Composite fibrous glaucoma drainage implant

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Abstract. Glaucoma is a frequent reason of loss vision. It is usually caused by increased intraocular pressure leading to damage of optic nerve head. This work deals with the development of fibrous structure suitable for glaucoma drainage implants (GDI). Commercially produced metallic glaucoma implants are very effective in lowering intraocular pressure. However, these implants may cause adverse events such as damage to adjacent tissue, fibrosis, hypotony or many others [1]. The aim of this study is to reduce undesirable properties of currently produced drains and improve their properties by creating of the composite fibrous drain for achieve a normal intraocular pressure. Two types of electrospinning technologies were used for the production of very small tubular implants. First type was focused for production of outer part of tubular drain and the second type of electrospinning method made the inner part of shape follows the connections of both parts. Complete implant had a special properties suitable for drainage of fluid. Morphological parameters, liquid transport tests and in-vitro cell adhesion tests were detected.

1. Introduction
Glaucoma is an eye disease where the optic nerve becomes damaged primarily due to elevated intraocular pressure. It is the leading cause of blindness worldwide. Over 60 million people are affected, especially in developing countries [2]. For the treatment of glaucoma, it can be used various approaches. This work deals with drainage implants for discharging excess liquid from anterior chamber. Glaucoma drainage implants (GDI) are most commonly used when traditional methods such as medications or trabeculectomy operations are not sufficiently effective. In complicated glaucoma cases, it may be primarily chosen drainage implant surgery [3].

2. Materials and methods
Composite structure of GDI was prepared by the electrospinning technology [4]. The main conditions for the selection of material were conditioned as follows: biocompatible material, non-degradable, resistant to cell growth to prevent blockage of the channel and suitable for electrospinning. Ideally material was combination of outer part made from polyvinylidene fluoride (PVDF) with polyethylene oxide (PEO) and inner part made from cross-linked PVA.
3. Chemicals and preparation of solution

Polyvinylidenfluorid (PVDF; Kynar 720) was obtained from Arkema, polyethyleneoxide (PEO; Mw: 900 000 g/mol), polyvinyl alcohol (PVA; Mowiol 8-88, Mw: 67 000 g/mol), glyoxal (40 wt.% in water) were obtained from Sigma Aldrich. Dimethylacetamide (DMAC; purity ≥99 %), phosphoric acid (purity 84-87 %) were obtained from Penta Chemicals. Polymer solutions were prepared as follows: PVDF 17 wt.% with PEO 1 % was dissolved in DMAC, PVA 20 wt.% was dissolved in distilled water. PVA solution was cross-linked by using 4 wt.% of phosphoric acid and 3 wt.% of glyoxal to provide water-insolubility. PVDF/PEO polymer solution was magnetically stirred at 60 °C for 4 hours and PVA at room temperature for 24 hours to allow complete dissolution before electrospinning.

4. Electrospinning

In the first phase was prepared outer part of implant by electrospinning method of PVDF/PEO solution. Fibers produced from the solution located in a 10 ml syringe and heated to 60 °C was collected on a rotating metallic rod with 1.5 mm diameter to create the tubular implant. The syringe was shifted equally over the entire length of the collector by pneumatic shift of linear pump (KDS 100, KD Scientific). Distance from the end of the needle (with 1.2 mm of internal diameter) to the rotating collector was 15 cm. Electrospinning was carried out for 40 minutes. The speed of rotation of the collector was 500 rev./min. Voltage on the needle was 10 kV positive, powered by DC high voltage supply (Spellman SL 150). All experiments were carried out at 21 °C and relative air humidity 60 %.

In the second phase were produced parallel nanofibers, which were subsequently twisted to yarn. Electrospinning was carried out onto a special type of collector, see on Fig. 1. PVA solution was pushed from 10 ml syringe to an opposite charge rotation collector for 30 minutes. The voltage on the tip of needle was 20 kV positive and on the collector was 3 kV negative. The distance between arms of the collector was 10 cm and between needle and collector was 15 cm. The speed of rotation of the collector was 60 rev./min. Collector was powered by DC Regulated Power Supply (model RXN-302D-3). All experiments were carried out at 20 °C and relative humidity of 40 %.

![Figure 1. 3D model of special type of collector for production of parallelized nanofibres [5]](image-url)
Into the tubular drain made of PVDF/PEO was inserted nanofiber yarn from PVA in various numbers. This special prepared structure of PVDF/PEO implant with three nanofibrous yarns made from PVA is shown on Fig. 2.

