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Application of Electrospinning for Vascular Prothesis Design – Preliminary Results

Abstract

One of the novel technologies for vascular prosthesis design is melt electrospinning. The solvent–free approach allows to model electrospinning without the complications associated with solvent evaporation or the risk of a potential adverse, toxic reaction, both local and systemic. However, many tissue-engineers wish to combine various cells and electrospin material for clinical use. Preliminary research of flat fibrous structures made by melt electrospinning and the processing influence of parameters on the resulting fibre properties was performed. Surface analysis of planar structures by Scanning Electron Microscopy (SEM) and determination of the fiber diameter using SEM microphotographs and Lucia G software were realised.

Key words: vascular prosthesis, electrospinning, melt – electrospinning, fibre diameter, SEM images.

Introduction

Cardiovascular diseases (CVD), often resulting from arteriosclerosis, remain the leading cause of death in the whole world. A report issued by the American Heart Association shows that cardiovascular diseases are one of largest causes of mortality [1-2].

Mortality data show that cardiovascular diseases are a significant cause of death, accounting for 34.3% (831 272) of all the 2 426 264 deaths in 2006 or 1 out of every 2.9 deaths in the United States [3]. However, in the USA more than 151 000 of those people who died of a cardiovascular disease in 2006 were older than 65 years of age. Every year since 1900, cardiovascular disease has accounted for more deaths than any other major cause thereof in the USA and Europe [4].

Active research on cardiovascular diseases and vascular surgery is still being conducted. It shows a need for research on new ways of therapy, techniques and surgical procedures. Minimally invasive technologies (stents, stent-grafts, etc.) are constantly being developed, but a broad group of patients still requires alternative grafts with acceptable patency rates [5]. Therefore, for 60 years enormous efforts have been made to solve this. Voorhees et al. [6] demonstrated that porous synthetic materials can serve as an effective substitute for a vascular vessel.

The design of prosthetic vascular graft material, such as polyethylene terephthalate (trade name: Dacron®) and polytetrafluoroethylene (PTFE) is required. The clinical application of man-made vascular grafts to treat coronary or peripheral artherosclerosclerotic diseases

has shown continuous progress in modern vascular and cardiovascular surgery, resulting in a reduction in postoperative complications, thereby improving patients' lives. The use of vascular prostheses (both in invasive and non-invasive surgery) has become almost routine and is generally successful surgery [7].

Man-made vascular prostheses are suitable for the replacement of high-diameter arteries or vessels (with an internal diameter higher than 10mm) where blood flow is relatively high. However, when the diameter of the vascular vessel is small (less than 6 mm) and the blood flow is low, the success rate of the reconstruction is significantly lower. The causes of this failure are multi factorial and very complex; the exact mechanisms are not yet understood. Small-diameter, textile vascular prostheses fail rapidly due to occlusion. In the case of reconstruction, a by-bypass showed rates of thrombosis greater than 40% after 6 months [8]. Therefore, there is a massive clinical need for an alternative artificial supply of vessels to replace diseased or defected arteries [9].

The factors responsible for the abovementioned occlusion can be divided as follows:

A) related to the material used for the manufacture of a vascular graft:

- a difference in compliance between the natural artery and artificial vascular prosthesis;
- post-implantation changes in the material as well as the macro- and microstructure of the artificial vascular prosthesis;
- surface properties of the artificial vascular prosthesis;

B) related to the patient:

- state of the inflow and outflow vessels:
- progression of atherosclerosis;
- other illnesses (such as diabetes, etc.) The need for a prosthetic graft that performs as a small-diameter conduit has led investigators to pursue many avenues in vascular biology [10]. Some investigation groups have continued to use manmade graft materials as a base for coating with bioactive materials (such as heparin, prostaglandins, various growth factors, anticoagulant peptide sequences, dextran derivates, and antibiotics) or as a scaffold for supporting embedded cells (endothelial cells, smooth muscle vascular cells, etc.) [11].

Electrospinning of a vascular substitute and melt – electrospinning application for tissue engineering and cardiovascular surgery

The idea of the research was connected with clinical needs for the elaboration of a novel, effective-in-clinical use, biometic prostheses design to be applied in vascular and cardiovascular surgery.

Electrospinning using solutions of polymers has been extensively studied because it offers quicker success and significant effectiveness in the race to develop fibres with diameters less than 100 nm, which are necessary as a result of the low viscosities that need to be overcome to produce a continuous jet. The technology above for the generation of nonordered fibrous structures has been used as an effective method for the formation of biomimetic scaffolds comprising of a large network of interconnected fibres and pores [12]. The prosthetic vessels designed can be altered to closely match the properties of native vessels [13].

