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Chapter 6. STUDY OF THE ANGIOGENESIS PROCESS ON THE SURFACE OF BIODEGRADABLE POLYESTERS BASED ON ISOSORBIDE

6.1. Introduction

The main challenge of tissue engineering is to allow for a full restoration or repair of a damaged tissue, using a combination of chemistry, materials engineering, and molecular biology [1]. Design of multifunctional tissue scaffolds is an essential aspect of progress in tissue engineering, as a variety of health conditions requires the use of implantable biomaterials. Tissue scaffolds can be used to rebuilt body functions in different locations, e.g., skin scaffolds can facilitate wound healing, and bone scaffolds can improve bone tissue regeneration. Apart from a specialistic function, every tissue scaffold needs to enable the regenerating tissue to function properly, including its proper nutrition, for which the formation of blood vessels is necessary [2]. Therefore, the design of tissue scaffolds should take into consideration their ability to affect the formation of blood vessels. A major challenge of tissue engineering is to create a scaffold that will be

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penetrable by the blood vessels [3]. This is why it is crucial that tissue scaffolds exhibit a porous structure, and a large surface area per scaffold volume. This allows blood vessels to passing through, to provide the transportation of cells, as well as nutrients [4].

The selection of appropriate biomaterials plays an important role in the process of developing tissue scaffolds. Of particular interest are biodegradable polymers, such as polylactide (PLA), polyglycolide (PGA), poly(lactide-*co*-glycolide) copolymer (PLGA), polycaprolactone (PCL), as well as bioactive ceramics, such as calcium triphosphate (TCP) [5]. Biomaterials can be processed with the use of different techniques to form a porous structure, that will promote angiogenesis. One of these techniques is electro-spinning, which helps to fabricate micro- and nano-fibers. This process involves pushing the polymer through an electrostatic field, which leads to the formation of a flux, which then breaks down to form fibers whose diameters can be adjusted [1]. The use of nano-fibers enables the formation of 'nano-scaffolds' that are not only lighter, but also more mechanically robust [6]. Other common methods of producing porous scaffolds include thermally induced phase separation (TIPS), or phase inversion in the solvent-insolvent system using supercritical CO_2 [7]. Phase separation methods are very sensitive to many parameters (solution concentration, polymer molecular weight, etc.), which makes it difficult to obtain a repeatable microstructure of the material [4]. Observations using a confocal fluorescence microscope are used to confirm whether cells are able to penetrate such materials. Using a confocal microscope, it is possible to visualize both, a 2D plane and a 3D structure of a biomaterial.

Angiogenesis is a process responsible for the expansion and reconstruction of the blood vessel network. It consists of two mechanisms: endothelial germination and intussusception of microvascular growth (IMG) [8]. The progression of the angiogenesis process can be monitored by the analysis of different biomarkers. For instance, vascular endothelial growth factor (VEGF) is known to induce a strong angiogenic response and lymphangiogenesis in various *in vivo* models. VEGF acts as a survival factor for endothelial cells both *in vitro* and *in vivo*, and its level determines the rate of formation of blood vessels [9]. There are several forms of VEGF proteins in the human body, for instance VEGF-A, which induces the angiogenesis process and produces arterial blood vessels, VEGF-B responsible for the development of coronary arteries, and VEGF-C and VEGF-D, thanks to which the lymphatic system develops. As all these forms have a significant impact on the angiogenesis process [10]. The process of angiogenesis can

be also studied by analyzing an expression profile of particular genes. VEGF-A expression is regulated in different tissues and conditions by a number of factors including hypoxia (e. g. via the HIF-1 pathway), other cytokines and growth factors (e. g. TGF-β), various hormones (e. g. estrogen and progesterone) and, importantly in cancer, by oncogenes and tumor suppressor genes. VEGF-A produces several different transcripts by alternative splicing; three of these, the 189, 165, and 121 amino acid forms, are thought to be of the greatest importance (mouse isoforms are one amino acid shorter). The product of VEGF-B expression is a pre-pro-protein, which in the process of post-translational processing is transformed into two isoforms, containing 167 and 186 amino acids, respectively, while VEGF-C is the product of expression of a gene located in the long arm of chromosome 4 (4q34). This factor participates in the stimulation of proliferation and migration of endothelial cells and contributes to increased vascular permeability. It also causes hyperplasia of the lymph vessels of the skin. VEGF-D stimulates the proliferation of lymphatic endothelial cells. It exhibits angiogenic properties both *in vitro* and *in vivo*. Increasing the expression of this factor in keratinocytes stimulates the formation of lymph vessels in the skin. Observations showed the presence of VEGF-D expression products also in melanocytes, fibroblasts and lung mesenchyme [12]. In addition, the angiogenesis process is regulated by the enzymes MMP-2 and MMP-9. At the same time, MMP-2 and MMP-9 may also contribute to the formation of angiogenesis inhibitors (TIMP-1, TIMP-2). The correct expression of MMPs is regulated at the level of gene transcription, translation, activation of proenzymes and by activators and tissue inhibitors (TIMPs – tissue inhibitors of metalloproteinases). Moreover, the growth factor VEGF is an important regulator of MMPs expression.

