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Biodegradable and antimicrobial polycaprolactone nanofibers with and without silver precipitates

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ABSTRACT

Purpose: The purpose of the paper is to present the results of own researches, including the study of the structure and the properties of new obtained single- and double-component polycaprolactone polymer nanofibers as well as of composite nanofibers with and without silver precipitates produced by electrospinning including the results of biological research, proving the usefulness of the newly developed nano-engineering materials and their applicability in regenerative medicine, as well as tissue engineering.

Design/methodology/approach: On the basis of the data available from the fundamental literature and based on the criteria of potential and attractiveness, polycaprolactone was selected for research from among a number of polymer materials, using a method of procedural benchmarking and weighted scores. The obtained nanomaterials undergone the following examinations to confirm the assumed aim of the work: infrared spectroscopy FTIR, Wide-angle X-ray scattering (WAXS), BET, Langmuir specific surface area and DTF porosity assessed with the gas adsorption method, in a transmission electron microscope (TEM), a scanning electron microscope (SEM), a fluorescence microscope, antibacterialness and antifungalness investigations and examinations of biological properties in vitro.

Findings: The applicability of polymer fibers in medicine depends on biocompatibility and non-toxicity of the applied material, which is influenced by the chemical purity of the materials applied and the toxicity of the input solvents. The potential toxicity of nanofibers should therefore be eliminated, starting with selection of materials used for obtaining solutions. Many other factors fundamental for the quality and properties of polycaprolactone nanofibers need to be taken into account to create single- and double-component and composite nanofibers.

Practical implications: The obtained composite materials, due to their non-toxicity resulting from the components applied, including solvents, bacteriocidity and bioactivity, may find their applications in tissue engineering as membranes in controlled regeneration of bone tissue, as carriers of medicinal agents in bone surgery, as implantable surgical meshes and as scaffolds for a tissue culture. In turn, the composite core-shell nanofibers, by combining the antibacterial properties of the coating with bioactive properties of the core, are attractive materials for three-dimensional tissue scaffold.

Such materials can be used as a carrier of medicine, a treatment of hard healing wounds, invasive surgery, neurosurgery, as substrate for the culturing of a retina, material to reconstruct nerves and in dentistry or oncology, to replace the natural tissue removed because of a cancer with the possibility of applying a therapeutic agent, e.g., an antibiotic or a medicine used in cancer therapies, released after the dissolution of the coating of nanofibers.

Originality/value: The present paper is the original report from a personal own research and explains the concept and scope of own research of a new obtained single- and double-component polycaprolactone polymer nanofibers as well as of composite nanofibers produced by electrospinning for application in regenerative medicine, the presentation of technological conditions and methodology of own research into polymer nanofibers, and above all very detailed description of the results of own investigations

Keywords: Nanocomposites; Polymer nanofibers; Electrospinning; Biocompatibility; Non-toxicity; Regenerative medicine; tissue engineering

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MATERIALS

1. Introduction

A market potential analysis indicates clear development trends of the global medical product markets, and directly relevant are, most of all, development tendencies of the biomaterials market, medical bionic implant/artificial organs market, orthopaedic devices market, orthopaedic soft tissue repair market, orthopaedic trauma fixation devices market, orthopaedic soft tissue repair market, and 3D Printing in Medical Applications Market. The development of a complementary market should also be expected, associated with the popularisation of the concept and newly established technical and clinical solutions of hybrid implants featuring a porous zone, acting as scaffolds for the ingrowth of living tissues and hybrid engineering-biological implants, according to the authors' original concepts [1-6]. Implantable biomedical devices are currently aggregately considered to be medical bionic implants where bionics is understood as the fabrication and investigation of biological systems to create and implement artificial engineering systems which can restore the lost functions of biological systems [7]. In general, medical bionic implants encompass numerous solutions eliminating various dysfunctions of a human organism, among others orthopaedic prostheses (bone grafts, bone plates, fins and connecting and stabilising devices, including screws applied in the area of ankles, knees and hands, bars and pins for stabilising fractured limbs), screws and plates in skull-jaw-face reconstructions, dental implants, and also scaffolds of bones and tissues in tissue engineering [7].

The undertaken foresight research shows that the global the medical bionic implant and artificial organs market is a potentially growing one with a global market of USD 12.67 billion in 2012 as it is expected to grow at a CAGR of 7.1% to reach USD 17.82 billion in 2017 [8], and the market of tissue engineering and regenerative medicine will grow in the USA only from USD 6.9 billion in 2009 to USD 32 billion in 2018 [9]. This constitutes a real and tangible reason for the intensification of research and implementation works, which inevitably leads to higher industrial spendings for investments associated with know-how acquisition and expansion of manufacturing capacities in this field. The current methods of organ and tissue replacement employ primarily autographs, allografts or metal devices or such made of other engineering materials [10]. The problem of organ transplantation proves how important are activities consisting of the creation of state-of-the-art biomaterials and how much there is still to do in this area. The necessity to perform another operation, with such an operation being a discomfort for patients and an additional burden for health service finances, will be avoided by replacing non-biodegradable implants with materials undergoing complete resorption. Tissue scaffolds have been intensively developed for several years apart from transplantable implants, and in fact instead of them. Very high demand for various types of organs, a low number of donors, the necessity to take medicines after implantation, have become a driving force for tissue engineering, allowing to fabricate organs without risking rejection after transplantation. Tissue engineering seeks

innovative methods of restoring a natural tissue and provides an alternative solution to the currently used conventional methods [11]. Tissue engineering, as a field of technical sciences using medical knowledge and materials engineering methods [12,13], has been involved in construction and fabrication of scaffolds, maintaining the developing tissues, in manipulation of somatic and stem cells, in influencing the tissues growth conditions and their structure and in maintaining the physiochemical conditions of the environment supporting this growth, in order to produce functional substitutes of damaged tissues or entire organs.

Polymer materials are used in many prostheses such as percutaneous transluminal coronary angioplasty (PTCA) catheters, heart valves, contact lenses, intraocular lenses and for constructing rigid bases for dental prosthesis and dentures, in ophthalmic applications, and in urology and gastroenterology. The content of polymers, including nanofibres made of biodegradable polymers, as skin and soft tissue scaffolds, will grow fastest between 2013 and 2019. The majority of current works is focussed on designing and fabricating a scaffold using various fabrication technologies, including also materials of natural origin, such as: chitosan (a derivative of chitin), collagen or elastin, as well as of synthetic origin, such as PCL (polycaprolactone), PLA (polylactide), PEO (poly(ethylene oxide). The polymers mentioned, after implantation into a recipient's body, are subject to degradation to products easily removed by a human organism, in particular in the citric acid cycle called the Krebs cycle [14]. Methods are commonly applied in tissue engineering, in which three-dimensional engineering constructions are employed permitting ex vivo tissue transplantation, injection or implantation for the initiation of stem cells regeneration. Opposite to pure therapies, in which stem cells are injected directly into peripheral circulation or located in particular tissues, in numerous clinical cases it is necessary to use stem cells carriers to transport them and scaffolds for three-dimensional grouping in a particular place of an organism. A treatment strategy developed these days includes tissue engineering methods, respectively, scaffold-based vascularised bone tissue engineering (SBV BTE), Vascular Tissue Engineering (VTE) or scaffold-based tissue engineering (SBTE). Promising outcomes are achieved by the application of tissue scaffolds in combination with autologous bone marrow stem cells and growth factors (mainly BMP-2).

Special focus is laid in this work on the possible use of polymer nanofibers and composite materials, in which they can be used, as scaffolds in tissue engineering.

2. Methodological approach

The primary aim of this work is to investigate the properties of the obtained single-component and double-component and composite nanofibers in terms of their applicability as scaffolds in tissue engineering. The preliminary results were presented in a few own earlier papers, patents and also as lectures on scientific conferences around the word [15-27]. A research thesis was proposed concerning the possibility of obtaining composite nanofibers with a bactericidal coating and a bioactive core for tissues scaffolds for application in tissue engineering. Tissue engineering inscribes itself in the area of regenerative medicine and seeks innovative methods of restoring a natural tissue and provides an alternative solution to the currently used conventional treatment methods.

It is the goal of this work to fabricate completely bioresorbable tissue scaffolds using polymer nanofibers which, owing to their properties, support natural regeneration processes of a natural tissue and temporarily substitute its function, by undergoing gradual degradation until recovering its full functionality.

Table 1.
Potential and attractiveness criteria (own study)

Criterion	Objective values (potential)	Weight
K_P1	Tensile strength of material	0.2
K_P2	Stability of shapes achieved	0.1
K_P3	Enzymatic or hydrolytic bioresorbability	0.3
K_P4	Material transformability into a fibre from solution (fibre-forming ability)	0.2
K_P5	Applicability of non-toxic solvents	0.2
	Subjective values (attractiveness)	
K_A1	Material applicability as carrier of medicinal substances	0.25
K_A2	Number of publications concerning the material (acc. to Science database)	0.1
K_A3	Composite material manufacturing cost	0.15
K_A4	Potential range of application at industrial scale	0.25
K_A5	Availability of material acquisition sources	0.2

It is not a sufficient condition, however, to restore the structure of a natural tissue, as it is required from a material from which scaffolds are made, to be nontoxic, depending, in particular, on the solvents used for conversion into nanofibers. If toxic solvents are used, there is a risk that they are transmitted into an organism, even if only in residual content. A solvent, in the entire period of degradation, may adversely affect the surrounding cells, which may lead to changes at the molecular level of cells, therefore poses a risk of carcinogenesis. For this reason, special emphasis was laid on the necessity to use non-toxic materials only, including solvents, which, even if they enter an organism as a result of polymer degradation, will be metabolised to final compounds such as water and carbon dioxide and will not create a risk of carcinogenesis. Many more factors fundamental for the quality and properties of polymer nanofibers need to be taken into account to create double-component nanofibers.

The above-mentioned polymer materials, on the basis of the data available obtained from the fundamental literature, based on the criteria of potential and attractiveness (Table 1), were subjected to an analysis using a method of procedural benchmarking and weighted scores [28,29]. The details on results of analysis are given in the own work [4,17-20].

The relevant potential and attractiveness criteria were ascribed to the above polymers, and summary of results of analysis of these polymer materials after multiplying the particular criteria by weight were presented in Table 2 and graphically in Figure 1.

Table 2.
Summary of results of analysis of relevant polymer materials

	PLA	PCL	CHITO-SAN	PA6	KH	PMMA	PP	PVA
SYMBOL	A	B	C	D	E	F	G	H
POTEN-TIAL	6.9	8.2	5.7	5.7	8	5.6	4.1	7
ATTRA-CTIVE-NESS	7.05	8.4	7.55	3.4	6.8	3.7	4	6.6

The following polymer materials have the highest level of attractiveness and potential: polylactides (A), polycaprolactone (B), chitosan (C), hyaluronic acid (E) and polyvinyl alcohol (H) found in the most promising quarter of the matrix (wide-stretching oak). For the materials listed, polycaprolactone (B) has revealed the greatest attractiveness and potential, while chitosan (C) has proved to be another polymer with the highest potential (C). Hyaluronic acid (E) proved to be most attractive in the study and for this reason such materials (B, C and E) were selected for research as the basic materials. The key advantages and disadvantages of the selected polymer materials (PCL, chitosan, hyaluronic acid) applied in medicine (Table 3) are listed according to the literature data.

