Skin tissue engineering and wound healing

Skin tissue engineering and wound healing are other important areas of research for our lab. We used both natural and synthetic polymers as substrates for skin and stem cell growth. An important natural polymer, **nanocellulose**, **either of bacterial or plant origin**, was obtained in collaboration with the University of Chemistry and Technology in Prague (Prof. Václav Švorčík), Central European Institute of Technology (CEITEC) in Brno (Assoc. Prof. Lucy Vojtová), and the University of Tampere, Finland (Prof. Pasi Kallio).

Cellulose is the most abundant polymer on Earth, produced by biological synthesis, i.e., green technology, and there is therefore considerable interest in its widest possible use in various technologies, including regenerative medicine. However, as a linear glucose polymer, cellulose does not contain specific binding sites for cell adhesion receptors in its molecules. It is also not degradable in the human body, which is problematic for modern tissue engineering. Therefore, it requires further modification, such as modification with plasma (**Fig. 1**), conversion to water-soluble carboxymethylcellulose or oxidized cellulose, mixing with animal extracellular matrix proteins such as collagen (**Fig. 2**), mixing with or exposure to cellulase enzymes (**Fig. 3**), all of which have been achieved within the above-mentioned collaborations.

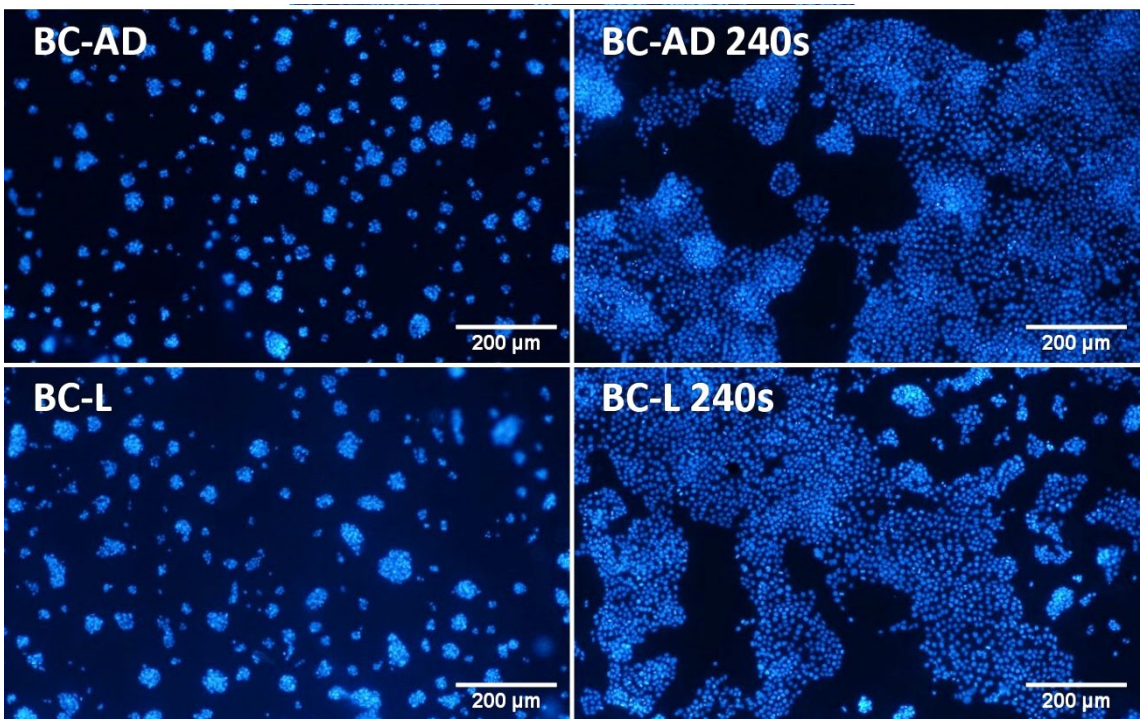

***Figure 1**. Human HaCaT keratinocytes on day 4 after seeding on bacterial nanocellulose, a promising material for "smart" wound dressings, i.e., dressings that deliver drugs and cells for active wound healing. **Left**: Small islands of keratinocytes on bacterial nanocellulose dried by air (BC-AD) or lyophilization (BC-L). **Right**: Formation of continuous areas covered with keratinocytes on BC-AD and BC-L modified with argon plasma for 240 s (**collaboration with the University of Chemistry and Technology, Prof. Václav Švorčík, Dr. Anna Kutová**).*

This research has resulted in the following publications:

*Kutová A, Staňková L, Vejvodová K, Kvítek O, Vokatá B, Fajstavr D, Kolská Z, Brož A, Bačáková L, Švorčík V. Influence of Drying Method and Argon Plasma Modification of Bacterial Nanocellulose on Keratinocyte Adhesion and Growth. **Nanomaterials** (Basel). 2021;11(8):1916. doi: 10.3390/nano11081916.*

*Staňková, L'., Kutová, A., Doubková, M., Kvítek, O., Vokatá, B., Sedlář, A., Idriss, H., Slepička, P., Švorčík, V., Bačáková, L. Argon plasma-modified bacterial nanocellulose: Cell-specific differences in the interaction with fibroblasts and endothelial cells. **Carbohydrate Polymer Technologies and Applications**. 2024, 7, 100470. doi: 10.1016/j.carpta.2024.100470.*