Vascular tissue engineering attempts to create functional small-diameter scaffolds by employing natural and/or artificial materials with endothelial and smooth muscle cells, resulting in a tubular construct that can be used *in vivo* [14]. These vascular scaffolds are combined with viable cells to allow the graft to be remodelled when implemented *in vivo*. Electrospinning has been used to form biocompatible, infection resistant structures which have appropriate biomechanical properties.

However, electrospinning directly from solution can give a few disadvantages [15]:

- a necessity for the dissolution of polymers in volatile and often toxic solvents as well as their removal/recycling:
- a lower throughput occurs due to a loss of mass by solvent evaporation – most solutions are expensive and extremely hazardous;
- sub-micron scale fibres of polymers which do not have appropriate solvents at room temperature, such as polyethylene and polypropylene cannot be obtained;
- multi-component systems, such as blends and composites, which in many cases is due to the fact that no solvent for all of the components may be found.

These faults have forced research on nonwoven technologies which do not need solvents, being the main reason for the application of the melt spinning technique for the design of novel vascular prosthesis showing a significant decrease in local and systemic adverse effects (related to solvent residues), as well as biometic behaviour, corresponding to high clinical effectiveness.

There are a few literature publications about the application of the melt blown technique for small-diameter vascular prosthesis design [16, 17]. However, as a result of studies on nonwovens, the authors obtained fibres of $6 - 10 \mu m$ diameter [16]. Other authors studied melt blown structures, whose fibre diameter ranged $10 - 50 \mu m$ [17].

An alternative method of designing small-diameter vascular prostheses could be melt electrospinning. Some publications regarding melt electrospun nonwoven structures report a design of an implant from a collection of fibres of a mixture of nano- and micro-scale diameters [18, 19, 25, 31]. Polymer melt-electrospinning may allow new approaches to certain aspects of electrospinning, particularly overcoming the technical restrictions governed by the solvent accumulation and resulting residual, long-term local and systemic toxicity [19].

Melt electrospinning also has some disadvantages:

- the technique can only be used for thermoplastic polymers;
- the viscosity of melt is higher, affecting higher fibre diameters;
- the high temperature of the process.

Most of the research published chose polymers for the electrospinning effects of one or two processing parameters on the fibre diameter and morphology [14, 22 - 32].

Lorrondo and Manley [23] studied the effect of polymer viscosity on melt-electrospun fibres. The critical viscosities and electrical fields applied were significantly higher than those required during electrospinning from solution. The viscosity of the polymer melt corresponds to the melt flow index: a high melt flow index means lower viscosity during the spinning process [42]. The above-mentioned parameter affects the reduction in surface defects, and finally more uniform fibres have been formed [20, 41].

One of the parameters of melt electrospinning most studied is the spinning voltage. In electrospinning, the electrical voltage applied affects jet stability and fibre morphology to a remarkable degree. Every polymer that is electrospun possesses unique properties. Consistent with past electrospinning research [33], an increase in the electric field strength decreases the average fibre diameter for all of the polymers examined.

Many other parameters have been studied, yet their exact effect on the electrospinning process is unknown. Literature studies [12 - 13, 15 - 26] have shown varying results when discussing aperture diameter, working distance, the feed rate or electrostatic potential. In the articles published [23, 34 - 34], the authors argue that these parameters vary inversely with the diameter. Some of them argue that they vary inversely, whereas others have said that they have no effect on the final product.

There have been only a few researches carried out to obtain a ready-to-use vascular implant by melt electrospinning, especially in the case of small-diameter vascular implants made to order (for an individual patient), which will correspond in shape and curvature to the individuality of the surgical replacement. However, Sung Jin Kim et. al. [37] presented the possibility of designing composite scaffolds using a combined melt electrospinning and solution electrospinning process.

The literature [37] suggests making a layered design in which some layers would be from low-diameter fibres, which

should also have lower porosity. On the other hand, others suggest constructing a fully resorbable implant [38].

A review of the literature leads to the conclusion that melt electrospinning could be an appropriate technique for small-diameter (less than 6 mm) vascular prosthesis design. Melt electrospinning allows to model small diameter vascular prosthesis without using solvent, which can cause a toxic reaction, both local and systemic [15, 19]. This technology gives the possibility to obtain textile structures with various fibre diameters and controlling mechanical properties [18 - 22].

