Polytetrafluoroethylene/Polylactide-co-Glycolide Laminate Containing Dexamethasone Allows Delayed Adjustable Strabismus Surgery in a Rabbit Model

Jun-Pil Jee,1,2 Ho-Kyung Choubang,2,3 Chong-Kook Kim,1 and Jeong-Min Hwang4

PURPOSE. To determine the usefulness of polytetrafluoroethylene/polylactide-co-glycolide (PTFE/PLGA) laminate containing dexamethasone in delayed adjustable strabismus surgery.

METHODS. A prospective, masked-observer, controlled study was performed in rabbits. Fifty-two rabbit eyes were divided into three groups. After a recession of the superior rectus muscle (SRM), a PTFE/PLGA containing or not containing dexamethasone or balanced saline solution was applied beneath and over the SRM in the three treatment groups: the PTFE/PLGA-dexamethasone group (the P-D group), the PTFE group (the P group), and the control group (group C). Delayed adjustment was performed once on each SRM at 3 or 5 weeks after surgery by a masked observer. Adjustment lengths, the forces required, and degrees of adhesions were evaluated.

RESULTS. In the control group, adjustment was possible in no eyes at 3 or 5 weeks after surgery. In group P, adjustment was possible in 6 of 10 eyes at 3 weeks after surgery and in 4 of 9 eyes at 5 weeks after surgery. In group P-D, adjustment was possible in 7 of 9 eyes at 3 and 5 weeks after surgery.

CONCLUSIONS. PTFE/PLGA containing dexamethasone was found to allow delayed adjustment in most eyes for up to 5 weeks after surgery without instillation of anti-inflammatory agent. (Invest Ophthalmol Vis Sci. 2006;47:2485–2490) DOI:10.1167/iovs.05-0796

Since Jampolsky’s introduction in 1975,1 adjustable-suture strabismus has become an effective method of adjusting binocular alignment in the immediate postoperative period.2 However, binocular alignment may drift over time, even after the eyes are placed in a suitable position by adjustable strabismus surgery.3 Therefore, delayed adjustment may be desirable for better postoperative results.4,5 However, the postoperative healing process causes adhesions that inhibit such a delayed adjustment.

From the 1National Research Lab for Drug and Gene Delivery, College of Pharmacy, Seoul National University, Seoul, Korea; the 2Department of Ophthalmology, Seoul National University College of Medicine, Seoul National University Boramae Hospital, Seoul, Korea; and the 3Department of Ophthalmology, College of Medicine Seoul National University, Seoul National University Bundang Hospital, Seongnam, Korea.

2Contributed equally to the work and therefore should be considered equivalent first authors.

Supported by Grant 02-03-008 from the Seoul National University Bundang Hospital Research Fund.

Submitted for publication June 23, 2005; revised December 9, 2005; accepted April 20, 2006.

Disclosure: J.-P. Jee, None; H.-K. Choung, None; C.-K. Kim, None; J.-M. Hwang, None

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Corresponding author: Jeong-Min Hwang, Department of Ophthalmology, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam, Gyeonggi-do 463-707, Korea; hjm@snu.ac.kr.

Delayed adjustment has been attempted by implanting physical barriers such as silicone,3,6 viscoelastic material,7 oxidized regenerated cellulose barrier (Interceed TC7; Johnson & Johnson Medical Inc., Arlington, TX)9 polyglactin 910 mesh,8 polytetrafluoroethylene (PTFE),9 or bioresorbable adhesion barrier film (SurgiWrap; Cytori Therapeutics, San Diego, CA)10 or by using antiproliferative agents such as mitomycin C11 or combinations of these various physical barriers and antiproliferative agents.12–14 In animal experiments, adjustment could be delayed by using silicone for up to 11 days in humans and for up to eight weeks in rabbits.15 However, silicone can cause discomfort because of its thickness and rigidity and may trigger infection, extrusion, or granuloma formation. PTFE barriers have been shown to allow delayed adjustment for up to 4 weeks and to have some advantages over other physical barriers.9,15 However, all barriers require the frequent instillation of an anti-inflammatory agent.

