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Nano- and Microfiber PVB Patches as Natural Oil Carriers for Atopic Skin Treatment

Zuzanna J. Krysiak, Łukasz Kaniuk, Sara Metwally, Piotr K. Szewczyk, Ewa A. Sroczyk, Petra Peer, Paulina Lisiecka-Graca, Russell J. Bailey, Emiliano Bilotti, and Urszula Stachewicz*

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ABSTRACT: Atopic dermatitis (eczema) is a widespread disorder, with researchers constantly looking for more efficacious treatments. Natural oils are reported to be an effective therapy for dry skin, and medical textiles can be used as an alternative or supporting therapy. In this study, fibrous membranes from poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (PVB) with low and high molecular weights were manufactured to obtain nano- and micrometer fibers *via* electrospinning for the designed patches used as oil carriers for atopic skin treatment. The biocompatibility of PVB patches was analyzed using proliferation tests and scanning electron microscopy (SEM), which combined with a focused ion beam (FIB) allowed for the 3D visualization of patches. The oil spreading tests with evening primrose, black cumin seed, and borage were verified with cryo-SEM, which showed the advantage nanofibers have over microfibers as carriers for low-viscosity oils. The skin tests expressed the usability and the enhanced oil delivery performance for electrospun



patches. We demonstrate that through the material nano- and microstructure, commercially available polymers such as PVB have great potential to be deployed as a biomaterial in medical applications, such as topical treatments for chronic skin conditions.

KEYWORDS: PVB, electrospun fibers, biocompatibility, oil carriers, atopic skin patches

1. INTRODUCTION

Skin diseases are one of the most common human illnesses, which cause a huge burden on global healthcare. The multiplicity and complexity of the involvement of the skin as a clinical presentation of disease can be caused by a variety of factors. These include immune system disorders, medications, and infections. Current eczema treatments are not able to provide a long-term cure; therefore, the development of new strategies for skin regeneration is needed.¹ Atopic dermatitis (AD) frequently affects young children, and the number of people affected by eczema has increased over recent decades.² It is characterized by dry and itchy skin and increased transepidermal water loss^{3,4} and thus increased permeability to allergens and irritants.⁵ Lipids are one of the major components of the stratum corneum, the outer layer of the skin. Treatment of AD is centered around rehydrating the skin with standard emollients such as petroleum jelly and the cautious use of topical steroids to reduce inflammation and itching.⁶ With AD, the skin becomes extremely itchy and inflamed, causing redness, swelling, vesicle formation (minute blisters), cracking, weeping, crusting, and scaling.⁷ Often, wet wraps are applied as a night dressing, which can be bought or made from articles of clothing, soaked in water, and applied to the affected skin on the body. Wet wraps are best used in the evening after bathing, moisturizing, and applying medication. You can use clean cotton clothing as a dressing and pajamas on top if the eczema is widespread and cotton gloves or socks if it is not. Goodyear et. al. reported on 30 children with acute

erythrodermic eczema treated with wet wraps soaked in weak topical steroid creams with a 90-100% decrease in eczema severity;⁸ however, in many cases, the improvement without using steroids was moderate.

During recent years, nanotechnological approaches have been recognized as a promising way for new product developments.9 Among the technologies that are used to realize nanoscale materials or that are able to manipulate the matter at the nanoscale, electrospinning is one of the most promising and fast-growing ones.¹⁰ Electrospinning as a technique is widely considered to be simple, cost-effective, and also easily scalable from laboratory to commercial production.^{11,12} The increased surface area of nanofibers gives them unique abilities for increased release of treating substances compared to the standard bandages.^{13,14} Electrospinning can produce fibers at the nano- to microscale,¹⁵ with random or aligned orientation¹⁶ and a smooth or porous surface structure through alteration of processing parameters,¹ which allows for the control of membrane porosity and pore size,^{18,19} both of which are important in transdermal patch applications. Within this study, we aim to take advantage of

