PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science
Department of Physical Chemistry

Study of Preparation and Degradation of Biodegradable Polymer Fibres Prepared via Electrospinning Method

Bachelor thesis

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I declare, that I elaborated my bachelor's paper entitled "Study of Preparation and Degradation of Biodegradable Polymer Fibres Prepared via Electrospinning Method" independently under the supervision of RNDr. Jana Soukupová, Ph.D. using sources included in the bibliography.

In Olomouc _____ _____

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BIBLIOGRAFICKÁ IDENTIFIKACE:

Jméno a příjmení:	Hanna Dilenko
Název práce:	Studium přípravy a degradace biodegradabilních vláken připravených elektrostatickým zvlákňováním
Pracoviště:	Katedra fyzikální chemie
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Abstrakt:	Předmětem předkládané bakalářské práce byla příprava biodegradabilních vláken generovaných v systémech obsahujících různý procentuální podíl poly(laktid-co-glykolové) kyseliny (PLGA) v různých směsných rozpouštědlech, a to v tetrahydrofuranu (THF), acetonu, chloroformu a N, N, dimethylformamidu (DMF). Po vlastní přípravě byla vlákna inkubována v kyselém prostředí, v destilované vodě, ve fyziologickém roztoku a v silně alkalickém roztoku při zvýšené teplotě (37°C a 42°C). Proces degradace vláken byl sledován pomocí skenovací elektronové mikroskopie (SEM).
Klíčová slova:	biodegradabilní polymerní vlákna, PLGA, elektrostatické zvlákňování, obvazové krytí, nanočástice stříbra
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Abstract:	In this bachelor paper, fibres were generated on the basis of various percentages of poly(lactic-co-glycolic acid) (PLGA) in different solvents such as N, N, dimethylformamide (DMF), tetrahydrofuran (THF), acetone, and chloroform. After their generation, the fibres were incubated in acid medium, distilled water, physiological solution and in a strongly alkaline solution at elevated temperature (37°C and 42°C). The process of fibre degradation was monitored by means of scanning electron microscopy (SEM).
Keywords:	biodegradable polymer fibres, PLGA, electrospinning, wound dressing, silver nanoparticles
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Contents

INTRODUCTION

Nanotechnology is today considered one of the amazing branches of modern science, capable of allowing a great number of discoveries to be made. Typically, it represents a set of methods and technologies that enables to monitor synthesis and modification of objects which include components with sizes below 100 nm in at least one dimension. These objects exhibit fundamentally new qualities. Despite the fact that nanomaterials have gained a special popularity in the last couple of decades, their history can be traced back to ancient times – natural asbestos nanofibers, for example, were used more than 4,500 years ago as reinforcement of a ceramic. [1]

This submitted paper is focused on a detailed study of such a field of application of nanotechnological products, namely nanofibres, as perspective degradable wound dressings or materials applicable in tissue engineering. In general, this interdisciplinary area is aimed on creating fibres, sometimes also labelled as textiles, with such structures that can be capable of supporting, improving or replacing the normal functioning of living body tissues, including skin. This is one of the important problems of modern medicine, because according to data from the US Wound Registry, about one third of patients with chronic wounds are not able to achieve full healing even after a long period of treatment. The consequences of such situations can be even fatal and include amputation and death of patients. [2]

In the course of this thesis, biodegradable fibres of poly(D,L-lactic-co-glycolic acid) will be generated and consequentially studied with respect of their usage as perspective scaffolding materials or simply materials used in "active" wound dressings. The fibres will be generated using the device working a principle of electrospinning. The influence of various parameters (such as the choice of solvent/solvent mixtures, the concentration of the polymer in the solution, the voltage used, etc.) on the electrospinning process and the nature of the generated fibres will be studied. Consequently, the as-generated fibres will be tested with respect to their degradation under such conditions that can define different model states of skin. The characterization of the results will be primarily performed with microscopic techniques – scanning electron microscope (SEM).

1. NANOTECHNOLOGY

Nanoscience and nanotechnology represent today progressively developing interdisciplinary research fields. They have a potential to change the way, in which we create and understand materials and products and expand its functionality. Nanotechnology is developing worldwide with increasing speed and has a substantial commercial impact, which will be undoubtedly increased in future [3].

Surprisingly, the history of existence and generation of nanoparticles began earlier than we commonly believe and their synthesis does not proceed only in modern scientific laboratories. Nanoparticles appeared on planet Earth with first volcanic eruptions, wildfires and as a result of the vital activity of microorganisms. Tailored production of nanoparticles was primary connected with preparation of dyes. Ornaments made of glazed ceramics, which appeared in Mesopotamia in the 9th century, had surprising optical properties due to the presence of silver and/or copper nanoparticles in the upper layers of the glaze. [1]

Nanomaterials are materials formed with materials in nanoscale range – with nanoparticles and/or through nanotechnological processing and according to the Official Journal of the European Union nanomaterial could be any natural or manufactured material which contains 50 % or more of particles that have one or more dimensions in the size of 1-100 nm. [4]

Nowadays, nanoparticles of different kinds are widely used in almost all fields. They are present in food, modern cosmetic products, electronics and used to purify water. In addition, nanomaterials represent one of the best candidates in the field of renewable energy sources. Artificially generated nanoparticles are particularly important in medicine. Magnetic nanoparticles are often used as a drug-delivery system (e.g. in treatment of cancer) and due to the hyperthermic effect (when high-frequency electric fields are applied, energy is released and it heats tissues) the nanoparticles can produce in the field of breast cancer treatment. [6] Silver nanoparticles exhibit high antimicrobial activity and catalytic properties; they are involved in tissue repair. [7]

1.1. Classification of Nanoscale Materials

1.1.1. Classification by Pokropivny and Skorokhod

Due to the synthesis of a large amount of nanoscale materials, it was necessary to create a specific classification of the obtained products. The first classification was proposed by N. Gleiter in 1995. But it was incomplete, because did not take into account fullerenes,

nanotubes and needed changes. The final version in 2005 was provided by Pokropivny and Skorokhod. Their classification includes such structures as 0D, 1D, 2D and 3D nanoscale structures. [8; 9]. If the diameters of the structure in all dimensions are within the range of 1-100 nm, then it belongs to 0D structures. This is, for example, nanoclusters and quantum dots. Nanowires belong to 1D structures because their length is much greater than 100 nm. Nanocomposites on thin films will belong to the group of 2D materials because both length and width is more than 100 nm. Finally, the bulk materials have all three dimensions above 100 nm and therefore refer to 3D. The whole classification can be found in the Figure 1. [8; 9]

1.1.2. Classification based on the physical and chemical properties of the particles

In addition, as far as every material consists of some sort of particles, there are classifications depending on physical and chemical properties of the particles. Based on this, we can distinguish the following main classes of nanoparticle (NP) materials, i.e. *carbon*-based NPs, *metal* NPs, *polymeric* NPs, and *lipid*-based NPs. [10]

1.1.2.1. Carbon-based Nanoparticles (NPs)

The two main classes include fullerenes and carbon nanotubes (CNTs). [10] The era of *carbon*-based NPs begins with the synthesis of fullerenes, named after the architect Buckminster Fuller, who designed geodesic domes. [11] They represent a globular carbon hollow cell that is mostly hydrophobic but it is possible to prepare a stable colloidal solution. [12] There are data on the antioxidant properties of water-soluble fullerenes and the absence of acute and subacute toxicity. Over the past decade one of the main directions has been the synthesis of fullerenes with atoms and molecules of metallic nitrides, sulphides, etc. captured inside of the cell. [13] The second class, carbon nanotubes are cylindrical graphene sheets with unique optical, mechanical and electrical properties. Their diameter is usually a couple of nanometres. Depending on the number of tubes present in the CNTs, they are divided into several types: single-walled CNTs, double-walled CNTs and multi-walled CNTs. [3; 12] They are used as sensors, as field-emission sources, filters or for other purposes. [12]

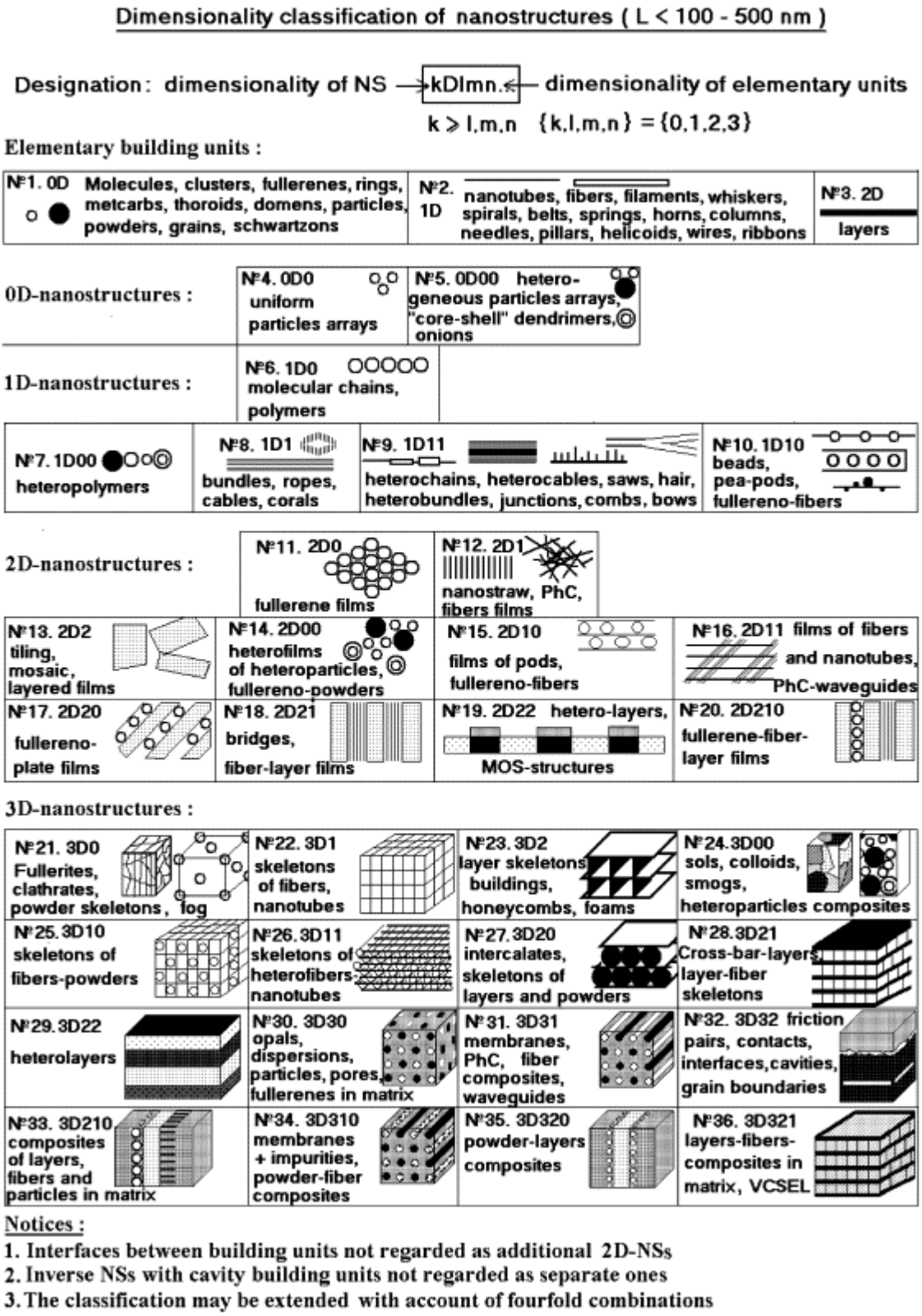


Figure 1. Dimensionality classification of nanostructures by V.V. Pokropivny and V.V. Skorokhod. [5; 9]

1.1.2.2. Metal Nanoparticles (NPs)

Metal nanoparticles (NPs) are obtained from metallic precursors. They are widely known for their optical and electric properties, they exhibit strong absorption in visible region of the

electromagnetic spectrum. [10] Metal NPs can be classified according to their size as ultradispersed, highly dispersed and micrometric particles. [7] The uniqueness lies in the formation of quantum dots (semiconductor particles) and magnetic properties. Metal NPs are widely used in environmental studies for water purification and in medicine in diagnostics and therapy. [10]

1.1.2.2. Polymeric Nanoparticles (NPs)

Usually organic nanoparticles could be classified into nanocapsules or nanospheres. [10] Nanocapsules have a matrix-like structure while the nanospheres have a core-shell structure and contain active elements in its cavity. [10] Both kinds of polymeric particles are commonly synthesized from polymers such as poly (lactic acid), polyvinyl alcohol, poly(D,L-lactic-co-glycolic acid) and others. They find their applications in medicine in the diagnosis and therapy of various diseases. For example, Abraxane®, which is protein-based drug delivery system (contains paclitaxel 100 mg in the form of nanoparticles bound to albumin), is prescribed for the treatment of breast cancer. [13] Biodegradation is one of the most important properties of polymer NPs because it allows the drug to be efficiently released and the particles themselves can be eliminated from the body by natural metabolic pathways. [14]

2. NANOFIBERS

Natural fibres are found everywhere – in animal bodies and plants. In animals, they usually consist of proteins and could be found, for example, in wool and hair, while plant fibres usually consist of α-cellulose, hemicellulose, lignin, pectin and could be found in stems, seeds and wood. [15] Recently, they are particularly interesting because of their unique combination of properties – strength, recyclability, and relative low cost of materials.

Nanofibers are solid fibres, differing in diameter, located in the nanometric scale and their length can reach up to a couple of meters. If only pure polymer is used to create fibres, they will not have any specific properties and can only be used as scaffolds. However, if other NPs or drugs are added to the polymer solution, nanofibers of a specific nature can be formed. [15]

Nowadays, nanofibers are of a great interest due to their wide application in numerous areas and especially in medicine. They are used as drug-delivery systems, in the field of tissue engineering, pharmacology, implant synthesis, etc.

2.1. Types of Polymers used for Preparation of Nanofibres

Typically, polymers are divided into two large groups, depending on their degradation: hydrolytically degradable polymers and enzymatically degradable polymers. [16] Polymers occurring in nature, belong to the second group and they can be considered the first bio-degradable biomaterials used in medicine. However, the rate of their decomposition varies greatly depending on the site of implantation and the concentration of enzymes. In addition, a large number of difficulties associated with their cleaning, which implies the possibility of transmission of diseases and immunogenic response. On contrary, synthetic biomaterials are supposed to be biologically inert and there is a possibility to predict their properties, but when a certain level of bioactivity is required, they come to the development of hybrid materials consisting both of synthetic polymers and natural ones. [16]

2.1.1. Hydrolytically Degradable Polymers

The group of hydrolytically degradable polymers usually include poly(a-esters), polyurethanes, poly(esteramide), and others. This class is distinguished by the fact that it has hydrolytically labile chemical bonds in its chain. [16] For their synthesis, two methods are usually used:

condensation (or step) polymerization and chain (additional) reaction. The latter mentioned also includes a ring-opening reaction (ROR). In addition, there is a radical polymerization, but in the course of this synthesis for the most part non-degradable polymers are synthesized. [16]

2.1.1.1. Poly(α-esters)

Poly(*α*-esters) are polymers that contain hydrolytically labile ester bonds. The greatest attention has been lately paid to poly(anhydroxy acid) s, such as PLA and PGA. Polyester can be synthesized with polycondensation as well as ROR. However, the first method is not used now in practice, not only because of the difficulty to obtain high molecular weight polymers with its help, but also because of commercial disadvantage. ROR provides requires milder conditions for the reactions to take place and the time needed for the synthesis is much shorter. In addition, specific initiator molecules can control the final molecular weight of the polymers. [16]

Degradation of poly(*α*-esters) are routinely considered to be bulk erosion, i.e. degradation occurs throughout the whole material equally and its speed depends on the initial volume of the material. In addition, the result of erosion does not have a linear dependence. [16]

One representative of poly(a-esters) is polyglycolide. This is a highly crystalline polymer, with a high tensile modulus (about 12.5 GPa) and low solubility in organic solvents. Despite the low solubility, this polymer was used in medicine as one of the first. In 1969, the first suture material was approved based on Polyglycolide - Dexon®, monofilament absorbable sutures. [17] At the moment, Dexon® is used to approximate soft tissue and/or ligation, including ophthalmic procedures. In addition, it is used as orthopaedic fixation devices. [16]

In terms of degradation, polyglycolide is a classic poly(a-esters) and undergoes bulk erosion. The polymer loses its strength after 1-2 months, and the loss of mass occurs for 6-12 months. (16) In human body it can be excreted in urine in the form of glycine or enter into the Krebs cycle and be excreted in the form of carbon dioxide and water.

The next representative of the group is polylactide. Its structure and the structure of the corresponding cyclic lactone, from which polymers could be obtained is showed in the Figure 2. Unlike polyglycolide, this chiral molecule can exist in two forms, L and D. Polymerization of monomers leads to the synthesis of semicrystalline or amorphous structures. Poly(L-lactide) is an ideal biopolymer for orthopaedic fixation devices, because it has high strength and high tensile modulus - 4.8 GPa, but degrades at a slower rate. [16] Complete *in vivo* degradation can take from 2 to 6 years, despite the fact that in approximately 6 months the polymer loses its

strength during hydrolysis. At the same time, poly(DL-lactide) is amorphous and its tensile modulus is approximately 1.9 GPa. [16] But considering this and the high rate of degradation, it is an excellent candidate for drug delivery vehicles.

