

Experimental use of a castor bean (*Ricinus communis* L.) oil derived polymer on lamellar, interlamellar and penetrating implants in rabbit's cornea

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Abstract

The purpose of this study was to investigate the feasibility of using membranes made of a castor bean oil polymer as a new material for corneal repair or keratoprosthesis. The polymer shows good biocompatibility when used as prosthesis material on various sites (e.g. testis, bone, teeth). Twenty-eight rabbits weighing from 2.5 to 3.5 Kg were submitted to lamellar, interlamellar and penetrating keratoplasties. Clinical and histopathological evaluations were performed from the 2nd to the 120th post-operative day. Lamellar and penetrating implants presented good corneal cicatrization, although all membranes were extruded around the 15th day. All globes submitted to penetrating keratoplasties kept the integrity of the anterior chamber, with some of them showing some degree of synechiae. Interlamellar implants did not present important clinical reactions. The histopathological study demonstrated few inflammatory signs in all implants, with some epithelial lining appearing over the penetrating grafts. It was concluded that the castor bean oil polymer presented good biocompatibility and may be used in emergencial situations for temporary closure, as a globe salvage procedure. It is suggested that the castor bean oil polymer should be further studied in keratoprosthesis, due to its advantages (good biocompatibility, lower price and vast shape, porosity, thickness and refractive index possibilities).

Key-words:

Carbamates.
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Rabbits.

Introduction

The cornea is considered to be a privileged site for transplantation, because of its avascularity and of the almost complete absence of immune cells.¹ Under normal circumstances, corneal transplantation presents good results and graft rejection rarely occurs. Many kinds of grafts are also used repairing the cornea with good results.^{2,3,4,5} However, in certain situations, such as after alkali burns, the cornea is so vascularized that corneal

transplantation is almost always unsuccessful and the only option to restore the patient sight is a keratoprosthesis implant.

Keratoprosthesis, artificial corneas and intracorneal lenses have various applications in ophthalmology and the discovery of a really inert material would be enormously advantageous. Numerous attempts have been made to develop an artificial material inert to the cornea and that would stay in place for long periods. Nevertheless, when artificial material is implanted into the cornea, generally its

collagen begins to suffer lysis and the graft is condemned to fail, in a process that is not yet completely understood.

Various materials have been studied for keratoprosthesis or intracorneal lenses, such as glass, silicon, ceramics, and polymers, such as poly(methyl methacrylate) (PMMA)⁶, poly(2-hydroxyethyl methacrylate) (PHEMA)⁷, polytetra fluoroethylene (Gore-Tex®)⁸, presenting different rates of success. However, even PMMA, which is the polymer most widely used in the haptic portion of the keratoprosthesis, is successful for a limited period of time after implantation, and may be rejected even many years after the surgery.⁹ More recently, new microporous polymers are the main subject of study in order to enhance the integration of the keratoprosthesis and the cornea, showing better results.^{10,11,12}

Many trials on the biocompatibility of a new polymeric material derived from castor bean (*Ricinus communis L.*) oil have been performed. The product is a polyurethane which is highly biocompatible when implanted in bone, articular space, dental alveolar cavity^{13,14} and soft tissues as in testicular prosthesis.¹⁵ Based on such observations we decided to investigate the corneal reaction to the implantation of membranes made of this castor bean oil polymer.

Materials and Methods

Polyurethane membranes

Castor bean polyurethane production was described by Claro Neto.¹⁶ Membranes were prepared by extending the polioliol and pre-polymer mixture over a clean and straight glass surface until polymerization was complete. After that, the polymer was cut in squares measuring 2, 3 and 4 mm and then sterilized in a steam autoclave for 15 minutes. These squares of different sizes were used, respectively, in interlamellar, penetrating and lamellar implantations.

Surgical procedures

Twenty-eight New Zealand male and

female rabbits, were divided in two experimental groups: G1, with 18 animals, for lamellar and interlamellar implantations, evaluated at 2, 7, 15, 30, 60 and 120 days after surgery; and G2, with 10 animals for penetrating implants, which were evaluated at 2, 7, 15, 30 and 60 days after surgery. Rabbits were anesthetized by intramuscular kethamine-xylazine association and instillation of anesthetic eye drops.

For the animals from Group 1, after a 4mm square lamellar keratectomy in one eye, the polymer membrane was implanted in the lamellar bed using interrupted suture and 8-0 silk. In the other eye, using a Beaver's surgical knife, an intrastromal dissection was performed from the limbus to the center of the cornea, producing a pocket. The implant was placed at the end of the incision, in the central region of the cornea at midstromal depth. No suture was made. For Group 2, a 2.5 mm square penetrating keratectomy was performed at the center of the cornea, where the membrane was sutured in place using 8-0 silk interrupted sutures.