The following was used in order to investigate the influence of fabrication conditions and properties of solutions on the structure and properties of nanofibers: PCL (polycaprolactone) with M_w = 70,000-90,000 g/mol and M_w = 45,000 g/mol by Sigma Aldrich and the chemical reagents: 99.95% acetic acid, 99.95% formic acid, 99.95% dimethyl sulfoxide (DMSO) by Sigma Aldrich, 99.95% tetrahydrofuran (THF), methanol and chloroform by Chemland. The molecular mass of polymers was examined by means of gel permeation chromatography (GPC).

Polymer solutions with the content of 2-10%, containing polycaprolactone without additives, was prepared using polycaprolactone (PCL) with the molecular mass of M_w = 70,000-90,000 g/mol in a mixture of hydrochloric and formic acid solvents with a mass ratio of (70:30). For this purpose, the weighed polymer material was introduced into the prepared mixture of solvents and dissolved for 12 hrs using a magnetic stirrer by Chemland.

The following was prepared with polycaprolactone (PCL) with the molecular mass of M_w = 70,000-90,000 g/mol:

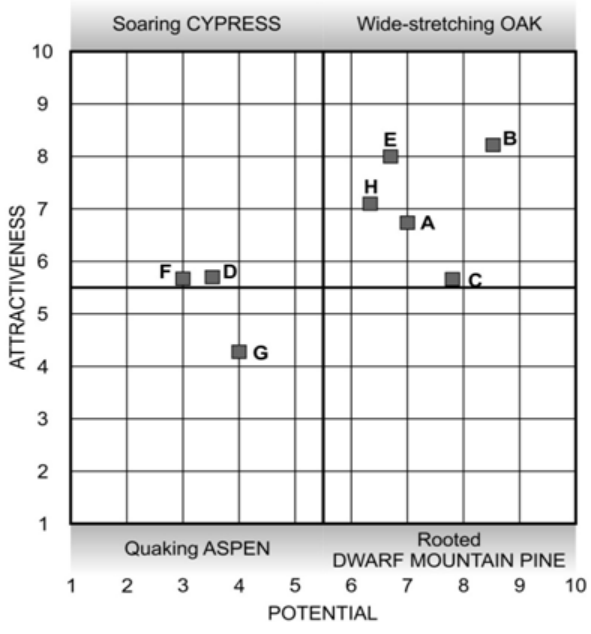

Fig. 1. Graphical representation of selected polymers' potential and attractiveness

Table 3.
Key advantages and disadvantages of the selected polymer materials

No.	Polycaprolactone	Chitosan	Hyaluronic acid
Key advantages	- high strength - high biocompatibility - biodegradable material - material with very good electrospinning properties - synthetic material, ability to achieve high material purity	- bactericidal material - hydrophilic material - material with antioxidation properties - very good adhesion with cells - material coming from renewable sources	- high biocompatibility - participates in all the phases of wound healing - activates the RHAMM receptor which contributes to enhanced mobility of cells - biopolymer present in majority of living organisms
Key disadvantages	- poor adhesion to cells due to hydrophobic surface - toxic solvents	- solutions possess high viscosity - very difficult to transform into nanofiber - limited number of solvents	- solutions possess high viscosity - difficult to transform into nanofiber - limited number of solvents

a) a polymer solution with the fraction of 10% in a mixture of chloroform and methanol with a mass ratio of (70:30),
b) a polymer solution with the fraction of 10% in a mixture of tetrahydrofuran and dimethyl sulfoxide with a mass ratio of (70:30),
c) a polymer solution with the fraction of 10% in a mixture of hydrochloric and formic acid with a mass ratio of (70:30).

A dependency 1 for calculating the necessary fraction of polymer materials with the accuracy of 0.01 g and of solvents measured according to their density was used for preparing the solutions. Dissolving was carried out in polypropylene or glass containers with the volume of 125 ml with a cap.

$$C_p = \frac{m_s}{m_r} \cdot 100\% \quad (1)$$

where:
C_p – fraction, %,
m_s – mass of the dissolved substance, g,
m_r – mass of the solution, g.

The polymer solution, from which fibers were obtained, was selected in the investigations with the method of weighted points with the potential and attractiveness criteria used (Table 4).

The statement of analysis results of particular PCL solutions after multiplying the particular criteria by weight were presented in Table 5 and graphically in Figure 2. A PCL solution obtained from a mixture of formic acid and hydrochloric acid at a rate of 70:30 m/m (A), ranked in the most promising quarter of the matrix (wide-stretching oak), has the highest level of attractiveness and potential (Fig. 2). The other solutions (B) and (C) were rejected from further investigations due to the properties obtained.

Table 4.
Potential and attractiveness criteria

Criterion	Objective values (potential)	Weight
K_P1	conductivity of 10% of solutions: high – 10 points low – 1 point	0.20
K_P2	viscosity of 10% of solutions: high – 10 points low – 1 point	0.20
K_P3	diameter of fibers achieved: diameter of 51% of fibers: below 500 nm – 10 points above 500 nm – 1 point	0.15
K_P4	presence of defects: high – 10 points high content of existing defects – 1 point	0.30
K_P5	process stability (electrospinning ability): very good – 10 points insufficient – 1 point	0.15
	Subjective values (attractiveness)	
K_A1	toxicity of mixture of solvents: non-toxic –10 points carcinogenic – 1 point	0.25
K_A2	evaporation rate of mixture of solvents used: high – 10 points low – 1 point	0.25
K_A3	dissolution rate of polymer by the mixture of solvents used: to 1h at room temp. – 10 points to 10h at room temp. – 1 point	0.10
K_A4	potential range of application at industrial scale: high – 10 points low – 1 point	0.20
K_A5	availability of solvents: high – 10 points low – 1 point	0.20

Table 5.
Statement of analysis results of particular PCL solutions

Size	Solution obtained from mixture (70:30) of		
	hydrochloric acid and formic acid	tetrahydrofuran and dimethyl sulfoxide	chloroform and methanol
SYMBOL	A	B	C
POTENTIAL	7.9	5.3	3.5
ATTRACTIVENESS	7.55	3.7	4.6

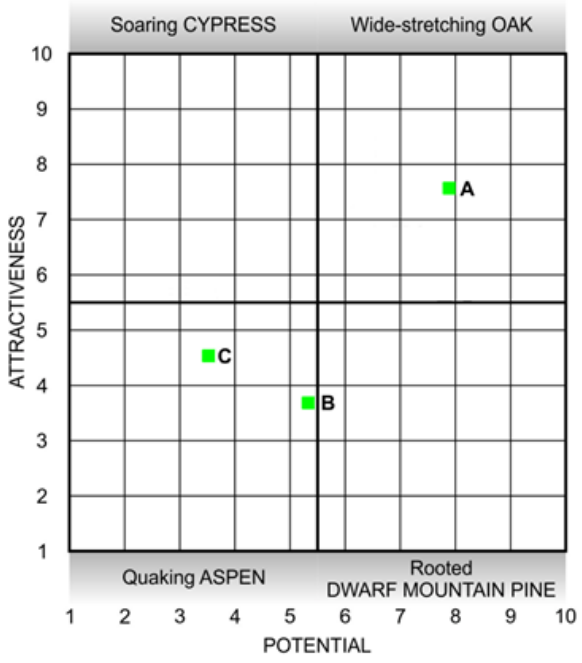

Fig. 2. Graphical representation of selected PCL polymers solutions' potential and attractiveness

The Electro–Hydrodynamic Atomization 2.2D – 500 device by Yflow Nanotechnology Solutions equipped with a working chamber, control panel, infusion pumps with flow adjustment for µl/min and ml/h, two systems maintaining solution temperature and nozzles for standard and coaxial electrospinning was used in order to transform the solutions obtained into fibers. After dissolving, the solutions were placed in containers of the Yflow Nanotechnology Solutions device, where a flow rate and solution temperature was controlled and then it was subjected to the activity of an electrostatic field converting the solutions into differently structured fibers.

The nanofibers were fabricated during electrospinning onto a flat collector dimensioned 40x40 cm or onto

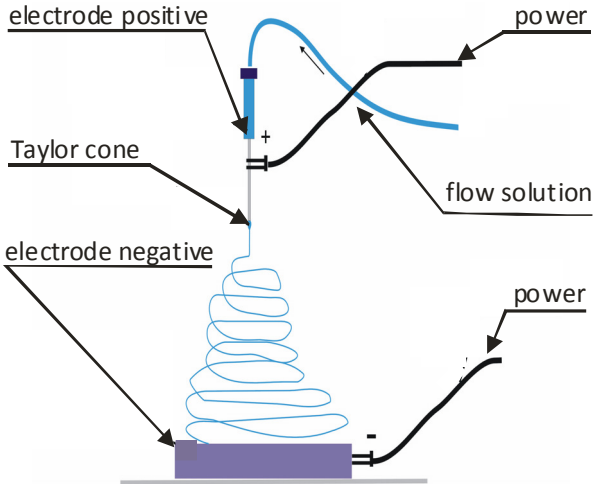

Fig. 3. Nanofibers electrospinning schematic

a rotating collector with the width of 40 cm and diameter of 20 cm each time for 0.25-12 h (Fig. 3).

The solutions obtained were subjected to the activity of an electrostatic field in the conditions described in Table 6, by converting the solutions obtained into differently structured fibers.

The properties and application possibilities of polymer nanofibers can be improved prior to their surface treatment by introducing additives into polymer solutions providing bioactive micro-biological properties, also into a solution with the fraction of 10% of polycaprolactone (PCL) with the molecular mass of M_w = 70,000-90,000 g/mol in a mixture of hydrochloric acid and formic acid with a mass ratio of (70:30); macromolecular chitosan with M_w = 100,000-300,000 g/mol, silver nitrate $AgNO_3$ or AlphaSan was, respectively, introduced with the fraction of 1-5%. In order to prepare such solutions, the weighed polymer materials and additives were introduced into the prepared mixture of solvents and dissolved for 12 hrs in polypropylene or glass containers with the volume of 125 ml with a cap. The polymer solutions containing silver nitrate $AgNO_3$ or AlphaSan, prior to adding polycaprolactone, underwent sonification using a Labindex homogeniser for 5 minutes, and were left for 12 hours after adding PCL to dissolve.

