Responses of the rabbit's soft tissue of the orbit and periorbital area, and orbital bone structures to the introduction of the polymer composition implant and PFTE implant

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Keywords:

periorbital area, scleral sac, auricle tissue, reconstructive surgery, polymer composite, polytetrafluoroethylene, experiment

Background: *The synthetic polymer materials to be used in plastic and reconstructive surgery for craniofacial injuries should have high compatibility with biological tissues. In cooperation with MEDBIOTECH LLC (Republic of Belarus), we have developed a carbon-polymer composite with thermo- and electrophysical characteristics close to those of viable biological tissues.*

Purpose: *To investigate experimentally the responses of the soft tissue of the orbit and periorbital area, and orbital bones to the polymer composite implants (PCI) and polytetrafluoroethylene (PFTE) implants.*

Materials and Methods: *Chinchilla rabbits received one of the two types of implants, PCI (Mt, Mt1, Mt2 or Mt2+ ceftriaxone versions), or PFTE implants, into the periorbital area, scleral sac, orbital tissue, or auricle tissue. The response of the soft tissues and bone structures to the implants was assessed based on changes in clinical and pathomorphological indices at days 10, 30 and 60.*

Results: *In any type of implant, post-surgical wound healing was found to occur by primary intention. Inflammatory responses of the rabbit's soft tissue of the orbit and periorbital area, and of the orbital bones to the PFTE implant were more pronounced than those to the PCI. We found histomorphologically that adjacent soft tissues grew into the PCI, and a capsule formed around the PFTE implant.*

Conclusion: *The polymer composite implants were found to have improved biocompatibility compared to polytetrafluoroethylene implants.*

Introduction

Craniofacial injuries are primarily caused by anthropogenic and criminal-related ocular and orbital trauma [1], and their incidence has been increasing in recent decades, with 11.6 to 27.0% of patients with penetrating trauma undergoing enucleation or evisceration [2, 4-7]. Surgeons are not always satisfied with using biological tissue as a ductile material, and legal requirements for harvesting of donor material have been made increasingly stronger. The development of synthetic polymer materials for restoration of soft tissue of the orbit and periorbital area, and orbit bone structures that have suffered anatomical and/or functional damage is therefore of primary importance.

Studies on the use of silicone, polyethylene, polytetrafluoroethylene (PFTE) and hydroxyapatite implants in ophthalmology have demonstrated that they have low biocompatibility and do not ensure ingrowth of biological tissue in any of them [8-11]. Non-biological implants with porous and hollow structure which tend to integration with adjacent orbital tissues offer a new opportunity for ophthalmology. A disadvantage of carbonic felt (Carbotekstim-M), a non-biological implant [3], is that it is loose, fragile, and, therefore, non-uniform and does not hold its shape. Therefore, the development of biocompatible and easy-to-use non-biological implants is of utmost importance.

In cooperation with MEDBIOTECH LLC (Republic of Belarus), we have developed a biocompatible carbon-polymer composite with thermo- and electrophysical characteristics close to those of viable biological tissues (patent application № a 20140613 issued 18.11.2014. Carbon fiber composite material for repairing soft tissue defects. Authors: Dubkova VI, Glinnik AV, Maletskiy AP, Maievskaia OI, Bigun NM).

The purpose of this study was to investigate experimentally the responses of the soft tissue of the orbit and periorbital area, and orbital bones to the polymer composite implants (PCI) and polytetrafluoroethylene (PFTE) implants.

Materials and Methods

Experimental studies were conducted at the vivarium of the Filatov Institute. All animal experiments were performed in compliance with the Law of Ukraine on Protection of Animals from Cruel Treatment No. 3447-

IV dated 21.02.2006 and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes from the European Treaty Series (Strasbourg, 1986), and approved by a local Bioethics Committee of the Filatov Institute.

A total of 30 Chinchilla rabbits (age, 5–6 months; weight, 2–3 kg) were used in the study and were maintained at the same conditions during the study period. We compared the response of rabbit's soft tissue and bones of the orbit and periorbital area, auricle tissue to two types of implant material, polymer composite (developed in cooperation with and MEDBIOTECH LLC, Republic of Belarus), and PFTE (manufactured by Ecoflon, St. Petersburg, Russia).

In the current study, the following PCI versions were used: first, IKVOBAN-Mt polymer composite implant (PCI), a macroporous implant with a dense structure, developed from carbonic material and designed as a flexible rectangular plate or small ball of a specified size; second, IKVOBAN-Mt1 PCI, comprising fibrous carbon composite (carbon fabric) and thermoplastic polymer (low density polyethylene or high-molecular polymer); third, IKVOBAN-Mt2 PCI, comprising fibrous carbon composite (nonwoven fabric) and thermoplastic polymer (low density polyethylene or high-molecular polymer); and, finally, IKVOBAN-Mt2-Ts PCI, comprising fibrous carbon composite and antibiotic (ceftriaxone) immobilized on the surface thereof. To investigate the response of rabbit's soft tissue of the orbit and periorbital area, and orbit bone structures to the PCI and PFTE implant, the two latter were incorporated into the following rabbit's structures: the periorbital area, scleral sac, orbital tissue and auricle tissue.