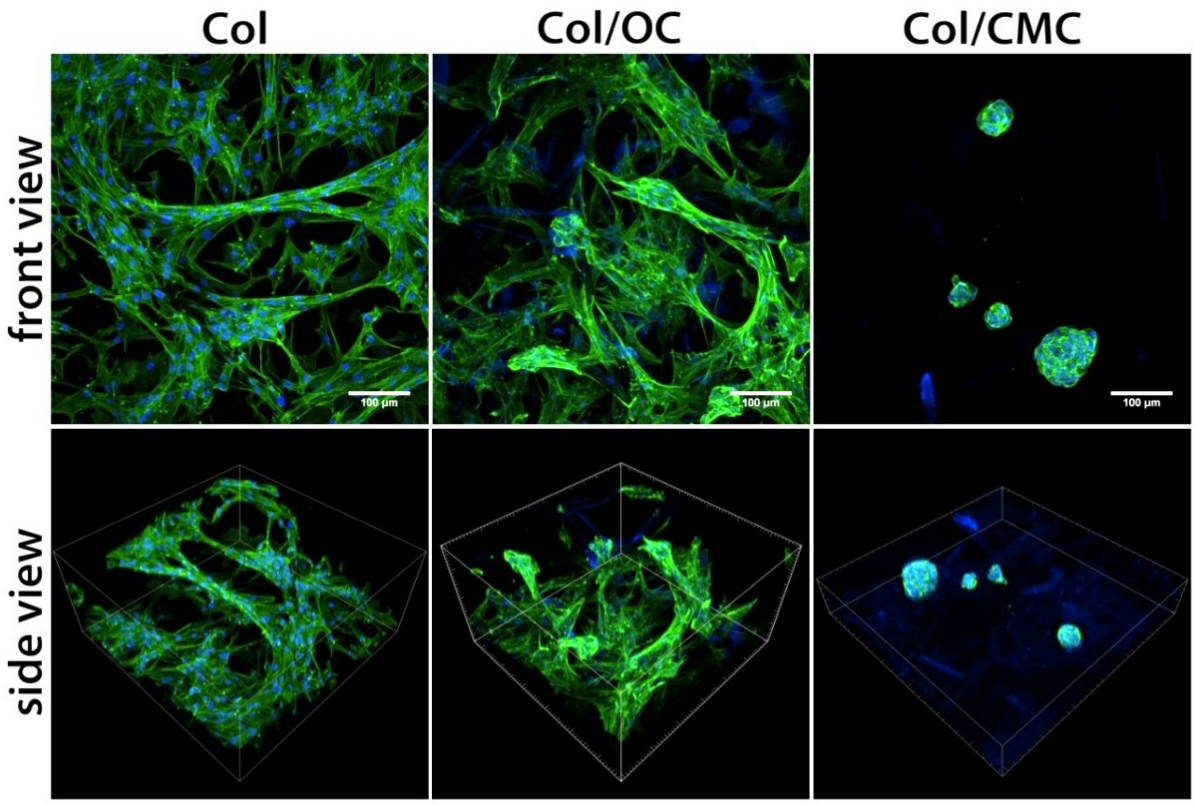

***Figure 2**. The morphology of human subcutaneous adipose tissue-derived stem cells on day 14 after seeding. Upper row: Maximal projection (front view). Lower row: 3D projection (side view). The tested materials were as follows: collagen (Col), collagen/oxycellulose (Col/OC), and collagen/carboxymethyl cellulose (Col/CMC). The F-actin of the cells is visualized with phalloidin-Atto488 (green) and the cell nuclei are counterstained with DAPI (blue). The photographs were taken using a Dragonfly 503 scanning disk confocal microscope with a Zyla 4.2 PLUS sCMOS camera and an objective lens of HC PL APO 20x/0.75 IMM CORR CS2.*

It is clear from **Fig. 2** that cell colonization on Col/CMC scaffolds is much lower than on Col/OC and pure collagen (Col) scaffolds. This is because Col/CMC has a higher swelling capacity, which provides less support for cell adhesion and growth. The cells compensate for the weaker cell-material adhesion by increasing cell-cell adhesion, leading to the formation of spheroid-like structures. Therefore, Col/OC offers a more stable scaffold suitable for skin tissue engineering, while Col/CMC is better for moist wound healing, such as in a mucoadhesive gel, where reducing cell adhesion is advantageous. (**Collaboration with Assoc. Prof. Lucy Vojtová, CEITEC, Brno**).

This research has resulted in the following publication:

*Kacvinská K, Trávníčková M, Vojtová L, Poláček P, Dorazilová J, Kohoutek M, Hlináková K, Pajorová J, Tirpáková M, Bačáková L. Porous cellulose-collagen scaffolds for soft tissue regeneration: influence of cellulose derivatives on mechanical properties and compatibility with adipose-derived stem cells. **Cellulose** 2022; 29:8329–8351. doi: 10.1007/s10570-022-04759-4.*