The process of angiogenesis is also related with the inflammation, since the presence of an inflammatory state induces angiogenesis. Therefore, when designing biomaterials intended to promote angiogenesis, one must pay attention to whether the same biomaterial induces inflammation. The protein IL-8 (Interleukin 8) can be used for checking whether the biomaterial induces a foreign body reaction. IL-8 is a chemotactic, and growth factor for endothelial cells. It plays an important role in tumor growth, angiogenesis and metastasis. It is correspondingly upregulated when inflammation occurs [11].

Consequently, the most effective biomaterial that will be able to serve as an efficient tissue scaffold should have a porous structure that allows cell penetration, promotes

the process of angiogenesis through the expression of appropriate genes, but at the same time prevents tissue inflammation. The main goal of our work is to develop biocompatible polyesters and to investigate their impact on the process of angiogenesis and inflammation. In our previous work [13], we have shown the synthesis of a biocompatible polyester based on poly(isosorbide sebacate) (PISEB) and poly(L-lactide-*co*-glycolide) (PLGA), as well as described the preliminary data concerning its biocompatibility. In this work, we present an in-depth analysis of its impact on the process of angiogenesis and inflammation by the analysis of microscopic images of HUVEC cells cultured on its surface.

6.2. Materials and methods

6.2.1. Materials synthesis

Poly(isosorbide sebacate) (PISEB) and poly(L-lactide-*co*-glycolide) (PLGA) were synthesized according to the previously described procedure [13]. A blend of PISEB and PLGA (1:1) was dissolved in dichloromethane and electrospun as described earlier [13].

6.2.2. Biological Characterization

The human umbilical vein endothelial cells, HUVEC (pooled donor, Lonza, Basil, Switzerland), were cultured in EGMTM-2 Endothelial Cell Growth Medium supplemented with all components from EGMTM-2 SingleQuots Supplement Pack in 75 cm^2 cell culture bottles. Once the cells reached confluency, they were removed from the culture bottle and washed with 3 mL of Dulbecco's phosphate-buffered saline, DPBS (PAN-Biotech, Aidenbach, Germany) before being treated with 3 mL of 0.25% trypsin--EDTA solution (Sigma Aldrich) for 5-10 minutes. The trypsinization was stopped by adding 6 mL of a complete culture medium. The cells were then seeded into 12-well plates at a density of 2×10^5 cells per well in a 2 mL volume of medium. After 48 hours of incubation, the cytotoxicity of the test materials was evaluated by measuring the ability of viable cells to reduce the tetrazolium dye MTT (3-[4,5-dimethylthiazol-2-yl]--2,5-diphenyltetrazolium bromide) to formazan. The cells were collected from the

12-well plates and exposed to 50 µL of MTT solution (0.05 mg/mL in phenol red- and FBS-free DMEM-F12; PAA). After 2 hours of incubation, the MTT solution was removed, and the resulting violet formazan crystals were dissolved in 400 µL of acidic isopropanol (0.05 M HCl). The absorbance of the solution was measured at 570 nm using a multi-well plate reader SYNERGY4 (BioTek Instruments, New York, NY, USA).

To conduct long-term microscopic observations, the HUVEC cells were seeded directly onto the test materials or a control polystyrene plate (Sarstedt, Numbrecht, Germany) in EGM-2 medium, supplemented with a complete Bulletkit of growth factors (Lonza). After incubating for 48 hours, the cells were live stained with Sybr Green dye (Life Technologies, Carlsbad, CA, USA) for nucleus visualization, and the signals were captured using a JuLI_FL™ apparatus (NanoEntek, Seoul, South Korea) with a magnification of 40× and collected in visible or FITC channels.

The expression of pro-angiogenic markers (IL8, VEGF, MMPs) and TIMPs was assessed by performing gene expression analysis using RT-qPCR. This analysis was conducted on both control cells and cells incubated with the tested materials for 48 h (HUVEC cell line). Following trypsinization and collection of the cells, total RNA was isolated using the phenol-chloroform extraction method with the Total RNA Isolation kit (A@A Biotechnology, Gdańsk, Poland). The efficiency of RNA isolation was measured spectrophotometrically. Gene amplification was carried out with help of commercially available kits (Real-Time 2xPCR Master Mix SYBR A; A@A Biotechnology) and a set of primers for IL8, VEGF, MMP2, MMP9, TIMP1, and TIMP2 (Genomed, Warsaw, Poland) was created with an online tool (OligoAnalyzer™ Tool, IDT, Coralville, IA, USA). The quantitative PCR reaction, preceded by reverse transcription using the NG dART RT kit (EURx, Gdańsk, Poland) was performed with the CFX96 Touch™ Real-Time PCR Detection System thermocycler (Bio-Rad, Hercules, CA, USA). Data are presented as mean ± SD, from 3 experiments, calculated with formula $R = 2^{-\Delta\Delta C_T}$ by Livak [17]. Statistical significance was calculated with a T-test and p-value < 0.05 is indicated with a star*.

