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Enhanced Endothelial Cell Growth on NaOH-Treated Electrospun PET Coated with Fibrin

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ABSTRACT

Polyethylene terephthalate (PET) hydrophobic nature may lead to problems, particularly protein absorption, when used for biomedical application. Thus, NaOH surface treatment was introduced towards electrospun PET fiber, to improve the hydrophilicity of the fiber. This investigation involved treating electrospun PET fiber with sodium hydroxide (NaOH) at 65°C for one hour. Fibrin gel was subsequently applied to NaOH-treated samples and uncoated PET for comparative analysis prior to the seeding of human umbilical vein endothelial cells (HUVECs) for durations of 2, 4, and 6 hours. The NaOH treatment led to increased hydrophilicity attributed to the presence of carboxyl and hydroxyl groups, as verified by EDS mapping data. Following the application of fibrin gel to NaOH-treated PET, in vitro culture with HUVEC cells demonstrated enhanced cellular growing up to 6 hours of culture. The PET fibers treated with NaOH and coated with fibrin were demonstrated to attract HUVEC cells and enhance endothelialization.

1. Introduction

The creation of a synthetic material that accurately replicates the small diameter of vascular grafts (≤6 mm) remains unpatented, hence generating interest in addressing this significant gap in the cardiovascular device sector. The primary cause of long-term failure in small-diameter vascular grafts is a low patency rate [1].

Synthetic materials such as polyethylene terephthalate (PET) are utilised for large diameter blood vessels (ID > 6mm); however, they exhibit limitations when applied to small diameter blood vessels due to the absence of an endothelial layer. This results in noncompliance, thrombogenicity, intimal hyperplasia, aneurysms, calcium deposition, and infection, which further restricts growth for implantation and consequently diminishes their efficacy [2]. This occurs because PET is hydrophobic and possesses low surface energy, which makes it difficult for other materials to adhere effectively to its surface [3].

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The hydrophobic nature of PET has prompted researchers to address this limitation by applying various coatings to its surface. Dutoya *et al.*, for example, used elastin-derived protein and elastin as a coating substance on PET to increase the patency and yielded structure. This fabrication is also mimic the natural arterial wall where elastin fibers are an important role in the blood vessel wall [4]. This study shows endothelial cells gives positives respond also growing well and its phenotype is stable on ESP-coating (with elastin or not) with the concentration of 100 μg/cm^2 [5]. Li *et al.,* show layer by layer (LBL) coating which is hyaluronic acid (HA) and cationized gelatin (CG) coated on PET improved cell adhesion, cell growth and blocked unnecessary genes on the sample. The *in-vivo* testing in rabbit shows this LBL coating effective in inhibit the inflammatory cell infiltration and encourage the development of the new ligament among the graft fibers. The existence of collagen I in HA-CG coating also greater than control group thus makes PET grafts coated with HA–CG possible material for artificial ligament grafts [6].

In addition, Recek *et al.,* found that PET surfaces treated with oxygen plasma, which are hydrophilic, exhibited the highest rate of protein adsorption when compared to untreated samples. In addition, the total protein adsorption was significantly greater on the treated surfaces, and the adsorption layer displayed viscoelastic characteristics. Moreover, these treated surfaces were more conducive to cell adhesion [7].

Similarly, Deutsch *et al.,* developed a method for autologous in-vitro endothelialization of ePTFE prostheses (ID = 6-7 mm) to address the limited patency of synthetic grafts. Specifically, the inner surface of the ePTFE prostheses was coated with fibrin to enable the seeding of autologous endothelial cells under in-vitro rotating conditions. After approximately 9 days of culture, 341 grafts were implanted as infrainguinal bypasses in 310 patients. Over a 15-year clinical follow-up, endothelialized ePTFE prostheses maintained endothelial presence for 2–4 years post-surgery and exhibited patency rates comparable to vein grafts. Notably, the patency rate of 7mm prostheses was significantly higher than that of 6mm grafts (78% vs. 62% at 5 years, 71% vs. 55% at 10 years). Despite these findings, vascular tissue engineering has emerged as a promising strategy to overcome the limitations of autografts, such as morbidity and limited availability, as well as the suboptimal properties of synthetic grafts [8].

Furthermore, a study investigating fibrin-coated PET single monofilament fibers revealed that the presence of angiogenesis directed the physical interaction between HUVEC cells and the polymer fiber. This interaction facilitated the formation of the microvascular wall structure and enabled lumen formation [9]. In addition, Ma *et al.,* demonstrated that gelatin grafting onto PET fibers enhanced endothelial cell (EC) behavior, including spreading and proliferation, while preserving the EC phenotype, which is crucial for blood vessel applications [10].