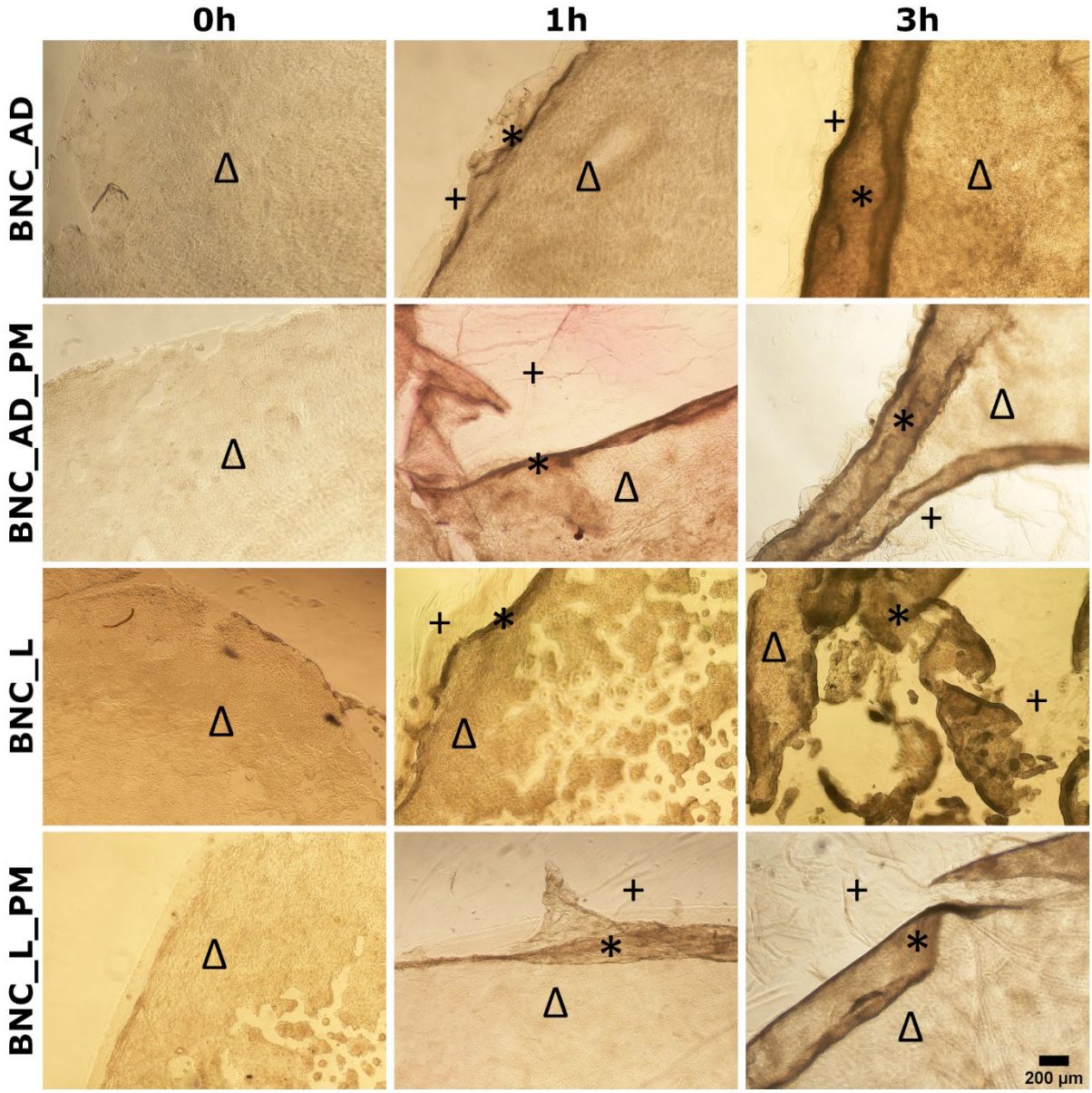

Figure 3. *Human HaCaT keratinocytes on day 10 after seeding on BNC samples (AD: air-dried, AD_PM: air-dried plasma-modified, L: lyophilized, L_PM: lyophilized plasma-modified) before (0 h) and after 1 h and 3h exposure to cellulase to cellulase from Trichoderma reesei ATCC 26921 (Sigma Aldrich, Cat. No. C8546), at a concentration of 37.5 units/ml in phosphate-buffered saline (PBS), pH 6.5, 37°C,* humidified air atmosphere containing 5% of CO_2.

Δ*: cell population layer;* *******: start of separation of self-standing cell sheets, which could be used for skin tissue engineering or to support wound healing;* ***+****: degrading BNC.*

*Olympus IX51 microscope, DP70 digital camera, objective 4×, scale bar 200 µm. (****Collaboration with the University of Chemistry and Technology, Prof. Václav Švorčík, Dr. Anna Kutová****)*

This research has resulted in the following publication:

Staňková L', Kutová A, Doubková M, Kvítek O, Sedlář A, Švorčík V, Bačáková L: Cellulases and their promising use in tissue engineering and cell sheet technology. ***Materials and Design****. 254, June 2025, 114111. doi: 10.1016/j.matdes.2025.114111.*

An interesting regulator of cell adhesion and growth on nanocellulose is its electrical charge. We found that a positive electrical charge tends to repel cells, forcing them to compensate for the cell-matrix adhesion deficit by cell-cell adhesion, resulting in the formation of cell spheroids that can be further developed into organoids. On the other hand, a negative charge promotes cell adhesion and growth and the formation of confluent cell layers. Under the action of external cellulases, the material can then be processed into self-standing, scaffold-free, continuous cell sheets for use in skin tissue engineering and wound healing (**Fig. 4**).