Core-shell composite nanofibers were also fabricated by a technique of co-axial electrospinning. A special type of a co-axial jet enabling the flow of two polymer solutions needs to be applied to obtain the core-coating nanofibers in an electrostatic field. The 'core in core' is the dominant type, in which an internal jet can be distinguished, responsible for the flow of the solution forming the core of nanofibers consisting of two parts, and a surrounding external

Table 6.
Conditions of obtaining single-component PCL, double-component and composite nanofibers of the core-coating type

Fiber type		Single-component	Double-component	Core-coating
Type of nozzle applied		standard nozzle		co-axial jet
Process type		single-stream process		co-axial process
Solution flow rate, ml/h	of core	1.00		0.04-0.50
	of coating	not applicable		0.3-1.00
Type of collector applied		flat 40x40 cm; rotating with diameter of 20 cm and length of 40 cm		flat 40x40 cm
Collector rotational speed, rev./min		200, 500, 600, 1400	500	not applicable
Electrostatic voltage, kV/cm		0.95-1.00	0.95-1.63	0.95-1.20
Solution temperature, °C		25		
Gas temperature in working chamber, °C		23		
Gas humidity in working chamber, %		20-30		

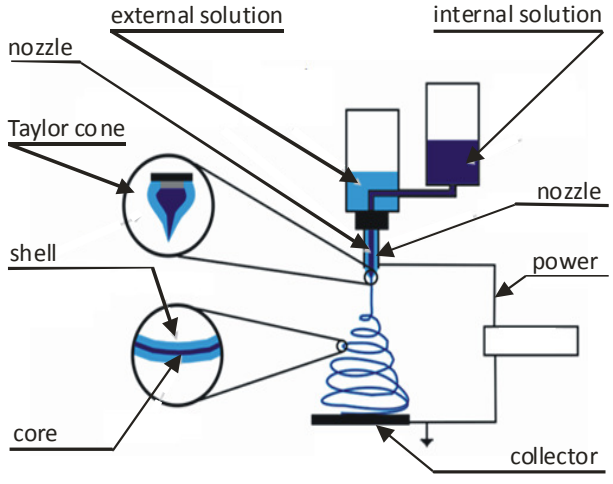

Fig. 4. Simplified co-axial electrospinning

jet, responsible for formation of a coating surrounding the core from each side (Fig. 4).

The shell was prepared with a polycaprolactone solution with the molecular mass of M_w = 70,000-90,000 g/mol with a 3% additive of silver nitrate and with a solvent of hydrochloric acid and formic acid, whereas the core was made of a polycaprolactone solution with addition of 5% of low molecular hyaluronic acid in the conditions described in Table 6. Co-axial jets were used to obtain core-shell nanofibers in an electrostatic field enabling the flow of two 'jet in jet' polymer solutions, in which an inner jet can be distinguished, responsible for the flow of a solution forming the core of nanofibers consisting of two parts and a surrounding external jet responsible for creating a shell surrounding the core from each side.

This work presents the results of own research concerning the structure and properties of polymer nanofibers from those selected as described above with the intention to apply them for medical purposes. The nanomaterials obtained underwent the following examinations to confirm the assumed aim of the work: infrared spectroscopy FTIR, Wide-angle X-ray scattering (WAXS), BET, Langmuir specific surface area and DTF porosity assessed with the gas adsorption method, in transmission electron microscope (TEM), scanning electron microscope (SEM), fluorescence microscope, antibacterialness and antifungalness investigations and examinations of biological properties in vitro. The details of the made research are presented in another own publications [1,15-20].

3. The results of own investigations

The outcomes of own investigations of single- and double-component polymer nanofibers as well as of composite nanofibers are presented.

When the fraction of caprolactone is increased in a solution with three mixtures of solvents, this has increased solution viscosity (Fig. 5). The highest increase in viscosity

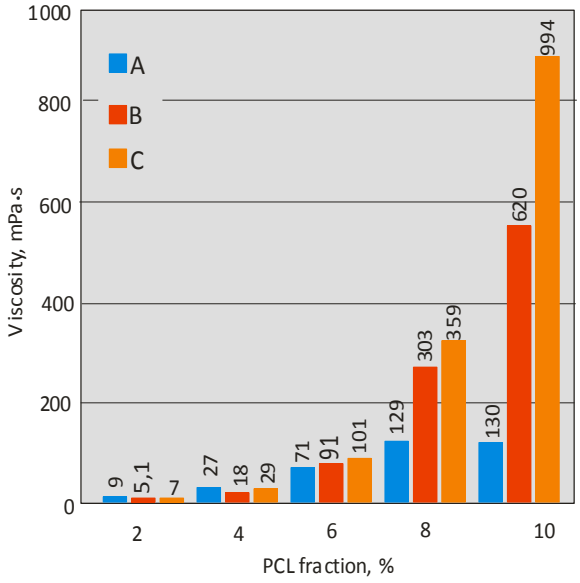

Fig. 5. Change of viscosity of polycaprolactone solution with fraction of 2-10% dissolved in mixture of hydrochloric acid and formic acid (A), chloroform and methanol (B) and a mixture of tetrahydrofuran and dimethyl sulfoxide (C)

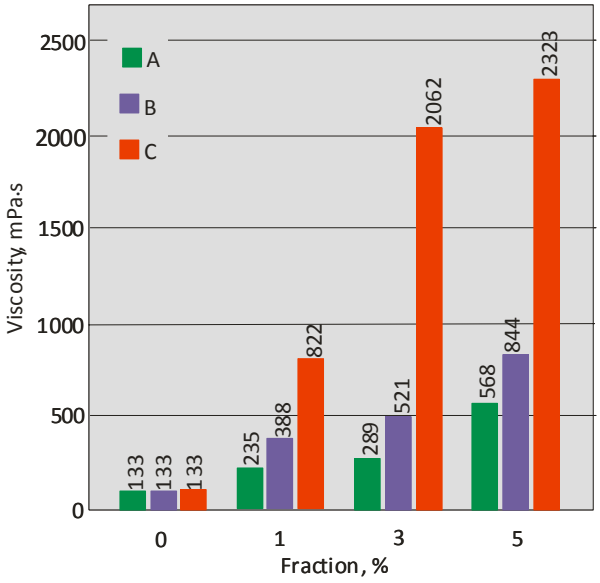

Fig. 6. Change of dynamic viscosity of polycaprolactone solutions (M_w = 70,000-90,000 g/mol[1]) with the fraction of 9.5-10%, obtained by dissolving PCL granulate in the mixture of formic acid and hydrochloric acid (mass ratio of 70:30) and by adding 0-5% of $AgNO_3$ (A), AlphaSan (B), chitosan (C)

is characteristic for a solution in the mixture of tetrahydrofuran and dimethyl sulfoxide from 7 to 933 mPa·s, and a much smaller increase occurs in the case of a mixture of hydrochloric acid and formic acid from 9 to 133 mPa·s.

The viscosity of the solutions obtained is also changed by introducing the additives of chitosan, AlphaSan and silver nitrate into the initial polycaprolactone solution. If the fraction of the abovementioned additives is increased, viscosity is increased from 133 mPa·s for a solution not containing additives to, respectively, up to 568 mPa·s, 844 mPa·s and to 2323 mPa·s for solutions containing 5% of silver nitrate, 5% of AlphaSan, and 5% chitosan (Fig. 6).

The electric conductivity of the solutions is also changed (Fig. 7), which decreases as the fraction of polycaprolactone, being an isolator, is rising. The initial conductivity is decreased by 66.6% from 4.5 to 1.6 µS/cm in case of PCL solution in a mixture of chloroform and methanol with the fraction of, respectively, 2 and 10% of PCL. The smallest reduction is seen for a mixture of hydrochloric acid and formic acid, the conductivity of solutions falls by 30.2% of the initial value of 43 to 30 µS/cm, respectively, with the fraction of PCL of 10 and 2%. The influence of silver nitrate additives, AlphaSan and chitosan on electrical conductivity of polycaprolactone solutions is diverse.

The electric conductivity of the solutions is increased after adding chitosan from 28 µS/cm in case of polycaprolactone not containing chitosan, to 366 µS/cm with the fraction of 5%, and silver nitrate to 840 µS/cm with the fraction of 5%, whereas it is decreased – for solutions containing AlphaSan, to 23 µS/cm for fraction of 5% (Fig. 8), which is the lowest electric conductivity for all the studied solutions.

The solvents used, with a varied evaporation rate, influence the obtainment of differently structured fibers. Solvents with high volatility support the creation of fibers with large diameter and disadvantageously do not support the creation of nanofibers. The slowly evaporating solvents influence the creation of nanofibers. The diameter and geometrical properties of polymer fibers obtained in an electrostatic field, deposited onto the surface of a flat collector, are therefore largely dependent on the mixtures of solvents employed: tetrahydrofuran and dimethyl sulfoxide; chloroform and methanol; and hydrochloric acid

[1] The molecular mass of polycaprolactone according to manufacturers' guidelines is given in the text of, respectively, M_w = 45,000 g/mol and M_w = 70,000-90,000 g/mol. Own investigations of the molecular mass of polymers used by means of gel permeation chromatography (GPC) show differences in the molecular mass of the tested polymer materials given by the manufacturer and are, respectively, M_w = 38,107 g/mol for the first polymer and M_w = 100,720 g/mol for the second polymer.

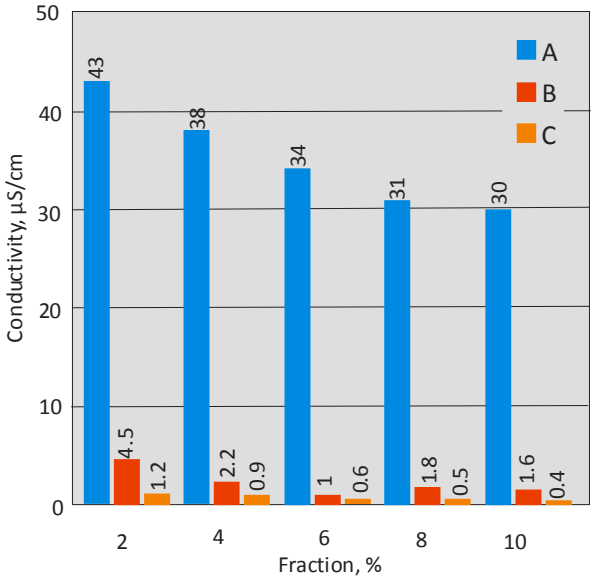

Fig. 7. Change of electric conductivity of polycaprolactone solution with fraction of 2-10% dissolved in mixture of hydrochloric acid and formic acid (A), chloroform and methanol (B) and a mixture of tetrahydrofuran and dimethyl sulfoxide (C)

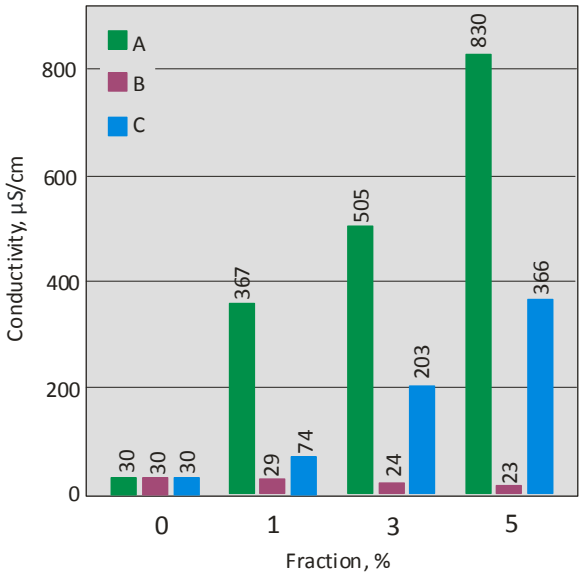

Fig. 8. Change of electric conductivity of polycaprolactone solutions (M_w = 70,000-90,000 g/mol) with the fraction of 9.5-10%, obtained by dissolving PCL granulate in the mixture of formic acid and hydrochloric acid (mass ratio of 70:30) and by adding 0-5% of $AgNO_3$ (A), AlphaSan (B), chitosan (C)

and formic acid (Fig. 9). 76% of the nanofibers obtained using a mixture of chloroform and methanol exhibit the diameter of 1.0-2.1 µm, while the remaining 24% are nanofibers, and the thinnest ones are nanofibers with the diameter of 500-600 nm, as indicated by the results of SEM investigations. Fibers with a high irregularity of diameters are formed by employing this mixture of solvents and numerous surface defects occur, the fibers are glued; the similar geometric features are exhibited by fibers obtained from a mixture of solvents of tetrahydrofuran and dimethyl sulfoxide (Fig. 9). 68% are microfibers with the diameter of 1.0-2.5 µm, whilst the remaining 32% are nanofibers, and the thinnest ones are nanofibers with the diameter of 400-500 nm. The fibers obtained using tetrahydrofuran and dimethyl sulfoxide are also characterised by gluing