The aim of this study is to examine the synergistic effects of surface modifications on protein adsorption dynamics and endothelial cell signaling. The electrospinning process was used to generate nanoscale fibres with porosity, effectively simulating the extracellular matrix (ECM) as a basement membrane. The scaffold was inoculated with Human umbilical vein endothelial cells (HUVEC) to facilitate cell growth through ligand-receptor interactions after PET was treated with NaOH. Modified PET nanofibers may mitigate certain thrombosis-related challenges in current vascular prostheses.

2.Methodology

2.1 Fabrication of the PET Nanofiber

Polyethylene terephthalate (PET) with an intrinsic viscosity of 0.82 g/dl was obtained from Espet Extrusion Sdn Bhd, and trifluoroacetic acid (TFA) from Millipore was used as the solvent. PET pellets were dissolved in 20 wt% TFA and stirred at 350 rpm for 2 hours to ensure complete dissolution. The solution was loaded into a 5 mL syringe with a 0.21 mm needle and ejected at a flow rate of 1 mL/hr,

based on optimal conditions from previous studies. A voltage of 13 kV (up to 15 kV) was applied, with the current and frequency set to zero. The fibers were collected on an aluminum collector during a 4-hour electrospinning process.

2.2 PET Nanofiber Surface Treatment with NaOH

The PET nanofibers were treated with NaOH solution and heated in an oven at 65°C for one hour. They were then rinsed with distilled water until the pH reached approximately 7 and left to dry at room temperature (25°C) for 24 hours. The dried nanofibers were cut into 17 mm diameter circles to fit a 24-well plate and soaked in 70% ethanol for 15 minutes. Afterward, they were washed five times with PBS, each wash lasting 3 minutes, and dried in a sterile environment at room temperature.

2.3 PET Nanofiber Surface Treatment with NaOH Coated Fibrin Gel

Figure 1 illustrates the process of fibrin gel coating. A fibrinogen solution was prepared by dissolving fibrinogen powder in HBSS, while thrombin was prepared at a concentration of 100 U/mL in HBSS, supplemented with 350 KIU/mL of aprotinin. Both the fibrinogen and thrombin solutions, which were not sterile initially, were warmed in a water bath at 37°C for approximately 15 minutes before use (Figure 1(a)). The solutions were then sterilized using a 0.22 µm syringe filter (Figure 1(b)). A moderate fibrin gel, with a concentration of 2 mg/mL, was formed by rapidly mixing the fibrinogen-thrombin mixture and stirring for 3 minutes in a 24-well plate (Figure 1(c)). The fibrinogen polymerized and embedded the sterile PET nanofibers within the gel (Figure 1(d) and (e)). The fibrin-coated PET nanofibers were incubated at 37°C in endothelial growth media overnight. Afterward, the samples were dried on filter paper at room temperature in a sterile environment (Figure 1(f)).

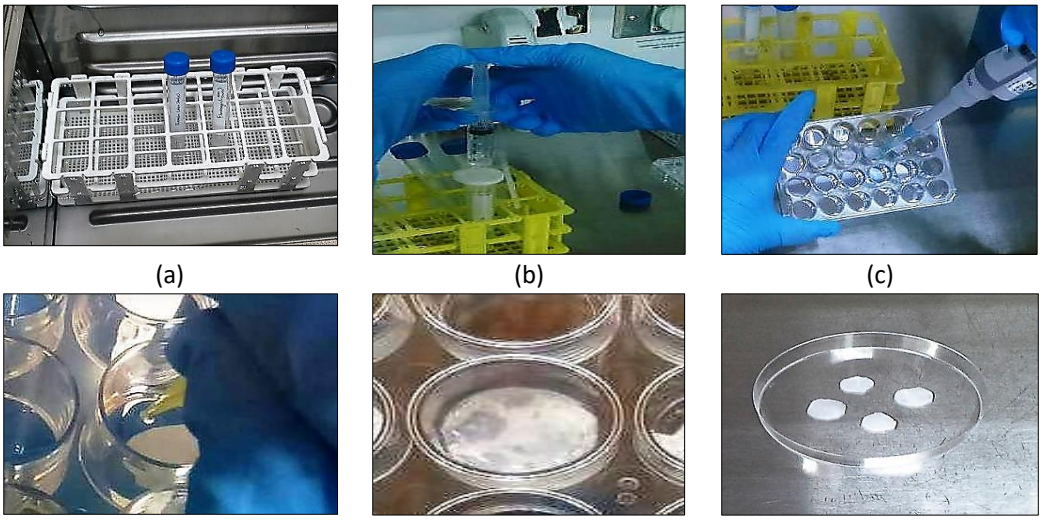

Fig. 1. Fibrin gel coating process

2.4 FESEM

The morphology of all samples was captured using field emission scanning electron microscope (FESEM). Fiber was coated via gold plated for 5 minutes before mounted on the holder and was run at the acceleration voltage of 20 kV under low vacuum. The images were taken at several different magnifications 10K and 25K.