Prior to surgical procedure, animals were anesthetized with thiopental sodium 0.1% (1.0 mL/kg, intramuscularly). Carbon-polymer composite implants of various versions (IKVOBAN-Mt, Mt1, Mt2 and MT2 with ceftriaxone) and PFTE, 10.0 mm x 10.0 mm x 0.5 mm in size, were incorporated into soft tissues. Group 1 involved 15 rabbits. After evisceration was performed and a 13.0 to 15.0-mm long cheek skin incision was made, each rabbit received an implant (IKVOBAN-Mt, Mt1, Mt2, MT2 with ceftriaxone, or PFTE; each of the subtypes for three rabbits) into the scleral sac and soft cheek tissue, and the wounds were sutured with interrupted 6-0 silk. Group 2 involved 15 rabbits. After the orbit was opened, and an auricle skin incision was made, each rabbit received an implant (IKVOBAN-Mt, Mt1, Mt2, MT2 with ceftriaxone, or PFTE; each of the subtypes for three rabbits) into the orbit and auricle tissue, and the wounds were sutured with interrupted 6-0 silk. Animals were euthanized by air embolism after anesthesia and immediate removal of implants with adjacent tissues at days 10, 30 and 60.

The response of soft tissues and bone structures to incorporated implants was assessed based on clinical and pathomorphological changes. Clinical signs (edema of orbit tissue, edema of cheek, auricle edema, state of sutures, presence of discharge) were scored on days 2, 5, and 10 and every 5 days thereafter until the end of the study as follows: edema of orbit tissue (0, no edema; 1, edema of postoperative suture site; 2, edema of postoperative suture site and adjacent conjunctiva; 3, marked conjunctival chemosis and edema of orbit tissue); state of sutures (0, no suture line disruption; 1, solitary sites of suture line disruption, up to 1.0 mm; 2, sites of suture line disruption, 1.0-5.0 mm; 3, disruption along the entire suture length); and conjunctival discharge (0, no discharge; 1, mild sanious discharge from the conjunctiva; 2, mild sanious and serous discharge from the conjunctival sac, at eyelid margins, and at the orbital area; 3, marked sanious and serous discharge from the conjunctival sac, at eyelid margins, and both at orbital area and outside the orbit). In addition, the tissue samples from the periorbital area, orbit and auricle were pathohistologically examined at days 10, 30 and 60.

Tissue samples were fixed in 10% formalin for 24 hours and then embedded in paraffin. They were stained with hematoxylin and eosin on day 5 and examined with Jenamed 2 microscope (Carl Zeiss, Jena, Germany).

Results

At days 2 and 5 after incorporation of PCI or PFTE implant, all animals demonstrated marked edema of the tissues of the eyelid, orbit, cheek and auricle. In addition, marked discharge from the conjunctival sac was noted in rabbits that received the implant in the scleral sac. In rabbits that received the PCI, edema reduced or resolved at day 10, whereas edema of the soft tissues of orbit, cheek and auricle was present in rabbits that received the PFTE implant until as long as day 30. This is evidence that soft tissue inflammation around PFTE implants is present much longer than around the polymer composition implants.

It is noteworthy that postoperative observation of the wound of the skin and conjunctiva after incorporation of the PCI or PFTE implant revealed that its healing occurred by primary intention. Given the fact that the inflammatory response around the implants varied between rabbits, we found it expedient to investigate pathohistological changes in the soft tissues of the orbit, ocular sclera and periorbital area after incorporation of the PCI or PFTE implant. As the pathohistological changes in the soft tissues of the periorbital area, scleral sac, orbit, and auricle were similar between rabbits that received PCI of various versions (IKVOBAN-Mt, Mt1, Mt2 or MT2 with ceftriaxone), we report only on the response of these tissues to incorporation of PCI Mt version.

At day 10 after incorporation of PCI (Mt) in the soft tissues of the orbit, moderate inflammatory infiltration of the structures around the implant was seen, and soft tissues (fat, connective tissue, peripheral nerves) were edematous and mildly infiltrated by lymphocytes (Fig. 1). If the implant was placed directly beside the optic nerve, no substantial structural changes in the nerve were observed. With the PCI incorporated into the scleral sac, edema of the subepithelial conjunctiva and diffuse lymphocyte infiltration in the fibrous fabric around elementary implant

components was observed. No destructive changes in the conjunctival epithelium were seen even in the presence of implant components in the subepithelial conjunctiva (Fig. 2). In addition, no destructive changes were seen with the PCI incorporated into the auricle near the auricular cartilage (Fig. 3).

