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In Vivo Application of Poly(1,3-trimethylene carbonate) as a Scleral Buckle in a Rabbit Model

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Summary: Retinal detachments can be repaired with the use of cerclages. Currently non-resorbable silicone scleral buckle implants are used to indent the eye and in that way increase the internal pressure of the eye. The use of resorbable implants has advantages over using non-resorbable materials. High molecular weight poly(1,3-trimethylene carbonate) (PTMC) is a flexible and biodegradable polymer that has great potential in soft tissue engineering and the preparation of resorbable implants like scleral buckles. To be able to use PTMC devices in clinical applications, a full biocompatibility study of the polymer was first evaluated. Then the indentation of the eye over time using a PTMC scleral buckle was assessed *in vivo* in a rabbit model.

Keywords: biocompatibility; biodegradation; *in vivo* implantation; poly(1,3-trimethylene carbonate) (PTMC); resorbable polymer; scleral buckle

Introduction

In the eye, retinal detachment (RD) occurs when the sensory retina (SR) separates from the retinal pigment epithelium (RPE) and when not assessed and treated can lead to total blindness.[1] In general, RD is most frequent in the middle-aged or elderly population and can occur without an underlying cause. It is often caused by trauma, diabetes, inflammatory disorder, myopia or cataract. There are several methods to treat retinal detachment: cryopexy, laser surgery and pneumatic retinopexy for healing small detachments, and the use of a scleral buckle to indent the wall of the eye or vitrectomy to remove gel or scar tissue pulling on the retina in more extensive detachments.

A widely used procedure for treating retinal detachment is scleral buckling surgery, which involves the use of encircling implants to indent the sclera. By increasing the inner eye pressure, the retina is contacted with the RPE.[2] The shape and placement of the implant is determined by the size and position of retinal tear. The first scleral buckling procedure was performed in 1937[3] using cotton gauze swab. Since that time various materials, shapes and styles were developed in order to improve the outcome of specific applications.[4,5] Now, a permanent silicon buckle is the ''gold standard'' in surgical practice. The buckle is kept in place with sutures and, while its function is only needed in the first two to four weeks after implantation it remains indefinitely around the eye.[6] This permanent placement may result in pain, infection, problems with vision due to myopia and diplopia, and implant displacement. In these patients and also in children's

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surgery, the buckle needs to be removed, necessitating a second surgical procedure. For this reason, bio-resorbable synthetic materials could play a major role in the treatment of RD by scleral buckling, in order to eliminate the need of a surgical removal operation and reduce the probability of late complications.[7]

In a previous study,[6] we used poly(1,3-trimethylene carbonate) (PTMC) to prepare a resorbable scleral buckle. Figure 1 shows the synthesis route of PTMC by ring opening polymerization of trimethylene carbonate (TMC).

This transparent polymer is an amorphous material at room temperature with a low glass transition temperature (approximately $-20\,^{\circ}C$). It degrades enzymatically *in vivo* by surface erosion without releasing acidic compounds.[8–10] High molecular weight PTMC can be cross-linked into form-stable, flexible and creep-resistant elastomeric networks under the influence of sterilizing gamma irradiation. This cross-linked PTMC shows good shape recovery after deformation. The cross-linked films have values of the Young's modulus and yield strength that are close to 5.0 MPa and 1.5 MPa, respectively. The stress at break of these networks is around 9 MPa, while the elongation at break is 1200%.[11]

The most important requirement in the development of a medical device is the biocompatibility of the materials used. Biocompatibility can be defined as the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient.[12] In the industry, biocompatibility of implantable devices entails the interaction of the material with living cells, tissues or systems without being toxic, injurious, or causing immunological reactions while performing or functioning appropriately. A comprehensive, general guideline on the testing of biocompatibility of materials for medical applications is specified in the International Standard ISO 10993-1:2009. The good biocompatibility of TMC copolymers has previously been described in the literature.[13,14] However, no full studies addressing the biocompatibility of homopolymeric gamma irradiated PTMC materials in accordance with ISO 10993 have yet been published.

