2. Polymer Nanofibers Materials, Fabrication Technologies and Research Methods

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2.1. Description of the selected polymer materials applied in regenerative medicine

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. Polymer materials applied most often in medicine are described further in this part of the work.

Poly(lactic acid) (PLA) is a cyclic dimer of lactic acid having two optical isomers – left-handed type L and right-handed type D. Owing to an additional methyl group, it is more hydrophobic than PGA, and thus is undergoing degradation slower. It decomposes into lactic acid following implantation in the hydrolysis reaction. It is eliminated from an organism as carbon dioxide and water at further stages. This polymer starts to degrade after 7 weeks from implantation and is characterised by high strength similar to PGA and a high Young modulus. It is used as a carrier of medicines [1],[2].

Collagen is a basic protein of a human organism, it dominates in connective tissue, which is a basis of the motor organ: bones, tendons and ligaments. This protein represents the one-third of all proteins of a human organism. It is an inhomogeneous protein, as there are at least 27 genetically separate types of this protein. They differ in subunit composition, molecular mass, composition and sequence of amino acids, the degree of hydroxylation and glycosylation and spatial structure. A collagen macroparticle is a right-handed helix in which three left-handed polypeptide chains are bound with chemical bonds. Collagen belongs to bioresorbable polymers, insoluble in water. It is undergoing denaturation under the influence of the activity of elevated temperature, detergents, salt solutions, organic solvents, ultrasounds, concentrated acids and bases [3]. Large content of collagen is present in bones, cartilages, tendons, ligaments, fascia and in skin. Collagen fills the space between cells, but its basic function is to ensure cell strength, hardness, stiffness and elasticity. Collagen takes part in multiple physiological and pathological processes: it binds water in a tissue, takes part in blood coagulation processes, in regenerative processes connected with wound healing, it creates
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scars, takes part in bone regeneration after fractures. Collagen, in different forms, is applied as: binding agents, porous structures in tissue engineering, filling of bone defects and soft tissue defects, for cosmetic purposes and in composition bone substitutes in connection with hydroxyapatite or calcium triphosphate [3].

Polycaprolactone (PCL) is a semicrystalline, linear, aliphatic and biocompatible polyester with good mechanical properties. Interest in this material has been gradually growing in the recent years. It derives from its applicational possibilities, including, in particular, in: tissue engineering, regenerative medicine, e.g. for regeneration of nerves, as medicine carriers, sensors and other biomedical applications [4]-[6]. The applications of this polymer material are hindered due to a possibility of employing few and usually toxic solvents after material transformation into the form of a solution. These include: CH$_2$Cl$_2$, a mixture of THF:DMF at a rate of (1:1), acetone, a mixture of DMF: CHCl$_3$, a mixture of MeOH methanol with CHCl$_3$ chloroform, but also tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO). PCL is a biodegradable polymer with very long decomposition time of up to 2 years. A copolymer with much faster decomposition time can be achieved by joining the following copolymers: CL ε-caprolactone and DL-lactide [5]. As biomaterial-biosystem integration depends to a high extent on chemical properties of the surface, actions are taken with the additives of, notably: calcium carbonate and hydroxyapatite (HAP), to improve the surface properties of PCL, which may support the adhesion of cells to the surface of the so obtained composite. Unfortunately, despite introducing the additives mentioned, the adhesion of cells to the biomaterial surface has not been substantially accelerated [6].

Chitin is a second polysaccharide, after cellulose, most often occurring in nature. A chitin particle, in chemical terms, is similar to a cellulose particle, the difference between the macro-particles is that the acetamide group [-NH(C=O)CH$_3$] occurs in chitin in the position of atom C2. Chitin may occur in two crystalline polymorphic variants, namely α and β. Similar to chitosan, it is an antibacterial, biocompatible biopolymer creating chelate compounds with metals, and is also biodegradable and hydrophilic. It is a compound with poor solubility, which considerably restricts its applications. One of few chitin solvents is 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP).

Chitosan is a derivative of chitin and a copolymer in which the D-acetylglucosamine group (typical for chitin) and glucosamine group (typical for chitosan) can be differentiated. The
origin of chitin (fungi, crustaceans, fish) is mainly decisive for the properties of chitosan. Chitosan is obtained by deacetylation of groups (D-acetylglucosamine) naturally occurring in chitin, as a result of which glucosamine groups are created. The degree of chitosan deacetylation (DD) is the fraction of the glucosamine group to the total fraction of the groups of D-acetylglucosamine and glucosamine. The degree of deacetylation and length (mass) of the macroparticles are the main conditions determining the properties of chitosan. For macroparticles whose $M_n$ is:

a) higher to 220,000, chitosan assumes the shape of compact molecules,

b) lower than 220,000, chitosan creates the conformations of accidental helices.

![Structure of chitin and chitosan macroparticles](image)

*Figure 2.1. Structure of chitin and chitosan macroparticles*

It is related mainly with the creation of hydrogen bonds whose share increases as the molecular mass of polymer is rising. This polymer belongs to the group of biodegradable, non-toxic materials, and owing to the presence of the NH$_2$ group, it is much easier to solve than chitin [7]. In practice, to dissolve chitosan, such solvents are used as: trifluoro acetic acid
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(TFA), hexafluoro-2-propanol (HFIP) [8], trichloromethane (TCM) and dichloromethane (DCM). Unfortunately, the solvents are very expensive or toxic and may lead to polymers degradation. Hydrochloric acid CH₃COOH or formic acid HCOOH solutions are mainly used for this reason to dissolve chitosan. The presence of acids is to ensure an acidic reaction for the aquatic medium below 6.5 pH, in which it is possible to transform chitosan into the form of a solution. Chitosan is composed of two units:

a) 2-acetamido-2-deoxy-β-D-glucopyranose and

b) 2-amino-2-deoxy-β-glucopyranose (Fig. 2.1).

The material contains approx. 6.89% of nitrogen taking part in the creation of chelates, i.e. complex chemical compounds consisting of a ligand (Fig. 2.2). For chitosan, these are groups –OH, groups –O⁻ situated on D-glucosamine residues and attached to it with several central ion bonds (metal cation). Haemoglobin in blood platelets and chlorophyll in plant leaves exhibit the similar property [9]-[11].

