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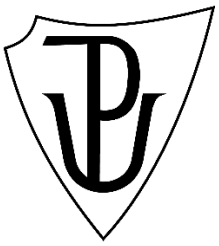

Preparation of Multilayer Composites via Electrospinning

Diploma thesis

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I declare, that I elaborated my bachelor's paper entitled "Preparation of Multilayer Composites via Electrospinning" 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
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Abstrakt:	Předmětem předkládané diplomové práci byla příprava biodegradabilních vláken generovaných na bázi poly (laktid-co-glykolové) kyseliny, polyurethanu a polyethyleniminu v různých rozpouštědlech, jako je N, N, dimethylformamid, aceton, a hexafluoroisopropanol. Vlákna byla studována jako samostatně existující vrstva a jako sendvičová struktura. Po jejich vytvoření byla vlákna inkubována v destilované vodě, fyziologickém roztoku a v silně alkalickém roztoku při zvýšené teplotě (40 °C). Proces degradace vláken byl monitorován pomocí rastrovací elektronové mikroskopie (SEM).
Klíčová slova:	biodegradabilní polymerní vlákna, sendvičová struktura, PLGA, PU, PEI, elektrostatické zvlákňování, obvazové krytí
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Abstract:	In this master's thesis, fibres were generated on the basis of *poly(lactic-co-glycolic acid*) (PLGA), *polyurethane* (PU), and *polyethyleneimine* (PEI) blend in different solvents such as N, N,-dimethylformamide, acetone, and hexafluoroisopropanol – HPIF. Fibres were studied as both a self-standing layer and a sandwich structure. After their generation, the fibres were incubated in a distilled water, physiological solution and in a strongly alkaline solution at elevated temperature (40°C). The process of fibre degradation was monitored by means of scanning electron microscopy (SEM).
Keywords:	biodegradable polymer fibres, sandwich structure, PLGA, PU, PEI, electrospinning, wound dressing
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INTRODUCTION

Achievements in science, research and technology in the last quarter of the last century have convincingly demonstrated what tremendous opportunities the use of specific phenomena and properties of materials in the nanometer size range promises. Words with the prefix "nano" like nanoworld, nanotechnology, nanomaterials, nanoelectronics, nanobiotechnology, nanomedicine and others have rapidly entered the lexicon of not only scientists and engineers, but also journalists, economists, ecologists, medical doctors, and even military. Obviously, this is due to the intensive development of "nano" activities, the penetration of its results into the most important spheres of life and the enormous significance of this process for the present and the future.

Many sources, primarily English-speaking, associate the first mention of methods that will later be called nanotechnology with the famous speech of Richard Feynman "There's Plenty of Room at the Bottom", made by this Nobel Prize laureate in 1959 at the California Institute of Technology (CALTECH) at the annual meeting of the American Physical Society. However, we have known nanomaterials for a long time and e.g. glassblowers of antiquity and the Middle Ages were unconsciously the first nanotechnologists when they added gold chloride to molten glass, with a demand for characteristic optical properties of the glass – for the tint of ruby colour due to the appearance of gold nanoparticles. (1)

Our knowledge is, however, much further compared to the first touches made by the glassblowers. We do not prepare nanoobject in order to use extraordinary optical properties. We find the beauty in this morphology and premium characteristic feature the shift from macro- to nanoworld can bring on bio-physico-chemical level. Therefore, this work is focused mainly on one of the fiber synthesis technologies (electrospinning) and the application of its principles on various polymeric materials for their further use in the field of wound dressings, since the healing of soft tissue injuries caused by various mechanical, thermal and other factors (purulent-inflammatory processes, dystrophic and others), remains an urgent scientific and practical task. Although there are many commercial products available on the market for wound treatment, the issues connected with high exudate volume, infection, decreased perfusion and maceration of the area around the wound has not been completely solved yet. Then another issue, that calls for solution, can be found in the formation of scars and the aesthetic side of the treated disorder. In this case,

both natural and synthetic polymers can be used, which are discussed in more detail in the following chapters of this thesis. However, a certain level of biocompatibility and biodegradability is required along with a high level of biomimicry for use as a wound dressing. The rate of decomposition should also be consistent with the wound healing process. These conditions can be met by creating more complex sandwich structures, consisting of several layers that perform different functions.

In this thesis, biodegradable fibers of poly (D, L-lactic-co-glycolic acid) were created, on the surface of which mixed fibers of polyurethane and polyethylenimine were then generated using the electrospinning technique already mentioned above. These structures were then tested for biodegradability under wound conditions (elevated temperature and varying acidity), and the results were characterized using a scanning electron microscope.

Normal skin structure

The skin is the largest human organ, which occupies about 2 m^2 and weighs up to 4 kg. It creates a barrier that protects all internal tissues and organs from both infections and mechanical damage. (2; 3) The skin consists of three layers: epidermis, dermis and subcutis or hypo-dermis, which is represented by subcutaneous fat. (2; 3)

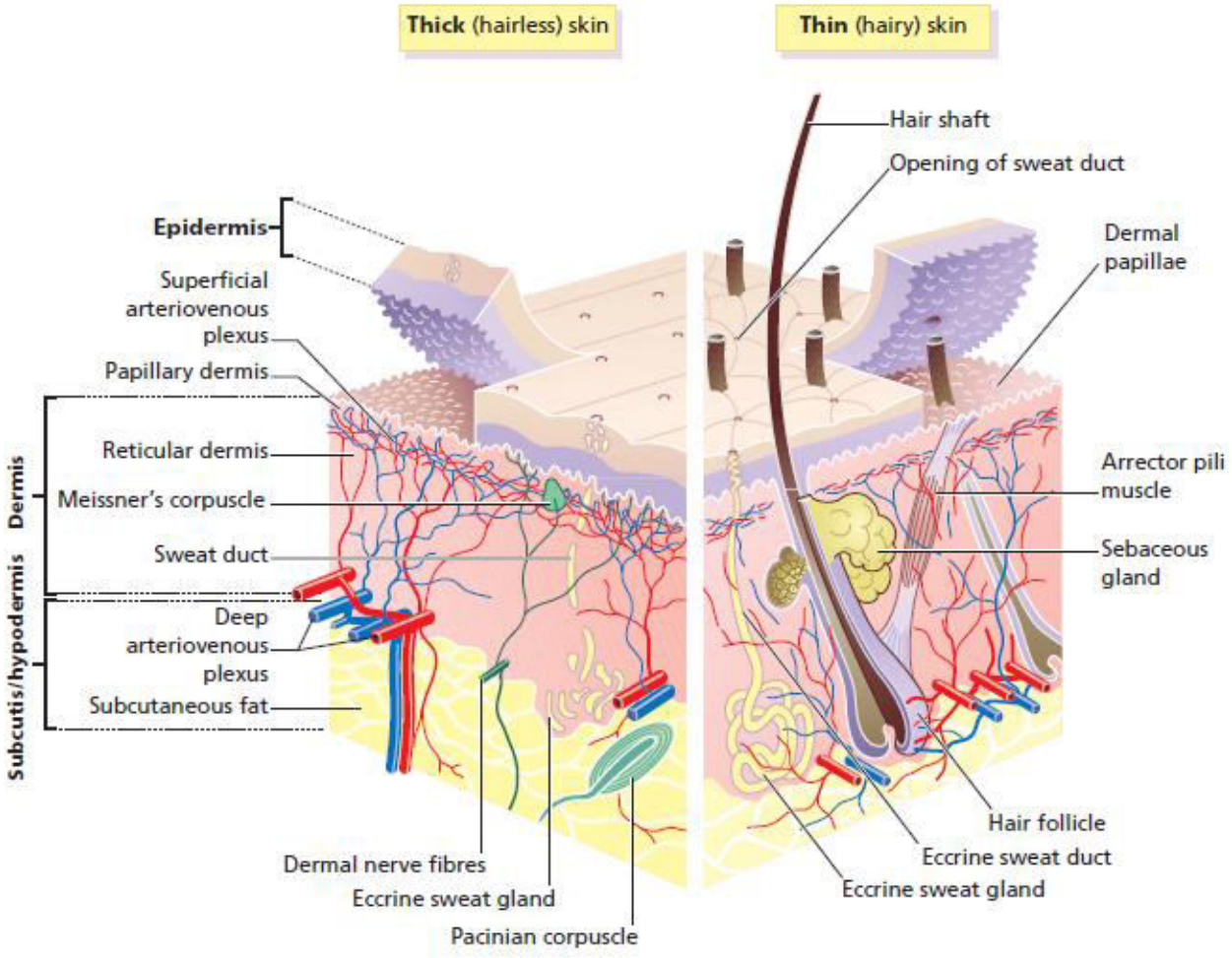

Figure 1: Skin structure. *(2)*

Epidermis

The epidermis is the outer layer of the skin. In thick skin (not covered with hair), it includes five layers: *stratum basale, stratum spinosum* (or prickle cell layer), *stratum granulosum* (or granular layer), *stratum lucidum* (can be found on the palms and the soles), and *stratum corneum* (horny layer). (2; 4) These layers are located above the dermis and performing a predominantly barrier function. In a thin skin (covered with hair) there is no shiny and sharply thinned granular layer. (2; 4)

The epidermis is constantly updated. A similar effect is associated with specific transformations and migration of keratinocytes from the deep to the outer layers during their differentiation. Together with exfoliating scales, chemical and biological pathogens are removed from the skin surface. The epidermis presents some components of the immune system. (2)

In the basal layer there are undifferentiated, proliferating cells and these same cells then differentiate into keratinocytes, which then within 4 weeks reach the upper border of the epidermis. (3) *Stratum spinosum* consists of keratinocytes, which produce keratin and fibrous protein, and also stick to each other through desmosomes (consist of transmembranous desmoglein – desmocollin pairs). (2; 3)

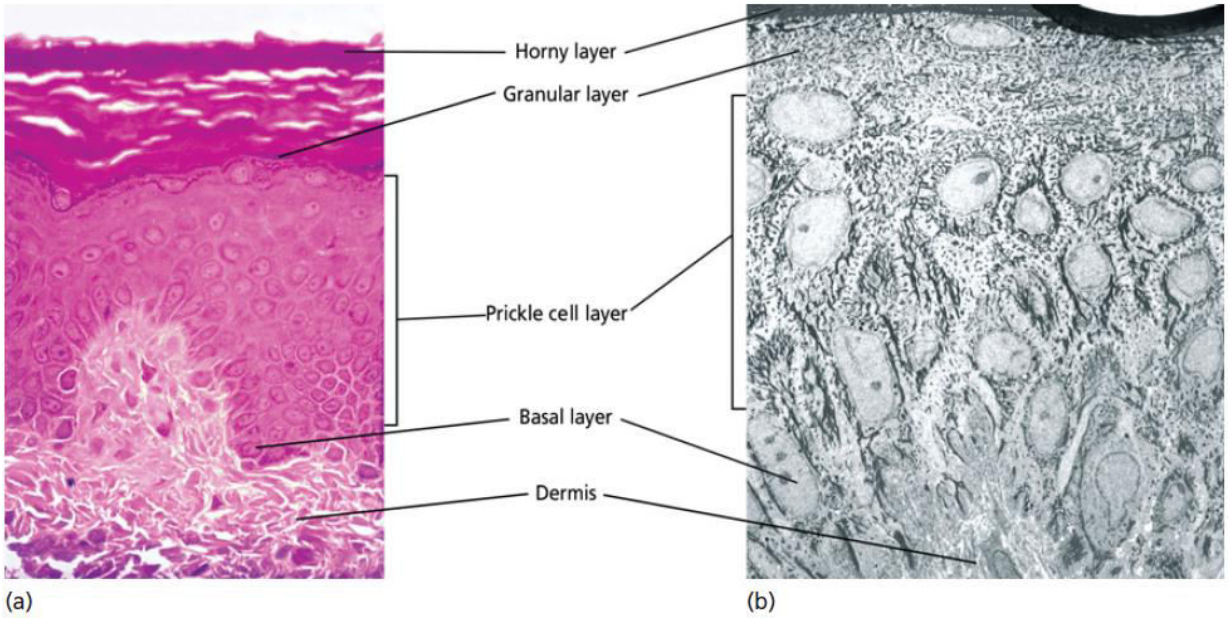

Figure 2: Skin layers. (1)

In *stratum granulosum*, cells continue their differentiation, accumulate specific granules and become flatter. (3) Granules, as set forth, of large size and irregular shape, they contain proteins, including involucrin, loricrin and profilaggrin. Such granules are called keratohyalin. (2) There is another type of granule – lamellar granules. They contain polysaccharides, glycoproteins and lipids. As the cells advance to the upper layer, they release these granules and become flatter, their contents forming the substance that binds the cells in the *stratum corneum*. (2; 3)

The last layer is dead flat, polyhedral, plate-like cells filled with keratin and these cells are held by the "cement" described above. In this way, they create a semi-permeable

barrier, which is the main physical barrier of the whole organism. (3) It is important to note that “keratin” is a group of proteins and different keratin is present in different layers of the skin (for example, basal cells contain keratins 5 and 14), while the others can be considered markers of a disease (keratins 6 and 16 in psoriasis). (2) The natural moisturizing factor (NMF) and antimicrobial peptides (AMPs) that can be found on the epithelial surfaces help maintain barrier functions. The most important of them are defensins and the cathelicidins, which are the first line of immune defence. (2)

As mentioned above, the horny layer is partially permeable. The rate of penetration of a substance depends on its concentration and on the thickness of the horny layer itself. It is generally permeable to water, but not to large ions. (2) Regulation of epidermopoiesis occurs through cytokines, transcription factors and integrins.

Although keratinocytes represent the majority of epidermal cells, there are other skin cells: melanocytes, Langerhans cells and Merkel cells (mechanoreceptors). (2; 3) Melanocytes are dendritic pigment-producing cells located in the basal layer. (3) Their number is approximately the same in people of all races; different amounts of melanosomes affect the skin pigmentation (granules containing melanin pigment). (3)

Langerhans cells are also dendritic cells that carry the class II histocompatibility complex (MHC), surface receptors for C3b, IgG and IgE. (2)They play an important role in immunity reactions — they take an exogenous antigen, process it and submit it to T-lymphocytes. Thus, they are considered highly specialized macrophages. (2)

Dermis

The dermis is a supporting structure that contains vascular and nerve bundles, muscles, and skin appendages. Its thickness is from 1 to 4 mm. (3) The main cells of the dermis are fibrolasts, but there are also phagocytes, lymphocytes, dermal dendritic cells and mast cells. Also, during inflammation, other blood cells can occur in the thickness of the dermis. (2) The dermis matrix itself consists mainly of collagen fibers (70 - 80 % of the dry weight of the skin), elastin, and an extrafibrillar substance (consists of two main glycosaminoglycans, hyaluronic acid and dermatan sulfate). (2; 3) The presence of these glycosaminoglycans is very important because they bind water, nutrients and work as a lubricant between the collagen and the elastic fiber.

Table 1: Dermal cells. (2)

Fibroblast	Synthesis of collagen, reticulin, elastin, fibronectin, glycosaminoglycans, collagenase
Mononuclear phagocyte	Mobile: phagocytose and destroy bacteria
	Secrete cytokines
Lymphocyte	Immunosurveillance
Langerhans cell and dermal dendritic cell	In transit between local lymph node and epidermis
	Antigen presentation
Mast cell	Stimulated by antigens, complement components, and other substances to release many inflammatory mediators including histamine, heparin, prostaglandins, leukotrienes, tryptase and chemotactic factors for eosinophils and neutrophils
Merkel cell	Act as transducers for fine touch

It is also important to note that the skin contains a part of the peripheral immune system – the skin immune system (SIS). Effects of acquired and innate immunities with coordinated immune cells of the skin (macrophages, dendritic cells and T cells). (2)

Wound Healing

For convenience, descriptions of wound healing are divided into several stages: haemostasis, inflammation, proliferation, contraction, remodelling. (5) These phases are very tentative because they often overlap in time. (5)

Haemostasis

It is important to understand that healing begins immediately after an injury occurs. (5) Thus, haemostasis is the first stage, which can be characterized by a general contraction of tissues, including vasoconstriction, which is caused by substances secreted by damaged cells. (6; 7) Thanks to this reaction, blood loss is reduced. (8) However, for the initiation of the

clotting cascade is necessary to lose a certain amount of it. (5) Damage to collagen in the skin stimulates adhesion and degranulation of platelets, as well as the work of the compliment system. (5) At the same time, fibrinogen is converted to fibrin, which, together with platelets, forms a scab on the surface of a wound. In addition, the presence of fibrin attracts fibroblasts. (5; 7) The aforementioned platelet degranulation releases a number of cytokines, which acts as chemoattractants for neutrophils that play an important role in the next phase of healing. (5; 6; 7) As a result, within a few minutes, the wound is filled with a haemostatic coagulum.(8)

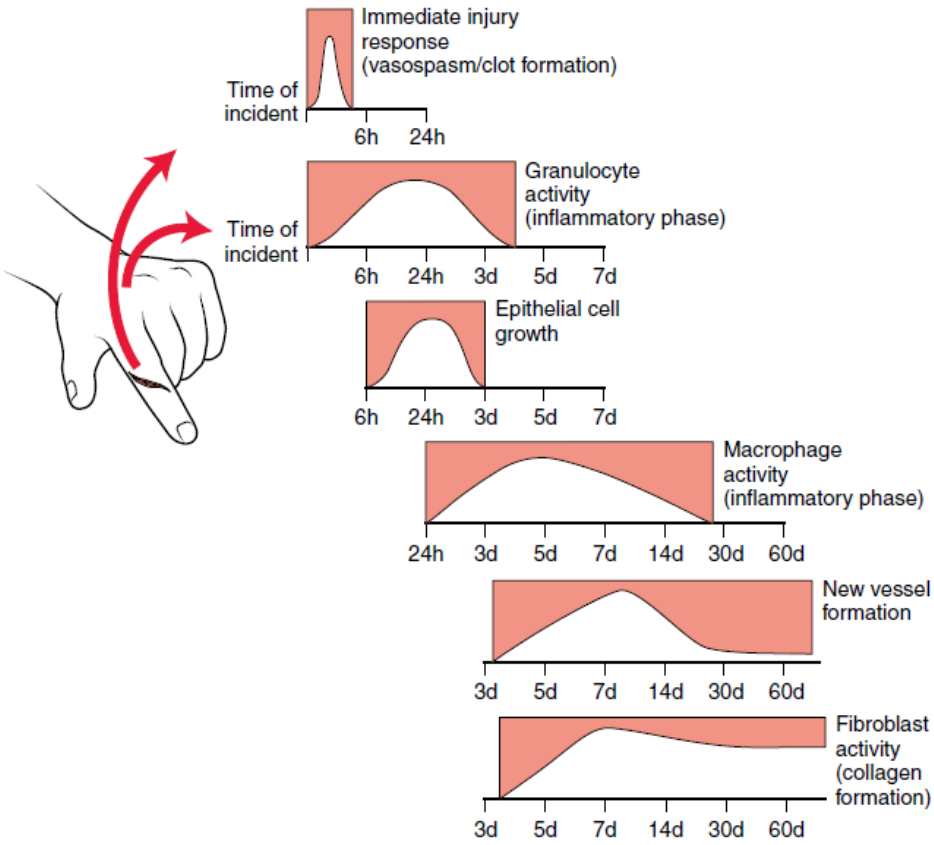

Figure 3: The various components of wound healing and their time frames. *(8)*

Table 2: *Cells involved in wound healing. (7)*

Cell type	Time of action	Function
Platelets	Seconds	Thrombus formation
		Activation of coagulation cascade
		Release inflammatory mediators (PDGF, TGF-β, FGF, EGF, histamine, serotonin, bradykinin, prostaglandins, thromboxane.
Neutrophils	Peak at 24 hours	Phagocytosis of bacteria
		Wound debridement
		Release of proteolytic enzymes
		Generation of oxygen free radicals
		Increase vascular permeability
Keratinocytes	8 hours	Release of inflammatory mediators
		Stimulate neighbouring keratinocytes to migrate
		Neovascularization
Lymphocytes	72–120 hours	Regulates proliferative phase of wound healing although exact mechanisms are unclear
		Collagen deposition
Fibroblasts	120 hours	Synthesis of granulation tissue
		Collagen synthesis
		Produce components of extracellular matrix
		Release of proteases
		Release of inflammatory mediators

Inflammatory phase

After reaching haemostasis, the inflammation phase begins. Chemotactic factors that attract granulocytes accumulate (their greatest concentration appears, as a rule, at 12-24 hours after injury, granulocytes disappear already on the third day). After 24 hours, macrophages are dominant. (7; 8) Macrophages, in turn, originate from monocytes, which attracts the cytokine IL-8 secreted by neutrophils. (9) Macrophages absorb bacteria, release interferons, cytokines (NF, IL-1, IL-6, bFGF) and prostaglandins. (5) In addition, macrophages release collagenases and elastases that help them break down dead tissue. Cytokines are chemoattractants for mesenchymal cells, which later differentiate into fibroblasts. (5; 7) It should also be noted that in this phase vasoconstriction is replaced by vasodilation. (5) The release of bradykinins and histamines increases vascular permeability, which causes plasma to flow into interstitial spaces. (5)

Proliferation

During the proliferation phase, cells such as macrophages and neutrophils are already aging, their activity decreases, and most undergo apoptosis. However, their goal was the release of biologically active substances - cytokines, chemoattractants and free radicals, etc. (5) Due to stimulation by such biologically active substances, fibroblasts rapidly proliferate and produce new collagen fibrils by the 2nd day after injury The greatest synthesis occurs between the 5th and 7th day, and the greatest amount of collagen is observed in 3 weeks. (8) Initially, collagen is a fragile substance/molecule but it is constantly being rebuilt. The balance between synthesis and breakdown of collagen creates the most vulnerable period approximately 7-10 days after the injury. (8; 9) The most common types of collagen in a wound are type I (80% - 90% of total collagen), type III, type IV (basal membrane) and type V (vessel wall).