The data analysis of real-time PCR reactions, as well as the control of the thermocycler and data collection, were performed using Bio-Rad's CFX Manager 3.1 software. For the obtained threshold values of Cq (Ct) determined by the software, the relative expression level of the target gene in cells was calculated compared to control cells. The 2-ΔΔCt method was used for this purpose. Measurements were conducted for a reference gene (RPL) and the target genes. This allows for the calculation of the ratio,

representing the relative increase in expression (R), based on the fluorescence intensity of the respective gene at the Cq cutoff points (Cq target and Cq reference), according to the formula.

$$\mathbf{R} = \frac{2^{\Delta Cq\ target}}{2^{\Delta Cq\ reference}}$$

R – relative expression ratio of the gene, representing the increase in transcript quantity in the sample compared to the reference gene.
ΔCq target – the difference in threshold values (Cq) for the target gene.
ΔCq reference – the difference in threshold values (Cq) for the reference gene.

To perform confocal microscopy observations, the HUVEC cells were seeded onto the test materials, or a control polystyrene plate (Sarstedt, Numbrecht, Germany) as described above. After incubating for 48 h, the cells were stained with DAPI dye (Invitrogen™, Waltham, MA, USA) for nucleus visualization, and the signals were captured using Olympus FluoView FV1000™ confocal microscope (Olympus LS, Tokyo, Japan). In a confocal microscope, a laser is used to excite the fluorescent label present in the sample. The laser beam is concentrated to a specific point and systematically moved across the sample, scanning it in a sequential manner, line by line. DAPI stands for 4',6-diamidino-2-phenylindole, which is a fluorescent dye that specifically binds to DNA molecules. The mechanism of DAPI staining involves the intercalation of the DAPI dye molecules between the base pairs of double-stranded DNA. This binding occurs due to the affinity of DAPI for the A-T-rich regions of DNA. Once bound, DAPI molecules emit blue fluorescence when excited by ultraviolet (UV) light. DAPI undergoes approximately 20-fold enhancement of fluorescence when associated with DNA, having an excitation maximum of 358 nm and an emission maximum of 461 nm.

6.3. Results and Discussion

The biocompatibility of PLGA, PISEB, and PLGA/PISEB was assessed in the previous experiments that have been already published [13]. The MTT assay results indicated that the investigated materials were biocompatible since the relative cell viability of PISEB (91 ± 3%) and PLGA/PISEB (91 ± 4%) was only slightly lower than that of the tissue culture plastic control (100%), and comparable to that of PLGA (94 ± 3%).

The JuLI_FL™ imaging system was used to observe the visible light and green fluorescence signals emitted by the nuclei of HUVEC cells seeded on investigated polymers during 48–72 h of microscopic observation. The HUVEC cells were cultured on polystyrene plastic plates coated with collagen type IV to improve cell adhesion and vein-like morphology formation. Compared to panels stimulated with vascular endothelial growth factor (VEGF) and cultured with a complete Bulletkit of growth factors, cells on the control plates exhibited a typical tube-formation morphology during the neo-angiogenesis process (Fig. 1) [14, 15].