Thus, a system that allows the slow release of an anti-inflammatory at a sustained therapeutic concentration over several days or weeks would obviate the need for multiple instillations. Biodegradable polymers have been used for site-specific controlled drug release over extended periods in various organs, including the eyes.15 Of these biodegradable polymers, poly(lactide-co-glycolide; PLGA) copolymers have been most extensively studied, because they are degraded by simple hydrolysis of ester bonds into lactic and glycolic acids, which are biocompatible and removed by normal metabolic pathways.16 Moreover, they are one of the few biodegradable polymers that are approved for specific human clinical use by the U.S. Food and Drug Administration.17 In this experimental study, we evaluated the effectiveness of PTFE, containing sustained-release dexamethasone for the prevention of postoperative adhesions, in enabling delayed adjustment after strabismus surgery without frequent topical steroid instillation.

MATERIALS AND METHODS

Twenty-six New Zealand White rabbits, weighing 2.0 to 3.0 kg, underwent 5-mm recession of both superior rectus muscles (SRMs) using double-armed nonabsorbable 5-0 polyester sutures (5-0 Ethibond; Ethicon, Piscataway, NY) in rabbit eyes and subsequent adjustment at 3 and 5 weeks after surgery. All study procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

PTFE (WL Gore & Associates, Inc., Flagstaff, AZ) containing sustained-release dexamethasone was used as a physical barrier in group P-D animals, whereas PTFE not containing dexamethasone was used in group P. In group C, the control group, no physical barrier was used. Fifty-two SRMs of 26 New Zealand White rabbits were allocated to group C (12 muscles; 6 for examination at 3 weeks and 6 at 5 weeks), group P (20 muscles; 10 for examination at 3 weeks and 10 at 5 weeks), and group P-D (20 muscles; 10 for examination at 3 weeks and 10 at 5 weeks). The 52 SRMs were allocated randomly to the three groups by the drawing of lots.
Preparation of Polymeric Film Fabrication
Poly(t-lactide-co-glycolide) 75:25 (lactide:glycolide) (PLGA; average molecular weight, 90,000–126,000; Sigma-Aldrich, St. Louis, MO) containing dexamethasone was cast onto PTFE film using a solvent-casting technique. Briefly, dexamethasone (141 mg) and PLGA (940 mg) were dissolved in 10 mL of acetone (Fisher Scientific, Suwanee, GA) with stirring for 20 minutes at room temperature. Blank and drug-containing PLGA solutions were cast onto glass plates and onto PTFE film-coated glass plates. The cast polymer films were dried in a refrigerator for 48 hours to allow the acetone to evaporate slowly and then dried under an oven for 24 hours at 40°C. The PLGA films and PTFE films coated with PLGA were peeled from the glass plates and cut into 1 × 2 cm pieces. Film drug concentrations were approximately 1.5 mg/cm².

Determination of Dexamethasone Loading PLGA
Polymer films were dissolved in 2 mL of acetonitrile with vortexing for 1 minute. The polymer solutions obtained were then filtered through a 0.45-μm membrane filter to remove precipitated material, and acetonitrile (4.5 mL) was then added to 0.5 mL of filtrate and vortexed for 30 seconds. One hundred microliters of this solution was diluted to 900 μL with mobile phase, described in the next paragraph, and a 100-μL aliquot was directly injected into a high-performance liquid chromatograph (HPLC). Dexamethasone loadings were then determined as previously described.18

The HPLC system used comprised a pump (LC-10AS; Shimadzu, Kyoto, Japan), UV detector (SPD-10A; Shimadzu), autosampler (717 plus autosampler; Waters, Milford, MA), and a C₁₈ reversed-phase column (Capcellpak UG 120, 5 μm, 250 mm × 4.6 mm; Shiseido, Kakegawa City, Japan). Data were analyzed on computer (dsChrom software; Donam Instruments, Gyeonggi-do, Korea). The mobile phase consisted of 60% (vol/vol) acetonitrile and 40% (vol/vol) 2 mM acetate buffer (pH 4.8). The volume injected was 100 μL, and the flow rate used was 1.0 mL/min. Under these conditions, the linear calibration curve of dexamethasone was obtained in the concentration range 0.5 to 100 μg/mL (r² > 0.999).

