

# Conjunctival Expansion Using a Subtenon's Silicone Implant in New Zealand White Rabbits

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**Purpose:** In the field of ophthalmology, the conjunctival autograft is a useful therapeutic material in many cases, but the small size of the autograft is a disadvantage. Therefore, we evaluated the feasibility of taking an expanded sample of conjunctival tissue using a subtenon's silicone implant.

**Materials and Methods:** We included a total of nine rabbits; eight rabbits were operative cases, and one was a control. A portion of conjunctival tissue from the control rabbit, which did not undergo surgery, was dissected and examined to determine whether it was histologically different from the experimental group. The surgical procedure was performed on eight rabbits via a subtenon's insertion of a silicone sponge in the left superior-temporal portion; after surgery, we dropped antibiotics into the eyes. We sacrificed a pair of rabbits every three days (on days 3, 6, 9, and 12) after surgery, removed the expanded conjunctival tissues with the silicone sponge implants, and measured their sizes. **Results:** The mean size of the expanded conjunctival tissues was  $194.4 \text{ mm}^2$ . On the third day, we were able to harvest a  $223.56 \text{ mm}^2$  section of conjunctival tissue, which was the most expanded sample of tissue in the study. On the twelfth day, we removed a  $160.38 \text{ mm}^2$  section of conjunctival tissue, which was the least expanded sample of tissue. Statistically, there were no significant differences in the mean dimensions of the expanded conjunctival tissues for each time period. Microscopic examinations showed no histological differences between the expanded conjunctival tissues and the normal conjunctival tissues. **Conclusion:** The results reveal that this procedure is a useful method to expand the conjunctiva for grafting and transplantation.

**Key Words:** Conjunctival expansion, subtenon's silicone implant

## INTRODUCTION

The conjunctiva is a thin, transparent mucosa that forms the mucous layer of tears, is involved in immune function, and functions to protect the eye from foreign materials such as microorganisms. The conjunctiva has many applications as a treatment means in the diverse field of ophthalmology. However, its utility is limited by the quantity of the conjunctiva that can be obtained. Therefore, we sought to take an expanded sample of conjunctival tissue using the subtenon's silicone implant.

We inserted a silicone sponge into one of the rabbits' eyes and determined whether the conjunctival surface that was obtained after the insertion varied over time by measuring the surface of the autologous expanded conjunctiva. If the surface varied, the time period when the maximal conjunctiva surface could be obtained was assessed. We also investigated whether the expanded conjunctiva was different from the normal conjunctiva upon histological examination.

## MATERIALS AND METHODS

### Subjects

Nine healthy, female New Zealand white rabbits were purchased for this study. The rabbits

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were without abnormalities in the extraocular or intraocular portions of the eye, the sizes of their eyes were comparable, and they weighed an average of 2 kg. Eight rabbits were divided into four groups of two animals each. In the one rabbit that did not undergo the silicone insertion surgery, a portion of the conjunctival tissue was dissected, stained, and examined to determine whether it was histologically distinct from the experimental groups.

### Procedure

We considered the Association for Research in Vision and Ophthalmology (ARVO) statement on the use of animals in ophthalmic and vision research. When we planned this project, we assumed that our bilateral ocular surgeries had the potential to affect vision bilaterally. Therefore, we performed a monocular procedure.

The systemic anesthetic agent ketamine chloride (50 mg/mL), 40 mg/kg, and the sedative Xylazine HCl (23.3 mg/mL), 23.3 mg/kg, were injected into the back muscles of the rabbits, and the rabbits were anesthetized.

Before the procedure, the area around the eye was sterilized with Betadine to prevent infection. Infraduction and adduction of the left eyeball of each of the eight rabbits was performed, the superior-temporal bulbar conjunctiva area was exposed maximally, and in an area 1 mm away from the corneal limbus, the superior-temporal conjunctiva and the Tenon's capsule were dissected carefully with a pair of spring scissors under a surgical microscope. For the cases in which infraduction and adduction were not performed sufficiently and the surgery was difficult, bridle suture suspension was performed with 10-0 nylon at the 12 o'clock position of the cornea, and the surgery was subsequently performed. An oval silicone sponge 5.0 mm × 15.0 mm × 3 mm in size was inserted into the inferior part of the dissected conjunctiva and the Tenon's capsule and fixated to the sclera using 5-0 Ethibond suture. Afterward, the previously-dissected conjunctiva and Tenon's capsule were sutured with 7-0 Vicryl suture (Fig. 1).