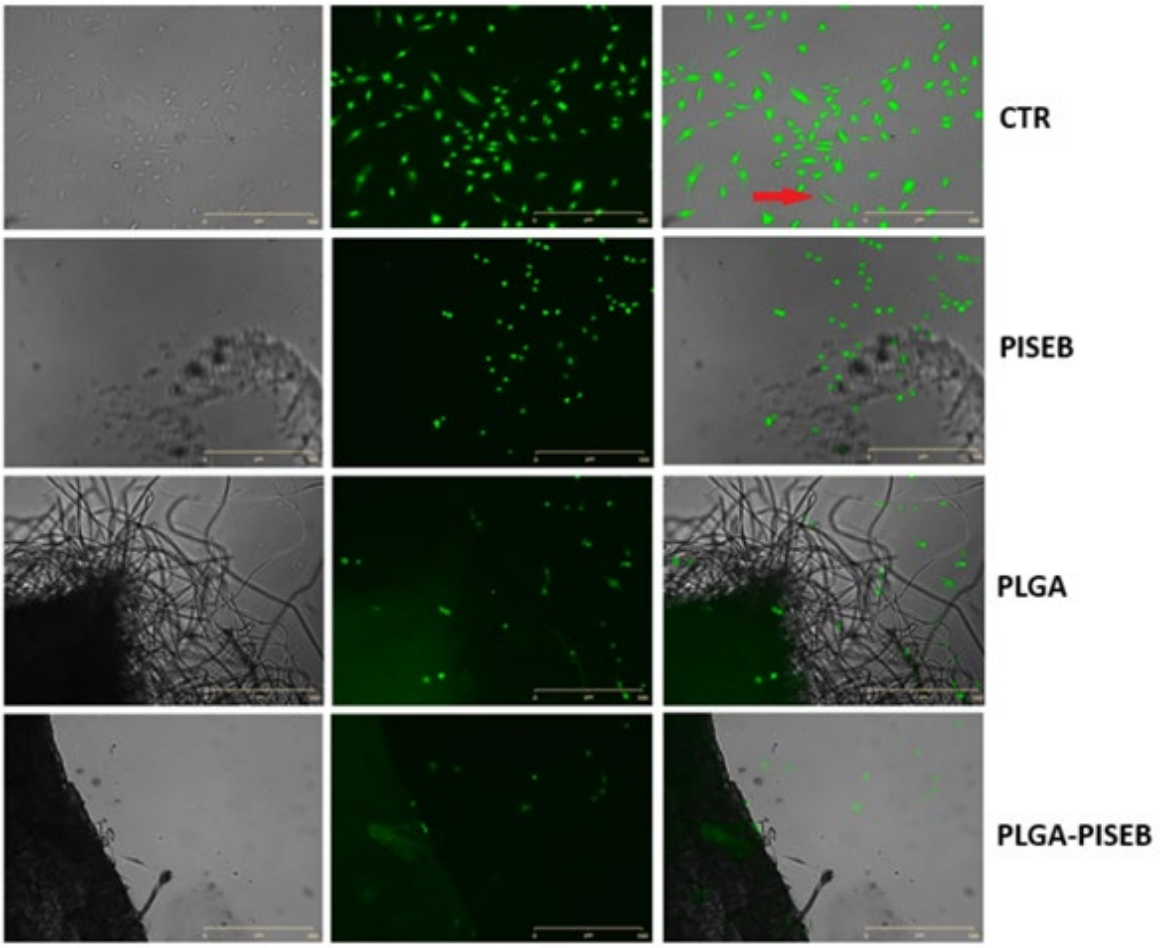

Fig. 1. Typical images of HUVEC cells cultured on VEGF-supplemented (w/v 0.5%) EGM-2 medium for 48 h on control polystyrene plate and tested materials: drop-casted PISEB, electrospun PLGA, and electrospun PLGA/PISEB, selected with fluorescein. Tubular shapes of the cells presented in visible light are indicated by red arrow (scale bar 500 μm; magnification 40×; JuLI_FL™ apparatus)

Rys. 1. Obrazy komórek HUVEC hodowanych na pożywce EGM-2 z dodatkiem VEGF (stężenie 0.5%) przez 48 godzin na kontrolnej płytce polistyrenowej oraz testowanych materiałach: osadzonym PISEB, elektroprzędzonym PLGA i elektroprzędzonym PLGA/PISEB, wybrane za pomocą fluoresceiny. Rurkowate kształty komórek prezentowane w świetle widzialnym wskazano czerwoną strzałką (skala 500 μm; powiększenie 40×; aparat JuLI_FL™)

Real-time qPCR reaction was performed to assess gene expression for HUVEC cell controls [13]. These cells were previously incubated with materials – PLGA, PLGA+PISEB, and PISEB for 48 hours. The ratio (relative gene expression fold change) is calculated using the formula mentioned in previous subsection – $R = 2^{-\Delta\Delta Ct}$. As a result, the control is always determined as a value of 1.

The results (Fig. 2), which have been previously presented in [13], show that both the presence of VEGF and its absence increases the expression of the MMP2 gene. In case of TIMP2 gene, an upregulation in absence of VEGF is observed. If a similar experiment was conducted for a longer period rather than just 48 hours, it would be possible to observe more clearly how VEGF suppresses the expression of MMP2.

In the case of MMP9 and TIMP1, MMP9 is significantly overexpressed in the presence of VEGF. The situation is reversed when VEGF is not present – the expression of the TIMP1 gene outweighs the expression of MMP9. From this phenomenon, it could be concluded that angiogenesis markers are produced only in the presence of VEGF, which is the first factor initiating this process.

Similarly to MMP9, significantly higher expression of IL8 was observed after the addition of VEGF – adding VEGF to the culture medium blocked its expression. The absence of VEGF can initiate VEGF expression, so that it may undergo slight stimulation in the following days.

