European Journal of Ophthalmology / Vol. 15 no. 5, 2005 / pp. 591-597

Effects of membrane adhesion barriers on wound healing reaction after glaucoma filtration surgery: A comparative study with Interceed and Seprafilm

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PURPOSE. To evaluate and compare the effectiveness of two adhesion barriers, Interceed and Seprafilm, on wound healing reaction after glaucoma filtration surgery.
MATERIALS AND METHODS. Full-thickness filtration surgery was carried out on three groups, each containing four rabbits. Interceed and Seprafilm prepared in 3x4 mm dimensions was put on and around scleral opening in Groups 1 and 2, respectively. All groups received tobramycin and dexamethasone drops tid for 14 days. Intraocular pressure (IOP), anterior chamber depth, and bleb appearance were checked on the first, third, seventh, and 14th days. The rabbits were killed on the 14th day and the trabeculectomy area with overlying conjunctiva was excised. The samples were fixed with 10% formalin, buried in paraffin, and stained with hematoxylin and eosin. The surgical site and surrounding subconjunctival area were evaluated histopathologically for cell counts (fibroblast, lymphocyte, eosinophil, and macrophage), presence of edema and foreign body reaction, and potency of the fistula tract.
RESULTS. Mean IOP at the first and third day examinations was significantly different between groups, but there was no statistically significant difference among the groups with respect to IOP, anterior chamber depth, or bleb appearance at the seventh and 14th days. The groups were similar with respect to number of fibroblasts, eosinophils, and neutrophils. Number of macrophages was significantly increased in Groups 1 and 2 and number of vessels was significantly decreased in Group 1.
CONCLUSIONS. Neither of these two adhesion-preventing substances seems to suppress wound healing reaction after glaucoma filtration surgery. However, a diminished wound healing reaction was expected with a decreased number of vessels, such as in Group 1. Increased number of macrophages in both groups may result in a decreased level of some inflammatory mediators. (Eur J Ophthalmol 2005; 15: 591-7)

KEY WORDS. Interceed, Seprafilm, Filtration surgery, Wound healing

Accepted: March 2, 2005

INTRODUCTION

Glaucoma filtration surgery (GFS) involves the creation of a new drainage channel for the aqueous humor to flow out of the eye and thus lower intraocular pressure (IOP) (1). Successful lowering of the pressure depends mostly on the nature of the healing response, which is also the single most important modifiable factor (2). Adhesions may develop as a normal consequence of wound healing process in any tissue. Subconjunctival adhesions are the leading causes of failure after GFS. Increased permeability of the blood vessels in the traumatized tissue produces inflammatory exudates rich in plasma proteins, such as fibrinogen. Under optimal conditions, the majority of fibri-

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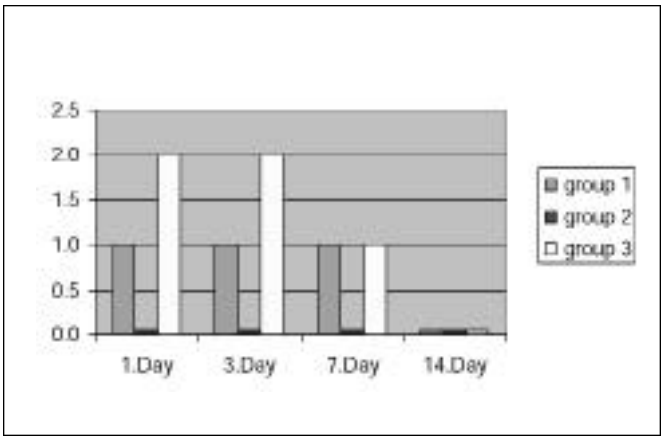

Fig. 1 - Number of elevated and functioning blebs in study groups.