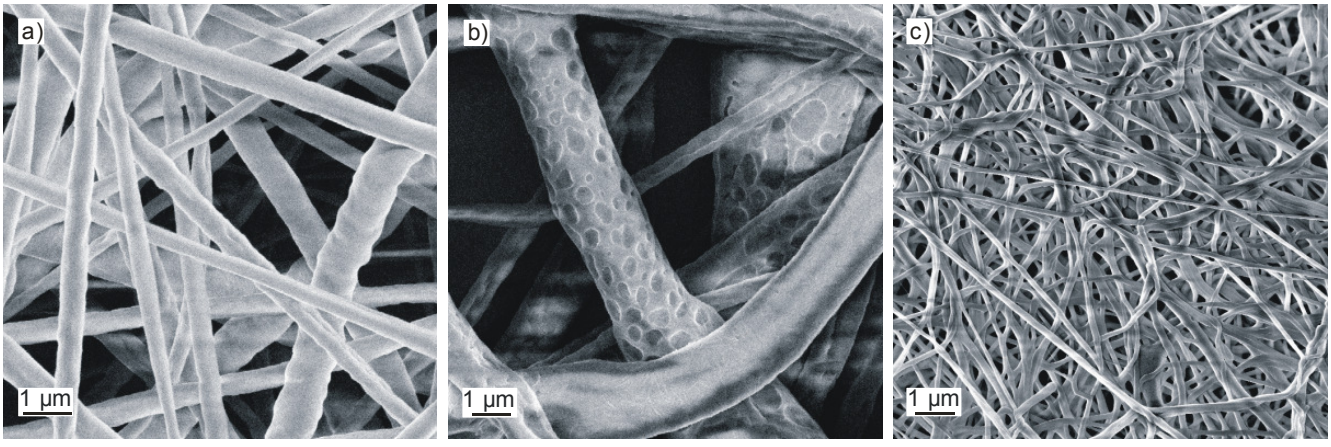

Fig. 9. Comparison of influence of solvents' properties on the structure of the obtained PCL fibers from the following solutions; a) mixture of formic acid and hydrochloric acid at a rate of 70:30; b) mixture of tetrahydrofuran and dimethyl sulfoxide at a rate of 70:30, c) mixture of chloroform and methanol at a rate of 70:30

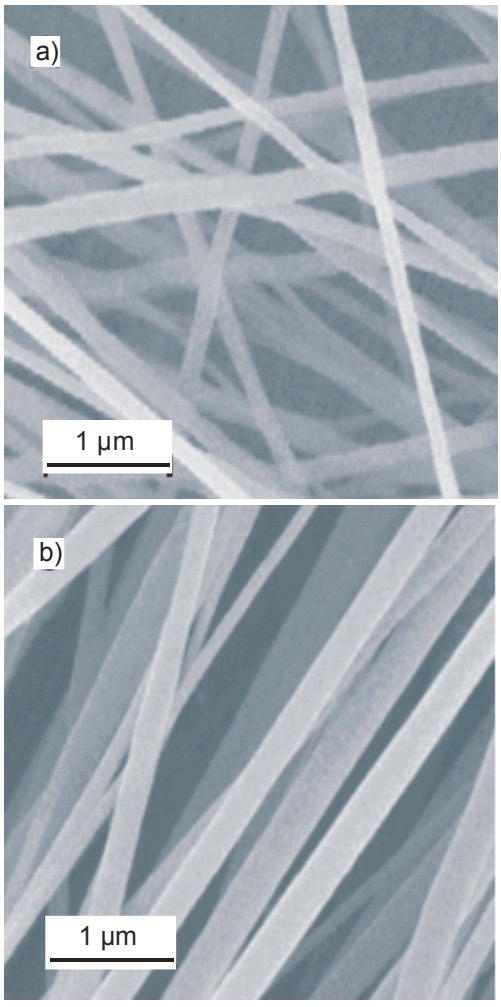

Fig. 10. Influence of rotational speed of collector on spatial arrangement of fibers deposited with the rotation speed: a) 200 rev./min, b) 1400 rev./min

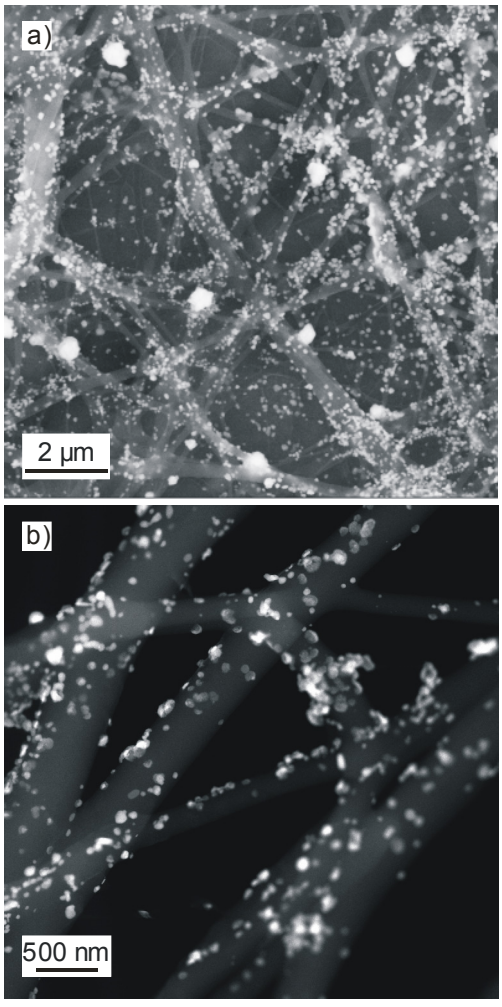

Fig. 11. Geometrical characteristics of double-component fibers obtained by dissolving PCL granulate using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with addition of $AgNO_3$: a) 3%, b) 25%; photographs taken after precipitation of silver in 2% ascorbic acid solution; a) SEM, b) TEM (HAADF)

tendency and are distinctive for their high geometrical irregularity of the diameter and surface. Completely different geometrical characteristics are exhibited by fibers achieved from a polycaprolactone solution using a mixture of formic acid and hydrochloric acid (Fig. 9), as nanofibers with the diameter of 1-500 nm occur then only, and the thinnest ones are nanofibers with the diameter of 1-100 nm, and, among them, a fraction of ultrathin nanofibers with the diameter of 20-30 nm, not existing in other cases. The nanofibers obtained are characterised by a smaller gluing tendency as compared to other samples, and – due to the properties of the solvents used – show much smaller toxicity.

The type of the collector used (rotating or flat) and rotational speed (for rotating collectors) is highly influencing the geometrical characteristics and isotropy of nanofibers obtained from a 10% polycaprolactone solution in a mixture of hydrochloric acid and formic acid. Fibers deposited onto a flat collector are characterised by isotropy, whereas the fibers deposited onto a rotating collector are anisotropic. The deposition of fibers onto the surface of a rotating collector is highly influencing their spatial arrangement (Fig. 10). The diameter of the obtained fibers depends on the rotation speed of the collector. In case of fibers deposited onto a rotating collector with the rotation speed of 200 rev./min, 72% of all nanofibers are those with the diameter of 100-200 nm, and 68% for rotational speed of

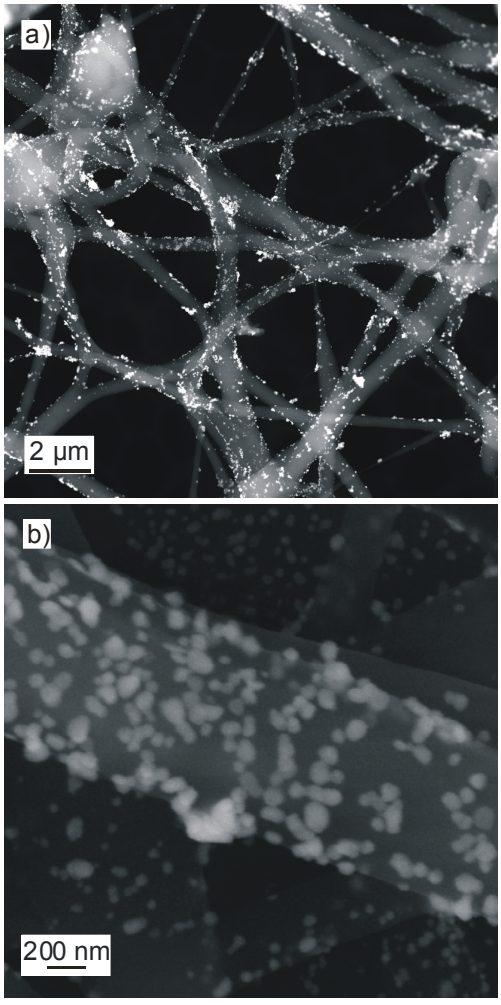

Fig. 12. Geometrical characteristics of double-component fibers obtained by dissolving PCL granulate with the molecular mass of M_w = 70,000-90,000 g/mol using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with addition of 25% of $AgNO_3$; photographs taken after precipitation of silver in 2% ascorbic acid solution; a) TEM (HAADF), c) SEM

600 rev./min. The biggest differences in the diameter of the fibers obtained with the diameter of 1 to 1000 nm occur for rotational speed of 1400 rev./min, although nanofibers with the diameter of 100-200 nm account for 50%. Fibers' anisotropy is increased as the rotational speed of the collector is increased. Opposite to the fibers obtained with a flat collector, there is no gluing tendency of fibers in case of employing a rotation collector.