Post-operative care involved gentamycin ointment TID during 5 days for all the animals, and atropine 1% eyedrops SID during 5 days for the G2 rabbits. Elizabethan collar was employed in order to prevent the animals from rubbing their eyes. Rabbits were examined for signs of inflammation, neovascularization, epithelialization, infection, and extrusion. At the end of the period of study, the animals were euthanized and histological sections of the cornea were produced and stained with hematoxylin and eosin (H&E).

Results

Clinical results

The eyes that received lamellar and penetrating implants all showed injected conjunctiva and mucous discharge during the first days following implantation. These signs subsided subsequently. These clinical signs of inflammation were not observed in the eyes that have gone through interlamellar

implantation.

Segmental vascularization and opacity of the cornea could be seen from 1 to 4 weeks after surgery in the eyes that received lamellar implants. These aspects gradually improved after extrusion, which took place between 5 and 28 days. Fluorescein stain test in these corneas produced negative results after the 7th post-operative day (Figure 1), showing corneal epithelialization beneath the implants, what was confirmed by the histological analysis.

Interlamellar implants showed better evolution, with no clinical signs of discomfort or inflammation, except for some slight neovascularization that occurred towards the implants in some of the corneas. Corneal deformation over the membranes was evident under slit lamp examination. Three of the membranes were extruded around the 27th, 30th and 55th days, showing no other clinical signs. Some corneal thinning was noted over the center of few implants (Figure 2), which was best demonstrated by histopathology.

Penetrating implants were extruded around the 18th day. The eyes, however, kept their integrity, with corneal reconstruction occurring under the polymer membranes, leading to some opacity and neovascularization that became clearer after some time. Anterior chamber was preserved in 9 of the 10 eyes. In 6 eyes there was no synechiae, and 3 eyes showed partial synechiae between the cornea and the iris. Nevertheless, vision was preserved, except in the eye where anterior chamber was lost. Some degree of uveitis was observed few days after surgery in all these eyes, but subsided spontaneously. Some corneal leukoma remained in the site of implantation (Figure 3).

Histopathological results

Epithelial inclusions were observed very early in lamellar implants. They increased until the 15th day, when total epithelialization of the cornea occurred (Figure 4). Inflammatory cells were rare or moderate in number, and some neutrophils and eosinophils appeared in large number. Later on, some mononuclear cells were also seen. Fibroblasts have taken part in stromal



Figure 1 - Clinical aspect of lamellar implant at the 7th postoperative day with negative fluorescein stain test, showing corneal epithelialization. See segmental vascularization (*)

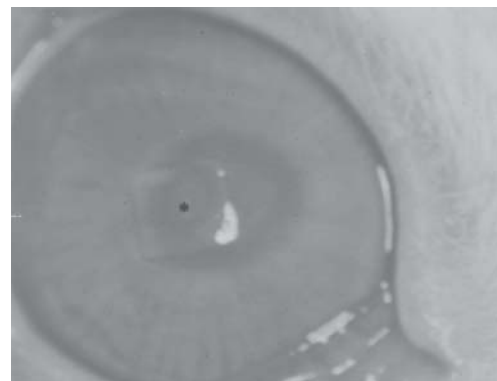


Figure 2 - Clinical aspect of interlamellar implant at the 17th postoperative day with no signs of inflammation. See corneal thinning over the center of the implant (*)



Figure 3 - Clinical aspect of penetrating implant at the 60th postoperative day, showing some opacity and neovascularization (arrow). The anterior chamber is preserved without synechiae

repair after the 15th day. Neovascularization was evident after 7 days of implantation.

Corneal deformation was an important finding in the interlamellar series of implants (Figure 5). Epithelial thinning was observed above the implants as early as the 2nd day and remained until the 120th post operative day. The progression of this aspect was seen as stromal thinning and even corneal ulceration with implant exposure in some cases. Cellular infiltration of eosinophils, mononuclear cells, macrophages and giant cells ranged from slight to moderate.

In the penetrating implants, soon after the surgery, the lesion was covered by a fibrin plug, which was gradually substituted by fibroblasts, collagen and neoformed fibers. As seen in the lamellar series, epithelial inclusion under the implants was observed after the 7th day, and the defect was covered on the 15th day. There was a moderate cellular infiltration. Neovascularization was moderate to major (Figure 6).

Discussion and Conclusions

Castor bean oil polyurethane is well tolerated by the cornea for periods of at least 120 days, when applied in the interlamellar form, being covered by corneal stroma and epithelium, and showing good biocompatibility. The finding that the castor bean polymer is tolerated by the cornea without toxic reaction is consistent with previous reports on its use in other surgical specialties.^{13,14,15,16}

Although the lamellar and penetrating implants have suffered dehiscence after variable periods of time, the great majority of the corneas were repaired at these moments. It is necessary, however, to try to understand the possible causes of the extrusion. Since there was no evidence of corneal necrosis and that the few inflammatory signs shown in histological examination indicated poor reaction to the material, it is possible to assume that the material was not rejected by corneal tissues.