At day 30, there were practically no signs of the inflammatory response, and no edema of the soft tissues of the orbit or external ocular muscles. In addition, no marked destructive changes were observed in the structures adjacent to the implant, and the sclera was normal with mild parenchimal edema (Fig. 4).

Moreover, at the sites of most abundant fibroblast-like cells with initially loose fibrous fabric, numerous thin-walled capillaries of various sizes were noted (Fig. 6).

No changes in the nerve trunk or cartilage tissue were observed at day 30 after incorporation of the PCI into the auricle. Destruction in the bone tissue was noted in one case. The following was characteristic at that time point: proliferation of the fibroblast-like cells located around the implant with their subsequent ingrowth in the PCI, moving apart the PCI structural components, with formation of the fibrovascular capsule around the implant (Fig. 5). In addition, abundant blood vessels were seen (arrows). Maturation of the connective tissue was accompanied by reduction in the number of cells and blood vessels. Until day 30, a similar process occurred only in the superficial layers of the implant. At several locations, fibrovascular tissue was detected also in deeper layers.

At day 60 after incorporation of PCI (Mt) in the soft tissues of the orbit, a band of dense fibrous fabric was found to form around the implant and spreading practically throughout it, thus evidencing the formation of a fibrovascular capsule. In addition, maturation of the connective tissue was observed, with reduction in the number of connective tissue cells and increase in the number and size of collagen fibrils, and partial obliteration of blood vessels was noted. Fibrous fabric, most often, was found to divide the implant into separate elementary structures, or sometimes into groups composed of numerous needle-like structures (Fig. 7).

At day 60 after incorporation of the PCI in the subconjunctival compartment or the auricle, neither acute nor destructive changes in the conjunctiva and/or cartilaginous tissue was observed.

Pathomorphological changes in the soft tissues of the orbit, periorbital area, intraocular structures and conjunctiva after incorporation of the PFTE implant into the orbit were different from those after incorporation of the PCI (Fig. 8). This is related primarily to the degree of inflammatory response, which is characterized by edema and marked infiltration of tissues and by the presence of eosinophilic white blood cells and reduced numbers of neutrophils. Such changes are more apparent in the subconjunctival tissue and orbital soft tissue. In the presence of marked inflammatory infiltration, signs of degeneration of transversal striated muscle tissue were detected, which was evidenced by muscle fiber edema and complete loss of transversal striated muscle structure (Fig. 9). Edema and degeneration of transversal striated muscle tissue were also observed (Fig. 9). Inflammatory changes characterized by focal lymphoid infiltration (arrows), were still present. Degenerative signs were found also in the peripheral nerves of the orbit, along with focal destruction of the bone lamellae of the orbital walls. After incorporation of the PFTE implant into the auricle near the auricular cartilage, vacuolar degeneration of chondrocytes was detected (Fig. 10).

Inflammatory and destructive changes were evident within 10 to 30 days.

At day 30 after incorporation of the PFTE implant into the auricle, a thin band of dense fibrous fabric (arrows) appeared; it contained a small number of cells and adhered tightly to the implant. No penetration of the fibrous fabric in between the structural elements of the implant was observed (Fig. 11). The optic nerve appeared compressed, with signs of deformation and loss of structure.

At day 60 after incorporation of the PFTE implant, the capsule surrounding the implant appeared to get thicker, and contained no blood vessels (Fig. 12). Formation of coarse fibrous capsule (with a small number of cells) (arrows) around the implant was observed (Fig. 12). No penetration of the fibrous fabric into the implant was noted.

It is noteworthy that there were signs of destruction of the elementary components of the implant, which was evidenced by variety of their shapes and loss of their tinctorial properties (Fig. 13).

Conclusions

First, we found experimentally that the inflammatory response (edema and discharge from the conjunctival sac) of the rabbit's orbital soft tissue, periorbital area, and auricle to the PFTE implant was more pronounced and was seen as long as 30 days, whereas that to the polymer composition implant was moderate and was seen during 10 days.

Second, at day 10, the clinical response of the rabbit's orbital soft tissue, external ocular muscles, sclera, optic nerve and auricular cartilage to introduction of the PCI was characterized by the development of edema in these structures, and neither marked inflammatory nor destructive changes were revealed by histomorphological study.

Third, at days 10 to 30 after introduction of the PCI into the orbit, a fibrovascular capsule was found to form around the implant, and to penetrate in between its elementary structural components.

