EXTENDED REPORT

Deposition of silicone oil droplets in the residual anterior lens capsule after vitrectomy and lensectomy in rabbits

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Aim: To examine the histology of preserved anterior lens capsule in vitrectomised and lensectomised rabbit eyes with and without silicone oil tamponade.

Methods: Forty adult Japanese albino rabbits received two port vitrectomy and lensectomy with or without silicone oil tamponade in one eye under both general and topical anaesthesia. Anterior lens capsule was preserved during operation. After healing intervals residual anterior capsule was histologically observed under light or electron microscopy.

Results: Immediately after operation, cuboidal lens epithelial cells were observed on the posterior surface of the preserved anterior capsule. During healing intervals in eyes with or without silicone oil tamponade, regenerated lens structure of Sommerring's ring and fibrous tissue formed in the peripheral and central areas of the residual capsule, respectively. Ultrastructural observation revealed the presence of many vacuoles amid matrix accumulation on the posterior capsular surface, suggesting the deposition of emulsified silicone oil droplets.

Conclusion: Lens epithelial cells produce regenerated lenticular structure and fibrous tissue on the residual capsule following vitrectomy and lensectomy in rabbits. Silicone oil droplets formed by its emulsification deposit in extracellular matrix accumulated on the posterior surface of the anterior capsule. Emulsified silicone may potentially enhance opacification of residual anterior capsule following pars plana vitrectomy by silicone oil deposition and subsequent activation of lens epithelial cells.

Complete removal of the vitreous base is an important step during vitrectomy for the treatment of proliferative vitreoretinopathy or diabetic retinopathy.[1] Excision of the crystalline lens may be useful to facilitate the complete removal of vitreous humour as well as to reduce the risk of developing anterior hyaloidal fibrovascular proliferation.[2] Crystalline lens may be removed by phacoemulsification and aspiration (PEA)[3 4] or pars plana lensectomy (PPL).[5 6] Although PEA in association with a suitable intraocular lens may suppress the formation of capsular opacification,[7–9] such an approach may potentially damage the corneal endothelium by ultrasound and/or irrigation flow. Moreover, preservation of the posterior capsule may prevent complete surgical removal of the peripheral vitreous, leading to a higher risk of late postoperative retinal tear formation. On the other hand, PPL may be beneficial in removal of the vitreous base as well as causing less damage in the corneal endothelium. Preservation of the lens capsule may reduce the risk of damage to the anterior chamber structure during vitreous procedures, as well as providing a mechanical support for the intraocular lens. Following PEA or PPL, the posterior or anterior capsule may be preserved, respectively.[10]

The vitreous cavity may be tamponaded with gas or silicone oil.[11–16] Following PEA, silicone oil injected into the vitreous cavity faces the posterior surface of the posterior capsule; thus it does not attach to lens epithelial cells. However, after PPL, such silicone oil immediately attaches to the residual lens epithelial cells remaining on the posterior surface of the preserved anterior capsule.

Although liquid silicone oil is hydrophobic, it can be emulsified when exposed to endogenous detergents such as protein.[17] Such emulsified oil may be engulfed by local macrophages, but non-digestive oil can be re-released following cell death of macrophages in local tissues and can be deposited there.[18–20] Moreover, emulsified silicone oil causes obstruction of the drainage route of aqueous humour in the anterior chamber angle, leading to the development of secondary glaucoma.[21–23] We have previously reported the presence of many vacuoles, which were presumed to be emulsified oil, in extracellular matrix accumulation in human healing anterior lens capsule following PPL, vitrectomy, and silicone oil tamponade in the treatment of proliferative vitreoretinopathy.[24] However, such human specimens might not be enough to evaluate the exact effect of silicone oil on the histology of residual lens capsule after PPL. In the present study, we examine the histology of residual anterior capsule following lensectomy and vitrectomy with or without silicone oil tamponade in rabbits by using light and electron microscopy.

