DEVELOPMENT OF FIBROUS IMPLANT FOR THE TREATMENT OF GLAUCOMA

KLAPSTOVA Andrea[1], VRSINSKA Klara[1], HORAKOVA Jana[1], SAMAN Ales[1], SHYNKARENKO Andrii[2], LUKAS David[1]

[1]*Technical University of Liberec, Faculty Of Textile Engineering, Department of Nonwovens and Nanofibrous Materials, Liberec, Czech Republic, EU*
andrea.klapstova@tul.cz
[2]*Technical University of Liberec, Faculty of Mechanical Engineering, Department of Manufacturing Systems and Automation, Liberec, Czech Republic, EU*

Abstract

This article deals with the development of fibrous non-biodegradable drainage device for the treatment of glaucoma disease. Currently produced implants are often associated with hypotony, undesired fibrosis, tissue damages around the implant and many others [1]. Nanofiber implant has great potential to alleviate or completely eliminate these problems. The main goal of this work was a creation of the drain for safety drainage of intraocular fluid from the patient eye to achieve a normal intraocular pressure. The small-diameter tubular implant (<1 mm) was developed by the use of the electrospinning technology. The structure was composed of two parts, by using special types of collectors. The outer part was formed of non-biodegradable material that is resistant to cell growth. The inner supporting part was formed of hydrophilic material which is also resistant to cell growth. This part prevents the compression of the outer material and a total blockage of the drain. The special structure supports the drainage of fluid. Material was subjected to morphological structures, liquid transport tests and in-vitro cell adhesion tests. First results show great potential in the use of the implant in treatment of glaucoma disease.

1. INTRODUCTION

Glaucoma is the leading cause of blindness worldwide, especially in developing countries. The main risk factor is primarily elevated intraocular pressure [2]. Consequently, it can lead to damage the optic nerve or to complete blindness. Several ways of glaucoma treatment exist but this paper heads toward a surgical method. Glaucoma that does not respond to medical or conventional surgical treatment is called refractory glaucoma (RG). The most common surgical method for RG is trabeculectomy with or without anti-fibrotic agents. Alternative and highly effective method of treatment RG are drainage implants [3]. It is basically a modification of trabeculectomy with the same efficiency, but with several of postoperative complications such as hypotonia, of postoperative inflammatory reactions or the need for local application glaucoma therapy postoperatively. Drainage implant allows drainage of aqueous humor from the anterior chamber into the suprachoroidal space [4].

2. EXPERIMENTAL PART

2.1. Materials

Chemicals and preparation of solutions: Polyvinylidenfluorid (PVDF; Mw: 180 000 g/mol), polyvinyl alcohol (PVA; Mowiol 8-88, Mw: 67 000 g/mol), glyoxal (40wt.% in water) were obtained from Sigma Aldrich. Acetone (purity ≥99.5%), dimethylacetamide (DMAC; purity ≥99%), phosphoric acid (purity 84-87%) were obtained from Penta Chemicals. Polymer solutions were prepared as follows: PVDF 26wt.% was dissolved in DMAC/Acetone (in ratio 8:2), PVA 20wt.% was dissolved in distilled water.

Solution was cross-linked by using 4wt.% of phosphoric acid and 3wt.% of glyoxal to provide water-insolubility. The polymer solutions were magnetically stirred at room temperature to allow complete dissolution before electrospinning.

2.2. Electrospinning

Outer and inner part of drain were prepared separately. Outer part from PVDF was prepared by electrospinning technology from special rotation collector. PVDF solution was electrospun from 10 ml syringe with a needle having internal diameter 1.2 mm to rotation bar with 1 mm diameter, 10 cm length for 60 minutes. The syringe was shifted equally over the entire length of the collector by pneumatic shift of linear pump (KDS 100, KD Scientific). Voltage on the needle was 10 kV positive, powered by DC high voltage supply (Spellman SL 150). The speed of rotation of the collector was 500 rev./min. The distance between the end of the needle and the collector was 15 cm. All experiments were carried out at 19°C and relative air humidity 60%. Device is shown on **Figure 1**.