Fourth, at day 60 after introduction of the PCI into the orbit, complete maturation of the connective tissue was observed around the implant and between its elementary structures.

Fifth, no substantial pathomorphological difference in the state of structural elements of the orbit, eye, and auricular cartilage after introduction of various PCI

versions (IKVOBAN-Mt, Mt1, Mt2 and MT2 with ceftriaxone) was found.

Sixth, the clinical response of the rabbit's orbital soft tissue, external ocular muscles, sclera, optic nerve and auricular cartilage to introduction of the PFTE implant was characterized by the development of marked inflammation by day 10, which lasted up to 30 days, with degenerative findings in these structures.

Finally, at day 60 after introduction of the PFTE implant into the orbit, complete maturation of the connective tissue surrounding the implant (without penetration of this tissue in between the elementary structures of the implant), and partial destruction of the implant was observed.

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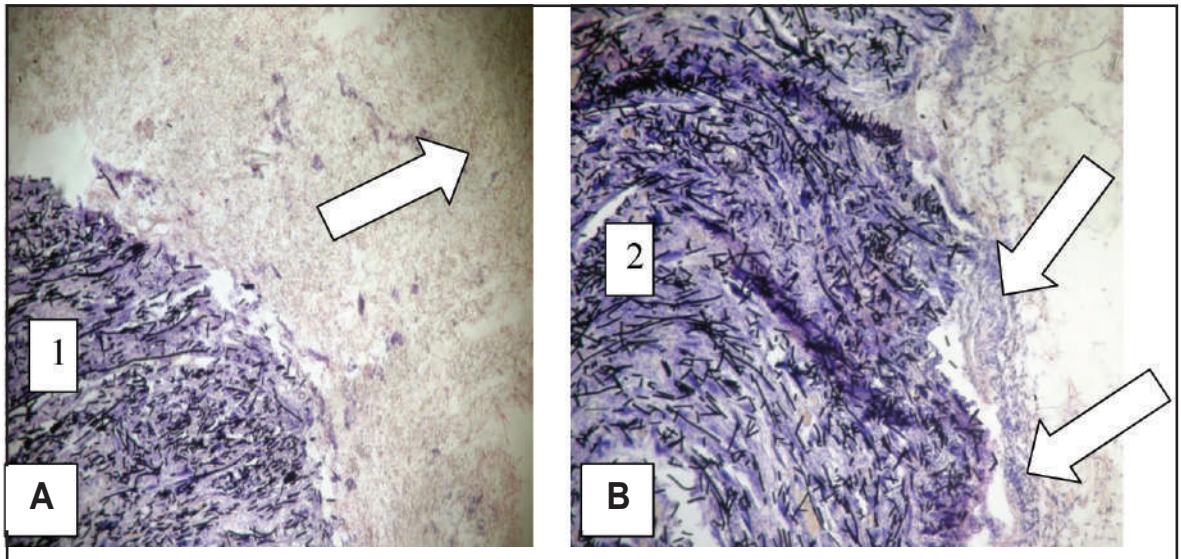

Fig. 1. Day 10 after incorporation of the Mt (A) or Mt2 (B) polymer composition implant into the orbit. A: Edematous soft tissues of the rabbit orbit (arrow) and external ocular muscles, and dilated or even thrombosed blood vessels are observed. Hematoxylin and eosin staining. Original magnification ×75. B: A band-like accumulation of fibroblast-like cells is seen at the border between the implant and adjacent tissues, with some of these cells being observed between individual structural components of the implant. A fibrovascular capsule has been formed around the implant. Hematoxylin and eosin staining. Original magnification ×75.

