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| Original Article |

The Effect of the Cell-derived Extracellular Matrix Membrane on Wound Adhesions in Rabbit Strabismus Surgery

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Abstract : The cell-derived extracellular matrix (ECM) membrane is a bio-absorbable membrane composed of aseptic ECM obtained from swine knee chondrocyte. The purpose of our study was to evaluate the inhibitory effect of the cell-derived ECM membrane in postoperative adhesion following strabismus surgery on rabbit eyes. Twenty four adult male New Zealand albino rabbits were used. Each rabbit has undergone 3 mm resection of the superior rectus muscle in both eyes. The cell-derived ECM membrane was applied between the conjunctiva and superior rectus muscle only to right eye in each rabbit. The eyes were divided into a surgery-ECM group and a surgery-no ECM group. The rabbits were sacrificed at 2, 4, and 6 weeks after the surgery. Each time, 16 eyes of 8 rabbits were enucleated to observe the adhesion in muscle and adjacent tissue grossly by blunt dissection. Then, histopathological sections were analyzed for inflammation and fibrosis by staining with hematoxylin–eosin and Masson trichrome, respectively. Inflammation and fibrosis were graded on a scale of 0 to 4. A researcher was blind to the experiments performed all the examinations. Comparing with the surgery-no ECM group, there was no significant difference with regard to inflammation at the area of superior rectus muscle resection at 2, 4, and 6 postoperative weeks (p = 0.52, p = 0.55, p = 0.82). However, a significant reduction in the degree of adhesion (p = 0.01, p = 0.03, p = 0.04) as well as decreased fibrosis (p = 0.04, p = 0.03, p = 0.02) between muscle and conjunctiva after strabismus surgery was observed in all surgery-ECM groups. The current results demonstrated that the cell-derived ECM membrane could inhibit the formation of postoperative adhesion and fibrosis between conjunctiva and muscle. The authors concluded that the use of ECM membrane could reduce postoperative adhesion around surgical area after strabismus surgery in rabbits.

Key words: *adhesion, cell-derived extracellular matrix membrane, strabismus surgery*

1. Introduction

Postoperative adhesion after strabismus surgery might be the most important cause of the failure of surgery. Formation of the severe adhesion may affect the function of extraocular muscles and result in motility problems.[1,2]

In addition, postoperative adhesion following strabismus surgery have made it difficult to undergo reoperation or adjustment after strabismus surgery. When diplopia caused by adhesion develops, reoperation requires removing as much scar tissue as possible. Nevertheless, complete elimination of diplopia is usually difficult. Therefore, prevention of postoperative inflammation and adhesion results in maintaining more stable angle as well as improvement of success rate in strabismus surgery.[3]

Adhesion after strabismus surgery is caused and deteriorated by tissue injury due to careless procedure and repeated operation, excessive hemorrhage, foreign body reaction to suture materials, and postoperative infection.[2,4] Thus, careful and atraumatic surgical techniques are the most effective means of preventing

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postoperative adhesion.[5] Nevertheless, traumatic tissue injury, hemorrhage, and inflammation secondary to surgery are inevitable, and some amount of postoperative adhesion is a natural result.[6]

Therefore, in several trials to decrease surgical adhesion, physical barriers such as amniotic membrane, supramide (Supramide Extra®; Jackson, Alexandra, VA, USA), silicone sleeve, polyglactin 910 mesh (Vicryl mesh®, Ethicon Inc., Somerville, NJ, USA), polypeptide sleeve, oxidized regenerated cellulose sleeve (Interceed®, Johnson and Johnson Medical Inc., Arlington, TX, USA), sodium hyaluronate, and Seprafilm® (Genzyme, Cambridge, MA, USA) as well as antimetabolites such as mitomycin C and 5 fluorouracil were applied.[7-19] However, the use of physical barriers has potency to lead to dislocation and re-adhesion. The application of antimetabolites has the potential risk of serious complication such as scleral necrosis and corneal epithelial defect. Therefore, any products could not achieve the properties as an ideal material convenient for routine strabismus surgery.

Now, taking another perspective, we have devised a new method to prevent excessive fibrous scar around extraocular muscles by using the cell-derived ECM membrane.