2.5 In Vitro Cell Culture

Sterilized PET and PET modified were put and fits nicely into the bottom of 24 well-plate with the rounded dimension (d=15mm). Next, ~ 1 x $l0^6$ cells/ml of HUVEC cells from passage 3 to 5 was seeded on the surface of the fibers. Integrin receptors on cells' outer membrane surfaces, in turn, ''recognize'' and bind to certain of these proteins as shown in Figure 2. This occurs because in aqueous solution at physiological pH values, the protonated amine group possesses a localized positive charge which helps attract the negatively charged biomolecules (proteins) and cells.

Live/Dead HUVEC were observed 2, 4 and 6 hours for cell attachment under inverted fluorescence microscopy (Carl Zeiss, Germany) and images were recorded. The percentages of live cells were randomly taken at the surrounding cells area. Briefly, 0.05 µmol l^{-1} Calcein AM and 4.0 µmol^{-1} ethidium homodimer (Live/Dead kit, Molecular) were stained on the test sample. The images were taken at 10X magnifications. The percentage area of cells was counted using Image J software.

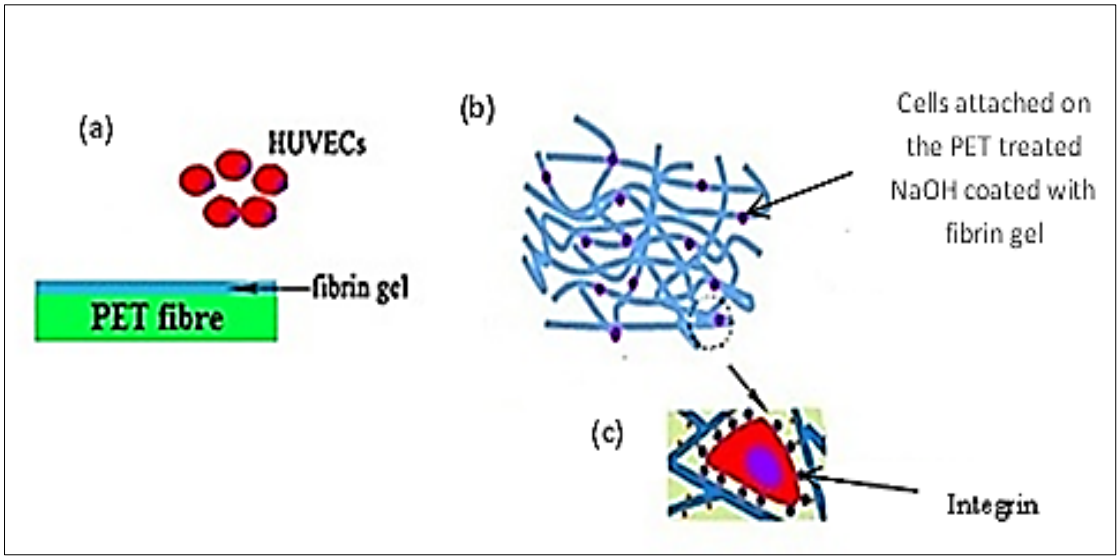

Fig. 2. Schematic diagram of static cell adhesion process on the coated sample (a) HUVECs were seeded on the PET surface treated with NaOH coated fibrin gel (b) Cell attached on the sample (c) cell attachment of HUVEC through integrin

3. Results

3.1 Microscopic Analysis of PET Nanofiber Before and After Modification

Figure 3(a) displays the micrographs of uncoated PET fibres produced using electrospinning. The morphology was homogeneous, characterised by a lack of features and a smooth texture. Figure 3(b) illustrates the electrospun PET coated with fibrin gel. The fibrin structures exhibited a smooth, flat surface and a translucent appearance following the coating process. The fibrin gel structures, after being coated with fibrin gel on PET nanofibers, were not visible due to the translucent nature of the hydrogels [11,12].

Consequently, surface treatment was performed to enhance the hydrophilicity of PET nanofiber. The hydrophilic surface facilitates the adsorption of organic substances, thus attracting cells to its surface [13]. Following the surface treatment depicted in Figure 3(c), PET exhibits a rough texture characterized by an irregular surface along the fibers. These fiber orientations are also crucial for increasing a fibre's strength and stiffness [14].The morphology of PET fibers treated with NaOH is superior to that reported by Hadjizadeh *et al.,* which indicates that the surface treatment of PET mats with 1N NaOH resulted in increased surface roughness and the formation of holes following

treatment durations of 2 and 4 hours [15]. The objective of this surface treatment is to enhance the hydrophilicity of the fiber surface thus period of 1 hour is enough for the treatment. This assertion is corroborated by further research [16,17]. It can be concluded that sodium hydroxide (NaOH) surface treatment at 65°C for 1 hour sufficiently enhances the hydrophilicity of PET nanofibers without significantly compromising their physical properties.