Naturally, for optimal wound healing, a stable supply of nutrients and oxygen is necessary – for this reason, neovascularization begins on the 3rd day and is most active by the 7th day. (8) Neovascularization occurs under the stimulation of cytokines such as FGF-2 and vascular endothelial growth factor (VEGF). (5; 7; 9) New vessels are loop-shaped, which are surrounded by growing fibroblasts. Because of these processes, the wound surface has a bright red colour of granulating tissue. (8)

The epithelialization stage is also often referred to as proliferation. The proliferation of epithelial cells begins with the migration of epithelial cells to the surface of the wound from skin appendages (for example, from hair follicles). (5) This process is also under the control of cytokines (TGF-β, EGF, KGF, FGF-7). (7) Part of collagen produced at this time becomes a new basement membrane for epithelial cells. (5; 7)

Contraction and Remodelling

Each wound undergoes a remodelling stage for several months or years. (5; 8) Remodelling, as such, is preceded by a phase of contraction, which is the attraction of the wound edges to the centre. In this process, cells such as myofibroblasts are sensed. (5; 8) The remodelling itself includes the already known collagen rearrangement (synthesis, lysis, cross-linking) and is regulated by interferon, TGF-β, PDGF, IL-1. (5) Wherein, TGF-β is divided into TGF-β1, TGF-β2 and TGF-β3, which are opposite in their action. As described earlier, the first two encourages the stem cell proliferation (they also prevail in adults), and TGF-β3 (inhibits stem

cell proliferation) and this type of transforming growth factor is observed to a greater extent in children (10) When such a regulation is out of control, keloid or hypertrophic scars are formed. (6) Matrix metalloproteinase (MMPs) are also responsible for the degradation of excess connective tissue. An imbalance between the tissue inhibitors of metalloproteinases (TIMPs) and MMPs also causes pathological wound healing. (6; 10)

There are also a number of other signalling systems besides TGF-β that regulate healing. These include: Wnt / β-catenin, Notch, Sonic Hedgehog, and ras/MEK/ERK signalling pathway. (11)

Wound dressings

Despite the fact that there are different types of wounds and their various classifications, wound dressings have several basic tasks: creating a protective barrier against bacterial infiltration and maintaining optimal moisture in the wound. (12)

Wound dressings are usually divided into occlusive and non-occlusive dressings. the first mentioned ones show better result, because they reduce bacterial infiltration in a wound. (12) In addition, they produce an acidic wound environment, which also promotes faster healing. (12)

Ideal wound dressing according to Kathryn and Peter Vowden should have a number of the following properties: maintaining moisture in the wound, removing excess exudate, maintaining gas exchange, reducing the likelihood of scar formation, impermeable to bacteria, not sticking to the wound bed. (12)

A wound dressing is selected depending on the type of wound. In the case of the choice of dressings, wounds are conveniently divided by their colour and a stage of healing: black (necrotic), green/yellow (sloughy), red (granulating) and pink (epithelializing).

There are several types of wound dressing, depending on their properties: low or non-adherent contact layer dressing, semipermeable film dressings, hydrogel dressings, hydrocolloid dressings, hydrofiber dressings, alginate dressings, foam dressings, deodorizing dressings, antimicrobial dressings. However, sandwich-structure composite membranes should also be mentioned, having several properties at once and containing two or more layers involved in one dressing. (12; 13)

Low or non-adherent contact layer dressings. As a rule, they are based on silicone and do not stick to the wound, do not disrupt or harm it. However, additional absorbent wound dressing is required. (12)

Semipermeable film dressings. Such dressings are usually made of polyurethane and are additionally coated with acrylic glue, which adheres to dry, intact skin around the wound. They are semi-occlusive, which maintains sufficient moisture in the wound and allows autolytic debridement of necrotic tissue. (12; 14)

Hydrogel dressings. As a rule, these are products based on water or glycerine. Application is similar to semipermeable film dressings – maintaining sufficient moisture in the wound and promoting autolytic debridement of necrotic tissue. Suitable for wounds with minimal or moderate exudate. Products can be in three different forms: gel, impregnated gauze and sheet hydrogel. They can also contain hyaluronic acid or antimicrobials. (12; 15) A typical product is Askina® Gel, which is intended for venous and arterial pressure ulcers, pressure ulcers and traumatic wounds. (15)

Hydrocolloid dressings. They contain absorbing ingredients (usually carboxymethylcellulose, pectin or gelatine) and are occlusive, because of which they do not allow bacterial agents or any fluids into the wound. They also lower the pH in the wound. However, it has its negative sides – they should not be applied in case of infected surrounding skin.

An example of a several component dressings containing a hydrogel may be Askina® Transorbent®, its structure is shown in Figure 4. (15) It can be used both for ulcers of various origins, and for traumatic wounds and burns. (15)

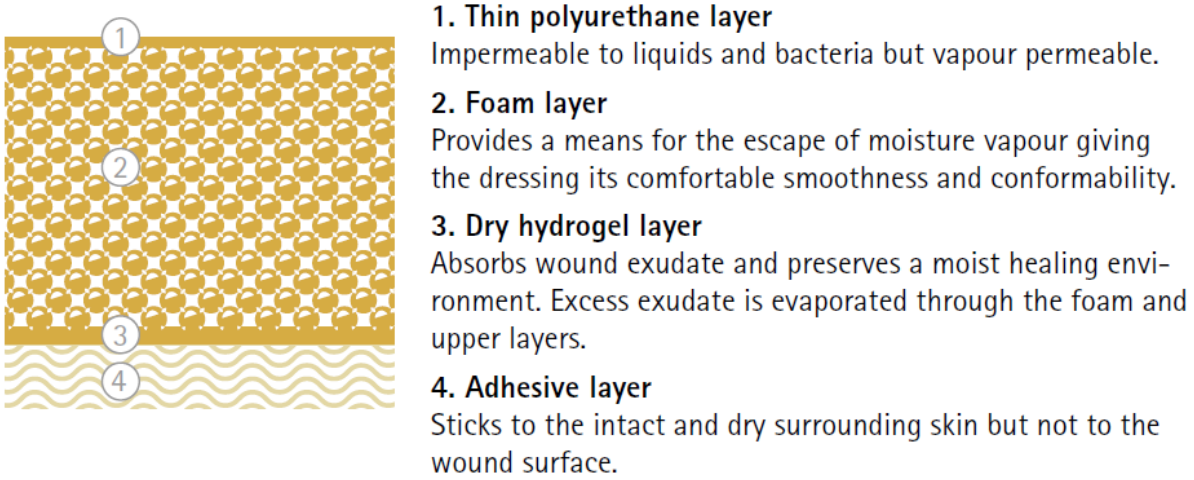

Figure 4: Askina® Transorbent® structure. *(15)*

Hydrofiber dressings. This is an improved version of a hydrocolloid, which as an adsorbent contains sodium carboxymethyl cellulose and can absorb up to 25 times its own weight. (12)

Alginate dressings. Alginates are fibrous products that form a gel upon contact with exudate. They can also be used in infected and non-infected wounds. Naturally, they should not be used in the case of dry wounds. Alginates, as a rule, need to have an additional dressing that protects them from drying out. (12)

Foam dressings. This is semipermeable dressings, based on polyurethane or silicon. They are non-adherent, create a barrier for bacterial agents, maintain moisture in the wound and create thermal insulation. There are also modified versions containing antimicrobial or anti-inflammatory drugs. (12)

Deodorizing dressings. They can be used as secondary dressing in cases of malignant wounds or chronic non-healing wounds, when the smell of a wound is a problem for patients. As a rule, these are Charcoal-based products. (12)

Antimicrobial dressings. We should also mention Antimicrobial dressings. They should be used when it is considered that bioburden is an obstacle to treatment. Currently, products containing iodine (cadoxomer iodine and povidone iodine) containing silver or antiseptic are being produced. (12) This group includes Askina® Calgitrol® Ag and Askina® Calgitrol® THIN. Askina® Calgitrol® Paste can also be noted. This paste increases its effectiveness over time as moisture absorption by the alginate facilitates the release of silver ions. (12) The mechanism of action is indicated in Figure 6.

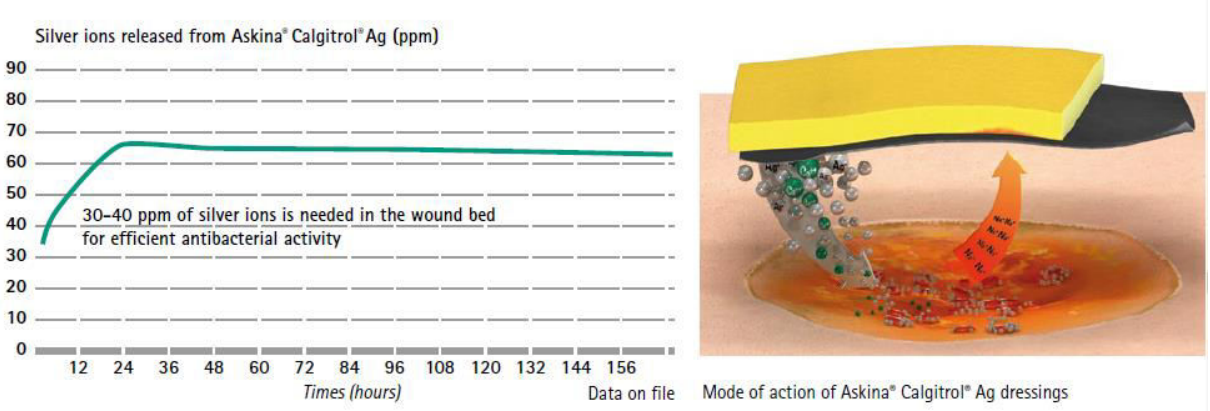

Figure 5: Silver ions release from Askina® Calgitrol® Ag. *(15)*

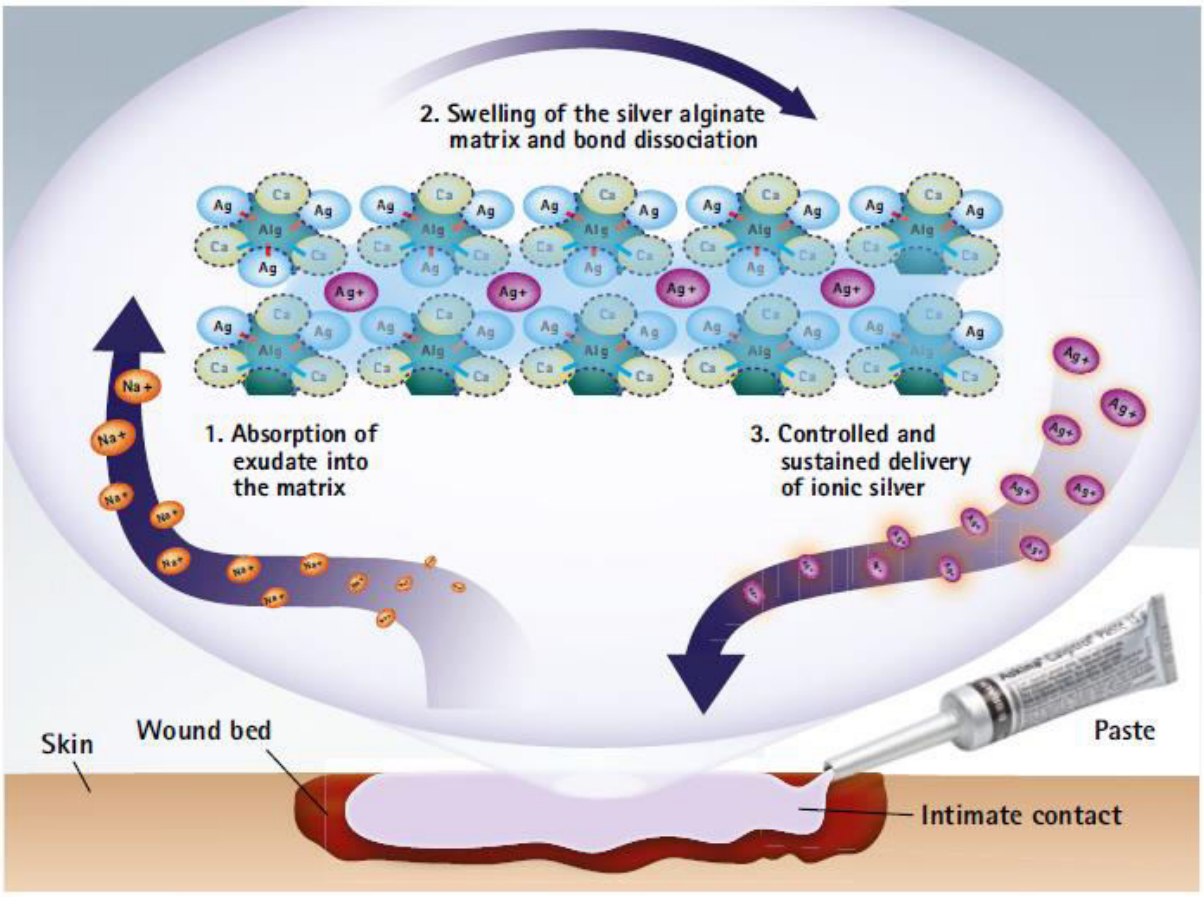

Figure 6: The mechanism of action of the Calgitrol® Paste. *(15)*

Sandwich-Structure Composite Membranes. One of the trends in the development of wound dressing is also the creation of a sandwich-structure composite membrane. They are a promising material for creating dressing since, as a consequence of the layering of fibres with different properties, it is possible to achieve the necessary multifunctionality: control of optimal moisture in the wound, antibacterial properties, the presence of growth factors and so on. (13; 16; 17; 18; 19) However, one should always monitor their biocompatibility, toxicity and biodegradability, which will ensure the necessary dosage of the medicinal substance. Such membranes are most often prepared with the help of electrospinning technology. (13; 19)

In addition, it is important that such membranes have dual properties: both hydrophilicity and hydrophobicity. Hydrophilicity provides tight adherence to the wound, and hydrophobicity – a barrier against the microbe-contained. (13; 18) An example of a membrane is shown in Figure 7. Commercial examples are Askina® Transorbent® or AQUACEL® Ag Foam.

Classification of Wounds

Wound is any damage to the normal anatomy or physiology of the human skin. (20) A clear classification of such damage does not exist, but there are a number of factors by which we can systematize skin defects. The easiest way to separate wounds is to divide into tidy and untidy wounds depending on the absence or presence of devitalized tissue. (21) Other factors can also be distinguished: the cause, the healing time, the depth of the lesion, the location of the injury, complexity, type, contamination and the risk of postoperative infection.

The classification of the alleged wound contamination during surgery is one of the most important in surgery. The damage to the skin is divided into clean (class I), clean/contaminated (class II), contaminated (class III) and dirty (class IV). Clean wounds are characterized by the absence of an infectious lesion and acute inflammation. (22) The clean/contaminated class wounds, as a rule, include those that damage the integrity of the respiratory, gastrointestinal, urinary tracts and other hollow organs, which are characterized by indigenous microflora. However, it is important to note that in these cases the spillage of the internal contents of such organs does not occur. (22) Contaminated wounds are those in which bacteria are infected in those areas of the body that are normally sterile. (22) The last class of wounds is characterized by a long delay in treatment and the presence of necrotic tissue.

Depending on the time spent in getting injured, we can talk either about acute wounds or about chronic ones. To the second we include those wounds that do not heal within 6 weeks. (21) The classification, depending on the conditions of occurrence, includes the division of wounds into incisions, bite, avulsions, lacerations, burns, ulcers and so on. In this thesis in a greater detail will be described wound in which treatment with sandwich structures will be useful.

Ulcers

An ulcer is a painful inflammatory process on the skin or mucous membrane, accompanied by tissue breakdown. (21) Ulcers lead to loss of the epidermis and at least part of the dermis, and in some cases subcutaneous fat, unlike fissures (crack) and erosion (a surface epithelium

defect that does not affect the basement membrane and the underlying layers that heal, unlike ulcers without scarring). (2)

Usually occur due to infections, mechanical, chemical or radiation damage, as well as as a result of circulatory disorders and/or innervation (a more detailed list is presented in table 5). For an ulcer, in contrast to a wound, there is a loss of tissue ("minus-tissue"). This type of skin lesion heals with scar formation. (2)

Table 3: Causes of skin ulceration. (23)

Category	**Condition**
Venous	Vein thrombosis, venous incompetence, varicose veins, venous stasis, lipodermatosclerosis, complication of sclerotherapy
Arterial	Arterial obstruction, atherosclerosis, thromboangiitis obliterans, cholesterol embolism, arteriovenous malformation, hemangioma, hypertensive ulcer, complication of sclerotherapy
Microcirculation	Diabetic microangiopathy, vasculitis
Neuropathic	Diabetes mellitus, Hansen's disease, tabes dorsalis, syringomyelia, spina bifida, paraplegia, amyotrophic lateral sclerosis, other neuropathic disorders
Hematologic	Sickle cell disease, thalassemia, hereditary spherocytosis, polycythemia vera, leukemia, dysproteinemias, disseminated intravascular coagulation, idiopathic thrombocytopenia, chronic graft versus host disease, acquired homocystinuria, warfarin necrosis, heparin necrosis
Immunologic	Bullous pemphigoid, cicatricial pemphigoid, pemphigus, epidermolysis bullosa acquisita, linear IgA bullous dermatosis, erythema multiforme, allergic contact dermatitis
Infectious	Erythema induratum/nodular vasculitis, mycotic, bacterial, leishmania, herpes
Metabolic	Diabetes mellitus, gout, pretibial myxedema, Gaucher's disease, prolidase deficiency, porphyria cutanea tarda, necrobiosis lipoidica, bullous diabeticorum, diabetic dermopathy, drugs
Renal	Kyrle's disease, reactive perforating collagenosis, calciphylaxis
Nutritional	Scurvy, malnutrition
Genetic diseases	Epidermolysis bullosa, Werner's syndrome, Klinefelter's syndrome
Neoplastic	Basal cell carcinoma, squamous cell carcinoma, keratoacanthoma, malignant melanoma, Kaposi's sarcoma, malignant eccrine poroma, angiosarcoma, cutaneous metastasis, cutaneous lymphoma
Chemical/physical	Caustic agents, decubitus ulcers, thermal injury, mechanical trauma, radiation, frostbite, factitial, Charcot's deformity, chronic osteomyelitis, insect bites, coral and marine life-induced ulcers
Lymphatic disease	Lymphedema, lymphangioma
Other	Pyoderma gangrenosum, panniculitis, Raynaud's disease

Ulcerations in the lower limbs are the most significant in the wound care area. Among them, about 80 % are venous, 5 % arterial and 2 % diabetic/neuropathic. (23) Among

the total number of ulcers, regardless of the anatomical position, 5–20 % are pressure ulcers.

Venous ulcers

Venous ulcers are most often found on the inner surface of the distal leg and ankle. Most ulcers lie along the *venae saphena*e system. (8) The exact mechanism of the development of venous ulcers is not currently known, although the basis of this disease is, of course, venous insufficiency. (23; 2) At the moment there are several hypotheses of the pathophysiological mechanism of disease development: the hypothesis 'trap', the hypothesis 'cuff 'and others.

The 'trap' hypothesis suggests that, due to venous hypertension and improper distribution of blood in tissues, fibrinogen and other macromolecules flow into the dermis, after which they bind growth factors that are no longer able to repair damaged tissues. (23) In addition, red blood cells and white blood cells are also captured, which release inflammatory mediators. (23) This accumulation of inflammatory cytokines, including TGF-β1, causes the release of oxygen free radicals and metalloproteinases, which causes local tissue destruction, fibrosis and ulceration. (2)

According to the 'cuff' hypothesis, fibrinogen, which seeps into the capillaries due to the action of venous hyperextension in the dermis, polymerizes, forming pericapillary fibrin deposits, reducing diffusion of oxygen and other nutrients. This violation leads to tissue ischemia and their death. (23) In addition, it is believed that dermal fibroblasts from venous ulcers become immune to the action of wound healing mediators and this undermines the wound healing process, forming a vicious circle. (23)

Macroscopically venous ulcers have an irregular shape, exude an abundant fibrinopurulent exudate greenish-yellow colour, which has an unpleasant odour. This condition is accompanied by pain and often superinfection. (23)

Venous ulcers naturally cause a number of complications and further developing conditions. They include deposits of hemosiderin (a dark yellow pigment consisting of iron oxide and is formed by the breakdown of haemoglobin and the subsequent denaturation and deproteinization of ferritin protein) or cayenne pepper purpura. Inflammation causes panniculitis. (23) Over time, panniculitis scleroses tissue and flows into lipodermatosclerosis. In addition, dystopic calcium deposits are noted. (23)

As mentioned above, superinfection is one of the most frequent complications. At the first appearance of pyrexia, lymphangitis, a rapid increase in the pain of the affected area, the use of systemic antibiotics is necessary. Allergic dermatitis and eczema are also typical complications. (2) Malignant transformations are also frequent (for example, squamous cell carcinoma). (2)

Arterial ulcers

Arterial ulcers are usually caused by narrowing of the lumen of the arteries. The latter, in turn, may be due to the accumulation of cholesterol plaques and blood clots. (23) Such a narrowing of the arteries causes collateral circulation to develop in the tissues to compensate for blood circulation. Most often, this process affects the entire femoropopliteal trajectory, and sometimes the small vessels, which leads to a heart attack of subcutaneous fatty tissue. (23)

Patients with arterial ulcers experience severe pain when walking, which is relieved by rest. Intermittent claudication, loss of hairy coat, cold skin and muscular atrophy are also noted. (23) Peripheral pulsation can be either weak or altogether absent. Usually, arterial ulcers located over pressure points, such as fingers and ankles. (23) Arterial ulcers have sharply defined edges with a small amount of granulation tissue. Often there are necrotic areas. (23)

In such cases, an integrated approach is used. On the one hand, this is the use of thrombolytics, and on the other, surgical intervention (wall installation or revascularization using the patient's own vessels or using transplants). (23)

Diabetic ulcers

Over 50% of lower limb amputations in the United States occur as a consequence of diabetes. (23) Such amputations are always preceded by foot ulcers. Diabetic foot ulcers, however, in turn, may also have a different origin. They are divided into neuropathic and ischemic. (23) In addition, there are other skin disorders: *necrobiosis lipoidica diabeticorum*, diabetic dermopathy, or diabetic bullae. (23)

Since diabetes causes neural pathology, patients tend to lose sensitivity and initially an ulcer appears as a pressure ulcer. Typical points for developing ulcers are the first and

fifth metatarsal heads, and the great toes. The very same foot can be a normal colour with a fairly strong pulsation. (23)

Pressure ulcers

As already mentioned, pressure ulcers is one of the five most common types of ulcers and one of the costly complications for bedridden patients. According to the Agency for Health Care Policy and Research (AHCPR), almost two-thirds of bed patients are hospitalized for pressure ulcers. (24) The death of soft tissues occurs as a result of constant pressure, as well as due to such complicated conditions as local circulatory disorders and nervous trophism. (24)

It should also be noted that such ulcers begin to develop not on the skin itself, but on the bone-tissue interface, since at these points the pressure is highest. Moreover, muscles and fatty tissue require more oxygen than the dermis, therefore, they are most susceptible to hypoxia. (24) There are also a number of risk factors. Among them are smoking (reduces tissue perfusion), low body mass, decreased sensory perception, moisture, and others. (24) For more accurate diagnosis, the classification of bedsores was introduced by stages. These stages are presented in table 6. (24)

Table 4: Decubitus stages. (24)

Stage	Characteristic
I	Observable pressure-related alteration of unbroken skin that differs from adjacent or opposite area of skin in: Temperature (warm vs. cool) Tissue consistency (firm vs. boggy feeling) Sensation (pain or itchiness) Induration, hardness, or edema may also be present.
II	Superficial ulcer with partial thickness skin loss involving epidermis, dermis, or both.
III	Full thickness skin loss involving damage or necrosis in subcutaneous tissue that may extend down *to*, but not *through*, underlying fascia.
IV	Full thickness skin loss with extensive destruction, necrosis, or damage in muscle, bone, or support structures such as tendons and joint capsules

Treatment

One of the most important factors for healing ulcers is the elimination of factors contributing to the development of ulcerations. In addition, it is necessary to eliminate such possible concomitant complications as an infectious lesion, a change in pH, and others. Given the above factors, typical treatment includes the following steps: cleansing the wound area, ligation, and bacterial control. (24)

To clean the wound from necrotic tissue and exudate, you must use warm saline or water. (24) Soaps and other antiseptics intended for intact skin should not be used because they are too aggressive for healing tissues and, moreover, damage fibroblasts, macrophages and other cells necessary for normal physiological wound healing. For example, iodine solutions that reduce the number of bacteria are toxic to fibroblasts, neutrophils, and red blood cells. (24) In case a detailed fabric remains after cleaning the wound, various debridement methods are used: autocatalytic, chemical, mechanical and surgical. (24)

Next, you need to choose the right Wound Dressings, depending on the condition of the wound. One of the main goals is to maintain optimal moisture in the wound, it contributes to faster cell migration, maintaining an appropriate level of phagocytic activity and proliferation of epithelial cells. (24)

Control of a bacterial infection is also an important part of the treatment of pressure sores. It is believed that all bedsores are contaminated. (24) However, the use of antibiotics may be questionable, since topical antibiotics are often ineffective against mixed microflora, and systemic antibiotics may not reach their destination at all due to impaired perfusion of tissues. (24) If the ulcer shows no signs of healing within 2-4 weeks, triple antibiotic or silver sulfadiazine can be used. (24) A flowchart for treatment selection is specified in Appendix 1.

