Non-penetrating very deep sclerectomy with a hydrophobic polymer implant in a rabbit model

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Purpose: The aim of the study was to evaluate the influence of an implant made of a terpolymer (PTFE-PVDF-PP) on the condition of rabbit eyes during a one year observation period. Methods: The implant in the shape of an equilateral triangle (3 mm side length) was manufactured from a thin hydrophobic porous membrane. There were evaluated 40 eyes of 20 rabbits. The animals had non-penetrating very deep sclerectomy (NPVDS) performed, with insertion of an implant in the form of a triangular thin membrane. The control group consisted of 20 eyes where the animals had NPVDS performed without implant insertion. The evaluations included the study of the anterior part of the eye together with photographic documentation. Histopathological examination of the eyes 52 weeks after NPVDS procedure has been made. The process of wound healing was comparable in both groups. Results: The evaluation of the rabbits did not reveal any acute process of intraocular inflammation. After 12 month period of observation, no statistically significant differences in the process of wound healing or status of eyes were found between the groups. An analysis of fibrous connective tissue attachment to the implant showed that its layer was not thick and did not differ significantly from the control. The procedure of very deep sclerectomy and insertion of a polymer implant were well tolerated by the rabbit eyes. Conclusions: The in vivo results indicate that the hydrophobic implant in the form of a membrane can serve as a sclera implant after further study.

Key words: glaucoma, biocompatible polymers, polymer membranes, non-penetrating very deep sclerectomy (NPVDS), rabbit model

1. Introduction

Despite progress in the fields of genetics, pharmacology, biochemistry and other sciences, ophthalmologists still do not have effective methods for neuroprotection and regeneration of the optic nerve. Currently, medicine is unable to effectively treat lesions of optic nerves. As a result, the essential method of therapy is prevention or delay of optic nerve atrophy, which can be achieved by decreasing intraocular pressure to a level causing neither further destruction of the optic nerve nor the progression of visual field alterations. When pharmacological and laser therapy are not able to maintain intraocular pressure, the only option is surgical treatment [1], [25].

Introduction of non-penetrating surgery and maintaining an additional barrier, i.e., an intact trabeculo-Descemet’s membrane, led to a decrease in the number of complications connected with hypotonia and penetration of inflammation as well as hemorrhages [10], [16], [21], [23], [33], [30]. In 2010, Mansouri et al. [20] published their results on a new method of non-penetrating very deep sclerectomy. The authors suggested exposure of the ciliary body while preserving the central bridge of the sclera to ensure support for the cylindrical implant made of lyophilized collagen of animal origin. After insertion of the implant...
into the intrascleral space, the implant absorbs water showing a two- to three-fold size increase.

In 2009, Leszczynski et al. [18] proposed another type of very deep sclerectomy. During the excision of the deep scleral flap, the ciliary body is partially exposed, leaving a band of sclera, 0.5 mm in thickness. Contrary to Mansouri’s modification in this type of surgery, the central deep bridge of sclera is not preserved. Almost complete exposure of the ciliary body allows for better utilization of the subchoroidal space aimed to increase uveoscleral outflow. The formation of uveoscleral cistern as a reservoir for aqueous humor stabilizes the outflow and provides a space for an implant. This creates a chance for the development of a new implant type an the need for a new material to manufacture such an implant, which should actively support the transport of aqueous humor. The implant should be made of a biostable material, not causing tissue irritation. Even in extreme situations, such as transport accidents, work or sport injuries, the trabeculo-Descemet membrane separating the intrascleral or uveoscleral space from the anterior chamber should not be damaged and affects ciliary body. The implants used so far, made of sodium hyaluronate (SK-GEL) or lyophilised collagen, were resorbed within a few months after implantation [7], [8], [15].

Various biostable materials have been studied to develop implants of long-term durability [3], [4], [9]. The first second generation implant accepted by clinicians is the T-flux implant (La Rochelle, France), made of the hydrophilic material POLY MEGMA®. The material is not biodegradable, which allows long-term intrascleral space maintenance in the recipient’s eye [2].