After the surgery, ofloxacin ointment was applied to prevent infection, and ofloxacin drops



**Fig. 1.** Insertion of a silicone sponge, and suture of the conjunctiva and Tenon's capsule.

were administered to the left eye four times per day.

### Outcome measures

Four groups of rabbits consisting of two animals each were sacrificed sequentially on the 3rd, 6th, 9th, and 12th day after surgery, then we marked the borders of the conjunctival tissues on the top of the silicone sponge with 7-0 Vicryl suture and performed the dissection of the conjunctiva by injecting saline into the subconjunctival area. Conjunctival tissues and a portion of Tenon's capsule tissue were dissected together. The harvested tissues were placed on a glass plate installed on the top of a grid scale ruler on which the scale of one grid is 1.8 mm, and the surface was measured. The sizes of the conjunctivae obtained from each group were compared, and the time when the maximum conjunctival tissue could be obtained was determined. The conjunctivae from each experimental group were examined under a light microscope after staining. The width and length of the silicone sponges were 5 mm and 15 mm, respectively. Therefore, we regarded the control as a 5 × 15 mm conjunctival area. In the one rabbit that did not undergo the silicone insertion surgery, a portion of the conjunctival tissue was dissected, stained, and examined to determine whether it was histologically distinct from the that of the experimental groups.

**RESULTS**

Conjunctival tissues were obtained from eight rabbits after the silicone sponge insertion was performed.

In our study, during the period of time from the insertion of the silicone sponge until the conjunctival tissues were obtained, none of rabbits developed impairment of eye movement, lagophthalmos, or conjunctival symblepharon. The eight rabbits were divided into four groups with three-day intervals until their sacrifice after surgery. The areas of the conjunctivae obtained from all eight eyes are shown in Table 1, Fig. 2.

The mean width of the conjunctival tissues obtained from the eyes of the eight rabbits was 9.0

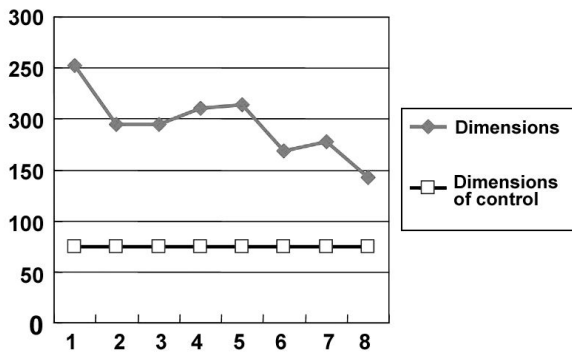


Fig. 2. The dimensions of the conjunctival tissue obtained from each rabbit.

± 1.36 mm, and the mean length was 21.6 ± 1.66 mm. The average surface area of the conjunctival tissues of all eight eyes was 194.4 ± 33.08 mm². Table 1 shows the average surface of the conjunctiva within each of the four groups.

The rabbits from which the largest conjunctival area was obtained were in the group that had the conjunctiva dissected three days after surgery, and the mean area was 223.56 mm² (a 298.1% expansion). The group from which the smallest area was obtained was from the rabbits in which 12 days had passed from the time of the insertion surgery to the dissection of the conjunctiva, and the mean area was 160.38 mm² (a 213.8% expansion).

The width, length, and mean area of the conjunctival tissues within each group of rabbits divided according to the sacrifice time were not statistically significantly different ( $p > 0.05$ ) when analyzed using the Kruskal-Wallis test.

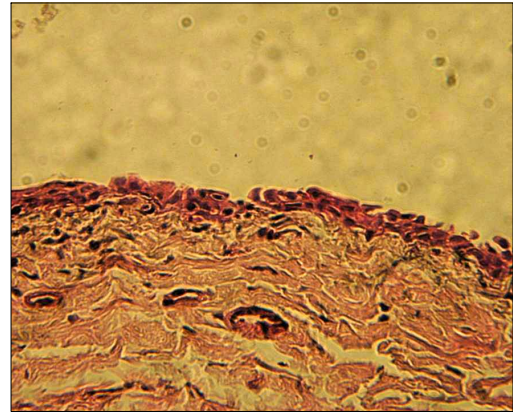
In one of the rabbits where 12 days had elapsed after the insertion procedure, a local scarring occurred in the conjunctiva (Fig. 3), and thus during the surgery performed to obtain the conjunctival tissue, a small buttonhole was generated in the end area of the conjunctival tissues. Upon histological examination of this case, we found many capillaries and fibrosis (Fig. 4). Conjunctivitis also developed in the eye of one rabbit 12 days after the insertion procedure, and