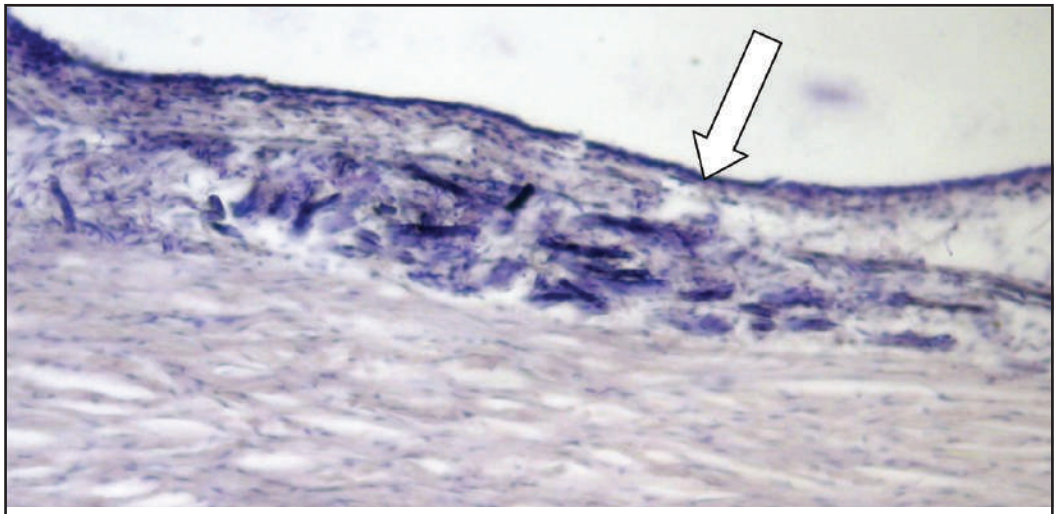

Fig. 2. Day 10 after incorporation of the Mt polymer composition implant into the scleral sac, with the presence of implant components in the subepithelial conjunctiva. Hematoxylin and eosin staining. Original magnification ×280.

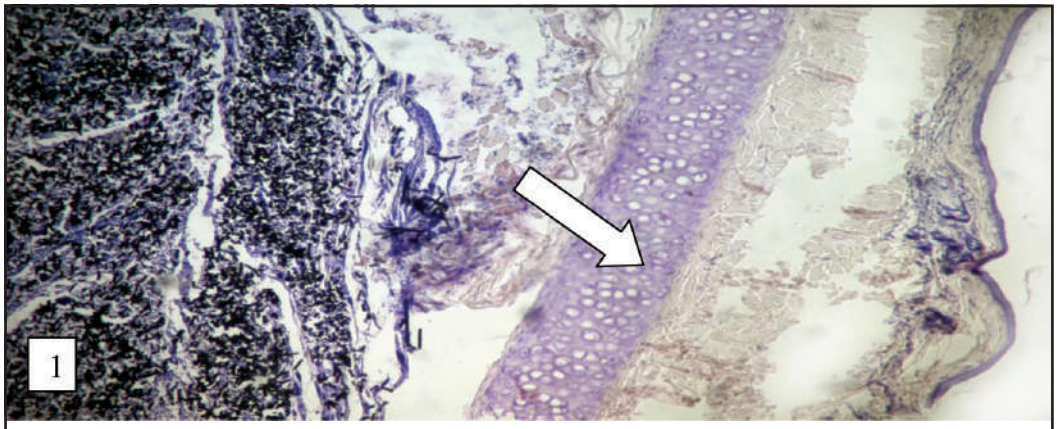

Fig. 3. Day 10 after incorporation of the Mt polymer composition implant (1) into the auricle near the auricular cartilage. No substantial structural changes are seen in the cartilage tissue (arrow). Hematoxylin and eosin staining. Original magnification ×75.

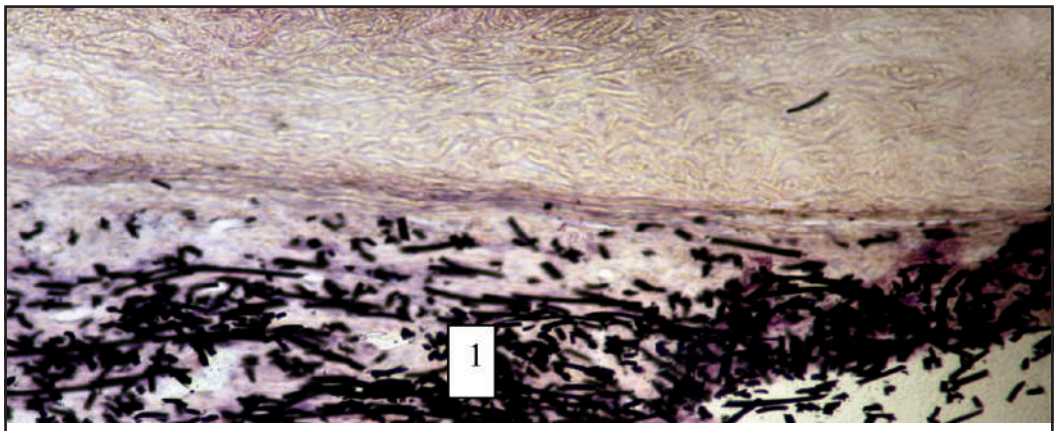

Fig. 4. Day 30 after incorporation of the Mt polymer composition implant (1) into the orbit around the globe. Hematoxylin and eosin staining. Original magnification ×240.

