An experimental technique for sutureless muscle surgery in the rabbit
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Citation

Abstract
Introduction: To evaluate in the rabbit model a technique for gluing the extraocular muscles to the sclera.

Methods: In 24 eyes of 12 adult rabbits, the superior rectus muscle was fixed to the sclera by means of octyl 2-cyanoacrylate adhesive, which was applied to both sides of a silastic strip inserted beneath the belly of the muscle with the proximal end adjacent to the scleral insertion. After treatment the muscles were subjected to histological examination.

Results: After 4 weeks, the muscles presented a tendon-like appearance, and histological examination revealed a leukocyte infiltration followed (8 and 10 weeks) by the appearance of fibrous tissue, which gradually replaced almost all of the muscle fibres in the segment attached to the strip.

Conclusions: This technique produces a progressive fibrosis of the portion of the extraocular muscle glued to the silastic strip. We hypothesize that the loss of contractile strength induced by this fibrosis may generate a muscle weakening effect.

INTRODUCTION
The use of tissue adhesives instead of sutures in ophthalmic surgery was first proposed in the 1960s (1). In a previous study the superior rectus muscle of rabbits was recessed 5 mm and reattached to the sclera with octyl 2-cyanoacrylate adhesive instead of sutures (2). The results were encouraging in terms of both the tensile strength and the absence of significant signs of tissue inflammatory response to the glue. Similar attempts in the past using methyl 2-cyanoacrylate (3) or iso-butyl 2-cyanoacrylate (4) yielded disappointing results in the rabbit model. However, in other similar studies (5-6) good anatomical results were obtained using a fibrin sealant.

In the present study we tested in the rabbit a surgical procedure for gluing the extraocular muscles to the sclera which does not involve sectioning of the muscle insertion.

MATERIALS AND METHODS
The study was conducted on both eyes of 12 adult New Zealand white rabbits. The procedures used were based on the Association for Researches in Vision and Ophthalmology's "Statements for the use of animals in ophthalmic and vision research". Every attempt was made to maintain asepsis during the surgical procedures. The animals were anesthetized with an intramuscular injection of Ketamine (30mg/kg) and Xylamine hydrochloride (5 mg/kg). One drop of antibiotic collyrium was instilled into each eye 10 minutes before treatment.

A conjunctival incision was made and the superior rectus and superior oblique muscles were exposed. The superior oblique was myotomized and allowed to retract. A strabismus hook was inserted beneath the scleral insertion of the superior rectus, and the muscle was isolated and elevated. The scleral surface beneath the muscle was carefully dried. A silastic strip (3 mm wide, 4 mm long, 0.5 mm thick) was grasped with thin forceps, and both sides were spread with a thin layer of octyl 2-cyanoacrylate surgical adhesive. The strip was then inserted beneath the belly of the muscle parallel to the longitudinal axis of the latter with the proximal end adjacent to the scleral insertion (Fig.1).
Figure 1
Figure 1: A silastic strip inserted beneath the muscle with the proximal end adjacent to the scleral insertion.

A light pressure was applied upon the upper surface of the muscle for two minutes to ensure good contact in the muscle-strip-sclera complex. The conjunctiva was then repositioned and reattached using a small amount of surgical adhesive.

Two, four, eight, and ten weeks after surgery, three rabbits were killed with an overdose of anaesthetic, and their eyes were removed. The conjunctival wound was reopened, and the muscle was examined under the operating microscope. A sharp blade was used to separate and isolated the muscle, the silastic band and the sclera. The muscle and the underlying complex sclera-choroid were then fixed in buffered formalin (pH 7.4) and embedded in paraffin. Sections 6 thick were stained with hematoxylin and eosin and examined under the light microscope.

RESULTS
None of the treated eyes displayed signs of inflammation at the time of enucleation. The conjunctiva overlying the treated muscle appeared mildly thickened but otherwise morphologically normal. When the conjunctiva was opened, the muscle and the silastic strip beneath it were found to be covered by a thin translucent membrane, which could be removed easily with sharp forceps in the eyes examined two weeks after surgery. This membrane was more adherent in those eyes examined during or after the fourth postoperative week. In the eyes examined at eight and ten weeks, the muscle was firmly attached to the silastic strip that had been glued to the underlying sclera. On gross observation, the surface of the muscle that had been attached to the strip appeared almost normal in the eyes examined at two weeks.

By four weeks, the glued surface of the muscle appears paler, and in the eyes examined at eight and ten weeks it presented a tendon-like appearance. On light microscopy, the muscles examined two weeks after surgery presented extensive inflammatory infiltrates with leukocytes in the areas corresponding to the glued strip.

By four weeks, fibroblast proliferation and signs of fibrosis were prevalent, and normal muscle fibres were present only in those parts of the muscle that had not been in contact with the silicon strip. At ten-week examination (Fig.2) there were extensive areas of fibrosis, which had replaced almost all the muscle fibers in the segment that had been attached to the strip.

Figure 2
Figure 2: Histological examination at ten weeks after treatment with extensive areas of fibrosis, which had replaced almost all the muscle fibers in the segment that had been attached to the strip.

The sclera underlying the silastic band showed a mild inflammatory response by two weeks after treatment. Histological changes were not found at the level of choroid.

DISCUSSION
Octyl 2-cyanoacrylate adhesive has been approved by US Food and Drug Administration for clinical use in repairing skin wounds. The glue itself is aseptic, slightly more viscous than water, and contains a monomeric preparation and a colouring agent (D & G Violet n. 2). When applied to a dry surface it polymerizes in approximately 45 seconds and the polymerization process does not involve crystallization, which can leave potentially irritating residues. Moreover, due to the presence of long chained alkyl groups, the adhesive is characterized by a very low level of histotoxicity (7).

In the present study we attempted to gluing an extraocular
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muscle by means of this adhesive using a silastic strip that was inserted between the muscle belly and sclera. The translucent membrane surrounding the muscle-strip-sclera complex (already evident two weeks after surgery) is probably the result of an inflammatory reaction to both the strip and the adhesive. Adherence between the muscle and the strip and the underlying sclera was good in all the eyes examined, even after 8-10 weeks. These findings, according to the results obtained in a previous study by using the same adhesive (2), suggest that the bond produced is compact and long-lasting. It is difficult to clearly separate the potential effects of the procedure which on the one hand could stem from the weakening of the muscle by an inflammatory transformation of the muscular structure and which on the other hand could stem from a fixation of the muscle belly primarily by the adhesive and secondarily by inflammatory reaction. In mechanical terms, the effects of this fixation is a new insertion point that is approximately 4 mm posterior to the original, just as if the muscle had indeed been recessed. Based on macroscopic examination of the muscle and the histological characteristics of that portion that had been attached to the silastic strip, however, we feel that the most important long-term effect of this procedure is probably represented by the progressive muscle fibrosis. Over time, we observed a leukocyte infiltration followed by progressive loss of muscle fibers and their gradual replacement by fibroblasts and connective tissue. Probably, the result of the changes obtained in our study should be a loss of elasticity and contractile force in the treated muscular segment. For this reason, the procedure can reasonably be expected to weaken the extraocular muscle without the need for recession. It is also reasonable to assume that the degree of weakening produced can be modified by varying the length of the strip that is inserted beneath the muscle.

Obviously, our preliminary results must be verified with additional studies before this experimental technique can be considered for use in humans.

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