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electrospun membrane porosity to capture oil droplets between fibers for the moisturizing effect on dry skin. Various oil behaviors depend on fiber diameter, roughness, and porosity of polymeric meshes.²⁰ The viscosity of oils is affected by the chemical structure, chain length, saturation, and position of the hydroxyl group in the chemical structure. It also influences the interaction of oils with any substrate material, especially porous ones; the higher the viscosity, the more the oil on the surface, rather than diffusing into the network.^{21,22}

Free fatty acids (FFAs), cholesterol (CHOL), and ceramides (CERs) belong to the group of skin lipids. These intracellular lipids prevent transepidermal water loss and CERs are the most important component in maintaining skin hydration. Linoleic acid (LA) and α -linoleic acid (ALA) are essential fatty acids (EFAs) as they cannot be synthesized by the human body but need to be absorbed from the food.²³ A metabolite of LA, ylinoleic acid (GLA), is an effective substance in the treatment of AD, by topical or oral administration. Importantly, GLA was found in natural oils such as borage²⁴ and evening primrose.²⁵ Dry skin is also caused by a deficiency of δ -6-desaturase, the catalyst of GLA synthesis from LA. Evening primrose oil contains both LA and GLA, and besides fatty acid supplementation,²⁶ it also affects immunological abnormalities in AD.^{5,27} Similar effects were also observed when black cumin seed oil was used in AD treatment.^{28,29} Emollients, topical corticosteroids, antihistamines, oral immunomodulators, antibiotics, and topical calcineurin inhibitors are frequently used in therapies for atopic skin treatment; however, they show a lot of adverse side effects.⁵ Various treatments can be applied, both pharmacological and nonpharmacological (e.g., anti-inflammatory agents, antibiotics, or textile-based therapies),³⁰ in which biomaterials can be used as patches.³¹ Textile-based therapies for AD are very beneficial as the moisturization time is elongated and occlusion by the material prevents scratching. Thus, new material developments and further processing into applicable products would be beneficial for medical therapies.³² Recent biomaterial studies have focused on existing material modifications, consisting of multistep and time-consuming processing,³³ manufacturing composites,³⁴ or core-shell structures.³⁵ In our studies, we focus on poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (PVB), which has not been previously applied as a porous membrane for use as a skin patch; however, the PVB thin film was assessed as a prospective dental material.³⁶

PVB is a hydrophobic polymer used commercially in wood and construction applications as a coating,³⁷ for electrode modification,³⁸ and in the automotive industry as a component in laminated safety glass^{39,40} because of its optical clarity. Its advantages are also low costs and good adhesion to many surfaces.⁴¹ To date, few cell culture studies have been performed on this material and were based on films made by solvent casting³⁶ and nanoparticles⁴² as perspective candidates in alveolar bone substitutes, drug delivery systems, or contrasting agents in cancer therapy. The direct electrospinning of PVB fibers on human epidermal fibroblasts was performed using a portable device for potential wound healing treatment; the culture study was only for 24 h and limited to testing a few PVB fibers, not patches based on thick porous membranes.43 PVB nanofibers manufactured via traditional electrospinning have been well-studied in terms of process parameters controlling the quality of the produced fiber but never specifically as a biomaterial.^{44–48} This encouraged us to investigate PVB membranes without any modification or postprocessing steps for biomedical applications, in particular for skin patches. The electrospun PVB membranes are highly porous patches that can be applied as a carrier of medical substances, such as atopic skin treatment. To electrospin membranes with controlled fiber diameter and spanning nanoand micrometer length scales, we used low molecular weight (LM_w) and high molecular weight (HM_w) PVB. The biocompatibility of these membranes was analyzed via a cell culture study. The detailed cell responses were visualized with scanning electron microscopy (SEM), which confirmed biocompatibility of PVB membranes, together with the longterm toxicity tests. To test the use of electrospun PVB fibers as natural oil carriers for atopic skin treatment, we incorporated the three oils evening primrose, black cumin seed, and borage into the fibers, to verify the wetting and spreading behavior. Finally, we performed the utility study of PVB patches with oils on the human skin.