![Complex compound](image)

**Figure 2.2. Complex compound**

Chitosan (CH) exhibits antioxidant properties consisting in the ability to remove a peroxide anion from the cell (a radical created as a result of chalcogens metabolism). The antioxidant properties of chitosan consist of a reaction of the superoxide anion with active atoms of hydrogen of quaternary ammonium salts [12]. One of the most valuable properties of chitosan, distinguishing it from other polymer materials, is its bacteriocidity, which depends on the degree of chitosan deacetylation (DDA), pH of the environment in which it interacts and on the molar mass of chitosan [13]. There are several concepts of the bacteriocidic activity of chitosan on Gram-positive and Gram-negative bacteria. One of them suggests that the electrostatic
interaction existing between amine groups of chitosan and the negatively charged groups of liposaccharides and proteins (forming part of cell membrane of a bacterium) contribute to interrupting the continuity of a microorganisms’ cell membrane, which is leading to the destruction of a given bacterium [14]. The properties of chitosan accelerate the healing of wounds. Materials with the content of chitosan are characterised by surface properties supporting the adhesion of bone or nervous cells. A special property of chitosan is its ability to improve blood coagulation. Research has been conducted for several years for this reason to employ this effect, notably for the removal of heparin (a compound reducing blood coagulation) used, among others, in surgical operations [15],[16].

**Hyaluronic acid** (HA) is a linear polysaccharide – glycosaminoglycan (GAG), created from the repeating disaccharide units containing D-glucuronic acid and N-acetyl-D-glucosamine bonded with β-1,4 and β-1,3 glycoside bond (Fig. 2.3). The arrangement of side groups in a hyaluronic acid macroparticle makes such type of the system very stable in terms of energy.

![Figure 2.3. Structure of disaccharide units present in hyaluronate](image)

**Hyaluronic acid** belongs to the family of glycosaminoglycans, however, compared to other glycosaminoglycans (which are bound with convalescent bonds to the protein core thus creating proteoglycans), hyaluronic acid creates gigantic skeletons to which binding proteins, other glycosaminoglycans or proteoglycans are attached in a noncovalent manner and create huge aggregates (Fig. 2.4). Hyaluronic acid aggregates are, apart from collagen fibres and non-collagen proteins, the main component of the extracellular matrix (ECM). A property of hyaluronic acid is its similarity in all living organisms, which allows to obtain it from different
sources. Bacterial cultures are currently the most popular method of obtaining hyaluronic acid [17],[18]. The name hyaluronic acid means a particle of undissociated character. In a physiological environment, the D-glucuronic group is dissociated and occurs as a polyanion bonded with Na⁺, such a particle is called sodium hyaluronate. Therefore, the correct term designating this compound is sodium hyaluronate. As this polymer enjoys immense popularity in trade, its name ‘hyaluronic acid’ is popular and hence this name is used in this work [19].

Figure 2.4. Hyaluronate aggregates in ECM

Hyaluronic acid particles (Fig. 2.4) may reach the length of 10,000,000 g/mol. The structure of the polysaccharide has large influence on the environment in which it is situated. Small particles, such as water, electrolytes or nutrients, can travel freely through a hyaluronic acid solution. Large particles (such as proteins) may also penetrate through the hyaluronic acid structure as this polymer is subject to constant movement in an aquatic environment, and creates pores of different type and size.

Hyaluronic acid is also the main component of the extracellular matrix of skin, joints and multiple other tissues, responsible for extracellular communication. A completely new discovery is a surprising multitude of biochemical processes in which hyaluronic acid participates, including, among others: proliferation, differentiation and migration of cells, angiogenesis, inflammatory reactions, activities of the immunity system cells, tumorigenesis
processes. Apart from skin, the largest concentration of hyaluronic acid is seen in the vitreous humour of the eye and in the synovial fluid of joints, in which the presence of hyaluronic acid contributes to viscoelastic properties of joint fluid. Hyaluronate has special hydrophilic, rheological and viscoelastic properties. It participates in all the phases of wound healing, and its decomposition time depends on the location and may be from several minutes to 3 weeks. It is estimated that a human organism contains 15 grams of this polysaccharide. 5 g of hyaluronic acid is constantly exchanged over a day (biosynthesis and degradation) [17],[19]. Hyaluronic acid is used in medicine, because:

- it is biodegradable, biocompatible and bioresorbable,
- it is characterised by an ability to absorb a high volume of water, which is decreasing the tension formed between the surfaces of joint cartilages creating friction between each other,
- its volume supports the division of cells,
- a hyaluronic acid macroparticle contains such function groups as: –COOH and –OH, which may support cross-linking and gelling,
- it participates in all the phases of wound healing,
- it maintains a moist environment, for this reason it is suitable as a material accelerating wound healing,
- hyaluronic acid does not bind with proteins, hence it is suitable for tissue cultures, besides, the surface of HA can be modified by introducing cellular receptors proteins on the surface of some cells enabling binding with hyaluronic acid CD44 and RHAMM (CD168), (CD44 is an integral hyaluronan receptor that can promote or inhibit motogenic signaling in tumor cells. RHAMM is a nonintegral cell surface hyaluronan receptor and intracellular protein that promotes cell motility in culture),
- the presence of HA activates the RHAMM receptor which contributes to the enhanced mobility of cells, what is very important in the wound healing process,
- low molecular weight hyaluronan (LMWH) plays a major role in controlling an inflammatory condition – it acts in a proinflammatory fashion,
- high molecular weight hyaluronan (HMWH) plays a role of fading an immunological response – has an anti-inflammatory effect.

Hyaluronate, due to its negative charge, creates multiple coordination bonds with H₂O particles and this property has influence on:
- high viscosity of hyaluronic acid solutions, which is proportional to the concentration,
- elasticity, which depends on a concentration or length of chains,
- flexibility.

The above-mentioned polymer materials, on the basis of the data available obtained from the literature, based on the criteria of potential and attractiveness (Table 2.1-2.4), were subjected to an analysis using a method of procedural benchmarking and weighted scores [20],[21]. The relevant potential and attractiveness criteria were ascribed to the above polymers, and were presented graphically after multiplying the particular criteria by weight (Fig. 2.5).