The molecular mass of polymers is also considerably influencing the diameter of micro- and nanofibers obtained from a 10% polycaprolactone solution in a mixture

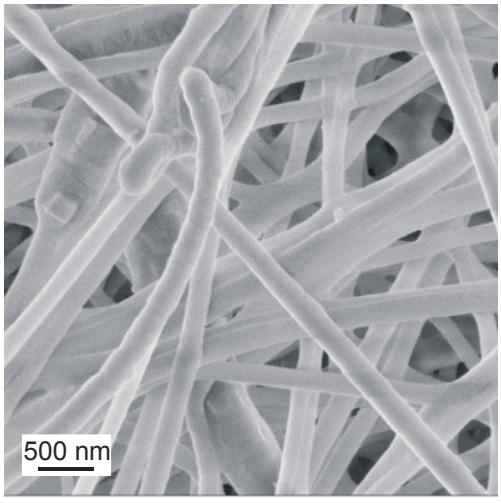

Fig. 13. Geometrical characteristics of double-component fibers obtained by dissolving PCL granulate with the molecular mass of M_w = 70,000-90,000 g/mol using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with AlphaSan addition with fraction of 5%; SEM

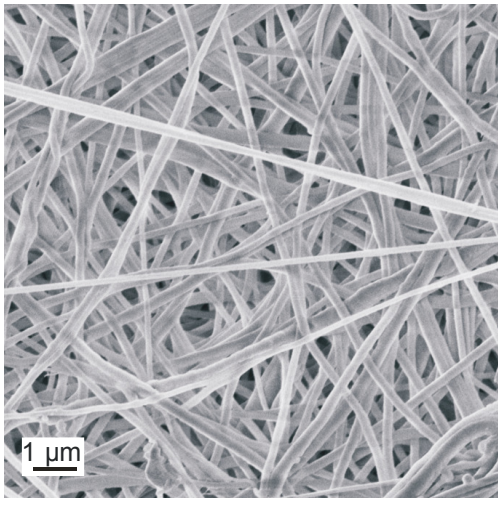

Fig. 14. Geometrical characteristics of double-component fibers obtained by dissolving PCL granulate with the molecular mass of M_w = 70,000-90,000 g/mol using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with addition of chitosan with fraction of 5%; photographs taken with SEM technique in InLens mode

of formic acid and hydrochloric acid. In case of using a PCL polymer material with smaller molecular mass M_w = 45,000 g/mol, larger differences exist in the diameter of the fibers obtained in the range of 1 to 800 nm as

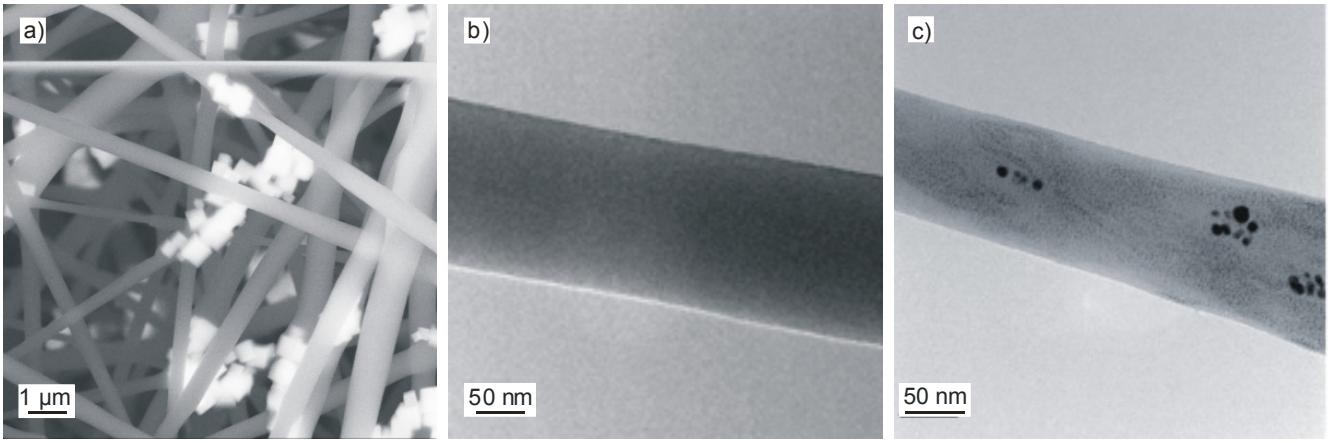

Fig. 15. Geometrical characteristics of double-component fibers obtained by dissolving PCL granulate with the molecular mass of M_w = 70,000-90,000 g/mol using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with addition of: a) 5% of AlphaSan b) 1% of chitosan, c) 1% $AgNO_3$, after precipitation of silver in 2% ascorbic acid solution; photographs with: a) SEM; b) and c) TEM

compared to the use of a PCL polymer material with higher molecular mass of M_w = 70,000-90,000 g/mol, when fibers with the diameter of 1 to 500 nm are formed.

By introducing the additives of silver nitrate, AlphaSan and chitosan into the solutions, the diameter of the fibers produced therein is influenced (Fig. 11 to Fig. 15). If an additive of 1-5% of chitosan is introduced, the dominant diameter of nanofibers is 200-300 nm. The diameter of fibers is increased by increasing the fraction of chitosan, but it also causes the existence of ultrathin nanofibers with the diameter smaller than 50 nm (Fig. 14). Silver particles with the diameter of 20 nm exist on the surface of fibers in case of double-component fibers containing an additive of silver nitrate (Fig. 11 and Fig. 12).

The tests of porosity and of the BET, Langmuir specific surface area and of porosity with the DTF method with the nitrogen adsorption method indicate that the largest specific surface area of 8.6 m^2/g is seen for PCL fibers fabricated with a mixture of hydrochloric acid and formic acid (Fig. 16 to Fig. 18). The specific surface area of fibers obtained using a mixture of tetrahydrofuran and dimethyl sulfoxide is 3.1 m^2/g. The smallest specific surface area of 0.96 m^2/g is seen for the fibers obtained with a mixture of chloroform and methanol, and the width of pores is within the range of 1.5-3.4 nm. In case of the fibers obtained using a mixture of formic acid and hydrochloric, their specific surface area of approx. 0.8 m^2/g is largest for pores with the diameter of 1.6 nm.

In case of fibers obtained using a mixture of tetrahydrofuran and dimethyl sulfoxide, the diameter of pores of 1.6 nm corresponds to the specific surface area of

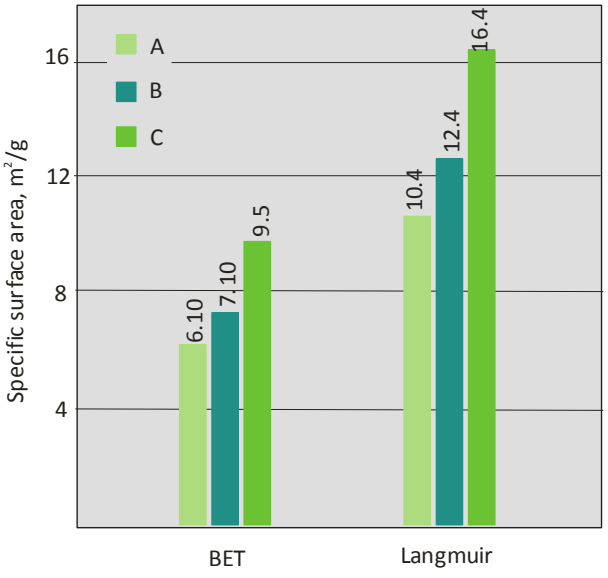

Fig. 16. BET and Langmuir specific surface area of PCL fibers with the molecular mass of M_w = 70,000-90,000 g/mol obtained from 10% mixture of formic acid and hydrochloric acid with mass ratio of 70:30 with addition of $AgNO_3$ with fraction of: A) 1%, B) 3%, C) 5%, after precipitation of silver with 2% ascorbic acid solution

0.45 m^2/g. The smallest specific surface area is exhibited by fibers obtained with a mixture of chloroform and methanol, and – for the pores' diameter of 1.6 nm, it is 0.05 m^2/g. The mentioned specific surface areas correspond to the adsorption ability of nitrogen.

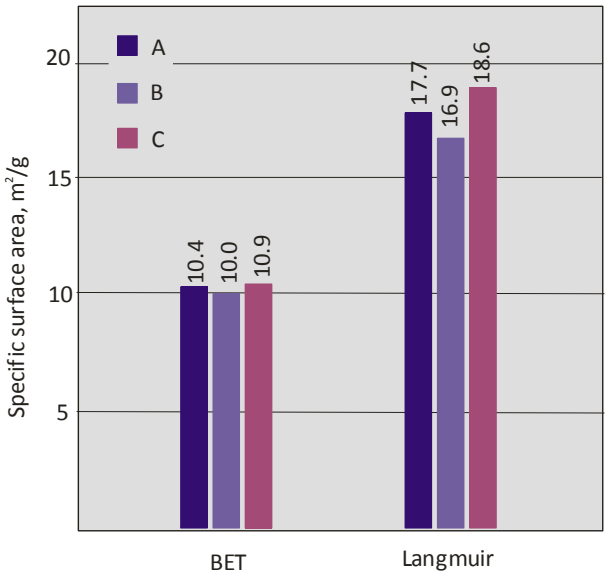

Fig. 17. BET and Langmuir specific surface area of PCL fibers with the molecular mass of M_w = 70,000-90,000 g/mol obtained from 10% mixture of formic acid and hydrochloric acid with mass ratio of 70:30 with addition of AlphaSan with fraction of: A) 1%, B) 3%, C) 5%

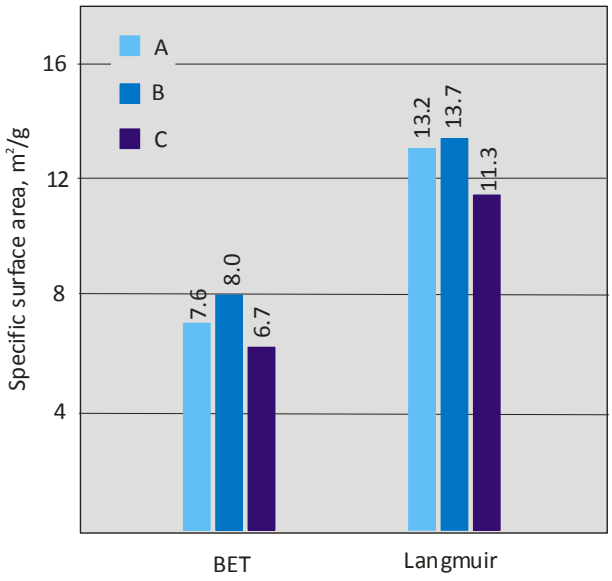

Fig. 18. BET and Langmuir specific surface area of PCL fibers with the molecular mass of M_w = 70,000-90,000 g/mol obtained from 10% mixture of formic acid and hydrochloric acid with mass ratio of 70:30 with addition of chitosan with fraction of: A) 1%, B) 3%, C) 5%

A specific surface area of the fibers produced is dependent on the presence of additives of silver nitrate, AlphaSan and chitosan (Fig. 16 to Fig. 18). As the fraction of silver nitrate and AlphaSan is growing, so is growing the BET and Langmuir specific surface area. A different tendency occurs if the fraction of chitosan is increased, as the specific surface area is then decreased. The highest porosity determined with the DFT method in the range of 1.4-5.4 nm for the fibers with an additive of chitosan, AlphaSan or silver nitrate is observed for fibers containing 5% of AplhaSan. After introducing a 5% additive of AlphaSan, an area of pores with the diameter of more than 1.4 nm is 5.97 m^2/g, and pores are dominant with the diameter of 1.58 nm, with their corresponding specific surface area of 0.66 m^2/g.