The cell-derived ECM membrane is a bioabsorbable film produced by sterilizing scaffolds formed after the cultivation of cartilage cell dispatched by knee cartilage tissue of a swine (Fig 1). It has many functions such as suppressing fibrovascular proliferation and prevention of tissue adhesion. Therefore, it has been used to decrease adhesions, mainly in orthopedic surgery under the name of Artifilm® (Regenprime, Suwon, Korea).[20,21]

We postulated that the cell-derived ECM membrane might be a potentially useful material for strabismus surgery. To the best of our knowledge, there are no reports about the histopathologic effect of this membrane in ocular surgery.

The current study evaluated and examined the histopathological effect of the cell-derived ECM membrane on postoperative adhesion formation in an experimental rabbit model to assess the potential benefit of the cell-derived ECM membrane in strabismus surgery.

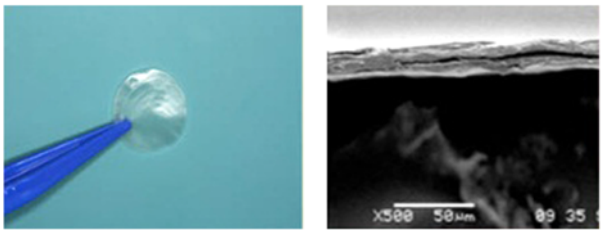

Figure 1. The cell-derived ECM membrane is round, thin film-like, aseptic membrane.

2. Materials and Methods

2.1 Animals

Twenty four adult male New Zealand albino rabbits weighing approximately 2.0 Kg at 12 weeks of age were purchased from Orient Bio Laboratory Animals (Daejeon, Korea). Animals had free access to deionized water and standard rabbit chow for 7 days after arrival. All experimental procedures were both designed to conform to the Institutional Animal Care and Use Committee of Inje University College of Medicine (No.; 2010-042).

2.2 Preparation of the Cell-derived ECM Membrane

The protocol of manufacturing the cell-derived ECM membrane and the gross structure were described elsewhere.[22-24] Briefly, porcine chondrocytes were isolated from the knee cartilage of 2-week-old piglets and cultured. A chondrocyte-derived ECM membrane was isolated from the culture plate then centrifuged to consolidate. Then this pellet-type construct has undergone cultivation. Cultured cartilage construct was freeze-dried to remove cellular contents and fabricated into an acellular porous membrane. The complete removal of cellular contents was confirmed by quantification of deoxyribonucleic acid (DNA) contents in the ECM membrane and 4',6-diamidino-2-phenylindole (DAPI) fluorescent dye staining (Invitrogen, USA). No detection of positive staining of DAPI represented that all of the porcine chondrocytes were effectively removed from the biomembrane. To apply it on ocular surface in this experiment, the ECM membrane was produced as a thin, semi-transparent membrane and sterilized by gamma radiation.

2.3 Experimental Procedures

A total of twenty four adult male New Zealand albino rabbits (body weight = 2 Kg) were used. For all procedures, rabbits were anesthetized with tiletamine/zolazepam (Zoletil®, Virbac, Carros, France: 50 mg/kg body weight), xylazine (Rompun®, Bayer, Leverkusen, Germany: 10 mg/kg body weight), and atropine sulfate (Jeil, Daegu, korea: 0.5 mg/kg) given intramuscularly. All surgical procedures described below were performed under additional topical anesthesia with 0.5% proparacaine hydroxychloride (Alcaine®, Alcon Laboratories, Inc., Fort Worth, TX, USA). In all cases, experimental manipulations were performed by a single experienced surgeon.

We carried out resection of superior rectus muscle of rabbits (24 right eyes of 24 rabbits, Surgery-ECM group). Surgical antisepsis with polyvinylpyrrolidoneiodine (Betadine) was applied to the eyelids before each operation. After a limbal peritomy from 10 o'clock to 2 o'clock, the superior rectus muscle

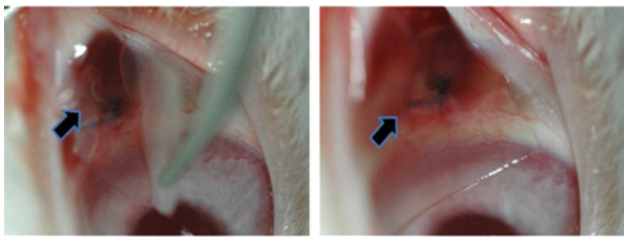

Figure 2. The cell-derived ECM membrane is seen before insertion between the conjunctiva and the resected superior rectus muscle in rabbit eye (arrow).