**Table 2.1. Potential and attractiveness criteria (own study)**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Objective values (potential)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_P1$</td>
<td>Tensile strength of material</td>
<td>0.2</td>
</tr>
<tr>
<td>$K_P2$</td>
<td>Stability of shapes achieved</td>
<td>0.1</td>
</tr>
<tr>
<td>$K_P3$</td>
<td>Enzymatic or hydrolytic bioresorbability</td>
<td>0.3</td>
</tr>
<tr>
<td>$K_P4$</td>
<td>Material transformability into a fibre from solution (fibre-forming ability)</td>
<td>0.2</td>
</tr>
<tr>
<td>$K_P5$</td>
<td>Applicability of non-toxic solvents</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjective values (attractiveness)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_A1$</td>
<td>Material applicability as carrier of medicinal substances</td>
</tr>
<tr>
<td>$K_A2$</td>
<td>Number of publications concerning the material (acc. to Science database)</td>
</tr>
<tr>
<td>$K_A3$</td>
<td>Composite material manufacturing cost</td>
</tr>
<tr>
<td>$K_A4$</td>
<td>Potential range of application at industrial scale</td>
</tr>
<tr>
<td>$K_A5$</td>
<td>Availability of material acquisition sources</td>
</tr>
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</table>
Table 2.2. Multicriteria analysis of attractiveness of particular polymer materials as porous materials and scaffolds (own study)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Weight</th>
<th>PLA</th>
<th>PCL</th>
<th>Chitosan</th>
<th>PA6</th>
<th>KH</th>
<th>PMMA</th>
<th>PP</th>
<th>PVA</th>
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</thead>
<tbody>
<tr>
<td>$K_p1$</td>
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<td>8</td>
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<td>6</td>
<td>5</td>
<td>8</td>
<td>6</td>
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<td>$K_p2$</td>
<td>0.1</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>$K_p3$</td>
<td>0.3</td>
<td>8</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>$K_p4$</td>
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<td>8</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>$K_p5$</td>
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<td>3</td>
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<td>4</td>
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<td>8</td>
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<td>$K_A1$</td>
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<tr>
<td>$K_A2$</td>
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<tr>
<td>$K_A3$</td>
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<td>10</td>
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<td>7</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>$K_A4$</td>
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<td>8</td>
<td>9</td>
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<td>9</td>
<td>1</td>
<td>0</td>
<td>8</td>
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<tr>
<td>$K_A5$</td>
<td>0.2</td>
<td>8</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>5</td>
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</table>

Table 2.3. Analysis results of individual polymer materials (own study)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>PLA</th>
<th>PCL</th>
<th>Chitosan</th>
<th>PA6</th>
<th>KH</th>
<th>PMMA</th>
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<th>PVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_p1$</td>
<td>1.6</td>
<td>1.6</td>
<td>2</td>
<td>1.2</td>
<td>1.2</td>
<td>1</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>$K_p2$</td>
<td>0.7</td>
<td>0.9</td>
<td>1</td>
<td>0.6</td>
<td>0.8</td>
<td>0.4</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>$K_p3$</td>
<td>2.4</td>
<td>2.7</td>
<td>0.9</td>
<td>1.5</td>
<td>3</td>
<td>1.8</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>$K_p4$</td>
<td>1.6</td>
<td>1.6</td>
<td>0.4</td>
<td>1.6</td>
<td>1</td>
<td>1.6</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>$K_p5$</td>
<td>0.6</td>
<td>1.4</td>
<td>1.4</td>
<td>0.8</td>
<td>2</td>
<td>0.8</td>
<td>0</td>
<td>1.6</td>
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<td>TOTAL</td>
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<td>8.2</td>
<td>5.7</td>
<td>5.7</td>
<td>8</td>
<td>5.6</td>
<td>4.1</td>
<td>7</td>
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<tr>
<td>$K_A1$</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>2</td>
<td>0.5</td>
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</tr>
<tr>
<td>$K_A2$</td>
<td>0.2</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>$K_A3$</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.1</td>
<td>1.05</td>
<td>1.05</td>
<td>1.2</td>
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<td>$K_A4$</td>
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<td>$K_A5$</td>
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<td>0.6</td>
<td>1.4</td>
<td>0.8</td>
<td>1.2</td>
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<td>1</td>
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<tr>
<td>TOTAL</td>
<td>7.05</td>
<td>8.4</td>
<td>7.55</td>
<td>3.4</td>
<td>6.8</td>
<td>3.7</td>
<td>4</td>
<td>6.6</td>
</tr>
</tbody>
</table>
Polymer nanofibers produced by electrospinning applied in regenerative medicine

Table 2.4. Summary of results of analysis of relevant polymer materials (own work)

<table>
<thead>
<tr>
<th>SYMBOL</th>
<th>PLA</th>
<th>PCL</th>
<th>Chitosan</th>
<th>PA6</th>
<th>KH</th>
<th>PMMA</th>
<th>PP</th>
<th>PVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>POTENTIAL</td>
<td>6.9</td>
<td>8.2</td>
<td>5.7</td>
<td>5.7</td>
<td>8</td>
<td>5.6</td>
<td>4.1</td>
<td>7</td>
</tr>
<tr>
<td>ATTRACTIVENESS</td>
<td>7.05</td>
<td>8.4</td>
<td>7.55</td>
<td>3.4</td>
<td>6.8</td>
<td>3.7</td>
<td>4</td>
<td>6.6</td>
</tr>
</tbody>
</table>

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 2.5) are listed according to the literature data.

Table 2.5. Key advantages and disadvantages of the selected polymer materials

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

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.
2.2. **Standard polymer nanofibers and their fabrication methods**

Nanotechnology, as a science dealing with objects sized from 1 to 100 nm, is regarded as one of the key fields of science in the 21st century and the main driving force of economic and technological advancements in the nearest future [13],[22]-[29]. Hopes are connected with nanotechnology that materials will be created featuring an increasingly larger specific surface area and properties characterised by a larger specific surface area, higher electric conductivity, higher thermal conductivity, higher strength properties and supermagnetism [25],[30],[31]. The reasons for such dynamic development of nanotechnology should be sought in the application potential of each of four groups of nanostructural materials the range of which is covered by nanotechnology [27],[30]:

a) **three-dimensional objects** (3D) in which at least one dimension is at a nanometric scale, an example can be a polycrystal containing nanograins,

b) **two-dimensional objects** (2D) in which one dimension of the matter is reduced, an example can be nanolayers and nanoplanes,

c) **one-dimensional objects** (1D) in which two dimensions of the matter are reduced, an example can be nanowires, nanotubes, nanofibers and

d) **zero-dimensional object** (0D) including nanoclusters, nanospheres or nanocrystals.

The BCC’s data shows the development dynamics of the nanotechnology market, according to which this market was worth USD 422 million in 2001, and will reach an unprecedented value of USD 48.7 billion until 2017. The nanotechnology market will be developing in the three main directions over the coming years, namely (Fig. 2.6) [32]-[35]:

a) **nanomaterials** whose value will grow from USD 15.9 billion in 2012 to USD 37.3 billion in 2017,

b) **nanotools** estimated in 2012 at USD 4.8 billion and will reach a value of USD 11.4 billion until 2017,

c) **nanorobots** estimated in 2012 at USD 45.3 billion and will reach a value of USD 176.2 billion until 2017.