For a 5% additive of silver nitrate, the area of pores with the diameter higher than 1.4 nm is 5.21 m^2/g, and pores with the diameter of 1.57 nm are dominant, with the specific surface area of 0.76 m^2/g. In case of 5% of a silver nitrate additive, the area of pores with the diameter higher than 1.4 nm is 3.36 m^2/g, and pores with the diameter of 1.61 nm are dominant, with the specific surface area of 0.40 m^2/g.

An attractive technical solution is the application of composite core-shell nanofibers for the outworking of three-dimensional scaffolds that can combine antibacterial properties of the coating with bioactive properties of the inner core, for example, is the introduction of a medicine, or an antibiotic released after the dissolution of the coating. Such a structure can give an additional function of a carrier of a medicine to the outworked material. Those nanofibers are obtained by co-axial electrospinning using a flat collector. The selection of materials which are components of the coating and the inner core of achieved fibers allows to design a tissue scaffold impacting on the body, including the elimination of microorganisms because of the presence of antibacterial silver in the coating of composite nanofibers, the disintegration of the coating and the unveiling of a bioactive core, supporting the development of tissues till the total rebuilt of appearing loss and the total decay of scaffolds to non-toxic products. The type and the properties of solutions used to manufacture core-shell nanofibers determine geometrical features and morphology, including the diameter of the produced fibers. Diversification of thickness of the inner core despite the continuous flow of both solutions results from the use of solutions of different viscosity, including the coating solution of 10% polycaprolactone without additives, as the solution of as the inner core of 4% solution of polycaprolactone without additives (Fig. 19). In the case of the solutions significantly

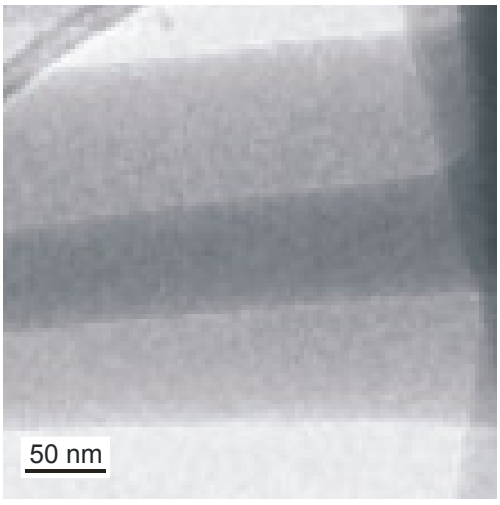

Fig. 19. Geometrical characteristics of core-shell composite obtained from: a) a coating solution of 10% polycaprolactone without additives and inner core solution of 4% of polycaprolactone without additives; TEM

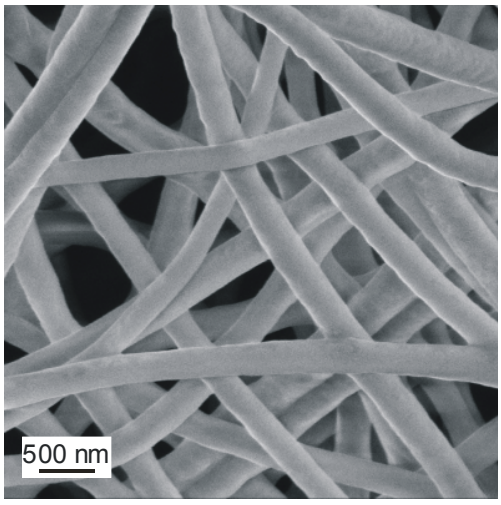

Fig. 20. Geometrical characteristics of core-shell composite obtained of 10% solution of polycaprolactone from which a core and a coating of fibers were obtained, the flow rate of 0.1 ml/h in the shell and the core; SEM

differing by electrical conductivity and viscosity, including in the coating solution of 3% polycaprolactone involving silver nitrate, and in the core of 10% polycaprolactone solution containing no additives, the fibers formed on the surface contains silver and have a diameter uniformity and shape. In the case of a core of a polycaprolactone solution containing 3% of silver nanoparticles in the coating 10% of polycaprolactone solution fibers with different geometric shapes and a tendency to agglomerate silver are formed (Fig. 19).

The structure of core-shell nanofibers was confirmed by scanning electron and transmission microscopy and as well by the use of confocal microscopy after the addition to the organic core of chemical compound DAPI (4',6-diamidino-2-phenylindole) as a fluorescent dye strongly binding to the acid deoxyribonucleic acid DNA on the principle of intercalation and commonly used to dyeing nuclei or chromosomes by visualization of DNA. In nanofibers coated with 12% solution of polyvinyl alcohol and a core of 4% polyvinyl alcohol solution, containing an additive DAPI as a result of which excitation by the laser beam in the confocal microscope followed by the illumination of fibers on the surface of a microscope glass (Fig.19).

Geometrical features and morphology of the produced fibers can be also adjusted by the flow rate of solutions. In the case of the application of 10% polycaprolactone solution, out of which the coating of the fiber core were achieved, at a flow rate of 0.1 ml/h a fiber diameter equals 200-600 nm, and at a flow rate of 0.5 ml/h is in the range of 200-1100 nm (Fig. 20) while in the case of the application of glycerine as a core solution at the flow rate 0.5 ml/h, the diameter of the fibers is 200-1300 nm. The diameter of the fibers of the coating with the solutions containing 12% hydrophilic polyvinyl alcohol and the core of polyvinyl alcohol with 4% contribution is in the range of 700-1300 nm, whereas the core of the natural oil, the interval is in the range of 300-1200 nm (Fig. 21). Relevant differences in a diameter also show nanofibers prepared out of 10% polycaprolactone solution without additives, polycaprolactone containing 3% additive of silver nitrate which were used for the construction of the coating and 5% polycaprolactone solution without additives, polycaprolactone with 5% low molecular weight hyaluronic acid, polycaprolactone with 5% contribution of silver nanoparticles, polycaprolactone with 2.5% mixture of the additive of low molecular weight hyaluronic acid, 2.5% additive of chitosan and colloidal gold, used to prepare the solution of the inner core (Fig. 21b).

In order to evaluate the bioactivity of selected nanofibers, including the ones generated out of 10% polycaprolactone solution in a mixture of formic acid and acetic acid and nanofibers containing, respectively, 5% of chitosan, 5% low molecular weight hyaluronic acid, a mixture of low molecular or high molecular hyaluronic acid with the participation of 2.5% chitosan with the participation of 2.5%, and colloidal gold, it caused that normal human

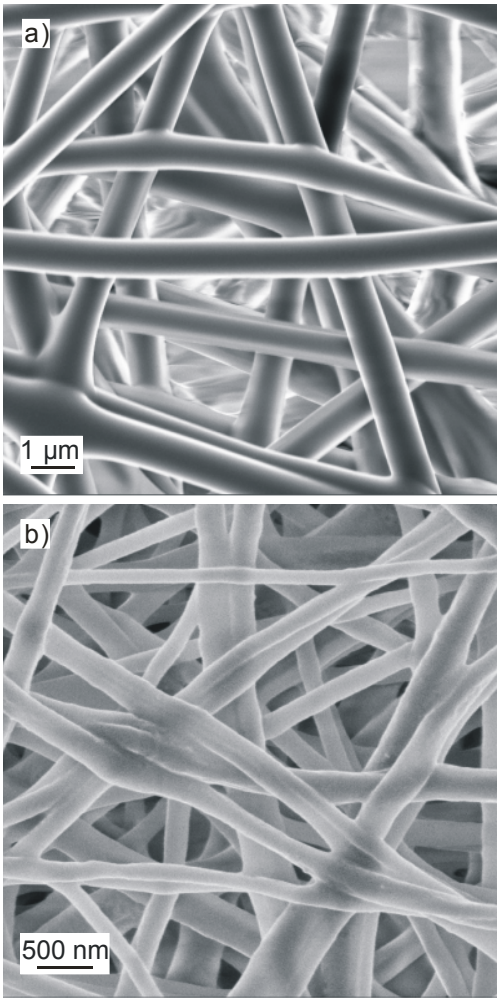

Fig. 21. Geometrical characteristics of core-shell composite obtained from: a) 12% solution of polyvinyl alcohol in the coating at a flow rate of 1 ml/h and the natural oil in the core at a flow rate of 0.1 ml/h; b) 10% solution of polycaprolactone with 3% additive of silver in the coating at a flow rate of 1.0 ml/h and 10% solution of polycaprolactone with 2.5% additive of chitosan and 2.5% additive of hyaluronic acid and gold in the core at a flow rate of 0.1 ml/h; SEM

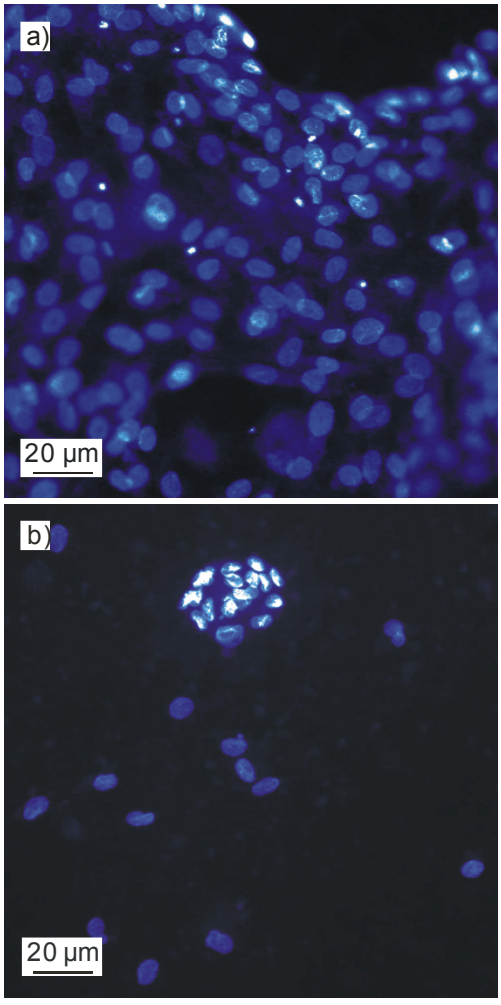

Fig. 22. The image of deoxyribonucleic acid DNA of cells cultured for 96 h and dyed by propidium iodide disclosed by the fluorescent method of a confocal microscopy on a substrate of nanofibers obtained from: a) 10% of polycaprolactone solution in a mixture of formic acid and acetic acid with 5% additive of low molecular weight hyaluronic acid; b) 10% of polycaprolactone solution in a mixture of formic acid and acetic acid with 5% additive of chitosan; confocal microscope

dermal fibroblasts NHDF were grown on its substrate by 96 h and surface density of cells grown on different substrates, as the number referred to the unit area (Fig. 22). Rating of surface density of cultured cells were made using fluorescent confocal microscopy after dyeing with propidium iodide of deoxyribonucleic acid DNA of the cultured cells. Nanofibers 10% polycaprolactone solution in a mixture of formic acid and acetic acid comprising the additive of 5% low molecular weight hyaluronic acid (Fig. 23), slightly higher than the surface density of cultured cells NHDF 206 on nanofibers obtained with 10% polycaprolactone solution in a mixture of formic acid and acetic acid have the highest average surface density of cultured cells NHDF 241. A significant difference in the effects of nanofibers containing low molecular weight and high molecular weight hyaluronic acid and weak bioactivity of nanofibers containing a combination of chitosan and polycaprolactone was shown.

