CHARLES UNIVERSITY

Second Faculty of Medicine

Summary of the Dissertation

Interactions of Skin and Stem Cells with Polymer Nanofibers for Construction of Skin Substitutes

Interakce kožních a kmenových buněk s nanovlákennými polymery pro konstrukci kožních náhrad

Júlia Tomšů (née Pajorová)

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The Dissertation was written during part-time doctoral study programme Human Physiology and Pathophysiology at the Department of Biomaterials and Tissue Engineering, Institute of Physiology of the Czech Academy of Sciences.

Author: Mgr. Júlia Tomšů (née Pajorová)

Supervisor: Doc. MUDr. Lucie Bačáková, CSc.
Department of Biomaterials and Tissue Engineering,
Institute of Physiology of the Czech Academy of Sciences

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The dissertation is available for inspection at the Department for Ph.D. Study of the Dean´s Office, Second Faculty of Medicine, Charles University, V Úvalu 84, 150 06 Praha 5 (phone 224 435 836).

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Abbreviations

3D	three-dimensional
ADSCs	adipose tissue-derived stem cells
CNFs	cellulose nanofibrils
cCNFs, aCNFs, c+aCNFs	cationic, anionic, combined cellulose nanofibrils
CK	cytokeratine
DAPI	4′,6-diamidino-2-phenylindole
ECM	extracellular matrix
ECs	endothelial cells
hKs	primary human keratinocytes
HUVECs	human umbilical vein endothelial cells
NHDFs	normal human dermal fibroblasts
PCL	polycaprolactone
PCR	polymerase chain reaction
PLA, PLLA	polylactic acid, poly-L-lactic acid
PLGA	polylactic-*co*-glycolic acid
SVF	stromal vascular fraction

Abstrakt

Úvod: Kůže je nejrozsáhlejší orgán lidského těla, a proto rozsáhlá kožní poranění mohou způsobit vážné zdravotní komplikace. Užití auto-, alo- i xeno-transplantátů doprovázejí komplikace v podobě nedostatku náhradní tkáně a jejího odhojování. Proto se vytvoření artificiální kožní náhrady v laboratorních podmínkách zdá být jedním ze slibných způsobů hojení rozsáhlých ran.

Cíle: První obsáhlejší část práce je zaměřena na zhotovení dvouvrstevné prevaskularizované kožní náhrady sestávající z kolagenového hydrogelu podloženého biodegradabilní nanovlákennou membránou. Druhá část této práce se pak zabývá odlišnou strategií, a sice vývojem dočasného krytu ran na bázi celulózy.

Výsledky: (i) V první části této práce bylo zjištěno, že lidské fibroblasty a kmenové buňky tukové tkáně upřednostňují fibrinové nano-vrstvy, zejména homogenní síť z fibrinu na povrchu podkladové membrány. Keratinocyty pak lépe adherují a také stratifikují na kolagenových podkladech. (ii) Tyto poznatky motivovaly další vývoj dvouvrstvých konstruktů, kde dermální fibroblasty migrovaly z nanovlákenných membrán potažených fibrinem do kolagenového gelu a na povrchu gelu byly kultivovány epidermální keratinocyty. Tento nový přístup byl rovněž využit pro pre-vaskularizaci dvouvrstevného konstruktu, ve kterém migrující kmenové buňky tukové tkáně podporovaly formování tubulárních struktur z endotelových buněk zalitých v gelu. (iii) Druhá část této práce byla zaměřena na celulózové materiály jakožto slibné přírodní krytí kožních ran. Výsledky ukázaly, že negativně nabitá celulózová nanovlákna na rozdíl od pozitivně nabitých celulózových nanovláken zlepšuji růst fibroblastů a kmenových buněk tukové tkáně. Tento výsledek však závisel na typu buněk a na složení vrstvy proteinů ze séra kultivačního média, zprostředkujících adhezi buněk na materiál.

Závěr: Výsledkem této práce jsou dva uměle vytvořené konstrukty na bázi nanovlákenných membrán použitelné k hojení kožních poranění. Prevaskularizovaný kolagenový hydrogel, podložený biodegradabilní nanovlákennou membránou, by mohl být perspektivní kožní náhradou v případech rozsáhlejších destrukcí kožní tkáně. Celulózové textilie potažené elektricky nabitou nanocelulózou se jeví jako slibné a relativně snadno dostupné kryty pro rychlejší hojení povrchových zranění kůže..

Klíčová slova: Nanovlákenná membrána, fibrinová nanovrstva, kolagenový hydrogel, fibroblasty, keratinocyty, tukové kmenové buňky, endotelové buňky, prevaskularizace, dvouvrstevný kožní konstrukt, celulózová nanovlákna, krytí ran, kožní náhrada

Abstract

Introduction: The skin is the largest organ of a human body, therefore any extensive skin injury leads to severe complications. Since the application of auto-, allo- and xeno-grafts is accompanied by severe problems like the source limitation and the graft rejection, a bioengineered skin substitute seems to be one of the promising healing approaches.

Aims: This work is focused mainly on the construction of a pre-vascularized skin substitute consisting of a collagen hydrogel reinforced by a biodegradable nanofibrous membrane. The second part of this work is then focused on the development of temporary cellulose-based wound dressings.

Results: (i) In the first more extensive part of this work, it was found that normal human dermal fibroblasts (NHDFs) and adipose tissue-derived stem cells (ADSCs) preferred fibrin nanocoatings, mainly thin fibrin homogeneous mesh on the surface of the membrane, while human keratinocytes (hKs) adhered and stratified on collagen substrates. (ii) These observations further motivated the construction of a bi-layered construct, where the NHDFs migrated from the fibrin-coated nanofibrous membrane into the collagen hydrogel, and the hKs were cultivated on the surface of collagen. This novel approach was also utilized for pre-vascularization of the bi-layered construct in which the migrating ADSCs supported the formation of a tubular structures from human umbilical vein endothelial cells (HUVECs) embedded in the collagen. (iii) The second part of this work is focused on a cellulose-based material as a promising nature-derived wound dressing. The results of this part showed that the negatively charged cellulose nanofibrils (CNFs), in contrast to the positively charged CNFs, enhanced the growth of NHDFs and ADSCs. However, the extent of this effects depended on the cell type and on the composition of the cell adhesion-mediating proteins.

Conclusion: The result of this work are two artificially created constructs based on nanofiber membranes that can be utilized for the healing of skin wounds. The prevascularized collagen hydrogel supported by a biodegradable nanofiber membrane is a promising skin replacement in cases of more extensive skin tissue destruction. The cellulose fabrics coated with charged nanocellulose appear to be promising wound dressings for accelerated healing of superficial skin injuries.

Keywords: Nanofibrous membrane, fibrin nanocoatings, collagen hydrogel, fibroblasts, keratinocytes, adipose-derived stem cells, endothelial cells, pre-vascularization, bi-layered skin construct, cellulose nanofibrils, wound dressing, skin substitute

1 Introduction

The skin plays a crucial role in the maintenance of body homeostasis, therefore any severe skin injury might cause various complications. The self-repairing process of the skin is sufficient for small defects, but chronic wounds or deep and extensive dermal injuries have to be treated with skin grafts, dressings or substitutes. However, currently available grafts and commercial products are not sufficient and their exogenous or allogenic origin can cause an immunological response. Therefore, the novel approach is focused on development of autologous bio-artificial skin equivalents, which should mimic all functions of the native skin.