Polymer nanofibers with the diameter of below 1 µm are especially important in the field of nanostructural materials. The simplest nanofiber fabrication method is drawing. The method allows to obtain single nanofibers by drawing (at a rate of 100 µm/s) a micropipette submerged
in a chosen polymer solution. Drawing is accompanied by solvent evaporation and fibre formation. Regrettably, the method, due to low efficiency, has not been applied industrially [4].

Template synthesis is another nanofiber fabrication method. The method consists of applying nanoporous membranes made of aluminium oxide Al₂O₃ or silicon oxide SiO₂ as a template for producing nanofibers, nanowires or nanotubes. Such a membrane has channels with the diameter of several to more than twenty nanometres. Structures made of polymers, arranged parallel to each other, are formed in such channels in electrochemical deposition, although the method can also be used for producing nanofibers made of metallic or ceramic materials. Precursor absorption takes place in the membrane pores, and then its conversion to the final solid state, in particular by a chemical reaction or pyrolysis. The last stage is the release of the nanofibers obtained by dissolving the material the matrix was made of [36]-[39].

Another nanofiber fabrication method is phase separation. It is a simple method of manufacturing three-dimensional structures consisting of submicron fibers. A homogenous and multi-component system becomes thermodynamically unstable in this process in the predefined conditions. In order to attain a privileged energy state featuring a low free energy value, decomposition occurs into a phase enriched with a polymer component and a phase.
Polymer nanofibers produced by electrospinning applied in regenerative medicine

impoverished with this component. The solvent selected and the input polymer content is decisive for the morphology of the structure obtained, characterised by high repeatability [40].

Molecular self-assembly is the process of spontaneous arrangement of molecules in extramolecular structures, also called supramolecular, in the conditions of thermodynamic balance. The process is carried out by low forces of intermolecular interactions, among others: electrostatic interactions (of the Coulomb type), hydrogen bonds, permanent dipole-permanent dipole interactions and Van der Waals' interactions. Despite the low energy of the bonds mentioned, it is feasible to form durably arranged structures. A good example of self-assembly are peptides made of hydrophilic and hydrophobic amino acid residues. In aqueous solutions, amino acids have a tendency to adopt a structure of the β-sheet. Nanofibers with the diameter of 10-20 nm and also nanofibers consisting, in their cross section, of 6 to 7 molecules [4],[6] can be achieved with the molecular self-assembly method.

Although diverse nanofiber fabrication methods are available, the dominant process is electrospinning [5],[7],[8],[10],[36],[41]-[48] also referred to as nanofiber fabrication in electrostatic field (Fig. 2.7). This results from the high process efficiency as compared to other methods.

The method enables to obtain nanofibers with full and empty cross section, core-shell nanofibers consisting of two parts and porous nanofibers [42],[43], dual- and multicomponent nanofibers [36]-[38],[44], composite nanofibers containing metal particles or ceramics, as well as organic materials [37],[41], hybrid nanofibers being a combination of polymers of the natural and synthetic origin [45]. It is possible to obtain polymer, but also carbon, metallic or ceramic nanofibers in an indirect or direct manner depending on the input material type and an attachment method of materials [49].

The electrospinning method includes:
- melt-electrospinning where the following polymers are melted in advance: PP, PE, PMMA or PET and then by subjecting the liquid to the activity of a strong electrostatic field in order to transform into a nanofiber,
- solution-electrospinning where polymer is transformed into a solution and then the liquid is subjected to the activity of an electrostatic field to stretch the stream and evaporate the solvent, which consequently leads to the formation of nanofibers [6].

Five phases of transformation of a polymer solution into a nanofiber can be distinguished in the typical solution electrospinning process.

2. Polymer Nanofibers Materials, Fabrication Technologies and Research Methods
The flow of a polymer solution though a metal jet, which is a positive electrode, represents the initial phase of electrospinning. This is accompanied by the activity of a unipolar electrostatic field on macroparticles in the solution causing their mutual repulsion. When the solution is flowing through the jet, stream orientation takes place in the liquid leaving the jet channel, which increases as the flow rate and channel length increases.

The creation of a Taylor cone is a prerequisite for the success of the entire process. A solution flowing out of the jet is subjected to the activity of an electrostatic created between the positively charged jet and the negatively charged collector. The charges collected on the surface of the initial solution are balanced energetically by surface tension of the solution (Fig. 2.7).

If the tension applied is too small, because smaller than critical polarisation $V<V_c$, a Taylor cone is not created and the solution condenses onto the collector surface. Upon exceeding critical polarisation $V_c$, corresponding to overcoming the surface tension forces by the electrostatic field $V>V_c$, viscoplastic flow of the solution occurs from a Taylor cone to the negative electrode – collector. Charges on the stream surface are reduced, and the solution assumes the form of a stationary stream which is stretched in an electrostatic field. The exceedance of critical polarisation, during which from several to more than ten Taylor cones are created, is called supercritical tension $V>>V_c$ and, similar to tension below critical
Polymer nanofibers produced by electrospinning applied in regenerative medicine

Figure 2.8. Electrostatic forces acting on a droplet of polymer solution

Figure 2.9. Influence of electrostatic field on solution

polarisation, is disadvantageous as it leads to the formation of a wide range of micro- and nanofibers (Fig. 2.9).

Rectilinear motion of the solution stream occurs directly after leaving the “Taylor cone”. A dipole electric layer is created in the surface part of the stream, in which one type of the generated electrostatic charges is directed outside the surface and the other one inside. A state of charging the solution stream, created as a result, is defined as surface charge density and the electric potential value. The surface charge density is differentiated along the rectilinear section of the stream, which results from Coulomb’s law. The solution stream is stretched at a rate of 1000 m/s over this section.

Disruptions in the rectilinear flow of the polymer solution occur in the next phase. The charges gathered on the rectilinear section of the stream interact with the electrostatic field created. As the polymer stream is moving towards the collector on the distance with its length depending on the process conditions and polymer solution conductivity, a critical value of an electrostatic charge is created, accompanied by abrupt solution flow and stream whirling.
Spiral motion of the polymer solution stream and fibre solidification is the result of exceeding the critical energy of internal cohesion of a rectilinear stream section by the electrostatic field value. The whirling of the rectilinear stream section occurs as a result of the charges, and the created diameter of spiral coils is increasing as approaching the negative electrode. An electrostatic field stretches the polymer stream at a rate of up to 25,000 m/s, accompanied by the sudden increase in the specific surface area, which means that the evaporation area of the solvent applied is enlarged, leading to the formation of fibers.