In the first part of this study, the biocompatibility of PTMC is addressed. In the second part, preliminary results of an *in vivo* study, where resorbable PTMC scleral buckles are used to indent the rabbit eye are presented. The indentation of the eye was monitored non-invasively over time and the tissue response to the implant was evaluated by histology.

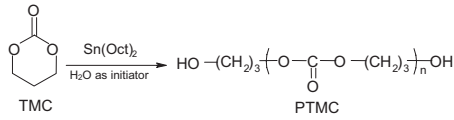

Figure 1.
Synthesis of PTMC by ring opening polymerization of trimethylene carbonate.

Materials and Methods

Materials

Under an inert atmosphere, PTMC was polymerized at $150\,^{\circ}C$ by ring opening polymerization of trimethylene carbonate (TMC, Boehringer Ingelheim, Germany) with stannous octoate as catalyst and water as an initiator (0,004 mol/L). The polymerizations were conducted for 6 hrs. The number average molecular weight of the obtained polymer was 300 kg/mol. The concentration of residual TMC was less than 2% as calculated from NMR spectra, the stannous octoate content was lower than 0.1 µl/g (<100 ppm). The obtained PTMC polymer was compression moulded into films without additional purification. For the biocompatibility tests, films with an average thickness of 125 micrometer were prepared. For preparation of the scleral buckles, strips with dimensions of $125 \times 2.5 \times 0.6$ mm were cut out from thicker compression moulded films. The specimens were sterilized and simultaneously cross-linked under inert atmosphere using gamma-radiation from a ^{60}Co source with

a dose of 25 kGy. Commercially available non-resorbable silicone buckles (MIRA® buckles from TPM, Luneburg, Germany) were used as controls. PTMC network films prepared in this manner are essentially endotoxin free.[10]

Full biocompatibility testing was performed by Toxikon (Bedford, MA, USA). The following evaluations were performed: cytotoxicity, sensitization, irritation/intracutaneous reactivity, systemic toxicity, toxicity, and implantation tests, genotoxicity, hemocompatibility, carcinogenicity, immunetoxicity. Full details of the test procedures are described in ISO 10993-1:2009.

Scleral Buckling Surgical Procedure

Eighteen New Zealand rabbits weighing 1.5 to 2.5 kg were used. The rabbits were anesthetized via intramuscular injections of 1.5 mL/kg of a 2 component mixture of a total of 1.25 mL Domitor Dexdomitor 0.5 mg/mL (1.00 mL) (Orion Pharma, Helsinki Finland) and ketaminehydrochloride (0.25 mL) (Alfasan, Woerden The Netherlands), followed by a local anesthesia Oxybuprocaine 0.4% (Chauvin Pharmaceuticals, Essex, UK) and iodine drops (Eurovet, Albrecht, Germany) for sterilizing the eye. Then they underwent a scleral buckling surgical procedure.

Different scleral buckle implantations using PTMC and silicone buckle were performed, as comparison of resorbable implant to the current non resorbable ''gold standard''. Non- operated eyes as well as silicone buckle served as a control. Evaluation of the indentation and histology was done after 2 and 4 weeks.

The conjunctiva of the left eye was opened and the PTMC or silicone encircling band was placed under two eye muscles and fixed to the eye. The ends of the buckle were laid over each and the suture were tightened in order to indent the sclera and fixed with long lasting resorbable sutures (PDSII, Ethicon Benelux, Amersfoort, Netherlands). The right eye served as a control. The conjunctiva was closed using a rapidly absorbing suture (Vicryl 8.0, Ethicon Benelux). The conjunctiva was then closed with resorbable sutures, and a chloramphenicol antibiotic ointment (Ratiopharma, Ulm, Germany) and a subcutaneous analgesic were administered. The rabbits received antibiotic eye drops twice a day for two weeks. The eyes of the rabbits were visually inspected (externally) after 1, 2 and 4 weeks (before enucleating). At the designated time points, the animals were sacrificed and the eyes were enucleated and prepared for histology.