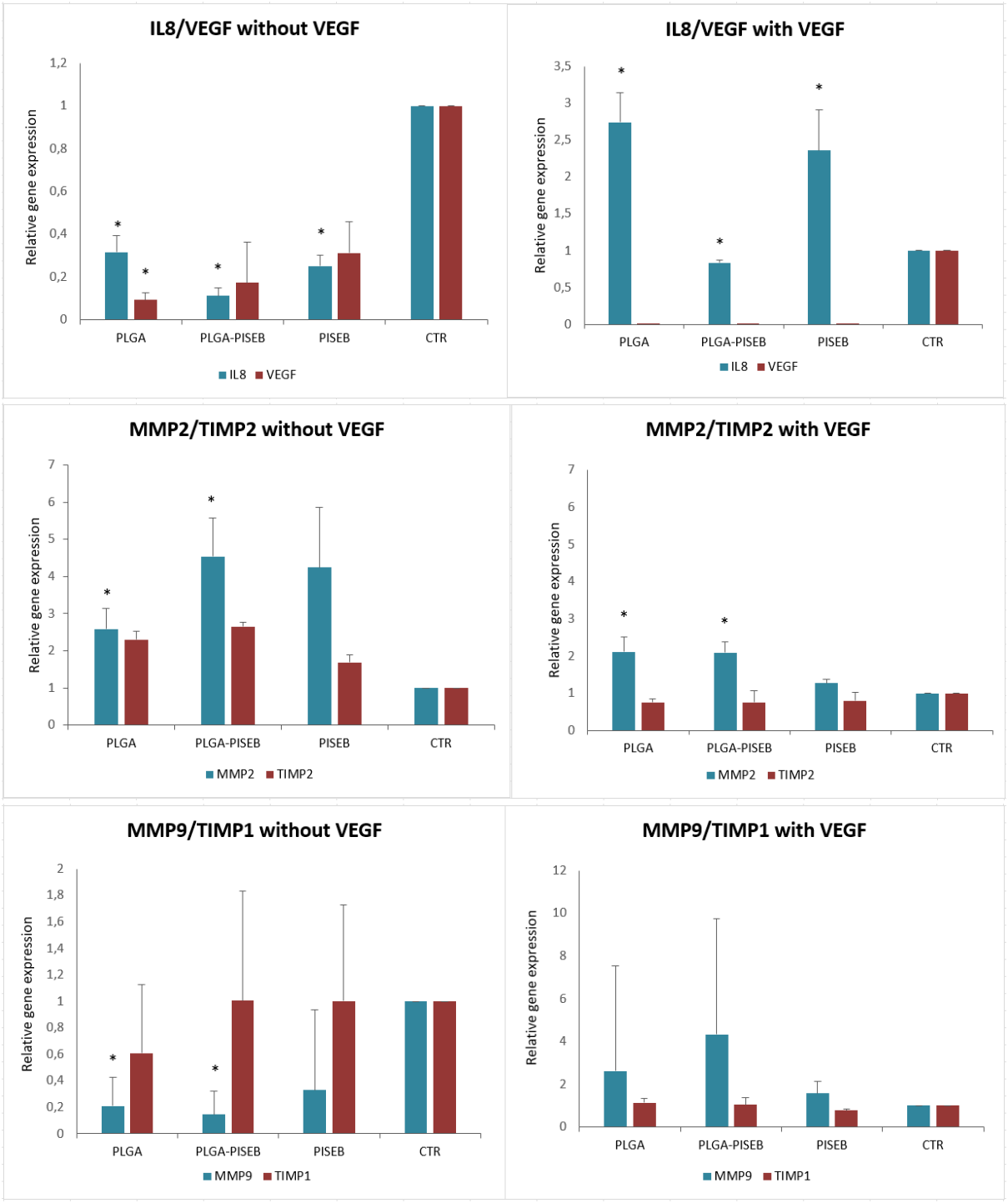

Fig. 2. Relative gene expression level, for pro-angiogenic markers: MMPs, IL8 and angiogenesis inhibitors TIMPs in HUVEC cells after 48 h of incubation on materials with, or without VEGF. Data are presented as mean ± SD from 3 experiments. Statistical significance in comparisons to controls was calculated with a Student's t-test and p-value < 0.05 is indicated with a star *

Rys. 2. Poziom względnej ekspresji genów, dla markerów pro-angiogennych: MMP, IL8 oraz inhibitorów angiogenezy TIMP, w komórkach HUVEC po 48 godzinach inkubacji na materiałach z lub bez dodatku VEGF. Dane przedstawione są, jako średnia ± SD z 3 eksperymentów. Istotność statystyczną względem kontroli policzono testem T-Studenta, gdzie wartość p przyjęto na poziomie p< 0.05 zaznaczono gwiazdką *

Observations using confocal microscopy and DAPI dye allowed for the visualization of HUVEC cell nuclei and the analysis of growth and penetration of these cells into the interior layers of the tested materials. As it can be seen in Fig. 3 and Fig. 4, the cells exhibited penetration and growth between the upper layers of the materials. However, it should be noted that the incubation lasted for 48 hours, which means that the cells did not have enough time to migrate into deeper areas of the materials. The presented images suggest that the cells showed the best growth in the case of electrospun materials such as PLGA and PLGA-PISEB, while it was poorer in the case of drop-casted PISEB material. This relationship will be analyzed in future studies.