Conventional Treatment:

Deep dermal wounds are usually accompanied with a slow and inadequate healing process due to lack of epidermis. Therefore, the conventional way of treatment utilized the porcine xenografts or the human skin allograft that are surgically extracted and placed on the wound side in order to cover the damaged area. Human skin allografts can be obtained either from living donors or from cadavers, and the supplies can be stored in skin banks that can be used immediately (Vig *et al.* 2017). Although the allograft is considered to be an appropriate skin analog, it is usually transplanted as a temporary cover due to a possible immunological rejection (Oualla-Bachiri *et al.* 2020). For example, allogeneic dermal substitute was implanted onto the intractable skin ulcers in order to cover the wound and to improve the wound conditions for subsequent application of autologous skin grafts (Hasegawa *et al.* 2005). Another strategy utilized a placental amnion which is a suitable source of collagen and growth factors. Covering the wound by the amniotic membrane reduces pain, minimizes the loss of fluids, decreases the infection risk and accelerates the epithelization and closure of the wounds. In comparison with classical allografts, the amnion lacks immune markers that are responsible for the immunological rejection (Eskandarlou *et al.* 2016). However, the lowest immunological reactivity is obtained using a split-thickness autograft. A thin layer of epidermis and dermis can be shaved by dermatome from the healthy area and it is immediately placed to the wounded area of the skin. For example, positive results were obtained by treating chronic wounds with a meshed split-thickness autograft in the combination with an amniotic membrane as the wound dressing (Lavor *et al.* 2018).

Commercial Products:

Commonly used tissue-engineered products can be divided into temporary, semi-permanent and permanent, and they can be further classified according to the presence of cells, type of materials and the origin. These various tissue-engineered products are being developed in order to replace desired layers of the damaged skin, e.g. epidermis, dermis or full-thickness skin (Vig *et al.* 2017).

(i) The easiest way how to enhance the healing process is to cover the wound by acellular material. There are several commercially available products. For example, Integra® and Biobrane® combined the synthetic and biologic materials, while Alloderm® is composed of an acellular human dermis. Permacol™, Matriderm® and Oasis® are made from decellularized xenogeneic materials (Oualla-Bachiri *et al.* 2020).

(ii) The examples of dermal substitutes containing living cells are Dermagraft® and Transcyte® with neonatal foreskin fibroblasts. Apligraf®, OrCel® and StrataGraft™ are the full-thickness skin composites containing the allogenic dermal fibroblasts and keratinocytes (Vig *et al.* 2017; Oualla-Bachiri *et al.* 2020).

(iii) Due to the temporary character of allogenic products, the novel tissue-engineered autologous products are being developed. The commonly available autologous epidermal products are based on cultured keratinocytes in the form of confluent cell sheets (EpiDex® or Epibase®) or in a combination with some supported material (MySkin®, Bioseed®-S, Epicel® or Laserskin®) (Vig *et al.* 2017; Oualla-Bachiri *et al.* 2020). Keratinocytes can also be sprayed into the wound in the form of suspension (CellSpray) (Magnusson *et al.* 2007). The autologous dermal products combined the autologous fibroblasts with various scaffolds, such as silicone membrane (Hyalograft 3D or Hyalomatrix®) or biodegradable nanofibers in combination with hyaluronic acid, collagen or chitosan (Ajalloueian *et al.* 2014; Vig *et al.* 2017). These products can also be used together; for example, the TissueTech autograft system combines dermal Hyalurograft 3D and epidermal Laserskin® (Uccioli *et al.* 2011). The examples of commercially available autologous equivalents of both skin layers, so-called full-thickness skin substitutes, are Permaderm™, Tiscover™ and DenovoSkin™. All of them are promising, but due to their relatively high price and long preparation time, they are still not commonly available for clinical applications (Vig *et al.* 2017; Oualla-Bachiri *et al.* 2020). The vascularization of these products is also limited, which leads to integration failures. Therefore the current research is focused

mainly on the optimizing the manufacturing process and on revascularization of full-thickness skin substitutes (Klar *et al.* 2014; Klar *et al.* 2016; Oualla-Bachiri *et al.* 2020).

Novel Research Approaches:

The novel strategies are aiming at modeling the physiological conditions for the cells, which mimic the natural three-dimensional (3D) tissue environment (Duval *et al.* 2017). The most advanced approaches utilized the following strategies: creating the cell sheets, supporting the cells with scaffolds and embedding the cells into hydrogels (Chaudhari *et al.* 2016).

(i) The cell sheet technology combines the sheets of the cells with extracellular matrix (ECM) molecules between them using a layer-by-layer technology. For example, the cell sheets can be easily produced on thermally-responsive polymeric substrates and separated without trypsinisation by lowering the temperature (Lee *et al.* 2018).

(ii) The scaffolds for the cells can be manufactured from a wide range of biodegradable or non-biodegradable materials that can be synthetic, nature-derived or composite. Based on the fabrication protocols, the scaffolds can be produced in different shapes for various applications, e.g. porous, fibrous or microspheres (Chaudhari *et al.* 2016).

The synthetic polymers, such as polylactic acid (PLA) polylactic-*co*-glycolic acid (PLGA), polycaprolactone (PCL) and many others, are commonly processed to nanofibrous scaffolds by conventional electrospinning (Chen *et al.* 2017) or by direct deposition of nanofibers into the wound using *in situ* electrospinning technique (Dong *et al.* 2016). The cells can be either seeded on the nanofibers (Bacakova *et al.* 2016) or encapsulated inside the fibers (Chen *et al.* 2015). In order to enhance the cell adhesion and proliferation, the synthetic scaffolds are coated or loaded with nature-derived substances that attracted and stimulated the cells (Bacakova *et al.* 2016; Fu *et al.* 2016). Moreover, the nanofibers are able to absorb exudate from wounds (Chen *et al.* 2017) and also to release growth factors (Xie *et al.* 2013) or other therapeutic agents with anti-bacterial, anti-inflammatory and anti-oxidant effects (Shababdoust *et al.* 2018).

Natural scaffolds are made either from polysaccharides, such as chitosan, hyaluronic acid, heparin and cellulose, or from proteins, mainly collagen, fibrin, fibronectin, silk fibroin, keratin and laminin (Raveendran *et al.* 2017). The cellulose extracted from plants or bacteria is a promising biomaterial commonly used in the form of nanoparticles, nanocrystals or nanofibrils (Bacakova *et al.* 2019a). Cellulose nanofibers can also be prepared by electrospinning technology (Park *et al.* 2015). Collagen is the most abundant ECM protein that is extensively used in the form of sponge, nanofibers, powders or hydrogels for the wound healing

(Chattopadhyay and Raines 2014). It has been discovered that the collagen nanofibers, collagen containing composites or collagen-coated nanofibers are more appropriate for the cells than synthetic scaffolds due to their higher biocompatibility (Zhou *et al.* 2016; Lai *et al.* 2014; Bacakova *et al.* 2017). In addition, the collagen in the form of hydrogel provide physiological 3D environment to the encapsulated cells (Smithmyer *et al.* 2014; Tavakoli and Klar 2020).