Evaluation of the Scleral Buckles

The indentation on the sclera caused by the scleral buckle was evaluated non-invasively by echography. (B-5500; Sonomed Inc., Lake Success, NY, USA) After systemic anesthetization of animals, Chloramphenicol ointment (Ratiopharma, Ulm, Germany) was applied to the eye as a contact fluid for echography. The depth of the greatest indentation was measured as the buckling effect.

For histological analysis, the eye cups were fixated and dehydrated. After fixation and dehydration, the samples were embedded in Technovit T7100 (a HEMA-based resin, (Heraes-Kulzer GmbH, Wehrtheim, Germany), cut into 3 μm thick coupes and stained with toluidine blue. The evaluation of the tissue response was based on the presence of granulocytes, macrophages, multinucleated giant cells. Also phagocytosis, vascularisation and the presence of a fibrous capsule was assessed. The number of cells was evaluated semi-quantitatively by scoring the number of cells present in microscopic images of 5 different histological coupes at the different time points, visualized at 40× magnification. The tissue response was rated according to the following scoring system: − = not present, sp to +++ = sporadic to high infiltration of granulocytes, macrophages, multinucleated giant cells.

Results and Discussion

Biocompatibility of PTMC

For medical application of PTMC devices such as a scleral buckle, demonstrating the

biocompatibility of the polymer (network) is of great importance. A biocompatible material is non-toxic, non-carcinogenic, non-pyrogenic and does not affecting living tissue in and adverse manner. Biocompatibility is an essential requirement before being able to perform any preclinical experimental implantations that might proceed to human clinical trials if successful. Therefore, a complete biocompatibility study on PTMC films that included evaluation of: cytotoxicity, sensitization, irritation/intracutaneous reactivity, systemic toxicity (acute), toxicity (sub-acute to chronic), and implantation tests, genotoxicity, hemocompatibility, carcinogenicity, immunetoxicity was performed. In Table 1, details of the conducted tests and the outcomes in the different categories are presented for the PTMC films.

The cytotoxicity tests involve the exposure of substances extracted from the test material to one of two cell culture lines that are extremely sensitive to minute quantities of leachable chemicals and readily display characteristic signs of toxicity in the presence of potentially harmful components. The cytotoxicity of the PTMC films was classified as grade 0 because the extract caused no toxicity to the cell line. Very good results were also obtained for PTMC in evaluations like the testing of genotoxicology, which evaluates the ability of a material to cause mutation or gross chromosomal damage, or the hemolysis test which measures the ability of a material or material extract to cause red blood cells to rupture.

Most crucial in assessing biocompatibility are implantation tests which assess the local effects of a material or implant device when in contact with living tissue. The properties of the materials, *e.g.* shape, size, surface chemistry, morphology and porosity, composition, sterility, the duration of contact with body and material degradation need to be considered during development of medical device.[15,16]

The most comprehensive evaluation of the biocompatibility of PTMC was obtained from intramuscular implantations performed for times of up to 13 weeks. Implantation of a PTMC film in the paravertebral muscle of the rabbit and did not induce any local toxic effect nor did it cause a strong local response of the tissue. Macroscopic evaluation of the implant

Table 1.
Evaluation of the biocompatibility of gamma irradiated PTMC films according to ISO 10993-1:2009.