MATERIALS AND METHODS

Surgical procedure in rabbits

Forty adult Japanese albino rabbits (2.0–2.2 kg body weight) were used, under both general and topical anaesthesia with intramuscular injection of ketamin hydrochloride and xylazine, and oxybuprocaine eyedrops as previously reported.[25] Scleral incision was made at two points 1.0 mm apart from limbus, and then PPL and vitrectomy were performed using the 2 port system under infusion of balanced salt solution (BSS plus, Alcon, Fort Worth, TX, USA). Anterior capsule was preserved and posterior capsule was excised in one eye of each animal. Lens epithelial cells on the inner surface of the anterior capsule were not aspirated. The vitreous cavity in 20 eyes of 20 animals was filled with the irrigation solution, and that of the other 20 eyes was tamponaded with liquid silicone oil following fluid-air exchange technique. Scleral wounds were sutured and an antibiotic solution was administered subconjunctivally. After a specific healing interval of 2, 3, 4 weeks, and 12 months, the animals were sacrificed with an

Abbreviations: PEA, phacoemulsification and aspiration; PPL, pars plana lensectomy.

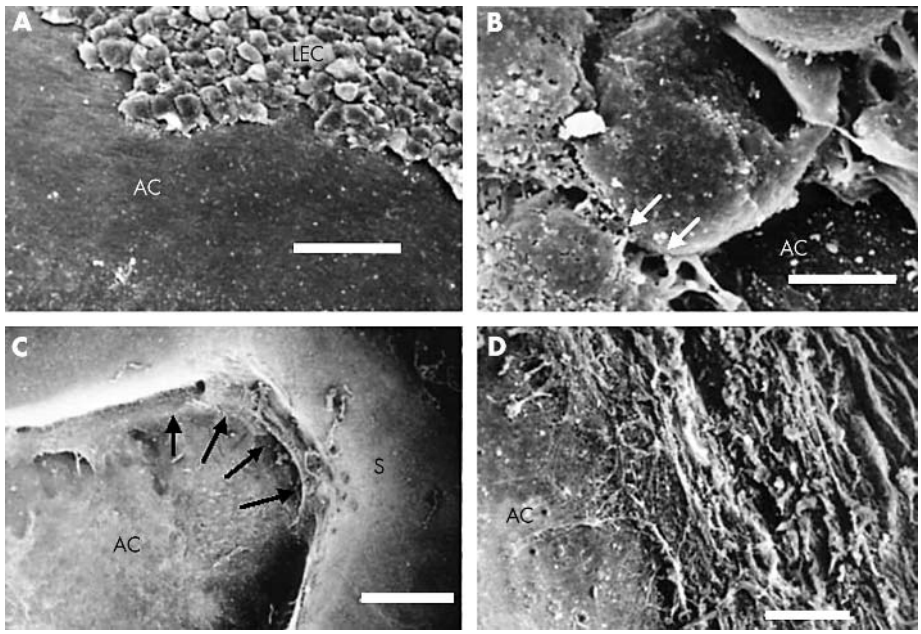

Figure 1 Scanning electron microscopy of the posterior surface of the residual anterior capsule of a rabbit after PPL and vitrectomy. (A) Immediately after the operation, islands of the remaining columnar lens epithelial cells (LEC) are detected on exposed posterior surface (AC) of the residual capsule. (B) At a higher maginification, intercellular junctions are observed (arrows). (C) A posterior surface of the capsular specimen at week 2. Regenerated lenticular structure of Sommerring's ring (S) is observed in the peripheral region. No columnar cells are seen in the central area. (D) A higher maginification picture of the central area shows accumulation of fibrous extracellular matrix structure on the capsular surface (AC). Bar: 50 μm (A and C); 5 μm (B and D).

intravenous overdose of pentobarbital sodium. Each globe was enucleated and processed for histology as described below.