Decellularized collagen-rich scaffolds are produced by removal of the cells from the whole tissue in order to reduce the immunological response to the implant. These acellular scaffolds can be recellularized by autologous cells. Several commonly available commercial products are based on acellular scaffolds, but they are made from the xenogenic porcine or bovine skin. However, Benny *et al.* developed an allogenic organotypic cultured skin that is made by decellularization of the ECM molecules deposited by fibroblasts and keratinocytes (Benny *et al.* 2015). Greaves *et al.* found out that allogenic human decellularized dermis enhance angiogenesis during the healing process (Greaves *et al.* 2015).

(iii) The most recent approach is based on embedding the cells into the nature-derived hydrogels made mainly of collagen, fibrin, hyaluronic acid, chitosan and gelatin (Franco *et al.* 2011; Miron-Mendoza *et al.* 2012; Braziulis *et al.* 2012; Smithmyer *et al.* 2014). The hydrogels are able to self-assemble from the liquid monomeric phase to the polymeric mesh network under a certain temperature, pH, and enzymatic activity (Chaudhari *et al.* 2016). The hydrogels enable the encapsulation of living cell that can degrade the hydrogel and synthetize their own ECM molecules (El Ghalbzouri *et al.* 2009; Miron-Mendoza *et al.* 2012). Although the hydrogels have excellent biocompatibility and biodegradability, their mechanical properties are weak with tendency to contract under traction forces of the embedded cells (Achilli and Mantovani 2010; Antoine *et al.* 2015). In order to increase the stability and to improve mechanical properties of the material, the hydrogel can be compressed (Braziulis *et al.* 2012), cross-linked (Lotz *et al.* 2017) or strengthened by some scaffolds, such as nanofibrous membranes or knitted meshes (Hartmann-Fritsch *et al.* 2016; Franco *et al.* 2011). The cells embedded in the hydrogels, especially fibroblasts and other mesenchymal cells, tend to be more spread with typical spindle-like morphology and to create a network with cell contacts in all three dimensions (Hartmann-Fritsch *et al.* 2016; Miron-Mendoza *et al.* 2012). On the other hand, keratinocytes with their polarity prefer flat surfaces of the hydrogels (Fujisaki *et al.* 2008; Klar *et al.* 2018). Although the encapsulated cells are separated from the cells growing on the surface, their communication can be mediated by cytokines and growth factors released from the cells by a paracrine manner or by cell-hydrogel mechanosensation (Wojtowicz *et al.* 2014; Doyle and Yamada 2016).

2 Objectives and Hypothesis of the Work

(i) The first main objective of this work was to construct a pre-vascularized skin substitute consisting of a collagen hydrogel reinforced by a biodegradable nanofibrous membrane. This general aim was subdivided into the following specific aims:

- Comparison of protein-coated nanofibrous membranes with the non-coated membranes in terms of the cell adhesion, morphology and growth. The hypothesis was that the biodegradable protein-coated nanofibrous membrane is suitable substrate for cell growth and for the construction of bioengineered skin substitute.

- Creating an optimal 3D environment for cell colonization and growth from collagen hydrogel and to analyze the migration capability of the cells in the hydrogel. The hypothesis was that the NHDFs and ADSCs are able to migrate from the substrate into the whole volume of the collagen hydrogel representing the dermal part of the substitute.

- Constructing a bi-layered skin substitute containing the collagen hydrogel reinforced by nanofibrous membrane pre-seeded with NHDFs and hKs on the surface of collagen hydrogel. The hypothesis was that the collagen hydrogel is the suitable substrate for adhesion, growth and stratification of hKs and for the formation of an artificial epidermis.

- Co-culturing the ADSCs with HUVECs in the bi-layered skin construct in order to support the formation of capillary-like network. The hypothesis was that the ADSCs support the pre-vascularization of collagen-based skin substitutes.

(ii) The second objective of this work was to develop temporary cellulose-based wound dressings. For this purpose, the cellulose-based materials will be coated with fibrin or with charged CNFs to improve the material properties for cell attachment. The hypothesis was that the modified cellulose-based materials enhance the adhesion and proliferation of the cells by specific adsorption of cell adhesion-mediating proteins.

3 Materials and Methods

Material Preparation and Characterization:

Experiments were carried out either on nanofibrous membranes prepared by needle-free electrospinning technology from synthetic degradable polymers (PLA, PLGA and PLLA) or on cellulose meshes (nonwoven cotton fabric PurCotton®). The materials were further coated by proteins, such as fibrin, collagen and fibronectin; see Bacakova *et al.* (2017, 2018b and 2019b) and Pajorova *et al.* (2018 and in preparation). The pure cellulose meshes were either carboxymethylated and converted to a sodium salt to produce a Hcel® NaT textiles for fibrin coatings (Bacakova *et al.* 2018b) or they were directly coated by positively- or negatively-charged CNFs (cCNFs and aCNFs); see Pajorova *et al.* (2020).

The material topography, roughness, elasticity and the morphology of the protein nanocoatings were evaluated using various microscopy techniques, i.e. scanning electron microscopy, atomic force microscopy and confocal microscopy; see Pajorova *et al.* (2018 and 2020) and Bacakova *et al.* (2017, 2018b and 2019b). The wettability, swelling ratio and the pre-adsorption of the serum-derived proteins on the CNF coatings were also analyzed (Pajorova *et al.* 2020).

Cell Isolation, Characterization and Culture Conditions:

The NHDFs purchased from Lonza (Switzerland) were used in all our publications; see Bacakova *et al.* (2017, 2018b and 2019) and Pajorova *et al.* (2018, 2020). The human aneuploid immortal keratinocytes of the line HaCaT purchased from Cell Lines Service (Germany) were utilized in Bacakova *et al.* (2017). The primary hKs were isolated from skin of a young adult donors. The hKs were used for the construction of collagen-based 3D skin constructs in Bacakova *et al.* (2019b) and Pajorova *et al.* (in preparation). The human ADSCs were isolated from subcutaneous adipose tissue from 15 healthy donors; see Travnickova *et al.* (2020). The liposuctions were conducted on the thighs or on the abdomen using a low negative pressure (-200 mmHg; referred to as “low”) and a high negative pressure (-700 mmHg; referred to as “high”). The yields of isolated cells were characterized, see Travnickova *et al.* (2020). The ADSCs that were more positive for CD146 than the others, i.e. “low” abdominal ADSCs from a single donor, were utilized for the construction of pre-vascularized 3D skin construct (Pajorova *et al.*, in preparation). In order to decrease the variability in the cell behavior between individual donors, the ADSCs from 10 donors isolated from the same region under the same

pressure were mixed. The behavior of pooled ADSCs isolated from thighs under the "low" pressure on different CNF coatings was compared with NHDFs, see Pajorova *et al.* (2020). The HUVECs were purchased from Lonza and they were used for preparation of pre-vascularized 3D skin construct (Pajorova *et al.*, in preparation).

