Histological figure of wound gap 10 minutes after surgery in suture technique

Description: Konj = conjunctiva; skl = sclera; Bar = 10 μ m

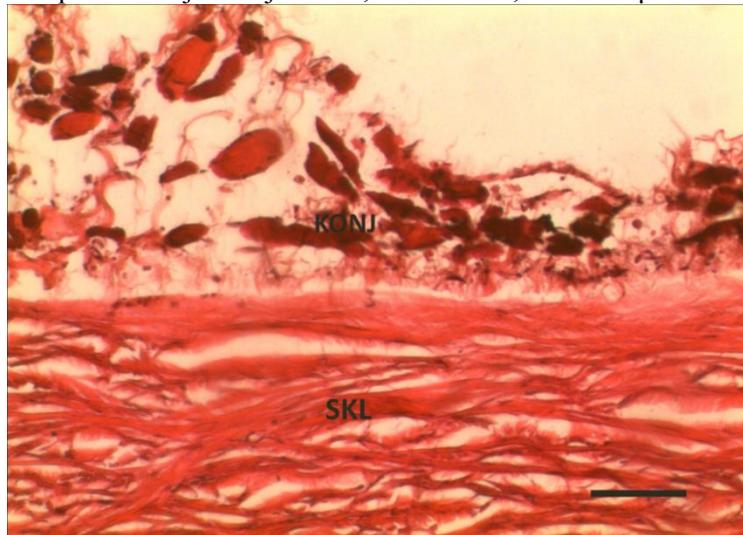


Figure 3. Histological figure of wound gap on day-7 after surgery in albumen glue technique

Description: Konj = conjunctiva; skl = sclera; Bar = 10 μ m

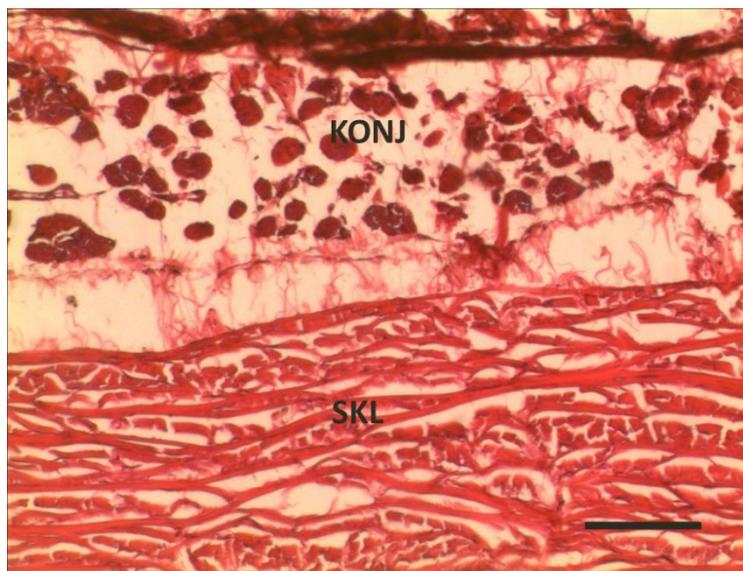


Figure 4. Histological figure of wound gap on day-7 after surgery in suture technique

Description: Kon = conjunctiva; skl = sclera; Bar = 10 μ m

In this study, there were six albumen glue technique eye preparations that did not have wound gap on 10 minutes after surgery observation. Very small gap wound was observed at 10 minutes after surgery in one eye preparations of albumen glue technique, probably was caused by an irregular applicator of albumen glue or air contaminated, so the graft did not attached well. While one preparation cannot be assessed, possibly due to the thick albumen applied to the conjunctival graft had changes in protein viscosity due to temperature changes. Albumen is a gel or a viscous-elastic matrix, and it has thermolabile characteristic, so it can be transformed into a dilute when the temperature changes [18].

The wider size of the wound gap in suture technique was possibly due to less exposure of both tissues. Both tissues did not attach well maybe caused by manipulation that slower graft tissue

adhesion and healing process and cause a gap between the tissues [4]. Healing process includes four phases : hemostasis, inflammation, proliferation, and remodeling. In the early stages of wound healing, vessel is having vasoconstriction about 10-15 minutes, then going vasodilation and increased capillary permeability, so plasma proteins and neutrophils go out into the wound area in 24 hours followed by lymphocytes and macrophages. Macrophages produce growth factors and new ECM, accelerate the process of angiogenesis that is critical to the further healing process. The process of epithelialization, granulation and angiogenesis is an important process in the proliferation stage. Granulation tissue formation phase is characterized by the activity of fibroblasts and new blood vessel formation. Fibroblasts are mostly active at the wound edges at 3-5 days post-traumatic and around the end of the first week. Fibroblasts are the main cells in the the wound. Granulation tissue is needed to fill wound gap during inflammatory process and continues until the base wound is healed, and it reaches maximum state on the fifth day of the wound. When there is a large wound gap, then more granulation tissue are needed, and more infiltration of inflammatory cells there and granulation tissue formation process will take longer [14]. Gilang et al. presented the histological figure that showed the greater grade of epithelialization, thinner granulation tissue, larger angiogenesis in fibrin glue technique rather than suture technique on first week of postoperative observation. This finding is related to the function of granulation tissue injury, which is known as a gap filler. In smaller wound gap of autologous fibrin glue technique, then the granulation tissue is thinner, while suture technique wound gap that larger will have more granulation tissue [16]. This condition can also be seen in wound healing between albumen glue and suture technique, which smaller wound gap showed in albumen glue technique compared to suture technique. In this study only observed the histological wound gap between albumen glue and suture techniques, not observing the thickness of granulation tissue.

In remodeling phase, there is balance change of extracellular matrix composition. Imbalances can be happened due to excessive collagen production by fibroblasts that can be happened because of mechanical pressure and leads to excessive delays of wound healing or tissue fibrosis or even keloid scarring [14]. Based on the theory, it can be said that the excessive formation of collagen by fibroblasts due to suture will delay wound healing or excessive fibrotic tissue formation, so that wound gap in suture technique is larger than albumen glue technique. This theory is in accordance with this study results in Table 4 that showed the average difference (delta) between 10 minutes after surgery wound gap and on day-7 after surgery albumen glue technique was 0.05 μm , while for suture technique was 8.96 μm . It shows that the wound gap in albumen glue technique is smaller than suture technique. In suture technique, complete epithelialization may not fully occur in the first week of observation because wound gap in suture technique that cause unification tissue process and vascularization did not successfully complete, so the graft was in a loose attachment.

4. Conclusion

There was a conjunctival graft tissue attachment grade in albumen glue, which was better than suture technique. Wound gap between the sclera graft conjunctiva and the layer beneath was smaller in albumen glue compared to suture technique.

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