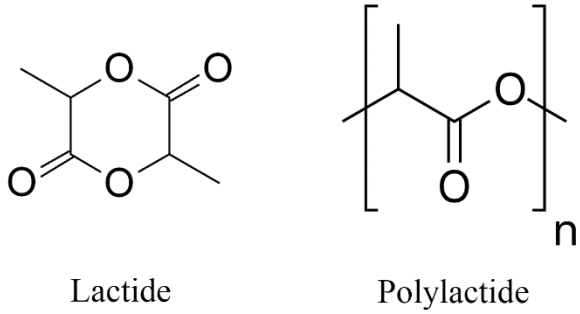


Figure 2. Structure of cyclic lactone lactide and corresponding homopolymer polylactide. [16]

Polydioxanone represent another polymer in the polyester group. It is a semicrystalline polymer, usually obtained by ROR from p-dioxanone. It was first used for monofilament suture, which benefited from a low risk of developing infections from multifilament sutures, under the commercial name PDS in the 80's. [16; 18] It is excreted from human body in a form of glycoxylate with urine or in a form of carbon dioxide and water, after passing a cycle of tricarboxylic acids. Like polyglycolide, it loses its strength after 1-2 months during hydrolysis, and the loss of mass occurs for 6-12 months. [16]

Polycaprolactone is another member of the polyester group. It is a semicrystalline polymer soluble in organic solvents and which is normally obtained by the use of ROR from ε-caprolactone. [16] The rate of degradation is low, about 2-3 years, and due to this fact, it is a good candidate for a long-term drug delivery vehicle. [16] One of the commercial examples is Capronor® – subdermal contraceptive that releases levonorgestrel over a 12- to 18-month period. In addition, e-caprolactone is often used in combination with other monomers (e.g., glycolide, lactide and poly (ethylene glycol)). [19] In addition, these biopolymers, which are synthesized by bacteria as a source of energy, are also used. [16]

2.1.1.2. Polyurethanes

Polyurethanes are polymers with carbamate (urethane) links. Most of them are thermosetting polymers. Polycondensation is usually performed via the reaction of di- or poly-isocyanate with a polyol. However, several studies conducted recently have focused on reduction of isocyanates

with respect to toxicity. Polyurethanes are often used as long-term medical implants, for example – cardiac pacemakers. [16] In addition, the commercial version of elastic poly (ester urethane) of Italian manufacture – DegraPol® is already used for research purposes for cardiac tissue engineering and in the field of cartilage repair. [16]

2.1.1.3. Poly(ortho esters)

The next group is polyorthoesters. These polymers are divided into four generations, which are showed in the Figure 3. They have found a wide application in the field of drug-delivery because of one of its distinctive features - undergo surface erosion (degradation occurs layer by layer, and not throughout the whole material equally, as in the case of bulk erosion). Polyorthoester type I is usually obtained by the transesterification of the reaction between diol and diethoxytetrahydrofuran. In an aqueous medium, it is autocatalytically hydrolyzed in an uncontrolled manner. Therefore, it should be stabilized when used as an implant material. Its commercial application was unsuccessful because of above-mentioned reason and complex synthesis. [16]

The synthesis of poly(ortho ester) II (POE II) was aimed on overcoming the negative aspects of the synthesis of the first generation. In this case, the polyaddition reaction between diols and diketene acetal 3,9-bis (ethylidene 2,4,8,10-tetraoxaspiro [5,5] undecane) was used. Polymers of this type are hydrophobic and less acid sensitive than polymers of the first generation. The rate of degradation can be changed by adding special modulators (e.g., adipic acids). [16]

Polyorthoester type III is synthesized using direct polymerization. However, their use in medicine is limited not only because of their propensity to form a gel-like material, but also because of prolonged synthesis and poor reproducibility. [16]

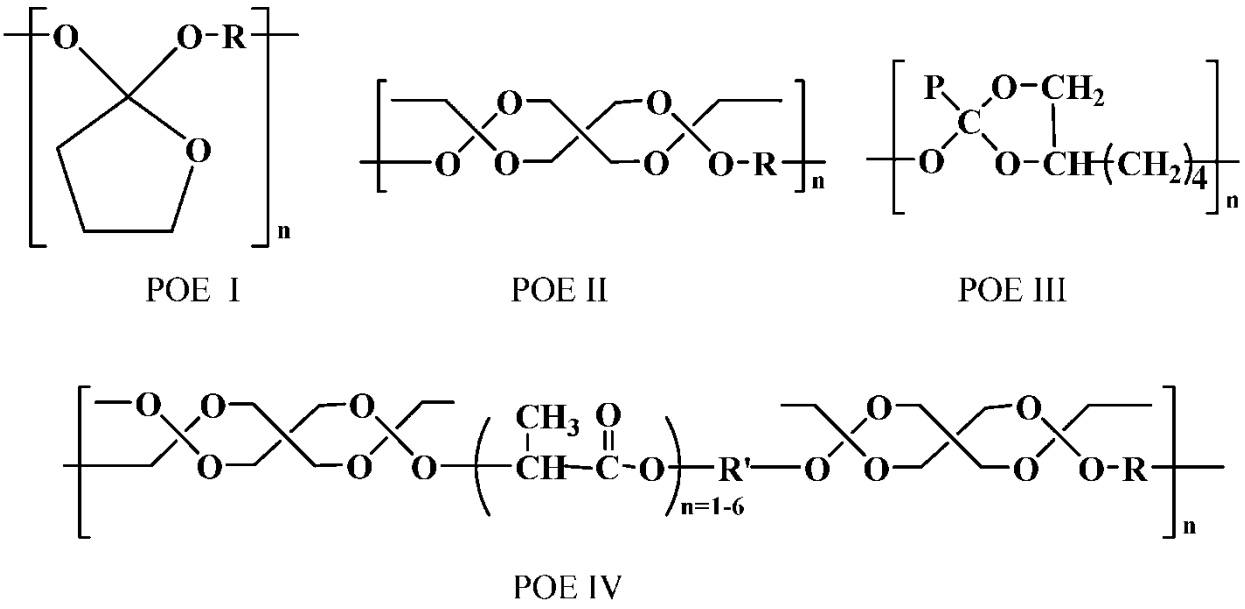


Figure 3. Structures of different poly(ortho ester)s. [20]

This all led to the synthesis of poly (ortho ester) IV, which are a modification of the second generation. The rate of degradation can vary from a few days to a couple of months, depending on the amount of glycolic or lactic acid in the sequence. In addition, changing the diols included in the polymers can be obtained as solid materials, and soft gel-like. [16]

2.1.1.4. Polyanhydrides

A group of polyanhydrides is one of the most studied groups due to a number of distinctive properties. Synthesis of polymers from this group includes one step and comes from readily available cheap resources. They are hydrolytically labile and the rate of degradation is easy to predict, since it has a linear dependence. It is derived from the human body completely. [16] This group of polymers include poly[(carboxy phenoxy propane) - (sebacic acid)], poly(anhydride-co-imide), poly(propylene fumarate), pseudo poly(amino acid), polyphosphazenes and some others that are not necessarily to be named.

The most studied one is poly [(carboxy phenoxy propane) - (sebacic acid)] (PCPP-SA). In the case where the polymer is loaded with a drug, the release of the substance can occur from a couple of days to several years, depending on the weight of the polymer and the ratio of its monomers in the formulation. It was approved for use as a localized delivery vehicle for the treatment of brain cancer (known by the commercial name Gliadel®). [16]

2.1.2. Enzymatically Degradable Polymers

2.1.2.1. Proteins and Poly (amino acids)

Proteins are one of the main biopolymers, which are natural components of any organism. They form a three-dimensional structure, passing four stages of synthesis in the human body. And being a natural component of any tissue, proteins have become the basis for a variety of different scaffolds and drug delivery vehicles. Protein structures include such polymers as collagen, elastin, elastin-like peptides and others. [16]

Collagen. Collagen represents a group of the most important polymers in the human body, usually found in connective tissues. At the moment, more than 20 types of collagen are known, but only one of them predominates in the human body - type I collagen. [21] It is mainly located in skin, tendon, bone, and dentin and performs the function of resistance to tension. [21] The length of each molecule is 300 nm, and its width is 1.5 nm. [22] Under the action of such enzymes as, for example, collagenases, collagen decomposes to the corresponding amino acids.

Since it is a natural polymer in human body that supports such cell functions as adhesion to the substrate and proliferation, collagen has been used in medicine in the field of tissue engineering and wound dressing. In addition, it has another pair of important properties – non-immunogenic and hypo-allergenic. When used as wound dressing, collagen acted as a mechanical support for damaged tissues, thereby reducing swelling and inflammation. [21]

One of the commercial representatives - Promogran® matrix wound dressing, consists of 45% oxidized regenerated cellulose (ORC) and 55% collagen, is used for diabetic ulcers, pressure ulcers, ulcers caused by mixed vascular aetiologies and in a number of other cases. [23] Currently, there are couple of products available on market such as Biobrane®, Alloderm®, TransCyte®, etc. [16]

Recently, bovine or porcine skin is used as a source of collagen, which complicates its biomedical application. Cleaning increases the cost of collagen, variable physico-chemical and degradation properties make it difficult to work with it, and there is a risk of transmission of infectious diseases. [22; 24]

Elastin. Elastin is usually located in the extracellular space and is mainly inherent in the connective tissue of elastic organs, such as the lungs, skin, vessels and others. [21] Similarly to collagen, the name "elastin" refers not to one particular molecule, but to a group of proteins and peptides.

This polymer is directly related to the smoothing of the skin and the appearance of wrinkles. In humans, elastin biosynthesis slows down with age and this fact has been used by many cosmetic companies that have begun adding it to their products. [21] In addition, elastin is often used to synthesize heart valve prostheses. However, their use is often complicated by calcification. [22]

One of the interesting characteristics of elastin is the inverse temperature transition (ITT), which characterizes how elastin is transformed from a disordered form to an ordered form. This allows us to use elastin as a smart, injectable drug delivery systems. [22] Natural human elastin, however, is insoluble, which is why the recombinant has been created, which has expanded the limits of the use of elastin with biomedical purposes.

Fibrin. The next known biopolymer of protein origin is fibrin. It is similar to collagen and takes part in the blood clotting process. [22] It is also one of the biopolymers first used as biomaterials. The first product was a fibrin sealant, used for various surgical operations as a haemostatic. [22] In addition, it was studied as a transport system for biologically active substances. In combination with keratinocytes is a product of Bioseed®, used to treat chronic wounds. [16]

2.1.2.2. Polysaccharides

Polysaccharides are macromolecules formed by a great number of monosaccharides, linked together by glycosidic linkages, which play an important role in providing vital activity to the cell. But, as a rule, polysaccharides are not basically biodegradable due to lack of digestive enzymes because of this they often need further chemical modification. [16]

The most important representative is the group glycosaminoglycans (GAGs). GAGs are unbranched polymers consisting of a recurring disaccharide unit, which in turn, usually consist of an amino sugar with uronic sugar or galactose. In addition, they are an integral part of proteoglycans. [16]

Hyaluronic acid, which structure is presented in the Figure 4, is the main representative of the glycosaminoglycans group, water-soluble, and forms highly viscous solutions, is the basis of connective tissue, being present in large quantities in cartilage, the cervix and skin.

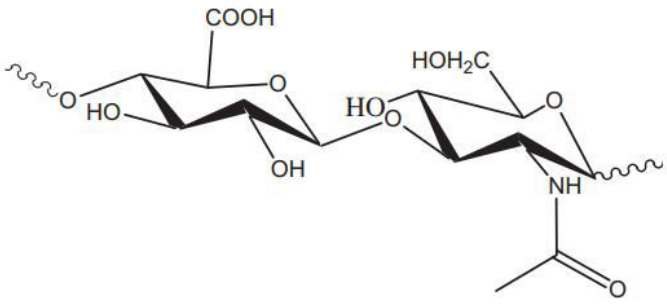


Figure 4. Structure of HA. [16]

Like collagen, hyaluronic acid can be easily modified (for example, by esterification). By regulating the rate of degradation and accelerating angiogenesis and cell migration, hyaluronic acid can be widely applied in wound dressing application. [16; 22] There are products based on hyaluronic acid, such as Ossigel®, Hyaff®, Amvisc® and many others are known.

We should also mention cellulose and starch. They are not biodegradable polymers, which virtually excludes their use in biomedical applications, but many researches are conducted to modify them and to study the possibility of their use. [22]

2.1.3. Hybrid Materials

It is also worth mentioning hybrid materials, which are mainly composites from polymers and inorganic particles. Looking at the huge number of possible combinations, this group is very extensive. A typical example of used particles is silver nanoparticles (Ag NPs), which are especially important in medicine, namely wound management. The AgNPs possess promising antimicrobial properties. When AgNPs interact with a bacterial membrane, the formation of "pits" occurs, because of this the permeability of membranes changes and the bacterium dies. In addition, these nanoparticles are prone to the formation of free radicals (which also damage the bacterial membrane) and to a violation of DNA replication. In the light of the emergence of many antibiotic-resistant strains of bacteria, the use of AgNPs is a good option. Polymer materials that are already used as wound dressings, because of their unique properties, can be improved by applying them in combination with Ag NPs. [25]

Studies of such a composite as chitin / nanosilver for wound dressing applications have shown in addition to its' antibacterial properties, also a blood clotting ability that was much higher than that of chitin without nanoparticles or commercial collagen. [26]

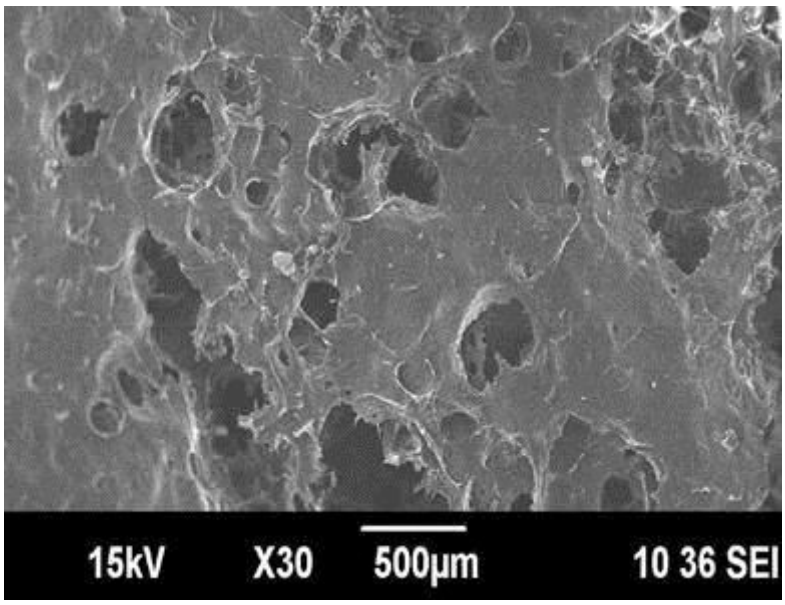


Figure 5. SEM image of chitin / nanosilver composite scaffold. [26]

Collagen/chitosan scaffolds loaded with AgNPs, studied by Chuangang You et.al showed similar anti-inflammatory properties, regulated the migration of fibroblasts and the activation of macrophages, which promoted wound healing. [27]

2.2. Preparative Methods of Fibre Formation

Once the polymer is ready, there are a number of techniques to create its fibers. This is self-assembly, phase separation, spinning and others. Self-assembly is a method inspired by the natural formation of peptides from amino acids in a living body. [28] There exist various driving forces to synthesize proteins – the vary from van der Waals forces to pH change. [28] Phase separation is the production of fibres by means of thermodynamic changes. At the moment, this method produces porous membranes for filtration and studies the possibility of using this method for scaffold fabrication. [28]

2.2.1. Spinning Methods

Spinning methods are devoted the greatest attention. Their essence lies in the fact that the polymer must be initially transformed into a liquid state (dissolved or melted), and then forced through the spinneret, then it cools to a solidified state. [28] There exist such techniques as wet spinning, dry spinning, melt spinning, electrospinning and others.

Wet spinning and dry spinning are used when the polymer cannot be melted. When using wet spinning, the spinneret is submerged in a chemical bath filled with another fluid that

is not a solvent for the polymer and the resulting fibres in it are solidify, as it emerges. When dry spinning solidification of fibres occurs it is connected with the evaporation of the polymer solvent. [29]

The most important type of spinning methods is electrospinning, which is used in this work. The meaning of the technique is that fine fibres are generated from a solution or a melt of a polymer using an electrostatic force. This method will be discussed in more detail in the next chapter.

2.3. Applications

Nanofibers can be used in numerous end-products. They find their application in water purification, air purification, and can be used as energy sources and drug-delivery systems. In this part, only a couple of areas of their application will be considered.

2.3.1. Environmental Engineering Applications

Non-woven fibres have become one of the materials for the preparation of membranes used in environmental engineering applications. They display high porosity and can be easily modified for different tasks. [30]

The problem of water purification is one of the most important global up-to-date problems and the use of such membranes can help to solve it. Membranes are used as a carrier of active nanoparticles, which attract toxic heavy metal ions by adsorption and electrostatic attraction mechanisms. [30] In addition, such membranes can be used in air purification. By controlling the pore size and additive substances, it is possible to purify air from both solid and gaseous pollutants. Last but not the least, these kinds of membranes are used due to their unique high surface area and because they have the ability to increase the conductometric properties of the sensors. [30]

2.3.2. Energy generation applications

Given that natural energy sources are depleted, it is necessary to seek new ways of obtaining energy. [30] Nowadays, nanofibers are investigated due to a possibility to create polymer batteries that can replace lithium batteries, and photovoltaic cells. [30]

2.3.3. Defence

Protective clothing used today is not practical enough and mobile, so there are many studies of the use of nanofibers in the field of protection. [30] Due to their properties, it is possible to create a lightweight material that allows detecting chemical and biological warfare agents and also that can protect a person from toxic substances. [31] Moreover, they have the ability to catalyse decomposition of organic chemical warfare stimulants. [31]

2.3.4. Healthcare Applications

Drug Delivery. The field of drug delivery deals with the direct transport of a drug substance to a specified area of the body. The development of biomedical science and biotechnology has led to the creation of new means of packaging and delivery of medicinal substances, for example, nanosomes and nanocapsules. [32]

Nanofibres can be generally classified into three groups: nanofibers with a core-shell structure where the core is responsible for carrying the encapsulated substance; fibres carrying the medicine on their surface; and fibres where the medicine is scattered throughout the fibre matrix. [33] The fibre structure, its size, mass and diameter can be easily adjusted by changing the synthesis parameters, which is an important plus in the work, since all these characteristics influence the release of the drug. [33]

Tissue engineering. Nanofibrous scaffolds are a matrix for the natural formation of tissues. If we compare nanofibrous and other types of scaffolds, they favourably differ in that they support the adhesion of cells and their proliferation. In addition, recent studies focus on creating matrices with pharmacologically active substances and growth factors. Thus, this area closely intersects with drug delivery and wound healing.