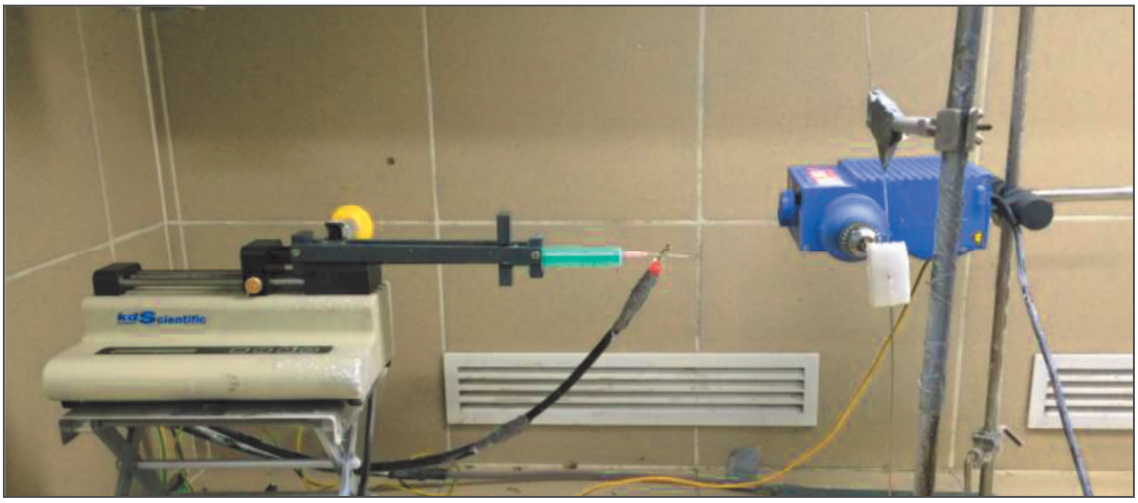


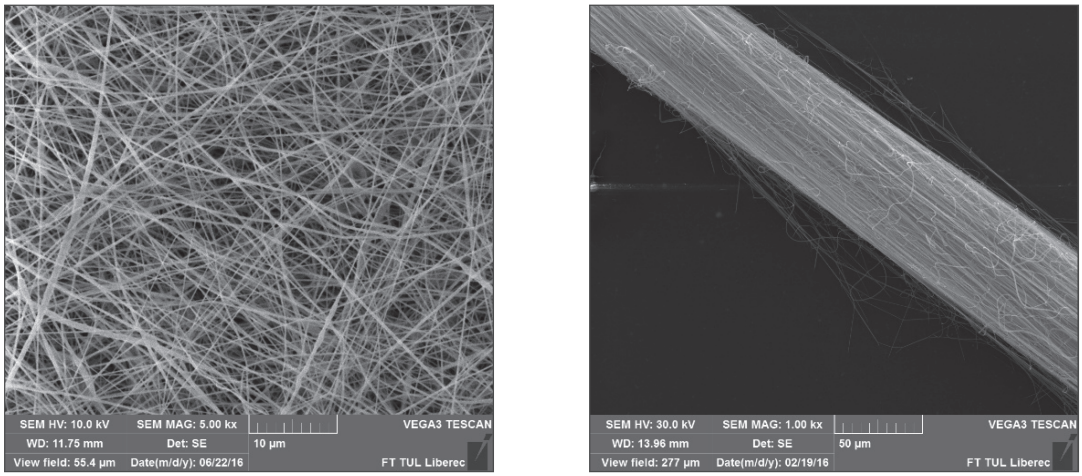
Figure 1 Electrospinning device for production of PVDF outer part of fibrous drain

Inner part from PVA was prepared also by electrospinning technology to another rotation type of collector with specially shaped arms (**Figure 2**). This rotation collector allows the production of parallelized fibers. Solution was pushed from syringe to an opposite charge rotation collector for 20 minutes. The voltage on the tip of needle was 22kV positive and on the collector was 3kV negative. The distance between needle and collector was 15 cm and the distance between arms of the collector was 10 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 23°C and relative humidity of 38%. Fibers were caught between the arms of the collector and subsequently twisted into yarn. After remove of the fibers the crosslinking procedure for 7 minutes at 130°C followed.