An in vivo study, performed in rabbits by Kaluzny et al. [14] showed that implants made of sodium hyaluronate (SK-GEL) were more biocompatible than biostable implants made of either polymethacrylate, hydrogel or silicone. This histopathological effect was also confirmed in clinical studies; however, the latter did not reveal significant improvement of in vivo results after implementation of T-flux implants made of POLY MEGMA [27].

Despite many scientific research dealing with various biostable and bioreabsorbable polymer biomaterials for glaucoma treatment, with varying extent of durability, there is still no optimal implant allowing for fully acceptable long-term functionality.

The aim of the study was to evaluate the efficacy and biocompatibility of an implant in the form of hydrophobic porous membrane made of a terpolymer (PPTFE-PVDF-PP) on the condition of rabbit eyes during a one-year observation period. We evaluated biocompatibility of the material as intrascleral implant, its efficiency and stability after fixation in rabbit eye model.

2. Material and methods

Implant manufacture and its characteristics. For the manufacture of the implant, the terpolymer (polytetrafluoroethylene-co-polyvinylidene fluoride-co-polypylene, PTFE-PVDF-PP) was used, containing 56% PTFE, 27% PVDF and 17% PP. The samples in the form of porous membranes were prepared. The procedure for the manufacture of porous membrane and its detailed characteristics have been published elsewhere [19], [31], [32]. Open porosity and the pore-size distributions of the membranes were determined by using a mercury porosimeter PoreMaster 60 of the Quantachrome Instruments Company. The material displays a bimodal microporous structure with the size of pores ranged from 8 to about 72 micrometers, and a mean pore value of 32 μm (Fig. 1, Fig. 2). The mechanical characteristics of the membrane was assessed by tensile stress-strain test with a universal testing machine Zwick (model 1435) PC controlled by TestXpert (v.8.1) software, with the strain rate of 10 [mm/min]. The membranes were further submerged in phosphate buffered saline (PBS) solution and incubated at 37 °C. Stress-strain examination of the membrane samples was carried out after different time of incubation. The materials characterized by highly hydrophobic properties, without any changes in its microstructural parameters after long-term incubation in PBS. The implant was tested in vivo, according to the regulations of EU ISO 10993-5 for materials to be used as medical implants. The selected properties of the membrane are listed in Table 1. The implant cut from a polymer membrane was designed as an equilateral triangle (side length: 3.0 mm, thickness 0.6 mm) with a circular opening in the center, 0.5 mm in diameter, which allowed for a good fixation to the sclera. Due to the presence of the circular opening the total volume of implant inserted into the eye was decreased (Fig. 2). Before implantation the implants were sterilized with a plasma technique (40 °C, H₂O₂), Sterrad 120, ASP, Johnson & Johnson, USA.

In vivo study. In vivo studies were performed according to the regulations of EU ISO 10993-6, which specify recommendations concerning both animals selection and evaluation of early and late tissue reactions. The animal experiments were performed according to the regulations of ethical aspects concerning
studies involving animals (PN-EN 30992, Animal Welfare Requirements). The schedule and protocol of the experiments were approved by the local bioethics committee in Kraków, Poland. The surgery was performed in 40 eyes of 20 white New Zealand rabbits. The age of the rabbits was between 9 and 12 months; they weighed 3–4 kg. Taking into consideration the type of implant and the site of implantation, the experimental group was divided into five subgroups (each subgroup consisted of four animals) and evaluations were made at 2, 4, 12, 24 and 52 week of surgery. NPVDS was performed under general anesthesia. In one eye of each rabbit, NVPDS and implant insertion were performed; the other eye only underwent NPVDS (control group).