Burns

Burns are skin or other tissue damage caused by heat, or due to radiation, radioactivity, electricity, or contact with chemicals. (25) Burns are a fairly global problem, since about 180,000 deaths are recorded annually. In addition, they are a common cause of disability-adjusted life-years (DALYs). (25)

At the site of the damaged area, an increase in capillary permeability occurs and a large part of the plasma, like macromolecules (albumin and others), goes beyond the limits

of the vessels in the interstitial spaces. (26; 27) This continues for 48 hours after receiving the burn, with a maximum in the first 8. (26) Due to the release of macromolecules beyond the capillaries, additional oncotic pressure is created, which draws even more fluid, thereby causing dehydration and even collapse. Burns affecting more than 15% of the body of an adult and more than 10% of the body of a child always land up in hypovolaemic shock, unless appropriate medical assistance is provided. (26; 27) If more than 35% of the adult's body surface is damaged and more than 30% of the child's body, such burns can be considered life-threatening. (26) Moreover, with more severe burns, pulmonary edema and myocardial dysfunction can occur as histamine and other inflammatory mediators are released, which increase the vascular permeability systemically. (27) After 48 hours, the capillaries thrombose and no longer participate in the blood circulation. (26) In addition, the level of endogenous steroids increases, which contributes to a decrease in immunity and the frequent occurrence of sepsis in patients.

In describing the damage, you can use Jackson's burn model, in which he singled out the zone of necrosis, the zone of stasis and the zone of hyperemia. (27) The zone of coagulative necrosis is the zone of greatest heat exposure, in which proteins are denatured due to the high temperature, which results in necrosis. (27) The stasis zone is characterized by high edema, however, this tissue can usually be saved. (27) The next zone – the zone of hyperemia is characterized by inflammation, this tissue can almost always be saved, except for cases of sepsis. (27) However, for clinical purposes, it is most convenient to determine the extent of damage by the depth of penetration of the burn into the skin.

Thus, the following degrees of burns are most often distinguished: superficial, partial thickness (superficial partial thickness and deep partial thickness), full thickness. (8; 27) Superficial (or First-degree burn) affects only the epidermis. Such burns are very painful and erythematous, however, they heal without scarring, within 5-7 days. (26) A typical example of such burns is sunburn. (27)

Partial thickness can be divided by classification into Superficial partial thickness and deep partial thickness. Superficial partial thickness (second-degree superficial) are characterized by the appearance of blister (may occur within 24 hours after the end of the exposure to the heat factor), usually between the epidermis and the dermis. (8; 27) Such burns, like superficial, are also painful and erythematous. However, unlike them, they are not dry, but wet due to leakage of liquid from the blister. Heal within 3 weeks and rarely

cause scarring. However, there is a risk of infection that will inhibit healing. (27) In the case of first and second-degree burns, the primary intention should be used. Second-degree superficial burns heal on the basis of the epithelium of residues of hair follicles, which are abundant in the dermis. (26)

Deep partial thickness (second-degree deep) penetrates deep into the dermis, affecting the skin follicles and skin glands. (8; 27) Are characterized by the appearance of blister, because of which infections can often join. Such burns heal over 10 weeks with scarring. Moreover, there are clear contracture over joint lines. (27)

In the case of full thickness (third-degree burn), the name speaks for itself and such burns affect the entire thickness of the dermis, as well as sometimes subcutaneous fatty tissue. However, due to damage to the nerves in the dermis, they are practically painless. (8; 27) This type of burns can be characterized by the absence of blisters, as well as the appearance of burn eschar (inelastic, dry skin) from denatured dermis, which can interfere with blood circulation in the underlying tissues. Typically, this type of burns requires surgery and grafting for healing. (27)

Healing of burn wound

Healing occurs as in the case of other skin lesions according to a typical principle, which includes three stages: Inflammatory (reactive), proliferative (reparative) and maturation (remodelling). Distinguishes burns only the duration of each phase.

Inflammatory phase. Inflammatory phase is the same in all types of wounds. Almost immediately after injury, an inflammatory reaction begins in the body, which has vascular and cellular components. The vascular response has already been affected above. Immediately after receiving the burn, dilation of the vessels occurs and the plaque leaks into the interstitium. (26)

Cellular response is also typical. Neutrophils and monocytes migrate to the site of inflammation first. After some time, macrophages, attracted by kallkireins, fibrin and substances released by the cells, proteases, leukotreins and cytokines, come to the place of neutrophils. (26)

Proliferative phase. As mentioned above, with burns with a partial depth of damage, re-epithelization begins with the migration of keratinocytes from viable skin appendages in the

dermis within a few hours after injury. A new layer of epithelium, as a rule, appears after 5-7 days. (26) The basement membrane is formed after the restoration of the epidermis.

Remodeling phase. The remodeling phase is the final stages of wound healing. During this phase, fibrous structural proteins (collagen and elastin) are first released around the epithelial, endothelial and smooth muscles as an extracellular matrix, which is later reconstructed into scar tissue, and the fibroblast becomes myofibroblast, which causes the contracture of the scars. (26)

In the case of burns on the full thickness of the dermis, this phase may take several years. In addition, melanocytes can react to such thermal damage, causing hyperpigmentation of the burn site. This is also often seen in patients with skin grafting. (26)

Treatment

Naturally, when providing first aid, it is very important to stop the spread of thermal damage. It is usually achieved by the fact that the site of the lesion is washed with tepid water (about 15 degrees Celsius) for 20 minutes. In some cases, to minimize fluid loss, the place of burns is covered with a cling film. (27)

Upon arrival at the hospital, doctors follow a clear protocol of action when providing immediate hospital management. As a rule, they check the airway patency (the need for intubation is assessed), disability, and study the dream of a burn and collect additional history. (27) Also, fluid resuscitation is one of the important points of care, since severe burns greatly reduce intravascular volume. For these purposes, Hartmann's solution or Ringer's lactate is most often used. (27)

In addition to those actions that are aimed at systemic improvement of the state of the body (such as restoring the volume of fluid in the body or systemic antibiotics), there are a number of techniques aimed directly locally at the affected site. In addition to surgical closure through the primary healing process or by suturing, skin grafting, Skin substitutes, various Wound dressings and Negative pressure wound therapy (NPWT) can be used. (28)

Table 5: Skin substitutes. *(28)*

Skin substitute	Composition	Cells incorporated
KaroSkin	Human cadaver skin with dermal and epidermal cells	
GraftJacket®	Human acellular pre-meshed dermis	
StrataGraft™	Human dermal fibroblasts and stratified epidermis derived from Near-diploid Immortalised Keratinocyte S (NIKS)	
GlyaDerm®	Glycerol preserved acellular dermal collagen-elastin matrix	
OASIS® Wound Matrix	Porcine acellular lyophilized small intestinal collagen matrix	
XenoDerm	Lyophilised acellular porcine dermis	
Permacol™	Porcine acellular diisocyanite cross-linked dermis	
PermaDerm™	Autologous keratinocytes and fibroblasts cultured with bovine collagen	
OrCel®	Bilayered type I collagen matrix	Allogenic neonatal foreskin keratinocytes and fibroblasts
RenoSkin®	Bilayer dermal matrix – silicone film and porous crosslinked bovine collagen	
TransCyte®	Porcine collagen-coated nylon mesh	Allogenic neonatal human foreskin fibroblasts
Integra®	Cross-linked bovine tendon collagen and glycosaminoglycan, and polysiloxane (silicone)	
Pelnac®	Porcine tendon derived atelocolla	
Hyalograft 3D	Hyaluronic acid membrane	Autologous fibroblasts
Dermagraft®	Bioabsorbable polygalactin mesh matrix	Human neonatal fibroblasts
TissueTech Autograft System	Microperforated	Autologous keratinocytes and fibroblasts
Suprathel®	Synthetic copolymer – dl-lactide (> 70%), trimethylenecarbonate and ε-caprolactone	
Laserskin®	Hyaluronic acid membrane	Autologous keratinocytes and fibroblasts
MySkin™	Silicone support with a specially formulated surface coating	Autologous keratinocytes

As mentioned above, with burns affecting the entire thickness of the dermis, eschar may occur. In cases of eschar in the torus area, its surgical removal is necessary – escharotomy, since a dense burn threatens tissue perfusion. (27)

Usually, surgical wound closure is best performed within the first 6 hours, however, if we are talking about burns, then as a rule, the surgeon does this after 72 hours after receiving the burn. (26)

There are also a number of promising technicians who are only at the development stage. For example, it is possible to use a keratinocyte patient to accelerate epithelialization and reduce the likelihood of scarring or improving their condition in those cases where their appearance cannot be avoided. Kerotinocytes can be used as a complete graft (keratinocyte sheets), or on such carriers as chitosan, alginate, fibrin or collagen, or even in the form of a spray. (29; 30) Despite good results, among the minuses of the technique is the long period between the biopsy and the possibility of using grown keratinocytes. (29)

Infection and Immune Profile

Given such factors as hypovolemic shock and weakened immunity, infection is one of the main problems in the treatment of serious burns. It was noted that in the accession of infectious diseases of burn patients an important role is played not only by bacterial agents, but also by viruses and fungi. (26) The most common disease is sepsis (as well as pneumonia, as one of the manifestations of sepsis). The most common cause of pneumonia is *Staphylococcus aureus* and *Pseudomonas*. The rest of the infection is other gram-negative organisms, such as *Kleibsiella*, *Escherichia coli, Salmonella, Acetinobacter bomnii* and *Haemophillus*. (26)

Candida albicans, as well as some species of *Candida nonalbicans* (*C. krusei and C. glabrata* and *Aspergillus*) can be distinguished among the main fungal agents. (26) It has been observed that such fungal lesions are associated with very high mortality, as the organisms are sensitive only to echinocandins and amphoteracin B and are resistant to azoles. (26)

Immunity is weakened by the fact that polymorphonuclear and mononuclear phagocytes are ineffective in burn patients. (26) The specific immunity is also always markedly depressed. It is important to note that microorganisms are located not only on the surface of the skin, but also in the dermis - namely, near the sebaceous glands and hair follicles. (26) Such microorganisms often begin to multiply in the sub-spherical space, because eschar is, in fact, a dead tissue and a favourable environment for microorganisms. Conventional antimicrobial agents do not penetrate into the sub-axial space and therefore

are non-effective. It is believed that 1 % sulfadiazine silver cream is one of the best creams available for extensive burn treatment. (26)

Scars after burns and injuries

As a rule, the inevitable continuations of deep burns are scars and contractures. (26) Appearance of a scar at the site of the burn corresponds to the remodeling phase of wound healing. In the case of deep burns, the edges of which are not excised, and hypertrophic scars occur in the treatment of skin grafting. (26)

INTRODUCTION TO POLYMERS

The word polymer itself can have different meanings. The most important of these are (i) a polymer as a material and (ii) a polymer as a polymer chain. In this paper, polymers will be considered in these two senses. Polymers refer to macromolecules in which, by definition, there are more than a thousand atoms bound by a covalent bond. Such macromolecules can be linear, branched or other types of structures. (31)

For the description of polymers many parameters are used. One of them is: molar mass distribution (MMD) (or the molecular weight distribution is also found in some literature). This parameter indicates the average size of the molecule and can vary greatly depending on the method of synthesis of the polymer. As a rule, a polymer product contains many different lengths of chains, and all these possible options are subject to the same probability function. And for practical applications of a specific polymer, it is important to know not only the average mass of the molecule, but also mass distribution, since some properties (for example, tensile and impact strength) depend on the shortest molecules, and others (melt elasticity) on the longest. (31)

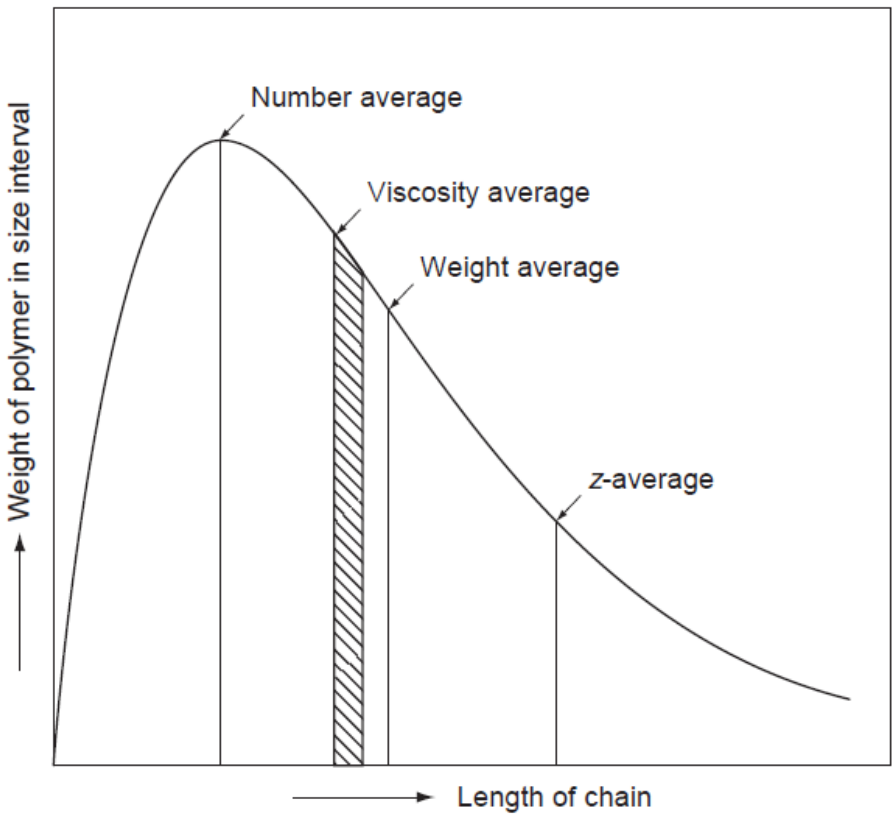

Figure 7: Molar mass distribution graph (1)

Such a curve is described by several parameters: M_n (Number-average molar mass), M_w (Weight-average molar mass) and M_z (z-Average molar mass), each of which can be calculated using a certain formula. (31) The most important are the following ratios 1 and 2, which are responsible for polydispersity index Q:

$$Q = \frac{M_w}{M_n} \quad (1)$$

$$Q' = \frac{M_z}{M_w} \quad (2)$$

In the case when Q = Q ’= 1 we speak of an ideal monodisperse polymer. (31) This index is also associated with the U — non-uniformity index. It, in turn, is directly related to the standard width of the distribution (S_n). Such characteristics as packing density, molecular mobility and others are also often used.

CLASSIFICATION OF POLYMERS

Polymers can be classified in various ways. For example, based on the polymer structure, polymerization mechanism and even thermal behavior. However, the most important classification is separation based on origin of the polymer. (32)

Classification based on origin

Polymers can be natural or purely synthetic (sometimes combined materials are also separated into a separate group). Naturally, the first polymers used were such natural polymers as cotton, starch and proteins. The advantages of their use include the renewability of resources, biodegradability and environmentally friendly degradation products. (33) Also, almost all processes in the human body depend on the presence of such polymers as enzymes and are regulated by them. Enzyme deficiency can lead to many diseases (for example, gout) and life-threatening conditions (e.g. diabetes mellitus). (32)

At the beginning of the twentieth century, synthetic polymers began to be produced and these include various families: fibers, elastomers, plastics, and so on. (31; 33) At the moment, a wide variety of polymers is available to mankind with different variations of either the main chain or the side branches of the polymer. Well-known household synthetic polymers include nylons in textiles and fabrics, Teflon in non-stick pans, polyvinyl chloride (PVC) in pipes, and others. Synthetic polymers are then divided into biodegradable (polyesters like poly (lactic acid), poly (glycolic acid)) and non-biodegradable (silicones, cellulose derivatives, etc.). (34)

NATURAL POLYMERS

Chitin and chitosan

Chitin is one of the most common polysaccharides in nature and is most often found in the exoskeleton of arthropods and crustaceans, as well as in mushrooms in the form of ordered microfibrils. (35) It plays the same role in the body of crustaceans as collagen in the human body and higher animals and cellulose in plants. (35) And chitosan, in turn, is the substance closest to chitin and actually has one structure with it since it differs only in the degree of acetylation. Chitin exists in three forms: α-chitin, β-chitin and γ-chitin, which correspond to an anti-parallel, parallel, and alternating sequence of polymer chains.

Chitin may be obtained in two stages, and chitosan in three. The extraction process includes demineralization and deproteinization for chitin, as well as subsequent deacetylation, for chitosan. (36)

Despite the structural similarity, chitosan is a much more popular material than chitin. It should be noted that chitin is rather difficult to process due to its low solubility in water and organic solvents due to strong hydrogen bonding network. (36) Chitin is much better dissolved in strong concentrated acids (sulfuric acid and phosphoric acid) and popular fluorinated solvents (such as hexafluoroisopropyl alcohol), but such solvents are corrosive and toxic, which complicates the use of chitin. (35; 37; 36) Naturally, there are methods, which enable to improve its processability. It is possible to use either one of the allomorphs of chitin, β-chitin, or with deacetylation of 0.5 chitin becomes water soluble. Such water-soluble chitin requires much less aggressive conditions in order to remain in solution, namely the pH of <5, which is not critical for living cells. (37)

Chitin and chitosan are in the focus of the interest due to the presence of a large number of amino groups, which can be appropriately modified to obtain the necessary properties, including solubility. Solubility of chitin can be affected by the introduction of water-soluble entities, hydrophilic moieties, and also hydrocarbon groups. (35) A detailed study of modifications of chitin and chitosan was also conducted by Hitoshi Sashiwa and Sei-ichi Aiba. (38) Dibutyryl chitin, for example, is soluble in common solvents and was prepared by esterifying native chitin with oily anhydride in the presence of perchloric acid. The spinned fibers were porous. (35)

Naturally, it is possible to spin fibers of chitin in combination with other polymers. Substances such as polyvinylpyrrolidone, methyl cellulose, and sulfite cellulose have been used to modify the properties of chitin by adding to the spinning solution. (35) It is also a positive feature that chitin is considered highly biodegradable and is easily excreted from the body through urine. (35)

Chitosan fibers are much easier to obtain, since they are soluble in dilute acid solutions. However, the cost of making such fiber is very high. Therefore, it is necessary to study composites and mixtures based on chitosan and other polymers. (35)

Chitosan, like chitin, is labelled as highly biodegradable and excreted in the urine, and also has no tendency to accumulate in the body. (35) It has been shown that the rate of decomposition is affected by the porosity of the fibers, as well as the mixing of chitosan with other polymers and the manufacture of compositions. (35) For example, adding a CS / PVA component to PLGA fibers by simultaneous electro-pinning from two syringes showed a significant change in the balance of hydrophilic / hydrophobic properties, which greatly influenced the degradability and mechanical properties of the fibers. Such a membrane is also a remarkable base for human embryo skin fibroblasts (hESFs) culture, since the cells not only were located on the surface of the membrane, but also infiltrated it. This behavior makes it possible to better repeat the natural extracellular matrices. (39)

Chitosan itself is an antimicrobial agent and has anti-inflammatory properties, making it an excellent candidate for its usage in the field of wound healing. (40) Photo-crosslinked fibers obtained by the electro-pinning method and containing quaternary chitosan (QCS) have also shown their effectiveness in inhibiting the growth of gram-positive bacteria and gram-negative bacteria. Similarly, photo-cross-linked chitosan composites with other polymers may have similar properties, such as, for example, QCS / PVA. (41) It was shown that chitosan hydrogel crosslinked upon ultraviolet light irradiation, covering the transplants, showed resistance against E. coli. (40) Such hydrogels also support cell adhesion and proliferation. (40) In addition, the joint use of chitosan and silver has a synergistic effect. A coating like chitosan/silver-doped hydroxyapatite shows good biocompatibility and does not have toxic effects on cells. (42) The chitin/CS and cellulose composite showed excellent moisture retention and these fibers are shown for keeping to dry. In addition, they do not cause irritation, which makes them indispensable for the elderly and children. (35)

Moreover, in some literary sources it is mentioned that the degradation of chitin and chitosan itself can stimulate tissue healing. Chitin and chitosan during decomposition are gradually depolymerized with the release of N-acetyl-β-D-glucosamine, which positively affects the orderly deposition of collagen, which not only accelerates healing, but also reduces the likelihood of keloid scars. And such degradation products as chito oligomers stimulate the work of macrophages. (41)

It should also be mentioned that chitin and chitosan composites can also be used for bone tissue engineering (especially after strengthening with materials such as Bioglass ceramic (BGC), Silicon dioxide (SiO_2), Titanium dioxide (TiO_2)). (43; 44)

Nanofibers based on chitin and chitosan can also be used for filtration (both water and air purification are possible), it is possible to use both biosensors or drug delivery systems. (41) Jiang H et al. reported poly (lactide-co-glycolide) / poly (ethylene glycol) -g-chitosan membrane synthesis, which was ibuprofen-loaded. It was described that the duration of the release of ibuprofen could be up to two weeks. (45)

Hyaluronic acid

Hyaluronic acid is a member of the glycosaminoglycans family. Just like the whole group, this is a linear polymer consisting of two components — N-acetyl-d-glucosamine and glucuronic acid. The molecular weight of this polymer can be from 20 kDa to 4000 kDa. (46; 47) Also this polymer in solutions can be characterized as very a viscous and elastic one. (47) Hyaluronic acid is synthesized in cells using three glycosyltransferases: Has-1, Has-2 and Has-3. (46)