**Table 1.** The Area of the Conjunctival Tissue Obtained from Each Rabbit

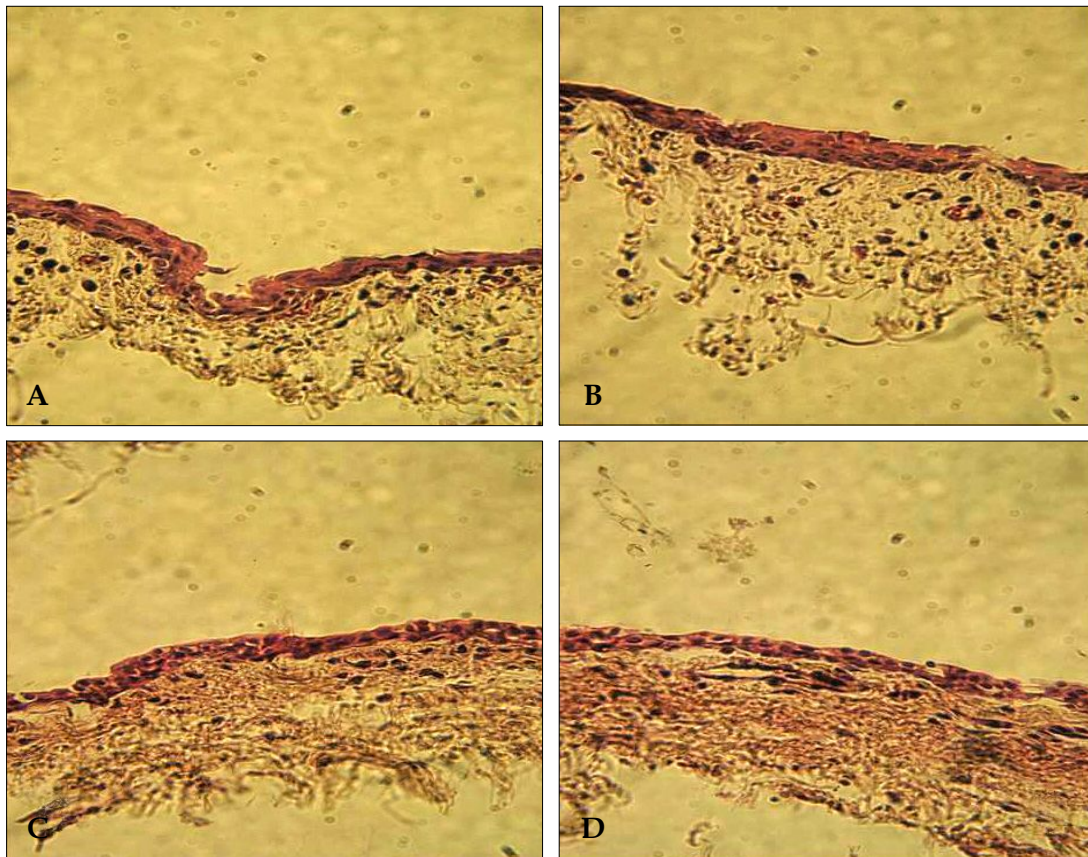
Rabbit number	1	2	3	4	5	6	7	8
Width of control (mm)	5	5	5	5	5	5	5	5
Width (mm)	10.8	9.0	9.0	9.0	10.8	7.2	9.0	7.2
Expanded width rate (%)	216	180	180	180	216	144	180	144
Length of control (mm)	15	15	15	15	15	15	15	15
Length (mm)	23.4	21.6	21.6	23.4	19.8	23.4	19.8	19.8
Expanded length rate (%)	156	144	144	156	132	156	132	132
Dimensions of control (mm²)	75	75	75	75	75	75	75	75
Dimensions (mm²)	252.72	194.40	194.40	210.60	213.84	168.48	178.20	142.56
Expanded dimensions rate (%)	337.0	259.2	259.2	280.8	285.1	224.6	237.6	190.1
Post operative date (days)	3		6		9		12	
Mean average of dimensions (mm²)	223.56		202.5		191.16		160.38	