Figure 2 Special type of rotating collector for production of PVA inner part of fibrous drain

2.3. Characterization

The morphology of produced fibers was studied to achieve best structure of the fibrous layer. Fibers were sputter coated by 7 nm of gold. Morphological structure was observed by scanning electron microscopy (SEM; Tescan Vega 3SB Easy Probe). Fiber morphology evaluation was carried out a software program NIS Elements AR 3.2. Images of fibrous layers are shown in **Figure 3**.



(a) *(b)*

Figure 3 Morphology of fibrous structures: (a) 26wt.% PVDF in DMAC/Aceton (8:2); (b) 20wt.% PVA in water. SEM microscopy, magnification: (a) 5.000x; (b) 1.000x

Planar samples of electrospun materials were tested in vitro for biocompatibility and resistance to cell growth. Materials were cultured with 3T3 mouse fibroblast for 8 days. Samples of 6 mm diameter were sterilized by immersion in 70% ethanol for 30 minutes, then 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 96-well plates. Cell growth was investigated by fluorescent 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 then stained by DAPI in the dark, washed in PBS again and analyzed by fluorescence microscopy.

2.4. Liquid transport

The capillarity of liquids through the tubular drain was measured on the device Microtensiometr Krüss K121. The sample was immersed into the ethanol and the increment of the liquid was measured. Also the fibrous tubular drain was evaluated for the liquid flow. The laboratory constructed equipment is shown in **Figure 4**. The container was filled with 0.8% sodium chloride.

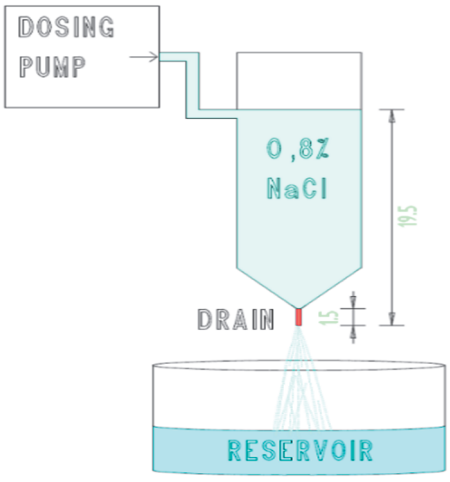


Figure 4 Scheme of the device for measuring of liquid flow. Dosing pump holds the fluid at the same level. Red color represents the drainage device

RESULTS AND DISCUSSION

2.5. Electrospinning

Both materials have been electrospun with various concentrations. The best results showed 20wt.% PVA and 26wt.% PVDF. Morphology and fibrous structure, include fiber diameters were studied by SEM. The average fiber diameter of PVA was 275±78 nm and PVDF was 352±103 nm. The thickness of outer PVDF drain was 100 µm and inner diameter was 1 mm. PVA nanofibrous yarns were manually

inserted into the PVDF channel. Optimal number of yarns into the channel was three. **Figure 5** shows the resulting composite fiber drain composed of an outer PVDF channel and an inner PVA nanofiber yarns.