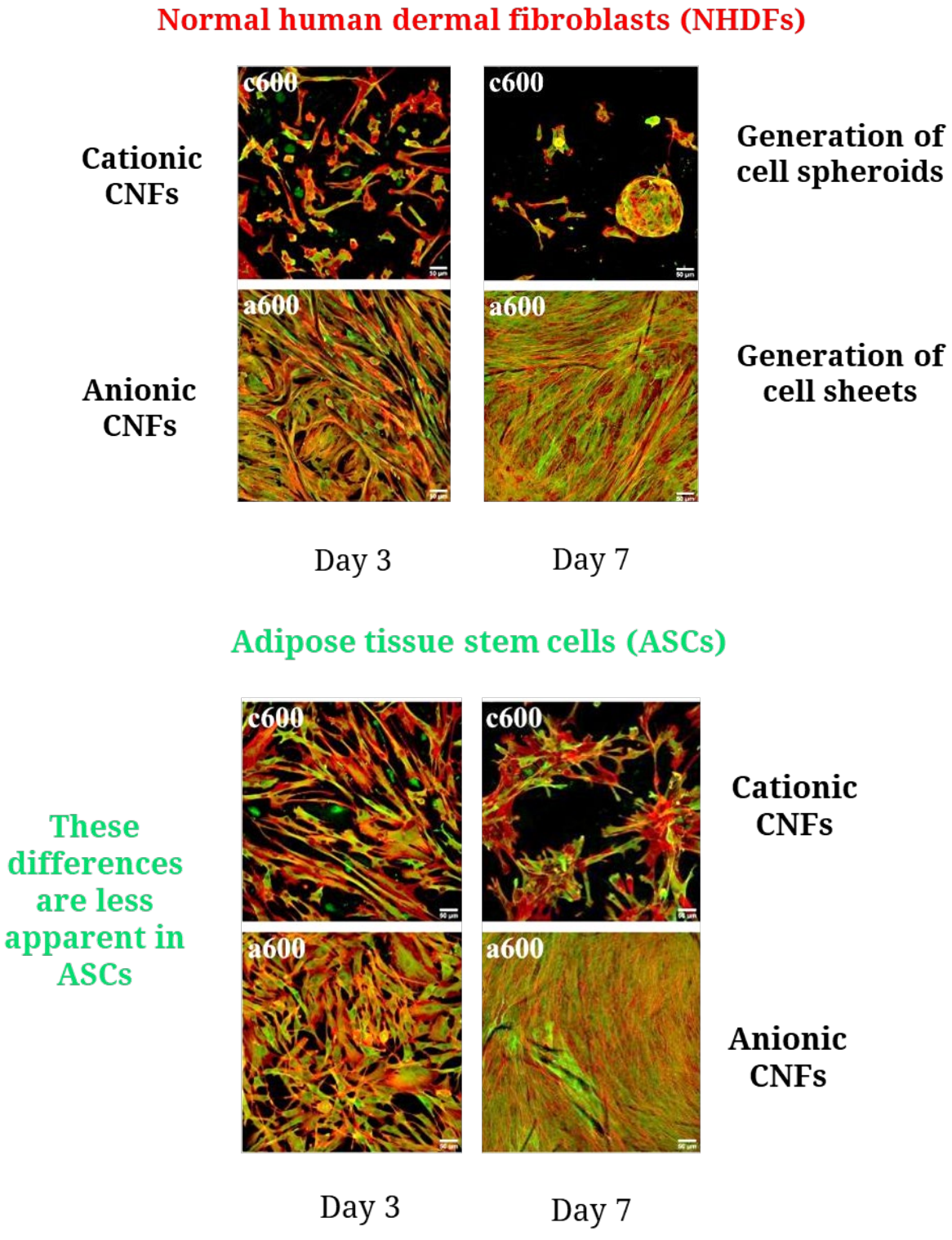

Figure 4. *The behavior of normal human dermal fibroblasts (NHDFs) and subcutaneous adipose tissue-derived mesenchymal stem cells (ASCs) on positively charged (cationic) and negatively charged (anionic) nanocellulose (**Collaboration with Prof. Pasi Kallio, Dr. Anne Skogberg, Tampere University, Finland**)*

This research has resulted in the following publications:

*Pajorova J, Skogberg A, Hadraba D, Broz A, Travnickova M, Zikmundova M, Honkanen M, Hannula M, Lahtinen P, Tomkova M, Bacakova L, 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. doi: 10.1021/acs.biomac.0c01097.*

- *this article won the 2020 competition for the best publication in a prestigious impact journal affiliated with the IPHYS, where the corresponding author is from the IPHYS – age under 35: https://fgu.cas.cz/en/research-and-laboratories/research/competition-for-the-best-iphys-publication/*

Bacakova L, Pajorova J, Tomkova M, Matejka R, Broz A, Stepanovska J, Prazak S, Skogberg A, Siljander S, Kallio P. Applications of Nanocellulose/Nanocarbon Composites: Focus on Biotechnology and Medicine. ***Nanomaterials*** *(Basel). 2020;10(2):196. doi: 10.3390/nano10020196.*

Bacakova L, Pajorova J, Bacakova M, Skogberg A, Kallio P, Kolarova K, Svorcik V. Versatile Application of Nanocellulose: From Industry to Skin Tissue Engineering and Wound Healing. ***Nanomaterials*** *(Basel). 2019;9(2). doi: 10.3390/nano9020164.*

Nanocellulose can also be used as a carrier for biologically active molecules (e.g. curcumin) for controlled drug delivery into wounds (**Fig. 5**).