Figure 4 - Light micrograph of lamellar implant at the 15th post operative day. Epithelialization of the cornea (arrow) and presence of eosinophils (small arrows). (hematoxylin-eosin –1000x)

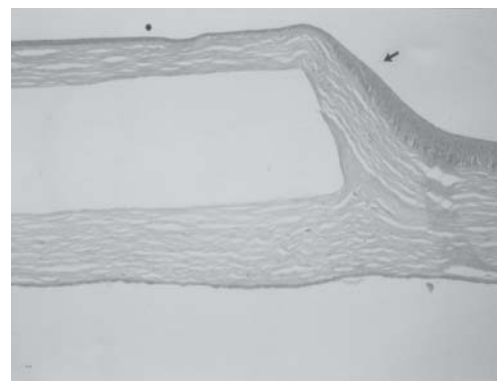


Figure 5 - Light micrograph of interlamellar implant 7 days after the surgery. Corneal deformation caused by the membrane (arrow) and epithelial thinning above the implant (*) (hematoxylin-eosin –100x)



Figure 6 - Light micrograph of penetrating implant 60 days after the surgery. Corneal integrity with neoformed fibers (arrow) and neovascularization (small arrows). (hematoxylin-eosin –500x)

Barber, Feaster and Priour⁹ suggested that exposure of the material to external or aqueous environment tends to facilitate the occurrence of infection, epithelial inclusion, formation of retroprosthetic membranes, glaucoma and corneal ulceration, what may lead to implant extrusion. This might be the major cause of dehiscence of the lamellar and penetrating implants, which had taken place after corneal epithelialization occurred under the membranes. On the other hand, since the material was not incorporated by the stroma, it may be another factor which facilitated extrusion, once this process has started. Many researchers advocate the use of porous materials in the implants for fibrovascular ingrowth, what may enable a better incorporation of the material to the cornea.^{7,8,10,11,12,17}

In interlamellar implants that were extruded, exposure began in the center or on the edge of the material, probably as a result of mechanical erosion in areas that were under pressure. After that, epithelial inclusion took place, facilitating dehiscence. Some

authors suggested that in any implantation of material into the cornea, it is important to place the material following the anatomical shape of the surrounding tissue in order to prevent pressure necrosis.⁷ Epithelial thinning and loss of keratocytes are among the most common findings in the implantation of intrastromal lenses^{18,19}, and are attributed to oxygen and nutritional deficits as well as to mechanical stress.^{7,9,19} Since rabbit cornea is relatively thin (0.37 to 0.45mm), a 0.26 mm membrane may be considered too thick for this implantation, suggesting that a thinner membrane should be used in other studies with this kind of animal.

The preservation of the integrity of all the eyes in this study and the poor reaction of the cornea to the material are encouraging results which may lead to further studies with the castor bean oil polyurethane. This polymer, as many others, has great versatility in relation to shape, thickness, porosity, and even structure, what enables a multiplicity of studies on the field of artificial corneas and keratoprosthesis.

Emprego experimental da poliuretana derivada de óleo de mamona (*Ricinus communis* L.) em implantes lamelares, interlamelares e penetrantes na córnea de coelhos

Resumo

Há muito que se estudam métodos e materiais reparadores de córnea, em busca de uma melhor reposição tecidual e, principalmente, manutenção ou recuperação da visão. A pesquisa de novos biomateriais tem permitido produzir próteses capazes de desempenhar a função requerida, sem reação importante. Conhecendo-se os bons resultados obtidos com a utilização da poliuretana vegetal derivada de óleo de mamona (*Ricinus communis* L.) em vários procedimentos, estudou-se sua implantação, em forma de membrana, na córnea, objetivando oferecer novo material para a reparação de lesões corneanas e a confecção de ceratopróteses biologicamente inertes. Utilizaram-se 28 coelhos, divididos em dois grupos (G1 e G2) e estes em subgrupos para avaliações de implantes lamelares e interlamelares aos 2, 7, 15, 30, 60 e 120 dias, e implantes penetrantes, aos 2, 7, 15, 30 e 60 dias de pós-operatório. Estudaram-se parâmetros como neovascularização, inflamação, transparência de córneas e implantes, bem como a aderência e viabilidade destes, através de exames oculares, e histopatológicos à microscopia óptica. Observou-se reação inflamatória branda em todos os períodos. Os

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implantes lamelares e penetrantes permitiram reparação da córnea e manutenção da integridade dos globos oculares, embora sofressem deiscência entre 5 e 28 dias de pós-operatório. Concluiu-se pela boa biocompatibilidade do material e pela possibilidade de empregar-se o polímero na reparação corneana e, possivelmente, em ceratopróteses.

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