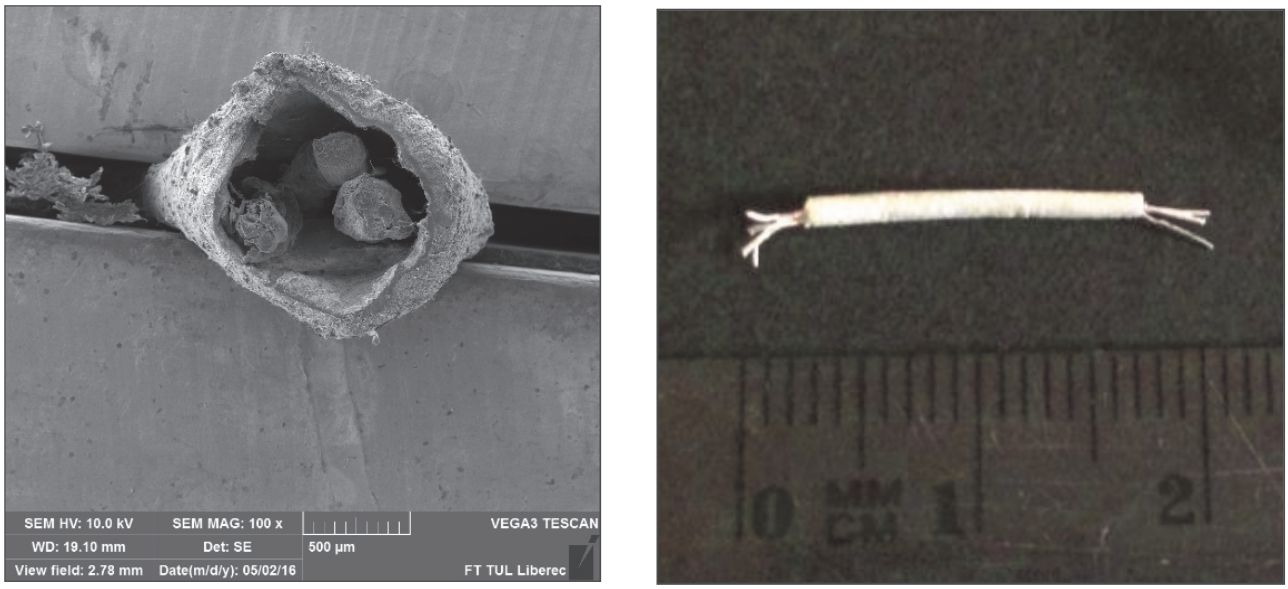
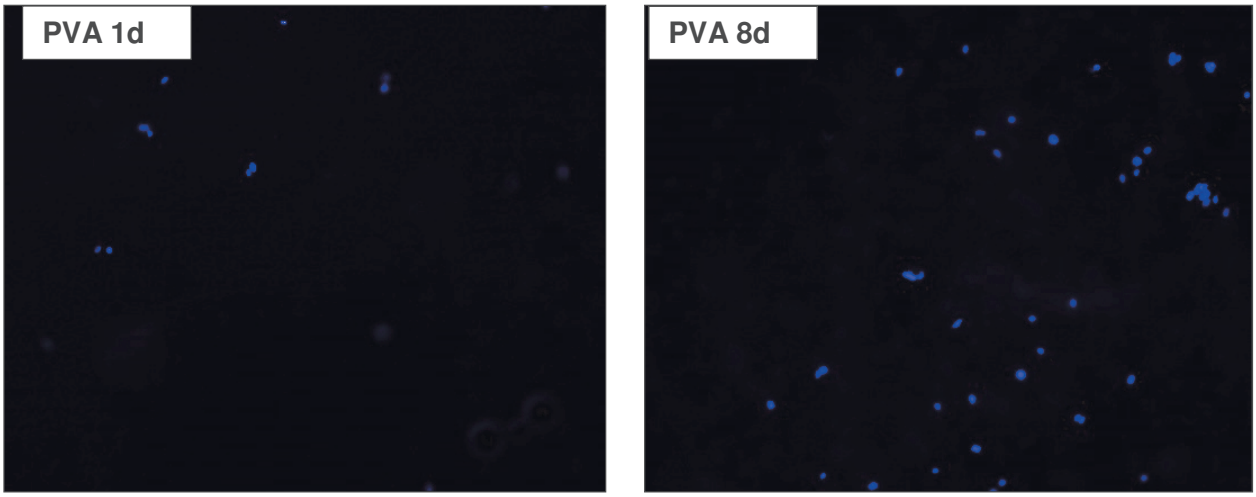


Figure 5 Composite fiber drain composed of an outer PVDF channel and an inner PVA nanofiber yarns; SEM microscopy, magnification 100x