Preparation of Collagen-Based Skin Constructs:

The PLLA membranes were coated with a homogenous fibrin mesh based on our protocol published in Pajorova *et al.* (2018), and they were seeded with NHDFs. The collagen hydrogels were prepared on the membranes after 3 days of cultivation on the NHDFs using collagen type I isolated from rat tails. The collagen hydrogels strengthened by the fibrin-coated membrane were seeded with primary hKs after 4 days of NHDF migration into the hydrogels. In order to stimulate the remodeling of the collagen hydrogels by the cells, the ascorbic acid was added into the culture medium. The behavior of both cell types in co-culture was observed for 14 days; see Bacakova *et al.* (2019b).

In case of the pre-vascularized skin construct, the fibrin-coated membranes were seeded with abdominal ADSCs isolated under "low" pressure. The hydrogels were prepared using collagen type I isolated form the porcine skin, similarly as it was described in Bacakova *et al.* (2019b). The hydrogel was polymerized with or without homogeneously dispersed HUVECs. The behavior of the ADSCs and HUVECs in the collagen hydrogels was observed after 7 and 14 days of cultivation without hKs. The primary hKs were seeded on the pure collagen hydrogel or on the collagen hydrogel with embedded HUVECs after 4 days of ADSC migration. Some samples were transferred into the air-liquid interface on day 3 after hK seeding to stimulate their stratification. The skin constructs were evaluated after 14 days. The data from these experiments will be published in an upcoming article (Pajorova *et al.*, in preparation).

Characterization of the Cell Behavior:

The metabolic activity of the cells was measured by MTS assays (Bacakova *et al.* 2017, 2018b, 2019b; Pajorova *et al.* 2018) or by the conversion of resazurin sodium salt into resorufin by mitochondrial enzymes (Pajorova *et al.* 2020). The cell viability on the protein-coated nanofibrous membranes was determined using a Live/Dead viability/cytotoxicity kit; see Bacakova *et al.* (2017).

The adhesion, spreading and morphology of the cells were visualized by staining proteins of the cell cytoplasmic and membrane cytoskeleton (F-actin, vinculin) and cell nuclei. Images of the cells were acquired by epifluorescence or confocal microscope (Bacakova *et al.* 2017,

2018b, 2019b; Pajorova *et al.* 2018, 2020). The morphology of the cells on the CNF coatings was also visualized by scanning electron microscopy (Pajorova *et al.* 2020). The production of extracellular collagen I and fibronectin by the cells was evaluated by immunofluorescence staining, and the expression of these proteins at mRNA level was measured by real-time polymerase chain reaction (PCR); see Pajorova *et al.* (2018).

The migration of the NHDFs from the membrane into the collagen hydrogel was evaluated by the confocal microscope after staining the cells, and also by MTS assay after separating the hydrogels from the membranes. The shrinkage of the collagen hydrogels caused by the cell traction was evaluated by measuring the diameter of the collagen hydrogel circles after their separation from the membranes; see Bacakova *et al.* (2019b).

The special 3D-printed air-liquid system was developed for the differentiation and stratification of the primary hKs on the collagen surface of pre-vascularized 3D skin models under *in vitro* conditions. In order to visualize the stratification of hKs, the specific cytokeratins (CK14, CK5 and CK10) in the cell cytoskeleton were stained by immunofluorescence. The images were acquired by the confocal microscope; see Pajorova *et al.* (in preparation).

The ADSCs and HUVECs were co-cultured in 3D collagen hydrogels for 14 days to initiate the capillary-like network formation in pre-vascularized 3D skin models. The specific CD31 marker of endothelial cells (ECs) was stained using primary and secondary antibodies. The actin filaments in the cells were stained with phalloidin conjugated with Atto 488 florescent dye, and the cell nuclei by 4′,6-diamidino-2-phenylindole (DAPI). The images were acquired by the confocal microscope (Pajorova *et al.*, in preparation).

In Pajorova *et al.* (2020) work, the dependence of the cell adhesion on the composition of the pre-adsorbed serum-derived proteins was evaluated on cCNF coatings using confocal microscopy. The speed of adhesion of NHDFs and ADSCs on cCNF coatings was evaluated by live cell imaging after distinguishing the cells by different fluorescent cell trackers.

Statistical Analysis:

The statistical significance of the non-Gaussian distributed data was evaluated using the nonparametric analysis of variance (Kruskal-Wallis), with Mann-Whitney U test or Tukey's *post hoc* test for pairwise comparison; see Bacakova *et al.* (2019b) and Pajorova *et al.* (2020). The normal distributed data were evaluated using parametric analysis of variance with Student-Newman-Keuls test or Tukey's *post hoc* test for pairwise comparison; see (Bacakova *et al.* 2017, 2018b, 2019b; Pajorova *et al.* 2018, 2020). Values of $p \leq 0.05$ were considered as statistically significant.

4 Results

Single-Layered Skin Constructs Based on Protein-Coated Nanofibers:

(i) Bacakova M., Pajorova J., Stranska D., Hadraba D., Lopot F., Riedel T., Brynda E., Zaloudkova M, Bacakova L. **Protein nanocoatings on synthetic polymeric nanofibrous membranes designed as carriers for skin cells.** *Int J Nanomedicine* 2017; 12:1143-1160.

(ii) Pajorova J.*, Bacakova M., Musilkova J., Broz A., Hadraba D., Lopot F., Bacakova L. **Morphology of a fibrin nanocoating influences dermal fibroblast behavior.** *Int J Nanomedicine* 2018; 13:3367-3380.

Both studies follow up on the work of Bacakova *et al.* (2016). In Bacakova *et al.* (2017) work, we compared fibrin nanocoating with collagen nanocoating as a potential substrate for fibroblast and keratinocyte growth. Fibrin coated the individual nanofibers in the membranes, and also randomly formed a thin nanofibrous mesh on the top of the membranes. Collagen also covered the fibers of the membrane and randomly created a soft gel on the membrane surface. Fibrin greatly improved the attachment, spreading, and proliferation of NHDFs, while collagen nanocoating enhanced the adhesion and growth of human HaCaT keratinocytes. In addition, fibrin stimulated the synthesis and deposition of fibronectin by the NHDFs. Furthermore, in Pajorova *et al.* (2018) work, we improved the preparation of the fibrin nanocoating that was described in our previous works (Pajorova *et al.* 2018). Depending on the mode of preparation, fibrin either covered the individual fibers in the membrane (referred to as "fibrin coating"), or covered the individual fibers and also formed a fine homogeneous nanofibrous mesh on the surface of the membrane (referred to as "fibrin mesh"). We discovered that the morphology of the fibrin nanocoating is crucial for the adhesion of fibroblasts, and consequently for their phenotypic maturation. The NHDFs on the "fibrin coating" remained in their typical spindle-like shape, while the cells on the "fibrin mesh" were spread mostly in a polygon-like shape, and their proliferation was significantly higher. Moreover, the "fibrin mesh" increased the gene expression and protein production of collagen I and fibronectin.