Most of the hyaluronic acid is in the extracellular matrix (ECM) of connective tissues. One of the functions of HA is intracellular signalling, and therefore there is a huge amount of receptors for this polymer in the human body. (47)

Hyaluronic acid is secreted by cells in the same way during wound healing, so it is of particular interest for wound dressings. HA is able to stimulate angiogenesis, promote the migration and differentiation of mesenchymal and epithelial cells. (46) These processes occur through the action of HA on the CD44 receptor. (47) This polymer is also immunoneutral, which makes it an excellent conditioner for creating drug delivery systems and for use not only as wound dressings, but also in tissue engineering. (46; 47) It is also

known that the different length of the polymer chain affects the biological processes of the body in different ways. There is a theory that, depending on the length of the HA molecule, it can function as a tumor suppressors or tumor growth agent. (47)

Based on hyaluronic acid using electrospinning, it is also possible to synthesize fibers, which can subsequently be loaded with a drug and cross-linked, as shown by Morgane Séon-Lutz et al. It is important to note that the team also used hydroxypropyl-βcyclodextrin to stabilize electrospinning. (48)

The creation of multilayer composites using layer-by-layer assembly technique is also mentioned in the literature. However, despite such properties of hyaluronic acid as the ability to stimulate epithelial cell migration and angiogenesis, an increase in the percentage of HA in such multilayer composites reduces air permeability and relative water vapor permeability, which can delay healing. (49)

Recently, HA began to be used as a drug delivery system. Using HA provides certain advantages: improved pharmacokinetic features, an improved solubility, and better bioavailability to follow, protects drugs from deactivation, prolonged plasma half-life, directs the medication specifically to the site of action. (47; 50; 51) Various combinations of HA with other polymers to create nanocapsules are also known, which later could be used as delivery systems. Among them the most interesting are: HA / poly (ε-caprolactone), HA / polylactide or poly (lactide-co-glycolide), HA / PEG, HA / chitosan and others. It is also quite effective to combine HA with magnetic nanoparticles, with graphene oxide and carbon nanotubes, or with quantum dots. (47; 51; 52)

In addition, HA and HA derivatives are widely used in tissue engineering applications. Such scaffolds show great practical potential. HA-based hydrogels have the ability to maintain a hydrated environment that promotes cell infiltration, which is ideal for the treatment of chronic non-healing wounds. The presence of pores and the ability to adjust their diameter depending on the method of synthesis of scaffolds (in the case of electro-pinning, the control of pore size is difficult) is also an important aspect, since the possibility and number of cells adhered on the surface depends on it. (53; 54; 55)

Collagen

Collagens are the most common proteins in the human body that have fibrillar structures and are the main skin proteins. However, it is possible to find them in other organs, these proteins perform the function of maintaining tissues. In the human body, 26 different types of collagen were found, the most common of which are types I-IV, and collagen I is the most studied protein. (46; 56)

All collagens form a characteristic triple helix of three polypeptide chains, although their functions and locations can vary greatly. Based on their supramolecular structure, they can be divided into the following types: fibril-forming collagens, fibril-associated collagens (FACIT), network-forming collagens, and others. About 90% of all collagens belong to the fibril group -forming collagen. (32)

Collagen, like other polymers, can be used for electrospinning and used in curing and other biomedical areas. For the first time, a nanofibrous matrix for wound dressing collagen was synthesized in 2006 by Rho et al. (57; 58) As a result, such fibers had a high porosity and high surface area-to-volume ratio. In addition, they were suitable material for the adhesion of cell culture. (57)

Moreover, it is possible to create combined fibers containing, in addition to collagen, other polymers – for example, PCL. However, such natural polymers suffer from batch-to-batch variations, which complicates the selection of parameters for electrospinning and, in fact, the process itself. Solution viscosity had the greatest impact on the fiber spinning process. As indicated in the article, the most successful was the case of 12wt.% PCL: Collagen solutions with spinning parameters such as: 15kV, 15cm needle-collector distance, 2cm bar distance and 1ml / h flow rate. (59)

Also, the combinations with other polymers are mentioned in the literature: Collagen/ZN and PLGA/collagen. Also marked tripolymer structure - PCL/gelatin/collagen. Sneh Gautam et al. In the course of the work, PCL/gelatin nanofibrous scaffold was prepared using electrospinning, which was later modified using collagen type I (0.2–1.5 wt.%) grafting. It is worth noting that, depending on the amount of collagen added, the pore size of the fibers varied during the experiments. This structure also showed good results as a matrix for cell culture. (60)

It is also possible to combine a polymer with antimicrobial agents (antibiotics such as irgasan and levofloxacin, for example) or growth factors (vascular endothelial growth factor, platelet-derived growth factor and others). Fibers of this type are also prepared using electrospinning, as described in Huan-Ju Lai et al. (57; 61; 62)

Silk fibroin

Silk fibroin (SF) is a natural protein polymer that has recently attracted a lot of attention in the field of tissue engineering, because it has high biocompatibility, biodegradability and low immunogenicity. Also, this polymer almost does not cause inflammation, it is not cytotoxic and, moreover, SF is approved by the FDA. (63) Currently, surgical sutures are the most widely used (e.g., SOFSILK ™, PERMA-HAND ™). (64)

Silk proteins spun into their metamorphosis during arthropods (e.g. silkworms or spiders). They perform many different functions in a living organism, including the constituent part of cocoons that protect eggs or larvae. The structure of these proteins depends largely on how exactly the kind of arthropods they synthesized, since some of the spiders are able to create up to 7 different types of silk. (65) Silk fibers in cocoons are interconnected with sericin, which is a serious allergen. Because of this, it is necessary to carry out the process of degumming, those. cleaning silk fiber. (63)

The most studied silk is silk produced by B. mori. (63) This silk has a sufficiently large molecular weight (about 200-350 kDa), has large hydrophobic and small hydrophilic domains, and consists of heavy (H) and light (L). The aforementioned sercin holds these chains together. (63; 66) The complex of the heavy and light chains is also bound by glycoprotein P25 through hydrophobic bonds, as a result of which the elementary micellar unit is formed. (64)

The amino acid sequence of this polymer provides ample opportunities for chemical modification and functionalization. They can change the hydrophilicity and / or charge of the SF molecule, which will also change the interaction between SF and other biomolecules. (63; 64) So transgenic silkworms are used to create a fluorescent coloured silk, which has excellent mechanical strength from normal fiber. Or another example of intrinsic functionalization is feeding silkworms with certain functional substances, however, a certain

degree of their hydrophobicity is required for penetration into the molecules in the silk gland. (64)

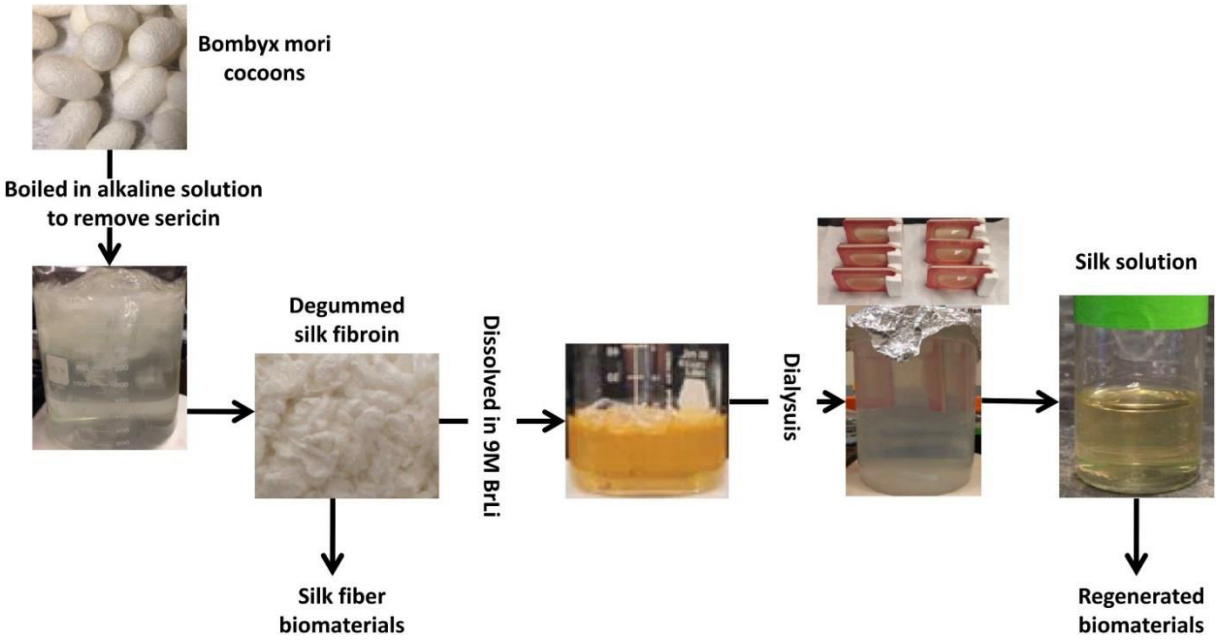

Figure 8: Scheme of Silk fibroin preparation. (43)

Postfunctionalization is also possible, which means the functionalization of the finished fibroin fibers or regenerated fibroin. This can occur through the inclusion of functional components in silk fibroin, either through physical adsorption or chemical crosslinking. The result naturally depends on the way of functionalization. Since, for example, chemical crosslinking of TiO_2 on silk led to a significant decrease in tensile strength, and the physical adsorption of TiO_2 on silk, on the contrary, led to a significant improvement in mechanical properties, including modulus and tensile strength. (64) Also, with the help of various crosslinking options, it is possible to adjust the pore size in the fibers and improve hemocompatibility in comparison with pure silk fibroin. (67)

Before starting the preparation of a silk solution to work with further forms of silk, it is necessary to remove sercin, as mentioned earlier. For these purposes, concentrated solutions of such salts as LiBr, LiSCN, or ionic liquids are commonly used. Then using different techniques it is possible to prepare various forms of silk. Such as hydrogels, films, fibers and many others. (63)

Electrospun SF fibers are an excellent candidate for use in tissue engineering because of their large surface area and excellent porous structure. In particular, because of the listed properties, they are an excellent material for cell seeding (adipose-derived stem cells (ASCs,

for example), adhesion and proliferation. (66) Covalently attached antibacterial agent coating (for example, silver nanoparticles. (64) For the preparation of these fibers, hexafluoroisopropanol (HFIP) and formic acid are most often used as a solvent. When using silk fibroin electrospun nanomatrix in burn rat model, the expression level of pro-inflammatory cytokine (IL-1α) is significant decreased compared with medical gauze, which suggests that silk fibroin nanomatrix effort flushes burn healing. There are also data and that the SF may be effective in the resolution of burns and skin defects, even when oral submission. (68)

It is also important to menation the combined materials for wound dressing. For example, scaffolds based on TEMPO-oxidized cellulose nanofiber showed a high degree of swelling, absorbing exudate and promoted wound healing. (66) And in the case of composite chitosan/silk fibroin chitosan improves the mechanical properties of SF, as well as they have an excellent porous structure (which means that in the future they can become a matrix for the stems of cells), biocompatibility and antimicrobial properties without any further modification. Shanyi Guang et al. This composite was tested for Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Monilia albicans. (69) Another example would be a mixture of SF and elastin (EL). The SF/EL scaffolds, after lyophilization, formed porous structures that were then crosslinked with genipin. Histological tests indicated migration and proliferation of connective tissue cells in the area of the burn, namely fibroblast and keratinocytes. (69; 70)

SYNTHETIC POLYMERS

Poly(lactide-co-glycolide)

PLGA is one of the most promising material. Nanostructures based on it have the potential to be used in drug delivery and in tissue engineering. The main advantage of using PLGA in biomedicine is that it completely decomposes in an aqueous medium. Also, this polymer is fully biocompatible and not only allowed by the Food and Drug Administration (FDA), but also by the European Medicine Agency. (71)

This polymer is a copolymer and consists of two monomers - glycolic acid and lactic acid. PLGA can be synthesized in various ways: polycondensation process, ring opening polymerization, segmer assembly polymerization, but the first two are essential. It is also soluble in a wide range of organic solvents: acetate, dichloromethane, tetrahydrofuranchloroform, acetone, and others. Also PLGA decomposes faster than PLA or PGA. The molar ratio of individual monomers in the chain directly affects the properties of PLGA. In front of them are such as the degree of crystallinity, mechanical strength, and the rate of decomposition. (71) Poly (lactide-co-glycolide) 50/50 (which means that it contains 50% lactide and 50% glycolide) is very hydrolytically unstable, decomposes in about 1-2 months. Accordingly, PLGA 75/25 - after 4-5 months and 85/15 - after 5-6 months. Different poly (lactide-co-glycolide) ratios have been commercially developed and are being studied for a wide range of biomedical applications, for example, PuraSorb® or Vicryl®. Currently, there is also a modified version of Vicryl Rapid® suture, which is an irradiated version of the material to increase the rate of degradation. (46)

PLGA, as well as PLA and PGA, is eroded in bulk by hydrolyzing ester bonds randomly. With a decrease in the length of the polymer chain and molecular weight, an increase in hydrophilicity occurs. The final decomposition products are glycolic and lactic acids, which are derived by classical metabolic pathways. The decomposition rate is influenced by many factors (71) With a decrease in molecular weight, the rate of decomposition (and the release of a drug when it is used), the rate of decomposition, as a rule, increases. (71; 72) Porosity also affects, with it, as a rule, the decomposition rate increases. There is information in the literature that PLGA-based microspheres could decompose for either 3 weeks or 20 depending on whether they were porous or not, respectively. (72)The following factors also influence: cross-linking density (negative correlation), crystallinity (negative correlation),

glycolic acid content (positive correlation), and many others. (71) Degradation rates can also be affected by such environmental characteristics as: temperature, pH, and ion strength. pH is one of the most important parameters since acidity varies greatly between healthy and diseased organs. Given this, it is possible to create systems for the delivery of drugs based on PLGA, sensitive to changes in pH. This is possible in three different ways: using polymers with ionizable fragments, by coupling acid-cleavable bonds, or incorporating gas-generating entities. (73)

The advantage of micro- and nanoparticles for drug delivery is also the ability to pass through the body's physiological barriers. A huge number of attempts have been made to create particles loaded with insulin, growth factors and vaccines. (73) To improve the bioavailability of medicinal substances it is also possible to use PLGA in combination with other polymers. For example, Bin Xu et al. in their work, they synthesized a PLGA / FA-CS composite (folic acid modified chitosan) using electrostatic self-assembly for oral insulin delivery. (74)

Thus, many studies are being conducted on its use as a biomaterial for medical applications, such as the drug delivery systems described above and as scaffolds for tissue engineering. (46) However, PLGA has no cell recognition sites and, as a result, poor cell affinity due to which it is necessary to use a lot of surface modification techniques. Among them are the following: plasma treatment (can generate functional groups); It is also possible to modify fibers by means of fibroblasts. (75) Another way to influence the success of cell adhesion is to fabricate composite fibers. Such fibers are made most often using electrospinning and then can be used in wound dressing, vascular tissue engineering, bone tissue engineering and so on. Using the co-axial electrospinning, which represents two solutions are coaxially electrospun through different capillary channels into one nozzle, it is possible to create a core-shell structure, which is especially important for fibers loaded with some medicinal substance. In such a structure, the core consists of one polymer (or drug substance) and a shell of another polymer. (76) In addition to electrospinning, many methods are available, and even the manufacture of scaffolds for tissue regeneration using 3D-printing. (77)

In addition, the polymer itself, without any drug loading or combination with other polymers and nanoparticles, can act as a therapeutic agent due to its hydrolytic products, namely lactate and H +. This is argued for the fact that lactate and H + tend to increase

angiogenesis and promote healing. This occurs through interaction with the following G protein-coupled receptors (GPR81 for lactate and OGR1, GPR4, TDAG8 for H +) and specific ion channels (MCT1, MCT2 for lactate and H + and TRPV1, ASICs for H +). This mechanism is presented in diagram 22. (78)

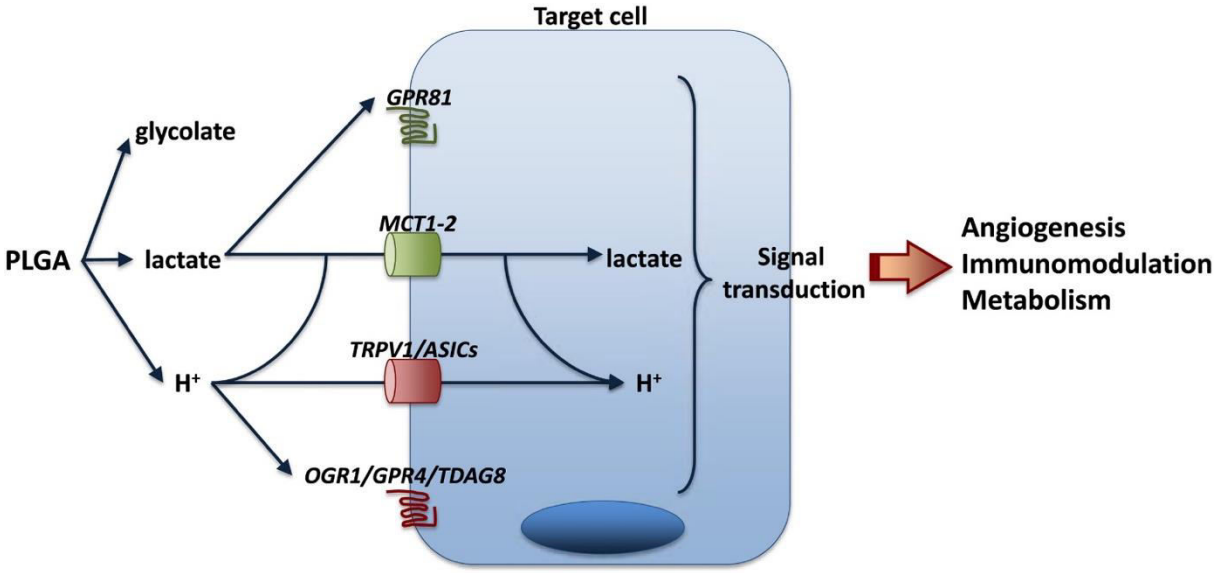

Figure 9: PLGA exert biological effects through its degradation products. (78)

Polycaprolactone

Polycaprolactone (PCL) is of great interest, since it can be obtained quite easily with ROP and cheap ε-caprolactone monomer, moreover it is approved by the FDA. It is a hydrophobic, semi-crystalline polymer and, at room temperature, it is highly soluble in solvents such as: chloroform, carbon tetrachloride, benzene, cyclohexanone, 2-nitropropane, toluene. (46; 79) On the basis of PCL, a huge number of different products can be created: nanofibres, nanospheres, foams, knitted textiles, and many others. (79)

Like previous polymers, PCL undergoes hydrolytic degradation due to the presence of ester bonds, but degradation occurs rather slowly (2–4 years). From the existing literature it is possible to conclude that PCL undergoes two-step decomposition. The first stage is the non-enzymatic hydrolytic cleavage of ester groups, and then, when the polymer has a low molecular weight, intracellular degradation in the phagosomes of macrophages and giant cells follows. Therefore, there is a theory that PCL can be completely resorbed and decomposed by the intracellular mechanism. (79) Due to the slow degradation of PCL, it was initially investigated as a long-term drug delivery vehicle, such as, for example, the

Capronor® long-term contraceptive device. (46) Due to the rather slow degradation of PCL, it can also be used to protect highly reactive drugs. Micheli Zanetti et al. used PCL to encapsulate geranyl cinnamate. The release of the drug in such a case can be observed only after the destruction of the polymer matrix, which can be triggered by certain enzymes in the human body. (80) However, it should be mentioned that PCL can cause inflammation in living organisms, but the level of cytocompatibility strongly depends on the degree of purification of devices based on the polymer and the tissue that interacts with it. (79)

It is also possible to create scaffolds for applications in the field of tissue regeneration (for example, bone tissue). Rui M. Duarte et al. in their work, they created a three-dimensional framework using CO2 as a foaming agent to impart porosity to the structure. The skeleton variants, loaded with ß-tricalcium phosphate and dexamethasone to induce bioactivity, were also produced. (81)

It is also possible to create PCL fibers for the subsequent manufacture of wound healing material. Tra Thanh Nhi et al. used these fibers as a base, covering them with a solution of gelatin and silver nanoparticles. As a result, a promising multi-coated PCL membrane with antibacterial properties and not sticking to the wound site was obtained, which makes it promising wound dressing. (82)

Polyurethanes

Polyurethanes are a fairly stable group of polymers. Due to their properties such as high elongation capacity, thermal stability, chemical resistance, high energy absorption capacity and excellent biocompatibility, they have been extensively studied as implants (pacemakers and vascular grafts). (46; 83) It is believed that this is blocked by the copolymer and it consists of “soft” and “hard” segments. It is one of the largest polymer products and based on it is possible to create products such as fibers, films, foams, coatings and others. All products can be divided into two groups: elastic PU, such as elastomers and rigid PU such as rigid foams. There is a literature on the use of PU in improving the durability of cementitious structures, polyurethane adhesive, polymer concrete polyurethane and many other applications. In addition, the PU has the support cell adhesion and proliferation property. (83; 84)

This polymer can be synthesized using various approaches. There is, for example, step growth polymerization (polyaddition), which can be carried out in one or two stages. In this case, the synthesis requires the following components: olygodiol, polyisocyanate and a chain extender. (84) The biocompatibility and the rate of decomposition of a polymer product can strongly depend on its composition of the polymer. The control of these two parameters is possible through careful selection of each component of polyaddiation (olygodiol, polyisocyanate …), as well as through the synthesis of various structures (an important role is played by the degree of crosslinking, etc.). For example, PUs synthesized based on aromatic isocyanates are less biocompatibility than polyurethanes based on aliphatic diisocyanates. And the increase in the number of hard segments provoked a decrease in the rate of hydrolytic degradation. This may be explained by the fact that in this case, due to increased crystallinity, water absorption is reduced. (85) Biodegradable polyurethanes are synthesized, usually on the basis of PCL, PGA and PLA. It is also important to note that the decomposition products of the polymer do not cause a change in the pH of the environment. (84)

The biodegradable elastic Degrapol®, for example, is already used for development of highly porous scaffolds for the use in tissue engineering. (46) The ability of these polymers to calcify in vivo (those maintaining the formation of calcium phosphate crystals) makes them an excellent candidate for bone tissue engineering. It is believed that the hydrophilicity of the polymer and the presence of ether oxygen most affects this property. It is possible to increase PU calcification with the help of PEG modification, which increases hydrophilicity and provides ether groups. (84) An important aspect can also be the pore size of porous scaffolds. It is possible to adjust the pore size using various fabrication of scaffolds techniques, from solvent casting/particle leaching (SC/PL) to electrospinning. (86) Also, composite materials such as natural and synthetic polymer blends (namely, composites with collagen and chaitosan) can be potential materials of choice for regeneration or rejuvenation of bone tissue. (87; 88) To enhance PUs bioactivity, polymer loading is possible with such substances as growth factors or composites. (84) Biodegradable scaffold for injection has also been developed. (89)