For these purposes, a large number of polymers are suitable. So, for example, for the regeneration of bone tissue synthetic polymers (poly (α-hydroxyl acid)) and natural (collagen, silk and chitosan) can be used. In the study by H. Yoshimoto poly (*ε*-caprolactone), scaffolds (PCL) coated with mesenchymal stem cells (MSCs) of rats were created. Further, these constructs were cultured with osteogenic supplements for 4 weeks. At the end of this time, the scaffolds were covered with cell multilayers and contained the mineralization and synthesis sites of type I collagen. [34]

Wound healing. Acute and chronic wounds represent one of the global healthcare problems. Wound dressing types can be classified into three groups – passive (gauze and tulle),

interactive (semi-permeable films, semi-permeable foams, etc.) and bioactive. The latter group includes collagens, hydrofibres, hydrocolloids. [35] Their important distinctive feature is the possibility of modification. Thus, biopolymers can contain important biologically active substances-various antibiotics (gentamicin in collagen foams and ofloxacin in silicone gel sheets), growth factors (for example, factors such as epithelia growth factor (EGF), fibroblasts growth factor (FGF), granulocyte- macrophage colony-stimulating factor (GM-CSF)), vitamins (such vitamins as A, E and C). [35]

Nanofibers have a number of properties that distinguish them from conventional Methods. They accelerate the first phase of wound healing (hemostasis), because of the high surface area to volume ratio they absorb water and exudates, the presence of pores flow moisture, repeat the edges of the wound, promising in healing wounds without scars. [35]

3. POLY (LACTIC-CO-GLYCOLIC) ACID

Poly (lactic-co-glycolic) acid (PLGA) is one of the most commonly used hydrolysable polymers used in medicine. Despite the success of the clinical use of PGA, studies of the synthesis of PGA and more hydrophobic polymer were carried out to expand the range of its application and one of them was PLA. The resulting polymer, PLGA, has been studied in various ratios of its homopolymers and has been applied in all sorts of industries. A widespread fame to this polymer came after the approval of the Food and Drug Administration (FDA) for use in humans. [29] Its biocompatibility and biodegradability make this polymer an ideal candidate for use in all biomedical fields. [23]

3.1. Chemistry of PLGA

As we can see from the text above, poly(lactic-co-glycolic) acid consists of two monomers – glycolic acid and lactic acid. [29] To produce high molecular weight polymers, ring-addition polymerisation is used as it is shown in the Figure 5, since the condensation reaction is reversible.

In addition, for the reaction, catalysts must be used. Usually tin compounds act in their role – e.g. tin (II) 2-ethylhexanoate and tin (II) alkoxides. [29] Unfortunately, their full elimination from the prepared polymer is impossible. It is demonstrated in the penetration of toxic tin compounds into the blood circulation of patients. Several attempts have been made to change the catalyst (for compounds such as zinc lactate, for example), but this did not give satisfactory results and the attempts to synthesize polymers of sufficient molecular weight were unsuccessful. [29]

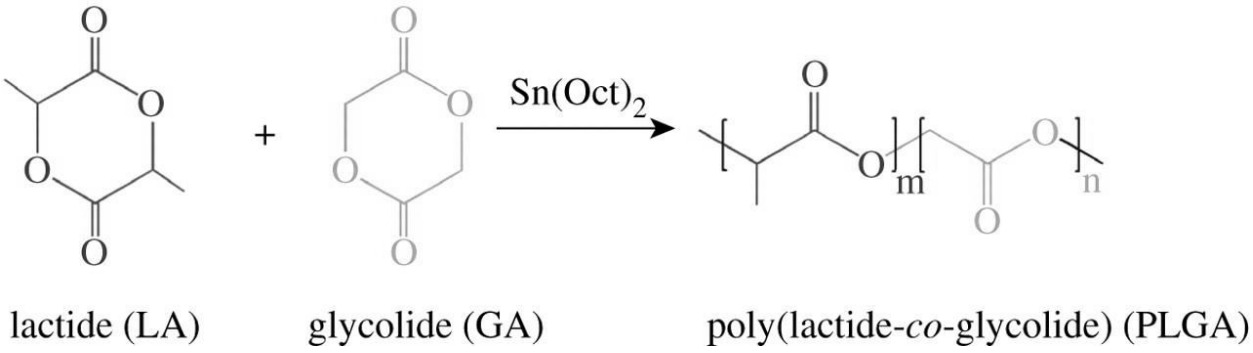


Figure 6. Ring opening polymerization of lactide and glycolide. [36]

3.2. Properties of PLGA

A significant advantage in using this group of polymers is that they can be synthesized with any necessary property. This is mainly achieved by manipulating three factors – the molecular weight of the polymer, the enantiomer of the lactide ester and the ratio of these acids in the resulting polymer. [29]

As mentioned above, the properties of PLGA depend on the ratio of the monomers used for its synthesis, and it should be noted that there is no linear relationship between them and the percentage of monomers. The surprising fact is that the PLGA degrades faster than the PLA and PGA separately. [30] This is due to the loss of crystallinity of the PLGA, since this property is responsible for the rate of hydration and hydrolysis. The mechanical strength and absorbability of the polymer also depend on this characteristic. Mechanical strength also depends on the high (45°C) glass transition temperature. [37] Unlike its monomers, PLGA shows good solubility in various solvents- for example in chlorinated solvents, tetrahydrofuran, acetone, dioxan, or ethyl acetate.

3.3. Degradation

3.3.1. *In vitro* Degradation

The polymer degrades according to the principle of bulk degradation, which scheme is seen in the Figure 7, which means that degradation occurs throughout the polymer matrix. [29; 37]

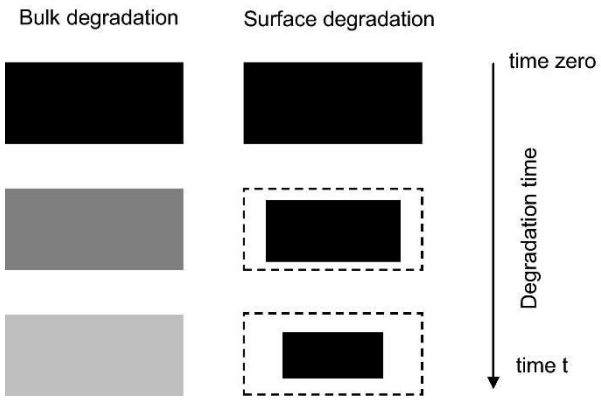


Figure 7. Schematic illustration of bulk degradation. [38]

It has been thought that PLGA undergo homogeneous bulk degradation, however, according to the latest data, large-sized PLGA devices are experiencing a heterogeneous process of degradation, namely - the core degrades faster than the surface of the device. At the Figure 8 the example of this type of degradation is pointed out. [37] The core of the tablet after

20 days of incubation in phosphate buffer was completely degraded. Vert et al. in their work, "Hydrolytic degradation of devices based on poly (DL-lactic acid) size-dependence", suggest that this effect is associated with "diffusion-reaction-dissolution" phenomena. This phenomenon is that at the beginning of degradation soluble oligomers slip from the surface of the matrix device faster than from the core, there the most gradually reducing the autocatalytic effect at the carboxyl-depleted surface. [37]

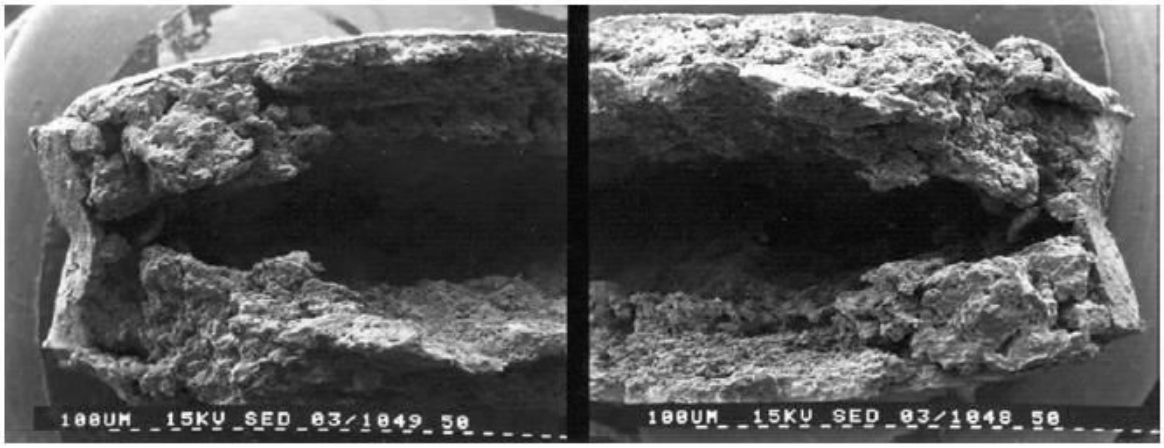
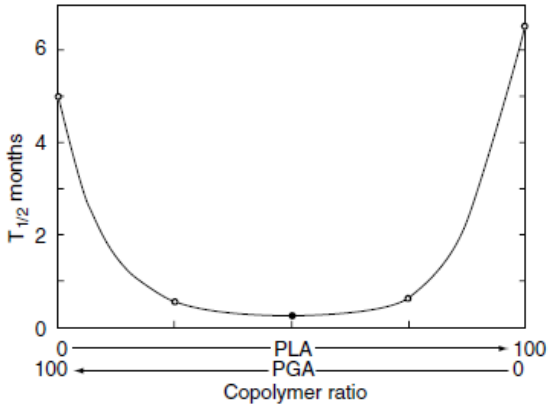


Figure 8. Scanning electron microscope picture of a DL-PLGA (50 : 50) tablet after 20 days of incubation in phosphate buffer, pH 7.4 at 37°C. [37]

Degradation is also influenced by many other factors such as crystallinity, molecular weight, the percentage of each of the monomers, the glass transition temperature and, last but not least, the environment, in which the degradation occurs. A polymer containing glycolic acid and lactic acid in a 50:50 ratio undergoes the fastest degradation – approximately two months. [37] Other degradation times and its relation on time is shown in the Figure 9.



Polymer	Biodegradation time (months)
L-PLA	>24
DL-PLA	12–16
DL-PLGA (85:15)	5–6
DL-PLGA (75:25)	4–5
DL-PLGA (50:50)	1–2
DL-PLGA (50:50)*	<1
PGA	6–12

*Copolymer with free carboxyl end groups

Figure 9. On the left: half-life in months of lactide/glycolide polymers implanted in rat tissue. On the right: approximate resorption times of commercially available lactide and glycolide homopolymers and copolymers. [37]

3.3.2. *In vivo* Degradation

In vivo, this polymer decomposes according to an identical principle, as well as *in vitro*. It is believed that none of the cells of a living organism or enzymes participate in the decomposition. The only difference is the emergence of a foreign body reaction. Cells participating in this reaction can produce radicals or acid metabolites, which results in faster hydrolysis. In addition, effects such as, for example, the absorption of lipids on the surface of the PLGA can influence the course and rate of degradation. [37]

However, at the stage when the polymer matrix undergoes fragmentation, these fragments are absorbed by phagocytes and are intracellularly hydrolysed to lactate and glycolate, which is the final product of the decay of this polymer. Elimination of these metabolites occurs in various ways. L-lactate is converted to pyruvate, which enters the Krebs cycle, and D-Lactate is found in unmodified form in the excreta. Glycolate can also join different processes. One of the ways of its elimination is directly with urine in unaltered form, like D-lactate, the other is the transformation into glycine, serine, and pyruvate. [37]

Various studies provide different data on rates of *in vitro* and *in viv*o degradation. At some speeds are equal, while others say that the rate of decomposition in living tissues is higher.

3.4. Biocompatibility and Tissue Reactions

As with any other implant, the body's response to implants based on the PLGA depends both on its volume and surface properties, and on the very organ and tissue in which implantation occurs. All the studies performed show that the body's response is either absent or there is a mild inflammatory response, which disappears with time. Allergic reactions were not observed in any of the studies. [37]

Response to PLGA implants occurs, as a rule, in three phases. The first phase involves the initiation of acute and chronic inflammation. There is minimal inflammatory reaction involving polymorphonuclear leukocytes, lymphocytes and other cells. During the second phase monocyte accumulation and neoangiogenesis are observed. Besides, new formed vessels grow into the implant. The third phase usually means the fragmentation and phagocytosis of polymer fragments. [37]

There are many factors that influence the development of foreign body reactions and the most important of them is the purity of the implant used. Clinical trials have confirmed the

biocompatibility of PLGA, but there are data of severe foreign body reaction that occurs in 5 - 8 % of patients with PGA-based implants. Tiainen et al. noted that basically, these immune responses mostly repent of homopolymers (PGA and PLA). [37]

3.5. Spinning of PLGA Fibres

Synthesis of woven structures from a polymer can occur in a variety of ways, as discussed earlier in Chapter 2. However, Electrospinning of PLGA fibres remains the most convenient and functional way.

Electrospinning is an old method, first observed in 1897 by Rayleigh and then studied by Zeleny in 1914. Taylor's "Electrically Driven Jets" in 1969 became the basis for the entire method. Despite its age, this method has found the greatest popularity only now, with the development of nanomaterials. [39] This method uses electrostatic force, applying it to polymer solutions or melts, for the production of fine fibres. Thus, using a current of a couple of kilovolts, it is possible to overcome the weak surface tension forces of the polymer. [39]

Electrospinning occurs at atmospheric pressure and at room temperature. Despite the fact that the researchers create different equipment for fibre spinning, two types of setups – vertical and horizontal - are still the most commonly used at the moment. Their set-ups are shown in the Figure 10.

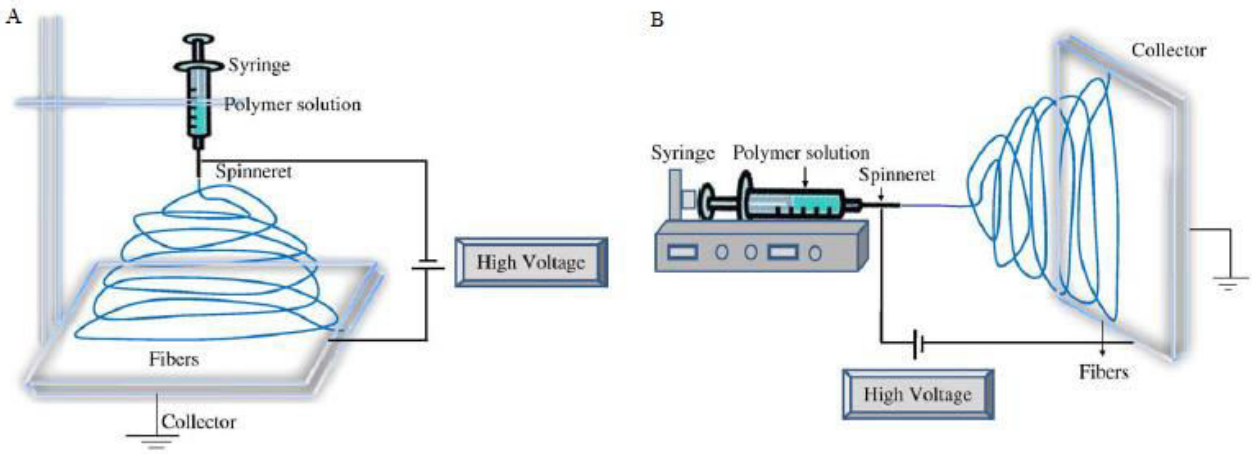


Figure 10. Schematic diagram of set up of electrospinning equipment. A – vertical set up and B - horizontal set up. [39]

But any setup contains three main elements – a source of high voltage, a spinneret (a syringe) and a collector. The last can act as stationary plates or rapidly rotating wheels, which must necessarily be conductive and grounded. [39]

If we consider this process in more detail, then when the voltage is applied to the polymer, the electric charge accumulates on the surface of the liquid and the so-called Taylor cone (deformation of the liquid surface) is formed. When the electrostatic force reaches a critical value, surface tension is overcome and, as a result, jet of liquid is emitted.

The result obtained with the use of this method is influenced by a huge number of different parameters both of the polymer solution itself and the setup of the apparatus. So, the molecular weight of the polymer, the concentration of polymer in solution, viscosity, surface tension and the choice of solvent play an important role. [39]

3.5.1. Processing Parameters

3.5.1.1. Applied Voltage

The choice of the applied voltage represents one of the most important parameters, since only after overcoming the critical value of tension does the synthesis of fibres take place. Some researches proves that when using high voltages, a strong stretching of the polymer solution occurs and, as a consequence, finer fibres. However, there is no consensus on this issue. [39] In different published research papers we can find that with an increase in the electrostatic force, the opposite is the excitation of more polymer and, as a consequence, the opposite result – the fibres become thicker. [39]

3.5.1.2. Flow Rate

The next important factor is the rate, at which the polymer solution passes through the syringe. It was shown that the best choice is the minimum possible rate under which the fibres are formed since then the solvent has sufficient time for evaporation. In addition, at higher flow rates, the fibres tend to thicken and increase the pore diameter. [39]

3.5.1.3. Types of Collectors

There are two fundamental types of collectors – a rotatory and static one. Typically, aluminium foil is commonly used as a finishing layer covering any type of a collector, which is commonly used. However, different researchers in their experiments use conductive paper, conductive cloth, wire mesh, rotating wheel and even liquid methanol coagulation bath. [40] Having different conductivity, all these collectors differently affect both other parameters of jetting and the characteristics of synthesized fibres.