was isolated on a Jameson hook and intermuscular connections were dissected. The incision was standardized to create incisions of the same size and shape. Two single arm sutures using 6-0 polyglactin (Vicryl®, Ethicon, Somerville, NJ, USA) suture material were placed at the muscle border 3 mm posterior from the muscle insertion and secured with a square knot. After resection was accomplished, the superior rectus muscle was sutured to the original insertion. The cell-derived ECM membrane measuring 6 × 6 mm was hydrated. Then, it was placed between superior surface of the resected muscle and conjunctiva without any additional sutures for preventing slippage of the cell-derived ECM membrane (Fig 2). The conjunctival wound was closed by suturing with 8-0 polyglactin (Vicryl®, Ethicon, Somerville, NJ, USA). As controls, the same surgical procedure was performed on 24 left eyes of 24 rabbits (Surgery-no ECM group) without applying the cell-derived ECM membrane.

At the end of each procedure, ofloxacin ointment (Tarivid®, Santen Pharmaceutical Company, Osaka, Japan) was applied topically to surgically treated eyes in both groups and 2 mg of gentamicin was injected in the thigh muscle immediately. Then, topical instillation of levofloxacin (Cravit®, Santen Pharmaceutical Company, Osaka, Japan) was administrated to each eye 3 times a day during 1 week. The rabbis were sacrificed at 2, 4, and 6 weeks after surgery, by using rapid perfusion pentobarbital sodium with a total dose of 250 mg through the auricular vein. To help in identifying the location of superior rectus, a conjunctival suture was placed at the site of it. Each time, 16 eyeballs of 8 rabbits were enucleated with conjunctival and subconjunctival tissues with the anterior part of the resected superior rectus muscles attached.

2.4 Evaulation of Adhesions

Gross adhesion between the muscle, sclera and conjunctiva was evaluated and recorded when enucleating the eyes of the rabbits. The adhesion was classified as following: C/M (conjunctiva/superior rectus muscle) if it was located above the superior rectus muscle, M/S (superior rectus muscle/sclera) when located below the superior rectus muscle. Adhesion severities were scored from 0 to 4 according to the criteria in a previous report[15] where 0 = no adhesion, 1 = adhesion easily separable with blunt dissection with cotton tip applicator, 2 = mild adhesion with freely dissectible plane, 3 = dense adhesion with difficult dissection, and 4 = non-dissectible plane.

2.5 Histological Preparation

Eyeballs of rabbit were histopathologically studied to evaluate the inhibitory effect of the cell-derived ECM membrane on postoperative inflammation and fibrosis formation around the surgically treated muscle. Then the eyeballs were fixed in buffered formalin solution overnight and embedded in paraffin. Anterior segments of eyes containing cornea, sclera, conjunctiva, and superior rectus muscle were cut vertically at 4-μm thickness, mounted on subbed slides, and then dried.

2.6 Histological Analysis

One pathologist examined all of the specimens in a blinded fashion. The pathologist got serial 3 slices of 2 mm posterior from the insertion of resected superior rectus muscle in each eyeball. The sections, 4 μm in thickness, were stained with hematoxylin–eosin for histopathologic examination for inflammation. The specimens were also stained with Masson trichrome to demonstrate collagen fibers and were graded for the amount of scar tissue formation. Both inflammation and fibrosis were graded on a scale of 0 to 4 according to classification used by Özkan *et al.*[19]

The inflammatory reaction is composed of lymphocytes, plasma cells, histiocytes, and polymorphonuclear leukocytes. The grading for inflammation was as follows: 0 = no inflammation; 1 = a few lymphocyte and plasma cell beneath the epithelium; 2 = mild inflammatory infiltrate composed of lymphocytes, plasma cells, and polymorphonuclear leukocytes beneath the epithelium and congestion; 3 = grade 2 plus neutrophils in the epithelium; and 4 = high concentrations (collections) of lymphocytes, plasma cells, polymorphonuclear leukocytes, and histiocytes—both intraepithelial and subepithelial—and ulceration.

A scale of 0 to 4 for fibrosis was based on the amount of collagen formation, and the grading was as follows: 0 = no fibrosis; 1 = mild perimuscular fibrotic reaction (stained collagen was detectable only in thin bands immediately adjacent to muscle); 2 = easily detected thick bands; 3 = well-developed, dense bands of collagen; and 4 = a severe fibrotic response replacing large areas.

Table 1. Comparison of postoperative adhesion scale obtained from gross obervation by blunt dissection in both groups at 2, 4 and 6 postoperative weeks.