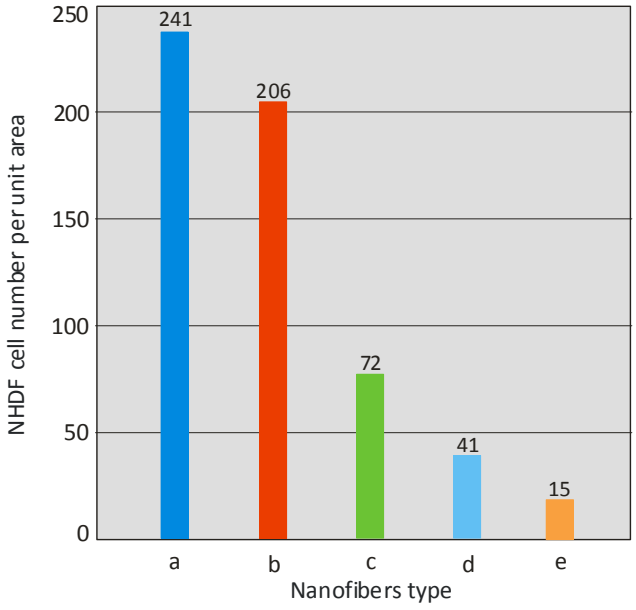

Fig. 23. The average surface density of deoxyribonucleic acid DNA of a cell cultured for 96 hours on substrate of nanofibers: a) are obtained with a 10% of polycaprolactone solution in a mixture of formic acid and acetic acid including the 5% additive of low molecular weight hyaluronic acid; b) obtained with 10% of polycaprolactone solution in a mixture of formic acid and acetic acid; c) comprising a mixture of 5% low molecular weight hyaluronic acid involving 2.5% contribution of chitosan and colloidal gold; d) containing 5% of chitosan; e) containing a mixture of 5% high molecular hyaluronic acid involving 2.5% contribution of chitosan, and colloidal

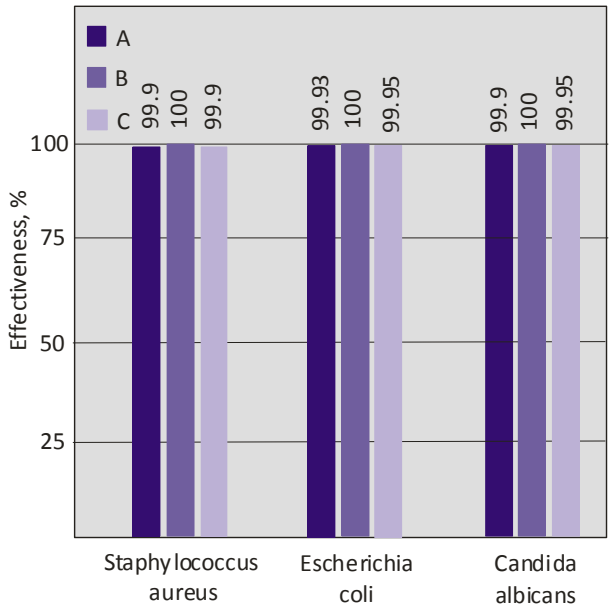

Fig. 24. Antibacterial efficiency (ABE) and antifungal efficacy (AFE) of double-component fibers obtained by dissolving PCL with the molecular mass of M_w = 70,000-90,000 g/mol obtained using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with AlphaSan additives with fraction of: A) 1%, B) 3%, C) 5%, after 17 hrs of incubation in the environment of microorganisms

The efficiency of the additives of silver nitrate, AlphaSan and chitosan introduced into double-component nanofibers in fighting Gram+ *Staphylococcus aureus* bacteria in antibacterialness examinations made in *in vitro* tests is diversified. The introduction of chitosan, regardless its fraction, does not improve antibacterial properties of the fibers obtained. The highest average antibacterial efficiency (ABE) of 99.93% in material groups containing 1 and 5% of the additive is recorded for nanofibers containing AlphaSan, while in the group with 3% of additives, the highest average antibacterial efficiency of 99.93% is characteristic for nanofibers containing AlphaSan (Fig. 24), and nitrate silver (Fig. 25).

There are also differences in how additives of silver nitrate, AlphaSan and chitosan with 1-5% fraction influence the antibacterialness of double-component nanofibers in *in vitro* tests on Gram- *Escherichia coli* bacteria. The highest antibacterial efficiency in relation to *Escherichia coli* bacteria for double-component nanofibers containing 1% of additive is indicated for nanofibers containing AlphaSan, analogously as in case of 5% of this additive, when it is 100%. The highest average antibacterial efficiency of 100% relative to *Escherichia coli* bacteria in a group of materials containing 3% of the additive is indicated for nanofibers containing 3% of silver nitrate and AlphaSan additive. Nanofibers containing 1-5% of chitosan do not show antibacterial properties in relation to *Escherichia coli* bacteria.

Double-component nanofibers containing 1-5% additives of chitosan do not exhibit antifugal properties, either, in an environment containing *Candida albicans* fungi. From among double-component nanofibers containing 1% of additive, the highest antifungal efficacy (AFE) in this environment of 99.9% is distinctive for nanofibers containing silver nitrate or AlphaSan. As the fraction of the additive is growing to 3%, the highest AFE in fighting fungi is exhibited by nanofibers containing AlphaSan. The highest AFE in a group of materials containing 5% of the additive is indicated for fibers containing AlphaSan.

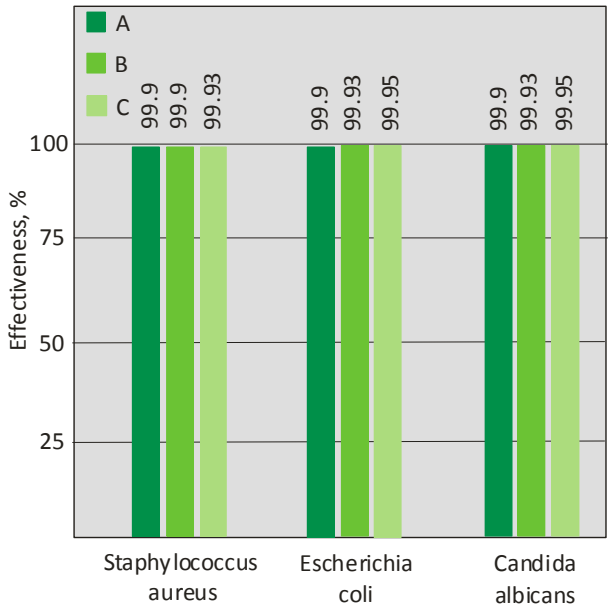

Fig. 25. Antibacterial efficiency (ABE) and antifungal efficacy (AFE) of double-component fibers obtained by dissolving PCL granulate with the molecular mass of M_w = 70,000-90,000 g/mol using 10% mixture of hydrochloric acid and formic acid with mass ratio of 70:30 with addition of $AgNO_3$ with fraction of: A) 1%, B) 3%, C) 5%, after precipitation of silver with 2% ascorbic acid solution and 17 hrs of incubation in the environment of microorganisms

4. Final remarks

The applicability of polymer fibers in medicine depends on biocompatibility and non-toxicity of the material applied, which is influenced by the chemical purity of the materials applied and the toxicity of the input solvents. The potential toxicity of nanofibers should therefore be eliminated, starting with selection of materials used for obtaining solutions. Many other factors fundamental for the quality and properties of polymer nanofibers need to be taken into account to create single- and double-component nanofibers. The influence of conditions of obtaining nanofibers, environmental conditions and properties of a solution influencing the diameter of fibers obtained is shown schematically in Figure 26.

Two solutions need to be used in co-axial electrospinning. The use of two solutions with different properties allows to isolate materials sensitive to atmospheric conditions by closing them in a core surrounded with a protective coating, by encapsulating various medicinal agents, enzymes or DNA in protective coating, and by determining the decomposition rate of the external coating, by releasing the encapsulated substances in specific time and at a specific rate, by obtaining new composite materials used, notably, as a reinforcing phase, which are a combination of a high-strength core with good external coating wettability in contact with a matrix material, by producing 3D scaffolds for the purpose of tissue engineering. Two solutions with differing properties are employed most frequently in coaxial electrospinning. For this reason, the number of conditions to be considered to achieve core-shell nanofibers, is doubled as compared to the standard electrospinning process, which substantially hinders to control the process. Moreover, interactions taking place between the core solution and the shell solution once they touch, which takes place at the end of the nozzle, and which additionally complicates the fabrication of core-coating nanofibers. Figure 27 shows schematically the conditions underlying the creation of core-shell nanofibers, including such acting on the core solution and external shell solution, as well as interactions between the solutions.

In the case of creating a single- and double-component nanofibers the solvents with moderate volatility, such as, e.g. a mixture of formic acid and hydrochloric acid, support the transformation of a solution under the influence of an electrostatic field in the form of a nanofiber, as their evaporation time equals the time necessary for its production. In case of solvents with considerable volatility, e.g. a mixture of chloroform and methanol or tetrahydrofuran and dimethyl sulfoxide, they support the fabrication of microfibers, as the time, in which solvents are evaporated, is shorter.

The type of the collector used may also influence the solvent evaporation rate. In the case where a collector is flat, the fibers tend to stick together, which is not the case if a rotating collector is used, in connection with the movement of the gas over the surface of such a collector, in particular as a result of synergic interaction of rotation motion of the cylinder and a gas flow system in the chamber. During electrospinning, the solvent is evaporated in the space between the electrodes, and solvent vapours coming from the solution constantly enter the gas situated between electrodes. In case of a flat collector, gas flow over its surface is insufficient to evacuate vapours of the solvents created between the flat collector and nozzle, even despite an installed system of evacuation of volatile products from a working chamber. For this reason the fibers stick together if a flat collector is used. The type of the collector used is also decisive for the spatial arrangement of fibers. The higher isotropy of fibers is obtained

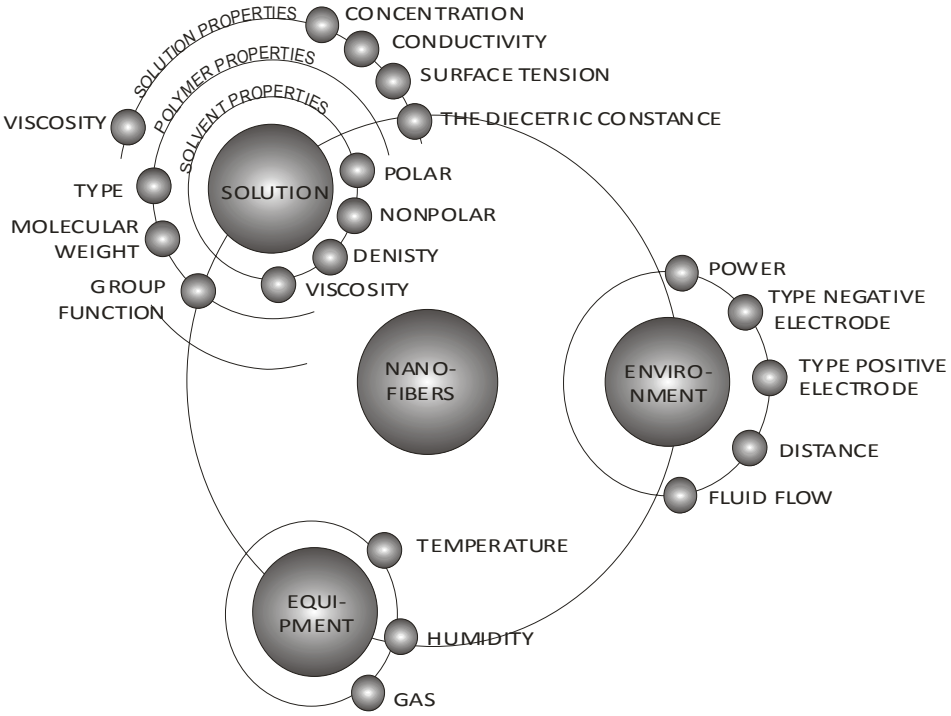

Fig. 26. Graphical presentation of process conditions, environmental conditions and properties of solution influencing the diameter of fibers obtained

by using flat collectors, whereas fibers anisotropy when using rotating collectors is increasing with the rotation speed of the collector.