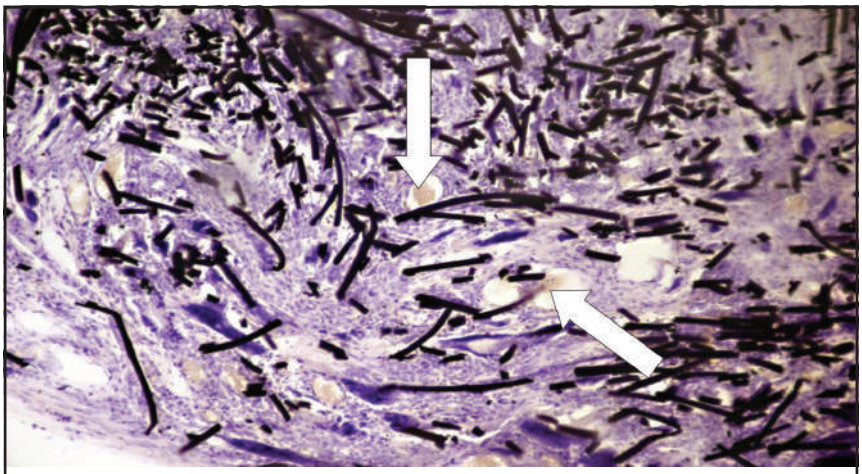

Fig. 5. Day 30 after incorporation of the Mt polymer composition implant into the soft tissues of the orbit. Hematoxylin and eosin staining. Original magnification ×240.

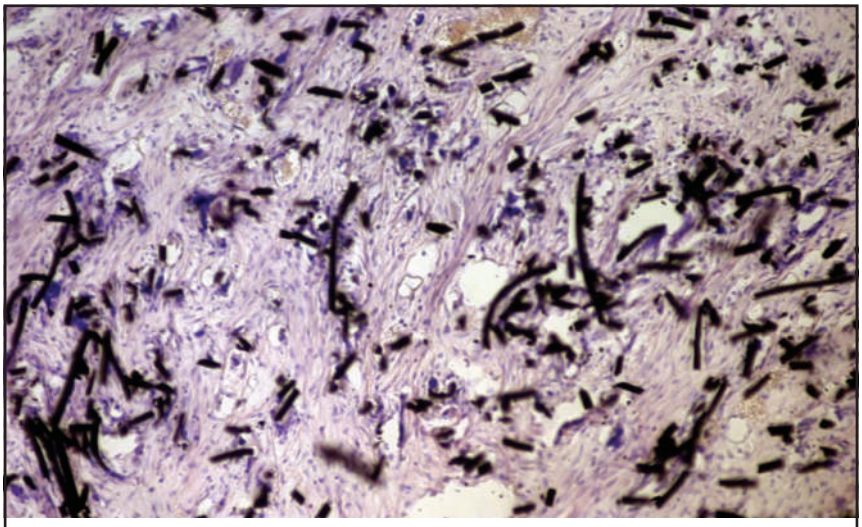

Fig. 6. Day 30 after incorporation of the polymer composition implant into the orbit. Hematoxylin and eosin staining. Original magnification ×240.

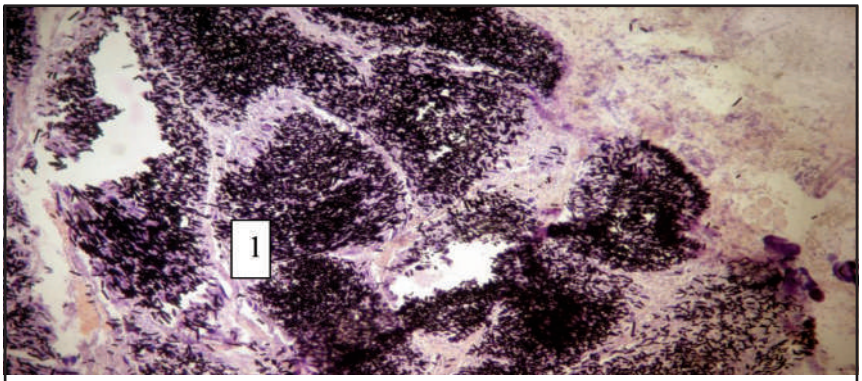

Fig. 7. Day 60 after incorporation of the polymer composition implant (1) into the orbit. Dense fibrous fabric divides the implant into sites with partial fabric penetration in between implant components. Neither signs of inflammatory infiltration nor destruction of the orbital soft tissue, external ocular muscles, optic nerve or sclera are observed. Hematoxylin and eosin staining. Original magnification ×40.