Polyethylenimine (PEI)

Polyethylenimine (PEI) is one of cationic polymers with high cation density (due to positive charge per 43 Da) and consists of a repeating units of an amine group and a CH_2CH_2 spacer. (90; 91) It can also be synthesized in two different forms: branched PEI and linear PEI. Branched PEI is usually synthesized using acid-catalyzed polymerization of aziridine, and for the preparation of the linear form, ring opening polymerization of 2-ethyl-2-oxazoline and subsequent hydrolysis is usually necessary. It is important to note that lPEI contains only secondary amines in its backbone, while bPEI contains primary, secondary and tertiary amines, and in a certain ratio (1: 2: 1). PEI molar masses can be found in a wide range, namely from 1 to 1600 kDa. (90)

This polymer is considered the gold standard of gene transfection, after Boussif et al. in 1995, PEI as a vector for gene transfection was first used. (90; 91) PEI condenses the negatively charged DNA, creating complexes and protecting DNA from degradation. The optimum molecular weight of the polymer for these purposes is between 5 and 25 kDa. (90) The best form for gene delivery is the linear form, which may be associated with an inherent kinetic instability. (91) Various polymer modifications are also possible to improve its qualities. For example, to improve the positive charge it can be masked by coating the surface with other hydrophilic polymers such as PEG. And in order to improve transfection efficiency, it is possible to introduce a request of alkyl linkage or, for example, attachment of hydrophobic ligands such as RGD peptide, or galactose. Also, the non-degradability of this polymer can be a problem, since non-degradable cationic polymers destabilize the cell membrane. In this case, it is necessary to introduce biodegradable bonds into cationic polymers. (90) Also, this polymer can have a cytotoxic effect, the mechanism of action of which is that the positive charges of the polymer induce necrotic cell death. (91) However, toxicity decreases after complexation with nucleic acid. (92)

Also, nanoparticles in unmodified native forms of PEI showed their antimicrobial activity, but the mechanism of the antimicrobial action of the polymer remains unclear. It should also be mentioned that PEI-based hydrogels for use in surgery (namely, surgical sealants) have been approved by the US FDA. (93)

Due to the ability of PEI to enter cells, it is mainly utilized as a drug carrier and there is very little literature on the creation of fibers based on this polymer, and especially using electrospinning. Semih Çalamak et al. first attempted to fabricate PEI/fibroin using electrospinning to create wound dressings with antibacterial activity. To create a PEI and fibroin solution, they dissolved into formic acid in various ratios, and the spinning parameters were as follows: volume rate of 0.100 mL/min, voltage was 17 kV, and distance tip-to-collector distance (collection plate was covered with aluminum foil) was of 13 cm. Later, some of the fibers were treated with methanol or were treated with glutaraldehyde vapor. These fibers had antibacterial activity against both of S. aureus and P. Aeruginosa, and also did not show cytotoxicity on the L929 cell line. Some of these fibers are shown in Figure 19. (94)

MATERIALS AND METHODS

Chemicals

The following chemicals were used to prepare polymeric solutions: N, N,-dimethylformamide – DMF (anhydrous, 99.8 %; Sigma-Aldrich), acetone (ACS reagent, ≥99.5 %), poly (D, L-lactide-co-glycolide) – PLGA (ester terminated, Mw 50,000-75,000), polyethylenimine (branched, average Mw ~25,000), polyurethane – PU (Permuthane EVO EX-SU-96-603 and Permuthane SU-22-542, both via Stahl), polycaprolactone – PCL (average M_w ~14,000, Sigma-Aldrich), hexafluoroisopropanol – HPIF (≥99%; Sigma-Aldrich), dichloromethane – DCM (anhydrous, ≥99.8%, Sigma-Aldrich). For other tests were used polyacrylate-polyalcohol (Sigma-Aldrich), sodium chloride – NaCl (PENTA s.r.o.), sodium hydroxide pearls G. R. – NaOH (Lach-Ner s.r.o.). All chemicals were used without further purification.

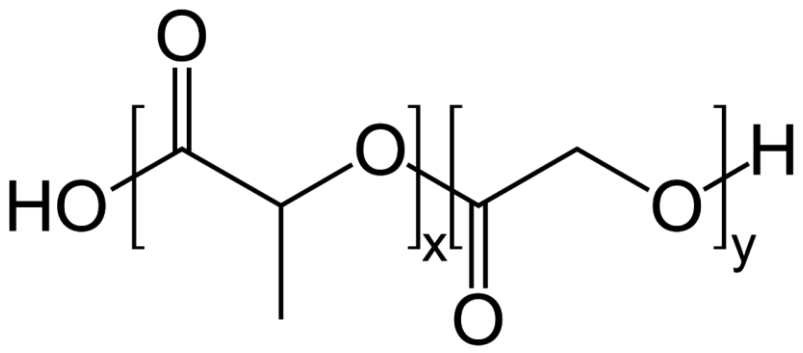

Figure 10: Structure of PLGA.

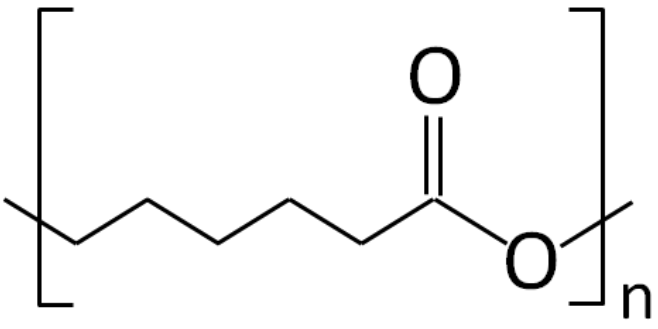

Figure 11: Structure of PCL.

Figure 12: Structure of PEI.

Equipment

PLGA, PU and PEI 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 24 hours. The solution was transferred into a syringe, and then into a syringe pump (NE-1000 Programmable Single Syringe Pump), which was used 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. For the preparation of SF and PCL fibres was used the same syringe pump but with metallic plate as a static collector.

The drying of the as-prepared samples of the generated fibres was carried out in a Binder 531 desiccator or in a fume hood in case of PCL and SF fibres prepared at the Aarhus University. Temperature tests were carries out with Binder furnace. The images of the fibres and images monitoring the degradation process were obtained from scanning electron microscope (SEM) Hitachi SU6600 and Hitachi TM3030Plus.

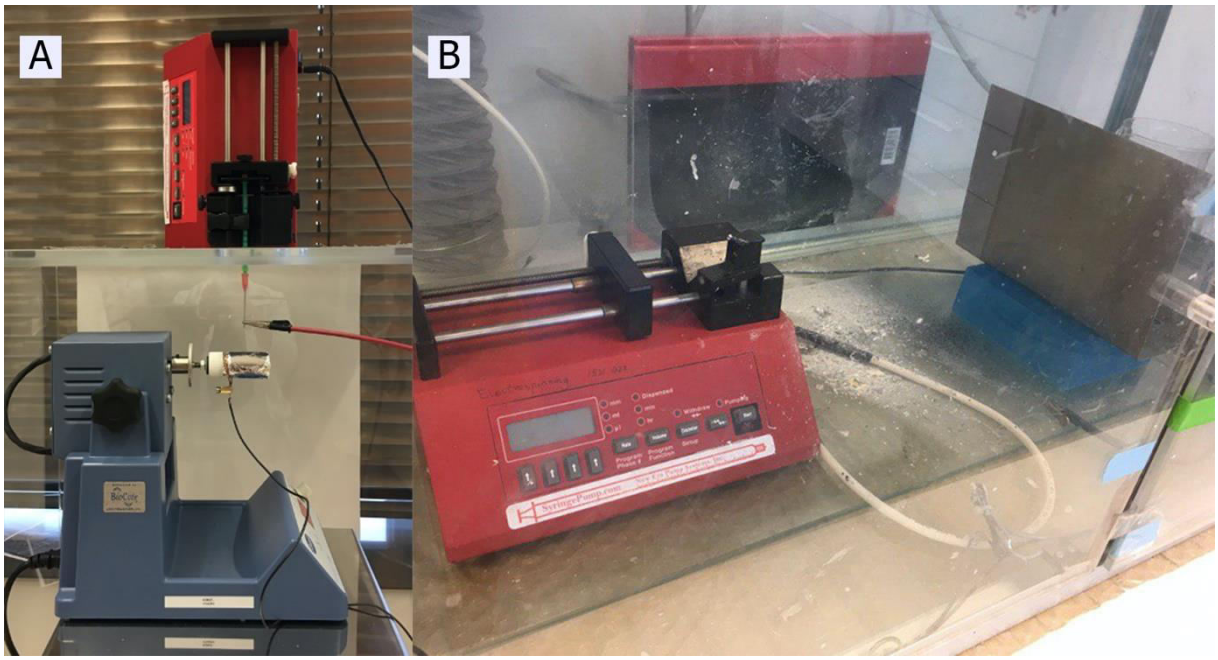

Figure 13: A. Design of the used electrospinning equipment for PLGA, PU and PEI fibres, UPOL; **B.** Design of the used electrospinning equipment for PCL and SF fibres, Aarhus University.

WORKING PROCEDURES

Preparation of fibers

In this work the electrospinning method was used to prepare all the following fibres. This is a method that uses electrical force to pull fibers from polymer solutions or polymer melts. This process does not require high temperatures and therefore is considered simple. It requires a power supply, syringe and conductive collector. During electrospinning, it first forms a drop that is squeezed out of the syringe. After that, it deforms into a Taylor cone, from which a charged jet is ejected. Then the jet propagates in a straight line and the subsequent thinning due to the presence of an electric field. After that, the jet begins to oscillate (Figure 14). Ultimately, the fibers solidify on the collector. (95)

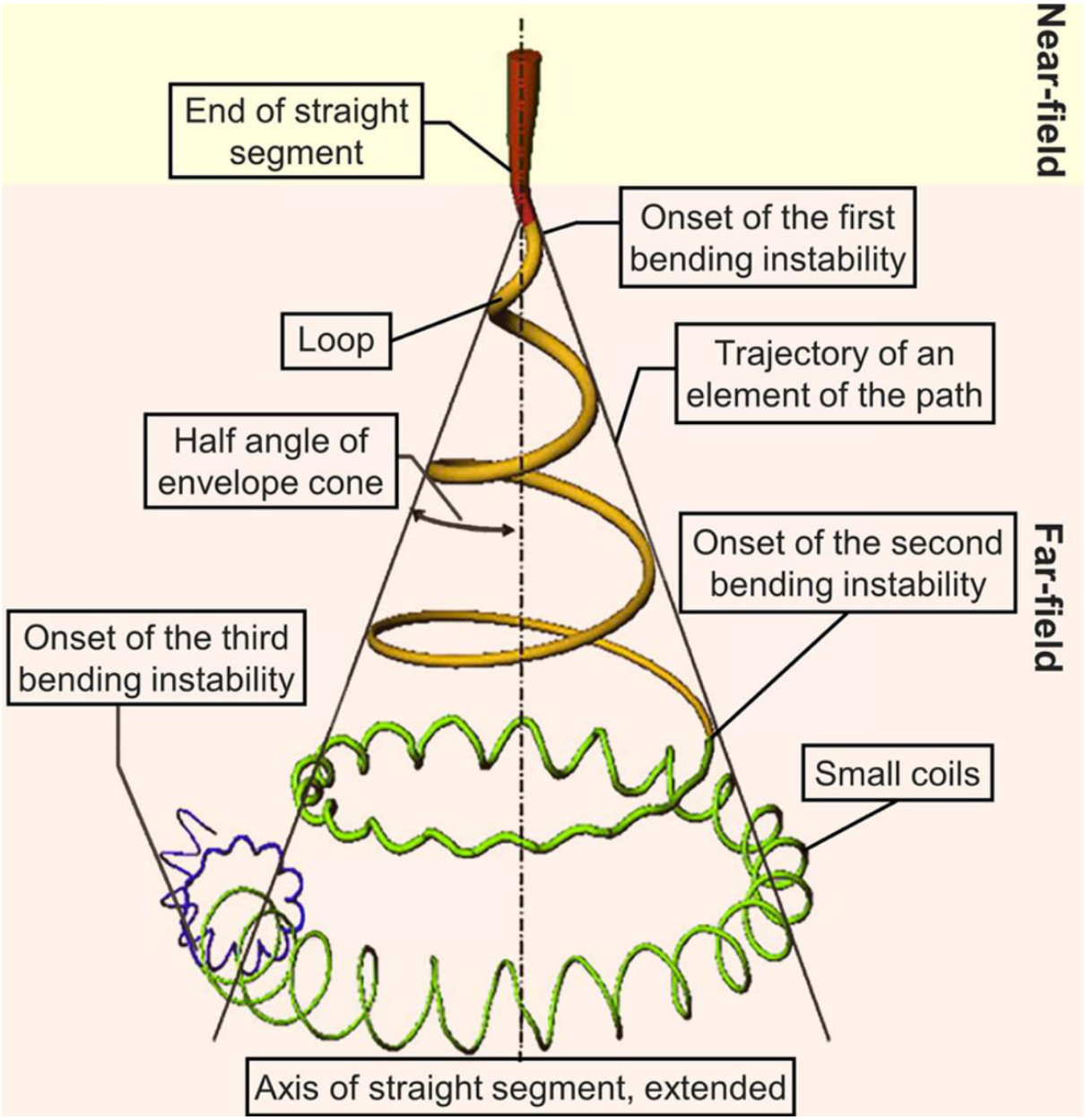

Figure 14: Structure of Taylor cone and stages of electrospinning.

The subsequent type of fibers depends on such factors as the properties of the solution (concentration, viscosity, surface tension of the solvent, elasticity, etc.), processing and the environment. The properties of the solution are the most decisive, namely the concentration of the solution is the key factor that influences the final macroscopic and microscopic appearance of the fibers. It is always necessary to select the ideal concentration of the solution; if the concentration is too low, drops will form, and a too thick solution, on the contrary, complicates the work of the spinner, and also increases the fiber diameter. An improperly selected concentration also prevents the production of uniform fibers. The next important factor of the choosing of the right concentration is the entanglement of polymer

chains. It occurs only at a certain concentration, which is called the entanglement concentration or C_e. (96) It is also necessary to choose the correct voltage. The voltage required for electrospinning can be calculated using the following formula:

$$V_c^2 = 4(\frac{D^2}{Le^2})(Ln\left(\frac{2Le}{R}\right) - \frac{3}{2})(0{,}117\pi\gamma R) \quad (3)$$

In this formula V_c is the voltage required for electrospinning or critical voltage; D is the distance between the tip of the needle (capillary) and the collector; Le is the length capillary; R is the radius of a needle; and γ stands for the surface tension. (96)

The next important factor is the polymer flow rate. It is generally believed that reducing the polymer flow rate allows for complete evaporation of the solvents from the fibers. In addition, such a factor as the distance between the syringe needle and the collector is decisive for the fiber diameter. The greater the distance, the finer the fibers are produced. (96)

Antibacterial tests

The antibacterial tests were performed in accordance with the modified ISO 22196:2011 standardized testing procedure – The evaluation of the antibacterial activity of plastic surfaces and other non-porous materials – in combination with the ČSN EN ISO 20743:2014 standardized testing procedure – The evaluation of antibacterial activity of textile products. The modification of the above mentioned standardized methods of antibacterial activity evaluation was necessary because of the nature of the samples. The modification dwelled in the following:

- the size of the samples was 20 x 20 mm (i.e. the minimally acceptable area according to the ISO 22196:2011 standardized testing procedure);
- all of the samples were covered with a polypropylene foil in order to eliminate evaporation of the liquid bacterial suspension and better distribution of the suspension over the entire surface of the tested sample (i.e. in order to enable the best possible contact of the suspension with the surface of the samples);
- the ISO 22196 states that the bacterial suspension should be washed off the surface but because of the nature of the samples it was impossible; therefore, the samples

were placed into sealable vessels with a doubled amount of SCDLP solution and shaken.

The samples and the cover foils were prepared in the size of the above stated 20 x 20 mm. All of the samples were disinfected prior the antimicrobial tests using UV radiation (for the period of 30 minutes from both sides). Standardized disinfection employing either ethanol or autoclave could not be used due to the nature of the samples. Especially the autoclave had a destructive impact on the nanostructures. The cover foils were disinfected with 70% ethanol. This way sterile samples were placed into a sterile Petri dishes. In the next step, the samples were evenly covered with the bacterial suspension (using 0.1 ml per a sample) and covered with the sterile cover foil. All of the samples we closed into the Petri dishes and inserted into incubator (temp.: 35±2 °C and relat. humidity 95 % for 24 hours). A half of the samples was always (at the zero contact time) was further utilized as follows: The samples (incl. the cover foils) were transferred into sealable vessels (three/parallel samples into on vessel). 15 ml of the neutralizing medium (SCDLP) was pipetted into each vessel (i.e. 5 ml of the medium per each of the samples), the vessels were closed and vigorously shaken for the 30 s period of time. Consequently, the subsequent dilution method, pouring into agar (PCA) and incubation at 35±2 °C for 24 hours) was employed in order to determine the concentration of living bacteria (CFU/cm^2). Comparable procedure was performed with the samples after 24hour incubation. The teste organisms were: *Staphylococcus aureus* CCM 4516 (gram positive bacteria) – the concentration of the suspension was $1.0 \cdot 10^5$ CFU/mL and *Klebsiella pneumoniae* CCM 4415 (gram-negative bacteria) – the concentration of the bacterial suspension was $1.4 \cdot 10^6$ CFU/mL

These tests were performed in order to prove that the as-prepared polymer structures do not exhibit any antibacterial activity itself. Possible “fake” antibacterial activity could have been caused by the residues of the solvents that were used to dissolve the polymers and in order to approach suitable electrospinning conditions reflected in required structures. These tests were performed, in the course of the thesis elaboration, in two microbial laboratories – either Tomas Bata University in Zlín (UTB, Zlín) or in Textile Testing Institute, Brno. None of the tested samples exhibited antibacterial activity which filled us with a optimism that the tailored structures can be used without any limitations in the targeted wound treatment and if any antimicrobial activity was required, the addition of e.g. silver nanoparticles (either into the fibres or on the fibre surface), antibiotics or any other

therapeutics, is possible. However, the final composite material would require a new evaluation.

Cell viability assays

The viability tests were performed following a standardized MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) procedure (Sigma Aldrich). The MTT is based on the conversion of colourless MTT to violet formazan in living cells, which reflects their mitochondrial activity. Concerning majority of cell population, the overall mitochondrial activity is directly proportional to the number of viable cells. I tis the reason why is this test quite commonly used for the cytotoxic effect evaluation.

The tests were performed with NIH/3T3 line of mice fibroblasts. The NIH/3T3 lines originated from the American-type Culture Collection (ATCC, USA) and were cultivated in Dulbecco's Modified Eagle medium (DMEM, Life Technologies) with a low content of glucose at 37°C and in v 5% CO_2 atmosphere.

The cells were inserted into 24 place microtiter plates (TPP, Biotech) in density equal to $2 \cdot 10^4$ cells per a place. At the bottom of each of the 24 positions, the tested material precisely cut for the size of the place was placed. After 24 hours, under the cultivation conditions – 37°C and in 5% CO2 atmosphere, the medium was gently eliminated and replaced by a freshly prepared DMEM medium with MTT solution. After the incubation (4 hours), also the MTT solution was gently eliminated and 500 µl of DMSO was added into each of the tested sites. DMSO is supposed to dissolve the crystals of formazan (viable cells transform the originally colourless MTT solution to violet formazan, which is reflected in an increased absorbance value compared to the non-viable cells with zero mitochondrial activity. The absorbance is detected at 570 nm using the instrumentation of Infinite PRO M200 multiplate reader (Tecan, Austria).

The degree of cell viability was calculated from the absorption, i.e $A_{sample}/ A_{blank}) * 100$

and plotted as the viability percentage. All measured samples were tested in triplets. Although the absorption is usually measured directly in the plates, where the cultivation proceeds, in this case it was, due to the nature of the samples, impossible due to the fact

that the material is not fully transparent. After the incubation with DMSO, the liquid was properly stirred and transferred to a 96 position plate (3x100 µl) and the measurement was performed as described above – thanks to the multiple reader.

The lowest viability of the cell was in the case of the PU and PEI fibers. The viability value was calculated to be 98.00%. It is obvious that even such controversial composition, involving PEI can be non-toxic for the cells. The charm probably dwells in the amount of PEI in the fibers – obviously the amount of 5 w/w % is sufficiently low to contribute to toxicity against cells and sufficiently high to give us an effective tool how to firmly attach e.g. silver nanoparticles.

RESULTS AND DISCUSSION

Preparation and analysis of PLGA fibres

In case of PLGA fibres, a mixture two solvents (acetone and DMF in a ratio of 50:50) was used to prepare a solution. The percentage of PLGA in solution was 40 % (w/w). To fully homogenize the solution of PLGA, it was left on the shaker for at least 30 minutes. The prepared solution did not require additional purification and was immediately injected into the syringe.

Following, via electrospinning method, which is described above from the resulting solution, nanofibers were synthesized on a foil covering the vial or at the static collector with a foil as well. The distance from the end of the syringe needle to the surface of the collector was 10 cm in the both cases. The speed of rotation of the vial was 40 rpm. The voltage was 10 kV and 15 kV. The rate of extrusion of the polymer from the syringe is 1 ml/hr in all of the variations of the experiment. The as-prepared fibres were in the desiccator for additional drying for at least 24 hours.

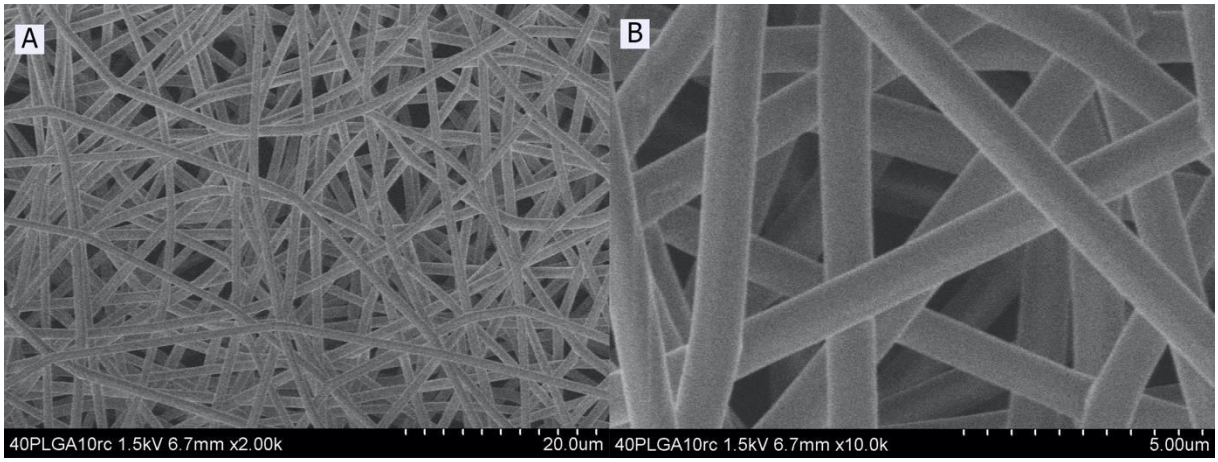

Figure 15: A. PLGA fibres, 40 % solution in acetone and DMF, rotating collector, 10 kV voltage, 10 cm distance from needle to the collector; **B.** closer look of the same fibres.