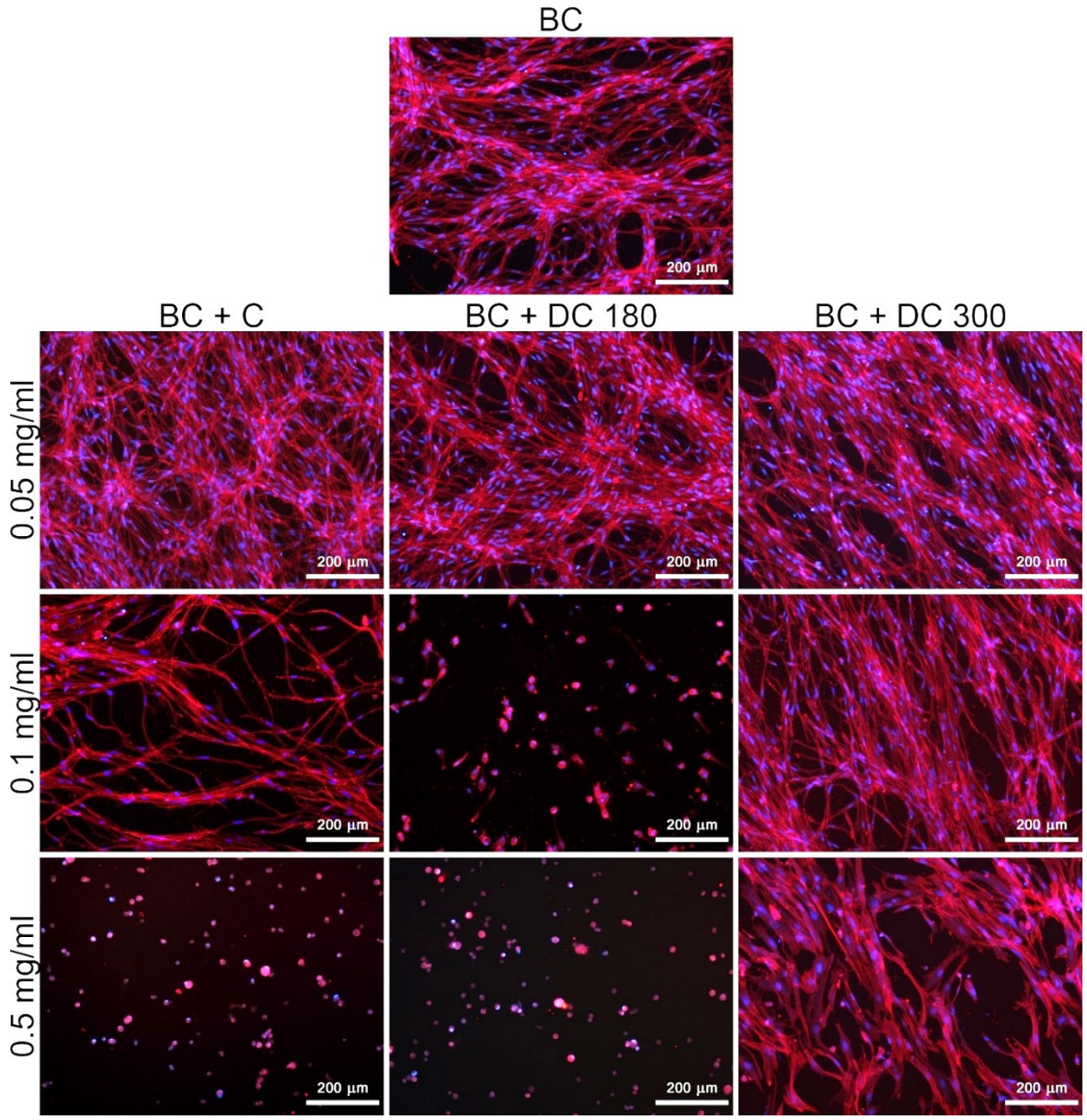

Figure 5*. Morphology of normal human dermal fibroblasts (NHDFs) on pristine bacterial nanocellulose (BC), and on nanocellulose loaded with pure curcumin (BC + C), or with curcumin degraded at 180°C (BC + DC 180) or at 300°C (BC + DC 300) at various concentrations (0.05, 0.1 and 0.5 mg/ml) on day 7 after cell seeding. The cells were stained with phalloidin-TRITC (red; F-actin cytoskeleton) and with DAPI (blue; cell nuclei). Olympus IX 51 microscope, obj. 10×, DP 70 digital camera.*

It is clear from **Fig. 5** that unmodified curcumin and curcumin degraded at 180°C inhibit the colonization of BC with NHDFs, while curcumin degraded at 300°C permits cell colonization. At the same time, curcumin degraded at 300°C retains some antibacterial activity (**Fig. 6**).

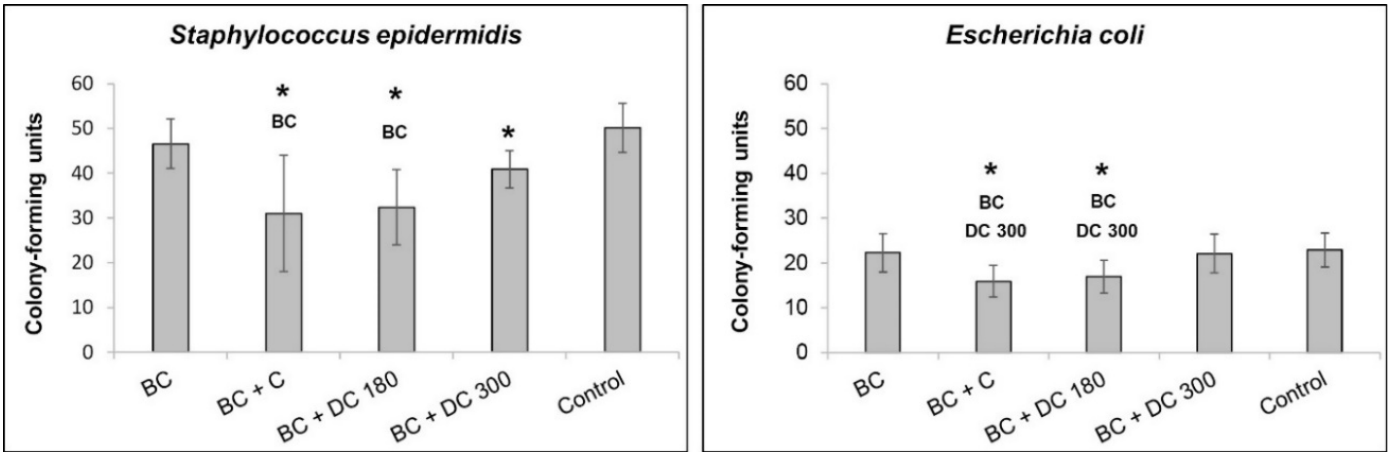

Figure 6. *Antimicrobial activity of pristine bacterial nanocellulose (BC) and nanocellulose loaded with curcumin (BC + C), or with curcumin degraded at 180°C (BC + DC 180) or at 300°C (BC + DC 300) at a concentration of 0.5 mg/ml. The pure medium with bacteria was used as the control sample. The antimicrobial activity is presented for S. epidermidis and for E. coli. Arithmetic mean ± SD from 12 – 16 measurements for each experimental group. Nonparametric Kruskal-Wallis One Way Analysis of Variance on Ranks, Dunn's Method, statistical significance (p≤0.05; depicted above the columns): *, **BC** and **DC 300** compared with bacteria cultivated in the control medium, in the presence of BC and BC + DC 300, respectively. (**Collaboration with the University of Chemistry and Technology, Prof. Václav Švorčík)***

This research has resulted in the following publication:

Zikmundova M, Vereshaka M, Kolarova K, Pajorova J, Svorcik V, Bacakova L. Effects of bacterial nanocellulose loaded with curcumin and its degradation products on human dermal fibroblasts. ***Materials*** *(Basel). 2020;13(21):4759. doi: 10.3390/ma13214759.*