Test Category	Test performed	Results
Cytotoxicity	Agar Diffusion Test MTT cytotoxicity test L929 MEM Elution Test	Non-cytotoxic Grade 0
Hemo- compatibility	Hemolysis using human blood	Non-hemolytic
Sensitization	Klingman maximization test	Grade 1 (weak)
Implantation	Intramuscular implantation 4 and 13 weeks	No difference compared to control
Irritation	Intracutaneous injection test	No difference compared to control
Systemic toxicity	90-day systemic toxicity in rats via subcutaneous implantation injection test	No reaction Negative
Pyrogen and Biological Safety Tests	Rabbit pyrogen test	Non pyrogenic
Genotoxicity	Chromosomal aberration *Salmonella* and *Escherichia coli* reverse mutation test	Non clastogenic Non mutagenic

sites of the specimens showed no significant signs of inflammation, encapsulation, hemorrhage, necrosis or discoloration. Moreover, microscopic evaluation indicated that no significant differences at the implant-tissue interface could be observed when compared to implantations of high density polyethylene controls. Some macrophages with vacuoles and some polymorphs and lymphocytes could be noticed.

A 90-day long term systemic toxicity evaluation, in which the PTMC polymer films were subcutaneously implanted in 20 rats, was conducted as well. Observations included body weights, clinical observations, hematology, clinical chemistry, coagulation parameters, organ/relative organ weight; histopathology, macroscopic and microscopic evaluation of implant sited and selected organs. When compared to implantations in control animals with high density polyethylene, the rabbits implanted with PTMC films did not show any differences with the controls that could attributed to the implant material. The bioreactivity evaluations did not indicate a difference with the control implantations. In vivo degradation behavior and degradation rates of gamma irradiated PTMC films were previously reported, the polymer networks erode at a relatively fast rate of 23,7 to 55,7 μm per day.[10]

From this it follows that according to ISO 10993 PTMC can be considered as fully biocompatible. Furthermore, it causes only a mild inflammation and a normal foreign body tissue reaction during its degradation in the body[10,17] process. These PTMC networks can therefore be considered safe and biocompatible.

Application of PTMC as a Scleral Buckle

Based on the favorable mechanical- and biological properties of PTMC materials and the excellent performance of the material earlier *in vivo* rabbit studies,[6] this more detailed *in vitro* and *in vivo* study using resorbable PTMC scleral buckles was performed in order to prepare for clinical trials in the near future.

The main purpose of applying a scleral buckle in treating retinal detachment is to create an indentation in the eye and increase the internal pressure between the detached retina (SR) and the retinal pigment epithelium (RPE). The required healing time is assumed to be a maximum of 4 weeks. While non-resorbable silicon buckles maintain the indentation continuously, this is not needed after the retina has attached to RPM and can cause late tissue response and other complications, a biodegradable scleral buckle should indent the sclera for no more than 4 weeks and resorb without excessive tissue reaction.

The surgical implantation of PTMC scleral buckles was performed in order to analyse the functionality of these resorbable implants. In Figure 2, implantation of a PTMC scleral buckle prepared by compression moulding is presented. It shows how the flexible, elastic and transparent PTMC polymer encircling band is placed around the eye.

The PTMC buckle was easy to handle in the surgery. No intra-operative problems occurred during the implantation proce-

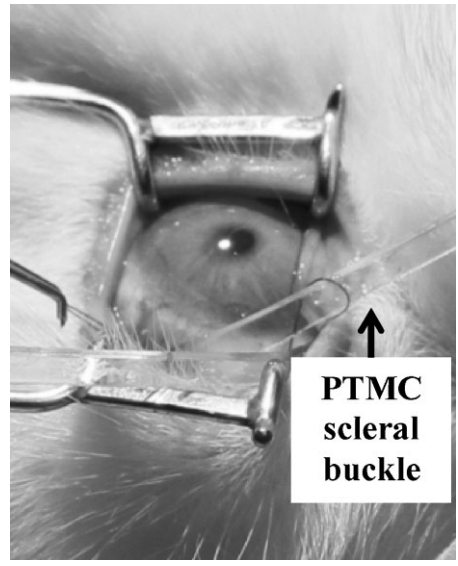

Figure 2.
Flexible and elastic PTMC scleral buckle implanted in the rabbit eye. The implant was prepared by compression moulding.