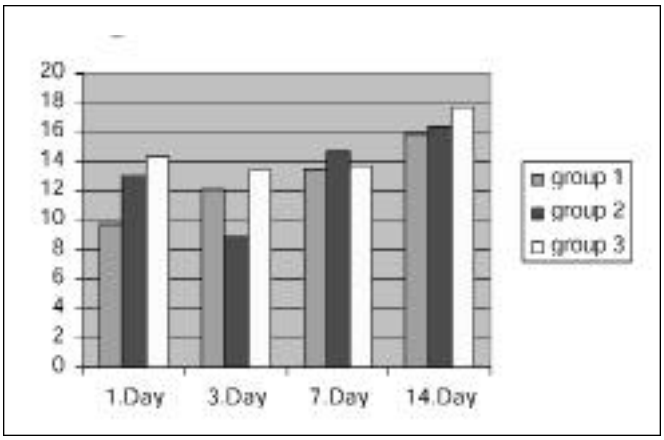

Fig. 2 - Mean intraocular pressures on the first to 14th days in the study groups.

nous attachments so formed are absorbed within a few days by fibrinolytic mechanisms. If they persist, fibroblastic proliferation may occur, causing adhesion formation (3).

Capability for adhesion formation is not identical for different tissues of the human body. GFS site differs from any ocular area in one fundamental aspect: it is bathed by aqueous humor and its contents can significantly affect the healing response (1, 4). Normal rabbit aqueous humor was shown to be powerfully chemoattractant to rabbit Tenon's capsule fibroblasts (5) and similar effects of human aqueous were also confirmed (6). The factors reported to stimulate fibroblasts in the aqueous humor are fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF) beta-1, insulin-like growth factor (IGF), fibronectin, transferrin, and interleukin 6. All of these substances stimulate fibroblast proliferation, migration, and collagen production to some degree (1).

Wound healing modulation after GFS already has been achieved by a growing number of approaches (6-9). Research in this area has strongly focused on inflammatory response following surgical trauma. Most used anti-inflammatory and antimetabolite derivatives inhibit the synthesis of inflammatory mediators leading to decreased granulocyte and mast cell degranulation, fibrin formation, and fibroblast proliferation (7, 8). Subconjunctival adhesion prevention with adhesion barriers may also control wound healing response and reduce failure.

Oxidated regenerated cellulose (ORC; Interceed), the first barrier agent to be used, was found to be effective in many clinical studies in gynecologic and abdominal surgery (9, 10). Sodium hyaluronate/carboxymethylcellulose (NaH/CMC; Seprafilm), a polymer film based on a combination of modified hyaluronic acid and carboxymethylcellulose, has been used to cover the abraded area after gynecologic and abdominal surgery for the prevention of adhesions (11, 12).

In this study, we evaluate and compare the effectiveness of two adhesion barriers, ORC and NaH/CMC, on wound healing reaction after GFS.

TABLE I - CELL COUNTS AS MEAN ± STANDARD DEVIATIONS IN STUDY GROUPS

	Group 1	Group 2	Group 3
Fibroblast	20.75±5.74	10.75±7.46	14.75±8.62
Macrophage	4.50±3.42	1.25±1.50	0.5±0.58
Lymphocyte	5.75±0.96	14.5±2.65	15.5±10.79
Eosinophil	0.0±0.0	1.0±0.82	3.25±1.71
Neutrophil	1.75±1.26	2.50±2.65	1.75±0.96

MATERIALS AND METHODS

This study was performed by the Ophthalmology and Pathology Departments of Fırat University School of Medicine. The experiments adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Twelve 6-month-old male albino rabbits were used for this study, with mean body weight of 2.87±0.36 kg. Three study groups were formed, each consisting of four rabbits.

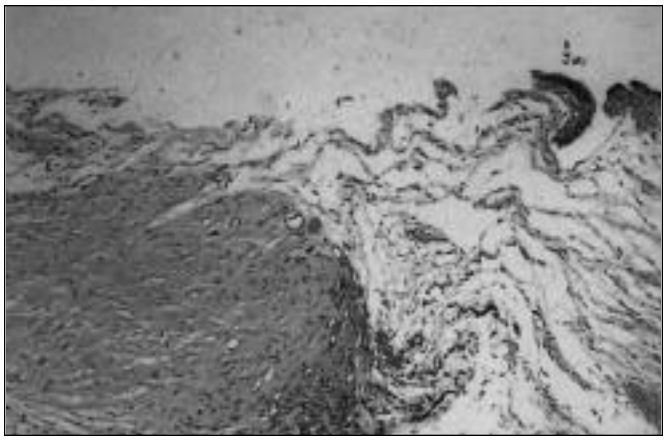

Fig. 3 - Photomicrograph of the operation site from a Group 1 (Interceed-treated) animal. Under the thickened conjunctival epithelium, newly formed connective tissue contains numerous activated fibroblasts and macrophages. Inflammatory infiltration is moderate. Moderately increased vascular patterns are seen (hematoxylin and eosin stain, original magnification x40).