3.5.1.3. Tip to Collector Distance

The distance between the collector and the tip dosing polymer plays an important role because if it is too large or if the needle is too close to the collector, fibres cannot be synthesized and instead of the fibres, beads start to be generated. In addition, this parameter is closely related to the voltage applied to the polymer solution. The dependency is graphically shown in the Figure 11 it is necessary to competently combine the strength of the electrostatic field and the distance to the collector.

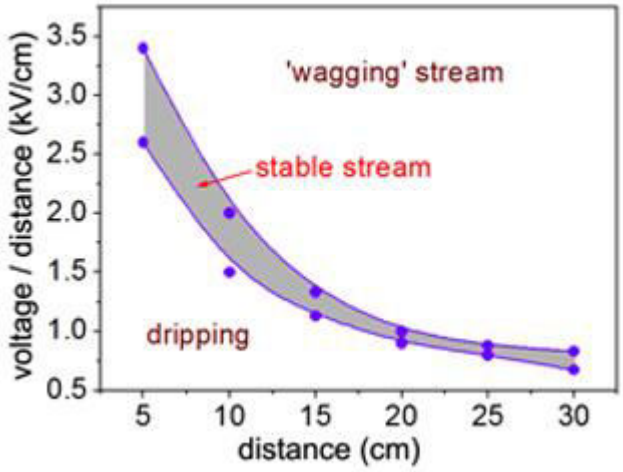


Figure 11. The relationship between the distance between the syringe tip and the collector must and the applied voltage to obtain a steady streaming jet. [40]

In addition, it is necessary to add that there are other factors that can influence process of fibre generation. Let´s mention at least humidity and temperature in the laboratory. These factors influence the behaviour of the polymeric solution, namely the behaviour of the solvent used. [39]

3.6. Medical Applications of PLGA Fibres

Taking into account such properties as biodegradability and biocompatibility, the ability to control the physical and chemical properties and the ability to adhere to the surface of subsequent nanoparticles and bioactive substances, PLGA has found wide application in medicine.

3.6.1. Pharmaceutical

A lot of researches performed with PLGA include the creation of drug-delivery vehicles. Since the property of biodegradability this polymer is ideally suited for the controlled release of medicines. The study of the release of antiepileptic drug Levetiracetam in PLGA 85:15 and PLGA 75:25 polymers, depending on the amount of drug loading confirmed that such fibres tend to release the drug to several months after their implantation and the percentage of the drug that enters the blood depends on the initial dose. [41] Moreover, fibres containing less than a drug substance were more easily released. [37]

3.6.2. Tissue Engineering

Tissue engineering is an application field dedicated to the formation of scaffolding materials for the maintenance and restoration of normal function of living tissues. Ideal material, for these purposes, should fulfil several functions, most important of which is the supporting cell attachment. This condition is completely satisfied when using hydrolysed biopolymers of synthetic origin, namely, PLGA. An important advantage of its application is also the possibility of manipulating the rate of degradation, enabling it to be adjusted to a specific tissue. In addition, the ability to produce it in any form (non-woven fibres or films) facilitates its use in various organs. [29]

3.6.3. Implants

Biodegradation allows the use of PLGA nanofibers as implants without the need for repeated surgical intervention. So, for example, studies were carried out on the delivery of growth factors to the area of osteochondral lesions in rabbit. In this case, the results were not only in favour of successful application of the implant, but also of the possibility of using the PLGA as a scaffold for tissue neogenesis. [29]

3.6.4. Wound Dressing

Fibres as a means of wound dressing have been used for several centuries, but now natural polymers are mostly replaced by synthetic ones. As mentioned above, PLGA is one of the polymers approved by Food and Drug Administration(FDA) and on its basis, there are already a number of commercially available drugs. For instance, the product under commercial name Vicryl® (10:90 PLGA) appeared in the 70s and became one of the starting points for studying the application of biodegradable polymers in the field of wound dressing. [29].

Since many fibre-based drugs are already available in stores, the main emphasis in modern research is put to attach bioactive drugs on the surface of fibres. For example, Intra et al synthesized an immunostimulatory suture (containing CpG oligodeoxynucleotides, which are short synthetic DNA molecules containing only one chain) based on the PLGA 75:25 for patients with neoblastoma, which helped not only to close the surgical site, but also to prevent recurrence of the disease. [42]

4. MATERIALS AND METHODS

4.1. Chemicals

The following chemicals were used to prepare PLGA polymer solutions: N, N,-dimethylformamide – DMF (anhydrous, 99.8 %; Sigma-Aldrich), tetrahydrofuran – THF (anhydrous, ≥99.9 %; Sigma-Aldrich), acetone (ACS reagent, ≥99.5 %) , chloroform (by LACHEMA, a.s.), poly (D, L-lactide-co-glycolide) – PLGA (ester terminated, Mw 50,000-75,000), poly [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)], which structure is shown at Figure 12, silver nanoparticles (patent PV-2017-806, "způsob přípravy koncentrované, agregačně stabilní disperze nanočástic stříbra, disperze a její použití"), sodium chloride G. R. – NaCl (Lach-Ner s.r.o.), acetic acid – $C_2H_4O_2$ (99,8 % G.R.; byLach-Ner), sodium carbonate – Na_2CO_3 (PENTA s.r.o.). All chemicals were used without further purification.

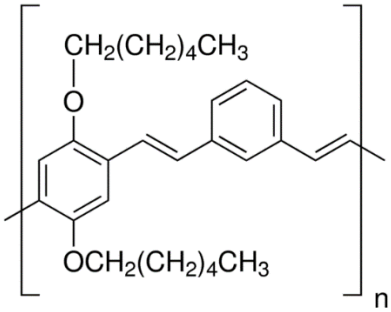


Figure 12. Poly [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)].

4.2. Equipment

Pelettes of poly (D, L-lactide-co-glycolide) (PLGA) were weighed on the analytical scales of Schoeller Pharma Prague. The polymeric solution was homogenized using a shaker MS 3 basic (IKA), shaking the solution from 30 minutes to 5 hours. The solution was transferred into a syringe, and then into a syringe pump (NE-1000 Programmable Single Syringe Pump), which was responsible for dosing the required amount in the process of electrospinning. As the collector a vial wrapped into a tin foil was used. The vial was attached to a rotator with tuneable speed of rotation (Stuart) and to the high voltage source (ES30, by Gamma High Voltage Research), the Figure 13 shows the design of the device.

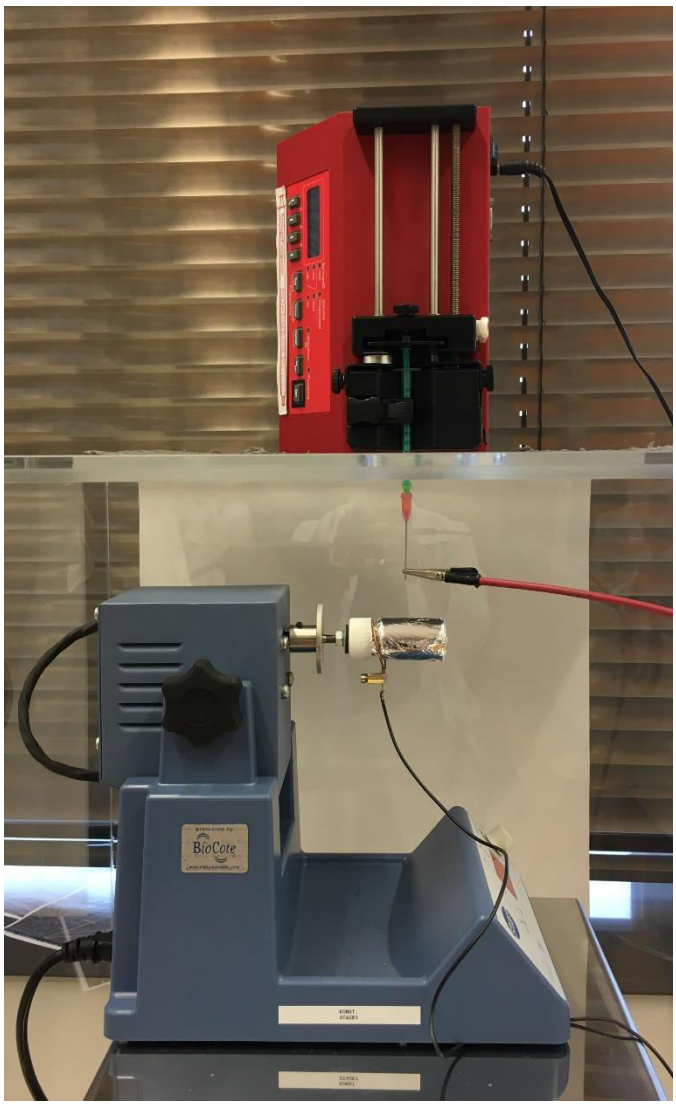


Figure 13. Design of the used electrospinning equipment.

The drying of the as-prepared samples of the generated fibres was carried out in a Binder 531 desiccator. The LAUDA M12B thermostat was used for the subsequent study of fibre degradation. To characterize the samples containing a mixture of fibres and nanoparticles an instrument working on a principle of dynamic light scattering (Zetasizer Nano ZS by Malvernpan Alytical) was used. Further processing of the samples was carried out with a Hettich Eba 20 centrifuge and an FB11201 ultrasonic bath (by Fisherbrand). The images of the fibres and images monitoring the degradation process were obtained from scanning electron microscope (SEM) Hitachi SU6600.

5. WORKING PROCEDURES

5.1. Preparation of Fibres

To generate all types of fibres (both oriented and mesh), an electrospinning method was used, which was described in detail in the theoretical part. Its set up was a classic version and consisted of three main elements – a source of high voltage, a spinneret and a collector. In addition, a similar method can be used for the synthesis of nanoparticles (in this case, it will be called "electrospraying"). [43] During this process, the jet of liquid under the influence of electric forces disrupts into droplets.

5.1.1. Generation of Oriented Fibres

In this case, a mixture of solvents - acetone and THF in a ratio of 50:50 was used to prepare a solution of PLGA. The percentage of polymer in solution is 30 % (w/w). To fully homogenize the solution of PLGA, it was shaking on the shaker for at least 30 minutes. The prepared solution did not require additional purification and was immediately injected into the syringe.

Further, by electrospinning method from the resulting solution, nanofibers were synthesized on a foil covering the vial. The distance from the end of the syringe needle to the surface of the vial was 2.5 cm. The speed of rotation of the vial was 40 rpm. The voltage was 10 kV. The rate of extrusion of the polymer from the syringe is 0.15 mL/hr. Finished fibres were in the desiccator for additional drying for at least 24 hours.

5.1.2. Synthesis of Oriented Fibres with Poly[(m-phenylenevinyl)-alt-2,5-dihexyloxy-p-phenylenevinyl])

For better visualization of the fibre formation process and their degradation, poly[(m-phenylenevinyl)-alt-2,5-dihexyloxy-p-phenylenevinyl]) was incorporated into the fibres dye as a dye. The crystalline dye was first dissolved in one of the proposed solvents, and then, this coloured solvent was used to dissolve the crystals of PLGA. The resulting solution did not require additional purification.

The oriented fibres were generated using acetone and THF as solvents mixed in the ration 50:50. The percentage of PLGA in solution again was 30 % (w/w). All spinning parameters remained the same as in the synthesis of oriented fibres without a dye. The distance from the tip of the syringe needle to the surface of the vial was 2.5 cm, the vial was rotating at a speed of

40 rpm. The applied voltage was 10 kV. The rate of extrusion of the polymer from the syringe was 0.15 mL/hr. The as-prepared fibres (Figure 14) were left in desiccator for additional drying for at least 24 hours.

Figure 14. Macrophotograph of oriented fibres (30 % (w/w) PLGA with poly[(m-phenylenevinyl)-alt-2,5-dihexyloxy-p-phenylenevinyl]), generated using acetone and THF as solvents mixed in the ration 50:50. The distance from the tip of the needle to the surface of the vial – 2.5 cm, rotating speed – 40 rpm. The applied voltage was 10 kV. The rate of extrusion of the polymer was 0.15 mL/hr.

5.1.3. Synthesis of Oriented fibres with Silver Nanoparticles

Silver nanoparticles (AgNPs) are well known due to their antimicrobial properties and as such were selected as the substance for incorporation into the overall volume of the generated fibres. Their content was established for 1 % (w/w) of the polymeric solution. The rest of the polymeric solution, all the parameters were kept identical to the previous cases – oriented fibres without/with the dye. The concentration of PLGA was 30 % (w/w), the mixture of solvents used was acetone and THF in the above-mentioned ratio of 50:50. The spinning parameters also did not require any further correction.

5.1.4. Synthesis of Mesh Structures

For the alternation in the morphology of electrospun fibres, a couple of variables of the previously mentioned systems were required. The first version of the mesh was generated using solvents such as chloroform and DMF in a ratio of 95:5. The polymer content in the solution is 30 % (w/w). The parameters were adjusted as follows: the distance of the collector from the needle of the syringe was 2.5 cm, the applied voltage was 7.5 kV, the rotation speed of the vial was 30 rpm and the extrusion speed of the PLGA solution was 0.08 mL/h. (Figure 15) A similar fibre sample was prepared using poly [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] as a model of an incorporated drug substance.

Figure 15. Macrophotograph of mesh fibres (30 % (w/w) PLGA). As solvents was used a mixture of chloroform and DMF in a ratio of 95: 5. The distance from the needle to the collector – 2.5 cm, the applied voltage – 7.5 kV, the rotation speed– 30 rpm and the extrusion speed – 0.08 mL/h.

The consequential systems counted with variation of the PLGA content - 20% (w/w), 10 % (w/w), 5 % (w/w) in a solution of a mixture of solvents such as acetone and THF in a ratio of 50:50. In the case of fibre formation from a solution with 20 % (w/w) polymer content, a rotating vial was used, as before. In the course of the electrospinning process, the distance from the needle of the syringe to the coil was 2.5 cm. The collector rotated at a speed of 30 rpm. The applied voltage was 7.5 kV. The extrusion rate of the polymer was 0.15 mL/h. When generating PLGA fibres from polymeric solutions containing either 10 % (w/w) or 5 % (w/w) a static collector (in a form of a plate covered with a tin foil) was used. In this case, the

distance from the needle to the collector was 5 cm. All other parameters remained unchanged. The speed of the extruding solution was 0.15 mL/h, the applied voltage was 7.5 kV.

The last tested system was a 30 % (w/w) solution of PLGA in a mixture of acetone and DMF in a ratio equal to 50:50. Both the rotating collector and the static collector were used. In the case of a rotating collector, it was adjusted to 4.5 cm from the tip of the needle. The rotation speed was 30 rpm. The applied voltage was 10 kV, the polymer flow rate was 0.15 mL/h. When using the static collector, the distance from the tip of the needle to the foil was also 2.5 cm, the polymer flow rate was 0.15 mL/h and the applied voltage was 7.5 kV. The generated fibres formed a kind of a sprayed surface on the tin foil according to which they could be detected from the macroscopic point of view (Figure 16).

Figure 16. Macrophotograph of mesh fibres generated with the use of 30 % (w/w) solution of PLGA).

5.2. Decomposition Process

5.2.1. Incubation of oriented fibres of 30 % (w/w) PLGA Fibres with Poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] in Distilled Water, Physiological Solution and Acetic Acid Solution.

The first decomposition processes were performed with oriented PLGA fibres containing poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)]. These fibres were chosen because of its morphology as the allow us to handle them without the underlying tin foil. A bunch of these fibres was placed into a thermostat for 1, 5 and 9 days and kept under the

temperature of 37 °C. The setting/organization of the whole experiment can be consulted in Figure 17. Three series of three vials were used. One vial from each set contained water, the other one physiological solution and the last one acetic acid solution with pH equal to 3.6. These solutions were chosen as a typical environment in which the fibres could be applied as a device for, as it is assumed that the obtained fibres will be used in the future in living systems as drug-delivery systems. The acidity of the skin surface on the average is equal to 5.5. [44], so an acetic acid solution was needed as a model of the acidic environment.

After the suggested incubation process, the fibres were taken from the liquid environment and dried in a desiccator for at least 2 days, and after that they were weighed on an analytical balance. Then the samples were characterized with SEM and the structures were compared with the structures of fibres before the stay in the thermostat.

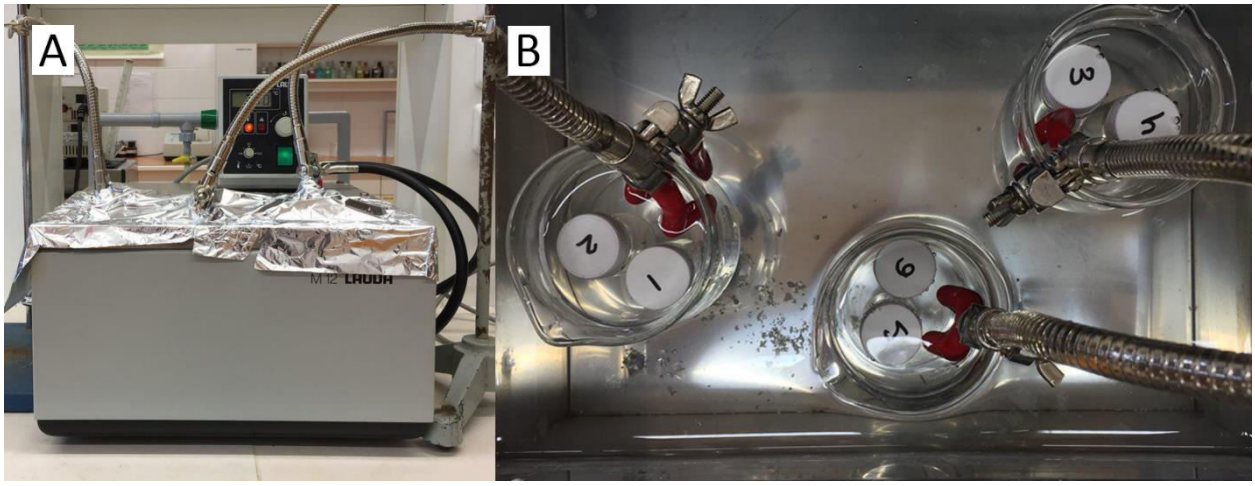


Figure 17. Set of the incubation test. **A** – view from the outside; **B** – view inside of the thermostat with a three series of two vials each.