Bi-Layered Skin Constructs Based on Collagen Gel and Nanofibers:

(i) Bacakova M.# and Pajorova J.#, Broz A., Hadraba D., Lopot F., Zavadakova A., Vistejnova L, Beno M., Kostic I., Jencova V., Bacakova L. **A two-layer skin construct consisting of a collagen hydrogel reinforced by a fibrin-coated polylactide nanofibrous membrane.** *Int J Nanomedicine* 2019; 14:5033-5050. *# contributed equally*

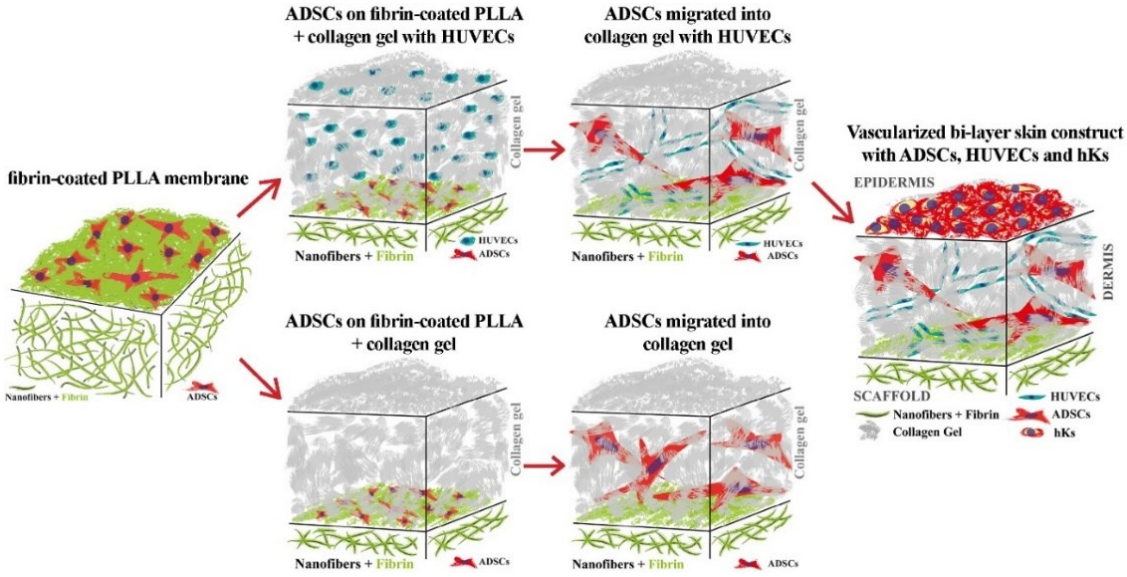
(ii) Travnickova M., Pajorova J., Zarubova J., Krocilova N, Molitor M., Bacakova L. **The influence of negative pressure and of the harvesting site on the characteristics of human adipose tissue-derived stromal cells from lipoaspirates.** *Stem Cells Int* 2020; 2020:1016231.

(iii) Bacakova L., Zarubova J., Travnickova M., Musilkova J., Pajorova J., Slepicka P., Kasalkova N., Svorcik V., Kolska Z., Motarjemi H., Martin Molitor M. **Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review.** *Biotechnol Adv* 2018; 36(4):1111-1126.

(iv) Pajorova J., Blanquer A., Broz A., Travnickova M., Matejka R., Suca H., Supova M., Bacakova L. **Pre-vascularized bi-layered skin construct based on collagen hydrogel reinforced by fibrin-coated nanofibrous membrane seeded with adipose-derived stem cells.** *Manuscript in preparation.*

All these studies partially contributed to the preparation of a pre-vascularized bi-layered skin construct. In Bacakova *et al*. (2019b) work, the NHDFs migrated from the fibrin-coated PLLA membrane into the collagen hydrogel that was subsequently seeded with hKs. This skin construct was further pre-vascularized using ADSCs and HUVECs that spontaneously formed capillary-like network (Pajorova *et al.*, in preparation). The ADSCs were isolated from the lipoaspirate of human donors (Travnickova *et al.* 2020; Bacakova *et al.* 2018a), and together with HUVECs were embedded into the collagen hydrogel. In Bacakova *et al.* (2019b) work, we demonstrated that the fibrin mesh accelerated the migration of the NHDFs from the substrate into the collagen hydrogel, and there was also a lower tendency to contract the hydrogel by their traction forces. The surface of the collagen was seeded with hKs that were able to form a basal layer of highly mitotically-active cells, and a suprabasal layer. In Travnickova *et al.* (2020) work, we isolated the ADSCs and we evaluated the influence of the negative pressure ("low" and "high") and also harvesting site ("inner or outer thighs" and "abdomen") on the yields and other cell parameters. We found out that the greater cell yields were obtained from the outer thigh than from the abdomen. However, in case of the abdomen region, the yields were greater under "high" pressure than under "low" pressure. These initial differences were equalized in subsequent subculture of the cells. In Bacakova *et al.* (2018a) review article, the abdominal ADSCs from single donor were cultivated on two different types of fibrin nanocoatings. Both "low" and "high" abdominal ADSCs adhered better and proliferated faster on PLLA membranes modified with "fibrin mesh" type of nanocoating than on "fibrin coating" type of nanocoating, see review Bacakova *et al.* (2018a).

In Pajorova *et al.* (in preparation) work, we combined the ADSCs with HUVECs in the collagen hydrogel in order to pre-vascularize the bi-layer skin construct. For this purpose, we selected the "low" abdominal ADSCs from a single donor that were highly positive for CD146 marker, i.e. the adhesion molecule that is essentially expressed on the vascular system, mainly on the pericytes (Wang *et al.* 2020). We cultivated the ADSCs on "fibrin mesh"-coated PLLA membrane for 3 days before we applied the collagen hydrogel. The hydrogel was polymerized either with or without embedded HUVECs. After 4 days of ADSC migration and their interaction with HUVECs, the hKs were seeded on the top of the hydrogel, and after next 3 days, the samples were exposed to the air-liquid interface. The overall cell interactions were evaluated after 14 days of cultivation (Scheme 1). We observed that the ADSCs were able to migrate through the hydrogel and create the monolayer on the surface when there were no other cell types. In case of the embedded HUVECs, the ADSCs interacted with the HUVECs that formed a capillary-like network. Interestingly, the HUVECs were also able to form a monolayer of the cells on the surface of hydrogel when the hKs were not present. However, when the hKs were seeded, the capillary-like network was slightly reduced, only some short branches and connections of HUVECs were still recognizable. The hKs created several layers of cells with overall thickness of approx. 50 µm. On the cell-collagen interface, the hKs visibly formed a basal layer with highly mitotically-active cells. This layer and also the next suprabasal layers expressed mainly the CK14, which is the cytokeratin of the non-differentiated cells. The size of the cells in the suprabasal layers gradually increased, and the most superficial layer of hKs on the air-liquid interface also expressed CK10, i.e. a marker of more differentiated cells.



Scheme 1. *Preparation of the pre-vascularized bi-layered skin constructs.*

Temporary Cell-Decorated Wound Dressings Based on Cellulose:

Pajorova J.#* and Skogberg A.#, Hadraba D., Broz A., Travnickova M., Zikmundova M., Honkanen M., Hannula M., Lahtinen P., Tomkova M., Bacakova L., and Kallio P. **Cellulose Mesh with Charged Nanocellulose Coatings as a Promising Carrier of Skin and Stem Cells for Regenerative Applications.** *Biomacromolecules* 2020; 21(12):4857-4870. # *contributed equally*

Bacakova M., Pajorova J., Sopuch T., Bacakova L. **Fibrin-Modified Cellulose as a Promising Dressing for Accelerated Wound Healing.** *Materials (Basel)* 2018; 11(11):2314.