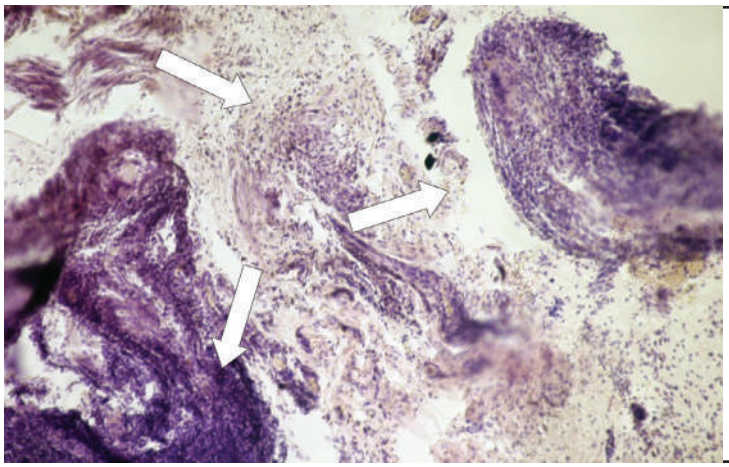

Fig. 8. Day 10 after incorporation of the PFTE implant into the orbit. Apparent diffusive and focal infiltration of the soft tissues of the orbit (arrows) with edematous and/or destructed collagen fibrils is noted. Hematoxylin and eosin staining. Original magnification ×70.

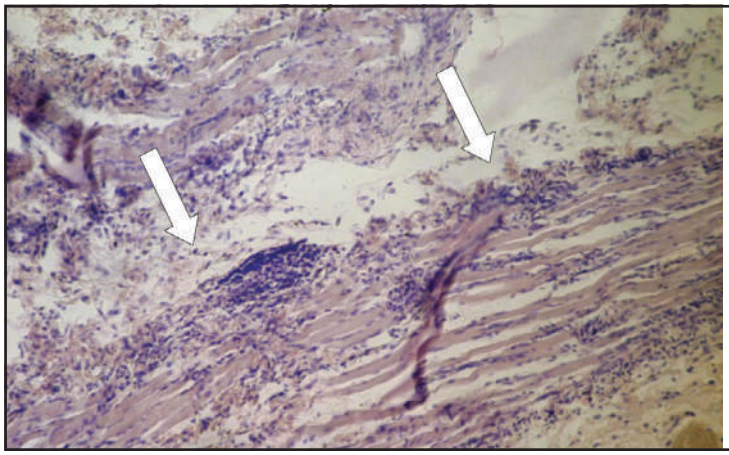

Fig. 9. Day 10 after incorporation of the PFTE implant into the soft tissues of the orbit. Hematoxylin and eosin staining. Original magnification ×70.

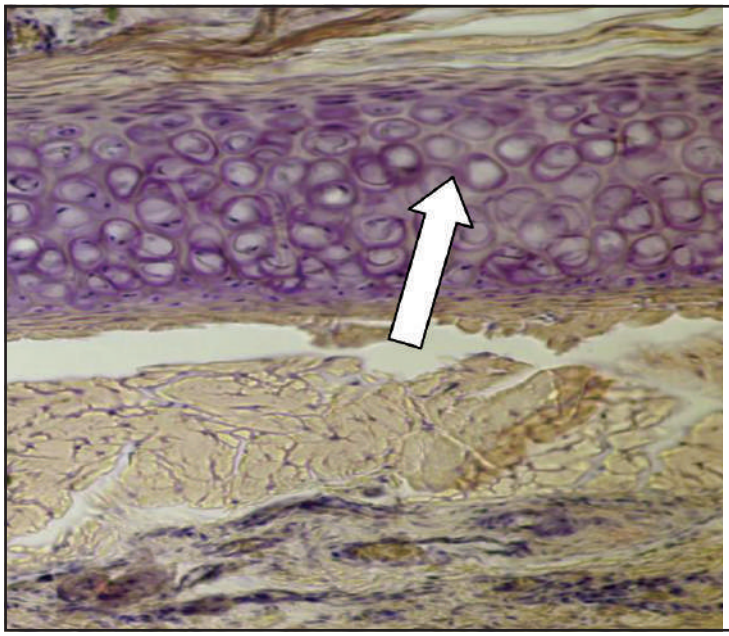

Fig. 10. Day 30 after incorporation of the PFTE implant into the auricle near the auricular cartilage. Hematoxylin and eosin staining. Original magnification ×120.