5.2.2. Incubation of 30 % (w/w) PLGA Fibres with Poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] in Distilled Water and Physiological Solution.

In contrast to the first experiment, in this case, the pH of the solution was also measured after incubation of the fibres in it. This decision was made regarding the fact that lowering the acidity helps to heal the wound. For this reason, it was necessary to fix the pH change.

The experiment was carried out with identical oriented fibres prepared based on 30 % (w/w) PLGA in acetone and THF in a ratio of 50:50. For the test, three series of two vials each were required, each containing a suspended sample of fibres and filled with distilled water or physiological solution. Physiological solution was prepared right before the experiment and its' acidity was 6.21.

From the thermostat, the incubated fibres were extracted on the 3, 7 and 10 days. After vial extraction, the pH of the solution was measured, and the fibre samples were dried in a desiccator and weighed. A sample for SEM was prepared from the dried fibres.

5.2.3 Incubation of 30 % (w/w) PLGA fibres in Strongly Alkaline Environment

Further, a study of similar fibres without a dye was carried out, but under more conditions to the situation on the wound surface. Since the pH varies depending on the type of wound but always remains in the alkaline region, an extreme case was chosen to begin the study. [44] To achieve this, a solution of Na_2CO_3 with a pH of 11.30 was used. The temperature value also equalled 37 °C. Three series of two vials were prepared – in the distilled water and in the above-mentioned Na_2CO_3 solution. Samples were withdrawn from the thermostat on the 3^{rd} , 7^{th} and 10^{th} day.

Further, by analogy with the experiments above, the change in the acidity of the solution in which the fibres were found was investigated, and the fibres themselves were dried in a desiccator for at least two days and then weighed on analytical scales. A sample for SEM was prepared from dry fibres.

5.2.4. Incubation of 30 % (w/w) PLGA Oriented Fibres in Strongly Alkaline Environment and with the Influence of High Temperatures

Considering the local increase in temperature in inflammation, it was decided to carry out a similar test again, but at 42 °C. To create an alkaline environment, Na_2CO_3 solution was used, its' pH was 11.21.

Samples were removed from the thermostat on the third, seventh and tenth days. After their removal the pH of the solution was measured, and the fibres themselves were dried in a desiccator. Then a sample was prepared for SEM.

5.2.5. Incubation of 30 % (w/w) PLGA Mesh Fibres in Distilled Water and Physiological Solution

The next experiment was the testing of the mesh structure, synthesized rotating collector. As before, three series of two vials were prepared. One of them was filled with distilled water and another one with physiological solution. These vials were placed in a thermostat set to 37 °C

and were taken out after three, seven and ten days. In this case, the measurement of acidity was not carried out. After drying, a sample for SEM was prepared in the desiccator.

6. RESULTS AND DISCUSSION

6.1. Influence of Parameters on the Characteristic Features of the Generated Fibres

As in the theoretical part, and from the previous chapter, it is clear that the most important role in the fibre synthesis is played by the solvent and the concentration of the polymer. By variation of these two factors, it is possible to generate different systems – both oriented fibres and the mesh structures. These parameters directly affect the surface tension of the solution, which determines the type of the synthesized fibres. [32]

To generate oriented fibres with a smooth surface and a diameter of about twelve micrometres, it is necessary to use acetone and THF as solvents and a concentration of PLGA – 30 % (w/w). The optimal distance from the needle to the collector was 2.5 cm in case of the rotating collector. For the continuous generation of such fibres the flow rate was equal to 0.15 mL/h and the rotation speed of the coil of 40 rpm are needed.

By decreasing the concentration of the PLGA, we obtain a mesh structure, which in addition to the fibres also contains polymer nanoparticles, as indicated in the Figure 18 and Figure 19 obtained with the Zetasizer Nano ZS which could be found below. For this analysis, the mesh was scratched off from the foil and several times centrifuged to separate the fibres from the particles, then exposed to an ultrasonic bath. The resulting colloidal solution was analysed by dynamic light scattering machine.

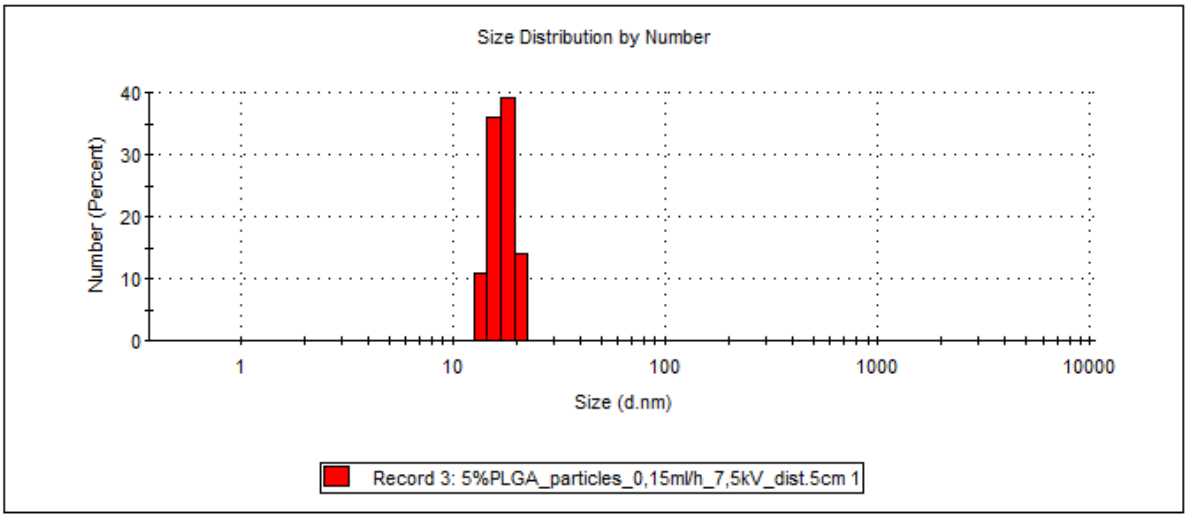


Figure 18. PLGA particles characterized with Zetasizer Nano ZS. The particles were prepared from the polymeric solution containing 5 % (w/w) PLGA, dispensed under the speed of 0.15 mL/h at the distance of 5 cm and applying 7.5 kV onto a static collector. The average size of the particles was 18 nm.

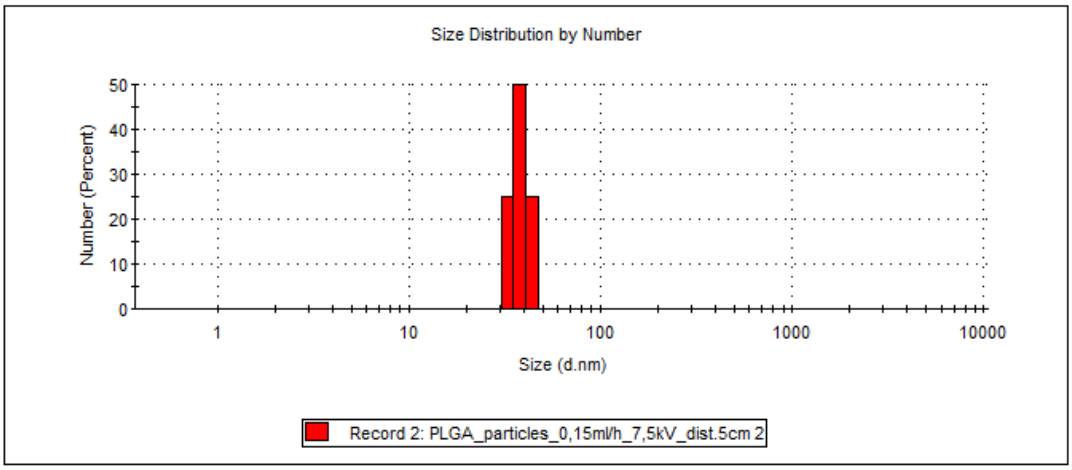


Figure 19. Zetasizer Nano ZS data of the particles were prepared from the polymeric solution containing 10 % (w/w) PLGA, dispensed under the speed of 0.15 mL/h at the distance of 5 cm and applying 7.5 kV onto a static collector. The average size of the particles was 38 nm.

The use of solvents such as chloroform and DMF in a 95:5 ratio, even with a polymer content of 30 % (w/w), did not provide a stable system and clearly oriented fibres. The obtained fibres, which can be seen in the Figure 15, could not be separated from the foil and used in similar incubation experiments in a thermostat, as acetone-based fibres. A solution of PLGA 30 % (w/w) in a mixture of acetone and DMF in a ratio of 50:50 also gave a homogeneous stable mesh in the macro analysis. The result can be seen in the Figure 16

6.2. Degradation

6.2.1. Study of Degradation of 30 % (w/w) PLGA Fibres with Poly[(m-phenylenevynilene)-alt 2,5-dihexyloxy-p-phenylenevynilene)] Incubated in Distilled Water, Physiological Solution and Acetic Acid Solution.

As described in the previous chapter, these fibres were analysed after 1, 5 and 9 days in a thermostat at 37 °C with SEM. The results are shown in the Figures 20-22. According to the SEM images, the initial fibres were about 12 μm in diameter and had a smooth surface (Figure 20).

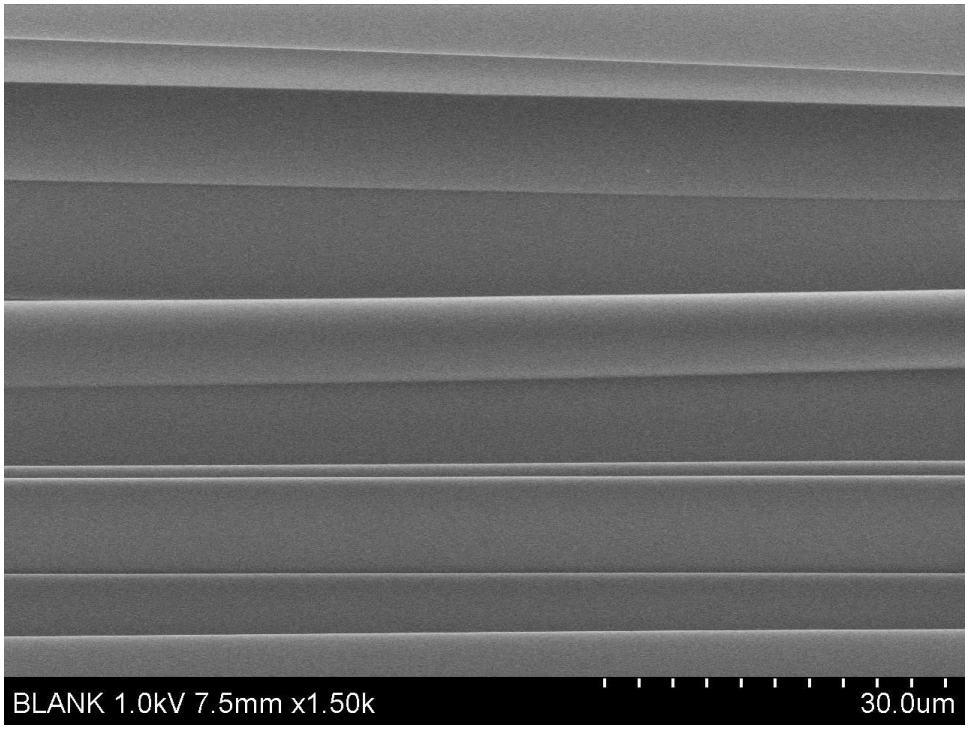


Figure 20. SEM image of 30 % (w/w) PLGA fibres with [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)].

Among the further results in this case, photographs of fibres incubated in distilled water are of the greatest interest. After 24 hours, appeared a lot of pores on the surface, indicating the beginning of the degradation process. The following Figure 21 shows the results of degradation at appropriate intervals. In addition, it should be noted that over time, the fibres tended to stick together, as it is clearly seen in the same figure.

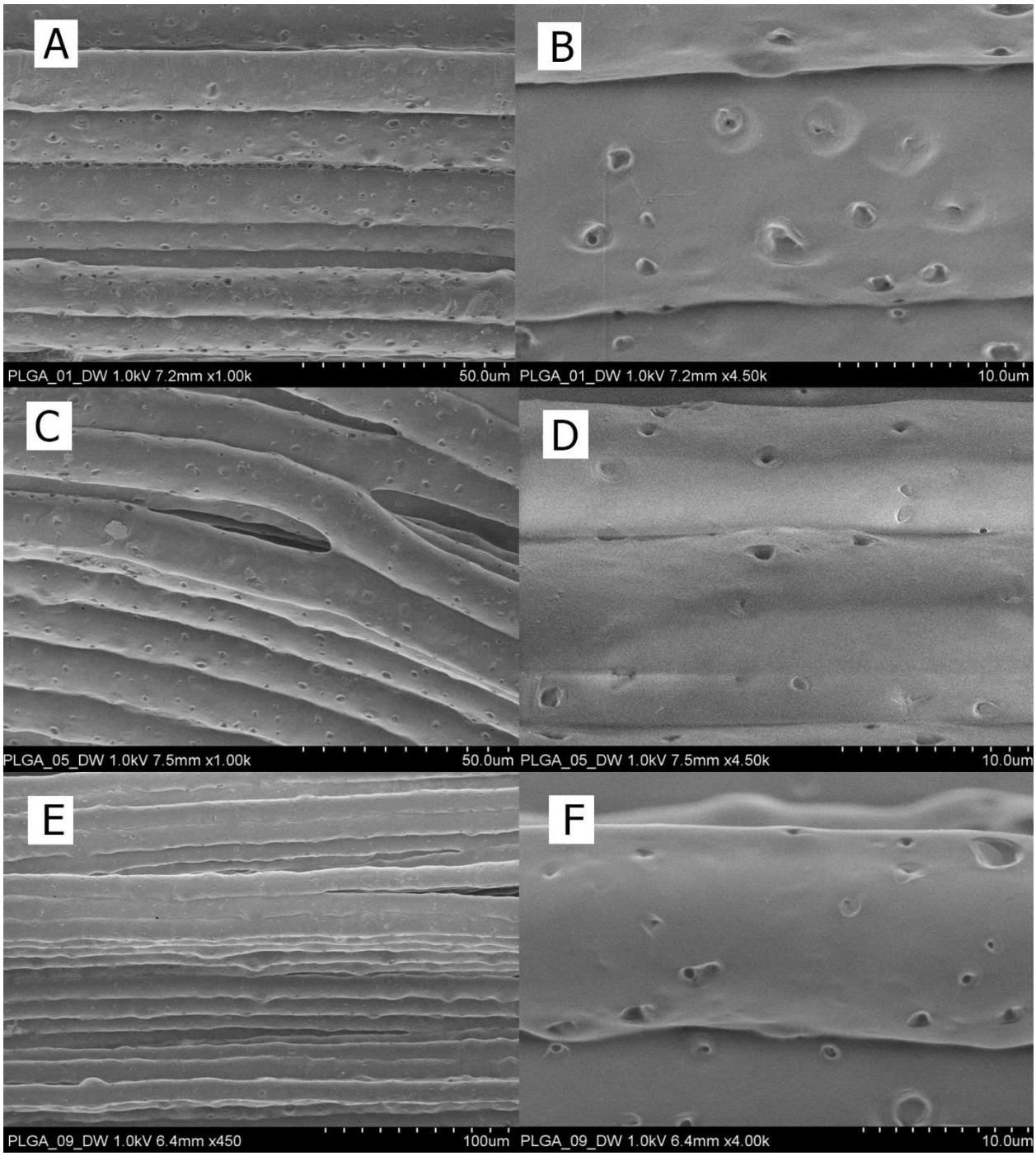


Figure 21. SEM image of 30 % (w/w) PLGA fibres with poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] incubated in the distilled water. **A, B** – fibres after 1 day of incubation; **C, D** – fibres after 5 days of incubation; **E, F** – fibres after 9 days of incubation.

When incubating fibres in physiological solution and acid solution, no comparable results were obtained and it is difficult to judge the course of the degradation process. A distinctive feature of fibres, which were in the acid, is the presence of adhered objects on the surface, (Figure 22)The results of weighing the weight of the fibres before and after incubation are not representative.

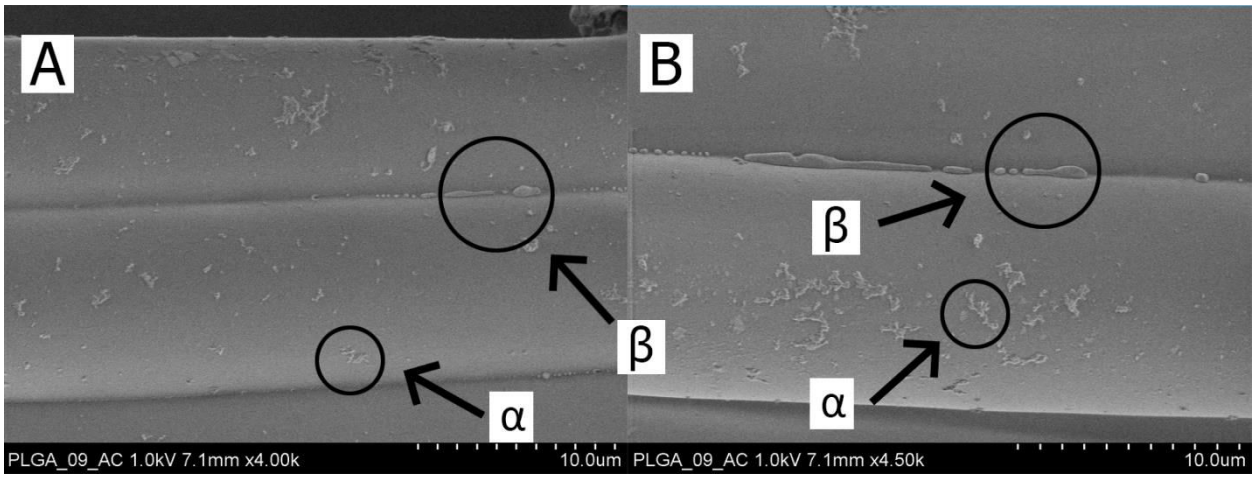


Figure 22. SEM image of 30 % (w/w) PLGA fibres with poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] incubated in the acetic acid solution 9 days. **A, B** represents different microphotographs; α - adhesion of fine particles on the surface; β – electrospinning artefacts.