Another important material for the creation of so-called "**smart" wound dressings**, i.e. for the delivery of drugs, biomolecules and even cells into wounds in our studies, were **polymeric nanofibrous meshes**, made of **synthetic polymers,** such as polycaprolactone (PCL) and polyvinyl alcohol (PVA), in cooperation with the **Technical University of Liberec** (Dr. Petr. Mikeš, Dr. Eva Kuželová Košťáková, Dr. Věra Jenčová) or **natural polymers**, such as chitosan combined with gelatin and elastin (in cooperation with the **University of Memphis, Memphis, USA**; Prof. Joel D. Bumgardner). The synthetic polymeric nanofibrous meshes were loaded, either directly during electrospinning, or indirectly through fibrin-based coatings (in collaboration with the Institute of Macromolecular Chemistry CAS; Dr. Tomáš Riedel), with growth and angiogenic factors, mainly in the form of human platelet lysate (**Figures 7 and 8**).

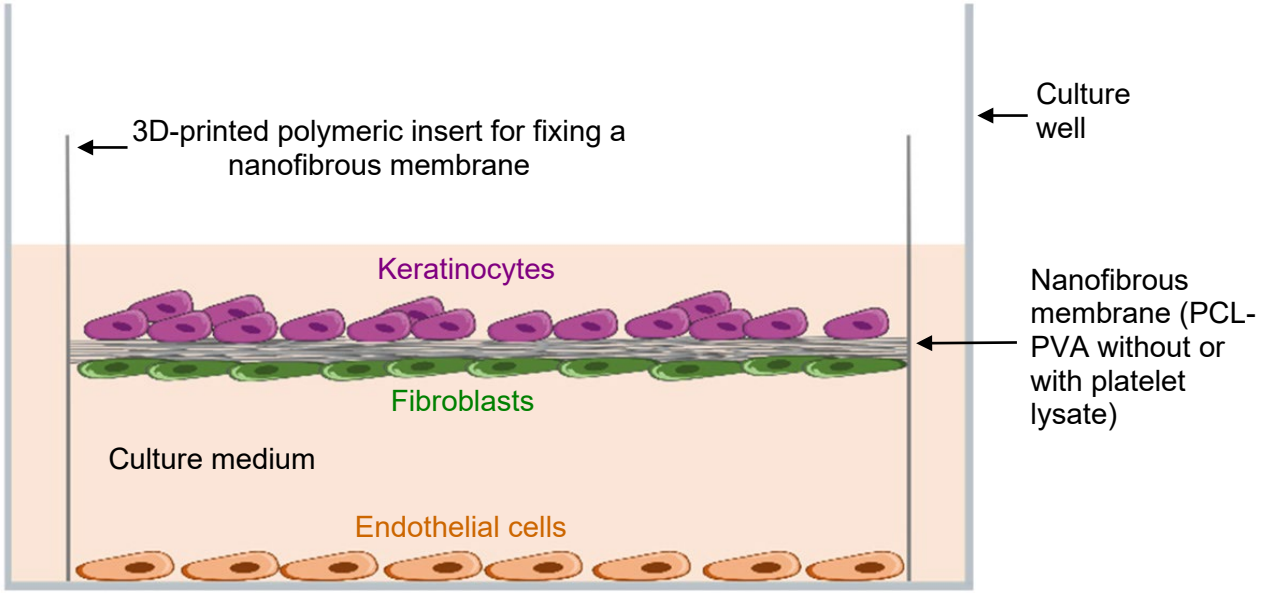

Figure 7*. A co-culture system for the three basic cell types found in skin, namely keratinocytes, fibroblasts, and endothelial cells.*