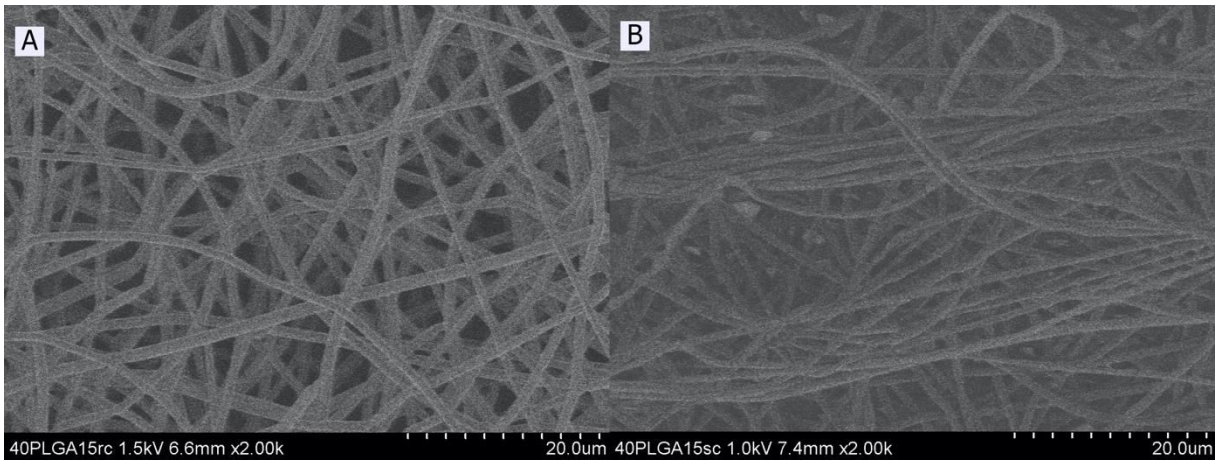

Figure 16: A. PLGA fibres, 40 % solution in acetone and DMF, rotating collector, 15 kV voltage, 10 cm distance from needle to the collector; **B.** PLGA fibres, 40 % solution in acetone and DMF, static collector, 15 kV voltage, 10 cm distance from needle to the collector.

From the images presented above, it is obvious that the use of 10 kV voltage and rotating collector gives us the most uniform and surface smooth fibers (Figure 15). Form the SEM images, the average diameter of the fibers was calculated to be approximately 500 nm. On the contrary fibres obtained with the static collector and under the voltage of 15 kV (Figure16) have irregular shape and rough surface. As well, we can notice beads formations. Therefore for our next experiments we choose PLGA fibres prepared on a rotating collector under the voltage of 10 kV.

Preparation and analysis of PU fibres

For the preparation of the PU fibres two kinds of PU precursor were chosen. One of them was Permuthane SU-22-542, solvent borne aromatic polyurethane, polyester based. It is a yellowish translucent liquid which is easy to dissolve in DMF. Fibres were obtained from 50 % and 75 % solutions (w/w) on a rotating collector.

For the spinning of 50 % solution next parameters were chosen: the distance from needle to the collector was 4 cm, the extrusion speed was 0,5 ml/hr, the voltage was 15 kV and the speed of rotation of the collector was 40 rpm. For the spinning of 75 % solution was used the same parameters except of the speed of extrusion, it was 1 ml/hr in this case.

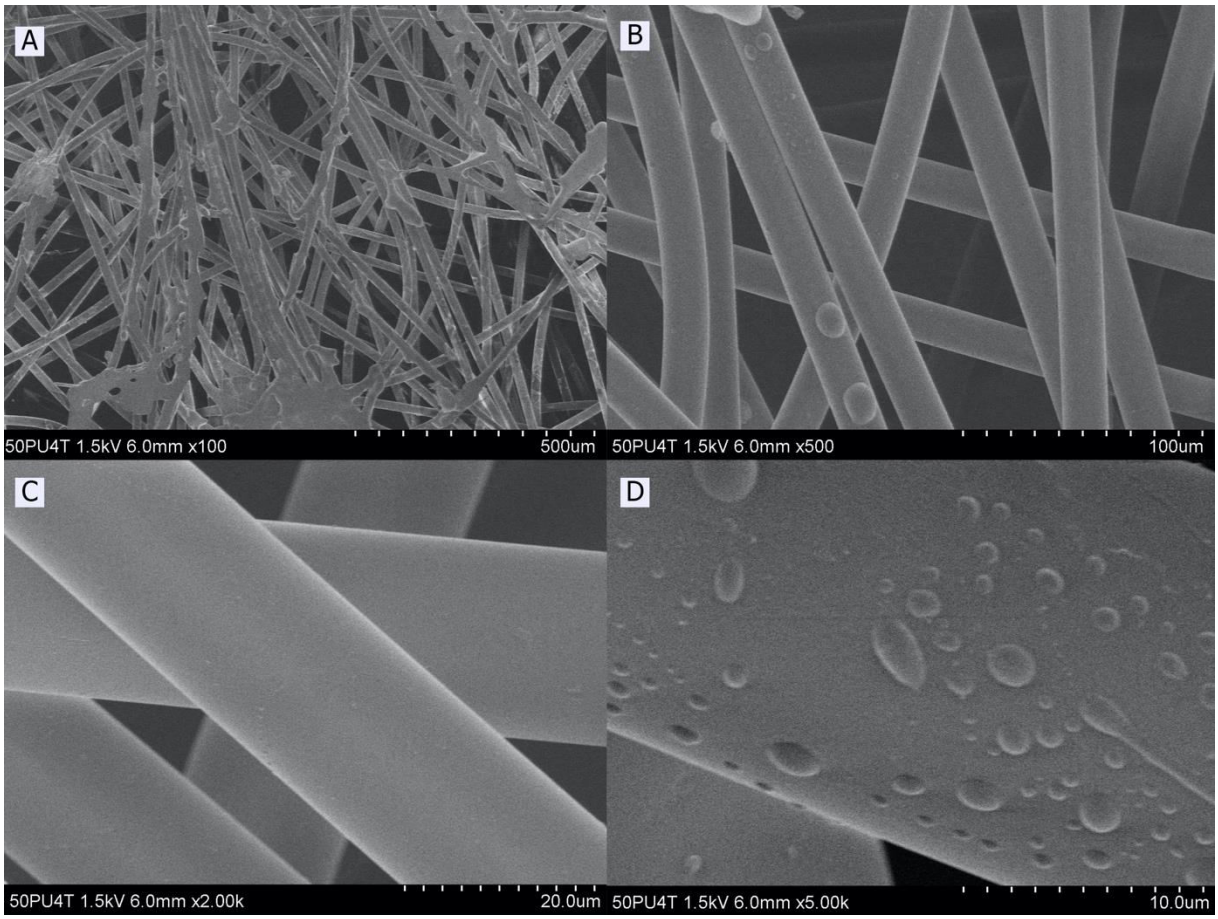

Figure 17: A. PU fibres (Permuthane SU-22-542), 50 % solution in DMF, rotating collector, 15 kV voltage, 4 cm distance from needle to the collector, speed of the extrusion is 0.5 ml/hr, 40 rpm; **B., C., D.** represent closer looks of the fibres.

From the SEM images, it is observable that almost all of the fibres are perfectly smooth and uniform and the average diameter is 20 µm. The artefacts, observable in the figure 17 B., C., D., and merged flattened areas on the figure 17 A. are probably due to the insufficient drying and presence of solvent in the sample. Operating with samples on the macroscopic level was difficult as well as the prepared sample was sticky and adhesive.

Following figures represent 75 % PU fibres. This kind of fibres are similar to the fibres obtained with the 50 % solution. They have smooth surface, all the fibres are uniform and even. Visible artefacts are probably due to the insufficient drying as in the case above.

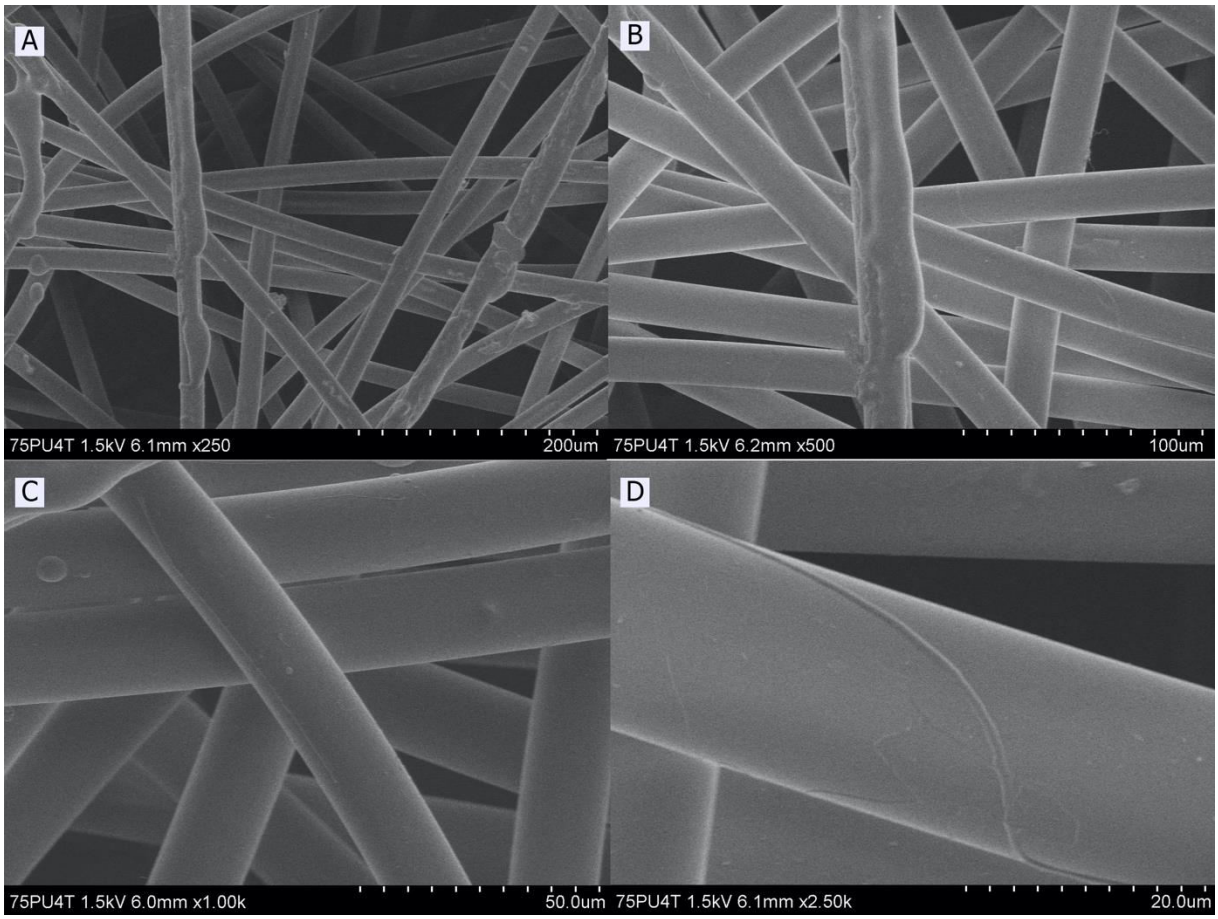

Figure 18: A. PU fibres (Permuthane SU-22-542), 75 % solution in DMF, rotating collector, 15 kV voltage, 4cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm; **B., C., D.** represents closer looks of the fibres.

The use of another PU precursor (Permuthane EVO EX-SU-96-603, solvent borne aliphatic polyurethane, polycarbonate based) did not give good results. It is imposable to dissolve in DMF, solvents for this polymer are isobutyl acetate and DMC. It was spinned in its initial form on the rotating collector, the parameters were: distance from the tip of the needle to the collector was 6 cm and 12 cm in the another case, voltage was 10 kV, speed of the flow is 1ml/hr and 40 rpm as the speed of the rotation.

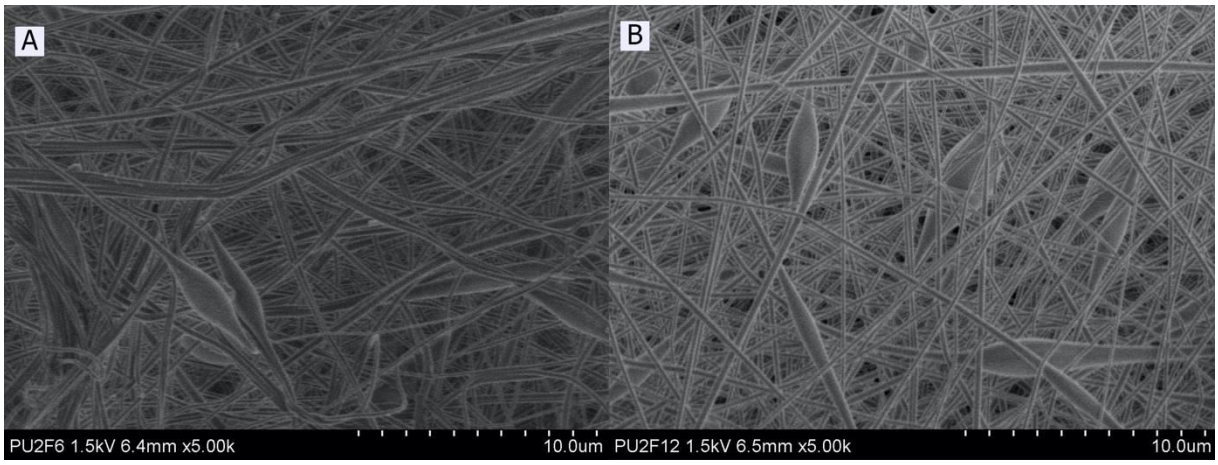

Figure 19: A. PU fibres (Permuthane EVO EX-SU-96-603), rotating collector, 10 kV voltage, 6 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm; **B.** PU fibres (Permuthane EVO EX-SU-96-603), rotating collector, 10 kV voltage, 12 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm.

From the figures above it is clear that the beads formation occurred at the both samples, fibres appeared to be very thin and without a clear orientation. Fibres obtained at the distance 12 cm give us a bit better results with a smooth surface but the uniformity is questionable. For the further tests this kind of fibres was not chosen.

Preparation and analysis of mixed fibres of PU and PEI

The idea behind this composition of the 2 polymer fibers was inspired by the patent *Polymer Substrate with Immobilized Silver Nanoparticle and Method of Preparation Thereof* (PCT/CZ2017/050002), where the molecules of PEI play the role of anchors for the, in the next step, generated silver nanoparticles. These particles are generated just on the surface and therefore offer complete active surface for antimicrobial action.

These tests were prepared with Permuthane SU-22-542 PU precursor in two variants: one of the solutions were PEI:PU:DMF in the concentrate of 5:45:50 and the other one was 10:40:50. Therefor solutions of 5 % PEI and 10 % PEI were obtained. Must be noted that Permuthane EVO EX-SU-96-603 was impossible to mix with PEI.

For the spinning of 5 % solution next parameters were chosen: the distance from needle to the collector was 4 cm and 6 cm, the extrusion speed was 0.5 ml/hr, the voltage was 15 kV and the speed of rotation of the collector was 40 rpm. The same parameters were chosen for the spinning of 10 % solution. After fibres were kept in the desiccator for additional drying for 24 hours. According to the following SEM images fibers have smooth

surface and a diameter around 20 µm as the PU fibres. No significant difference was noticed between fibres containing 5 % PEI and 10 % PEI, but the number of artefacts decreased compared to the pure PU fibres.

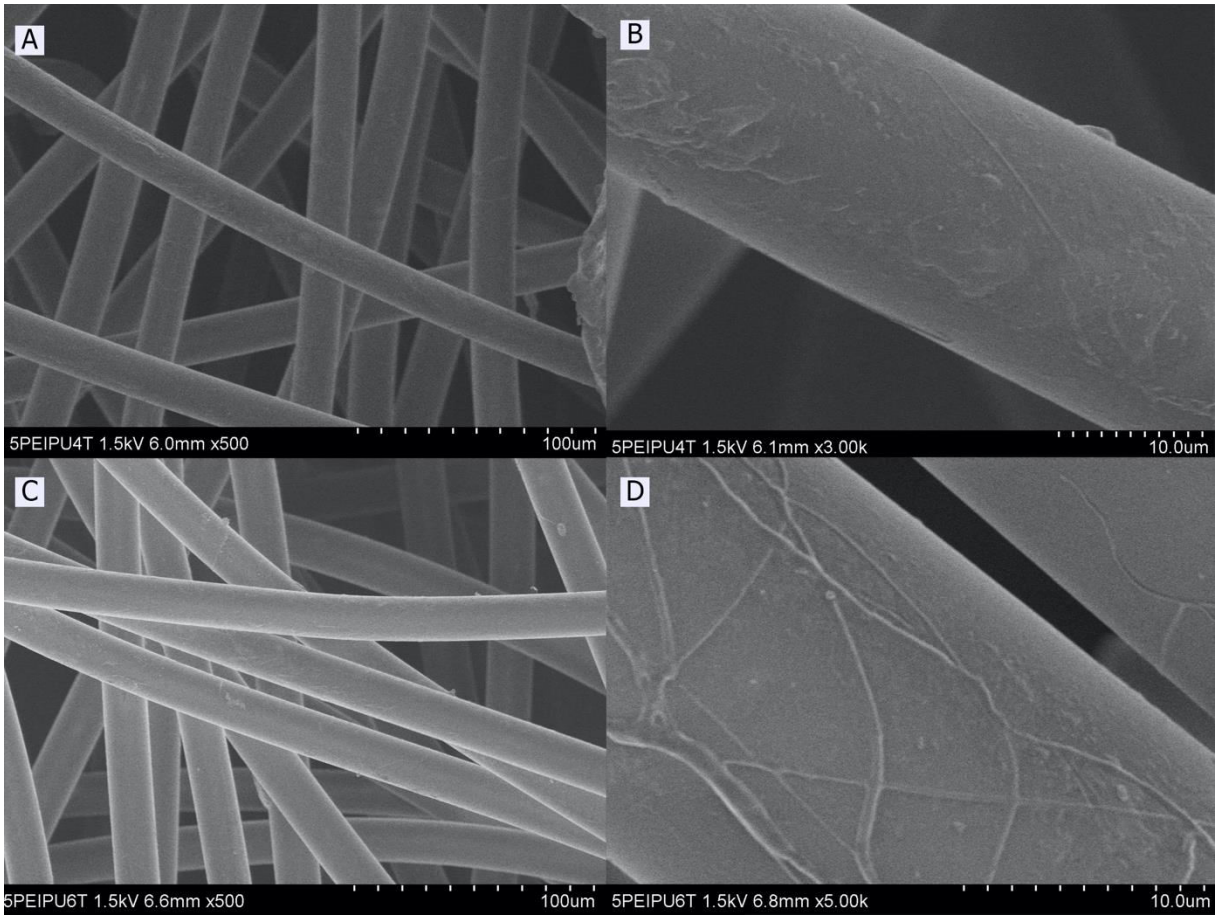

Figure 20: A., B. PU (Permuthane SU-22-542) and 5 % PEI mixed fibres, rotating collector, 10 kV voltage, 4 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm; **C., D.** PU (Permuthane SU-22-542) and 5 % PEI mixed fibres, rotating collector, 10 kV voltage, 6 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm.

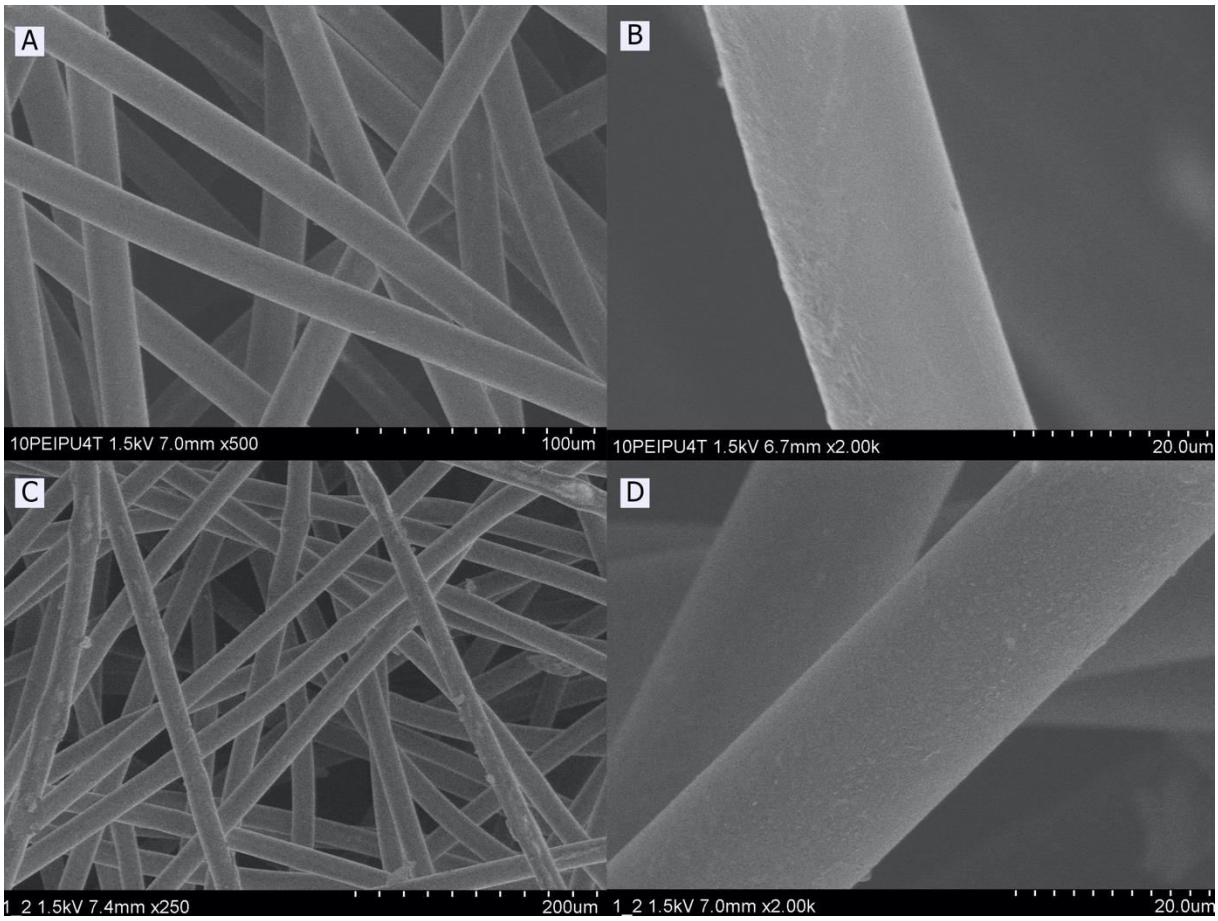

Figure 21: A., B. PU (Permuthane SU-22-542) and 10 % PEI mixed fibres, rotating collector, 10 kV voltage, 4 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm; **C., D.** PU (Permuthane SU-22-542) and 10 % PEI mixed fibres, rotating collector, 10 kV voltage, 6 cm distance from needle to the collector, speed of the extrusion is 1 ml/hr, 40 rpm.