6.2.1. Study of Degradation of 30 % (w/w) PLGA Fibres with Poly[(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] Incubated in Distilled Water or Physiological Solution

In this case, SEM fibres were not performed, since the experiment repeated the previous one and its purpose was to record the pH values. The summarizing results can be found in Table 1.The fibres of this type successfully reduce pH of even in a slightly acidic environment and can be used to return the normal skin acidity and with relatively small deviations.

Table 1. Degradation of 30 % (w/w) PLGA fibres with [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)] incubated in distilled water and physiological solution.

	Before		**After**		**Mass difference [%]**
	mass [g]	**pH**	**mass [g]**	**pH**	
Distilled water	0.0065±0.0006		0.0059±0.0009	-	-8.25
Physiological solution	0.0065±0.0008	**6.2**	0.0061±0.0007	**5.5**	-5.15

As in the case of the incubation of fibres in physiological solution, it can be noted that the fibres are fused together. The presence of pores represents a distinctive feature. In some images, we can note local superficial defects in a form of transverse grooves, which were not supposed to be the result of degradation processes. An example of such defects is shown in the Figure 23.

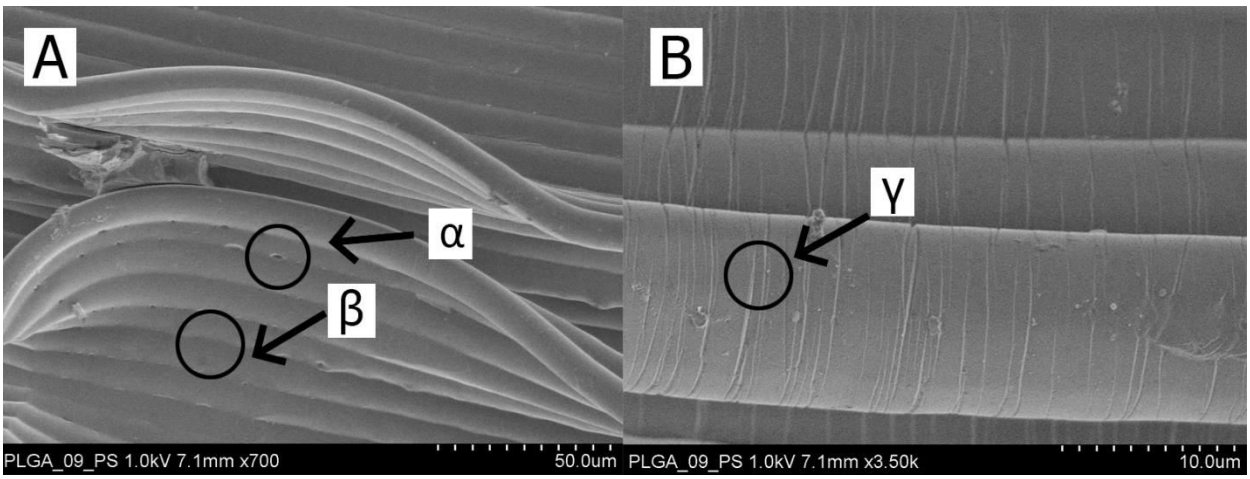


Figure 23. SEM image of fibres incubated in physiological solution for 10 days. **A, B** represents different microphotographs; **α** – pores in between fibres; **β** – process of fusion of fibres together; **γ** – transverse grooves on the surface of fibres.

6.2.2. Study of Degradation of 30 % (w/w) PLGA Fibres Incubated in Distilled Water and Strong Base

As described earlier, in chapter 2.5.3, during this experiment the extreme case of alkaline environment was studied with respect to the pH changes. This environment was chosen as a model of the typical environment in chronic wounds. Reduction of the pH value represents an important factor in the healing process. The results of the change in mass and acidity are shown in the Table 2. In this case, there is a tendency to reduce the weight of the fibres and lower the pH. In the sodium carbonate solution, after nine days, the weight of the fibres decreased by about 30 %.

Table 2. Degradation of 30 % PLGA fibres incubated in distilled water and strong base.

	Before		**After**		**Mass difference [%]**
	mass [g]	**pH**	**mass [g]**	**pH**	
Distilled water	0.0075±0.0001		0.0070±0.0003		-6.7
Sodium carbonate solution	0.0077±0.0003	**11.3**	0.0060±0.0006	**10.9**	-22.1

The SEM images (Figure 24 and Figure 25) confirm a massive degradation process in the sodium carbonate solution. Contrasting these two extreme cases – distilled water and strongly alkaline solution one can notice differences in the way in which the fibres decompose. In the case of a sodium carbonate solution on the 10th day of incubation, we can observe a huge number of small pores of irregular shape (the largest of them, at the widest point, are 1-2 μm in

width), without protruding edges and they almost completely cover the entire surface of the fibres. In addition, thinning of the fibres can be noted.

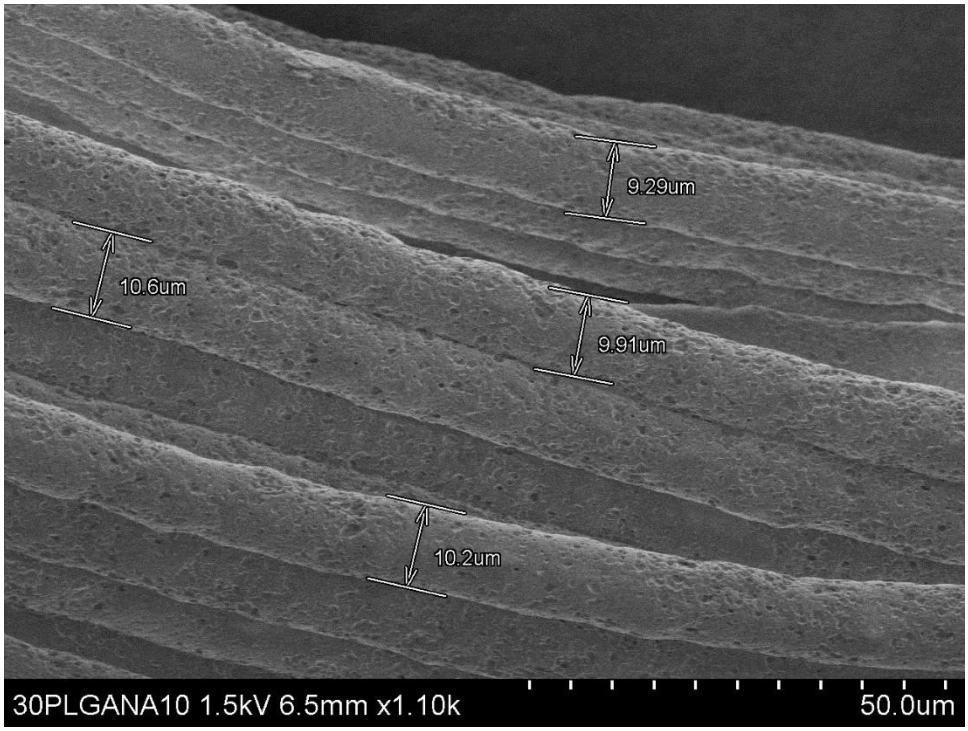


Figure 24. SEM image of 30 % (w/w) PLGA fibres incubated in Sodium carbonate solution for 10 days.

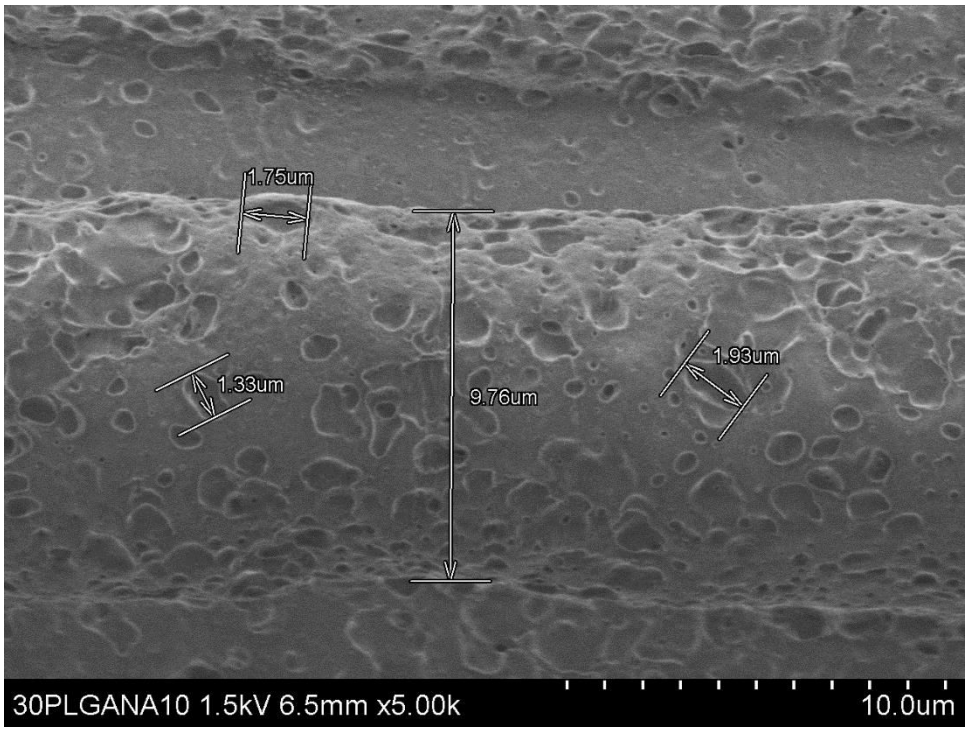


Figure 25. SEM image of 30 % (w/w) PLGA fibres incubated in Sodium carbonate solution for 10 days.

When we take the sample, which was kept in distilled water, it is necessary to mention that the case the situation looks different. On day 10, in this case the number of pores is much

smaller, and they differ in regular round shape and protruding above the surface of the fibre surface. Their size is, however, larger, on average their diameter is 2-4 µm. Examples of such pores can be seen in the following Figure 26 and Figure 27, and comparison of degradation processes in alkaline environment and in distilled water is presented in the Figure 28.

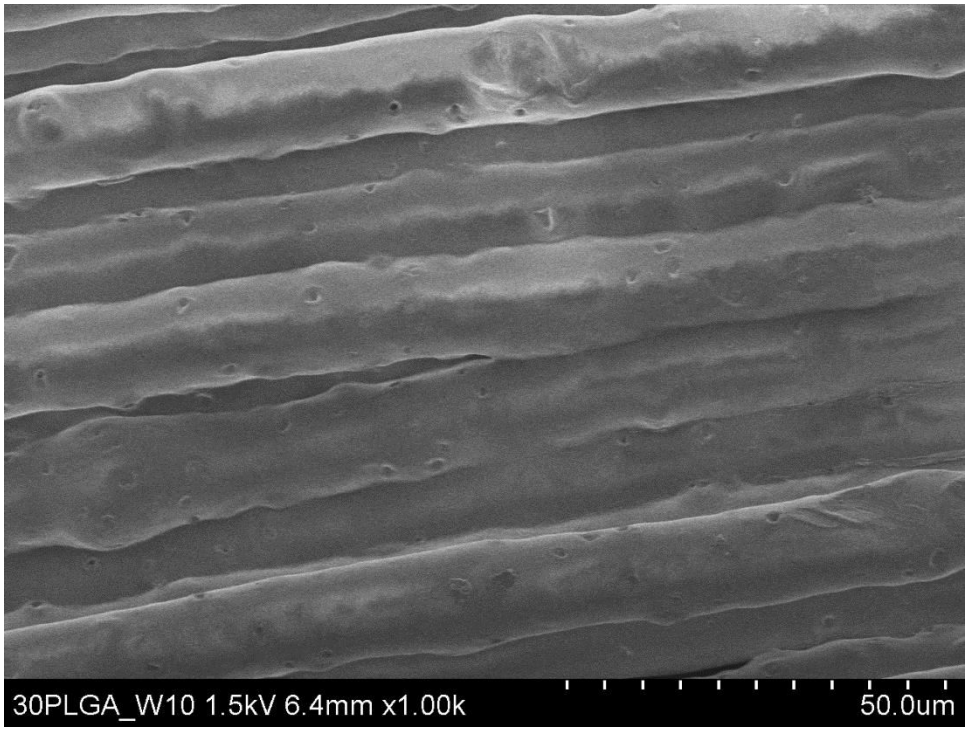


Figure 26. SEM image of 30 % (w/w) PLGA fibres incubated in distilled water for 10 days.

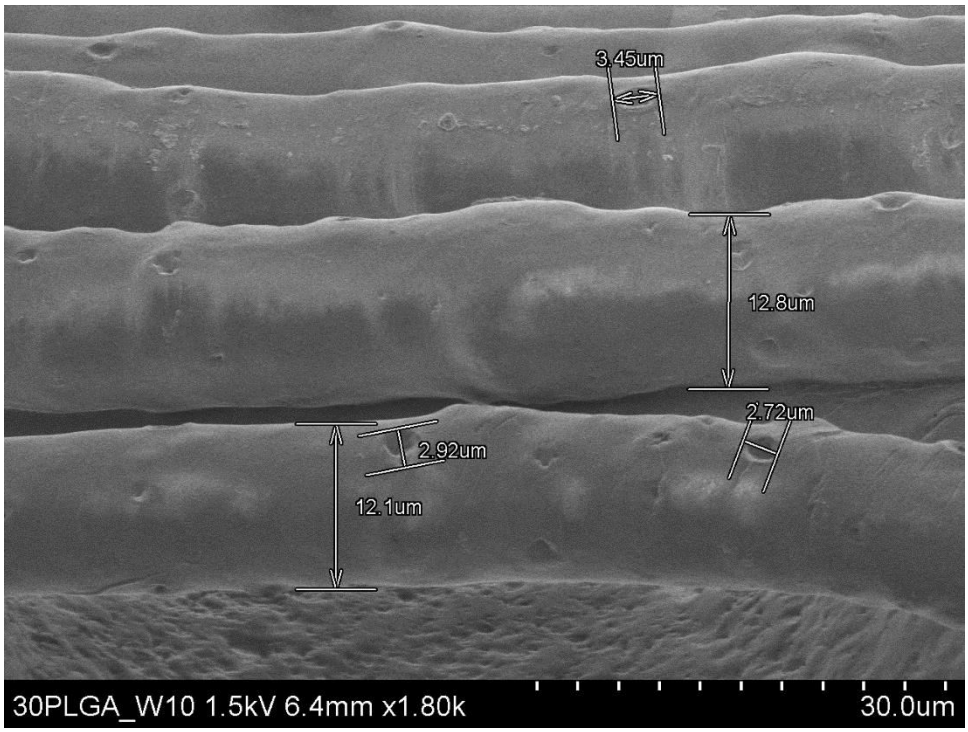


Figure 27. SEM image of 30 % (w/w) PLGA fibres incubated in distilled water for 10 days.

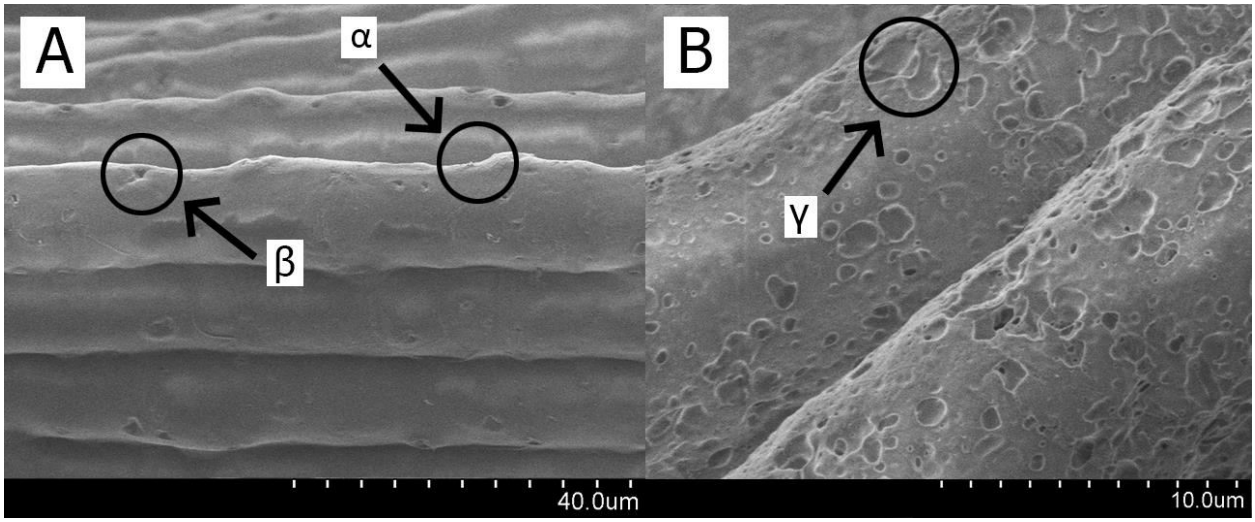


Figure 28. Comparison of degradation processes in alkaline environment and in distilled water of 30 % (w/w) PLGA fibres. **A** – degradation in the distilled water; **α** – elevation of pore edges above the surface of fibres; **β** – frontal view of the pore. **B** – the degradation process in a solution of sodium carbonate; **γ** – detailed view of pores in fibres.