Surgery. All operations on animals were performed by a surgeon experienced in performing human glaucoma surgery. In a single session, two operations were performed on the same rabbit. First, the NPVDS surgery with implant insertion was performed in one eye, and then the complete NPVDS surgery was repeated in the second eye, but without implant insertion. As opposed to human patients, Mitomicin C was not used. The surgery was initiated by flushing the operating field with a 5% solution of Betadine. Subsequently, a 7-0 (Nylon) traction suture was put into the cornea at the area of the limbus. The conjunctiva was incised at the limbus from 10 to 2 o’clock. Afterwards, a 5 × 5 mm superficial scleral flap of approximately one-half scleral thickness was dissected 0.5–1.0 mm into the clear cornea. During the excision of the deep scleral flap, the ciliary body was partially exposed (Fig. 3 and Fig. 4) leaving a band of sclera of 0.5 mm in thickness. Following that, an attempt was made to remove the inner wall of Schlemm’s canal and juxtacanalicular trabeculum. In one eye of each rabbit the implant was placed and secured with a single suture. The superficial scleral flap was then secured with two single sutures (9-0 Polypropylen). In the control group, the NVPDS surgery was performed using the same method but no implant was inserted.

Premedication and general anesthesia. A cannule 20GA (Venflon BD 1.0) was inserted in the left or right marginal ear vein. Before general anesthesia, all animals received an intramuscular injection of 0.06 mg/kg atropine (Atropinum Sulfuricum 0.5 mg, Polfa), 10 mg/kg xylazine and 3 mg/kg azaperon (Stresnil, Janssen Animal Health BVBA). Animals were anaesthetized with 5.0–15.0 mg/kg of IV ketamine (bolus dose) with a subsequent maintenance dose of 1/5–1/4 of the initial dose, depending on the anaesthetic effect. Immediately before surgery, two drops of 0.5% oxyymetacain hydrochlorate (Alcaine, S.A. Alcon – Courvereu N.V., Belgium) were added to the conjunctival sac.
Postoperatively, analgesic drugs (tramadol 1–2 mg/kg) and systemic antibiotics: amikacin and cefuroxim (10 mg/kg and 25–50 mg/kg, respectively) were administered over the course of wound healing, for a minimum of seven days. Two drops of Tobradex (Dexamethasone + Tobramycinum) were instilled to each conjunctival sac twice daily for 14 days.

Microscopic examination. The examination of rabbit eyes was conducted after receiving inhalation anesthesia with a mixture of isoflurane and oxygen at postoperative weeks 2, 4, 12, 24 and 52. A portable slit-lamp was adapted to exam the following clinical changes: leakage from conjunctival sac, hyperemia of conjunctiva, size of filtrative bleb, cornea oedema, anterior chamber discharge, anterior chamber depth, hyperemia of iris and lens opacification. The changes were recorded in 4-grade scale, depending on the severity of the examined change from 0 to 3 [9], [13]. The intraocular pressure (IOP) was measured with the Schioetz-Tonometer, three times per eye and averaged. The eyes were checked with an indirect ophthalmoscope, and microphotographic external documentation was made by a digital camera.

Histopathology study. For histopathological evaluation, the eyeballs of two rabbits after 52 weeks from NCVDS surgery were fixed in 10% buffered formalin for 24 hours. After tissue dehydratation and paraffin embedding, tissues 7-mm-thick sections from the site of implantation were stained with hematoxylin and eosin (H&E). Inflammatory infiltration, resorptive granulation around the implants, fibrosis and oedema tissue and stromal oedema as well as connective tissue attachment to the implant and adjacent tissues, neovascularization and haemorrhage were assessed. The histological reaction was evaluated around the implant, in the area of the corneal limbus, in the ciliary body, within the suture area and the lacrimal gland as well as in the whole observed specimen.

Statistical analysis. Depending on the severity of the examined lesions, a numerical value from 0 to 3 was attributed as follows: 0 – none, 1 – mild, 2 – moderate, 3 – severe, according to the Hoekzem [13] and Erkilie [9] grading scale. The obtained parameters evaluating the condition of the eyeball were performed as the means ± SD. The statistical analysis was performed by using non-parametric U Mann–Whitney test. A $p<0.05$ was presumed to be statistically significant.

3. Results

During the surgery, the ciliary body hemorrhage occurred in one eye in the implant group and in two eyes of the control group, most often during the deep sclera flap dissection (Fig. 5). No complications were found following implant insertion and its suturing to the sclera margins.