Aim of study

The design idea was to obtain individually shaped fibrous structures by applying melt-electrospinning only. The abovementioned structures would consist of the following polymers:

- polypropylene (PP) or polyvinylidene fluoride (PVDF), being the base of the porous, non-resorbable structure of the implant (middle layer);
- polylactide (PLA) or co-polyesters (BAP) [36], being the base of external and internal thin layers of the implant. Both resorbable layers should be characterised by significantly lower porosity than the basic middle layer and ought to be resorbable after implantation.

The choice of polymers was due to their suitability for the melt electrospinning method, the possibility of using them in the designing of vascular prostheses (i.e. potential toxicity as well as susceptibility to resorption) as well as their biocompatibility in the aspect of the initial polymer and potential products of the degradation. The appliance of two polymers for each layer required the screening of the most optimal polymer types and properties, as well as with respect to future application (incorporation of tissue, acceleration of neo-intima formation, etc.).

PP and PVDF show optimal properties for the melt technique, because they have high thermal stability, as well as a wide range of melt temperatures and melt flow index.

For many years PLA has been the most important resorbable polymer used in medicine in many applications, both internal or external.

The thermal properties of PLA (such as the melt temperature) is also appropriate for melt electrospinning. Moreover, the final degradation products of polylactide show no potential toxicity [55]. The controlling biodegradation of PLA could be controlled by the application of polymers of strictly selected molecular weight and crystallinity.

The most important factor is to apply polymers with a high grade of chemical purity, which will be a key point of the studies. BAP polymer was elaborated by the Centre of Polymer and Carbon Materials, Zabrze (Poland), to exchange the standard catalyser with another of significantly lower toxicity [36].

All of the above-mentioned polymers are often used for forming implants. However, the most important aspects of the suitability of a polymer for medical devices is its purity (i.e. quantitative and qualitative presence of leakage substances, processing aids, surfactants, catalysers, etc.), affecting the potentiality of adverse effects, such as local and systemic toxicity. The application of commercially found polymers for medical device design requires biocompatibility studies according to Standard ISO 10993-1, even when a similar polymer, but differing in chemical purity, has been used for similar medical device designs.

Our proposal was to design a novel, biometic structure using fibrous structures obtained by the melt electrospinning technique, with a full elimination of solvents of potential adverse effects over a local and systemic range, as well as those acting acutely and/or chronically.

The above idea of making a three layer porous structure came as a result of analysis of potential complications of conventional vascular reconstructions and risk analysis (acc. PN-EN 14971:2009 Standard). The resorbable outside layer has an effect on the reduction in the mass of the implant, allows to control the growth of tissue and is responsible for assuring the intraoperative surgical tightness of the graft, whereas the inside layers can act as carriers for endothelialisation and support the intraoperative surgical tightness. The middle layer would be the skeleton of the prosthesis, giving the long-term strength required, flexibility, suture strength against pull-out and resistance against dilatation. The thickness of the artificial vascular prosthesis

wall has to be 0.2 - 0.4 mm [39], meaning than the layers of PLA or BAP have to be about 0.1 mm thin and the layer of PP or PVDF about 0.2 mm.

The design of a novel vascular prosthesis is shown in *Figure 1*.

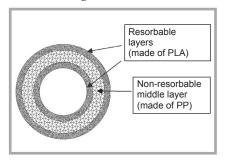


Figure 1. Idea of the vascular prosthesis design.

The research of the implant prototype was divided into three parts: the first stage - preparing flat fibrous structures and the optimisation of preliminary spinning parameters; the second phase - designing tubular structures of each polymer used, and the third phase – obtaining three- layer tubular structures designed individually for individual localisation of the implant. The work described in this article is related only to the first stage. Optimal flat and tubular structures should have the lowest average fibre diameter (in a submicro- or nano scale) and a reproductivity of the fibre diameter as high as possible due to the size of the resulting fibre effect on textile structure porosity and tissue growth after implantation.

All the above-mentioned phases of the research are important due to the following:

- 1. The preparation of flat structures is necessary for analysis of the influence of melt electrospinning process parameters of the resulting fibre diameters on the first-phase optimisation of the process parameters.
 - Preliminary research into electrospinning with the use of a flat collector will be helpful in the second stage of the research to elaborate electrospinning conditions for the tubular collector. The application of a tubular collector may affect the porosity of the fibrous structures and its final arrangement of fibres. Therefore, a second optimisation will be necessary; however, it will be based on the spinning output parameters obtained in the first optimisation.
- 2. The formation of single layers allows to analyse the mechanical and physi-

cal-chemical properties of each single layer.