2. EXPERIMENTAL SECTION

2.1. Solution Preparation and Electrospinning. 10 wt% poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) (PVB, LM_w —low molecular weight, 70,000–100,000 and HM_w —high molecular weight, 170,000–250,000, Sigma-Aldrich, UK) solutions were prepared by dissolving in *N*,*N*-dimethyloformamide (DMF), methanol, and dimethyl sulfoxide (DMSO), mixed in ratio 5:4:1 for the HM_w and for LM_w in ratio 4:5:1, with all solvents in analytical standard purchased from Avantor, Poland. Polymer solutions were stirred at 1000 rpm (IKA RCT basic, Staufen, Germany) for 3 h at 35 °C. Fibers obtained from LM_w PVB are referred as nanofibers and those from HM_w PVB are referred as microfibers.

Patches made of PVB fibers were produced *via* electrospinning (IME Technologies, The Netherlands). The climate conditions of the process were controlled and parameters were held at T = 25 °C and RH = 30%. The applied electrospinning parameters slightly differ for nano- and microfibers production; high voltage with positive polarity was 17–19 kV for nanofibers, while for microfibers, it was 11–13 kV. The flow rate of the polymer solution was in the range of 1.0–2.0 mL· h⁻¹. The distance between the needle (0.8 mm inner diameter) and collector was kept at 15 cm for both types. Fibers were deposited for at least 30 min on the collector covered with an Al foil for cytotoxicity study whereas on baking paper for the oil spreading tests.

2.2. Surface Characterization of Electrospun Membranes. The fiber morphology was analyzed using a scanning electron microscope (Merlin Gemini II, Zeiss, Germany). Prior to imaging, the samples were sputtered with a 5 nm gold layer (Q150RS, Quorum Technologies, UK). Fiber diameters were measured using ImageJ software (ver. 1.51 s, USA) and the average fiber diameter value was calculated from 100 measurements from the SEM micrographs.

The advancing contact angle on PVB electrospun fibers was analyzed with deionized water (DI water, Spring SUV purification system—Hydrolab, Straszyn, Poland). Images were taken with a Canon EOS 700D camera with EF-S 60 mm f/2.8 Macro USM zoom lens within 3 s after droplet (3 μ L) deposition on the membrane. The contact angle was analyzed using a MB-Ruler (ver. 5.3, Germany) and the mean value was calculated from 10 measurements.

For analysis of the roughness R_a measurements, a laser microscope (Olympus OLS4000, Japan) was used. Before measurements, the electrospun fibers were deposited on a glass surface and covered with a 5 nm gold layer. A total of 10 measurements of the 258 × 258 μ m area for both nano- and microfiber meshes were performed.

2.3. FIB-SEM and 3D Reconstruction. The PVB electrospun fibers were sliced and viewed with a focus ion beam-scanning electron microscope (FIB Zeiss Neon 40EsB CrossBeam, Carl Zeiss NTS GmbH, Germany) for nanofibers while for microfibers with a cryo focused ion beam-scanning electron microscope [Quanta 3D, Thermofisher (FEI), USA and Gatan, UK], which combines SEM

with a focused ion beam (FIB) to acquire cross-section images of frozen samples. Nanofibers were mounted on a SEM stub using a double-stick electrically conductive carbon tape and were coated with 10 nm of gold (Q150RS, Quorum Technologies, UK). Microfibers were flash-frozen using liquid nitrogen slush (Gatan, UK). The frozen samples were then placed in a vacuum airlock using a thermoconductive sled contained in a pressurized shuttle to ensure stability of the sample. Samples were then heated to -90 °C for 5 min to ensure sublimation of water and cooled down to -150 °C to ensure sample stability. Before imaging, samples were sputter-coated with approximately a 10 nm layer of Pt directly in the airlock. Samples were then placed into a cryo-stage (Gatan, UK) incorporated within the SEM chamber (FEI Quanta 3D, Thermofisher, USA) that was kept at constant -150 °C to ensure stability of imaged samples. SEM imaging was carried out at a working distance of 10 mm, 10 kV electron beam accelerating voltage, and beam current in the range of 10-60 pA. The collected 2D cross-sections of 144 images for nanofibers and 151 images for microfibers were stacked together using Avizo Fire (version 6.1, Amira, FEI, U.S.A.). All of these images were aligned to the spatial model to correct the SEM imaging being performed at a slicing angle of 52°. The next steps included manually selecting and marking fibers on the images first on the XY plane and further on the YZ and XZ planes to correct artefacts and reconstruction inaccuracies. Then, one more alignment of labeled slices was performed.