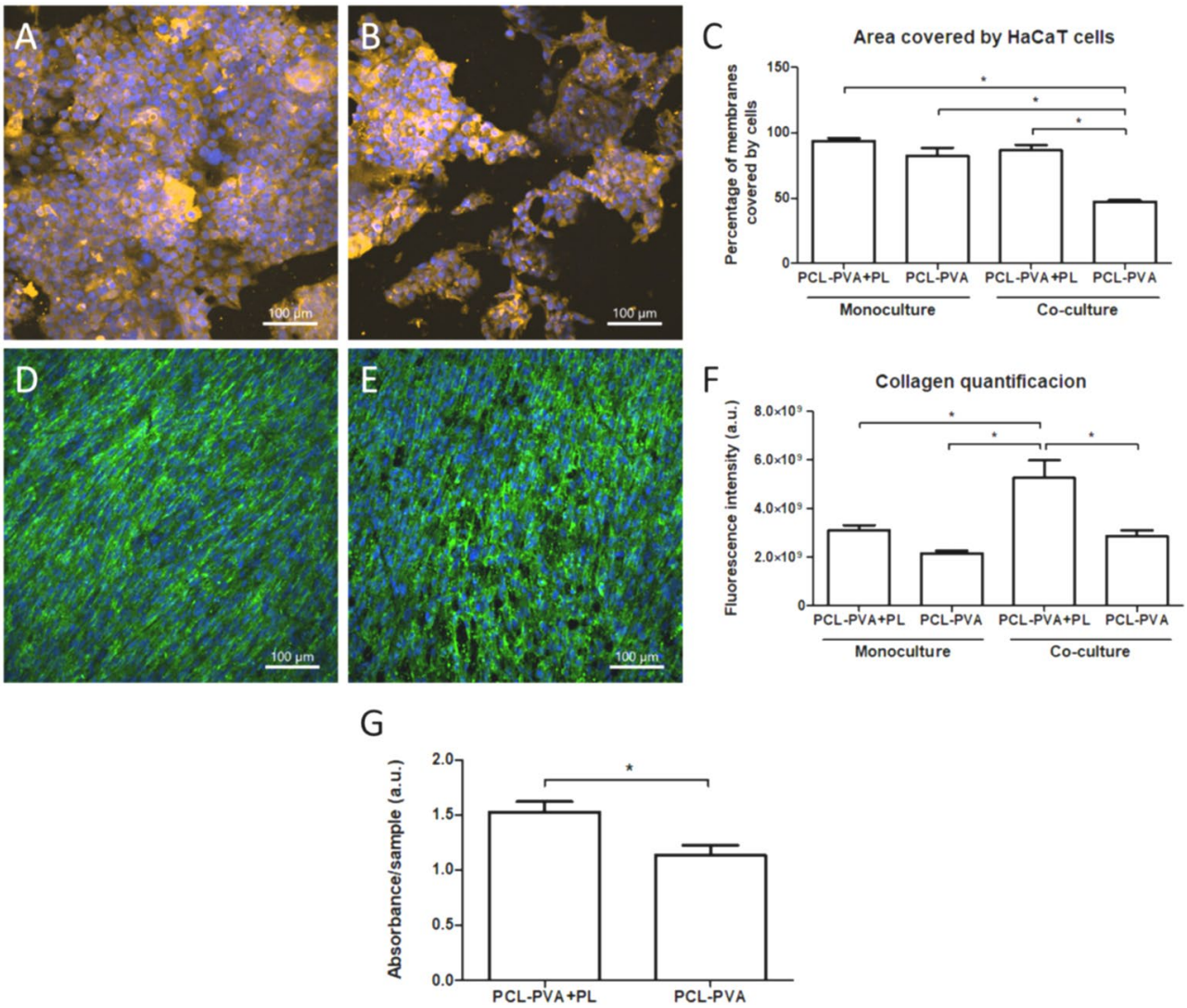

***Figure 8**. Human epidermal keratinocytes (**A, B**) and dermal fibroblasts (**D, E**) in cocultures on nanofibrous membranes made of a mixture of polycaprolactone (PCL) and polyvinyl alcohol (PVA), either containing platelet lysate (PL) (**A, D**) or without PL (**B, E**). The cells were further co-cultured with human endothelial cells, which were seeded at the bottom of the culture chamber. It is clear that both the presence of platelet lysate and co-culture with endothelial cells promote the formation of a continuous layer of keratinocytes (**C**), collagen production by fibroblasts (**F**), and the metabolic activity of both cell types (**G**).*

This research, which also included another foreign collaboration with the Universitat Autonoma de Barcelona, Spain (Dr. Andreu Blanquer, a former member of our lab), resulted in the following publications:

*Blanquer, A., Kuželová Košťáková, E., Filová, E., Lisnenko, M., Brož, A., Müllerová, J., Novotný, V., Havlíčková, K., Jakubková, Š., Hauzerová, Š., Heczková, B., Procházková, R., Bačáková, L., Jenčová, V. A novel bifunctional multilayered nanofibrous membrane combining polycaprolactone and poly (vinyl alcohol) enriched with platelet lysate for skin wound healing. **Nanoscale**. 2024, 16(4), 1924-1941. doi: 10.1039/d3nr04705a.*

Táborská J, Blanquer A, Brynda E, Filová E, Stiborová L, Jenčová V, Havlíčková K, Riedelová Z, Riedel T. PLCL/PCL Dressings with Platelet Lysate and Growth Factors Embedded in Fibrin for Chronic Wound Regeneration. Int J Nanomedicine. 2023;18:595-610. doi: 10.2147/IJN.S393890.

*Blanquer A, Musilkova J, Filova E, Taborska J, Brynda E, Riedel T, Klapstova A, Jencova V, Mullerova J, Kostakova EK, Prochazkova R, Bacakova L. The Effect of a Polyester Nanofibrous Membrane with a Fibrin-Platelet Lysate Coating on Keratinocytes and Endothelial Cells in a Co-Culture System. **Nanomaterials** (Basel). 2021, 11, 457. doi: 10.3390/nano11020457.*

Filova E, Blanquer A, Knitlova J, Plencner M, Jencova V, Koprivova B, Lisnenko M, Kostakova EK, Prochazkova R, Bacakova L. The Effect of the Controlled Release of Platelet Lysate from PVA Nanomats on Keratinocytes, Endothelial Cells and Fibroblasts. ***Nanomaterials*** *(Basel). 2021;11(4):995. doi: 10.3390/nano11040995.*

Mikes P, Brož A, Sinica A, Asatiani N, Bacakova L. In vitro and in vivo testing of nanofibrous membranes doped with alaptide and L-arginine for wound treatment. ***Biomed Mater.*** *2020;15(6):065023. doi: 10.1088/1748-605X/ab950f.*

Bryan A, Wales E, Vedante S, Blanquer A, Neupane D, Mishra S, Bačáková L, Fujiwara T, Jennings JA, Bumgardner JD. Evaluation of Magnesium-Phosphate Particle Incorporation into Co-Electrospun Chitosan-Elastin Membranes for Skin Wound Healing. ***Marine Drugs****. 2022;20(10):615. doi: 10.3390/md20100615.*

In collaboration with the **University of Memphis, Memphis, USA**; a chitosan-based matrix was printed together with cells on an Allevi bioprinter in our lab (**Fig. 9**):