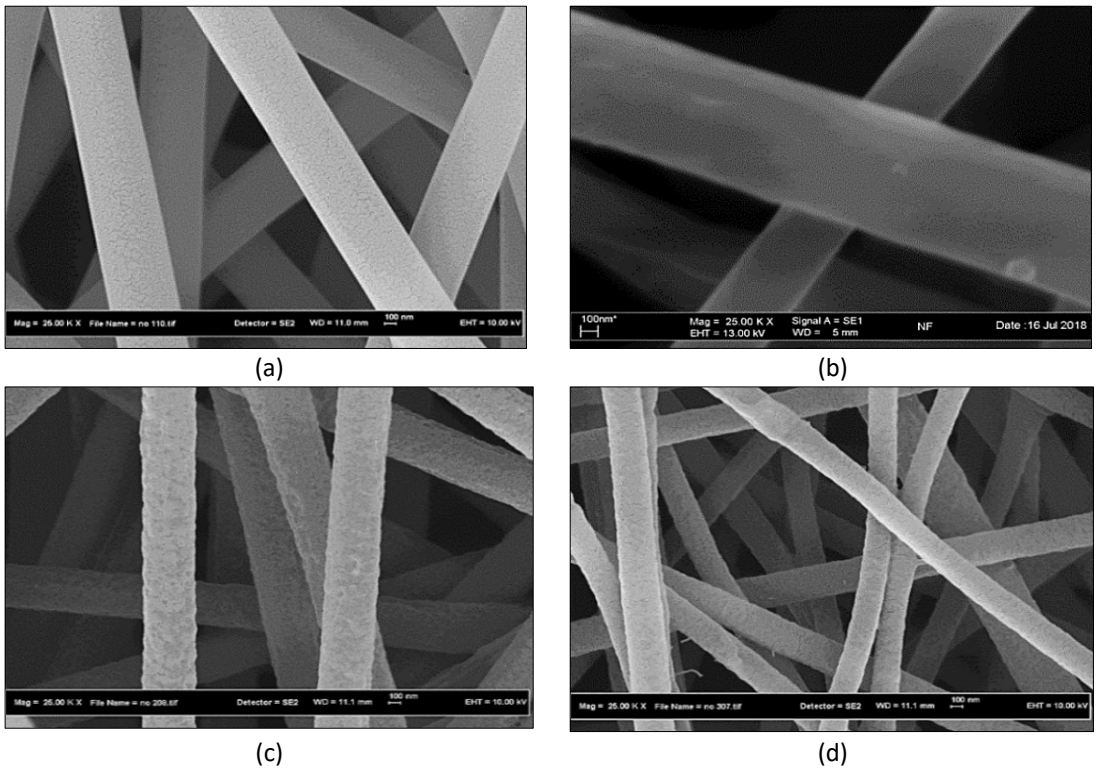

Fig. 3. PET Microscopic analysis of (a) PET nanofiber before coating (b) PET coated fibrin gel (10kX and 20kX) (c) PET treated NaOH (d) PET treated NaOH coated fibrin gel

It was reported before, surface treatment of NaOH towards as received non-woven PET matrices with the diameter size of 25µm increases the surface roughness when treated up to 120-hour. It was found that murine embryonic stem cells accumulated along with the fiber structure for 12-hour cell culture[17]. In this present study also shows the surface of PET electrospun nanofiber able being functionalization by NaOH surface treatment even though the fiber diameter range is between 300nm-600nm. The surface of PET nanofiber after functionalizing is not only able to attract the cells as mentioned before but also protein (fibrin gel) [18]. Results in Figure 3(d) also show PET fiber after treated with NaOH improved adhesions in the case of surface treatment. After PET treated with NaOH, nanofiber was subsequently coated with fibrin gel and the fiber surface became smooth, indicating that a fibrin gel was formed on the surface of the fiber.

3.2 EDS and Mapping Analysis of PET Nanofiber Before and After Modification

Figure 4 shows EDS result of PET coated fibrin gel with different elements exist of the sample including C, O, F, N, Mg, P, S, Cl, and Ca. These elements are referring to the PET and fibrin gel. Oxygen increases from 30.39% to 37.98% and the existence of nitrogen with 3.79% proved that fibrin gel was successfully coated on the PET nanofiber. Fibrin gel was coated unevenly on PET fibre. This is because PET is a hydrophobic and inert in nature [7,20,23]. This statement is proven by EDS mapping

indicating the distribution of the main element of fibrin gel are C, N, and O on the PET fiber. The distribution of N element is non-homogenous in the mat.