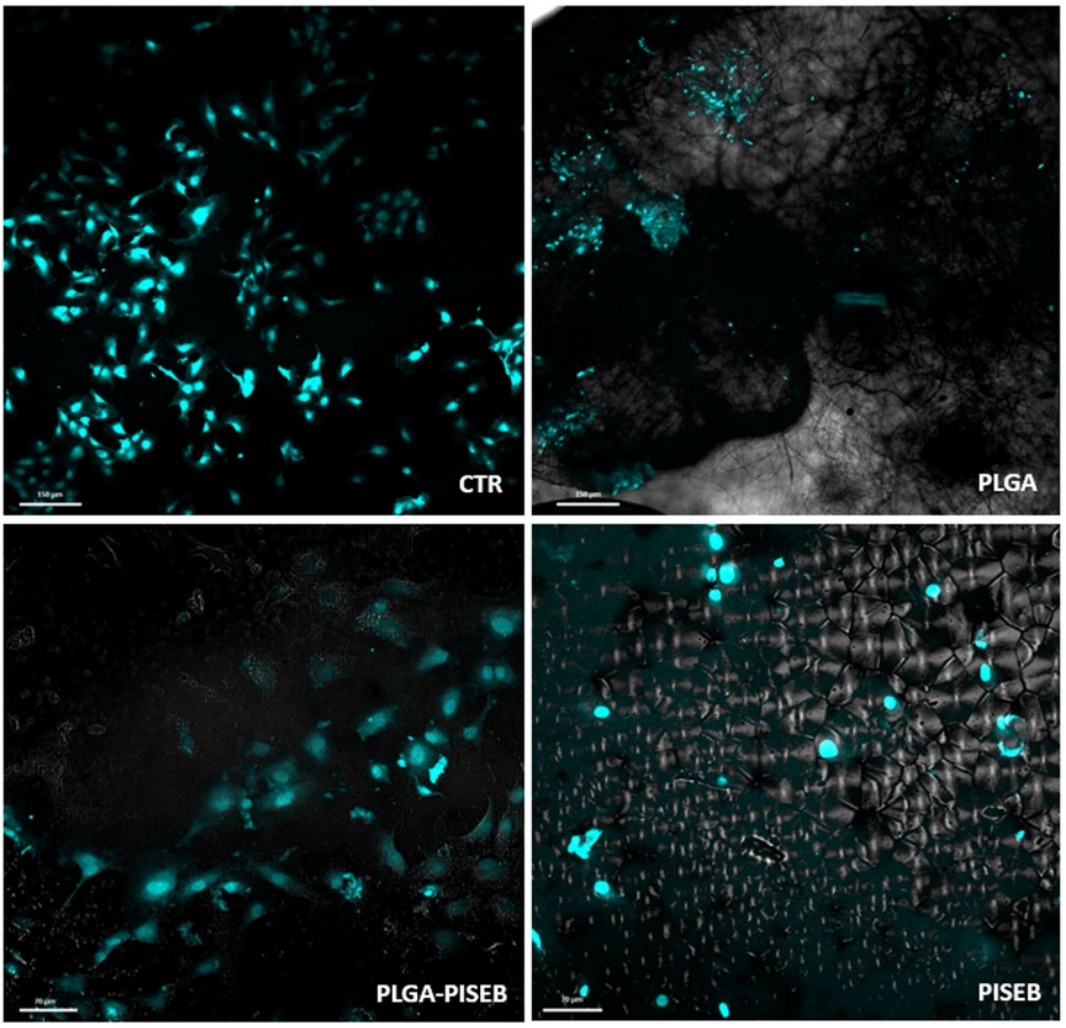

Fig. 3. Confocal images (2D) of HUVEC cells cultured on VEGF-supplemented (w/v 0.5%) EGM-2 medium for 48 h on control polystyrene plate and tested materials: drop-casted PISEB, electrospun PLGA, and electrospun PLGA/PISEB, selected with DAPI (scale bars 150 µm (top pictures), 70 µm (bottom pictures); magnification 120x, Olympus FluoView FV1000™ apparatus)

Rys. 3. Obrazy konfokalne (2D) komórek HUVEC hodowanych na pożywce EGM-2 z dodatkiem VEGF (stężenie 0,5% w/v) przez 48 godzin na kontrolowej płycie polistyrenowej oraz testowanych materiałach: osadzonym PISEB, elektroprzędzonym PLGA i elektroprzędzonym PLGA/PISEB, wybrane za pomocą barwnika DAPI (skala 150 µm – górne zdjęcia, 70 µm – dolne zdjęcia; powiększenie 120x, aparat Olympus FluoView FV1000™)