**Fig. 3.** One case developed local scarring in the conjunctiva.



**Fig. 4.** Histological findings of the conjunctival scarring (Hematoxylin-Eosin (HE) staining,  $\times 200$ ).



**Fig. 5.** Histological findings of the conjunctiva after the procedure. (Hematoxylin-Eosin (HE) staining,  $\times 200$ ). (A: Three days after the procedure B: Six days after the procedure C: Nine days after the procedure D: Twelve days after the procedure)

we found that the conjunctival vessels were dilated. On histological examination, comparable histological findings among each group were found (Fig. 5). In comparison with the control eye in

which the procedure was not performed (Fig. 6), noticeable histological differences were not seen with the exception of the case with conjunctival scarring.



Fig. 6. Histological findings of the normal conjunctiva (Hematoxylin-Eosin (HE) staining,  $\times 200$ ).

## DISCUSSION

In the field of ophthalmology, the autologous conjunctiva is very useful as a treatment means. It can be used to treat persistent ulcerative keratitis that has been unresponsive to drugs,<sup>1</sup> keratolysis of herpes keratouveitis,<sup>2</sup> neurotrophic ulcers,<sup>3</sup> peripheral corneal ulcers, exposure keratopathy, bullous keratopathy, chemical burns, descemetocele, anophthalmos,<sup>4-10</sup> and for other various reconstruction surgeries,<sup>11,12</sup> pterygium surgeries.<sup>13-18</sup>

Pterygium is a common disease. From the conjunctiva to the cornea, triangle-shaped fibrovascular tissues proliferate in the bulbar conjunctiva and invade the nasal cornea. The postsurgical recurrence rate of pterygium is high, and a recurrence rate of approximately 25 - 45% has been reported.<sup>19-20</sup> Surgery for recurrent pterygium cases requires the resection of the conjunctiva in a wide area, and if the area beneath the sclera is not covered by the conjunctiva after the recurrent pterygium surgery, necrotizing scleritis may develop. Visual acuity may also be lost in the worst cases, and thus a large quantity of the conjunctiva may need to be resected. Kenyon et al.<sup>21</sup> reported a low recurrence rate in pterygium surgery by using conjunctival autograft transplantation, and subsequently this has become an important surgery method in pterygium treatment. This surgery is used primarily as a treatment for recurrent pterygium, and it is commonly performed in patients in their 20's and

early 30's in whom it readily recurs.<sup>16,22</sup> The recurrence rate of pterygium after conjunctival autograft transplantation has been reported to be relatively low, from 5.3 - 14% depending on the investigators, and it is also esthetically appropriate. Therefore, it is a surgical method that has been performed commonly depending on the surgeons.<sup>16,23</sup> Conjunctival autograft transplantation has a great advantage in that it can reduce the recurrence rate without the use of Mitomycin C.<sup>22,24</sup>

The conjunctiva can be used in diverse cases, but the limiting problem is the quantity that can be obtained. In pediatric cases, the elasticity of the conjunctiva is excellent, and thus a certain amount of extra conjunctiva can be obtained, but as patients age this elasticity is decreased. In elderly patients requiring a large area of the conjunctiva for transplantation, a sufficient amount of the mucosa can not be obtained in many cases.

Recently, amniotic membranes have been used in place of the conjunctiva in many cases,<sup>25-40</sup> and for the reconstruction of the conjunctival surface in pterygium, conjunctival intraepithelial neoplasia, conjunctival scarring, conjunctival symblepharon, or conjunctivochalasia cases with large defects in the area of the conjunctiva, amniotic membrane transplantation has been performed. However, in such cases it is necessary to consider that amniotic membrane is expensive, its storage is complex, and amniotic membranes may be infected. Additionally, after transplantation, the grafted amniotic membrane can be torn relatively easily and detached again, and the mucus secreted from the mucosa is not sufficient for lubrication, so postoperative dry eye syndrome may also develop. Furthermore, although it is rare, cases of cornea melt after amniotic membrane transplantation have been reported.<sup>41</sup> In contrast, conjunctival autograft transplantation has the advantages that it is inexpensive in comparison with that using amniotic membrane, its outcome is good esthetically, complications are almost absent, and the risk of infection is markedly lower. Therefore, if more conjunctival autografts could be obtained, they would be widely used in clinics.