Figure 4 also shows the EDS spectrum of PET coated fibrin gel. Different elements exist on the sample including C, O, F, N, Mg, P, S, Cl, and Ca. The existence of C, N, and O in the EDS result is correlated with the main components of fibrin gel on PET nanofibers. Superior changes of oxygen element to 54.26% and the existence of nitrogen with 5.96% confirmed the existence of fibrin on the PET treated fiber after the coating process where the oxygen element represents carboxyl bonding and nitrogen is amide bonding [19]. The coating is fully covering the cellular fibers due to the increase in the hydrophilicity of the PET surface [18]. The distribution of C, N, and O which are the main element of fibrin gel deposited on the PET fiber was evaluated with the EDS mapping. The EDS mapping result of PET treated NaOH coated fibrin gel shows a uniform distribution of the C, N and O element throughout the surface matrix. This result is similar to the previous study where the fibrin regularly coated each fiber in the mat. The study also showed some of the fibrin randomly formed a thin fibrous mesh on the membrane surface [12].

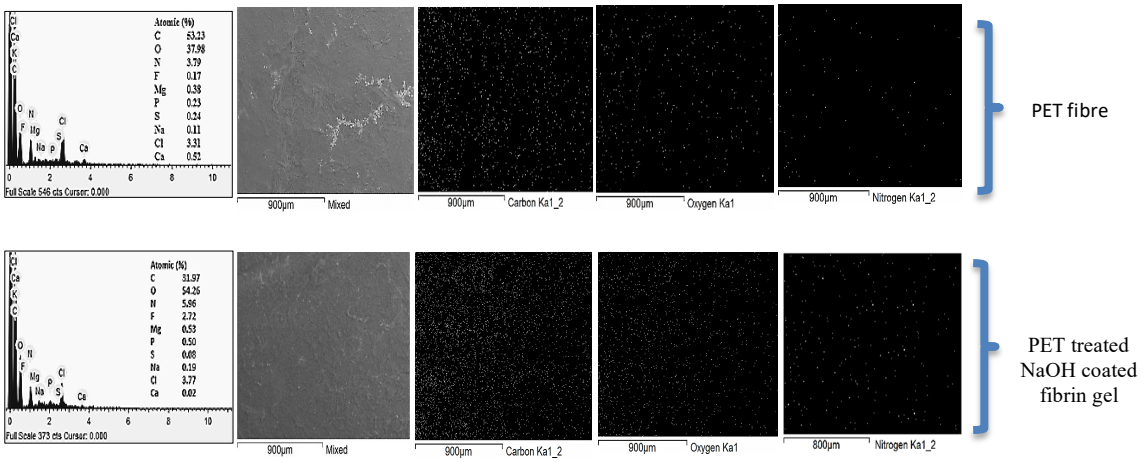

Fig. 4. EDS and Mapping results of on PET fibre and PET treated NaOH coated fibrin gel

This finding is consistent with the results of other research groups, which have demonstrated that PET functionalized by NaOH treatment and coated with fibrin gel can promote cell adhesion, growth, and proliferation on various electrospun substrates [25,26]. PET, when modified with appropriate chemical functionalities, effectively attracts and binds primary amine ($–NH_2$) groups from proteins in biological fluids or culture media. Integrin receptors on the outer membranes of cells subsequently "recognize" and bind to specific proteins. This interaction occurs because, in aqueous solutions at physiological pH, the protonated amine group carries a localized positive charge, which facilitates the attraction of negatively charged biomolecules (proteins) and cells.

3.3 Counting Initial Cells Attachment of HUVEC on PET Before and After Modification

For cellular attachment study, HUVEC were seeded at 1×10^6 cells/ml. At 2, 4 and 6 h after cell seeding, unattached cells were washed out and the attached cells were stained by the live-dead kit assay. The comparative morphology of HUVEC on the bare PET, PET coated fibrin gel and PET nanofiber after surface treatment with NaOH and coating with fibrin gel is presented in Figure 5. The viable cells are green while dead cells are red in color. Results showed more HUVEC were attached on PET treated NaOH coated fibrin gel up to 6 hours.

In the case of the bare PET surfaces, it is anticipated that the cells maintained a round shape even after 4 and 6 hours of attachment with 59% and 73% of live cells respectively, showing that cells were

able to attach but cannot spread in the shorter time. PET coated fibrin gel shows cells dense areas within 4 and 6 hours with 71% and 74%. The PET treated NaOH coated fibrin gel showed 71% live cell percentage for 4 hours cultures followed by 76% of live cell percentage after 6 hours.