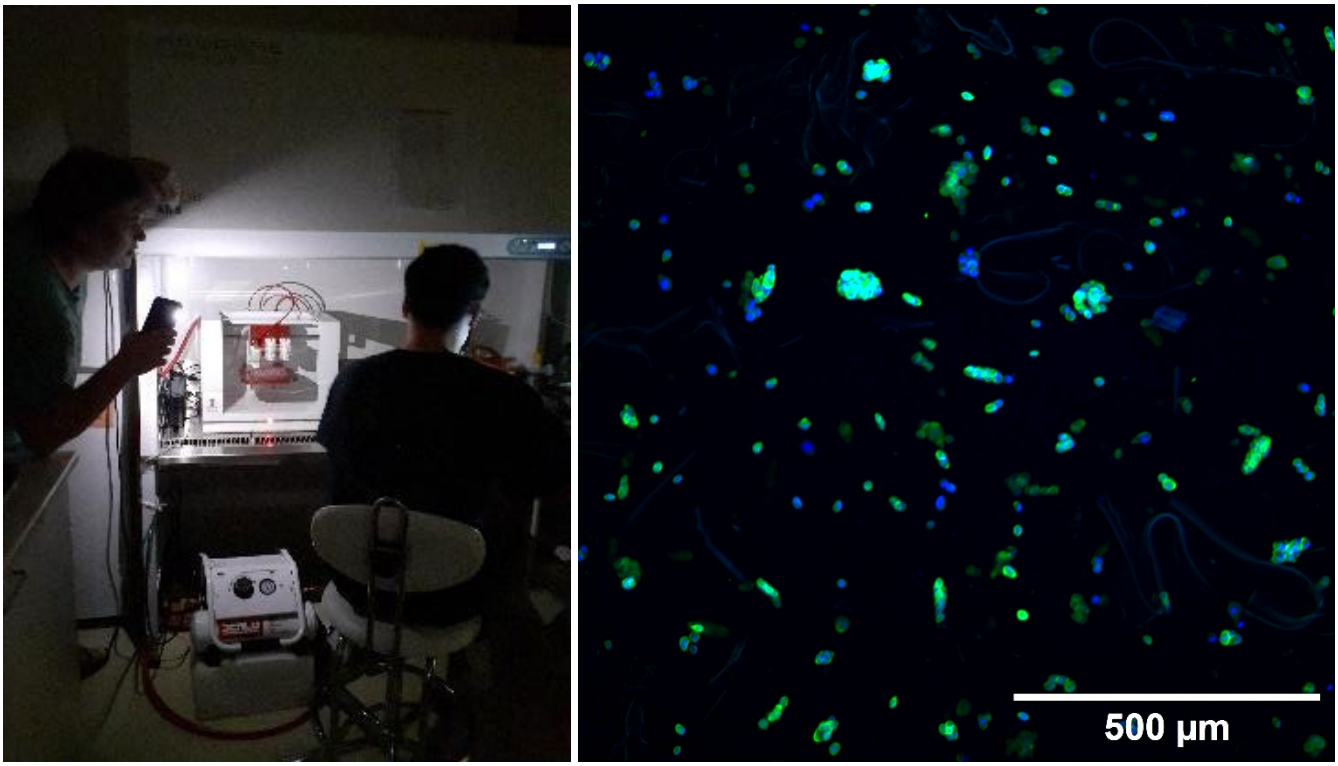

Figure 9*. **A:** 3D bioprinting of a chitosan-based matrix with skin fibroblasts during Prof. Bumgardner's visit from the UoM with his students, Alex Bryan and Nikken Malboeuf. N. Malboeuf is working together with Antonin Brož, a member of our team.* ***B:*** *Microscopic image of human dermal fibroblasts cultured in chitosan hydrogel, printed together with cells and cross-linked with UV light (cell nuclei stained with DAPI – blue, actin cytoskeleton – green).*

The following grant projects supported skin tissue engineering and wound healing in our laboratory in 2020-2024:

1. Czech Health Research Council (AZV), Ministry of Health of the Czech Republic, No. NV18-01-00332, "Treatment of diabetic wounds using nanofibrous dressings releasing platelet lysate components", duration 2018-2021, Principal Investigator: Elena Filová, Institute of Physiology of the Czech Acad. Sci., Pavel Klein, Faculty of Medicine in Pilsen, Charles University, Věra Jenčová, Technical University Liberec, Eduard Brynda, Institute of Macromolecular Chemistry.

2. Czech Science Foundation (GAČR), No. 20-01641S, "Degradable nanocellulose as a novel temporary scaffold for tissue engineering", duration 2020-2022, Principal Investigator: Lucie Bačáková, Institute of

Physiology of the Czech Acad. Sci., Co-Investigator: Václav Švorčík, University of Chemistry and Technology in Prague.

3. Technology Agency of the Czech Republic (TAČR), Programme DELTA 2, No. TM01000046, “Modular 3D bioprinting system for printing biocompatible hydrogel and polymer scaffolds for tissue engineering”, duration 2020-2022, Principal Investigator: Serhiy Forostyak, PrimeCell Bioscience, a.s., Co-Investigator: Roman Matějka, Institute of Physiology of the Czech Acad. Sci.; Taiwanese partner: Amber Shen, Life Star International Limited.

4. Mobility Plus, No. UofM-20-01, “Novel chitosan-based angiogenic nanofibrous scaffolds for skin tissue engineering”, duration 2020-2022. Czech partner: Lucie Bačáková, Institute of Physiology of the Czech Acad. Sci., American Partner: Prof. Joel D. Bumgardner, University of Memphis, Memphis, Tennessee, USA

5. Ministry of Education, Youth and Sports of the Czech Republic, co-funded by the European Union, OP JAC programme, project No. No. CZ.02.01.01/00/22_008/0004562 “Excellence in Regererative Medicine” (ExRegMed), duration 2023-2028. Principal Investigator: Pavla Jendelová, Institute of Experimental Medicine of the Czech Acad. Sci.; Elena Filová, Institute of Physiology of the Czech Acad. Sci.; leader of WP6 – Skin Tissue Engineering and Wound Healing.

6. Project CarDia, National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union - Next Generation EU. Principal Investigator: Institute of Clinical and Experimental Medicine (IKEM), duration 2023-2025. Co-Investigator: Institute of Physiology of the Czech Acad. Sci. (members of the laboratory of Biomaterials and Tissue engineering participated as professional colleagues; Elena Filová: Activity 3.3.: Wound healing using "smart" nanofibrous covers releasing platelet lysate components and serving as carriers for delivery of autologous adipose tissue stem cells into wounds.