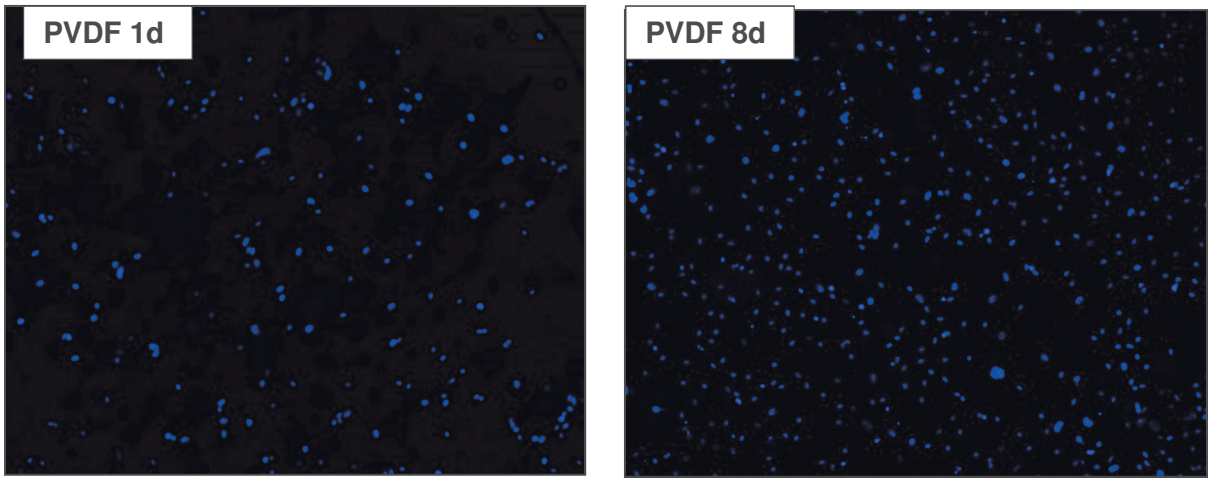
2.6. In vitro tests

Materials were tested for resistance to cell growth. Resistance to the growth of cells is very important for the possible blockage of the drain by cells. Material must be resistant to growth of the cells to ensure safe drainage of fluid from the posterior chamber of the eye to the front chamber. Both of materials were seeding by 3T3 mouse fibroblasts. Results are shown on the **Figure 6a** and **6b**.



(a) (b)

Figure 6a 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



(a) (b)

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

Nanofibrous materials made from PVA and PVDF are partially resistant to the growth of 3T3 mouse fibroblasts. Nanofibrous materials may not have ideal surface properties for cell adhesion, however, does not exhibit cytotoxic effects on fibroblast cell line used. Cells adhered poorly to the test material but gradually there was a proliferation of cells, which was very slow. For a complete resistance to growth of the cells would be appropriate to use anti-fibrotization agents.

2.7. Liquid transport

Fibrous drain with inner diameter of 1 mm, length 15 mm and three yarns inside the channel was used. The level in the tank was maintained at 19.5 cm (which simulate normal intraocular pressure about 15mm Hg or pressure 2 kPa) length of the drain was 1.5 cm. The flow rate was measured twenty times for each sample. Measurement results show good ability to drain the intraocular fluid, see in **Table 1**.

Table 1 The amount of fluid filtered through the drain

Sample	Liquid flow (ml/hr)
Device without the drain	315±25
Empty drain	80±12
Drain with three yarns	2±0.8

Average value secretion per day for a healthy patient is about 2.2 mm^3/min (0.4 ml/hr). 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.

3. CONCLUSIONS

Fibrous tubular drainage device allowing flow of intraocular fluid in glaucoma disease was created. Very simple method - electrospinning technology to produce the drain was used. For production have been developed a new rotary collectors enabling the production of very small tubular (<1mm) and yarns formations. The material exhibits good resistance to cell growth.

ACKNOWLEDGMENTS

The research was supported by the SGS - 21150 project for financial support and cooperation with prof. David Lukas (KNT). We would also like to thanks Petr Bulir, the chiefs of the Department of Ophthalmology at the Regional Hospital in Liberec.

REFERENCES

[1] WISCHKE CH, NEFFE A T, HANH B D, KREINER CH et al. A multifunctional bilayered microstent as glaucoma drainage device. J Control Rel 2013; 172: 1002-1010,

[2] QUIGLEY HA, BROMAN AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006;90:262-7.

[3] DUBEY S, et al. Safety and efficacy of Ahmed glaucoma valve implantation in refractory glaucomas in Northern Indian eyes. Saudi Journal of Ophthalmology 2014; Volume 29, Issue 2, Pages 103-108

[4] MINCKLER DS, HILL RA. Use of novel devices for control of intraocular pressure. *Exp Eye Res*. 2009 Apr;88(4):792-798.