Time	Adhesion site	Surgery-ECM group	Surgery-no ECM group	*p**
2 weeks	C/M	1.60±0.52	2.83±07.3	0.01
	M/S	2.49±0.71	2.95±0.67	0.72
4 weeks	C/M	1.73±0.66	2.89±0.55	0.03
	M/S	2.64±0.52	3.04±0.61	0.79
6 weeks	C/M	1.78±0.72	2.93±0.54	0.04
	M/S	2.71±0.63	3.10±0.59	0.82

*: Wilcoxon signed rank test.
C/M = between the conjunctiva and the superior rectus muscle, M/S = between the superior rectus muscle and the sclera.

Table 2. Comparison of inflammation and fibrosis scale obtained from histopathologic evaluation in both groups at 2, 4 and 6 postoperative weeks.

Time	Evaluation	Surgery-ECM group	Surgery-no ECM group	*p**
2 weeks	Inflammation	2.00±1.00	1.60±0.89	0.52
	Fibrosis	1.80±0.84	2.80±0.45	0.04
4 weeks	Inflammation	1.40±0.55	1.20±0.45	0.55
	Fibrosis	1.20±0.84	2.60±0.55	0.03
6 weeks	Inflammation	0.75±0.46	0.86±0.83	0.82
	Fibrosis	0.75±0.46	1.88±0.61	0.02

*: Wilcoxon signed rank test.

2.7 Statistical Analysis

The numeric values of postoperative adhesion, inflammation, and fibrosis were compiled from both two groups. To draw out the difference of scores between surgery-ECM group and surgery-no ECM groups, statistical analysis was performed using the Wilcoxon signed rank test. Statistical significance was determined at $p < 0.05$. Data were given as mean±standard error of the mean.

3. Results

To investigate the changes within the orbit of the rabbits, they were monitored daily. All rabbits survived healthy and ate normally until they were sacrificed to evaluate adhesion and to investigate histopathological examination. In some of the eyes mild redness in the surgical site of conjunctiva was observed. In contrast to that, no significant ocular, periocular, or orbital changes as well as no gross changes were visible in the treated muscles at necropsy. On harvested eyes in surgery-ECM group, we could find the cell-derived ECM membrane was properly placed and remained between superior surface of the resected muscle and conjunctiva at 2 weeks after surgery, whereas the cell-derived ECM membrane was totally absorbed and disappeared without a trace at 4 weeks postoperatively.

In the surgery-ECM group, the mean score for adhesion between the conjunctiva and the muscle was 1.60 at 2 weeks postoperatively, 1.73 at 4 weeks postoperatively, and 1.78 at 6 weeks postoperatively. In the surgery-no ECM group, the mean score for adhesion between the conjunctiva and the muscle was 2.83 at 2 weeks postoperatively, 2.89 at 4 weeks postoperatively, and 2.93 at 6 weeks postoperatively (Table 1). The mean score for adhesion between the muscle and the sclera was 2.49 at 2 weeks postoperatively and 2.64 at 4 weeks postoperatively, and 2.71 at 6 weeks postoperatively in eyes treated with the cell derived ECM membrane and 2.95, 3.04, and 3.10 respectively, in eyes treated without this membrane (Table 1). These results suggest that the cell derived ECM membrane can help to reduce postoperative adhesion between the conjunctiva and the muscle ($p = 0.01$, $p = 0.03$ and $p = 0.04$, respectively). However, the cell derived ECM membrane, had shown no effect on lowering the rate of development of adhesions between the muscle and the sclera ($p > 0.05$).

The mean values of inflammation and fibrosis scale obtained from microscopic evaluation in both two groups are listed in Table 2. There were no signs of muscle necrosis in any of the specimens. Histopathological examination of the superior rectus muscle and subconjunctival connective tissue showed