In Vitro Release of Dexamethasone
Dexamethasone release from the polymer laminate was studied in phosphate-buffered saline (PBS). A polymer film sample (1 × 2 cm) was placed in 20 mL of phosphate-buffered saline (PBS; pH 7.4) containing 0.1% sodium azide as a preservative under sink conditions and magnetically stirred in a circulating water bath at 37°C. At defined time intervals (0, 0.04, 0.08, 0.13, 0.17, 0.21, 0.25, 0.33, 0.42, 0.5, 1, 2, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, 27, and 29 days), 500-μL aliquots of the medium were collected, replaced with an equal volume of fresh PBS, and filtered through a 0.45-μm membrane filter, and 100 μL of the solution was directly injected into the HPLC for dexamethasone quantification, as just described.

Procedures
General anesthesia was achieved by administering 30 to 45 mg/kg of ketamine hydrochloride and 5 to 10 mg/kg of xylazine hydrochloride intramuscularly and topical anesthesia with proparacaine hydrochloride. The muscle recession procedures with or without a physical barrier were performed by HKC. To perform an adjustment later, the ends of the sutures were secured and a noose was placed around the sutures attached to the SRM. Polyvinylpyrrolidone-iodine was applied to the eyelids for preoperative antisepsis. A limbal peritomy was performed from 10 to 2 o’clock. SRM was isolated on a Jameson hook, and intermuscular connections were dissected. The superior oblique tendon was disinserted and allowed to retract from the surgical field. The SRM was then placed on a double-armed 5-0 polyester (5-0 Ethibond; Ethicon) suture close to the insertion and disinserted from the globe. In group P-D (20 SRMs) a separate sheet (1 × 2 cm) of PTFE/PLGA containing dexamethasone was placed between the sclera and the SRM (Fig. 1A) with PLGA and folded to separate the conjunctiva and superior rectus muscle. An identical process was used in the PTFE/PLGA without dexamethasone group (group P; 20 SRMs); no barrier was inserted in the group control (group C; 12 SRMs). Finally, the SRM was recessed 5 mm and reattached to the original insertion by using a hang-back suture technique. A bucket-handle suture was made for future traction. The edges of the conjunctival peritomy were approximated with interrupted 8-0 polyglactin sutures. At the end of each procedure, ofloxacin eye ointment was applied topically.

Delayed Adjustment
In a masked, random fashion, SRMs were adjusted under the anesthesia described above either at 3 or 5 weeks after surgery in all three groups. The delayed adjustment procedure was performed by JMH. The previous conjunctival incision site was opened with the tip of a curved needle holder. After the SRMs were fully exposed, the PTFE or PTFE/PLGA containing dexamethasone was placed between the sclera and the SRM (Fig. 1B) with PLGA and folded to separate the conjunctiva and superior rectus muscle.
PLGA membrane was visible and was removed before adjustment. A dial-tension gauge (DT-50; Teclock Inc., Okaya, Japan) was used for precise control of the amount of force applied. A noose suture attached to the SRM was hooked with a bar of the dial-tension gauge. SRMs were then moved anteriorly as much as possible (this distance was measured using a Castroviejo caliper) and the force required registered on the gauge. Adjustment distances and forces were recorded and the adjustment completed.

Evaluation of Adhesions

At the time of adjustment, adhesions between muscle, PTFE or PTFE/PLGA membrane, sclera, and conjunctiva were evaluated and recorded. Adhesions were classified as SRM/C (superior rectus muscle/conjunctiva) or SRM/S (superior rectus muscle/sclera), when located above or below the SRM, respectively. Adhesion severities were scored from 0 to 4, where: 0 was no adhesion; 1, a filmy adhesion easily separable with blunt dissection; 2, a mild to moderate adhesion with a freely dissectible plane; 3, moderate to dense adhesion with difficult dissection; and 4, nondissectible plane (Fig. 2). The animals were killed after the delayed suture adjustment by administering a 10 mL intravenous injection of sodium pentothal.