Scanning electron microscopy

Six globes excised at weeks 2 and 4 were fixed in 2.0% glutaraldehyde in 0.1 M phosphate buffer for 24 hours at 4℃. After hemisectioning the globe, the anterior segment of each globe was observed using the Miyake-Apple View[26] after preparation for scanning electron microscopic examination—that is, dehydration through an ethanol series, critical point drying, and cold coating by an ion spatter.[27]

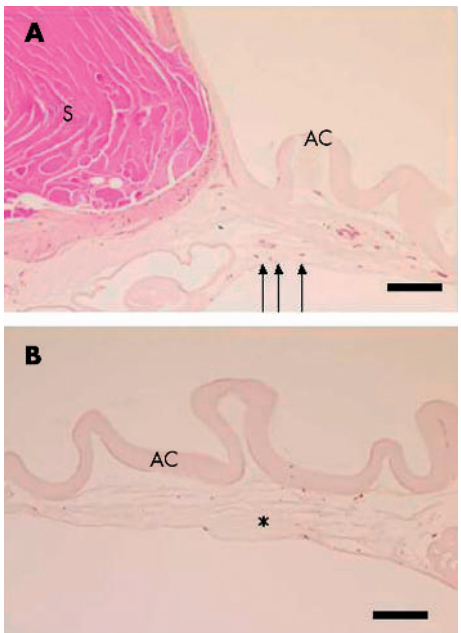

Figure 2 Histology with haematoxylin and eosin staining of a residual capsular structure at week 4. (A) Regenerated lenticular structure of Sommerring's ring (S) is observed in the peripheral region. Arrow indicates the area of adhesion of anterior capsule (AC) and the peripheral edge of the equatorial capsule. (B) The central area of the posterior surface of the remaining anterior capsule (AC) is covered with accumulation of fibrous extracellular matrix structure (asterisk) containing cells. Bar: 40 μm.

Light microscopy and immunohistochemistry

Globes obtained at weeks 3 and 4 were embedded in OCT compound or paraffin following a 48 hour fixation in 2.0% paraformaldehyde in 0.1 M phosphate buffer and dehydration. Cryosections (8 μm thick) or paraffin sections (5 μm thick) were processed for haematoxylin and eosin (HE) staining or indirect immunohistochemistry as previously reported.[28] Antibodies used in this study are listed in table 1.

Transmission electron miscroscopy

Four globes excised at months 1 and 12 were fixed in 2.0% glutaraldehyde in 0.1 M phosphate buffer for 24 hours at 4℃. Residual lens capsule was then removed and post fixed in 1.0% osmium tetroxide for 2 hours. Following dehydration through a graded ethanol series, specimens were embedded in Epon 812 mixture (Quetol 812, Nissin EM, Tokyo, Japan). Ultrathin sections were electron stained with uranyl acetate and lead citrate, and observed under transmission electron microscopy.

Table 1 Primary antibodies used for immunohistochemistry

Antibody	Animal	Dilution	Source
Anti-type I collagen	Goat	X200 in PBS	Fuji Yakuhin Kogyo†
Anti-type I collagen	Goat	X200 in PBS	Southern Biotechnology‡
Anti-type III collagen	Goat	X200 in PBS	Fuji Yakuhin Kogyo
Anti-type III collagen	Goat	X200 in PBS	Southern Biotechnology
Anti-type IV collagen	Goat	X200 in PBS	Fuji Yakuhin Kogyo
Anti-type IV collagen	Goat	X200 in PBS	Southern Biotechnology
Anti-type V collagen	Goat	X200 in PBS	Southern Biotechnology
Anti-cellular fibronectin	Goat	X200 in PBS	Southern Biotechnology
Anti-osteopontin	Goat	X200 in PBS	Santa Cruz Biotechnology§
Anti-α smooth muscle actin	Mouse*	X200 in PBS	Sigma¶

*Mouse monoclonal.
†Toyama, Japan.
‡Birmingham, AL, USA.
§Santa Cruz, CA, USA.
¶Saint Louis, MO, USA.