Fig. 5. Abundant hemorrhaging from the ciliary body during surgery

Fig. 6. External photographs of eyes after NVPDS surgery; 4 weeks (a)–(b), 12 weeks (c)–(d), 24 weeks (e)–(f), and 52 weeks (g)–(h)
External photographs and the degree of histopathological changes in the studied eyes after surgery are collected in Figs. 6 and 7. The diagrams compare the degree of changes in the anterior portion of eyes with implant and without implant during 52 weeks. The degrees of changes in leakage from conjunctival sac, conjugal hyperemia size of filtering bleb and corneal edema were evaluated according to the grading scale. The parameters are performed as means ± SD.

Other clinical signs, i.e., changes in discharge of anterior chamber, anterior chamber depth, hyperemia of iris and lens opacification were also assessed according to the grading scale. No noticeable differences between the two groups in the degree of those parameters were found, and the change degrees varied from 0 (none, \( p = 1 \)) to 1 (mild, \( p = 0.317 \)).

The evaluation on two week after surgery in the experimental group exhibited noticeable conjugal hyperemia in eyelid, higher than in control group (Fig. 7b). In the same time in the control group higher corneal edema was observed (Fig. 7d). In the experimental group with implants the conjunctiva of the eye in the area of the fissure were more congested and swollen (Fig. 7a) In both groups the presence of mucopurulent discharge, without significant differences between groups with and without implant (\( p = 0.096 \)) was observed. Evaluation of the area of the fissure revealed the presence of the flat filtering bleb, larger in the experimental group, and significant congestion of conjunctiva in the area of the operating field (\( p = 0.011 \)) (Fig. 7c).

In one eye of the experimental group and two eyes of the control group, edema and corneal opacification were observed, which occurred in the area of the traction suture (\( p = 0.19 \)). A slight congestion of the iris was also observed in both groups, without significant differences between groups (\( p = 0.13 \)). Lenses were clear, without any lesions in either group (\( p = 1.0 \)).

In the control group after 4 weeks, a significant decrease of eyelid and conjunctival edema were observed. In one eye, persistent edema and congestion of the third eyelid were observed. The congestion of conjunctiva in the area of the operating field was still visible and was more distinct in the experimental group with implant insertion (\( p = 0.011 \)) (Fig. 7b). In the evaluated animals, the presence of mucopurulent discharge was observed, which was also heavier in the experimental group (\( p = 0.032 \)) (Fig. 7a). In the group with inserted implants, in one eye irregular nodular lesions were found, with an area of thinning of the sclera in the area of the scleral flap. Petechiae in the area of the postsurgical wound in one eye were present. Filtrative blebs were present in all evaluated eyes, and were slightly larger in the experimental group (\( p = 0.04 \)) (Fig. 7c). The corneas were smooth and clear; in the area where the traction suture was placed, a linear inflammatory infiltration was found. Irises were normal, with slight congestion, without significant differences between groups (\( p = 0.495 \)).

Fig. 7. The degree of clinical changes after NVPDS surgery: leakage from conjunctiva sac (A), conjugal hyperthermia (B), size of filtering bleb (C), corneal edema (D)
Three eyes from the experimental group exhibited a more intensive inflammatory process in the operating field area. A significant difference in the intensity of inflammation was found between the control and experimental groups ($p = 0.011$). Upon physical examination, no inflammatory discharge was found in the anterior chamber. In one animal, bilateral exophthalmus was observed. After four weeks, no purulent discharge was found in any evaluated eye.

Superficial conjunctival congestion was observed in all eyes from the control group (Fig. 6a, c, e, g). In none of the evaluated eyes subconjunctival petechiae were found in the area of the operating field (12 weeks). At the position of the bleb, congestion was found without statistically significant differences between groups (Fig. 7b). In both groups a flat bleb was observed, larger in eyes with implants inserted ($p = 0.011$). In one eye from the control group an excessive filtering bleb, with a surface area of $0.8 \times 10$ mm, was found. In one eye from the experimental group a thinned sclera with translucent uvea was noted. In both groups the corneas were clear, without edema. In one eye in the area of implant insertion there was an inflammatory infiltration, with scar tissue in the limbal area. The anterior chambers were moderately deep, and without discharge. Neither congestion nor neovascularization of the iris were observed; there were also no changes in the lenses ($p > 0.05$). There were no significant differences either in the intensity of the inflammatory process or in the size of the scar in pairs of evaluated eyes. On physical examination no inflammatory discharge was found in anterior chambers.