In both studies we utilized a nature-derived cellulose mesh, which is commonly used for covering the wound beds. We optimized the physicochemical properties of this mesh for the cell attachment using wood-derived CNFs (Pajorova *et al.* 2020) and by fibrin nanocoatings (Bacakova *et al.* 2018b). In Pajorova *et al.* (2020) work, three different types of the coatings were proposed as the carriers of NHDFs and ADSCs: positively charged cationic cellulose nanofibrils (cCNFs), negatively charged anionic cellulose nanofibrils (aCNFs), and a combination of these two materials (c+aCNFs). The aCNF coating significantly improved the proliferation of both cell types, while the cCNF coating significantly enhanced the adhesion of ADSCs only. The number of NHDFs was similar on the cCNF coating and on the non-coated cellulose mesh. We found that the cell adhesion and proliferation on these coatings were highly dependent on the composition of the adsorbed adhesion-mediating serum proteins. The c+aCNF construct combined the benefits from both types of CNFs, therefore the c+aCNF-coated meshes might be a promising material for the skin tissue engineering. In Bacakova *et al.* (2018b) work, we coated the commercially available Hcel® NaT cellulose mesh with two types of fibrin nanocoatings according to the preparation protocol described in Pajorova *et al.* (2018). In order to increase the attachment of NHDFs, we covered the fibers of meshes with "fibrin coating" or "fibrin mesh" type of nanocoating. We confirmed the results obtained on PLA membranes, where the fibrin coatings significantly improved the material colonization with NHDFs (Pajorova *et al.* 2018). The number of adhered NHDFs on "fibrin mesh" was considerably higher compared to the cell number on "fibrin coating". However, after one week, the cell number of NHDFs on both coatings was almost at the same level.

5 Discussion

Single-Layered Skin Constructs Based on Protein-Coated Nanofibers:

The cell carriers made of various biodegradable synthetic nanofibrous polymers represent one of the current approaches in the treatment of the wounds. However, these membranes in their non-modified state do not provide sufficient properties for the cell growth. Desired physicochemical properties can be achieved by coating their surfaces with nature-derived biomolecules (Bacakova *et al.* 2016). In Bacakova *et al.* (2017) work, the results showed that the NHDFs preferred fibrin nanocoating, while the HaCaT cells preferentially adhered on collagen nanocoating. This behavior can be affected by the origin of the cells. The fibroblasts naturally migrate into the fibrin clot during wound healing, while the growth of keratinocytes occurs when the fibrin clot is particularly replaced with collagen. The positive effect of fibrin on the fibroblast behavior was also described in other works (Bacakova *et al.* 2016; Nair *et al.* 2014); however, these studies have not reached consistent conclusions in the role of fibrin on keratinocyte behavior. For example, Kubo *et al.* reported that the $\alpha_V\beta_3$ integrin receptors, important for binding the cells to fibrin molecules, were not present on the keratinocyte membrane, which made the fibrin unrecognizable by the keratinocytes (Kubo *et al.* 2001). However, in our study, the keratinocytes were able to adhere on fibrin nanocoating, even though their number was significantly lower compared to that on collagen nanocoating. In contrast to the fibrin nanocoating, the collagen nanocoating was not able to resist the traction forces generated by fibroblasts, while the attachment of keratinocytes was significantly increased. This was probably accelerated by the recognition of the Asp-Gly-Glu-Ala (DGEA) sequence in collagen molecules by α2β1 or α3β1 integrin receptors present on the keratinocyte membrane (Staatz *et al.* 1991; Symington *et al.* 1993). In Pajorova *et al.* (2018) study, we modified the preparation of fibrin nanocoating that was described in Bacakova *et al.* (2017) to create two different types of fibrin nanocoatings. The two-step fibrinogen adsorption was previously described by Riedel *et al.* and Bacakova *et al.*; however, in their studies, the fibrin mesh was formed randomly which resulted into nonhomogeneous fibrin nanocoatings (Riedel *et al.* 2009; Bacakova *et al.* 2016; Bacakova *et al.* 2017). Therefore, in this study, we also regulated the amount of the adsorbed thrombin by its controlled washing. In accordance with our previous results, the fibrin, mainly in the form of mesh, stimulated the adhesion and proliferation of NHDFs, and the synthesis and deposition of collagen I and fibronectin by these cells (Bacakova *et al.* 2016; Bacakova *et al.* 2017).

Bi-Layered Skin Constructs Based on the Collagen Gel and Nanofibers:

An advanced approach in the wound healing is the construction of bi-layered or even three-layered skin constructs to mimic the natural skin. For this purpose, we focused on collagen hydrogels that are commonly used in tissue engineering (Smithmyer *et al.* 2014). In Bacakova *et al.* (2019b) study, we co-cultured the NHDFs in the collagen hydrogel and hKs on the surface of collagen to create a bi-layered skin construct. The embedding of the fibroblasts and other mesenchymal cells into the collagen hydrogels has been previously reported by many other researchers (Miron-Mendoza *et al.* 2012; Sriram *et al.* 2015). Moreover, it has also been noted that the flat surface of a hydrogel supported the growth of keratinocytes (Sriram *et al.* 2015; Fujisaki *et al.* 2008; Klar *et al.* 2018). The separated layers of the cells are able to communicate by paracrine releasing of cytokines and growth factors or by cell-hydrogel mechanotransduction (Wojtowicz *et al.* 2014; Doyle and Yamada 2016). However, the mechanical properties of the hydrogels are usually inappropriate for tissue engineering. The physiological conditions that are required for the cell survival during polymerization caused the softening of the hydrogels which lead to the contraction of the hydrogel under traction forces of the spreading cells (Antoine *et al.* 2015; Achilli and Mantovani 2010). In order to improve the mechanical stability, we reinforced the hydrogel with the fibrin-coated nanofibrous PLLA membrane. In contrast to the works where the cells were embedded directly into the collagen hydrogel (Hartmann-Fritsch *et al.* 2016; Franco *et al.* 2011), we pre-seeded the fibrin-coated membrane with NHDFs and we let them migrate into the collagen. This novel approach reduced the contraction of the collagen hydrogel due to the optimal dynamics of collagen degradation and synthesis by NHDFs during their migration (Miron-Mendoza *et al.* 2012; El Ghalbzouri *et al.* 2009). In this study, we also discovered that the fibrin nanocoating accelerated not only the adhesion and growth but also the migration of NHDFs. Similar results were reported by Fu *et al.* with fibrinogen-coated PCL nanofibers (Fu *et al.* 2016). We confirmed that collagen in the form of hydrogel is a suitable substrate for the growth and stratification of the keratinocytes (Braziulis *et al.* 2012; El Ghalbzouri *et al.* 2009; Hartmann-Fritsch *et al.* 2016). In Bacakova *et al.* (2019) work, the hKs were seeded on a rat tail collagen hydrogel and they were cultured under a liquid surface, i.e. immersed in the culture medium (Bacakova *et al.* 2019b). However, in case of the pre-vascularized skin construct, the hKs were seeded on a porcine collagen hydrogel and the stratification of hKs into several layers was enhanced by their cultivation at air-liquid interface (Pajorova *et al.*, in preparation). The absence of a developed capillary network is still one of the major problems in construction of fully functional skin substitutes. In Pajorova *et al.* (in