Postmortem Histologic Examination

The involved tissues in each group were examined macroscopically and microscopically after death with hematoxylin and eosin (H&E) staining. A histopathologic study was performed to evaluate adhesions between sclera, SRM, and conjunctiva.

Statistical Analyses

Statistical analyses were performed with the Fisher exact test to identify intergroup differences with respect to adjustability. Advancement distances, the forces needed to advance SRMs, and adhesion severity were analyzed using the Mann-Whitney test. Statistical significance was accepted for \( P \leq 0.05 \).

RESULTS

During the follow-up period, before adjustment, two eyes in group C, one in group P, and two in group P-D were lost because of infection.

In Vitro Release of Dexamethasone

The release of dexamethasone from PTFE/PLGA film was found to occur in a triphasic manner, with an initial release burst followed by low- and high-release phases (Fig. 3). The initial release of dexamethasone was due to surface release. Dexamethasone was released from the PTFE/PLGA film in a triphasic manner, with an initial release burst followed by low- and high-release phases (Fig. 3).

![Figure 2. The degree of adhesions. (A) Grade 1, filmy adhesions easily separable with blunt dissection; (B) grade 2, mild to moderate adhesions with freely dissectible plane; (C) grade 3, moderate to dense adhesions with difficult dissection; and (D) grade 4, nondissectible plane.](image)

![Figure 3. In vitro cumulative release profile of dexamethasone from dexamethasone-PLGA-coated PTFE film. The release profile of dexamethasone from PTFE/PLGA film was studied in PBS at pH 7.4 and 37°C (n = 4). The total amount of dexamethasone released into the PBS was expressed as a percentage of the total amount of dexamethasone loaded into the PTFE/PLGA film.](image)
methasone release during the second and third phases is probably explainable by a combination of drug diffusion through the PLGA film and the hydrolytic degradation of PLGA.

Adjustability

In group C, adjustment was impossible in all eyes at 3 and 5 weeks after surgery. In group P, adjustment was possible in 6 of 10 eyes at 3 weeks after surgery and in 4 of 9 eyes at 5 weeks after surgery. In group P-D, adjustment was possible in seven eyes at both 3 and 5 weeks after surgery (Table 1).

On comparing adjustability at 3 weeks after surgery, a significant difference was found between groups P and C (P = 0.044) and between groups P-D and C (P = 0.021), but at 5 weeks after surgery, no significant difference was observed between groups P and C (P = 0.221), though a significant difference remained between groups P-D and C (P = 0.021). No significant difference in adjustability was observed between groups P and P-D at 3 weeks (P = 0.628) or 5 weeks after surgery (P = 0.355).

Adjustment Amount and Force Required

In group P, average adjustment (advancement) amount and force were 2.33 mm and 41.67 g at 3 weeks after surgery and 1.88 mm and 59.25 g at 5 weeks after surgery. In group P-D, the average adjustment amount and force were 2.71 mm and 37.29 g at 3 weeks and 2.50 mm and 41.29 g at 5 weeks after surgery (Table 1). On comparing the adjusted amount and force, no difference was found between groups P and P-D at 3 or 5 weeks after surgery (P > 0.05).

The Degree of Adhesion between SRM and Conjunctiva

The degree of adhesion was moderate, with a freely dissectible plane or with difficult dissection in most of the eyes in group C. In groups P and P-D, there was filmy adhesion easily separable with blunt dissection or mild to moderate with freely dissectible plane in most of the eyes (Table 2).

A significant difference was observed between groups P and C (P = 0.032) and between groups P-D and C (P = 0.014), with respect to degree of adhesion between SRMs and conjunctivae at 3 weeks after surgery, and between groups P and C (P = 0.009) and groups P-D and C (P = 0.003) at 5 weeks after surgery. No significant difference was observed between groups P and P-D (P > 0.05) at 3 or 5 weeks after surgery.