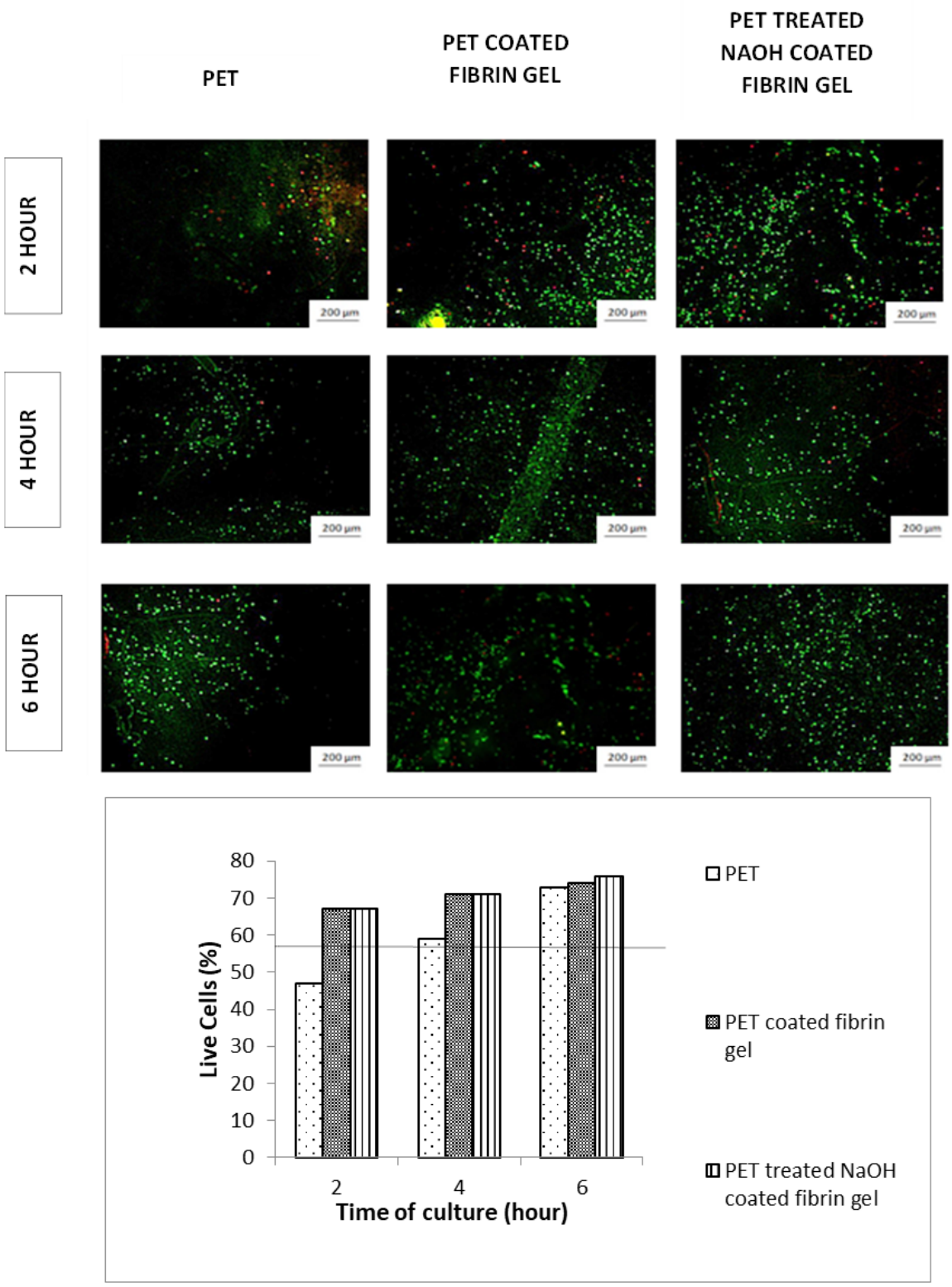

Fig. 5. Cell attachment of PET, PET coated fibrin and PET treated NaOH coated fibrin for 2, 4, and 6 hours

4. Conclusions

Surface modification techniques aim to enhance the surface properties of fibers by incorporating various functional groups. For that reason, significant increases in oxygen and nitrogen content were observed on the PET-treated fiber after the coating process, with oxygen indicating carboxyl bonding and nitrogen representing amide bonding. The results indicate that PET treated with NaOH and coated with fibrin gel promotes enhanced growth of HUVEC cells, as evidenced by improved cell attachment observed within the first 6 hours of culture. Consequently, this study suggests that PET treated with NaOH and coated with fibrin gel holds promise for biomedical applications, particularly in small-diameter vascular grafts.