Preparation and analysis of PCL fibres

This and the next part of experimental work was held at Aarhus University ElectroMed Laboratory, at the department of Biological and Chemical engineering. Electrospun PCL fibres in DCM:DMF (3:1) were prepared: 10 % and 12 % solutions were used, 2 hours of spinning process for each sample. For both of solutions following parameters were used: distance tip-collector – 12 cm, voltage – 15 kV, speed of extrusion – 1 ml/hr.

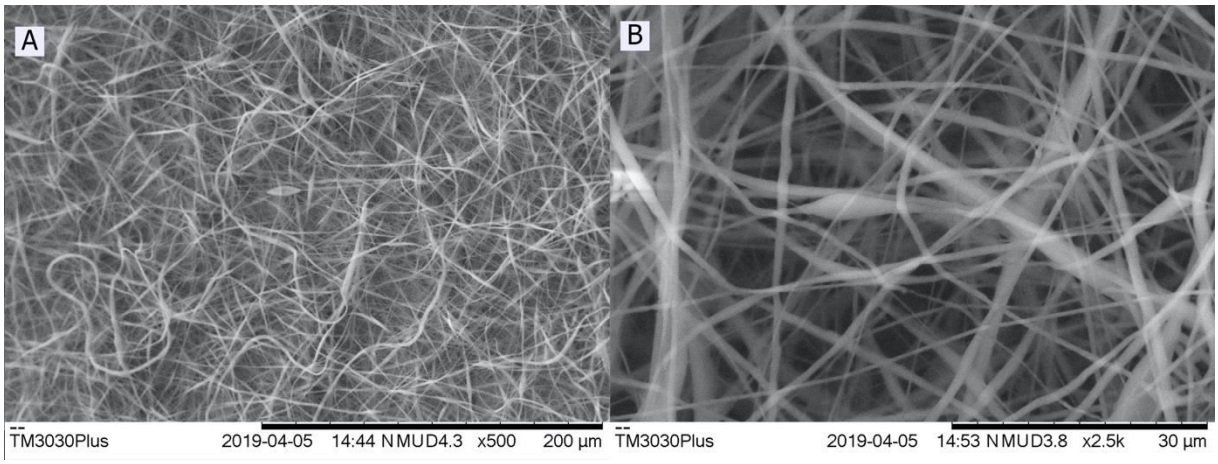

Figure 22: A. 10% PCL fibres in DCM:DMF (3:1), distance tip-collector – 12 cm, voltage – 15 kV, speed of extrusion– 1 ml/hr; **B.** closer look of the same fibres.

It is noticeable that this fibres are not uniform, although the surface is smooth they show beaded and meshed structure. According to the figure 22 B., the diameter varies from a few micrometers to almost 3 µm at the thickest locations.

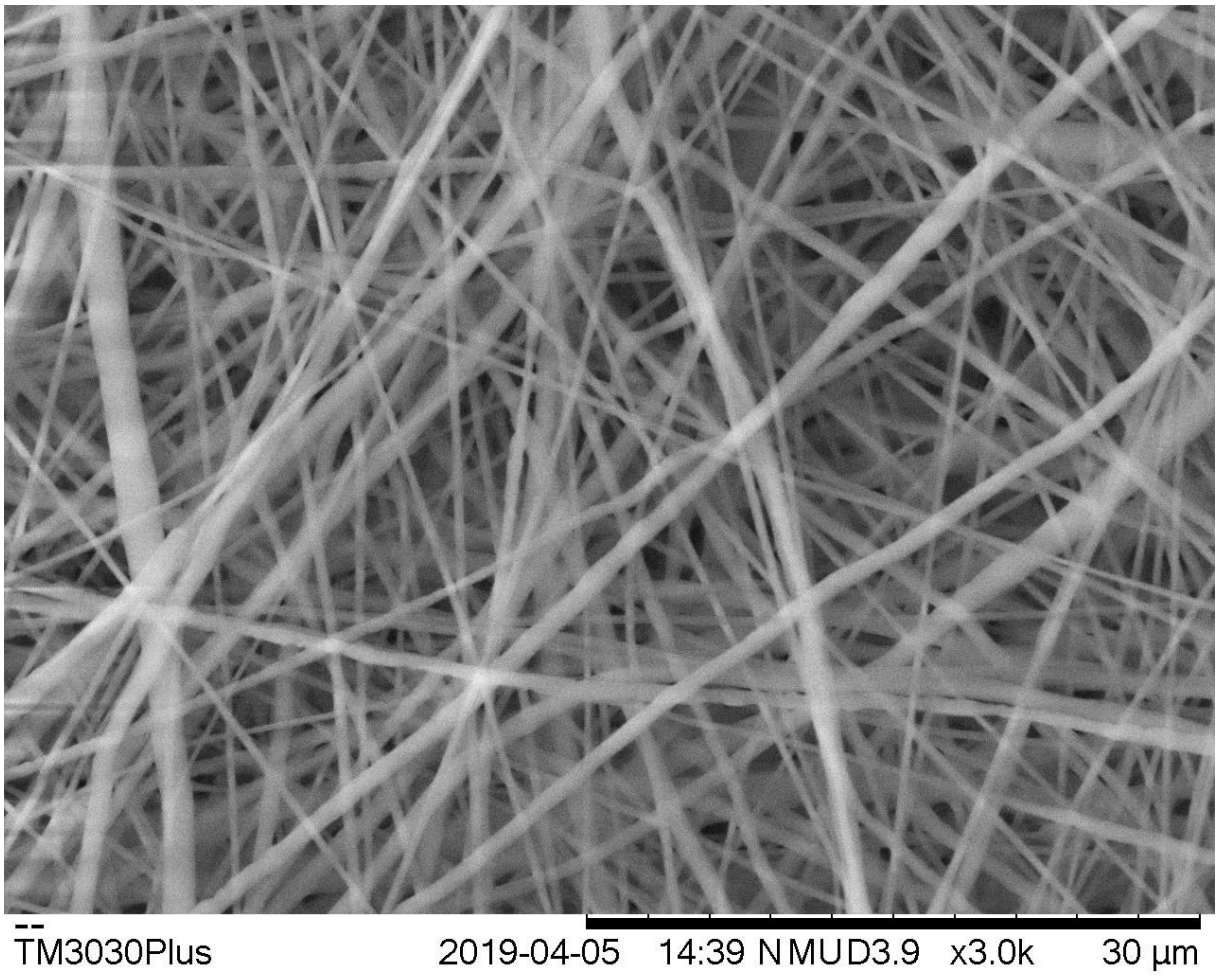

Figure 23: 12% PCL fibres DCM:DMF (3:1), distance tip-collector – 12 cm, voltage – 15 kV, speed of extrusion – 1 ml/hr.

From the SEM image above, it is obvious that with a higher concentration we can obtain more uniform structure. No beads are formed, fibres don't vary in a diameter in the same way as 10 % PCL fibres does.

As well HFIP solvent was tested. For this purpose 10% solution was used and the following parameters for electrospinning: distance tip-collector – 12 cm, voltage – 15 kV, speed of extrusion – 1.2 ml/hr. It is noticeable that change of the solvent can result in a tremendous difference in fibres appearance. The structure is more uniform and fibres have approximately same diameter, no beads can be seen. As well fibres are more curved comparing with the mixture of DCM and DMF as PCL solvents.

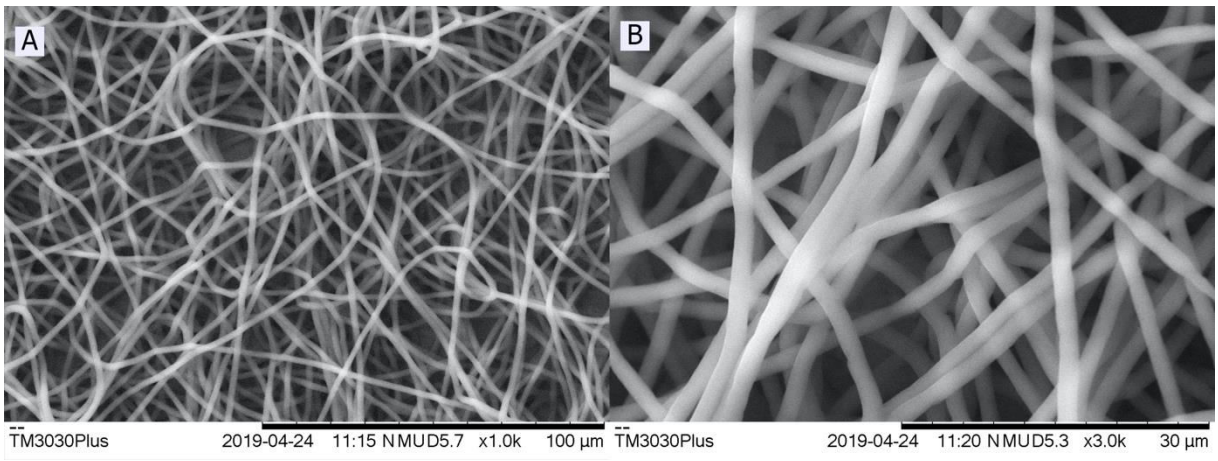

Figure 24: A. 10% PCL fibres in HFIP, distance tip-collector – 12 cm, voltage – 15 kV, speed of extrusion – 1.2 ml/hr; **B.** closer look of the same fibres.

Preparation and analysis of mixed fibres of SF and PCL

For the further work SF was prepared according to the protocol (see figure x). Firstly 5 g of cut cocoons were weighted and added to the boiling 0.02 M solution of Na_2SO_3. Boiling is a critical point as it degrades the fibroin. Further fibers were rinsed in ultrapure cold water for several times. After silk fibroin was dried in a fume hood overnight. At this point we have a silk fibroin without sericin. Further 20 % (w/v) solution of SF in 9.3 M LiBr solution was prepared. SF was placed first to the beaker and after topped with LiBr solution so SF dissolves fully in an oven at 60 °C for 4 h. Amber in colour appeared. As the next step solution was inserted to a dialysis cassette. Dialyze was held against 1 litre of ultrapure water. Water was changed after 1 h, 4 h, at the evening and two more times after at equal intervals. Further it was centrifugated to remove impurities. This resulting solution could be used as it is or lyophilized. Lyophilized form can be stable for years. (97)

After lyophilized SF was obtained solution of 10% PCL and 4% SF fibres in HFIP were prepared and SF was successfully dissolved, as far it was impossible to dissolve SF in DCM and DMF mixture. The next parameters were chosen as working: 15 kV as voltage, speed of extrusion was 0.1 ml/h, distance between the needle and static collector was 12 cm. The following figures represents the results.

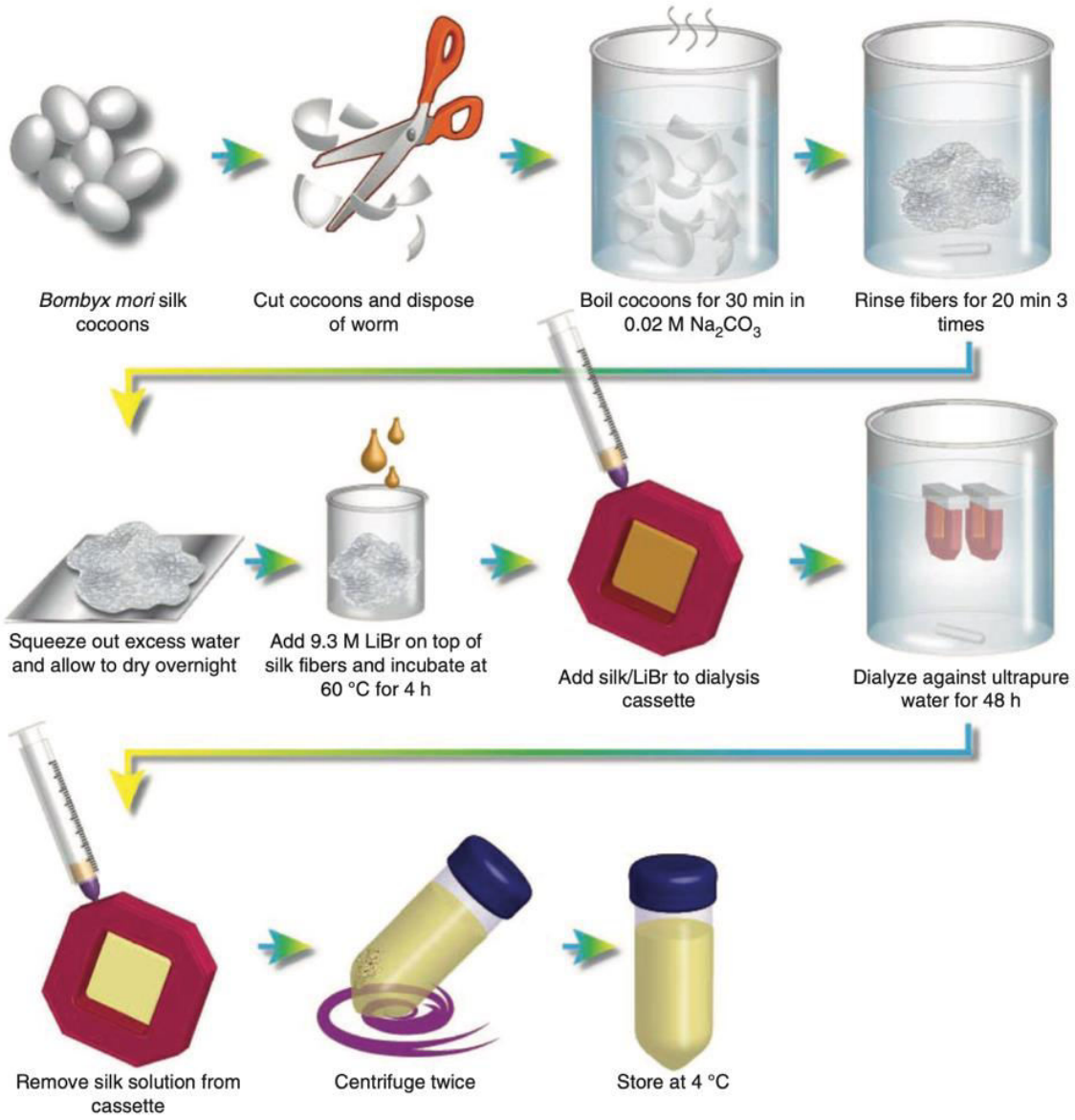

Figure 25: Preparation of SF for further use in blend fibres with PCL. (97)

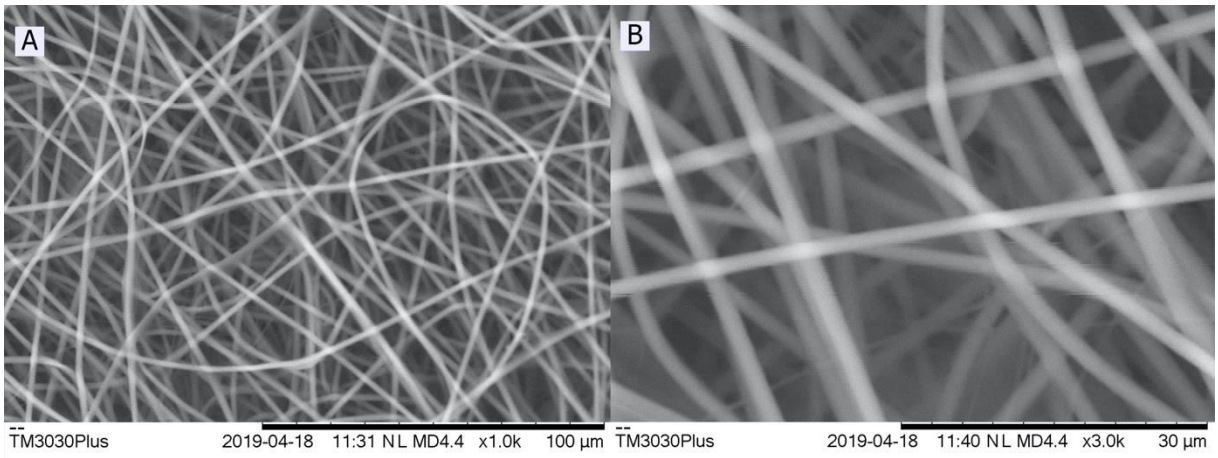

Figure 26: A. 10 % PCL and 4 % SF fibres in HFIP, 15 kV is voltage, 0.1 ml/h is speed of flow, tip-collector distance is 12 cm; **B.** closer look of the same fibres.

Preparation and analysis of a sandwich structure

A sandwich structure was prepared using PLGA fibres as a first layer and PU + PEI blend fibres as the second layer. Parameters from the previous experiments were used. This type of a sandwich structure was suggested in order to respect possible demand for different characteristics features in wound healing process. The PLGA fibers are referred to be biocompatible and biodegradable. As such they can carry and slowly release e.g. antibiotics. Therefore, in contact with the wound they can perform a long lasting dosing of any additive medicine. The PU fibers are supposed to be more degradation resistant and can probably play the role of a protective scaffold. Exactly this layer can be attributed with e.g. silver nanoparticles in order to prevent entrance of pathogens from outside. This suggested structure was also the first sandwich structure of this kind to be produced in our lab. The layer-by-layer deposition of differently composed fibers proved that there is no drawback in preparation and suggestion of such structures as the process of the second layer deposition was not anyhow negatively influenced by the presence of one already generated fibrous structure.

The PLGA fibres were prepared with the following parameters: the distance from the end of the syringe needle to the surface of the collector was 10 cm, the speed of rotation of the vial was 40 rpm, the voltage was 10 kV and the rate of extrusion was 1 ml/hr. After that fibres were dried at the desiccator. On top of this level bled fibres of 10 % PEI and PU were synthesised. The parameters were used from the chapter describing PU and PEI blend fibres: the distance from needle to the collector was 4 cm, the extrusion speed was 0.5 ml/hr, the

voltage was 15 kV and the speed of rotation of the collector was 40 rpm. To confirm the presence of the second layer the dye (poly [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)]) was used (Figure 27).

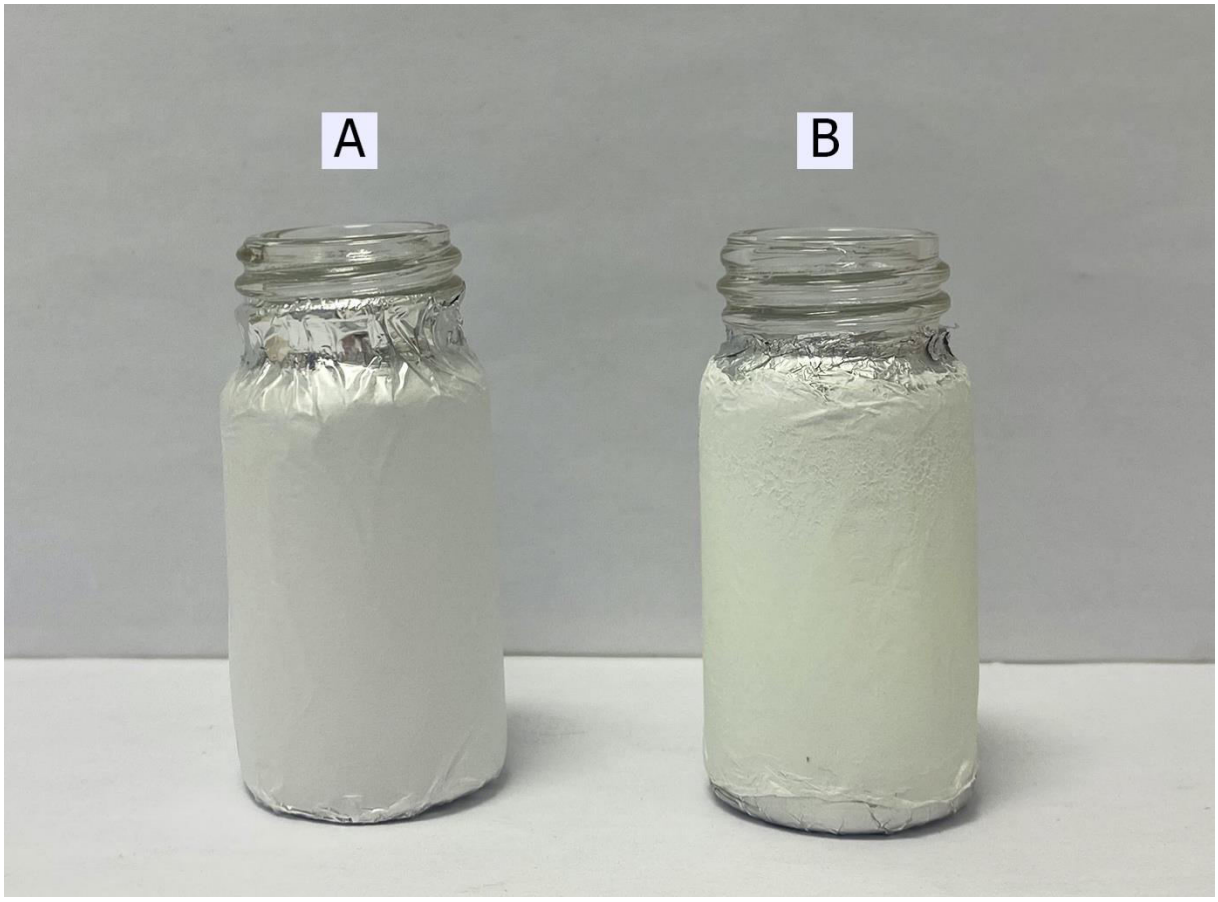

Figure 27: A. Sample with a pure PU and PEI blend on top; **B.** sample with a blend of PU and PEI mixed with yellow (poly [(m-phenylenevynilene)-alt-2,5-dihexyloxy-p-phenylenevynilene)]).

Further degradation tests took place. The tests were organized as follows. Petri dishes containing a hydrogel that absorbed liquids with different acidity were used. Water, physiological solution and NaOH solution with pH equal to 12 were used. The samples were placed on this hydrogel for 24 and 48 hours. In addition, one of the Petri dishes was kept at room temperature (21 °C), and the second was placed in a furnace at a constant temperature of 40 °C. Such conditions made it possible to simulate a moist wound surface. After the test samples were dried at the drying chamber again and placed for SEM analysis.