Degree of Adhesion between SRM and Sclera

The degree of adhesion was moderate, with a freely dissectible plane or with difficult dissection in most of the eyes in group C. In groups P and P-D there was filmy adhesion easily separable with blunt dissection or mild to moderate with a freely dissectible plane in most of the eyes (Table 2).

A significant difference was observed between groups P-D and C (P = 0.055) in terms of degree of adhesion between the SRM and sclera at 3 weeks after surgery, and between groups P and C (P = 0.012) and groups P-D and C (P = 0.002) at 5 weeks after surgery. However, no significant difference was observed between groups P and C (P > 0.05) at 3 weeks after surgery or between groups P and P-D at 5 or 5 weeks after surgery (P > 0.05).

Histologic Examination

Histologic examination showed inflammation with some fibrosis around the SRMs in all three groups (Fig. 4). No specific toxic reaction was observed in groups P or P-D.

DISCUSSION

PTFE has been used as a pericardial and abdominal patch graft material for several years, and as an alloplastic xenograft in eyelid and socket diseases or in glaucoma surgery.19–23 PTFE is chemically and biologically inert, nonantigenic and resistant to infection, and is easily cut, molded, and sutured.19 It is available as an expanded porous 1.0-mm-thick sheet, or as a 0.1-mm nonporous thin surgical membrane. Nonporous 0.1-mm PTFE is soft and flexible and can be applied to complex shapes without difficulty. Moreover, because of its thinness, it is tolerated well without causing patient discomfort. Given these advantages, we considered that PTFE would be the most suitable nonabsorbable material to delay the adjustment.

The ideal drug-release device for our purpose would fulfill the following criteria.24 First, it should provide a sustained and relatively uniform therapeutic drug release in a reliable and predictable manner over an adequate period. Second, the device should be easily implantable or inject-
able, but must remain stable and not migrate within the eye. Third, the device should have a long shelf life, and it should be easy to handle and sterilize. Moreover, because conjunctival incision must be reopened to adjust sutures, unlike other intraocular devices it does not have to be biodegradable. None of the sustained delivery systems introduced to date fulfill these criteria. In this study, 0.1-mm-thick PTFE film coated with PLGA was used. Compared with PLGA film, which is difficult to manipulate because it tends to roll up and stick to itself easily,14 PTFE coated with PLGA is much easier to handle.

Delayed adjustment may provide the surgeon with the opportunity to adjust eye alignment.4,5 but postoperative adhesions can prevent this delayed adjustment. Our previous study suggested that PTFE alone might allow adjustment to be delayed for up to 4 weeks after surgery in 40% of rabbits' eyes and that the combined use of PTFE and 5-fluorouracil or the addition of viscoelastic solution (Viscoat; Alcon Labs, Fort Worth, TX) could allow adjustment to be delayed for up to 4 weeks after surgery in 80%.15 Moreover, the present study produced better results without the need for frequent topical steroid instillation. In this study, both groups P and P-D were significantly more adjustable than the control group at 3 weeks after surgery, but at 5 weeks after surgery, only group P-D was significantly more adjustable than the control group. Therefore, the period of adjustability was extended from 3 to 5 weeks in group P-D, even though direct comparison of adjustability of group P versus P-D was not significantly different. These results demonstrate that PTFE/PLGA allowed sustained dexamethasone release and effectively prevented adhesion development after strabismus surgery in our rabbit model.

Delaying adjustment allows the surgeon to obtain a better picture of where a given patient's motility will stabilize. In humans, Hwang (Hwang JM, unpublished data, 1998) showed that delayed adjustable strabismus surgery is possible with a thin PTFE plate, and Shokida et al.25 found that delayed adjustment surgery using a silicone sheet produced better results in patients with exotropia who underwent reoperation than in those who underwent immediate adjustment. Moreover, the results of the present study suggest that PTFE/PLGA with steroid could delay adjustment without frequent postoperative anti-inflammatory drug instillation.

In summary, we have described a new drug-delivery system for the extraocular sustained release of dexamethasone from a PTFE/PLGA film. This drug delivery and tissue separation system was found to allow delayed adjustment in rabbit eyes for up to 5 weeks after surgery without topical steroid instillation.

References