The following is influencing the properties and structure of the micro- and nanofibers fabricated in the electrospinning process:
- properties of the solution (properties of polymer material, properties of solvent),
- environmental conditions (temperature, humidity, gas),
- process conditions (electrostatic field, type of positive and negative electrode, distance between electrodes, solution flow).

Each of the mentioned conditions is directly or indirectly related. The properties of a polymer material are crucial for the type of the solvent used. The molecular mass of the polymer applied influences the viscosity achieved after solving. Solution viscosity is decisive for the diameter of the fibers achieved as a result of electrospinning or for the particle size in the electrospray process. The properties of a solvent are to a large extent conditioning the conductivity achieved and the surface tension of the solutions obtained. Surface tension and conductivity are influencing an ability of charges to travel along the stream of the solution. Conductivity, viscosity, solvent volatility and solution pH vary according to temperature variations. On the other hand, an electrostatic field interacts with the surface tension of the solution, leading to the critical value \( V_c \), after exceeding which an electrospinning process begins.

Considering the conditions listed, the conditions can be classified to two groups in terms of their relevance related to the transformation of the solution into nanofibers:
- primary conditions, including, most of all, electrical conductivity of the solution, solvent content and the related solution viscosity;
- secondary conditions, comprising electrostatic field and solution flow in ml/h or µl/min.

The optimisation of the primary and secondary conditions is a prerequisite for achieving nanofibers free of defects [50]-[54].
2.3. Co-axial polymer nanofibers and their fabrication methods

The intensively evolving co-axial electrospinning technology enabling to achieve, notably, core-shell micro- and nanofibers [49] hollow nanofibers [45] and porous nanofibers [55] belongs to advanced nanofiber fabrication methods. Co-axial electrospinning, just like a typical fabrication process of nanofibers in an electrostatic field, can be carried out vertically or horizontally, both, using solutions and molten polymers [56]-[64].

Compared to typical electrospinning, the co-axial electrospinning technology enables to:

- isolate materials sensitive to atmospheric conditions by encapsulating them in a core surrounded with a protective coating,
- encapsulate various medicinal agents, enzymes, DNA in protective coating, and by determining the decomposition rate of the external coating, to release the encapsulated substances in specific time and with a specific rate,
- to obtain new composite nanofibers applied, notably, as a reinforcing phase of composites ensuring a combination of a high-strength core with good external coating wettability in contact with a matrix material,
- to obtain spatial 3D scaffolds for the purpose of tissue engineering ensuring a combination of a strong core with hydrophobic properties and long decomposition time, with another polymer material, among others, with good hydrophilic properties improving adhesion to cells in a living organism,
- to transform materials into the form of nanofibers, nanowires or nanotubes, which is unachievable in standard electrospinning,
- to encapsulate an electrically conductive core with a protective coating ensuring its protection against environmental conditions.

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 jet, responsible for formation of a coating surrounding the core from each side (Fig. 2.10).

Apart from the jets presented in Figure 2.11, jets are also used consisting of two separate jets, with a solution situated inside. The core-coating type fibers are obtained by placing...
a second jet over the main jet responsible for creating the Taylor cone, by means of which another solution is introduced, from which an internal core is obtained.

Co-axial electrospinning is subject to the same conditions as standard electrospinning. The difference between standard and co-axial electrospinning is related to the application of two solutions differing in properties, undergoing changes including the change of environmental conditions and process [45],[49],[55]. If the tension applied is smaller than the solution surface tension $V<V_c$, the process will not occur as the surface tension of the solution will balance the voltage resulting from the applied electrostatic field. In the $V>V_c$ optimum conditions, the electrostatic field is higher than critical tension after exceeding which the solution electrospinning process begins, and the interactions created between the electrostatic field and surface

**Figure 2.10. Simplified co-axial electrospinning**

**Figure 2.11. Co-axial core consists of, A – jet responsible for jacket formation, B – solution of external jet, C – second external jet situated in the place where the Taylor cone is formed, D – Taylor cone of internal solution, E – co-axial nanofibers**
field voltage are stretching the polymer solution stream, which causes the creation of micro- and nanofibers. The electrostatic field, which exceeds many times the minimum necessary to initiate electrospinning, is called supercritical tension and, similar as in the standard electrospinning process, is leading to the formation of several to more than ten separate Taylor cones [45],[49],[55],[56],[65]. The way an electrostatic field is acting on the stream of two polymers (Fig. 2.12) can be divided into:

- a critical field, in which the obtained Taylor zone is not disrupted in any way and spinning is performed optimally,
- a field below critical tension, which is too small, hence the both solutions start to drip from the jets',
- a supercritical field, which exceeds the voltage required for a given material and then the both solutions are divided and reverted towards the jet, which is accompanied by the formation of two independent streams, from which fibers with different diameter are obtained [66].

![Figure 2.12. Electrostatic field acting on the stream of polymer solution; V<V<sub>c</sub> – field below critical value; V>V<sub>c</sub> – optimum field; V>>V<sub>c</sub> – supercritical field](image)

An assumption of the EHD co-axial electrospinning is that the core solution and coating solution are stretched uniformly to obtain the core-coating nanofibers. The external solution, opposite to the internal one, acts as a guide for the charges created on the positive electrode, hence the solution should possess optimum electric conductivity [67].

The friction forces created between the solutions are more important than electric charges during core stretching. Stream flow continuity is interrupted in case of core solutions with insufficient viscosity. For this reason, it is important that viscosity of the solution used is sufficient to ensure uninterrupted continuity of stretching the internal stream. Interruption in core stream continuity is also prevented, apart from viscosity, by compressing the solution by the external coating as a result of the acting electrostatic field applied. This is additionally...
supported by the existence of much smaller forces acting in the internal core, as it is surrounded by the shell solution, as compared with the situation where the core solution would be surrounded by gas, as is the case in standard electrospinning [65]-[68]. One solution is used in conventional electrospinning, and as the content of polymer in the solution is growing, so is increasing the diameter of the fibers obtained, whereas decrease in this content is reducing the diameter of the fibers being obtained. In the co-axial electrospinning method, if polymer content is increased in the core solution while maintaining the constant content of polymer in the shell solution, the diameter of the core itself as well as of the entire fibre is increased. As polymer content is increasing in the core solution, the diameter of the coating is decreased as the same proportion of the shell solution is distributed over a larger and larger core area. Interactions taking place between the core solution and the shell solution once they touch, i.e. at the end of the co-axial nozzle, are crucial for fabrication of core-coating nanofibers. Successful electrospinning of fibers in majority of cases is seen when the solvents applied do not mix with each other. The investigations carried out using PEO (poly(ethylene oxide) in a water and ethyl alcohol solution prove, however, that the application of the same solvent is considerably lessening the tension created between solutions, which supports electrospinning. As surface tension between two solutions is decreasing, the probability of continuous, uninterrupted electrospinning is decreasing.