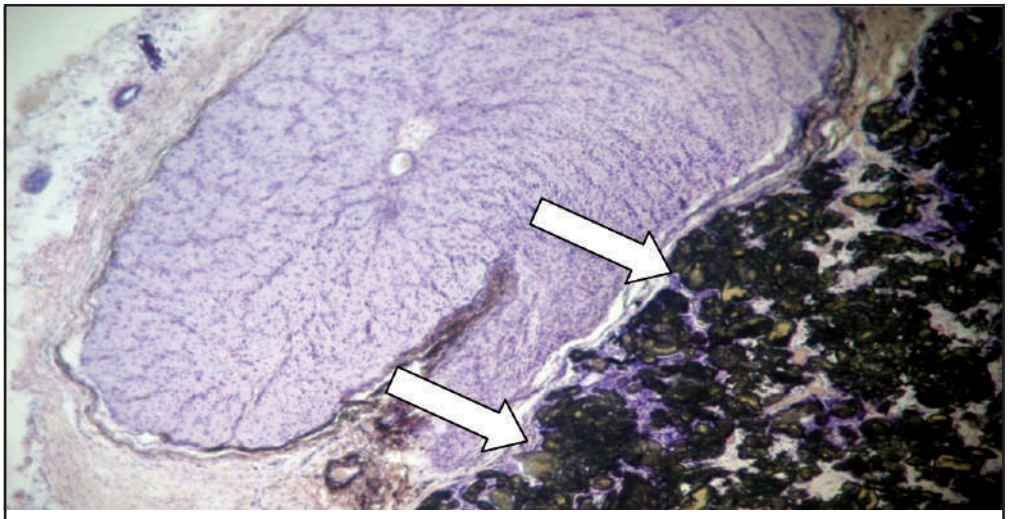

Fig. 11. Day 30 after incorporation of the PFTE implant near the optic nerve. Hematoxylin and eosin staining. Original magnification ×70.

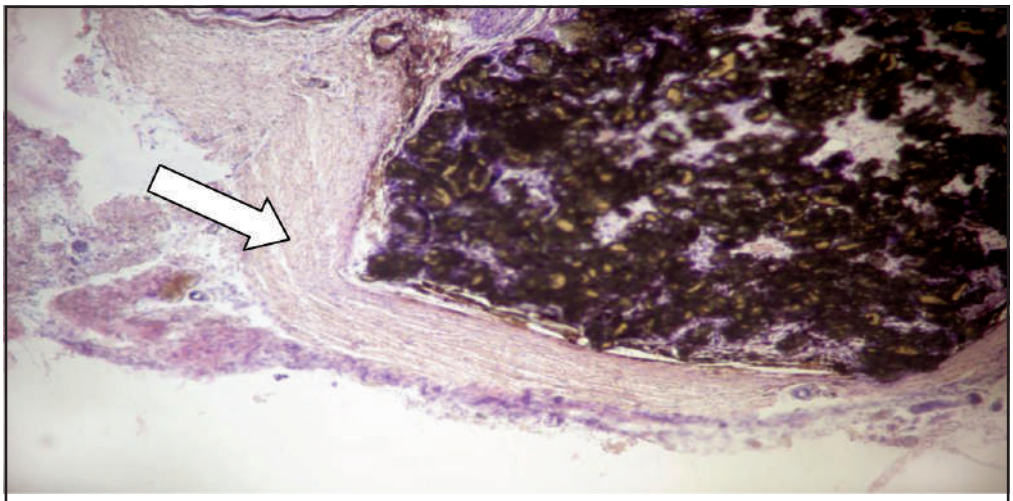

Fig. 12. Day 60 after incorporation of the PFTE implant into the soft tissues of the orbit. The capsule surrounding the implant appears to get thicker, and contains no blood vessels. Hematoxylin and eosin staining. Original magnification ×70.

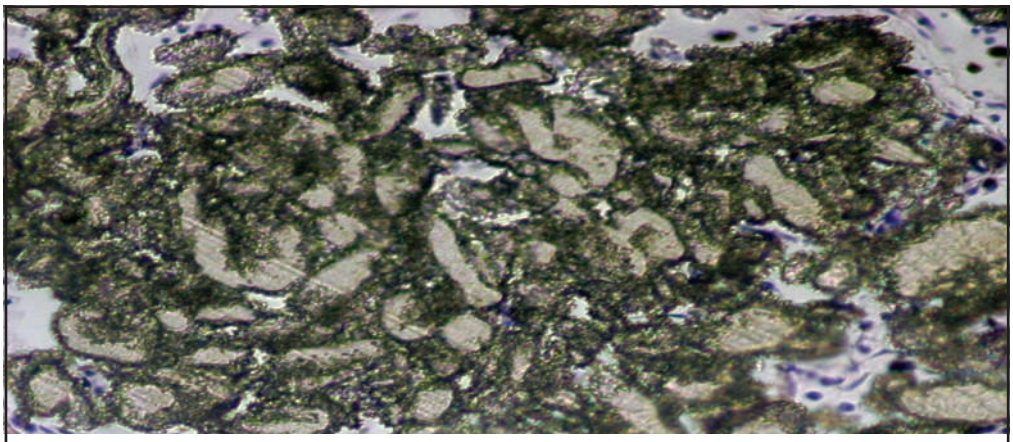

Fig. 13. Day 60 after incorporation of the PFTE implant into the orbit. Hematoxylin and eosin staining. Original magnification ×180.