6.2.3. Study of Degradation of 30 % (w/w) PLGA Fibres Incubated in Distilled Water and Strong Base + Temperature Impact

Initially, the fibres had a smooth surface and a typical size (about 12 μm) but the incubation at elevated temperature showed radically different degradation characteristics. Regarding incubation in distilled water, on the 10th day, we can note the complete fusion of individual fibres into a single structure (Figure 29). In the process of thermal fusion the fibres were almost tripled in their diameter (Figure 29).

In addition, we can note a much larger number of pores with the diameter from hundreds of nanometers to several micrometers, which indicates a constantly evolving process of decomposition. (Figure 30) Moreover, the structure indicated in Figure 31 deserves special attention. It also indicates an accelerated process of degradation, since it has a regular shape and smooth edges. However, to conclude what was a trigger for such a massive decomposition of fibres is difficult.

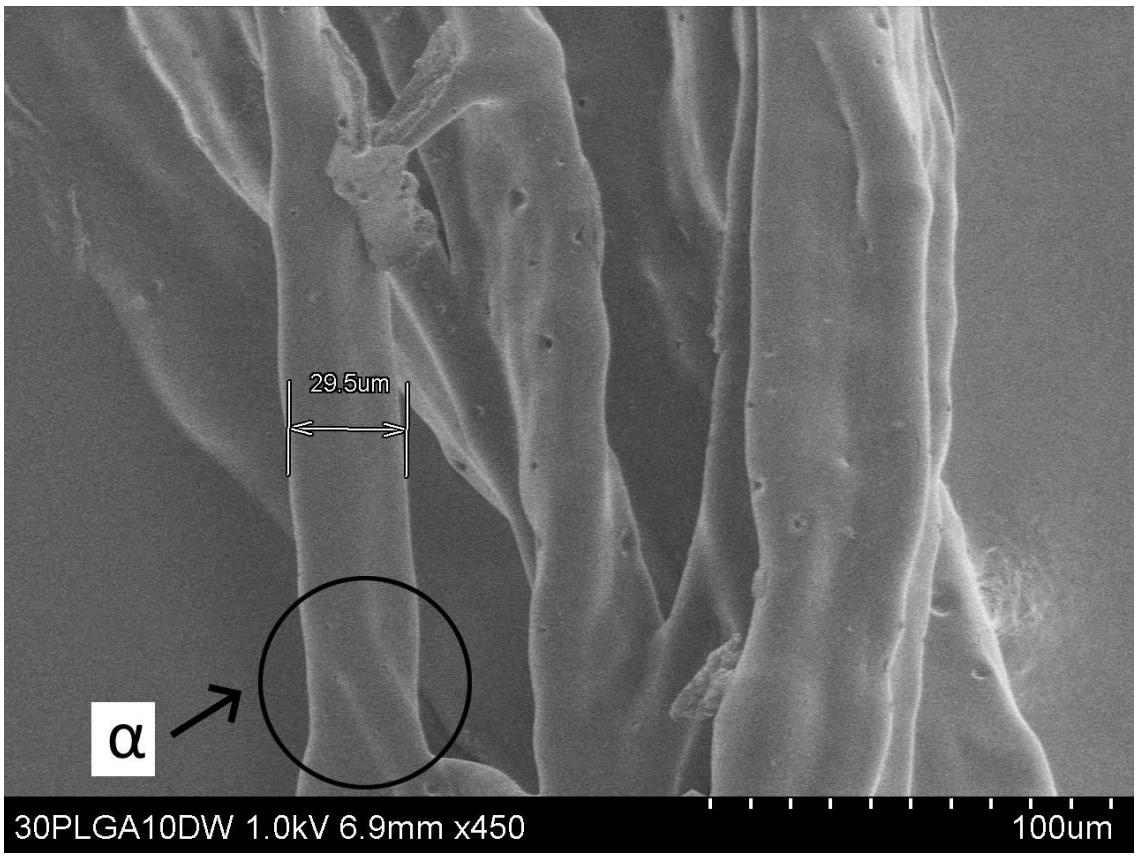


Figure 29. SEM image of 30 % (w/w) PLGA fibres incubated in distilled water for 10 days at the temperature 42 °C; **α** – place of fusion of fibres.

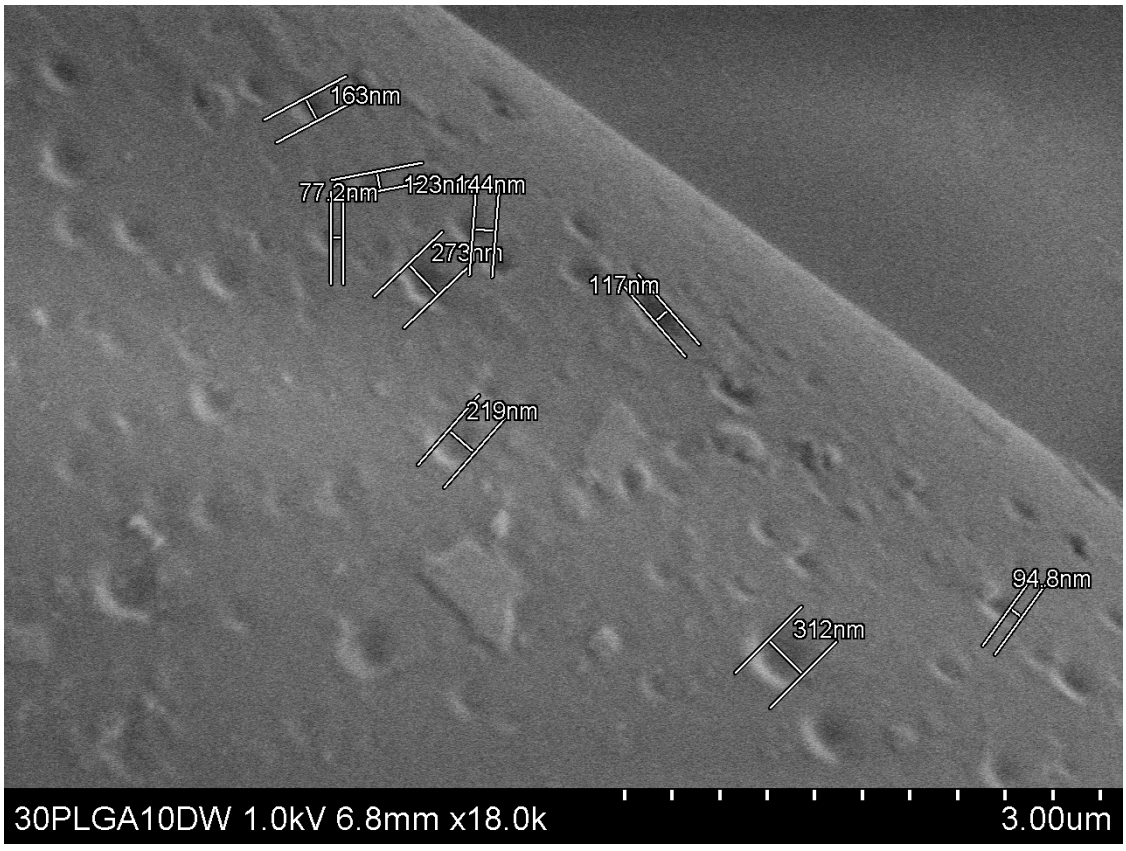


Figure 30. SEM image of 30 % (w/w) PLGA fibres incubated in distilled water for 10 days at the temperature 42 °C.

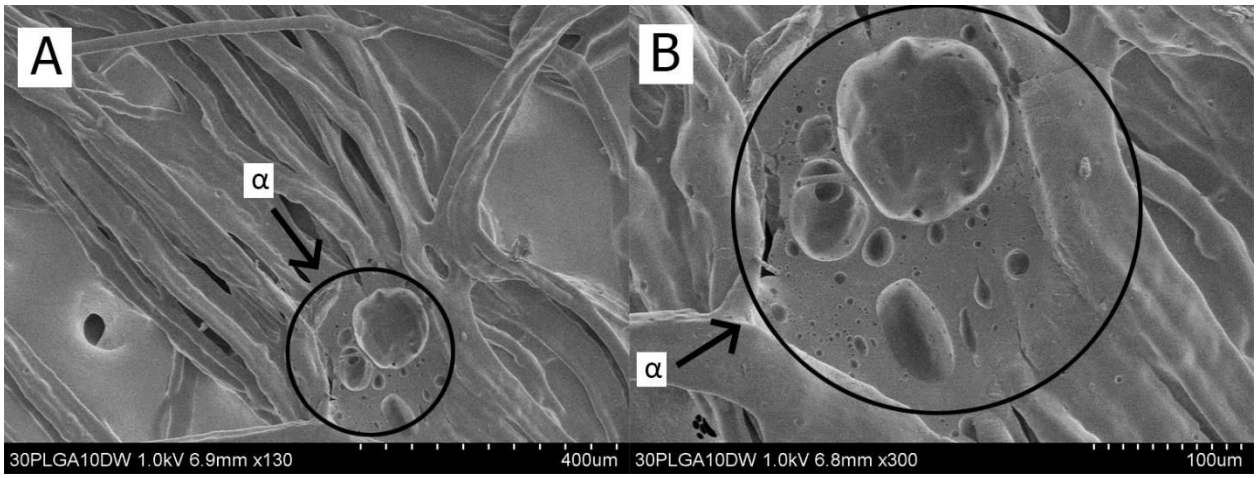


Figure 31. SEM image of 30 % (w/w) PLGA fibres incubated in distilled water for 10 days at the temperature 42 °C. **A, B** represents images with the different focus, **α** – local accelerated process of degradation.

Considering the degradation of fibres in alkaline environment, we can trace the above-mentioned tendency to fusion of fibres. In addition, compared with the experiment in deionized water, the number of pores of a regular round shape of different diameters (similar to the decomposition in distilled, the pores had a size of hundreds of nanometers to several micrometers) was larger (Figure 32 and Figure 33). The degradation at 37 °C was slightly more efficient as the percentage of the degraded mass was higher. The results also proved that the rate of the degradation is connected with the diameter of the fibres. In the solution tempered for 42°C the process of degradation is initiated through the fusion of the originally obtained fibres with diameter of approx. 12 μm into thick fibres having the diameter of approx. 30 μm. Therefore the whole process is hindered, which was also confirmed by the loss of mass. Therefore the mesh structure, with fibres in the diameter of hundreds of nanometers, would be probably more suitable for the purpose of inflammatory site compensation.

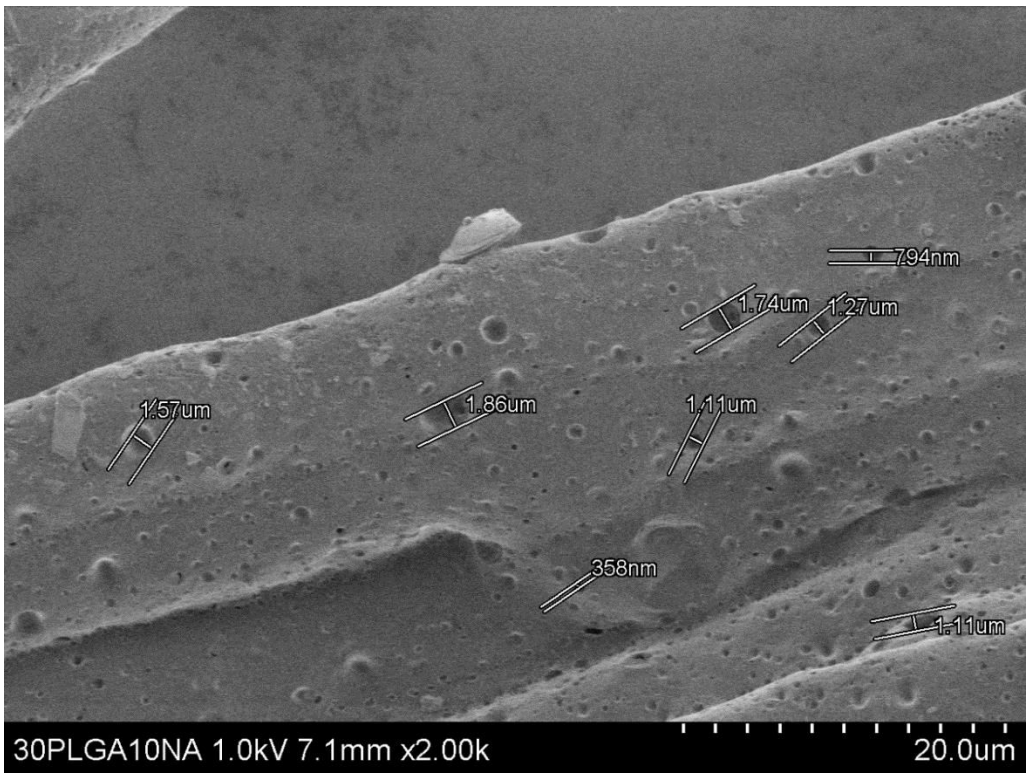


Figure 32. SEM image of 30 % (w/w) PLGA fibres incubated in Na_2CO_3 solution for 10 days at the temperature 42 °C.

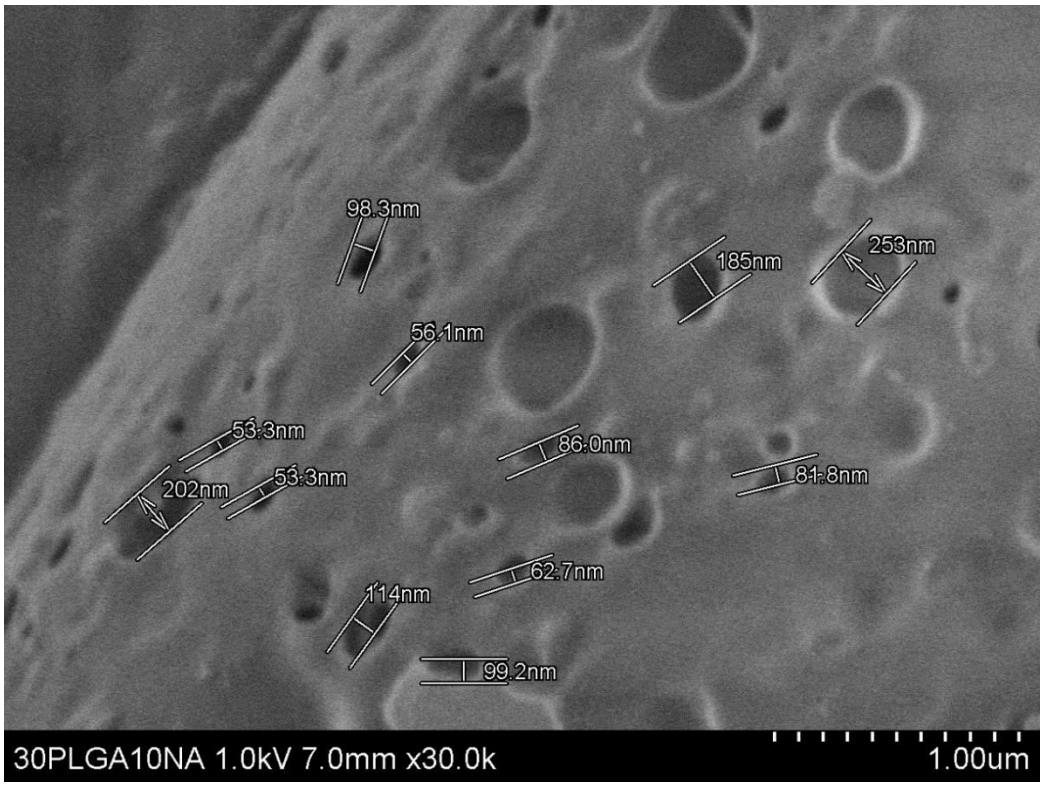


Figure 33. SEM image of 30 % (w/w) PLGA fibres incubated in Na_2CO_3 solution for 10 days at the temperature 42 °C.

Table 3. Degradation of 30 % PLGA fibres incubated in distilled water and strong base + temperature impact.

	Before		After		Mass difference [%]
	mass [g]	pH	mass [g]	pH	
Distilled water	0.0045±0.0002		0.0043±0.0001		-2,9
Sodium carbonate solution	0.0045±0.0001	**11.2**	0.0033±0.0002	**10.8**	-26,8

6.2.4. Study of Degradation of 30 % (w/w) PLGA Mesh Fibres in Distilled Water and Physiological Solution

The obtained fibres (Figure 34) had a smooth surface, were not oriented, and in some parts also involved defects like beads, which may occur as a result of imperfectly adjusted conditions of the electrospinning process. The study of decomposition in distilled water (Figure 35) showed quite reasonable results, since on the third day of incubation the fibres acquired a spongy-firm structure (Figure 35, a), which indicates the process of their degradation. On the tenth day of incubation, the swelling of the fibres and the separation of particles can be observed (Figure 35, c).

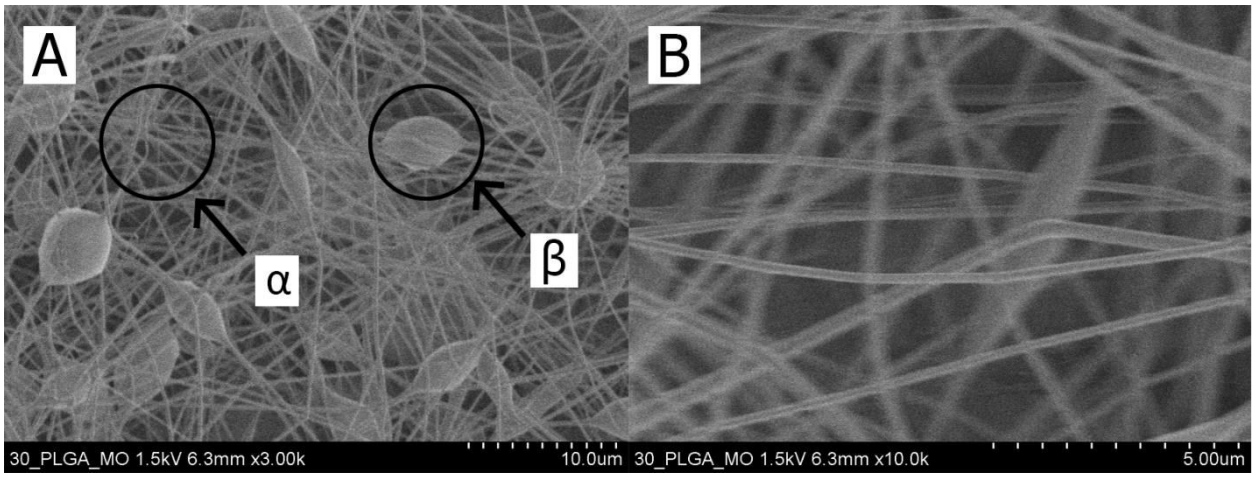


Figure 34. SEM image of the surface of 30 % (w/w) PLGA mesh fibres. **A, B** represents images with the different focus; **α** – area with fibres without bead defects; **β** – bead defects of the fibres represented by round thicknesses on the fibres.