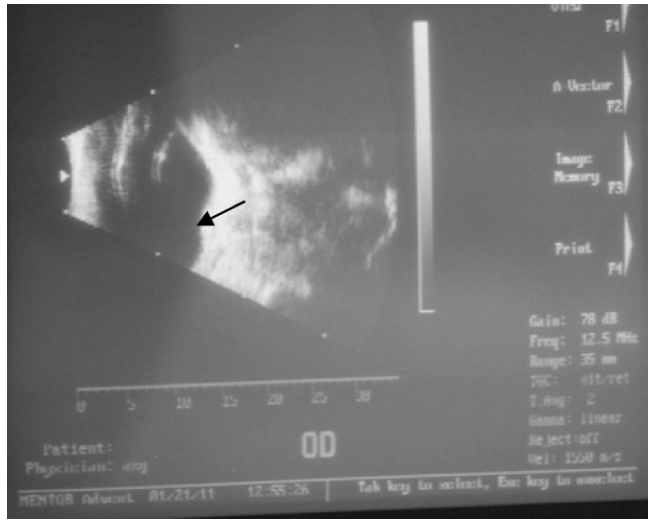

Figure 3.
Echography of a rabbit eye in which the indentation of the sclera (black arrow) with a resorbable PTMC scleral buckle is visible 4 weeks after implantation.

dure, and all animals recovered well. Healing after the surgery was normal. During clinical examination at the various experimental time periods, no reaction of the rabbit eyes to the PTMC buckle was observed.

The extent of indentation of the rabbit eyes using the PTMC scleral buckles was determined by echography at day 0, 14 and day 28 after implantation. As desired, indentation of the sclera of the rabbit eyes upon application of a PTMC buckle could be observed after 2 and 4 weeks. With this, a major requirement of using PTMC as a scleral buckle implant is fulfilled. In Figure 3 the indentation after 4 weeks using a PTMC implant, is shown. In the echogram the indentation is marked with an arrow. The implant itself cannot be visualized by echography.

The indentation achieved after 4 weeks with the bioresorbable PTMC implants was sufficient to achieve a therapeutic effect.

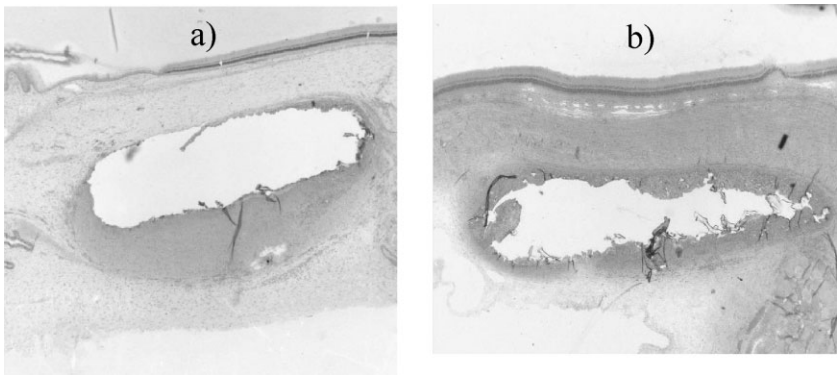

Figure 4.
PTMC scleral buckle after a) 2 weeks, b) 4 weeks implanted on the sclera of rabbit eyes. It can be seen that after 4 weeks of implantation, significant degradation of the PTMC implant has occurred.