preparation) work, we pre-vascularized our previously described collagen-based skin construct using ADSCs and HUVECs. We selected the most CD146 positive group of ADSCs that might have the most similar characteristics to the pericytes. The pericytes produce proangiogenic growth factors that lead the ECs into a capillary formation (Bergers and Song 2005). The similar strategy was described by Chan *et al.* who used only isolated ADSCs and a collagen–polyethyleneglycolated–fibrin hydrogel. They detected that ADSCs in a collagen layer exhibited spindle-like morphology, while ADSCs in polyethyleneglycolated-fibrin layer formed a network of tubular structures (Chan *et al.* 2012). Unlike to the Chan *et al.*, we additionally embedded the HUVECs into the collagen to accelerate the formation of the capillary-like network. The ADSCs that migrated from fibrin-coated membrane were used as the supporting cells for the capillary-like network formation from ECs. The similar cell setup was used by Baltazar *et al.* who used the 3D printing method to create a pre-vascularized skin substitute (Baltazar *et al.* 2020). We also noticed in our experiments that the embedded HUVECs were able to form a cell monolayer on the surface of the hydrogel when the hKs were absent. The same behavior of ECs on the collagen gel was described by Abe *et al.* (Abe *et al.* 2013). Karl *et al.* described another strategy, in which they used fresh stromal vascular fraction (SVF), i.e. a mixture of the cells isolated from human fat that spontaneously formed the capillaries (Klar *et al.* 2014). This enabled them to skip the isolation process, which made this strategy more convenient for clinical applications. However, the composition of the cells in each fraction of SVF might differ. Therefore, in their next study, these authors isolated and separated the ADSCs and ECs from SVF and they evaluated the behavior only of these two cell types in the collagen or fibrin hydrogels (Klar *et al.* 2016). In contrast to our study, Klar *et al*. in both works embedded the cells directly into the hydrogels. The tubular structures formed before the engraftment of the pre-vascularized skin substitute are capable to anastomose with the host vessel, which can increase the cell survival and decreases the rejection of a skin substitute (Laschke and Menger 2012).

Temporary Cell-Decorated Wound Dressings Based on Cellulose:

The innovative wound dressings can be enriched with gradually releasing drugs promoting the healing process or they can serve as temporary cell carriers (Bacakova *et al.* 2019a; Tavakoli and Klar 2020). These dressings regulate the amount of exudate and maintain the optimum moisture level, oxygen permeability, and sterility in the wound. In Bacakova *et al.* (2018) work, we coated Hcel® NaT cellulose mesh with fibrin nanocoatings. The beneficial effect of fibrin on the cell attachment and growth has been already discussed in our pervious works (Pajorova

et al. 2018; Bacakova *et al.* 2017) In Pajorova *et al.* (2020) work, we covered the cellulose-based meshes with CNFs with different surface charges and we evaluated the effect of the structure, surface chemistry and stiffness of the coatings on the cell attachment and growth. Based on the amount of the CNF solutions and the charge of CNF solutions, we were able to modulate the material surface properties. Although the studied CNF coatings differ in many parameters, we assume that the main cell adhesion-modulating factor is the surface chemistry, which determine the composition of proteins adsorbed on CNF coatings (Syverud 2017). We detected that positively charged trimethylammonium tails of cCNFs adsorbed more proteins, mainly bovine serum albumin (BSA), than negatively charged carboxyl functional groups of aCNFs. The positive charge probably attracted the negatively-charged proteins such as fibronectin, vitronectin, and the most abundant BSA (Hoshiba *et al.* 2018; Arima and Iwata 2007). This was also observed by Courtenay *et al.*, who quantified the proteins adsorbed from FBS, specifically BSA, on cCNF scaffolds (Courtenay *et al.* 2019). Although aCNFs adsorbed less proteins, they probably contained more cell adhesion-mediating proteins that can be perceived by the cells (Hoshiba *et al.* 2018). Therefore, the negatively charged CNFs promoted the cell attachment better than cCNFs, however it depended on the cell type. We found out that the adsorbed BSA on cCNF coatings decreased the amount of initially adhered NHDFs, but it did not affect the number of attached ADSCs. Based on the pre-adsorption tests, we suppose that the attachment of ADSCs was faster than the adsorption of BSA in a large quantity while the adhesion of NHDFs was slower. The adhesion of both cell types on combined c+aCNF coatings was high although there was adsorbed a lot of cell-non-adhesive BSA from FBS. This was probably due to a hydrophilic character of c+aCNF coatings, on which the BSA can be interchanged by cell adhesion-mediating proteins (Arima and Iwata 2007).

6 Conclusion

This work is focused mainly on the construction of a pre-vascularized skin substitute. In the first part, we showed that the cell colonization of the nanofibrous membranes (PLA, PLLA, PLGA) was accelerated by the protein nanocoatings. The proliferation of NHDFs and ADSCs was enhanced by the fibrin nanocoatings, while HaCaT keratinocytes grew better on the collagen nanocoatings. Moreover, we showed that the cell behavior depended also on the structure of the fibrin nanocoatings. The fibrin nanocoatings in the form of the fibrin mesh was selected as the most appropriate modification of nanofibrous membranes for creation of the bi-layered skin construct. In the second part, we proved that the collagen in the form of a hydrogel

with optimal mechanical properties attracted the NHDFs and ADSCs which grew on the fibrin-coated membrane. The collagen hydrogel also provided the keratinocytes an appropriate surface for their growth and stratification. In the third part, we demonstrated that the ADSCs migrated from the membrane into the collagen hydrogel. These cells were able to interact with embedded HUVECs that formed tubular structures in several layers of the collagen hydrogel. This layer, which can be referred to as dermal equivalent, was simultaneously seeded by keratinocytes that were able to create several layers of differentiated cells, which can be referred to epidermal equivalent.

The final part of this work was focused on the development of the temporary cellulose-based wound dressings. In this part, we found out that both fibrin and charged CNFs improved the cell adhesion on the cellulose mesh. We additionally observed that the negatively-charged aCNFs accelerated the cell adhesion and growth, while positively-charged cCNFs rather decreased it. However, it depended on the cell type and on the spectrum of cell adhesion-modulating proteins, adsorbed on the surface of different CNF coatings.

7 Summary

The creation of a bi-layered pre-vascularized skin constructs as well as the development of nature-derived wound dressings were the main areas of our focus. Partial results were written in seven impacted publications and in one prepared manuscript.

Firstly, we developed single-layered skin substitutes based on protein-coated biodegradable nanofibrous membranes. We demonstrated that the adhesion and growth of NHDFs and ADSCs were accelerated using fibrin nanocoatings, while the collagen nanocoating had a positive effect on keratinocyte behavior. These observations were further utilized for the preparation of the bi-layered skin constructs where the NHDFs or ADSCs migrated from a fibrin-coated nanofibrous membrane into the collagen hydrogel and hKs were seeded on the surface of the collagen. Moreover, we optimized the structure of the fibrin coating in order to improve the cell adhesion and growth. For vascularization of the bi-layered skin construct, we combined ADSCs and HUVECs. The HUVECs embedded in the collagen hydrogel were able to form a capillary-like network in the presence of the ADSCs that migrated from the nanofibrous membrane. This part of the construct can be considered as the dermal part, while the differentiating and stratifying keratinocytes on the surface of the collagen hydrogel represent the epidermal part.