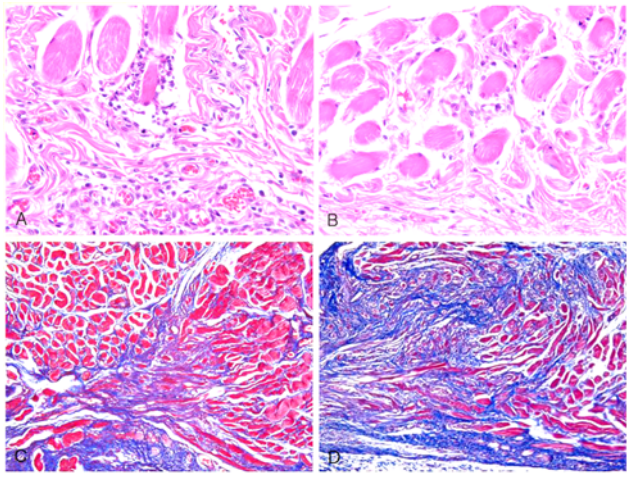

Figure 3. Light microscopic findings at 2 weeks postoperatively. (A, C): Surgery-ECM group; (B, D): Surgery-no ECM group. On hematoxylin-eosin stain (×400), the surgery-ECM group (A) showed slightly more inflammatory reaction than the surgery-no ECM group (B), but there was no significant difference between two groups. On masson's trichrome stain (×100), the surgery-ECM group (C) revealed less fibrosis compared to the surgery-no ECM group (D).

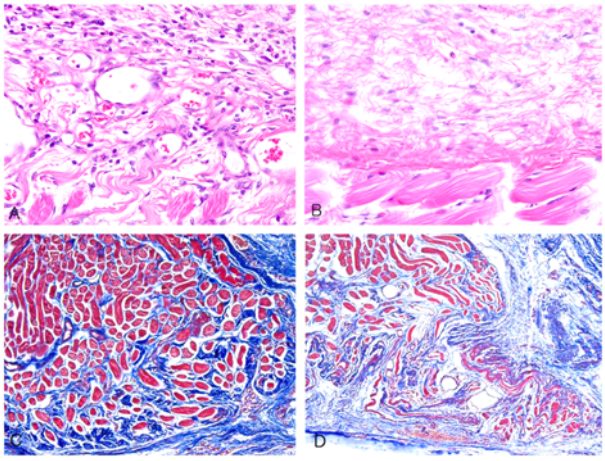

Figure 4. Light microscopic findings at 4 weeks postoperatively. (A, C): Surgery-ECM group; (B, D): Surgery-no ECM group. On hematoxylin-eosin stain (×400), the surgery-ECM group (A) showed slightly more inflammatory reaction than the surgery-no ECM group (B), but there was no significant difference between two groups. On masson's trichrome stain (×100), there was significantly less fibrosis on the surgery-ECM group (C) than the surgery-no ECM group (D).

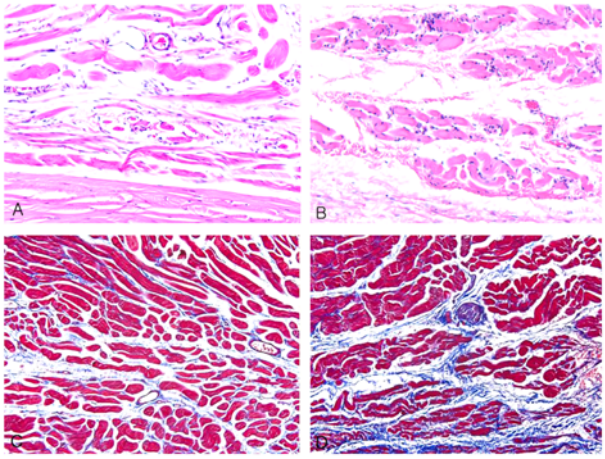

Figure 5. Light microscopic findings at 6 weeks postoperatively. (A, C): Surgery-ECM group; (B, D): Surgery-no ECM group. On hematoxylin-eosin stain (×200), there was no difference in inflammatory reaction between two groups (A, B). On masson's trichrome stain (×100), the surgery-ECM group (C) showed less fibrosis than the surgery-no ECM group (D), and there was significant difference between two groups.

the surgery-ECM group had slightly higher inflammation than the surgery-no ECM group at 2 and 4 weeks after surgery. However, there were no statistically significant differences at 2, 4, and 6 weeks ($p = 0.52$, $p = 0.55$ and $p = 0.82$, respectively). In the evaluation of fibrosis, the surgery-ECM group showed significantly less fibrosis formation than the surgery-no ECM group ($p = 0.04$, $p = 0.03$ and $p = 0.02$, respectively) (Table 2 and Figs 3 - 5).

4. Discussion

Postoperative adhesion between muscle and surrounding connective tissue after strabismus surgery is caused and increased by excessive hemorrhage, electrocauterization, and orbital fat prolapse. In addition, inflammatory reaction related to postoperative infection or suture reaction may also lead to formation of adhesions. Especially, multiple strabismus surgeries increase a cumulative risk for the development of postoperative adhesions.