We attempted to obtain an increased amount of the conjunctiva by inserting a silicone sponge into the subtenon space of rabbits and thus inducing conjunctival expansion. The silicone sponge for human eyes was relatively large for the rabbits'

eyes, but none of the rabbits developed lagophthalmos after the procedure in our study. Therefore, we conclude that in humans, whose eyeballs are bigger than those of rabbits, the insertion of a silicone sponge may be useful in obtaining a certain amount of conjunctival tissues without developing lagophthalmos.

To obtain useful conjunctival tissue, it is imperative not to make a buttonhole in the tissue. It is important not to pull the conjunctival tissues excessively with a forceps or manipulate a pair of spring scissors carelessly during the dissection. Above all, care must be taken to prevent the formation of adhesions, scars or granulomas. In our study, a scar developed locally in the conjunctiva of one eye, meaning that during the procedure to obtain conjunctival tissue a small buttonhole was made in the end area of the scarred conjunctival tissue. Clearly, during the insertion of the silicone sponge into the subtenon space, injuries to the tissue can be minimized by paying close attention to the manipulation. The surgeon has to be careful during the insertion of the silicone sponge and the resection of the conjunctival tissues, not only to minimize the formation of buttonholes in the conjunctiva, but also because the insertion of the silicone material itself can induce inflammatory reactions.<sup>42</sup> Consequently, inflammatory changes can develop in the conjunctiva, conjunctival hyperemia can persist, a thick conjunctival scar can form, and by developing a severe adhesion to other adjacent tissues, diplopia may develop, as well as other postoperative complications. When additional conjunctival tissues are required in the future, such changes render it difficult to obtain a satisfactory amount of conjunctival tissue. To prevent the development of such results, the tissue has to be manipulated carefully while performing the procedure to minimize damage. It is possible that subconjunctival injection of steroids and other anti-inflammatory agents may be helpful, and useful results may be obtained from studies that compare the group receiving injected anti-inflammatory agents into the subconjunctival after the completion of surgery with a group not receiving any injections.

The conjunctival tissue obtained 12 days after the procedure was not only the smallest, but it

was also found that buttonholes, conjunctivitis, conjunctival vessel dilation, and other complications had developed simultaneously. On the basis of such results, we conclude that leaving a silicone sponge in the eye for a long time is not desirable. However, the sample size in our study was small, and thus it was difficult to consider the results to be statistically significant. Inter-animal variability of response and size of the eyes may also have influenced our results. Studies on larger samples are required in the future.

In our study, during the period of time from the insertion of the silicone sponge until the conjunctival tissues were harvested, none of the cases developed an impairment of eyeball movement and conjunctival symblepharon. Because the rabbits were sacrificed immediately before obtaining the conjunctival tissues, we could not examine whether conjunctival symblepharon or other inflammatory changes developed after harvesting the conjunctival tissues. In the future, studies that obtain the conjunctival tissues without sacrificing the rabbits and that subsequently examine whether inflammatory changes such as conjunctival symblepharon or impairment of eyeball movement occur should be developed and should include a follow-up observation period.

In our study, one of the most important procedures was measuring the area of the conjunctiva on the top of the glass plates. In order to minimize the development of buttonholes while obtaining the conjunctival tissue, a resection including a part of Tenon's capsule was made. However, this may have introduced a measurement error compared to a situation in which only conjunctival tissue was harvested. Lee et al.<sup>17</sup> reported that in cases that developed necrotic scleral lesions, if the lesions were covered with the simple conjunctiva as well as Tenon's capsule, external stimulations to the necrotic area were reduced. Additionally, because of the supply of nutrients through blood vessels in the covered conjunctiva, the pathological lesions were stabilized and symptoms improved. Nevertheless, it is thought that the *in vivo* application of this technique to patients in the clinic is not necessary except in special situations. Clinically, most physicians use the conjunctiva alone without Tenon's capsule as grafting material.

With our procedure, a certain quantity of conjunctival tissue was obtained without severe complications. Therefore, we conclude that our method may be useful in obtaining conjunctival tissues in other settings such as clinics. Additionally, studies suggest that another method<sup>7</sup> used in congenital anophthalmos patients may be useful to obtain conjunctival tissues. This method involves inserting an ocular prosthesis into the orbit of the patient, and a self-inflating hydrogel expander is also inserted into the subconjunctival space to expand the conjunctiva. Patients are observed for 2-4 weeks before tissues are obtained.

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