The evaporation rate of the solvents used plays an important role in developing the morphology of core-coating polymer nanofibers. If a solvent with a high evaporation rate (such as chloroform or acetone) is contained in the core solution, being a mixture of two solvents, a thin layer is then produced in contact with the shell solvent, blocking the solvent which is diffused slower [68]. Negative pressure is then generated in the core, and it is collapsing under the atmospheric pressure and may assume the shape of a cylinder and strip [69].

The electrical conductivity of solvents is responsible for the stretching of fibers. The higher it is, the higher density of surface charges repelling each other under the influence of an electrostatic field, which are stretching the stream. If the conductivity of the core solution is higher than the conductivity of the coating solution, the continuity of core-coating nanofibers fabrication is interrupted. For this reason, the conductivity of the shell solution should be larger than the core solution, as it is leading to the intensive elongation of the coating solution, during which shear stresses are acting on the core solution [70],[71].
An electrostatic field is the primary factor of obtaining core-shell nanofibers leading to the formation of polymer nanofibers if the following conditions are met:
- the shell solution is an electrospun material,
- the shell solution should have higher viscosity than the core solution,
- small surface tension should exist between the shell and core solution,
- solvents with long evaporation time should be used,
- the shell solution should have higher electric conductivity than the core solution.

The flow of solutions is a prerequisite allowing to define the thickness of the micro- and nanofibers obtained. The diameter of the fibers obtained is also increased in standard electrospinning as the flow rate is growing. As a result of coaxial electrospinning, an increase in core solution flow entails an increase in the core diameter and the fibre diameter at the expense of decreasing the thickness of the external coating as the core flow is increasing. Core solution flow cannot be too small, though, as it may lead to interruptions in core continuity in the core-coating fibers. In order to ensure that core-coating polymer nanofibers are obtained, shell flow should be larger than core flow. Otherwise, the core solution flow is leading to Taylor cone destabilisation.
2.4. Key polymer nanofibers investigation methods

Polymer nanofibers can be investigated by various research techniques, among which the following should be distinguished: scanning electron microscope (SEM), confocal microscope, transmission electron microscope (TEM), atomic forces microscope (AFM), X-ray structure analysis (XRD), IR spectroscopy, FTIR, ASA and methods enabling to investigate strength properties and specific surface using gas adsorption.

The structure of the fibres obtained determined in the investigations presented further were observed using electron microscopes: a scanning and transmission microscope. SEM images were made using a Scanning Electron Microscope (SEM) Supra 35 by Carl Zeiss with the accelerating voltage of 3-25 kV equipped with the X radiation spectrometers: an energy dispersion EDS and wavelength WDS spectrometer and a system for analysing diffraction of back scattered electrons EBSD by EDAX. The high resolution and the precision imaging of the preparations viewed was achieved by applying a high performance In-lens SE detector working with low beam voltage and with a very small working distance of the preparation examined to the electron gun (Fig. 2.13).

**Figure 2.13.** The example of using SEM technique for the investigations of the morphology of obtained polycaprolactone fibres from a 10% solution in a mixture of formic acid and acetic acid of 70:30 m/m
Scanning Electron Microscopy (SEM) is based on the examination of a surface of a given material by means of a focussed beam of electrons. Secondary electrons, back scattered electrons, Auger electrons and X-ray radiation recorded by means of detectors are created as a result of the activity of the beam of electrons with the material surface. The observations were made with the magnification of 1000 to 100,000 times in order to determine the influence of the applied solutions and fabrication conditions on the nanofibers diameter, their spatial arrangement and formation of defects. Digital Micrograph software was used in the investigations. The qualitative and quantitative analysis of the chemical composition of fibres obtained was carried out using the X-ray energy dispersive spectroscopy (EDS) with the application of the EDS LINK ISIS spectrometer of the Oxford Company being a component of the scanning electron microscope (Fig. 2.14).

Transmission Electron Microscopy (TEM) is based on sample exposure with a beam of short-wave electrons, which improves microscope resolution (Fig. 2.15). A Tungsten fibre or LaB$_6$ crystal, from which electrons are released, is the source of electrons in transmission microscopy. Accelerating voltage in TEM microscope is much higher than voltage in SEM microscopy and is between 100 to 400 kV. The image obtained is a two-dimensional projection.

**Figure 2.14.** The example of using EDX technique for the investigations of the composite nanofibres containing silver on the surface; peaks for copper derive from the copper support mesh applied
Figure 2.15. The example of using high-resolution transmission electron microscope HRTEM for the investigations of the structure of obtained composite fibres with 5% addition of silver nitrate; a photograph taken after precipitation of silver with a 2% ascorbic acid solution of the internal material structure on the plane, and the following is used in practice for observations: dispersion contrast, diffraction contrast and phase contrast. The structure of fibres in the investigations presented further in the work was examined with an S/TEM TITAN 80-300 high-resolution transmission electron microscope HRTEM by FEI Company with the point resolution of $\leq 0.2$ nm. The microscope applied is fitted with an electron gun with XFEG field emission, a Cs condenser spherical aberration corrector, a STEM scanning system, and also Bright Field (BF) and Dark Field (DF) detectors and High Angle Annular Dark Field (HAADF), and also EDS. Imaging in the transmission mode (parallel beam) and scanning/ transmission mode (concentrated beam) was used during the examinations using BF, DF and HAADF detectors. An HAADF detector in the STEM mode could be used to assess a morphology and structure of the examined nanocomposites. This type of examinations is adequate for materials the components of which are strongly differing in their ordinal number (so-called Z contrast).

The operating principle of the Atomic Forces Microscope (AFM), also referred to as Scanning Probe Microscope (SPM), consists of interaction between the atoms of the surface of the examined sample with the test probe atoms. Coulomb and van der Waals forces should be distinguished here. As this microscope may work in several modes, in particular in the contact
mode and semi-contact mode, it allows to create images and measurements of the examined surfaces with very high resolution in relation to all three axes. The data obtained is transformed by means of a computer programme into an image of the examined surface with resolution reaching up to 0.1 nm relative to the axis x and y and 0.01 relative to the axis z.