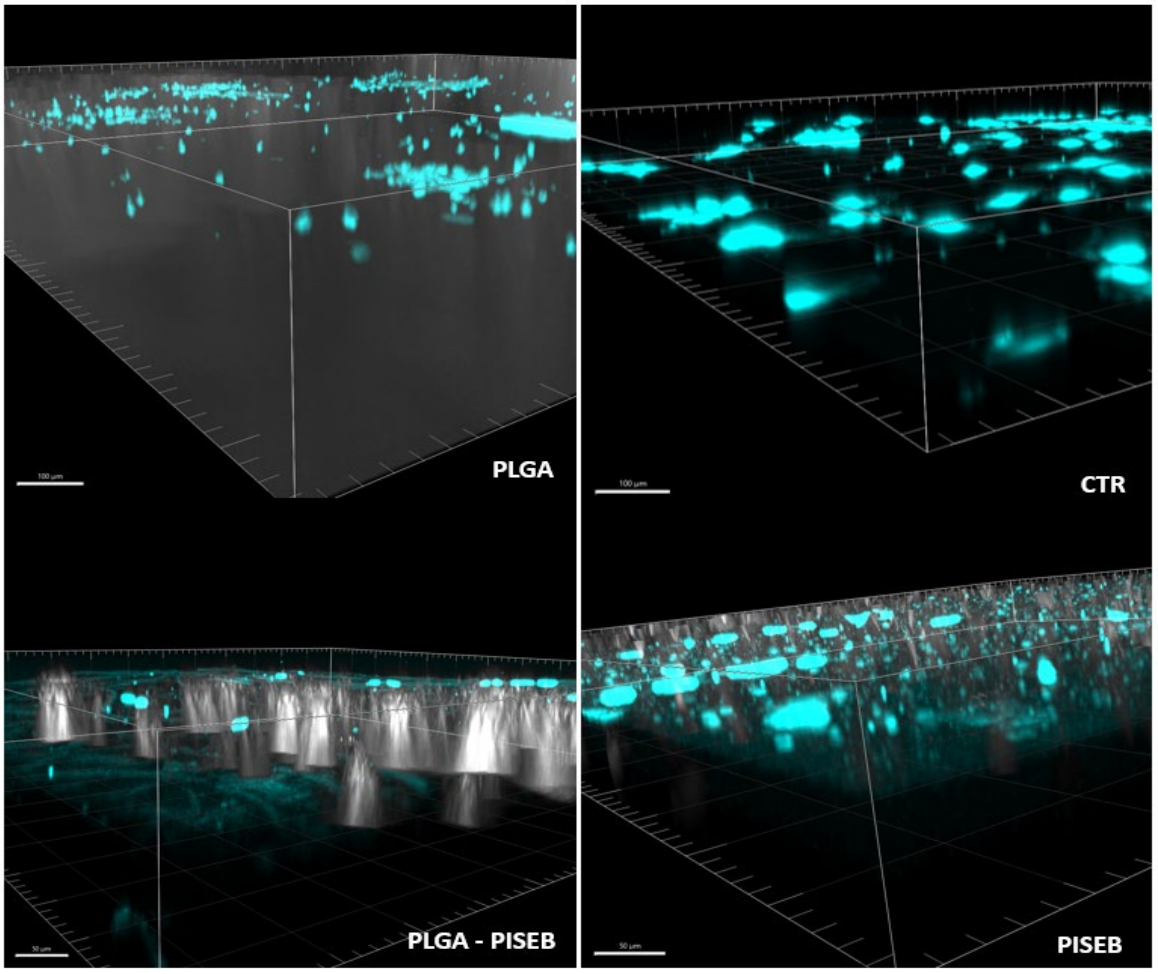

Fig. 4. Confocal 3D structure images of HUVEC cells cultured on VEGF-supplemented (w/v 0.5%) EGM-2 medium for 48 h on control polystyrene plate and tested materials: drop-casted PISEB, electrospun PLGA, and electrospun PLGA/PISEB, selected with DAPI (scale bars 100 µm (top pictures), 50 µm (bottom pictures); magnification 120x, Olympus FluoView FV1000™ apparatus)

Rys. 4. Obrazy struktury konfokalnej w trzech wymiarach (3D) komórek HUVEC hodowanych na pożywce EGM-2 z dodatkiem VEGF (stężenie 0,5% w/v) przez 48 godzin na kontrolowej płycie polistyrenowej oraz testowanych materiałach: osadzonym PISEB, elektroprzędzonym PLGA i elektroprzędzonym PLGA/PISEB, wybrane za pomocą barwnika DAPI (skala 100 µm – górne zdjęcia, 50 µm – dolne zdjęcia; powiększenie 120x, aparat Olympus FluoView FV1000™)

6.4. Conclusions

In this paper, we analyzed biological properties of polymeric materials: PLGA, PISEB and PLGA/PISEB. Genotyping of HUVEC cells collected after 48 h of incubations on PISEB, PLGA and PLGA/PISEB showed a potentially pro-angiogenic expression profile. The addition of VEGF to the culture medium activated the MMPs and IL8 expression and down-regulated VEGF expression (in a negative feedback loop

for itself). Levels of MMPs were higher in all three materials than in the control group, demonstrating the pro-angiogenic properties of polymers. Long-term live microscopic observations of living cells stained with fluorescein showed cell growth both on the surface of the culture plate and on the surface of polymers with typical tubular-shapes of HUVEC cells. Microscopic observations of fixed cells stained with DAPI showed the penetration of cells only the surface part of materials - 48h incubation was not long enough for cells to penetrate the lower layers. Further research plans include long-term and 3D cell culture experiments in co-cultivation of HUVEC and Normal Human Dermal Fibroblasts (NHDF). Experiments using 3D cell cultures will aim to mimic the human tissues in body.

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Bibliography

1. C. Vaquette i J.J. Cooper-White, *Increasing electrospun scaffold pore size with tailored collectors for improved cell penetration*, *Acta Biomaterialia*, t. 7, nr 6, s. 2544–2557, cze. (2011), DOI: 10.1016/j.actbio.2011.02.036.
2. J.P. Vacanti, R. Langer, *Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation*, *The Lancet*, t. 354, s. S32–S34, lip. (1999), DOI: 10.1016/S0140-6736(99)90247-7.
3. G. Lemon, D. Howard, F.R.A.J. Rose, J.R. King, *Individual-based modelling of angiogenesis inside three-dimensional porous biomaterials*, *Biosystems*, t. 103, nr 3, s. 372–383, mar. (2011), DOI: 10.1016/j.biosystems.2010.11.009.
4. M. Dziadek, K. Cholewa-Kowalska, *Wybrane metody otrzymywania porowatych rusztowań w inżynierii tkankowej*, Acta Bio-Optica et Informatica Medica. Inżynieria Biomedyczna, Vol. 20, nr 4, s. 193–203, (2014).
5. A. Laska, B*iomateriały stosowane w inżynierii tkankowej do regeneracji tkanek*, Zeszyty Naukowe Towarzystwa Doktorantów Uniwersytetu Jagiellońskiego. Nauki Ścisłe, t. 14, s. 187, (2017).
6. S. Blonski, *I Kongres Mechaniki Polskiej*, Warszawa, 28–31 sierpnia (2007) r.