In the second phase a tubular collector will be constructed with a spindle diameter of less than 6 mm, and the possibility of connecting high voltage.

3. The next step of the research was the preparation of tubular, three-layer fibrous structures made from the medical grade (mostly the VIth class acc. USP) of selected polymers with similar properties.

In the preliminary study structures were prepared using PP or PLA as a base model for the adaptation of melt electrospinning. Then PVDF (higher crystallinity, lower melt flow index as compared with PP) and BAP (lower melt temperature as compared with PLA) were applied to the multilayered fibrous structures formed.

The next stages of the studies will be the estimation of optimal processing parameters for other types of polymers (non-resorbable – PVDF and resorbable – aliphatic polyester) used for the formation of resorbable external/internal layers and a non-resorbable middle layer for the vascular implants designed (2-dimensional structures), as well as the elaboration of a novel partially resorbable, three-layered composite of a flat and tubular structure.

Materials and methods

The materials used for the preliminary design of the flat fibrous structures were polypropylene (Moplen HP 456J, Basell) and polylactide (4060D, NatureWorks [40]). In the research amorphous PLA 4060D was used because it is more suitable for the melt electrospinning method. The parameters of raw materials used are shown in *Table 1*.

Melt electrospinning was performed using a double screw extruder type EH – 16D (ZAMAK/ Poland) with a screw working length of 512 mm, screw nominal diameter of 15.8 mm, maximal screw speed within the range of 800 r.p.m and extruder spinning head type GWR (ZAMAK/Poland) with three nozzles of 0.5mm diameter and generator of high voltage type SWN 0650/2P (Elsikorski/ Poland). *Figure 2* shows a schematic drawing of the melt-electrospinning system used in this research.

The PP melt-electrospinning parameters were as follows:

Table 1. Materials used in the research and manufacturer specifications.

Raw polymer	Chemical structure	Density (g/cm³)	Melting point (°C)	Degradation temperature (°C)	Melt flow index at 230°C/2.16 kg (g/10min)
Moplen HP 456J polypropylene	[-CH ₂ CH(CH ₃)–]n	0,89	163	>300	3,4
4060D polylactide	[-CH ₂ (CH ₃)C(O)O-]n	1,24	150	-	15

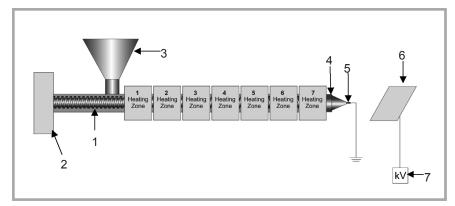


Figure 2. Scheme of the melt-electrospinning of polymers using an extruder: 1. Screws, 2. screw drive motor, 3. Hopper, 4. Spinneret, 5. Die, 6. Collector, 7. high-voltage power supply.

- temperature of head − 300 °C (The high temperature of the extruder head was chosen to obtain thin fibres);
- \blacksquare twist of screws 2 r.p.m;
- changeable parameters: distance of collector to spinneret (10, 15 or 20 cm) and the spinning voltage (25, 30 or 35 kV).

The PLA melt-electrospinning parameters were as follows:

- temperature of head -170 °C;
- twist of screws -2 rpm;
- changeable parameters: distance of collector to spinneret (10, 15 or 20 cm) and the spinning voltage (25, 30 or 35 kV).

Analytical methods

Scanning electron microscopy (SEM)

SEM microphotographs were taken on a Nova NanoSEM 230 scanning electron microscope (FEI Company).

Determination of fibre diameter

The fibre diameter of the flat fibrous structures designed was determined using Lucia G software.

Results and discussion

The first stage of the preliminary experiment was to determine the influence of the processing parameters, such as the voltage delivered and working distance on the properties of the final fibrous

structures, i.e. fibre diameter and mechanical properties.

Scanning electron microscopy (SEM)

The textile structure for vascular prosthesis design should be characterised by a low fibre diameter with high reproductivity and by the absence of physical defects, as any inaccuracy can influence the primary or secondary risk of infection.

The SEM analytical method was used to determine the distribution of fibres in the structure obtained, in the presence of physical pollution or any other physical or structural deformation.