Secondly, we contributed to the research of cellulose-based wound dressings. The commercially available cellulose mesh was coated by two types of fibrin nanocoatings or by CNFs. We confirmed that the fibrin nanocoatings increased the cell growth on the cellulose meshes. In order to create fully plant-based material, we coated the cellulose meshes by CNFs that are stable due to absence of cellulase enzyme expression in human cells. We observed that the aCNFs accelerated the colonization of the cellulose mesh by the cells, while cCNFs rather decreased it. However, this was strongly dependent on the cell types and the cell culture conditions.

8 Souhrn

Naším hlavním záměrem bylo zhotovení dvouvrstvé prevaskularizované kožní náhrady a vývoj kožního krytu z přírodního materiálu. Jednotlivé výsledky byly sepsané do sedmi impaktovaných publikací a do jedné připravované publikace.

Jako první jsme vytvořili jednovrstvou kožní náhradu z biodegradabilní nanovlákenné membrány potažené proteiny. Ukázali jsme, že fibrinová nanovrstva zlepšuje adhezi a růst lidských fibroblastů a kmenových buněk z tukové tkáně, zatímco kolagenní nanovrstva má pozitivní vliv spíše na keratinocyty. Tyto poznatky jsme využili pro konstrukci dvouvrstvé kožní náhrady. Fibroblasty a kmenové buňky z tukové tkáně migrovaly z nanovláken potažených fibrinem do kolagenového gelu, který byl následně osazen keratinocyty. Navíc jsme také vylepšili strukturu fibrinové nanovrstvy za účelem zrychlení adheze a růstu buněk. Za účelem prevaskularizace dvouvrstvého konstruktu jsme zkombinovali kmenové buňky z tukové tkáně s endotelovými buňkami. Endotelové buňky zalité v kolagenovém gelu v přítomnosti kmenových buněk z tukové tkáně formovaly útvary podobné kapilární síti. Tato část konstruktu byla vytvořena za účelem napodobení dermis, zatímco diferencující a stratifikující keratinocyty byly kultivovány na povrchu kolagenového hydrogelu za účelem vytvoření epidermis.

Ve druhé části jsme přispěli k výzkumu kožních krytů vytvořených z celulózy. Komerčně dostupnou celulózovou textilii jsme potáhli dvěma typy fibrinových nanovrstev nebo celulózovými nanovlákny (CNFs). Potvrdili jsme, že fibrinové nanovrstvy zlepšuji růst buněk na celulózových textiliích. Avšak pro vytvoření materiálů cistě rostlinného původu jsme celulózovou textilii potáhli celulózovými nanovlákny, které jsou stabilní díky absenci exprese enzymů celuláz v lidských buňkách. V této prací jsme také pozorovali, že vrstva anionických CNFs zrychluje kolonizaci celulózové textilie buňkami, zatímco vrstva kationických CNFs to spíš snižuje. Výsledky však závisely na typu buněk a podmínkách buněčné kultivace.

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10 Publications

Publications Related to Dissertation Thesis:

Publications with impact factor (IF):

Bacakova M, Pajorova J, Stranska D, Hadraba D, Lopot F, Riedel T, Brynda E, Zaloudkova M, Bacakova L. Protein nanocoatings on synthetic polymeric nanofibrous membranes designed as carriers for skin cells. *Int J Nanomedicine* **2017**; 12:1143-1160. **IF = 6.4 (2020)**

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Publications Non-Related to Dissertation Thesis:

Publications with impact factor (IF):

Przekora A, Vandrovcova M, Travnickova M, Pajorova J, Molitor M, Ginalska G, Bacakova L. Evaluation of the potential of chitosan/β-1,3-glucan/hydroxyapatite material as a scaffold for living bone graft production *in vitro* by comparison of ADSC and BMDSC behaviour on its surface. *Biomed Mater* **2017**; 12(1):015030. **IF = 3.715 (2020)**

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11 Conferences

International Conferences:

Pajorova J *et al.*: Nanofibrous Polymer Membranes Modified with Fibrin and Collagen Structures as Carriers for Skin Cells. *4th International Conference on Tissue Science and Regenerative Medicine*, Rome, Italy, 27 – 29 July 2015. Poster presentation.

Pajorova J *et al.*: The potential application of fibrin-coated polylactide nanofibers in skin tissue engineering. *NanoInBio*, Guadeloupe, France, 31 May – 5 June 2016. Poster presentation.

Pajorova J *et al.*: Effect of fibrin-nanocoating of nanofibrous polymer membranes on the adhesion and proliferation of human dermal fibroblasts. *Conference on Biomaterials in Medicine and Veterinary Medicine*, Rytro, Poland, 13 – 16 October 2016. Poster and oral presentation.

Pajorova J *et al.*: Fibrin-coated Nanofibrous Polymer Membranes as Carriers for Skin Cells. *International Conference on Mechanics of Biomaterials and Tissues*, Waikoloa, Hawaii, USA, 10 – 14 December 2017. Poster presentation.

Pajorova J *et al.*: Morphology of a fibrin nanocoating influences dermal cell behavior. *Summer School GENE2SKIN*, Porto, Portugal, 6 – 8 June 2018. Poster presentation.

Pajorova J *et al.*: A full-thickness skin construct made of a collagen hydrogel strengthened by a fibrin-modified nanofibrous membrane. *18th European Burns Association Congress*, Helsinki, Finland, 4 – 7 September 2019. Poster presentation. **The best poster presentation award.*

Domestic Conferences:

Pajorova J *et al.*: Nanofibrous polymer membranes modified with fibrin and collagen structures as carriers for skin cells. *PhD meeting in trest 2015*, Třešť, Czech Republic, 3 – 5 November 2015. Poster presentation. **The best poster presentation award.*

Bacakova L, Bacakova M, Pajorova J *et al.*: Skin Substitutes - Current state and future trends. *IX. Biomaterials and their surfaces*, Herbertov, Czech Republic, 20 – 23 September 2016. Oral presentation.

Pajorova J *et al.*: Potential applications of fibrin-modified nanofibrous membrane and adipose tissue-derived stem cells (ASCs) in skin tissue engineering. *V. International Conference – Stem*

Cells and Cell Therapy: From research to modern clinical application, Černá Hora, Czech Republic, 5 – 6 October 2017. Oral presentation.

Pajorova *et al.*: Morphology of a fibrin nanocoating influences dermal cell behavior. Scientific conference 2.LF UK 2018, Praha, Czech Republic, 25 – 26 April 2018. Poster presentation. **The best poster presentation award.*

Pajorova J *et al.*: Nanocellulose in skin tissue engineering. *XI. Biomaterials and their surfaces*, Herbertov, Czech Republic, 18 – 21 September 2018. Oral presentation.

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12 Internship

November 2017, June and September 2019: Faculty of Biomedical Sciences and Engineering, Micro and Nanosystems Research Group, Tampere University, Finland, Prof. Pasi Kallio, Funding: Institute of Physiology CAS (Development of HR capabilities, internationalization, popularization and IP utilization) and Charles University (Mobility).