7. A. Deng i in., *Porous nanostructured poly-l-lactide scaffolds prepared by phase inversion using supercritical CO2 as a nonsolvent in the presence of ammonium bicarbonate particles*, The Journal of Supercritical Fluids, t. 77, s. 110–116, maj (2013), DOI: 10.1016/j.supflu.2013.02.020.
8. M. Mashreghi, H. Azarpara, M. R. Bazaz, A. Jafari, A. Masoudifar, H. Mirzaei, M.R. Jaafari, (2018). *Angiogenesis biomarkers and their targeting ligands as potential targets for tumor angiogenesis*. Journal of cellular physiology, 233(4), 2949–2965. https://doi.org/10.1002/jcp.26049.
9. N. Ferrara, *The role of VEGF in the regulation of physiological and pathological angiogenesis*, *EXS*, nr 94, s. 209–231, (2005), DOI: 10.1007/3-7643-7311-3-15.
10. H.F. Dvorak (2005). *Angiogenesis: update* (2005). Journal of thrombosis and haemostasis : JTH, 3(8), 1835-1842. https://doi.org/10.1111/j.1538-836.2005.01361.
11. *IL-8 Directly Enhanced Endothelial Cell Survival, Proliferation, and Matrix Metalloproteinases Production and Regulated Angiogenesis1 | The Journal of Immunology American Association of Immunologists*. https://journals.aai.org/jimmunol/article/170/6/3369/71324/IL-8-Directly-Enhanced-Endothelial-Cell-Survival.
12. M. Namiecińska, K. Marciniak, J. Nowak, *VEGF jako czynnik angiogenny, neurotrofi czny i neuroprotekcyjny*, *Postepy Hig Med Dosw*, 59(0), grudz. (2005).
13. M. Śmiga-Matuszowicz, J. Włodarczyk, M. Skorupa, D. Czerwińska-Główka, K. Fołta, M. Pastusiak, M. Adamiec-Organiściok, M. Skonieczna, R. Turczyn, M. Sobota, K. Krukiewicz, (2023). *Biodegradable Scaffolds for Vascular Regeneration Based on Electrospun Poly(L-Lactide-co-Glycolide)/Poly(Isosorbide Sebacate) Fibers*. International journal of molecular sciences, *24*(2), 1190. https://doi.org/10.3390/ijms24021190
14. S. Jin, X. Xia, J. Huang, C. Yuan, Y. Zuo, Y. Li, J. Li, *Recent advances in PLGA-based biomaterials for bone tissue regeneration*. Acta Biomater. (2021), 127, 56–79.
15. M.T. Gentile, O. Pastorino, M. Bifulco, L. Colucci-D'amato, *Huvec tube-formation assay to evaluate the impact of natural products on angiogenesis*. JoVE (2019), e58591. https://doi.org/10.3791/58591.
16. K.L. DeCicco-Skinner, G.H. Henry, C. Cataisson, T. Tabib, J.C. Gwilliam, N.J. Watson, E.M. Bullwinkle, L. Falkenburg, R.C. O'Neill, A. Morin, J.S. Wiest, *Endothelial cell tube formation assay for the in vitro study of angiogenesis*. J Vis Exp. (2014) Sep 1;(91):e51312. DOI: 10.3791/51312. PMID: 25225985; PMCID: PMC4540586.
17. K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001, *25*, 402–408, DOI: 10.1006/meth.2001.1262.

STUDY OF THE ANGIOGENESIS PROCESS ON THE SURFACE OF BIODEGRADABLE SCAFFOLDS BASED ON ISOSORBIDE

Abstract

The need to regenerate blood vessels arises both in the case of congenital defects, cardiovascular diseases and accidents with accompanying hemorrhages. If the condition of blood vessels is poor, an auxiliary vascular scaffold needs to be used. The aim of our study was the synthesis of a biodegradable and bioresorbable blood vessel scaffold based on biocompatible polymers, followed by an in-depth analysis of the process of creating new blood vessels (angiogenesis). Electrospun scaffolds were made from a blend of poly(L-lactide-*co*-glycolide) (PLGA) and poly(isosorbide sebacate) (PISEB). PLGA was synthesized via a ring-opening polymerization of L-lactide and glycolide, while PISEB was synthesized via a polyesterification process. Human umbilical vein endothelial cells (HUVEC) were used for *in vitro* biological analysis. Viability of cells cultured on the obtained materials was determined by the MTT test. The process of angiogenesis was investigated by analyzing the morphology of HUVEC cells using confocal microscopy and examining the level of expression of marker genes for the angiogenesis process (MMP2, MMP9, TIMP1, TIMP2, VEGF), as well as selected biomarkers associated with inflammation (IL6, IL8). Cells collected after 48h of incubations on the surface of PLGA/PISEB scaffolds showed a potentially pro-angiogenic expression profile, as well as anti-inflammatory effects of this material.

Keywords: angiogenesis, biodegradable scaffolds, isosorbide, biocompatible polymers