**Figure 2.** Composite fibrous drain composed of an outer PVDF/PEO channel and an inner PVA nanofiber yarns; SEM microscopy, magnification 200x [6]

5. **Characterization**

Fibrous layers and yarns were studied by scanning electron microscopy (SEM; Tescan Vega 3SB Easy Probe) and evaluated by software program NIS Elements AR 3.2. Images of fibrous layers are shown in Fig. 3.

**Figure 3** Morphology of fibrous structures: (a) Outer part of drain made from PVDF/PEO in DMAC; (b) inner part of 20 wt.% PVA in water. SEM microscopy, magnification: (a) 3.000x; (b) 1.000x

The average fiber diameter of PVDF/PEO was 930±275 nm and PVA was 263±99 nm. The thickness of outer PVDF drain was 150 μm and inner diameter was 1.5 mm. PVA nanofibrous yarns were manually inserted into the PVDF channel.
6. **Liquid transport tests**

The composite fibrous tubular drains with various numbers of yarns were evaluated for the liquid flow. Inner diameter of drain was 1.5 mm and length was 15 mm. The scheme of laboratory constructed equipment is shown in Fig. 4.

![Diagram of the device for measuring of liquid flow](image)

**Figure 4** Scheme of the device for measuring of liquid flow. Dosing pump holds the fluid at the same level.

The tank was filled with 0.8 % of sodium chloride. Level was maintained at 19.5 cm, which simulate normal intraocular pressure 2 kPa. The flow rate was measured ten times. Results are shows in table 1.

| Sample (1.5 mm drain) | Liquid flow (ml/hr) |
|-----------------------|---------------------|
| Device without the drain | 315±25             |
| Empty drain           | 100±33              |
| Drain with one yarns  | 89±27               |
| Drain with two yarns  | 34±13               |
| Drain with three yarns| 9±4                 |
| Average human eye     | 0.4                 |

**Table 1** The amount of fluid filtered through the drain

Fibrous drain showed higher secretion of the fluid which is possible to influence by the size of the drain or by number of the yarns inside the channel. A greater number of yarns could not be possible to place into a small space of the drain.

7. **In-vitro tests**

Materials PVDF/PEO and PVA were separately tested for biocompatibility and resistance to cell growth by in-vitro tests using 3T3 mouse fibroblasts. Resistant to cell growth of the cell is very important for ensure safe drainage of fluid from the anterior chamber. For better handling, the samples were tested in a planar shape. Materials were seeded with 3T3 mouse fibroblast for 8 days. Samples were sterilized for 30 minutes by immersion in 70 % ethanol. Follows washed twice with phosphate buffered saline (PBS, Lonza) and incubated for 30 min in complete medium (DMEM + 10 % FBS + 1 % antibiotic + 1 % glutamine, Biosero). Materials were tested in 24-well plates. Cell growth was evaluated by fluorescence microscopy (NIKON Eclipse Ti-E) for 1 and 8 days.
The cells on the materials were fixed by the frozen methanol and twice washed in PBS. Samples were stained in the dark by DAPI, washed in PBS and again analysed by fluorescence microscopy. Results are shown on the Fig. 5 a Fig. 6.

**Figure 5** PVDF/PEO images of fluorescence microscopy after staining of cell nuclei with DAPI after 1 and 8 days of culture (magnification 10x): (a) PVDF/PEO after 1 day of cultivation; (b) PVDF/PEO after 8 days of cultivation

**Figure 6** PVA images of fluorescence microscopy after staining of cell nuclei with DAPI after 1 and 8 days of culture (magnification 10x): (a) PVA after 1 day of cultivation; (b) PVA after 8 days of cultivation

Materials made from combination of PVDF/PEO and made from PVA are biocompatible with 3T3 mouse fibroblasts and partially resistant to the growth of cells. Materials do not exhibit good adhesion to the cells and does not exhibit cytotoxic effects on fibroblast cell line used. Materials appears to be suitable for use in a place where it is necessary to resist against the cell growth.

8. Conclusions

Composite glaucoma fibrous tubular drain was created. This device is suitable for possible application in the treatment of glaucoma. Electrospinning technology for production of the composite drain was used. Materials were resistant to cell growth, which was confirmed by in-vitro tests. Liquid transport tests through the drain confirm its filtration properties.
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