References

[1] De Visscher, G., L. Mesure, B. Meuris, A. Ivanova, and W. Flameng. "Improved endothelialization and reduced thrombosis by coating a synthetic vascular graft with fibronectin and stem cell homing factor SDF-1α." *Acta biomaterialia* 8, no. 3 (2012): 1330-1338. https://doi.org/10.1016/j.actbio.2011.09.016

[2] Browning, M. B., D. Dempsey, V. Guiza, S. Becerra, J. Rivera, B. Russell, M. Höök et al. "Multilayer vascular grafts based on collagen-mimetic proteins." *Acta biomaterialia* 8, no. 3 (2012): 1010-1021. https://doi.org/10.1016/j.actbio.2011.11.015

[3] Catto, Valentina, Silvia Farè, Giuliano Freddi, and Maria Cristina Tanzi. "Vascular tissue engineering: recent advances in small diameter blood vessel regeneration." *International Scholarly Research Notices* 2014, no. 1 (2014): 923030. https://doi.org/10.1155/2014/923030

[4] de Valence, Sarra, Jean-Christophe Tille, Damiano Mugnai, Wojciech Mrowczynski, Robert Gurny, Michael Möller, and Beat H. Walpoth. "Long term performance of polycaprolactone vascular grafts in a rat abdominal aorta replacement model." *Biomaterials* 33, no. 1 (2012): 38-47. https://doi.org/10.1016/j.biomaterials.2011.09.024

[5] Melchiorri, Anthony J., Narutoshi Hibino, and John P. Fisher. "Strategies and techniques to enhance the in situ endothelialization of small-diameter biodegradable polymeric vascular grafts." *Tissue Engineering Part B: Reviews* 19, no. 4 (2013): 292-307. https://doi.org/10.1089/ten.teb.2012.0577

[6] Abdolahifard, M., S. Hajir Bahrami, and R. M. A. Malek. "Surface modification of PET fabric by graft copolymerization with acrylic acid and its antibacterial properties." *International Scholarly Research Notices* 2011, no. 1 (2011): 265415. https://doi.org/10.5402/2011/265415

[7] Bartnik, A., H. Fiedorowicz, R. Jarocki, J. Kostecki, M. Szczurek, A. Biliński, O. Chernyayeva, and J. W. Sobczak. "Physical and chemical modifications of PET surface usingáaálaser-plasma EUV source." *Applied Physics A* 99 (2010): 831-836. https://doi.org/10.1007/s00339-010-5596-1

[8] Dutoya, S., A. Verna, F. Lefebvre, and M. Rabaud. "Elastin-derived protein coating onto poly (ethylene terephthalate). Technical, microstructural and biological studies." *Biomaterials* 21, no. 15 (2000): 1521-1529. https://doi.org/10.1016/S0142-9612(99)00274-4

[9] Dutoya, S., F. Lefebvre, C. Deminieres, F. Rouais, A. Verna, A. Kozluca, A. Le Bugle, and M. Rabaud. "Unexpected original property of elastin derived proteins: spontaneous tight coupling with natural and synthetic polymers." *Biomaterials* 19, no. 1-3 (1998): 147-155. https://doi.org/10.1016/S0142-9612(97)00214-7

[10] Li, Hong, Chen Chen, Shurong Zhang, Jia Jiang, Hongyue Tao, Jialing Xu, Jianguo Sun, Wei Zhong, and Shiyi Chen. "The use of layer by layer self-assembled coatings of hyaluronic acid and cationized gelatin to improve the biocompatibility of poly (ethylene terephthalate) artificial ligaments for reconstruction of the anterior cruciate ligament." *Acta Biomaterialia* 8, no. 11 (2012): 4007-4019. https://doi.org/10.1016/j.actbio.2012.07.008

[11] Recek, Nina, Morana Jaganjac, Metod Kolar, Lidija Milkovic, Miran Mozetič, Karin Stana-Kleinschek, and Alenka Vesel. "Protein adsorption on various plasma-treated polyethylene terephthalate substrates." *Molecules* 18, no. 10 (2013): 12441-12463. https://doi.org/10.3390/molecules181012441

[12] Deutsch, Manfred, Johann Meinhart, Peter Zilla, Norbert Howanietz, Michael Gorlitzer, Alexander Froeschl, Andreas Stuempflen, Deon Bezuidenhout, and Martin Grabenwoeger. "Long-term experience in autologous in vitro endothelialization of infrainguinal ePTFE grafts." *Journal of vascular surgery* 49, no. 2 (2009): 352-362. https://doi.org/10.1016/j.jvs.2008.08.101

[13] Sukmana, Irza, and Joy Riski Pangestu Djuansjah. "Sandwiched polymer fibre in fibrin matrices for the dictation of endothelial cells undergoing angiogenesis." In *Journal of Physics: Conference Series*, vol. 423, no. 1, p. 012049. IOP Publishing, 2013. https://doi.org/10.1088/1742-6596/423/1/012049