After 24 weeks of observation in two eyes with inserted implants, significant thinning in the area of the superficial scleral flap with a large bleb was observed ($p = 0.046$) (Fig. 7c). In the area of the traction suture, a single nodular reaction was found. In one eye in the control group, a local infiltration was present at the location where the suture was placed. The anterior chambers in both groups were moderately deep, without pathological discharge. The pupils were normal, and symmetric in both groups. The lenses were clear and symmetric in both the study and control groups ($p = 1.0$).

In both groups there was no edema of the eyelids after 52 weeks; their movability and the width of the palpebral fissure was normal and symmetric. In the lower quadrants of the conjunctiva of lids and eyes there was no pathological congestion, with a slight amount of mucous discharge remaining ($p = 0.317$). In the upper quadrants, in the paralumbar area there was deep congestion of the conjunctiva, with no statistically significant differences between groups ($p = 0.317$). In both groups the filtering blebs were flat; evaluation of their thickness and surface revealed they were significantly larger in eyes with inserted implants ($p = 0.011$) (Fig. 7c). The anterior chamber was filled with watery fluid. In two eyes in the experimental group and one eye in the control group, a mild iris congestion was found. In one eye with an inserted implant a more significant iris congestion was found. No inflammatory discharge was found in the anterior chambers in any pairs of evaluated eyes.

Histopathological examination of the eyes 52 weeks after NPVDS procedure revealed an increased chronic inflammatory response and resorptive granulation and angiogenesis around the implant compared to the control (Fig. 8). In the experimental group, the space between the superficial scleral flap and the ciliary body was maintained and contained the implant. The implants did not show any shape changes, and also no changes were observed on their surface. In the control group the presence of chronic inflammatory process and granulation were almost absent which indicates a different wound healing mechanism in these eyes. Foreign body (i.e., implant) placement helped maintain the uveoscleral space but also induced an inflammatory response and connective tissue attachment to the implant. In the control group did not show any

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Fig. 8. Histology results after 52 weeks (A) eye with implant, (B) eye without implant. Legend: (a) inflammatory infiltration; (b) granulation tissue (c) fibrosis of ciliary body; (d) neovascularization (angiogenesis)
intrasceral space. The scleral flap firmly adhered to ciliary body surface; the healing process was faster and did not induce connective tissue attachment.

In the experimental group the healing process resembled healing by granulation, and in the control group it resembled healing by primary intention. An analysis of fibrous connective tissue attachment to the implant showed that its layer was not thick and did not differ significantly from the control.

The mean IOP before implantation was 11.5 mmHg, while after 2 weeks from surgery this value increased to 25.7 mmHg in the experimental group and 22.8 mmHg in control group. Then the IOP in both groups was decreasing with the time and reached the value at 52 week 15.7 mmHg and 13.5 mmHg in study and control group, respectively (Fig. 9).

![Fig. 9. Comparison of the IOP before and after NPVDS surgery in study and control groups](image)