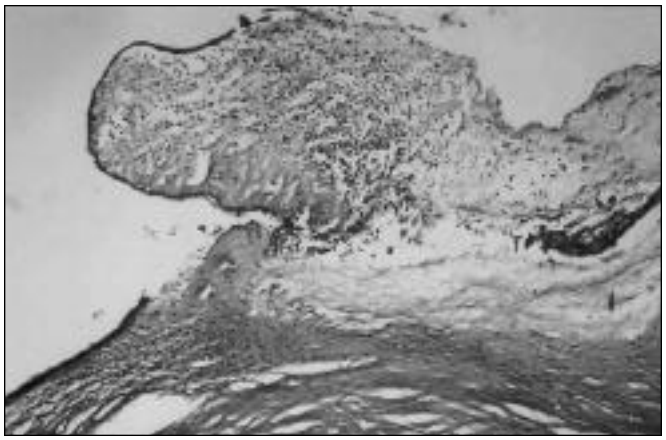

Fig. 4 - Photomicrograph of operation site of a Group 2 (Seprafilm-treated) animal shows the disorganized and comparatively hypocellular connective tissue. Subconjunctival area displays severe lymphocytic infiltration. Vascular patterns do not seem to increase (hematoxylin and eosin stain, original magnification x40).

Experimental filtration surgery

Full-thickness filtration surgery was performed in one eye of all rabbits in the following manner.

After the rabbits were anesthetized with intramuscular injections of xylazine hydrochloride 5 mg/kg (Rompun, Bayer, Istanbul, Turkey) and ketamine hydrochloride 25 mg/kg (Ketalar, Eczacýbasý, Istanbul, Turkey), the right eyes of the rabbits were cleaned and draped for surgery. A drop of oxybuprocaine hydrochloride (Benoxinate, Thilo&Co GmbH, Puurs, Belgium) was instilled and the traction suture was placed to the upper eyelid.

A limbal-based conjunctival flap was fashioned and a full-thickness scleral block excision was carried out next to the limbus in the superior quadrant of all eyes. A peripheral iridectomy was then performed and hemostasis was established.

At this point, Interceed membrane and Seprafilm, prepared in 3x4 mm dimensions, was placed to the opened sclera of the eyes in Groups 1 and 2, respectively. The membranes were draped with the conjunctival flap and left there.

No treatment was applied on the sclera in Group 3 (control group). At the end of the procedure, the conjunctiva was closed with a running 8-0 polyglactin suture and the eyes were patched after instillation of ophthalmic ointment consisting of oxytetracycline and polymyxin B sulphate. All of the eyes were operated by the same surgeon.