SEM microphotographs of the melt-electrospun fibres are shown in *Figures 3 - 4* (see page 50). The visual analysis of the flat polypropylene structures made at a working distance of 15 cm or 20 cm and spinning voltage of 25 kV or 35 kV showed the visual disturbance of fibres and a very wide spread of the fibre diameter (*Figure 3.b* and *3.f*). However, optimal structures were found for a working distance of 10 cm or 20 cm and spinning voltage of 25 kV (*Figure 3.a* and *3.d*).

All the PLA structures analysed did not show any defects. The SEM microphotographs present PLA structures characterised by a low fibre diameter, as shown in *Figure 4*.

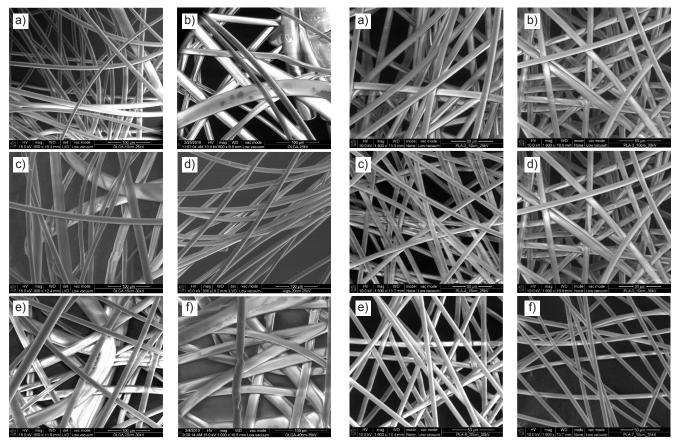


Figure 3. SEM microphotographs of melt-electrospun polypropylene fibers at the following processing parameters: working distance and spinning voltage a) 10 cm, 25 kV (magnification 800x), b) 15 cm, 25 kV (magnification 800x), c) 15 cm, 30 kV (magnification 800x), d) 20 cm, 25 kV (magnification 800x), e) 20 cm, 30 kV (magnification 800x), f) 20 cm, 35 kV (magnification 1000x).

Figure 4. SEM microphotographs of melt-electrospun polylactide fibers at the following processing parameters: working distance and spinning voltage: a) 10 cm, 25 kV (magnification 1600x), b) 10 cm, 30 kV (magnification 1600x), c) 15 cm, 25 kV (magnification 1500x), d) 15 cm, 30 kV (magnification 1500x), e) 20 cm, 30 kV (magnification 1600x), f) 15 cm, 35 kV (magnification 1500x).

Determination of fibre diameter

PP melt-electrospinning tests were carried out at a working distance of 10 cm and spinning voltage of 25 kV only (the collector was too close to the extruder to apply a higher voltage). Tests were also carried out at a working distance of 20 cm and voltage of 35 kV only.

An experiment of PLA melt-electrospinning was carried out at an applied voltage of 35 kV only for a working distance of 15 cm. Due to the disappearance of the Taylor cone at a working distance of 20cm, it was not possible to do tests at a spinning voltage lower than 30 kV.

The experiment to design PP or PLA structures at a spinning voltage less than 25 kV did not succeed as the voltage was too low for successful spinning. Meltelectrospun structures of PP were characterised by the lowest distribution of fibre diameters in the fibrous structures. The highest standard deviation ($\pm 14.33 \, \mu m$) was found for a working distance of 15 cm and spinning voltage of 30kV,

whereas the lowest ($\pm 1.44~\mu m$) was discovered at a working distance of 20 cm and spinning voltage of 25kV. Moreover, it was noted that the highest average fibre diameter of 23.17 μm was obtained for the following processing parameters: working distance – 15 cm and spinning voltage – 30 kV, while the lowest – 11.05 μm was attained at working distance – 10 cm and spinning voltage – 25kV.

PP structures prepared at a working distance of 15 cm are characterised by an increase in the average fibre diameter with a rise in the spinning voltage. When the spinning voltage was 25 kV, the resulting average fibre diameter amounted to 15.78 µm, whereas for a spinning voltage of 30kV the average diameter was 23.17 µm. The same phenomenon was observed for samples prepared at a working distance of 20 cm. In these cases, the average fibre diameter for a spinning voltage of 25 kV amounted to 11.46 µm, whereas for 30 kV it was determined as 16.38µm. The effect of processing parameters on fibre diameter is presented in *Figure 5*.