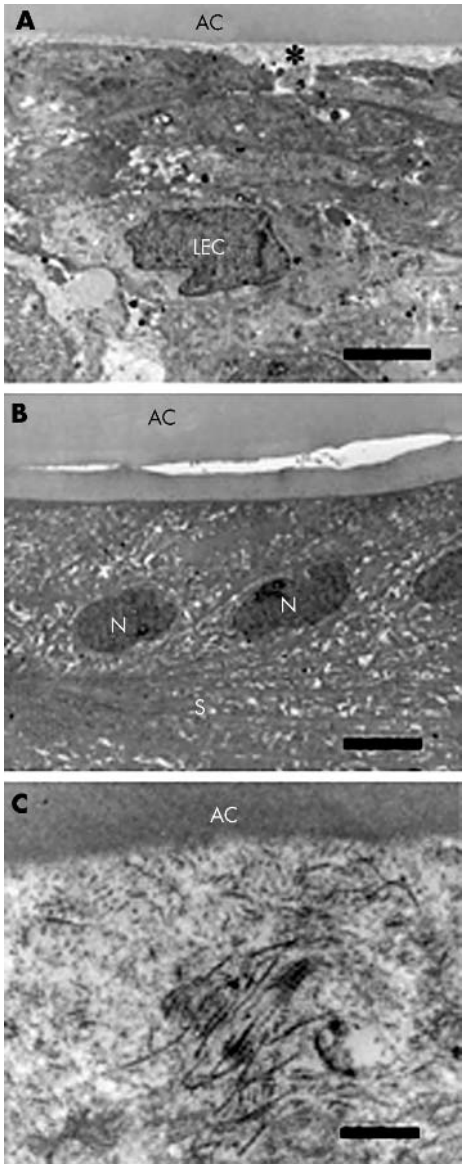

Figure 3 Transmission electron microscopy of residual capsular tissue after PPL and vitrectomy at week 4. (A) The central area of the posterior surface of the remaining anterior capsule (AC) is covered with accumulation of fibrous extracellular matrix structure (asterisk) containing fibroblast like presumed lens epithelial cells. (B) Regenerated lenticular structure of Sommerring's ring (S) is observed in the peripheral region. N indicates the nuclei of fibre differentiating cells. (C) A higher magnification picture of the asterisk area in frame A shows the presence of banded matrix fibres of collagen beneath the anterior capsule. Bar: 3 μm (A); 4 μm (B); 0.5 μm (C).

RESULTS

Light and electron microscopy of healing capsule without silicone oil tamponade

Scanning electron microscopy showed cuboidal epithelial cells lining the inner surface of the anterior capsule immediately after operation (fig 1A and B). The edge of the capsular bag was found to adhere to the peripheral area of the posterior surface of the anterior capsule, forming the regenerated lenticular structure of Somerring's ring (fig 1C). The central area of the posterior surface of the remaining anterior capsule was covered with fibrous connective tissue (fig 1D). Haematoxylin and eosin staining revealed similar findings as suggested by scanning electron microscopy. In healing capsular tissue, the capsular bag was found to adhere

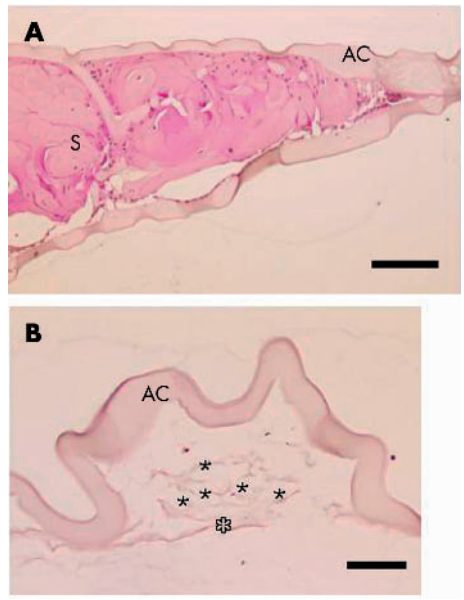

Figure 4 Histology with haematoxylin and eosin staining of a residual capsular structure in a silicone oil tamponaded eye at week 4. (A) Regenerated lenticular structure of Sommerring's ring (S) is observed in the peripheral region. (B) The central area of the posterior surface of the remaining anterior capsule (AC) is covered with an accumulation of fibrous extracellular matrix structure (open asterisk) containing cells. Many vacuoles can be seen amid the matrix (asterisks). Bar: 40 μm.