Once the adhesion develops, it is difficult to reverse their effect. Such adhesions may hinder the normal function of an extraocular muscle contraction which make strabismus surgery ineffective. Therefore, it may decrease the predictability of surgical outcome in strabismus surgery.[13] Furthermore, postoperative adhesion make it difficult to dissect previously operated field for adjustment after strabismus surgery. So although change of strabismus angle after strabismus surgery would be expected to happen, we have no choice but to carry out adjustment within 2 days postoperatively. Postoperative adhesion also render reoperation difficult because it make hard to control hemorrhage owing to excessive bleeding as well as to distinguish between muscle and connective tissue.[25] Once the adhesions develop, it is difficult to restore their effect. Therefore, it is important to hold down occurrence of postoperative adhesion so as to improve surgical outcome of strabismus surgery and to increase predictability for change of strabismus angle.

Control of hemorrhage, gentle surgical technique, and prevention of excess cautery are essential to decrease the occurrence of postoperative adhesions.[13] In addition to this, several operative methods have been reported as prevention for development of postoperative adhesions. Various materials and pharmaceuticals have been used in order to decrease the development of postoperative adhesion and scarring. For example, amniotic membrane shows characteristics such as basement membrane of conjunctival epithelium, therefore the application of amniotic membrane is considered to improve and promote regeneration and differentiation of epithelium. Furthermore, amniotic membrane prevent signal transduction of transforming growth factor-β and reduce conjunctival fibrosis.[8] But, disadvantage of amniotic membrane is that it is difficult to acquire and not convenient to use for surgeons. Supramide plastic sleeve (Supramide Extra®, Jackson, Alexandra, VA, USA) and silicone sleeve are nonabsorbable materials that act by forming a mechanical barrier.[2,12] Currently, they are not

in use because they are known to lead to infection, foreign-body reaction, moreover extrusion of material. Absorbable materials such as oxidized regenerated cellulose sleeve (Interceed®, Johnson and Johnson Medical Inc., Arlington, TX, USA), polyglactin 910 mesh (Vicryl mesh®, Ethicon Inc., Somerville, NJ, USA), and polypeptide sleeve have been used to reduce formation of adhesion. But, they have disadvantages such as feeling of irritation, improper placement, and granuloma formation. Now these materials are not recognized as having the ability to decrease adhesion formation.[14,15,17] Disadvantages of Interceed® are the requirement of complete hemostasis as well as significant elevation of postoperative adhesions formation.[18] If bleeding is not completely stopped, this may increase the risk of adhesions. Vicryl mesh® has the disadvantage of creating a rough implant tissue interface that can lead to technical difficulties in implantation and subsequent erosion of overlying tissue. The effect of a bioabsorbable membrane composed of sodium hyaluronate and carboxymethylcellulose (Seprafilm®, Genzyme, Cambridge, MA, USA) are controversial. Fulga *et al*.[26] reported Seprafilm® as ineffective in the prevention of adhesion formation in a rabbit model, whereas Searl *et al*.[13] and Yaacobi *et al*.[15] reported a favorable result. Experimental studies employing antimetabolites mitomycin C and 5-fluorouracil have shown positive results in decreasing adhesion formation.[9,16] However, these agents have the potential risk of serious side effects. Antimetabolites are known to cause severe corneal and scleral complications when used in glaucoma and pterygium surgeries.[27-29] They are known to decrease vascularity and are also believed to increase the risk of anterior segment ischemia in patients who have undergone multiple strabismus surgeries.[9,18,30]

The cell-derived ECM membrane is a bioabsorbable membrane composed of aseptic ECM obtained by chondrocyte. In biology, the ECM is the extracellular part of animal tissue that usually provides structural support to the animal cells. In vivo, previous studies showed the cell-derived ECM membrane could reduce tissue adhesion. the mechanism of this effect is not clear but one major possibility is the role of the chondrocyte components including proteoglycan that inhibit endothelial cell migration and vascularization.[31,32] Many studies suggested that angiogenesis and inflammation are codependent.[33] Angiogenic inhibitor effect of this membrane might reduce inflammation in acute wound healing phase on surgical field. Thus, the combined effects such as anti-angiogenesis and anti-inflammation of the cell-derived ECM membrane in acute wound healing phase might lead to reduction of tissue adhesion in late wound healing phase.[34] And, another minor possibility is the role as temporary physical barrier which separate tissues and hence decrease adhesions. Therefore, we anticipate it could be applied to decrease postoperative adhesion.