The XRD analysis of the phase composition of fibres and composites materials determined in the investigations presented further was carried out using PANalytical X’Pert PRO X-ray diffractometer equipped with the Xcelerator strip detector, in a goniometric system, using the filtered K radiation of the cobalt lamp at 40 kV voltage and 30 mA heater current. The reflected radiation intensity measurements were made in the 2θ angle range from 35 to 95° every 0.05° and counting time of 10 seconds (Fig. 2.16).

![Graph showing XRD analysis](image)

**Figure 2.16.** The example of using the XRD analysis of the phase composition for the investigations of the polycaprolactone nanofibres obtained using a rotational collector

Wide-angle X-ray scattering (WAXS) and wide-angle X-ray diffraction (WAXD) techniques were used to determine the crystalline structure of obtained fibres using apparatus DMAX RAPID II by Rigaku-Denki with installed rotating anode silver AgKα of wavelength λ = 0.05608 nm (Fig. 2.17). The degree of crystallinity was calculated using the Levenberg-Marquardt algorithm (LMA), also known as the damped least-squares (DLS) method, which could be used in many software applications for solving generic curve-fitting non-linear least squares problems. These minimization problems arise especially in least squares curve fitting.
Infrared spectroscopy (IR) and Fourier transform infrared spectroscopy (FTIR) are the methods enabling to examine the vibrations of molecules of chemical compounds. In infrared spectroscopy, a sample is irradiated with infrared radiation (1000–20000 nm). If radiation energy corresponds to the energy difference between the basic state and excited state, a photon will undergo absorption and a molecule transits into an excited state with higher energy. This energy difference is measured in infrared spectroscopy, as it has to be accompanied by a change of dipole moment; the spectra recorded during the investigation come from the vibrations of polar groups, in particular such as −OH, −NH, C=O (Fig. 2.18).

The inelastic scattering of radiation by a sample is used in Raman spectroscopy. The source of radiation in all Raman spectrometers are lasers with the emitted length of waves from the range of ultraviolet and visible light (dispersion spectrometers) to infrared (Fourier spectrometers). The spectra recorded presents lines with the frequency equal to the frequency of the incident radiation and the next, much weaker lines with frequencies moved in relation to the initial radiation, so-called Stokes and Anti-Stokes lines with frequencies respectively smaller and larger than the initial radiation. Stokes bands are analysed most often in Raman spectra in terms of their much higher intensity in a spectrum. In a Raman spectrum those
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vibrations are strong which, most of all, are coming from non-polar homonuclear groups, such as: C=C and N-N as well as from symmetric stretching vibrations, e.g. of aromatic rings. Moreover, Raman spectroscopy enables to record easily spectra in far infrared range (50-400 cm⁻¹), in which multiple bands exist characteristic for non-organic compounds.

Electrical conductivity (EC) tests are undertaken for single-component solutions and double-component solutions using an MM41 multimeter by Labindex. Calibration in a water solution of Crysolyt KCl 3M with the conductivity of 1413 µS/cm was performed before a conductivity measurement. An electrical conductivity measurement according to stability over the time of 4 seconds at the temperature of 25°C was employed in the tests presented further in the work.

\[ \text{Figure 2.18. The example of using the FTIR method for the investigations of obtained polycaprolactone fibres from a 10\% solution in a mixture of formic acid and acetic acid of 70:30 m/m deposited onto the rotational collector with the rotation speed 500 rev./min} \]

Viscosity tests of single-component and double-component solutions are performed by means of an Alpha L rotational viscometer by Labindex. An APM adapter for solutions with the volume of 8-13 ml was employed for measurements in the tests presented further in the work. The viscosity of solutions was measured with TL5, TL6 and TL7 spindles at room temperature. Spindle speed within the range of 0.3 to 100 rev./min was used for the tests.

The BET specific surface area and porosity was assessed with the gas adsorption method. This method assumes that:
active centres exist which may adsorb more than one gas molecule (multilayers are formed),
- interaction is avoided between adsorbent molecules in the surface layer of the adsorbent,
- the number of adsorbed molecules depends on the pressure,
- adsorption heat of the first layer differs from adsorption heat of the next layers.

A Geminii VII 2390t specific surface area analyser by Micrometrics was used for the examinations. The weighed samples with the mass of 0.2 g were subject to vacuum drying for 24 hours at the temperature of 30°C to remove humidity and the adsorbed gases. The dried samples were placed in the device and measurement conditions were defined. Pressure within the range of 0.1 to 1 was used for adsorption and desorption and nitrogen with the purity of 5.0 was used as a measuring gas.

The examinations of antimicrobial activity of the nanocomposites produced were carried out for the presence of:
- *Staphylococcus aureus* ATCC 25923,
- *Escherichia coli* ATCC 25922,
- *Candida albicans* ATCC 10231.

The reference strains of *Candida albicans* were used in the tests of anti-fungal activity. The samples of each of the examined composite sized 10×10mm were plasma sterilised and placed in 4 ml of a suspension of the reference fungal strain with the final density of 1.5·10⁵ CFU/ml (the units forming bacterial colonies in 1 ml) in tryptone water.

A sample of UG material in 4 ml of tryptone water and 4 ml of tryptone water was used as a negative control and 4 ml of a suspension of the reference microbial strain with the density of 1.5·10⁵ CFU/ml was used as a positive control.

The tested and control sample was made in 2 repetitions. After 17 hours of incubation at the temperature of 37°C in oxygen conditions, the volume of 20 µl was sifted from each test onto a permanent substrate for cultivating the fungi of Sabouraud agar. After 48 hours of incubation at the temperature of 37°C in oxygen conditions, the quantity of the cultivated fungus colonies was assessed to identify anti-fungal efficacy (AFE). The AFE of the examined samples was calculated according to the following dependency (2.1):

\[
AFE [%] = \frac{V_c - V_t}{V_c} \cdot 100\%
\]

where:
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\[ V_c \] – microbial suspension density in the positive control (blanc),

\[ V_t \] – density of the tested microbial suspension.

The reference strains of *Staphylococcus aureus* and *Escherichia coli* were used in the tests of antibacterial activity. The plasma-sterilised samples of each of the examined nanocomposites and UG material were placed in 4 ml of the suspension of the reference fungal strain with the final density of \( 1.5 \cdot 10^5 \) CFU/ml in tryptone water.