The properties of the solutions in which nanofibers are manufactured, including viscosity and conductivity, are closely linked to the type and properties of solvents. The nanofibers obtained from a polymer material with higher molecular mass (70,000-90,000 g/mol) are thinner as compared to those of the fibers obtained using polymer with considerably smaller molecular mass (45,000 g/mol). If electrostatic voltage is indeed applied in the both mentioned solutions, a Taylor cone is created, however, it is more stable in case of a solution with higher molecular mass as the interactions between macroparticles are increased. This, on the other hand, translates into the intensification of friction forces created between macroparticles and is supportive to the fabrication of fibers with their diameter similar to each other and to a decreased number of defects such as beads on the surface of fibers. The opposite situation takes place when the molecular mass of polymers is decreased. The destabilisation of a Taylor cone with the shorter length of a macro-particles chain is conducive to the formation of fibers with different diameter.

The diameter of the fibers obtained may change after introducing additives, e.g. silver nitrate and chitosan, which are enhancing electric conductivity. In the first case it is related to the presence of an atom of silver in a silver nitrate particle, while in the second case with the presence of polar chemical groups chitosan is made of, and which includes -acetamido-2-deoxy-β-D-glucopyranose particles and 2-amino-2-deoxy-β-glucopyranose groups containing, notably, an atom of nitrogen. Such groups support the formation of hydrogen bonds, thus impacting the change of electric conductivity of the solutions obtained. When AlphaSan is introduced into a solution, despite containing 10% of silver, it reduces the electric conductivity of solutions. By introducing any of the additives, the viscosity of solutions is increased due to a higher density of the solution achieved. The higher viscosity of the solutions obtained related to the activity between macroparticles, does not translate into smaller diameter of the fibers obtained. Such additives are impacting, however, the BET, Langmuir specific surface area and the area of pores. If chitosan is introduced, the specific surface area is decreased related to a larger diameter of the fibers produced. The opposite tendency occurs when the fraction of AlphaSan and silver nitrate is increased, leading to an increase in the specific surface area of fibers, as agglomerates of AlphaSan and silver crystals on the surface of fibers exist. The additives introduced have also substantial influence on the anti-bacterialness and antifungalness of nanofibers. Silver nitrate and AlphaSan show high efficacy in fighting

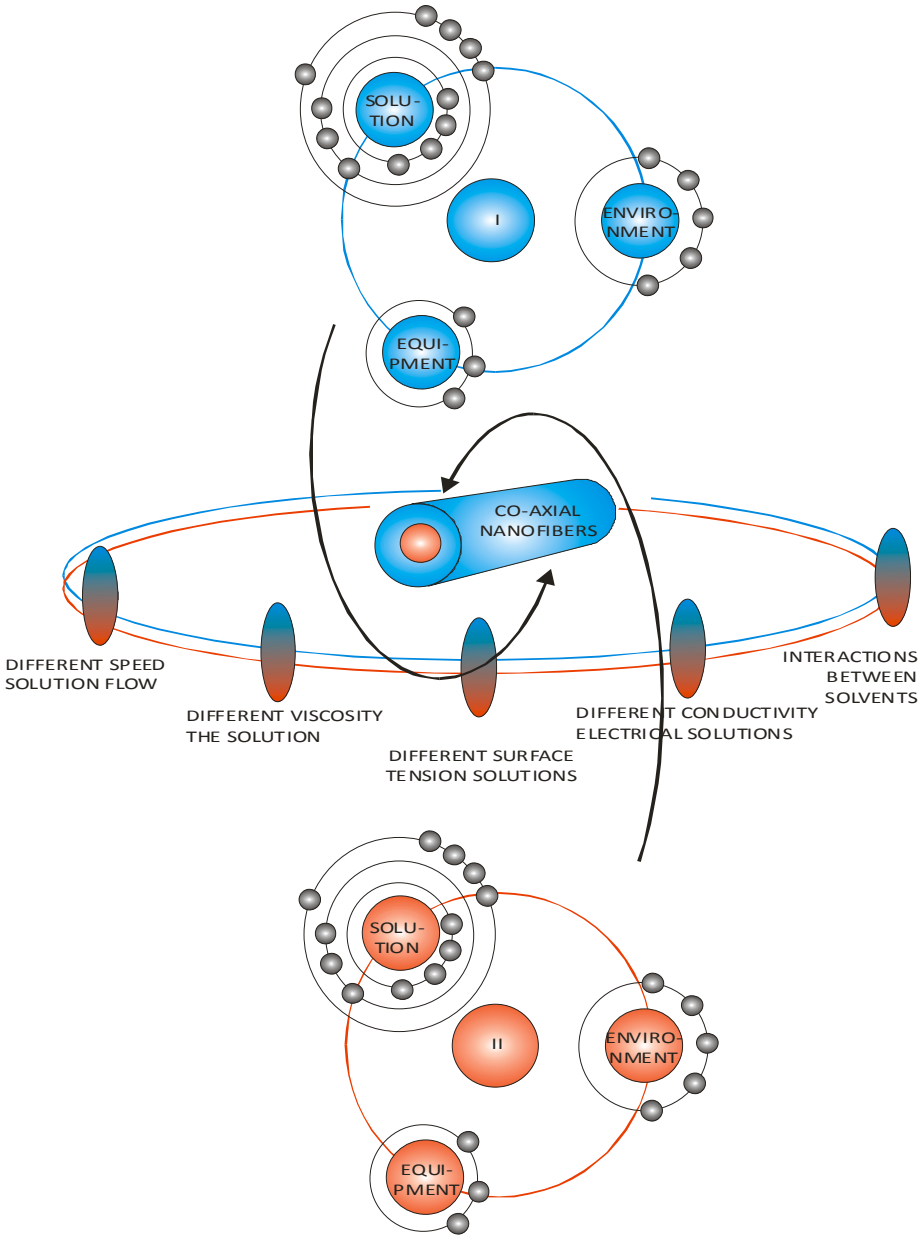

Fig. 27. Conditions underlying the creation of core-shell nanofibers: conditions influencing the core solution are shown in blue, conditions influencing the external shell solution are shown in orange, differences and interactions between solutions are shown in orange; own study

Gram+, Gram- bacteria and fungi, and macromolecular chitosan does not show antibacterial and antifungal properties. The additives mentioned are influencing the bioactive properties differently, by interacting with the cells of Normal Human Dermal Fibroblasts NHDF. The highest bioactivity is characteristic for nanofibers containing an additive of low molecular hyaluronic acid, whilst macromolecular hyaluronic acid is of much lesser importance.

Polymer nanofibers create significant applicational possibilities. The motivation for these works in close cooperation with medical doctors is encompassing numerous solutions eliminating various dysfunctions of a human organism. It is linked to sharp development of civilisational diseases, including cancer, because the incidence rate of malignant cancers e.g. in Poland has been regularly rising. The number of traffic accident victims has been growing

systematically [30]. Analogously, the number of fatalities in railway accidents is high. Followed by this, almost proportionally, grows the number of people injured in such accidents and requiring usually long-lasting medical care. Along with the intensification of sports activity, especially among young people, and with the promotion of leisure practise of sports by propagating a healthy lifestyle, more and more mature people start to practise sports, which is inherent to the growing number of sports accidents and the related serious bodily injuries of many people at a global scale. Globally, rise in aging population is playing a major role in increasing the incidence of sports injuries as aging diminishes body functions and movements which makes the body more prone to injuries. Annually the millions people are treated in hospitals for sports injuries. Patients' healthcare expectations are also growing, and economic aspects at the domestic scale call for the efficient elimination of disabilities, in particular motoric disabilities, and the restoration of previously handicapped persons to physical fitness and usually most often to full, or at least partial, professional activity, which considerably lessens pressure on the diminishing resources of social insurance funds. Of great importance is the shortened waiting time for service or therapy, a reduced price and availability of a medical product and service and therapy, a reduced risk of treatment failure, in particular with customised medical products according to a patient's individual anatomical features, and last but not least, a reduced therapy discomfort for the patient and his/her family. The people suffering from civilisational diseases, as shown above, including malignant cancers, often require bone reconstruction, e.g. of legs and hands and in the craniofacial area, as well as skin and other soft tissue reconstruction, and also oesophagus and/or blood vessels.

The outcomes of own investigations presented in this work are serving this purpose. The composite materials obtained, due to their non-toxicity resulting from the components applied, including solvents, bacteriocidity and bioactivity, may find their applications in tissue engineering as membranes in controlled regeneration of bone tissue, as carriers of medicinal agents in bone surgery, as implantable surgical meshes and as scaffolds for a tissue culture. In turn, the composite core-shell nanofibers, by combining the antibacterial properties of the coating with bioactive properties of the core, are attractive materials for three-dimensional tissue scaffold. Such materials can be used as a carrier of medicine, a treatment of hard healing wounds, invasive surgery, neurosurgery, as a substrate for the culturing of a retina, material to reconstruct nerves and in dentistry or oncology, to replace the natural tissue removed because of a cancer with the possibility of applying a therapeutic agent, e.g., an antibiotic or a medicine used in cancer therapies, released after the dissolution of the coating of nanofibers. The own detailed research in this area are continue *in vitro* and *in vivo* modes.

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Additionally selected issues related to the paper are planned to be presented in an inauguration lecture on "Electron microscopy as a synergic tool of the nanotechnology development" at the 22nd Winter International Scientific Conference on Achievements in Mechanical and Materials Engineering Winter-AMME'2015 in Zakopane, Poland in 6th-10th December 2015 in the framework of the Bidisciplinary Occasional Scientific Session BOSS'2015 celebrating the 10th anniversary of the foundation of the World Academy of Materials and Manufacturing Engineering WAMME, the Association of Computational Materials Science and Surface Engineering and of the Worldwide Journal of Achievements in Materials and Manufacturing Engineering.

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