In our experience, the cell-derived ECM membrane is relatively easy to handle it in surgical field. In this study, although it was placed between superior surface of the resected muscle and conjunctiva without any additional sutures, there was no displacement of this membrane by 2 weeks after surgery. However, if the material is hydrated, it glides easily and may move from its location. this property can increase the chance it may be located at inappropriate site. Therefore, the surgeon has to be careful during the initial positioning, and additional glue or sutures may be required—especially at the limbal area—to prevent slippage of the cell-derived ECM membrane through the corneal surface.

In this study, the placement of this membrane after superior rectus muscle resection significantly lowered the rate of developing adhesions between the conjunctiva and the superior rectus muscle ($p = 0.01$, $p = 0.03$ and $p = 0.04$, respectively) and reduced fibrosis formation at 2, 4, and 6 weeks postoperatively ($p = 0.04$, $p = 0.03$ and $p = 0.02$, respectively). However, the correct mechanism by which the cell-derived ECM membrane reduces postoperative adhesion and fibrosis remains unclear. It is possible that this membrane acts by establishing an inhibitory physical barrier to invading fibroblasts and preventing of fibrovascular proliferation, which results in less fibrosis. Although there was no statistical difference between two groups, the application of the cell-derived ECM membrane tended to increase postoperative inflammation rather than decrease at 2 and 4 weeks after strabismus surgery. However, at 6 weeks postoperatively, postoperative inflammation in the surgery-ECM group was less than in the surgery-no ECM group. Considering the total absorption of the cell-derived ECM membrane at 4 weeks postoperatively, the increased inflammation at 2 and 4 weeks after the operation may have been caused by foreign body reaction of this membrane.

Wound healing process is characterized by inflammation in the acute phase, granulation tissue in the intermediated phase and scarring in the chronic phase.[35-37] Collagen maturation during scarring in the chronic phase continues for 12-18 months.[38] Therefore, our study for evaluating effect of the cell-derived ECM membrane is partly limited by short term follow-up, 6 weeks.

In terms of physical characteristics, the cell-derived ECM membrane has some advantages. It stays longer than Seprafilm® within the tissue (approximately 14 to 28 days vs 7 to 14 days).[13,15] Thus, this membrane acts as a temporary physical barrier for a longer time than Seprafilm® between resected rectus muscle and conjunctiva in the surgical area,

result in functions reducing and delaying postoperative formation of adhesions. Furthermore, as the membrane is composed with aseptic ECM obtained from swine knee chondrocyte, no subsequent removal procedure is required because the materials are eventually absorbed into the body.

In our study, we observed grossly the cornea and sclera to verify the presence of corneal erosion or scleral melting before the histological examination. During the 6 week postoperative follow up, we could not observe any serious side effects such as corneal erosion or scleral melting. And, we could not identify any damage to the muscle fibers or the subconjunctival and scleral fibroblasts on histopathological examination. On animal experimental model studies, the cell-derived ECM membrane showed less antigenicity and no serious side effect on toxicity evaluation,[21] However, there are no data in the current literature evaluating the safety of this membrane use on human study. Thus, antigenicity and toxic effects on human eye must be further evaluated.

Rabbits have little subconjunctival connective tissue, so the original resection surgery was performed with little bleeding. It therefore seems quite possible that the operative procedures in rabbit eyes would induce less adhesion postoperatively than in human eyes after strabismus surgery. In addition, the wound recovery in rabbit eyes is much faster than in human eye. Therefore, it can be insufficient to apply our results, taken from animal research, to human studies.[5]

Furthermore, there is another limitation in our study design. Small resection hardly shows fibrosis nor side effects clinically rather than large resection. So experiment with the large resection could be done to overcome our limitation as fibrosis component becomes significant in large resection.

Through this rabbit experimental model study, we could carefully conclude that the use of the cell-derived ECM membrane to strabismus surgery in rabbits can provide a safer and more effective methods for preventing postoperative adhesions and fibrosis between operated rectus muscle and adjacent conjunctiva in the surgical area. Therefore, the cell-derived ECM membrane might be the future item that can be used as reducing postoperative adhesion in strabismus surgery, especially in cases where adhesion may be predicted to develope and recurrent conjunctival dissection may be needed.

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