to the posterior surface of the anterior capsule, forming the regenerated lenticular structure of Sommerring's ring (fig 2A). The central area of the posterior surface of the remaining anterior capsule was covered with fibrous connective tissue (fig 2B). Transmission electron microscopy in specimens at weeks 2 and 4 showed the presence of lens epithelial cells beneath the anterior capsule and regenerated lenticular structure in the peripheral area. Lens cells in the central area of the inner surface of the anterior capsular were elongated and fibroblast like (fig 3A), while regenerated lens fibres were seen in Sommerring's ring in the peripheral area (fig 3B). Extracellular matrix was found to be accumulated amid the lens cells with a fibroblastic appearance. An observation at a higher magnification revealed the presence

Figure 5 Ultrastructure of tissue newly formed on the central region of the posterior surface of the residual anterior capsule at month 12. Fibroblast like presumed lens epithelial cells and extracellular matrix accumulation are observed in the specimen with silicone oil tamponade. In the specimen with silicone oil tamponade, many vacuole like structures—suggesting the presence of emulsified silicone oil—were observed among cells (asterisks). Bar: 6 μm.

of fibrous components with the bundled characteristic of collagen (fig 3C).

Immunohistochemistry

Although light and electron microscopy showed the presence of presumed lens cells with a fibroblastic appearance and extracellular matrix accumulation, such morphological observations were not able to reveal the nature of these components. Immunohistochemistry revealed findings similar to those in previous reports[29–40]: the presence of type IV collagen in the capsule and vimentin positive lens epithelial cells. Lens epithelial cells were negative for type I collagen and cellular fibronectin. The specimens at week 3, with extracellular matrix accumulated on the central posterior surface of the remaining capsule, were labelled with antibodies against collagen types I and IV, cellular fibronectin, and osteopontin (data not shown). Presumed lens cells of a fibroblastic appearance were labelled with the antibodies against vimentin. Cells positive for α smooth muscle actin were also seen as previously reported[29–40] (data not shown).

Histology of healing capsule with silicone oil tamponade

Haematoxylin and eosin staining showed similar histological findings: Sommerring's ring was also seen as for cases without silicone oil tamponade (fig 4A). The accumulation of matrix and distribution of fibroblast like presumed lens cells were observed on the posterior surface of the residual anterior capsule. Vacuole like structures were seen amid the matrix (fig 4B).

Ultrastructural examination

Ultrastructural observation also showed similar histological findings: accumulation of matrix and distribution of fibroblast like presumed lens cells on the posterior surface of the residual anterior capsule (fig 5). Many vacuole like spaces were observed among the accumulation of cells and matrix (fig 5).

Immunohistochemistry

Immunohistochemistry detected collagen types I, III, and V and cellular fibronectin similar to the specimens without silicone oil tamponade (not illustrated).

DISCUSSION

In the present study, presumed lens epithelial cells of a fibroblastic appearance and accumulation of extracellular matrix—that is, collagen types I and IV, cellular fibronectin, and osteopontin—were observed on the posterior surface of the residual anterior capsule in eyes following PPL and vitrectomy. Fibroblast like lens cells were observed expressing α smooth muscle actin. The histological findings are similar to those of capsular opacification following cataract extraction and implantation of an intraocular lens.[29–40] Lens epithelial cells on the residual anterior capsule were exposed to aqueous humour, similar to those following cataract surgery, because the vitreous cavity was replaced by the aqueous humour after removal of the vitreous body. Thus, the cells were considered to be uninfluenced by the growth factors originally contained in the vitreous humour. More interestingly, in eyes with silicone oil tamponade, light and transmission electron microscopies revealed the presence of many vacuoles of presumed emulsified silicone oil amid extracellular matrix accumulation. The deposition of emulsified silicone oil in the iris, ciliary body, and retina tissue has been reported.[41] Deposition of perfluorodecarine in lens capsular tissue has also been reported.[42] In the present study, we did not observe emulsified silicone oil engulfed by macrophages, but found that oil droplets were there among presumed lens epithelial cells. It is likely that lens epithelial cells attaching to oil droplets might be stimulated to express many wound healing related molecules including extracellular matrix components. The deposition of emulsified silicone oil droplets may potentially enhance opacification of residual anterior capsule after PPL by both oil deposition and subsequent activation of lens epithelial cells. Further detailed study is needed to examine the effect on lens epithelial cells of exposure to emulsified silicone oil droplets.

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