A sample of UG material in 4 ml of tryptone water and 4 ml of tryptone water was a negative control and 4 ml of suspension of the reference microbial strain with the density of \( 1.5 \cdot 10^5 \) CFU/ml was a positive control (without the material samples).

*Figure 2.19.* The example of the image of deoxyribonucleic acid DNA of NHDF 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 10% of polycaprolactone solution in a mixture of formic acid and acetic acid with 5% additive of low molecular weight hyaluronic acid

The tested and control sample was made in 2 repetitions. After 17 hours of incubation at the temperature of 37°C in microaerophilic conditions, the volume of 20 µl was sifted from each of the tested and control sample onto a permanent substrate for cultivating the bacteria of Columbia agar with a 5% addition of sheep blood. After 48 hours of incubation at the temperature of 37°C in microaerophilic conditions, the number of the cultivated bacteria colonies...
was evaluated to determine anti-bacterial efficacy. The AFE of the examined samples was calculated according to the following dependency (2.2):

$$ABE[\%] = \frac{V_c - V_t}{V_c} \cdot 100\%$$

(2.2)

where:

- $V_c$ – microbial suspension density in the positive control (blanc),
- $V_t$ – density of the tested microbial suspension.

In order to evaluate the bioactivity of obtained nanofibres the normal human dermal fibroblasts NHDF were proliferated on their substrates by 96 h and surface density of cells grown on different substrates as the NHDF cell number referred to the unit area were compared. 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 (Fig. 2.19).
2.5. Final remarks regarding the application and research perspectives of polymer nanofibers

The polymers market has been developing since the 90’s. The BCC’s data reveals that the global consumption of polymer nanolayers will be growing since 2015 at a rate of 37% CAGR [72],[73], reaching the value of USD 2.2 billion in 2020. The dynamic advancement of polymer nanofibers is signified by year-to-year growth of a variety of research areas, the number of nanofibers-related publications, as well as the number of new enterprises specialising in industrial polymer nanofiber fabrication, in particular such as: SNS, Yflow, Elmarco FibeRio, eSpin [74]-[77].

Special characteristics of nanofibers justify a search for new uses of such materials. Nanofibers have been developing most intensively in the mechanical and chemical sector, including medicine, electronic and power industry, as they hold a great promise to develop products with unmet properties [78]-[88]. Polymer nanofibers are present these days also outside the textile industry and have been evolving in areas related to: medicine [34], filters [89], sensors [90], military industry, pharmacology, electronics [91], photovoltaics [92], environmental protection and as components of composite materials [93]-[95] (Fig. 2.20). The application potential of nanofibers appears to be most surprising, which, despite so diversified directions of research, is currently estimated at a level not exceeding 1% of hypothetical possibilities not yet determined in practice [39],[40].

As an example of possible custom applications of polymer nanofibers are presented the own patents [96],[97], and first of all the preparation of hybrid and multilayer composite materials consisting of biologically active cellular structures was presented [98], as well as a substrate having its matrix made of optimally selected nanostructural composite materials with the matrix consisting of polymer nanofibers, acting as a scaffold, permitting controlled engineering material delamination from the biological layer, upon achieving the therapeutic aims defined by medical reasons, as a result of one or several appropriately chosen and selected physiochemical factors, e.g. temperature, magnetic field, electric current, active enzymatic or chemical activity, by selecting appropriately the active medium, and – in necessary cases – as a result of natural biodegradation (Fig. 2.21).
Figure 2.20. Development directions of nanofibers obtained in electrostatic field

Such a hybrid, multi-layer composite biological and engineering material fulfills at the same time several functions, including:

- ensures the readiness of different types of artificially cultured cells, as a biologically active internal layer of a biological and engineering composite, for assimilation with natural humans cells, e.g. skin cells or cells of the internal surface of blood vessels; and an ability to ensure the function of cells within the time required to culture them on a substrate made of a composite engineering material, to deliver and to apply for therapeutic purposes, until detachment from the composite engineering material substrate, after meeting the clinical requirements related to the therapy applied,

- exhibits an ability of adhesion of a layer of different types of artificially cultured cellular structures to a multi-layer composite engineering material substrate as an external layer, ensuring the required mechanical properties, including rigidity and numerous, in some cases
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Figure 2.21. Scheme of engineering-biological scaffold structure, where A – amyotrophic transition layer (or several): photodegradable, thermodegradable, pH-degradable, enzyme-degradable, biodegradable layer, etc., subject to degradation or removal after fulfilling the therapeutic function by the entire composite or, after application to the wound, detaching from the substrate surface (D) and stiffening the wound by filling it, for faster in-growth of blood vessels or to improve adhesion to the wound, B – textured layer of an engineering material providing more space for cells culturing and enabling to create three-dimensional cellular structures, C – layer of three-dimensional structures of the grown cells, D – layer of a substrate engineering material ensuring strength properties required to transfer the grown tissues onto the damaged tissue surface

alternative physiochemical properties, allowing final delamination of each layer of this engineering material, and most of all the non-destructive separation of cellular structures from a composite engineering material substrate on which they are grown after meeting the clinical requirements related to the therapy applied,

- provides an ability of natural biodegradation of the substrate layer made of a composite engineering material, on which artificially cultured cells are located, being in direct contact with such cells, after meeting the clinical requirements related to the therapy applied or, alternatively, an ability to remove one or two indirect layers laid between a layer of artificially cultured cellular structures and a support substrate made of a composite engineering material, through controlled interaction of various physiochemical factors, including, e.g. temperature, magnetic field, electric current, active enzymatic or chemical activity, by selecting adequately the active medium, after meeting clinical requirements
related to the therapy applied, is dependent on the phenomena and mechanisms related to the artificial culturing of different types of cellular structures on a multi-layer composite engineering material substrate and on the surface phenomena and mechanisms connected with mutual adhesion of individual layers, on the technological process of fabrication of composite engineering material layers on which cellular structures are cultured and on the type, chemical composition and structure of each of the layers contained in this material and on their technological and operational properties in therapeutic conditions and during the influence of various physiochemical factors, including, e.g. temperature, magnetic field, electric current, active enzymatic or chemical activity, aimed at non-destructive separation of cellular structures from a composite engineering material substrate on which they are cultured, but after meeting the envisaged therapeutic function, e.g. after wound healing.

It should be concluded in the light of the information presented that further scientific and application research is profoundly substantiated and expected over the fabrication, structure and properties of polymer nanofibers and composite materials requiring their content. The next chapter of the work presents the results of own research concerning the structure and properties of polymer nanofibers fabricated with the intention to employ them for the purpose of regenerative medicine.
References to paper 2nd


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