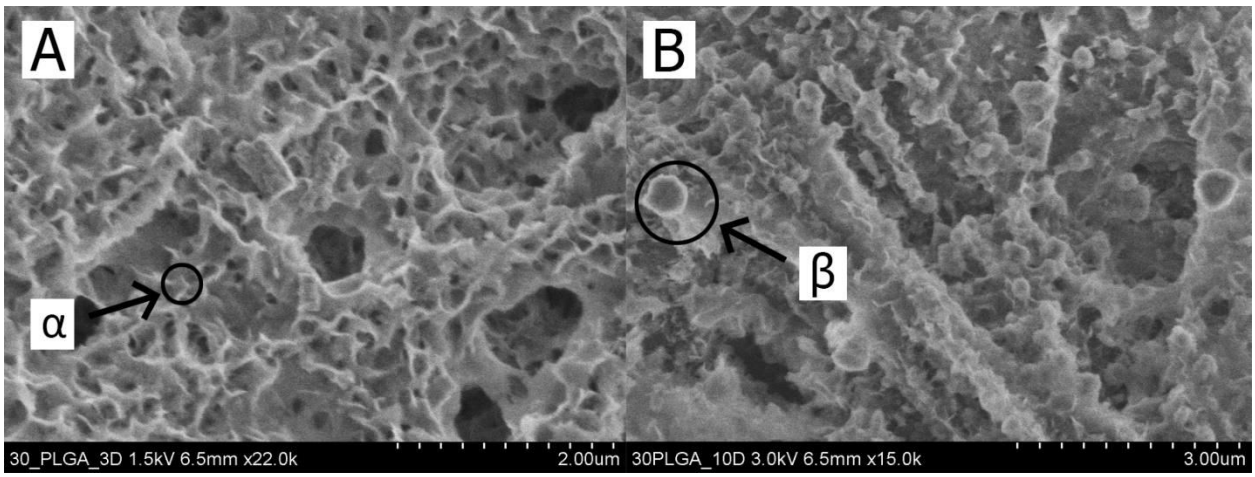


Figure 35. SEM image of the surface of 30 % (w/w) PLGA mesh fibres after 3 days of incubation in thermostat at 37°C in distilled water (**A**) and after 10 days (**B**); **α** – "branch" formation of the surface of fibres; **β** – space between the fibres.

The degradation process carried out in physiological solution (Figure 36) was complicated by the design of the experiment. Due to the impossibility to peel the fibres organized in a mesh structure off the tin foil, they were placed into the vials with either deionized water or physiological solution with it. In this regard, in SEM images we can observe a huge number of inorganic inclusions (Figure 36, a), which make it virtually impossible to perform a detailed analysis of the degradation of the mesh texture. Nevertheless, in some images we can note the thinning of fibres and the breakdown of their structure, which indicates their decomposition in physiological solution (Figure 36, b).

Additionally, we may note a completely different type of degradation which is undoubtedly connected with smaller diameter of the fibres in the mesh structured samples. Instead of fibres with a smooth surface, the fibres are already after 3 days fused and the penetration of the newly formed structure is commenced. (Figure 35) The penetration process is, however, more effective (Figure 35A) and the whole sample bears the structure of a spongy surface with numerous pores of different diameter.

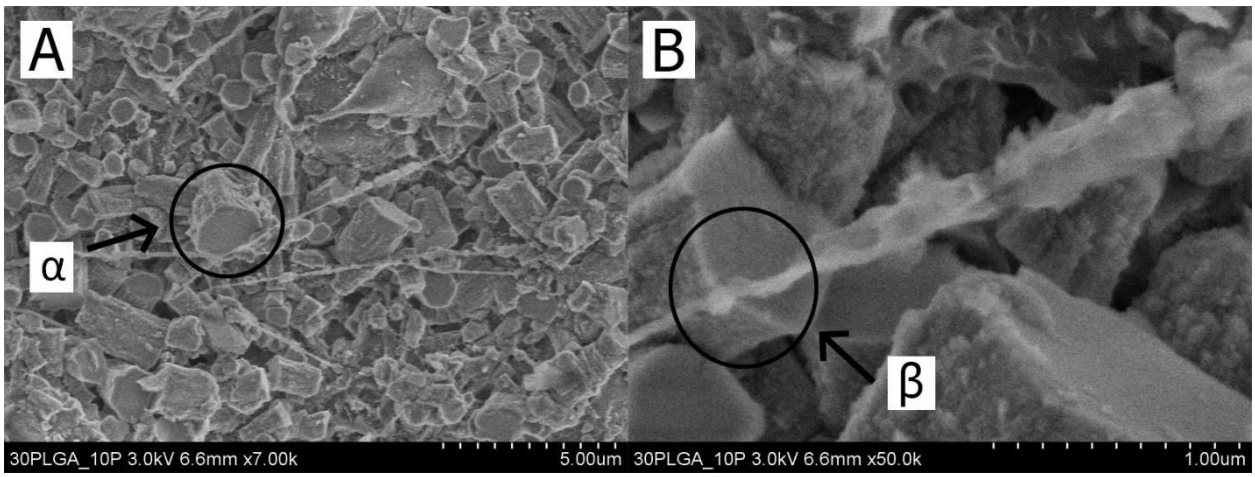


Figure 36. SEM image of the surface of 30 % (w/w) PLGA mesh fibres after 10 days of incubation in thermostat at 37°C in physiological solution. **A, B** represents images with the different focus; **α** – inorganic inclusions; **β**– thinning of fibres.

6.2.5. Study of 30 % (w/w) PLGA fibres with Silver Nanoparticles

The fibres generated via the method described in chapter 5.1.3. can be characterized as fibres with a smooth surface on which silver nanoparticles (AgNPs) are located. This fact is confirmed by the SEM images (Figure 37). On the surface of the fibres, the AgNPs can be detected (Figure 37 B). Some fibres are attached to one another already in the process of fibre generation. When these fibres are torn from one another the inner structures of the oriented fibres are also revealed. This way some of the AgNPs that are in the process of electrospinning located inside of the fibres are exposed (Figure 38 C, D).

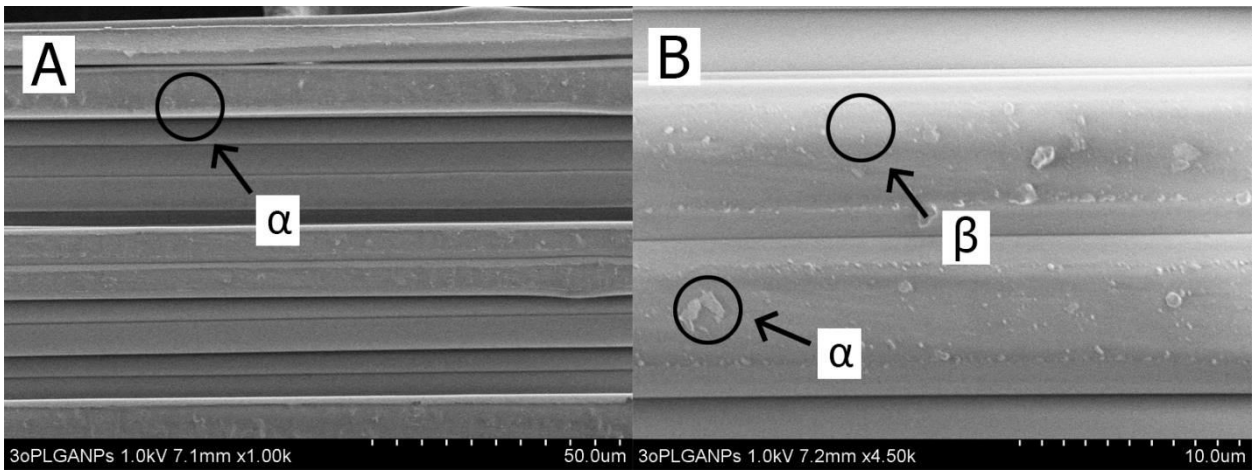


Figure 37. SEM image of the surface of 30 % (w/w) PLGA fibres with silver nanoparticles. The jetting polymeric solution involved acetone and THF as a mixed solvent in the ration equal to 50:50. A, B represents different microphotographs with the different enlargement; **α** – electrospininng artefacts attached to the surface of smooth fibres; **β** – silver nanoparticles.

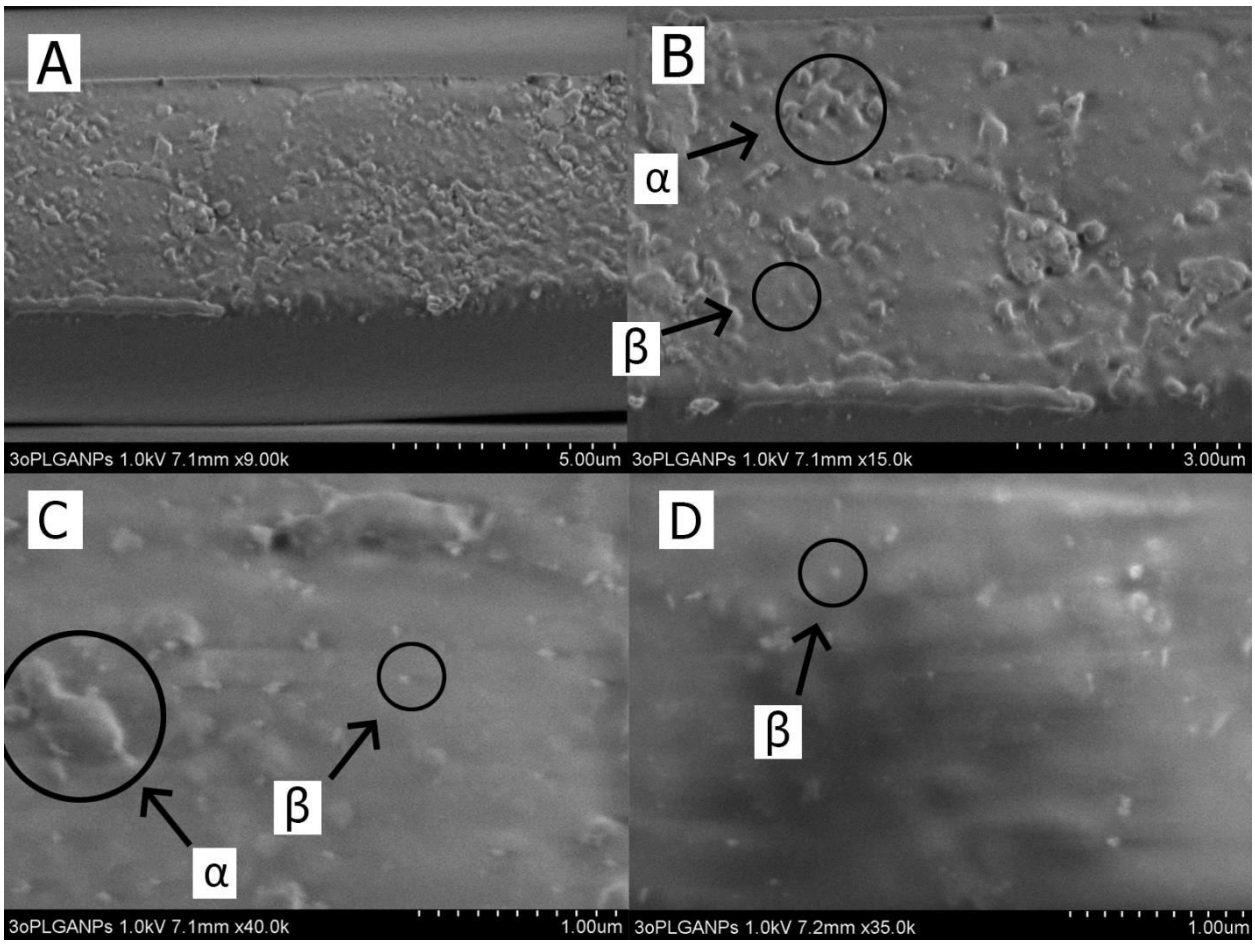


Figure 38. SEM image of the surface of 30 % (w/w) PLGA fibres with silver nanoparticles. The jetting polymeric solution involved acetone and THF as a mixed solvent in the ration equal to 50:50**. A-D** represents images with the different focus; **A** – the structure, which is left behind after the oriented fibre is torn from the other, originally attached fibres; **C, D** – AgNPs located inside of the fibres that were exposed after tearing of the fibres from one another; **α, β** - AgNPs.

SUMMARY

This topic of the submitted bachelor paper was to prepare biodegradable fibres of different kinds and to monitor how the fibres decompose in different environments and how does it affect the surrounding with the ambition to connect these results with problems connected with treatment of chronic skin disorders.

The imperfection of today's methods of treating disorders of the integrity of the skin leads to a deterioration in the quality of patients´ lives and to an increase in the number of strains of bacteria resistant to a larger spectrum of antibiotics. In this regard, in this paper, we investigated a possibility of using biodegradable polymers for drug release or as tools, which can play a role in the healing process for example as a scaffolding material. Namely, the parameters of the generation of such fibres are diameter of the fibre, their structure and the ability to undergo the degradation process. The last is an important property both for the release of drugs that can be contained inside the fibres, and for restoring the integrity of the skin.

In the course of the work, the oriented and mesh fibres were prepared on the basis of a biopolymer such as poly (D, L-lactic-co-glycolic acid) (PLGA) using an electrospinning method. The generated fibres could be divided into two different types depending on their structure – on the orientation and on the non-oriented. As all of the generated fibres were about 12 μm in diameter. For the generation of the oriented fibres, a mixture of solvents such as tetrahydrofuran (THF) and acetone was used, and the PLGA content was 30%. The best results were achieved using the following parameters: the distance from the end of the syringe to the surface of the vial was 2.5 cm, the speed of rotation of the vial was 40 rpm, the voltage was 10 kV. These fibres were then incubated in the thermostat at the elevated temperature (from the elevated temperature from 1 to 10 days. Temperature of 37°C and 42°C were chosen to model body temperature in inflammation. The incubations of fibres were carried out either in distilled water, physiological solution, or solutions of acetic acid or sodium carbonate. These solutions were also chosen on the basis of data that the human skin is normally weakly acidic (pH 5.5), and in inflammatory processes, on the contrary, it is alkaline. The results of degradation of these fibres in distilled water and in the alkaline environment, both at a temperature close to normal body temperature (37°C) and at elevated temperature (42°C), were of the greatest interest. The results of the performed experiments were monitored using scanning electron microscopy (SEM). The fibres were disrupted on their surfaces and the originally smooth surface was covered with pores or the fibres were fused together, which indicated an intensive

process of degradation. However, the type of the degradation processes differed with pH of the solution into which the fibres were immersed. At neutral pH, the pores were less numerous than under alkali region and their edges rose above the surface of the fibres. This tendency was observed under both temperature values. However, it is surprising that when the temperature was increased, the pore sizes of the fibres in the alkaline solution decreased to a few tens of nanometers and had a regular rounded shape, while at the same time, the pores were deeper and had irregular shapes at 37°C. In all cases, the pH of the solution was also studied and the results indicated a parallel shift of the pH to the acidic side, which is an important factor in the treatment of inflammatory disorders.

In addition, the oriented fibres containing silver nanoparticles on their surface and inside their matrix were synthesized. The distance from the tip of the needle to the surface of the vial is 2.5 cm, rotating speed is 40 rpm. The applied voltage was 10 kV. The rate of extrusion of the polymer was 0.15 mL/hr.

The non-oriented fibre system was generated using the following conditions: 30 % (w/w) solution of PLGA in a mixture of acetone and DMF (the best combination of generation parameters: 4.5 cm from the tip of the needle, the rotation speed was 30 rpm, the 10 %, the polymer flow rate was 0.15 mL/h), 30 % (w/w) solution of PLGA in a mixture of chloroform and DMF in a ratio of 95:5 (the synthesis parameters were as follows: The distance from the needle to the collector is 2.5 cm, the applied voltage is 7.5 kV, the rotation speed is 30 rpm and the extrusion speed is 0.08 mL/h) and the variation of the PLGA content is 20 % (w/w), 10 % (w/w), 5 % (w/w) in a solution of solvents such as acetone and THF in a ratio of 50:50. Only the first system was tested for the ability to undergo degradation, which unfortunately did not yield significant results due to the impossibility of peeling the fibres off the tin foil. The fibres were placed into the vials with either deionized water or physiological solution with it. In this regard, complications in the analysis of the images obtained. It should be noted that the latter, of the systems mentioned, contained nanoparticles, the size of which varied from 18 to 38 nm. Unfortunately, 30 % (w/w) solution of PLGA in a mixture of chloroform and DMF in a ratio of 95: 5 was not tested because of its instability already at the stage of fibre generation.

In conclusion, it has been proved that all of the generated fibres undergo the process of degradation. The process was well monitored by means of SEM images, and based on the obtained results it can be concluded that they are excellent candidates for the manufacture of

scaffolding materials and drug-delivery systems, especially for the treatment of skin diseases and disorders of the integrity of the skin.

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