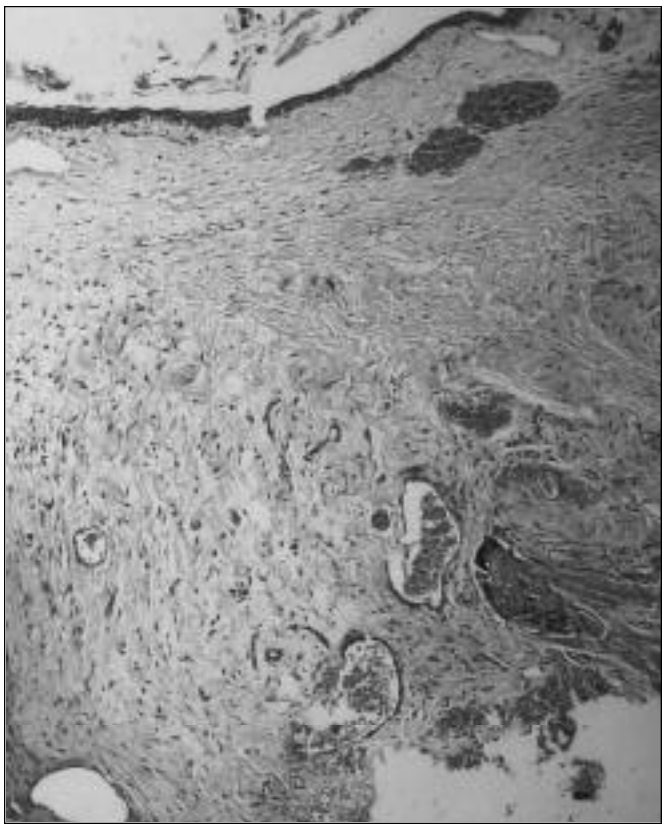

Fig. 5 - Photomicrograph of operation site of a Group 3 (control) animal shows numerous activated fibroblasts and newly formed collagen under the thickened conjunctival epithelium. Subconjunctival area displays mild to moderate lymphocyte infiltration. Vascular patterns seem to be more frequent than in the other groups (hematoxylin and eosin stain, original magnification x40).

Postoperative follow-up

All groups received tobramycin and dexamethasone drops tid for 14 days. IOP, anterior chamber depth, and bleb appearance were checked and recorded on the first, third, seventh, and 14th postoperative days. Schiotz indentation tonometer was used for IOP measurements. Anterior chamber depth was evaluated with the help of a penlight and estimated as grade 0 (flat chamber), grade I (narrow chamber), or grade II (chamber with a normal depth). Bleb appearance was also evaluated with inspection, and graded as grade 0 (no bleb or hardly visible nonfunctioning bleb), grade I (slightly elevated and functioning bleb), or grade II (grossly elevated and functioning bleb).

Histologic preparation

Animals were killed by overdose intravenous pentobarbital anesthesia at the end of the 14th day. After the placement of a blepharostat, a fixation suture was placed 3 mm behind the operation site, helping conjunctiva remain attached to the sclera. Then, 10x10 mm square corneoscleral blocks with overlying conjunctiva were dissected from all operated eyes having the trabeculectomy site on the center of the samples.

The blocks were immediately fixed with 10% formalin and then buried in paraffin. Five-micrometer-thick sections crossing the visible or estimated fistula site were cut using a microtome (Leica). The samples were stained with hematoxylin and eosin. Three sections were cut at a minimum of 20 µm apart to provide a different population of cells in each section.

Light microscopic analysis of the specimens was performed with the x40 objective of a standard light microscope (BH2 Olympus Photomicroscope) and x10 eyepieces.

Histopathologic analysis of the surgical site and surrounding subconjunctival area consisted of 1) cell counts per area (fibroblast, lymphocyte, eosinophil, neutrophil, and macrophage); 2) vessel count per area; 3) presence of edema and fibrosis, graded as absent (0), mild (1), moderate (2), severe (3); 4) presence of foreign body reaction, graded as absent (0) and present (1); and 5) potency of the fistula tract, graded as closed (0), semi-open (1), and open (2). All counts were made in two microscope areas and means±standard deviations were used. Only clearly identified cells with peculiar nuclear and cytoplasmic features were counted. Foreign body reaction was evaluated only in trabeculectomy site; reaction to suture area was omitted.

Statistical analysis

The values obtained from the clinical and histopathologic evaluation were statistically analyzed using nonparametric tests (Mann-Whitney U and Kruskal-Wallis tests) in SPSS for Windows. p Values were used to show statistical significance in comparisons and a p value less than 0.05 was considered significant.

RESULTS

The standardized surgical procedures and the administration of the protocols were well tolerated by the animals. None of the eyes showed infection signs. Anterior chamber depth was assessed as normal in all examinations of the groups. Figure 1 gives data about the appearance of filtration blebs by means of the number of the elevated and functioning blebs. Mean and SD of the grades in study groups were as follows: 0.75±0.96, 0.5±1.0, 0.5±1.0 (Group 1); 0.5±0.58, 0.5±0.58, 0.25±0.5 (Group 2); and 1.25±0.96, 1.25±0.96, 1.0±0.82 (Group 3) on the first, third, and seventh postoperative days, respectively. All of the filtering blebs failed on the 14th day and groups were found statistically similar with respect to the bleb appearance (Kruskal-Wallis test).