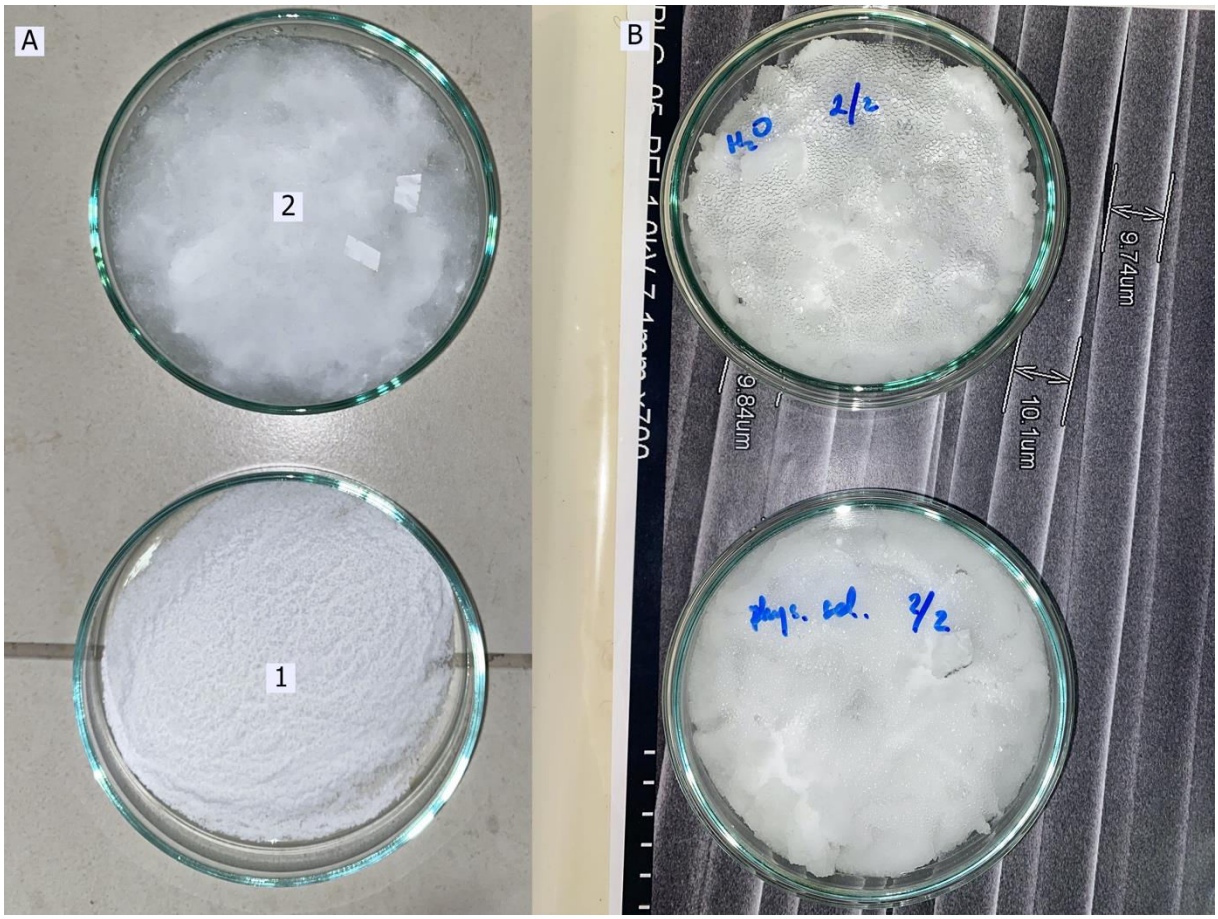

Figure 28: A. 1. Polyacrylate-polyalcohol before liquid adding; **2.** Polyacrylate-polyalcohol with liquid and fibre samples. **B.** Final design of the experiment.

The following photos will review the results of the experiment. For a more detailed analysis, SEM samples were prepared from both sides of the studied two-layer structure. The preparation of some of the samples was quite difficult due to the macroscopic changes in the fibers. In the case of elevated temperatures, the fibrous structures was practically distorted due to the hydrogel and, when dried, it formed an uneven surface with polyacrylate-polyalcohol particles, which creates certain difficulties for microscopic analysis. Despite this, it is possible to describe certain trends in the degradation of the sandwich structure.

Even in the case of water as a medium, it is possible to notice that the degradation of both sides is radically different from each other. After 24 hours at room temperature, the PLGA fibers did not show any noticeable changes, however, the polyurethane fibers, especially those that were on the surface, merged into a fairly homogeneous structure. This is noticeable on the figures 29 C. and 29 D. After 24 hours at a temperature of 40, a

noticeable change in the structure of the fibers is seen. The PLGA fibers swell noticeably, but retain their smooth surface. This can be characterized as the initial stage of degradation. On the other hand, polyurethane fibers formed a smooth surface with a certain number of pores, which again indicates a passing degradation (Figure 29 F.). The figure 29 C. is remarkable in that we can clearly see two different layers of the sandwich structure, fibrous PLGA and smoother polyurethane.

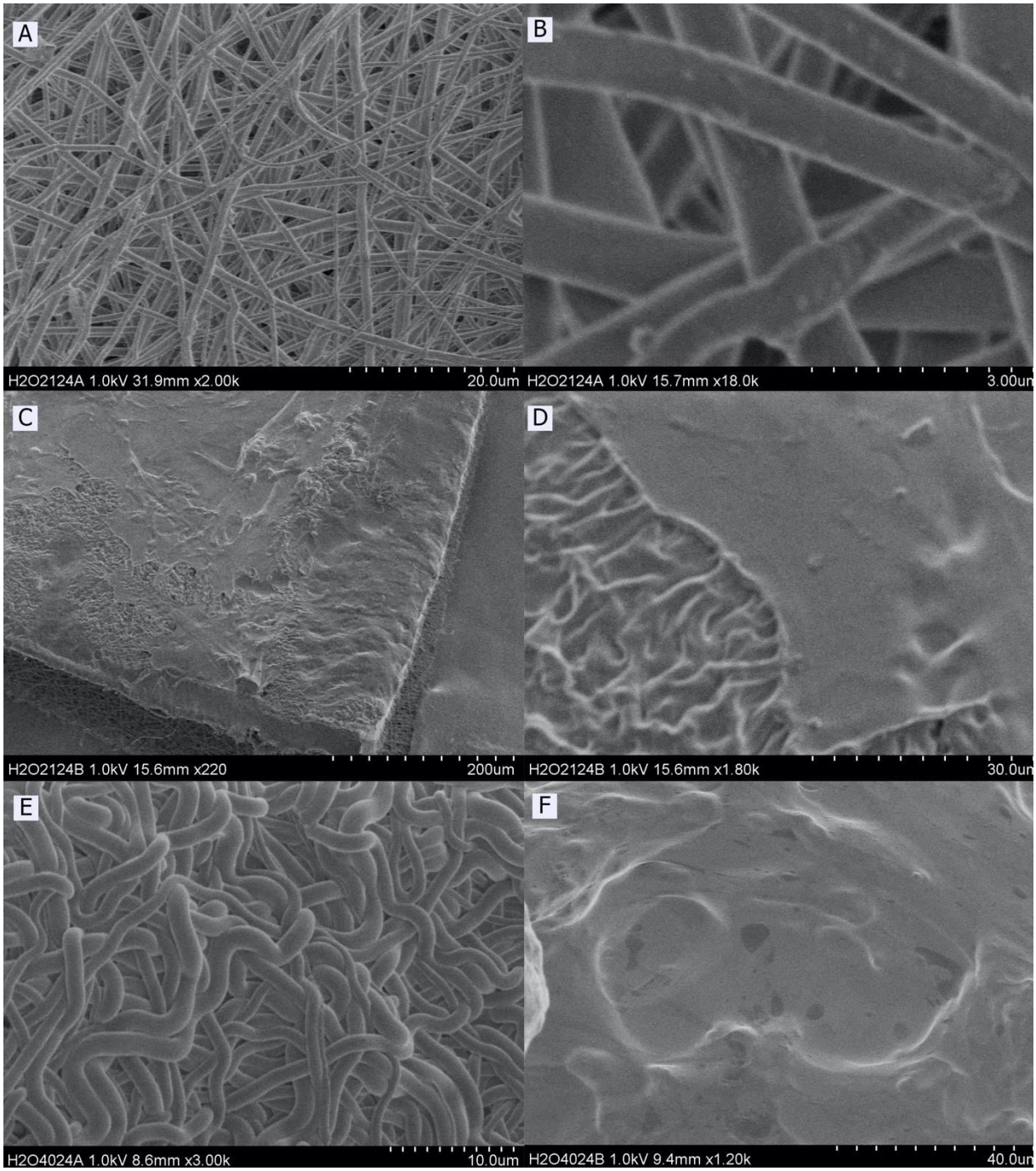

Figure 29: A., **B.** Sandwich structure after 24 hours of degradation in a distilled water at 21°C; **C.**, **D.** Sandwich structure after 24 hours of degradation in a distilled water at 21°C, another side of sample. **E.** Sample after 24

hours of degradation in a distilled water at 40°C; **F.** Sample after 24 hours of degradation in a distilled water at 40°C, another side of sample.

Next, we will consider the results of fiber degradation in saline. In this case, at room temperature, both after 24 hours and after 48 hours, the PLGA fibers retained their structure (Figure 30 C. and 30 D.). Fibers on the side of polyuretane fibers doped with PEI showed their degradation again. Certain images clearly show how the fibers that are closest to the hydrogel impregnated with physiological solution melt and merge into a homogeneous layer. Small point structures of light colour could be dried polyacrylate-polyalcohol granules. As well, as it is clear to see at the figure 30 F., PLGA fibres have the same behaviour at the elevated temperature in physiological solution as they had at elevated temperature at distilled water. They have swollen structure but with smooth surface. Figure 30 E. clearly shows the PLGA fiber under the layer of molten polyurethane fibers.

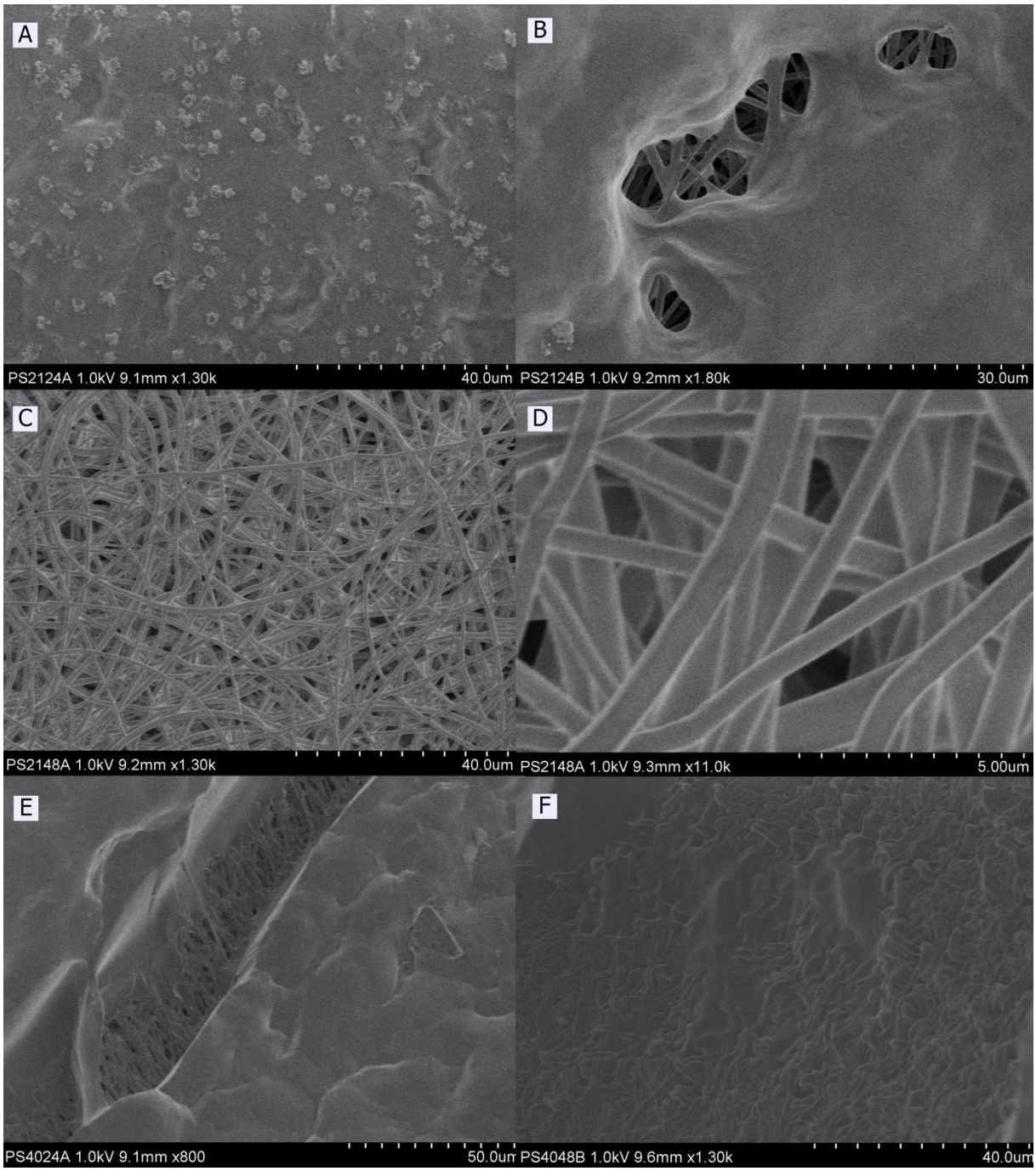

Figure 30: A., Sandwich structure after 24 hours of degradation in a physiological solution at 21°C; **B**. The other side of sandwich structure after 24 hours of degradation in a physiological solution at 21°C; **C., D.** Sample after 48 hours of degradation in a physiological solution at 21°C. **E.** Sample after 24 hours of degradation in a physiological solution at 40°C. **F.** Sample after 48 hours of degradation in a physiological solution at 40°C.

The most interesting option for us is, of course, degradation in an alkaline solution. The conditions created are an extreme case of skin surface conditions when a wound occurs. From the following images, it could be seen that at room temperature, the PLGA fibers retained their shape at our magnification, and 24 hours after placing them on a hydrogel

soaked in an alkaline solution. In this case, it is difficult to judge the progress of biodegradation, since the size of the fibers does not allow obtaining sharp micrographs; it is only possible to assume the appearance of insignificant pores on the surface of the fibers. In the figures 31 B. and 31 C. due to damage to the surface layer of the polyurethane, it is possible to see again the layered structure of the preparation and the melting of the upper layer. When the temperature rises by 40 degrees, the polyurethane deforms, shrinks and forms "convolutions", which are visible in the figures 31 D. and 31 F. Despite this, such a layer can be called homogeneous and capable of performing barrier functions. The PLGA fibers, in turn, swell again and completely fill the gaps between themselves. On some of them, we can note the appearance of transverse thinning, which can also be a consequence of degradation (Figure 31 E.).

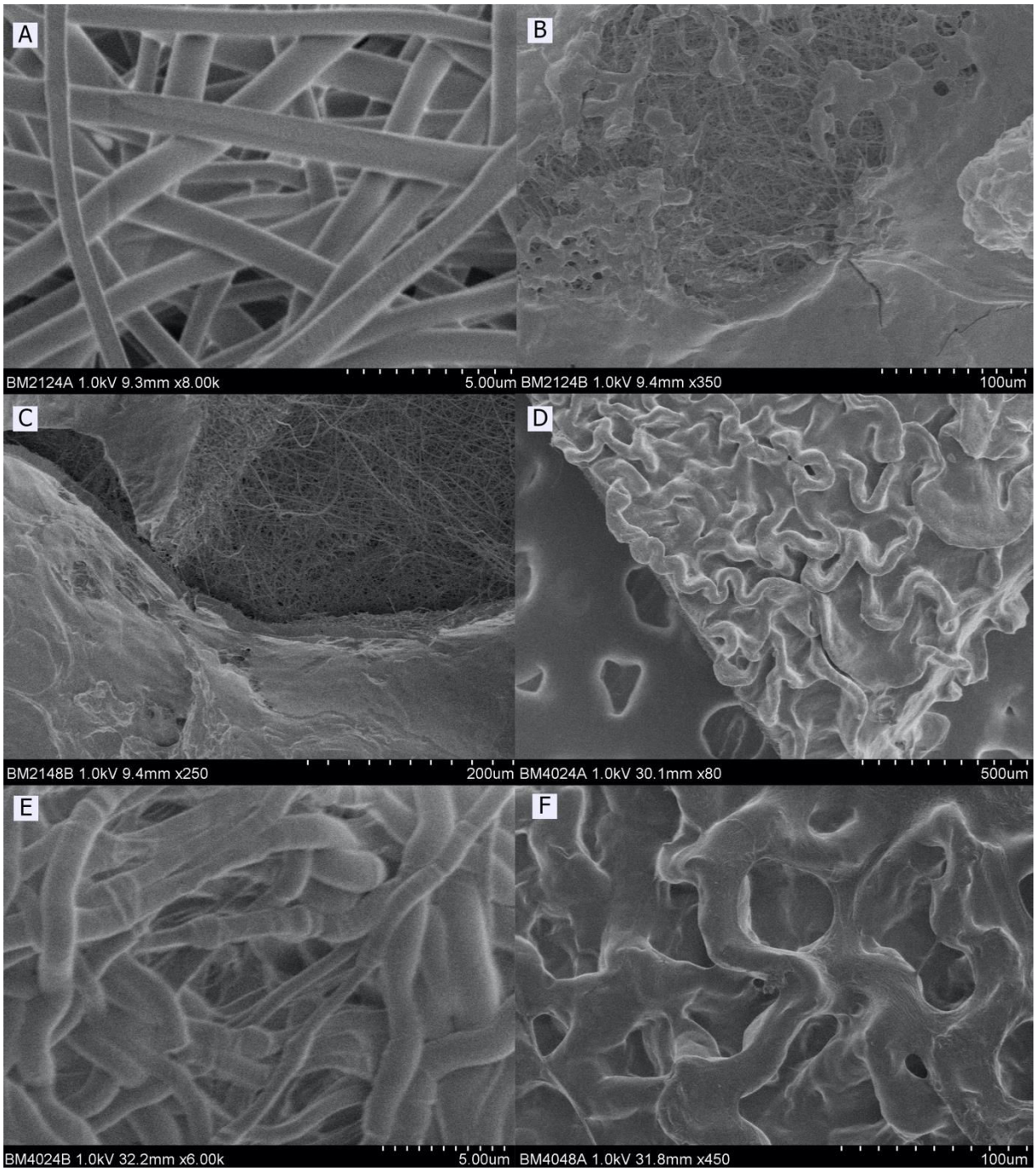

Figure 31: A. Sandwich structure after 24 hours of degradation in a basic solution at 21°C; **B**. Another side of sandwich structure after 24 hours of degradation in a basic solution at 21°C; **C.** Sample after 48 hours of degradation in a basic solution at 21°C. **D.** Another side of sample after 24 hours of degradation in a basic solution at 40°C. **E.** Sample after 24 hours of degradation in a basic solution at 40°C. **F.** Sample after 48 hours of degradation in a basic solution at 40°C.

Summary

The topic of this work was the preparation of fibers via electrospinnig using different polymers and different synthesis parameters with the aim of then combining them into one multilayer structure capable of performing different functions as wound dressing. Many commercial scaffolds for the treatment of the skin are still far from ideal, because they do not always provide protection against microorganisms and an infection develops in the wound surface, or do not maintain moisture in the wound, which slows down healing and does not provide sufficient conditions to avoid the formation of scar tissue, or cannot provide sufficient pain relief, and so on. All of the above problems can be eliminated by making one product containing several layers, each of which will perform a specific function.

In the course of the work, fibers were synthesized based on such polymers as PLGA, PU, PU and PEI blend, PCL, as well as PCL and SF blend using an electrospinning method. Then, the synthetic results were analysed using a scanning electron microscopy. Each of the fibers has been synthesized under specific parameters which are discussed in detail in the respective chapters of this thesis.

In this study, the most important part was the preparation of a structure consisting of two layers: PLGA fibers as a first layer and PU + PEI blend fibers as the second layer. The PLGA fibers were prepared using the following parameters: the speed of extrusion was 1 ml / hr, the distance from the tip of the syringe needle to the surface of the vial was 10 cm, the speed of rotation of the collector was 40 rpm and the voltage was 10 kV. Then it was necessary to dry the prepared fibers in a drying chamber. Thereafter, a layer of bled fibers of 10% PEI and PU was spinned on the surface of the PLGA fibers. The parameters of the electric spinning were already described in the previous parts of this work and were as follows: the extrusion speed was 0.5 ml/hr, the distance from the needle to the collector was 4 cm, the speed of rotation of the collector was 40 rpm and the voltage was 15 kV. In order to make sure that we were able to create fibers on the previous PLGA layer, the dye (poly [(m-phenylenevynilene) -alt-2,5-dihexyloxy-p-phenylenevynilene)]) was added to the PU + PEI mixture, which changed the colour of the next layer.

Subsequently, these fibers were tested on a hydrogel, which simulates the skin and the wound bed, under various conditions. The experimental conditions were chosen to mimic the wound surface and inflammation. It is known that when the integrity of the skin is

violated, the surface pH changes and the temperature rises locally. For this, a temperature of 40 ° C and a pH of 12 were chosen as extreme cases. In this case, we can see changes such as deformation of the polyurethane, and the formation of "convolutions". The PLGA fibers, swell and on some of them, we the appearance of transverse thinning can be noted, which can be a sign of degradation process.

These fibers have a high potential for wound dressing and quiet engineering as they consist of a degradable PLGA layer and a more stable PU + PEI. In the first mentioned layer, there is a perspective to give this structure additive function – the PLGA fibers and be additionally enriched by e.g. antibiotics that are slowly dosed into the wound. The PLGA fibers can be also filled with anti-inflammatory substances or pain relievers, creating a core-shell structure, which, when degraded, will release these biologically active substances and contribute to the normal course of wound healing and increase the patient's standard of living. The later mentioned PU + PEI structure was suggested with respect to cover the wounded site a prevent the entrance of pathogens into the treated site. PU is referred to be more stable polymer than PLGA and thanks to presence of the PEI molecules, antibacterial additive – namely silver nanoparticles – can be generated on the independent fibers. The structure of this layer will also help maintain optimal moisture in the wound, which is an important factor for treatment, since it provides ideal conditions for cell migration at all stages of healing of skin damage, which also affects the aesthetic side of the issue, reducing the likelihood of occurrence of complications like keloid scars and so on.

In conclusion, all of the above confirms that the synthesized two-layer structure can be used as wound dressing and is a candidate for the manufacture of modern and more advanced scaffolding materials.

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