[14] Ma, Zuwei, Masaya Kotaki, Thomas Yong, Wei He, and Seeram Ramakrishna. "Surface engineering of electrospun polyethylene terephthalate (PET) nanofibers towards development of a new material for blood vessel engineering." *Biomaterials* 26, no. 15 (2005): 2527-2536. https://doi.org/10.1016/j.biomaterials.2004.07.026
[15] Bacakova, Marketa, Julia Pajorova, Denisa Stranska, Daniel Hadraba, Frantisek Lopot, Tomas Riedel, Eduard Brynda, Margit Zaloudkova, and Lucie Bacakova. "Protein nanocoatings on synthetic polymeric nanofibrous membranes designed as carriers for skin cells." *International journal of nanomedicine* (2017): 1143-1160. https://doi.org/10.2147/IJN.S121299
[16] Bacakova, Marketa, Jana Musilkova, Tomas Riedel, Denisa Stranska, Eduard Brynda, Margit Zaloudkova, and Lucie Bacakova. "The potential applications of fibrin-coated electrospun polylactide nanofibers in skin tissue engineering." *International Journal of Nanomedicine* (2016): 771-789. https://doi.org/10.2147/IJN.S99317
[17] Hadjizadeh, Afra, Abdellah Ajji, and Martin N. Bureau. "Preparation and characterization of NaOH treated micro-fibrous polyethylene terephthalate nonwovens for biomedical application." *Journal of the mechanical behavior of biomedical materials* 3, no. 8 (2010): 574-583. https://doi.org/10.1016/j.jmbbm.2010.07.002
[18] Hadjizadeh, Afra, and Charles J. Doillon. "Directional migration of endothelial cells towards angiogenesis using polymer fibres in a 3D co-culture system." *Journal of tissue engineering and regenerative medicine* 4, no. 7 (2010): 524-531. https://doi.org/10.1002/term.269
[19] Ismail, Verawaty, Mohd Hilmi Ariffin, and Mohammad Syahadan Maksom. "Effect of Fibre Direction on Repairing Tensile Failure of Glass Fibre/Polyester Composite." *Journal of Advanced Research Design* 66, no. 1 (2020): 1-9.
[20] Hadjizadeh, Afra, Abdellah Ajji, and Martin N. Bureau. "Nano/micro electro-spun polyethylene terephthalate fibrous mat preparation and characterization." *Journal of the mechanical behavior of biomedical materials* 4, no. 3 (2011): 340-351. https://doi.org/10.1016/j.jmbbm.2010.10.014
[21] Inoue, Sachiko, Masaaki Imamura, Yoshiaki Hirano, and Yasuhiko Tabata. "Adhesion and proliferation of human adipo-stromal cells for two-or three-dimensional poly (ethylene terephthalate) substrates with or without RGD immobilization." *Journal of Biomaterials Science, Polymer Edition* 20, no. 5-6 (2009): 721-736. https://doi.org/10.1163/156856209X426600
[22] Ng, Robin, Xudong Zhang, Ning Liu, and Shang-Tian Yang. "Modifications of nonwoven polyethylene terephthalate fibrous matrices via NaOH hydrolysis: Effects on pore size, fiber diameter, cell seeding and proliferation." *Process Biochemistry* 44, no. 9 (2009): 992-998. https://doi.org/10.1016/j.procbio.2009.04.024
[23] Donelli, Ilaria, Paola Taddei, Philippe F. Smet, Dirk Poelman, Vincent A. Nierstrasz, and Giuliano Freddi. "Enzymatic surface modification and functionalization of PET: a water contact angle, FTIR, and fluorescence spectroscopy study." *Biotechnology and bioengineering* 103, no. 5 (2009): 845-856. https://doi.org/10.1002/bit.22316
[24] Chen, Wei, and Thomas J. McCarthy. "Chemical surface modification of poly (ethylene terephthalate)." *Macromolecules* 31, no. 11 (1998): 3648-3655. https://doi.org/10.1021/ma9710601
[25] Catelas, Isabelle, Nadjah Sese, Benjamin M. Wu, James CY Dunn, S. A. M. Helgerson, and Bill Tawil. "Human mesenchymal stem cell proliferation and osteogenic differentiation in fibrin gels in vitro." *Tissue engineering* 12, no. 8 (2006): 2385-2396. https://doi.org/10.1089/ten.2006.12.2385
[26] Sahni, Abha, and Charles W. Francis. "Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation." *Blood, The Journal of the American Society of Hematology* 96, no. 12 (2000): 3772-3778. https://doi.org/10.1182/blood.V96.12.3772.h8003772_3772_3778