4. Discussion

Glaucoma treatment considers surgery (deep sclerectomy – DS) to be applied in primary open-angle glaucoma after typical pharmacological therapy. Most of the surgical procedures cause various complications including hypotony, hyphema, a flat anterior chamber, hemorrhage and bulb failure [5], [10], [11], [17]. Such procedures are effective ways to reduce the IOP for a longer time in comparison to the pharmacological therapy. The DS procedures can be combined with implantation of collagen (DSCI), hiauronian acid (SK GEL, Corneal) or a non-resorbable implant (T-flux) [2], [28]. However, clinical trials related to these implantations are not coherent. Some authors state that a collagen implant favors subconjunctival filtration but induces blocking of pores in the implant by protein molecules [6], [12]. Other study reports a better surgical outcome when the collagen implant is used because long term control of IOP is possible and late complications, such as hyperfiltration, hypotony, endophthalmitis are decreased [26]. A paper documenting the use of SK GEL implant indicates that slow release of hyaluroran inside the decompression space may nourish the deprived tissues and improve their outflow function [29]. Commercially available non-degradable glaucoma implants, e.g., T-flux may evoke undesired fibrosis, a long term failure and damage of an adjacent tissue [27]. Our experiments with a new biostable implant designed for deep sclerectomy technique and its further modification, i.e., NPDS, were performed on a rabbit model. Due to anatomical differences between the rabbit and human eye DS surgery in rabbits seems to be more difficult compared with the same procedure in humans. The problems are associated with the muscle and orbit, the presence of the third lid, and the thickness of the sclera and ciliary body. The thickness of the rabbit sclera is between 0.2 and 0.5 mm, and the length of the eye is between 16 and 18 mm [24]. These values can vary and depend on the breed, age, health condition of a rabbit, and its eye status. In our animals, the sclera at a distance of 0.1 to 3.5 mm from the corneal limbus scleral limb was so thin as to thecause bluish discoloration of the eye, making the surgery significantly more difficult. It was also cause of higher number of complications during surgery. Complications related to surgery including excessive bleeding, inflammation or hypotony can occur in human as well as rabbit eyes. However, iatrogenic rupture of the ciliary body with vitreous prolapse is extremely rare in humans. During surgery on rabbit eyes we observed bleeding from conjunctiva and ciliary bodies more commonly. These bleedings were much heavier, but they could be stopped more easily than in human eyes. In our animals there was no need for diathermy. Due to the presence of the trabeculo-Descemet’s membrane, blood did not penetrate into the anterior chambers, and hemorrhages did not significantly influence subsequent phases of therapy. In the human eye the early postoperative complication with collagen implant included subtle hyphema, blood in the anterior chamber and hemorrhages did not significantly influence subsequent phases of therapy. In the human eye the early postoperative complication with collagen implant included subtle hyphema, blood in the anterior chamber and hemorrhages did not significantly influence subsequent phases of therapy. In the human eye the early postoperative complication with collagen implant included subtle hyphema, blood in the anterior chamber and hemorrhages did not significantly influence subsequent phases of therapy. 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both types of implants decrease the IOP approximately to the same level. Our study showed similar complications during NPDS, the intensities of which decreased with time, what was proved by in vivo assessment of rabbit eyes. The observed pressure changes remained on the same level. It also confirms other opinions that the degree of the clinical or histopathological changes depends, to some extent, on individual features of a patient (animal).

During the first days after surgery, apathy, moderate light intolerance and mydriasis were observed. In most of the animals, these surgery-related symptoms subsided after seven days. Despite the effort deployed to maintain asepsis, during surgery, we did have two cases of intraocular inflammation caused by lacrimal gland infection. However, this complication was not found in any of the eyes with implants (Fig. 6b–h). In no of the eye with an inserted implant rejection or purulent eye inflammation were observed.

The size of the anterior chamber was stable during the whole period of observation; no difference was found between the groups at 52 weeks of surgery. This may have been due to the fact that typical opening of the Schlemm’s canal and removal of its interior wall and juxtacanalicular portion of the trabeculum, which has an essential impact on human filtration, was not possible in rabbits [5].

Slight iris congestion was found in both groups. In these eyes deep hyperemia in the paralimbal area was found. Since this congestion occurred in both groups of animals, as well as in one rabbit that was not operated but was kept together with the experimental animals, these symptoms may be interpreted as a reaction to ammonia absorbed by cage bedding material.

5. Conclusions

Despite surgery-related complication, no acute or chronic inflammatory processes were observed that could be attributed to tissue response to the inserted implants. The surgery of very deep sclerectomy and insertion of the new type of intrascleral implant, were well tolerated by the eyes of rabbits evaluated over 52 weeks of observation.

The external examination of the selected clinical signs revealed no statistical differences between the study and control groups after 52 weeks from surgery. These promising in vivo results justify further study on the use of hydrophobic porous membrane as potential implant for glaucoma treatment.

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References


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