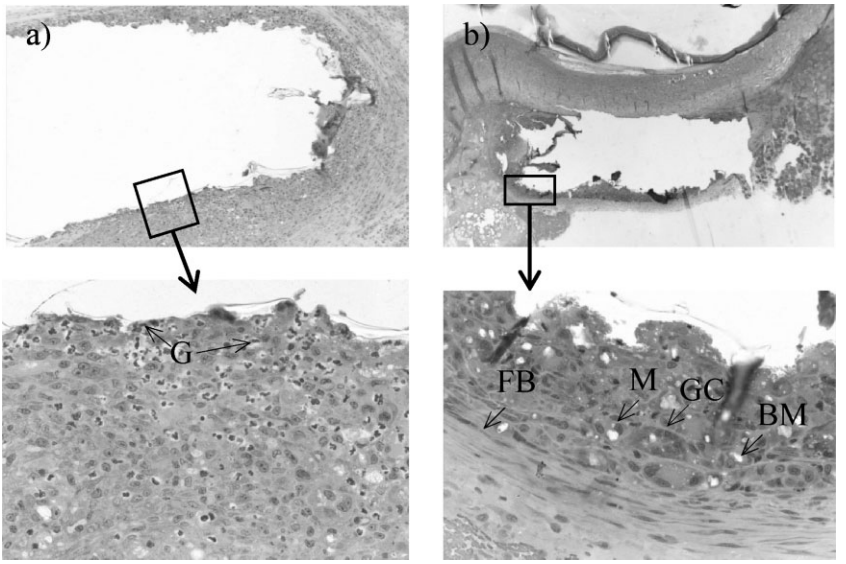

Figure 5.
Histological evaluation of PTMC scleral buckles after implantation a) 2 weeks- Granulocytes (G) are present b) 4 weeks- Fragments of degraded material (BM) are encapsulated, giant cells (GC) and Macrophages (M) engulfing the material are present, fibroblasts (FB) form a capsule around the implanted PTMC scleral buckle.

The tissue response to the PTMC implants was evaluated by histology after the eyes were enucleated at the appropriate time points (2 and 4 weeks). An overview of the histology is shown in Figure 4 and 5. With the histological images of the enucleated eyes, the immune response, inflammation, necrosis, fibroplasia, fibrosis, fatty infiltrate, and angiogenesis can be assessed. It gives important information regarding the tissue reaction and the general behavior of the body to the PTMC implants.

Figure 5a, shows that after 2 weeks of implantation numerous granulocytes could be found in the vicinity of the implant site of the scleral buckle, between 5–20 rows. After 4 weeks, granulocytes were no longer present and a thin capsule around the PTMC implant was formed by fibroblasts (Figure 5b). After 4 weeks significant degradation of the PTMC implant had occurred and erosion of the implant surface is extensive. At the higher magnifications, several layers of macrophages, giant cells and a fibrous capsule surrounding the implant can be seen at 4 weeks. The image shows that particulate debris originating from the PTMC material has been taken up by the macrophages and giant cells. These observations are indicative of a normal foreign body tissue reaction to implanted PTMC, and have been previously observed as well.[10,17]

Table 2 is a quantification of the tissue response to the implanted PTMC scleral buckle after 2 and 4 weeks. It can be seen that initially the tissue reaction is dominated by the presence of granulocytes, in

Table 2.
In vivo tissue reaction to the implanted PTMC scleral buckle

Time	Granulocytes	Macrophages	Giant cells	Vascularization	Phagocytosis
2 weeks	++	sp	−	+±	Yes (sp)
4 weeks	−	++	++	++	Yes

− = not present, sp to +++ = sporadic to high infiltration

time the role of phagocytosing macrophages and giant cells cells becomes more pronounced. Also vascularization becomes more evident.

Macroscopically, no signs of inflammation or pathological thinning of the sclera were seen.

Conclusion

In order to evaluate the safety of PTMC materials for clinical applications, the biocompatibility of gamma irradiated PTMC films was evaluated according to ISO 10993. No signs of cytotoxicity, irritation, mutation or sensitization were observed. These favourable results confirm that PTMC can be considered a biocompatible material. The use and efficacy of a resorbable PTMC scleral buckle in a rabbit implantation model was evaluated. Echography showed that considerable indentation of the sclera could be observed at two and four weeks after implantation. Histological analysis showed a normal foreign body reaction to the PTMC implants. From this we can conclude that PTMC is a safe material that can be used as a scleral buckle in a clinical study on treating retinal detachment.

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