Figure 2 shows the mean IOP at the postoperative examinations. Mean IOP at the first day examination was significantly different in Group 2 from the other groups. Also, mean IOP at the third day was significantly different in Group 3 from the other groups. On the other hand, no overall difference was found among groups at the seventh and 14th day examinations.

Histopathologic analysis of the sections from the operation site of the control group displayed one patent and three closed fistula tracts. The number of patent fistula tracts was two in Group 1 and one in Group 2. Groups were statistically similar with respect to the appearance of the fistula tract. Table I shows the mean cell counts in the light microscopic examinations. Mean number of eosinophils in Group 1 was significantly less than Groups 2 and 3 (p=0.046 and p=0.014, respectively; Mann-Whitney U test). Mean number of lymphocytes in Group 1 was significantly less than Group 2 (p=0.020; Mann-Whitney U

test). Histopathologic examination of the sections showed no foreign body reaction in any group.

A thick, dense layer of connective tissue lying under the epithelium was detected in all groups. The active fibroblasts of Tenon's layer were increased in number, elongated, and oriented parallel to each other. The elongated fibroblasts also invaded old scleral collagen. The fibroblasts in Group 1 seemed less infrequent than in Group 2 in spite of statistical insignificance. Active macrophages, which were widespread in Groups 2 and 3, appeared mostly around the fistula tract on which barrier membranes have been implanted. No statistically significant difference in the subconjunctival macrophage population was demonstrated between groups. Newly formed subepithelial connective tissue was rich with blood vessels. Mean numbers of vessels were 3.5±0.58, 6.0±7.62, and 9.75±2.36 in Groups 1, 2, and 3, respectively. The mean number of vessels in Group 1 was significantly less than in Group 3 ($p=0.019$). No statistical significance was found between the numbers of vessels of the other pairs of groups. Figures 3 through 5 show photographs of the light microscopic sections of Groups 1, 2, and 3, respectively.

DISCUSSION

Conjunctival wound healing initiates with the inflammatory phase, which is characterized by the movement of intravascular components to the extravascular area. By the end of this stage, a clot is formed and facilitates the movement of other cellular components into the wound. Among them, fibroblasts originating mostly from adjacent tissues (Tenon's capsule and episclera) appear on the third day and become the dominant cellular component of the wound in the second (fibroblastic) phase. Angiogenesis immediately follows the fibroblast migration and these two form a granulation tissue. Wound closure is achieved by the epithelialization with the migration and proliferation of the epithelial cells, and by contraction of the myofibroblasts, which originated from fibroblasts. Contraction starts at 5 to 7 days and is maximally observed at the fourth to fifth weeks. Remodeling, the last phase of healing, begins during the fibroblastic phase and may last for more than a year (7, 8).

Rabbits and monkeys are the most frequently used animal models for wound healing studies because of their suitably sized globes (6). We used rabbits because they are less expensive, docile, and easy to care for. Filtration surgery tends to fail in animal experiments, which indicates that the behavior of animal eyes is different from that of humans (6). The wound healing process generates in a relatively short time in animals. In the rabbit model, young fibrovascular tissue was seen in the fistula by the third day and it peaks within the first 2 weeks after experimental GFS (1, 13). Using these data, we chose to evaluate wound healing reaction at the 14th day.

The studies about wound healing modulation after GFS are far from accessing the endpoint (6-9). One of the important causes of the failure after GFS is subconjunctival adhesions. Adhesion formation in general may be reduced by several routes: reduction of the initial inflammatory response and subsequent exudation (i.e., topical corticosteroids), inhibition of fibroblastic proliferation (i.e., mitomycin-C), promotion of fibrinolysis, and mechanical separation of surfaces (14). The last one is achieved by barrier agents, which include mechanical barriers and viscous solutions (3). The ideal barrier should be nonreactive, bioabsorbable, and easy to use; also, it should persist during the critical stages of the wound healing. As far as we know from their intraperitoneal applications, both the ORC and NaH/CMC fit these requirements and they have been approved for clinical use (11, 15).