The results of the research lead to the conclusion that an optimal PP structure is attained at a working distance of 20 cm and spinning voltage of 25 kV (*Figure 5*).

The above-mentioned sample indicates the lowest average fibre diameter and the lowest standard deviation of the fibre diameter.

PLA melt-electrospun yielded fibrous structures characterised by an improved fibre diameter distribution as compared with samples made of polypropylene. The highest standard deviation were ±1.18 for a process carried out at a working distance of 15 cm and spinning voltage of 35 kV. The lowest standard deviation of the fibre diameter (±0.36 um) was determined for a working distance of 15 cm and spinning voltage of 25 kV. Moreover, the highest average fibre diameter - 8.41 µm was found at a working distance of 10 cm and spinning voltage of 20 kV. However, the lowest average fibre diameter (3.11 µm) occurred at a working distance of 15 cm and spinning voltage of 35 kV.

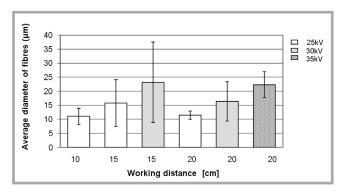


Figure 5. Effect of process parameters on the average fiber diameter of PP melt-electrospun 2D fibrous structures.

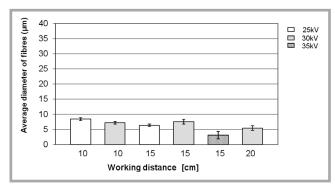


Figure 6. Effect of process parameters on the average fiber diameter of PLA melt-electrospun 2D fibrous structures.

When PLA samples were prepared at a working distance of 10 cm, the average fibre diameter decreased with an increase in the spinning voltage. In the case of a spinning voltage of 25 kV, the average fibre diameter amounted to 8.41 μ m, whereas the process carried out at a spinning voltage of 30 kV resulted in average fibre diameters of 7.20 μ m. The effect of process parameters on the fibres diameter is shown in *Figure 6*.

Preliminary research of the effect of the processing parameters of melt electrospinning on PLA structures shows that an optimal structure was obtained for a working distance of 20 cm and spinning voltage of 25 kV (*Figure 6*). This sample shows the lowest average fibre diameter and the lowest standard deviation of the fibre diameter.

Analysing the results obtained, it should be stated that although the process of electrospinning runs without disturbances, the fibre diameters obtained are not satisfactory, especially those of PP fibres. Research will be continued using PP and PLA of lower molecular weight and/or higher melt flow index.

Conclusions

The preliminary research presented showed that an increase in the spinning voltage and working distances caused an increase in the PP fibre diameter. The application of PLA for melt-electrospinning resulted in a reduction in PLA fibre diameter when the spinning voltage and working distance were increased.

The best results of PP fibrous structures occurred when the following processing parameters were used: a working distance of 20 cm and spinning voltage of 25 kV, whereas optimal preliminary PLA

fibrous structures were obtained for a working distance of 15 cm and spinning voltage of 35 kV.

A higher reproductivity of the fibre diameter was obtained for PLA fibrous structures, which was technologically connected with the higher melt flow index (15 g/10 min at 230°C/2.16kg in comparison to PP). Potentially, PP has a lower density (0.89 g/cm³), showing more reproductive results than PLA (density of 1.24 g/cm³). However, differences in the melt flow index between both polymers were significant, having a significant influence on fibre diameters.

The PP or PLA fibres obtained in this research showed a higher diameter than that of submicro and nano fibres, as initially assumed. Taking into account the statements above, a continuation of the optimisation process is necessary for obtaining lower diameters of fibrous structures with significantly higher reproductivity.

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Multifilament Chitosan Yarn

The Institute of Bioploymers and Chemical Fibres is in possession of the know- how and equipment to start the production of continuous chitosan fibres on an extended lab scale. The Institute is highly experienced in the wet - spinning polysaccharides, especially chitosan. The Fibres from Natural Polymers department, run by Dr Dariusz Wawro, has elaborated proprietary environmentlyfriendly method of producing continuous chitosan fibres with bobbins wound on in a form suitable for textile processing and medical application.



Multifilament chitosan yarn

We are ready, in cooperation with our customers, to conduct investigations aimed at the preparation of staple and continuous chitosan fibres tailored to specific needs in preparing non-woven and knit fabrics.

We presently offer a number of chitosan yarns with a variety of mechanical properties, and with single filaments in the range of 3.0 to 6.0 dtex.

The fibres offer new potential uses in medical products like dressing, implants and cell growth media.