To our knowledge, there is no study about the use of barrier agents to modulate the wound healing reaction after GFS in the ophthalmic literature. There are two controversial studies about the use of barrier agents (ORC) in strabismus surgery (16, 17). Yaacobi et al reported that the use of ORC sleeves significantly increases the formation of postoperative adhesions (16). However, Hwang and Chang reported that the combined use of ORC, 5-fluorouracile, and Viscoat could delay the adjustment time after adjustable strabismus surgery in rabbits (17). The most confusing point about the effects and biodegradation behavior of these products in GFS is the presence of aqueous humor in the environment.

The exact mechanism of action of ORC is unknown. Although it has been thought to function as a physical barrier in the beginning, there are convincing data about its breakdown products being biologically active. Interaction of ORC with macrophages may result in a decreased secretion of matrix components, inflammatory mediators like interleukin-1 beta, and cellular growth factors (18).

With our small study size, it is difficult to discuss any serious interaction of ORC with wound healing reaction. The statistically insignificant differences in overall IOP and

filtrating blebs allow little hope for this product. On the other hand, some results of the histopathologic examination may be convincing for the future use of ORC in GFS. The decreased number of vessels in the ORC group compared with controls may show less aggravated wound healing reaction. In addition, the statistically significant increase in macrophage counts may result in a decreased level of inflammatory mediators like interleukin-1 beta and cellular growth factors, as shown before (18).

In vivo and in vitro biodegradation and solubilization of ORC was well studied for the conditions in which it is used as intraperitoneal adhesion barrier. Dimitrijevich et al collected peritoneal fluid, serum, and urine from rabbits in whom ORC were surgically implanted on their uterine horns and analyzed for carbohydrate components utilizing high performance liquid chromatography with pulsed amperometric detection (19). They reported that oligomeric products were evident in peritoneal fluid from the implantation site, with no apparent accumulation in either the serum or the urine. The size and amount of products rapidly decreased and by day 4, peritoneal lavages were free of oligomers (19). In vitro solubilization was studied by the same authors and method, with the presence of serum/plasma and hydrolytic enzymes. They reported that ORC first undergoes chain shortening to give oligomers, which, in the presence of plasma or serum, are further hydrolyzed to smaller fragments, including glucuronic acid and glucose (20).

It is reported in two prospective randomized multicenter studies that NaH/CMC also reduced postoperative adhesions of the abdominopelvic cavity (11, 12). It acts as a temporary barrier that keeps the surfaces separated during the early days of wound healing. Although the exact mechanism of action of NaH/CMC is unknown, it has been thought to function as a physical barrier (21). Seprafilm treatment of normal peritoneal fibroblasts, adhesion fibroblasts, and mesothelial cells did not alter the expression of markers examined: TGF-beta 1, type I collagen, matrix metalloproteinase-1 and 2, tissue inhibitor of metalloproteinase 1, and tissue plasminogen activator (21). It hydrates to a gel in 2 days and then slowly resorbs from the abdominal cavity in about 5 to 7 days and is excreted from the body within 28 days (12). Foreign body reaction was not reported in abdominopelvic surgery (17). The unique disadvantage of the material in abdominal surgery was reported as a possible increased risk for anastomotic leak, which would be a disputable advantage for GFS (22).

Similar discussion as with ORC may be put forward for NaH/CMC about its effects of wound healing reaction. Despite lack of statistical significance, the number of vessels in the NaH/CMC group was decreased compared with controls. The number of macrophages was significantly increased, as in the ORC group. However, there were no statistically significant differences in overall IOP or filtrating bleb appearance. Because these two agents successfully prevent intraperitoneal adhesions, it would not be incorrect to say that they were interacting with wound healing reaction. On the other hand, it may not be possible to achieve a functioning bleb in an animal even with adjunctive therapy for wound healing modulation, due to the augmented healing reaction (6).

Barrier agents may have great potential in GFS. More studies on glaucomatous human eye should be performed to explore their effects.

The authors have no proprietary interest in any aspect of the article.

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