2.4. Mechanical Testing. The mechanical properties were analyzed with an Instron tension and compression testing machine (model 4502, USA) with a 10 kN load cell. The fiber membranes cut in rectangles $(0.7 \times 6 \text{ cm})$ were placed in the rubber pads to protect samples from slipping and then in the ceramic clamps; the extension speed was 5 mm·min⁻¹. Both nano- and microfiber patches were tested, six probes for each sample type. For the thickness' measurement, images of membranes were taken with a Dino-Lite digital microscope (Dino-Lite Europe/IDCP B.V., The Netherlands), and then, using ImageJ software (ver. 1.51 s, USA), thickness was measured and the average was calculated. From the stress–strain curves, maximum stress and strain were calculated and curves were smooth with the Savitzky–Golay method (200 point of window, 3 polynomial order) using the origin integrate function (ver. 2019 SR2, OriginLab, USA).

2.5. Cell Culture. Murine fibroblast (NIH 3T3) cells were used for cell culture. Cells were seeded on nano- and microfiber PVB membranes sterilized with UV light at concentration 2×10^4 cells per sample. Cell culture was carried out in an incubator at $37 \,^{\circ}$ C and an atmosphere with a concentration of 5% CO₂ and humidity about 90%. NIH 3T3 cells were cultured in a complete culture medium composed of Dulbecco's modified Eagle medium (DMEM with 4.5 g/L D-glucose, Gibco, UK), supplemented with 10% of fetal bovine serum (FBS, Sigma-Aldrich, USA), 2% of antibiotics (penicillin–streptomycin, Sigma-Aldrich, USA), 1% of L-glutamine solution (Sigma-Aldrich, USA), and 1% of aminoacids (Mem nonessential amino acid solution 100×, Sigma-Aldrich, USA). The medium was changed three times a week, except samples for lactate dehydrogenase (LDH) assay.

2.5.1. Cytotoxicity Test. Prior to the assay, a negative control was prepared as 1% triton, by mixing 100 µL of Triton X-100 (Sigma-Aldrich, USA) with 9.9 mL of complete medium. The nano- and microfiber PVB was incubated at 37 $^{\circ}C$, H = 90%, and 5% of CO₂ for 1, 3, 7, 14, 21, and 28 days with complete cell culture medium. After each time point, the medium was transferred to the test tube and stored at 4 °C until samples for all incubation times samples were collected. NIH 3T3 cells were seeded on a 96-well plate at the concentration of 5×10^3 cells per well and cultured for 24 h; then, the medium was discarded. Cells were incubated with 150 μ L of previously collected and fresh medium as a positive control. For the negative one, 150 μ L of 1% triton solution in the cell culture medium was used. Incubation took 24 h; for each incubation solution, five repeats were done. After that time, LDH (Roche Diagnostic GmbH, USA) was performed. A total of 100 μ L volume of the medium was transferred to 96-wells and 100 μ L of the LDH reagent was added to each well and incubated for 30 min at 20 °C. During the incubation

time, the plate was protected from the light. Then, the absorbance was measured at 492 nm using a microplate reader (LT-4000, Labtech, UK).

For another LDH (LDH, Roche Diagnostic GmbH, USA) and MTS (CellTiter 96 AQueous One Solution Cell Proliferation Assay, MTS, Promega, USA) proliferation assay cells were seeded on both nano- and microfibers and a 24-well plate (TCPS) as a reference with the concentration of 2×10^4 cell per sample. For each time point, two samples were tested for each type of membrane. LDH cytotoxicity assay was performed after 4 h and 1 and 3 days of cell culture. After each time point, the medium was transferred into a test tube and centrifuged (MPW—352, Poland) at 250 rpm for 5 min to remove traces of cells; then, 100 μ L of the supernatant was transferred to a 96-well plate in triplicates, 100 μ L of the LDH reagent was added, and test was performed as described above.

2.5.2. Proliferation Assay. Cell proliferation was analyzed using MTS assay after 1, 3, and 7 days of cell culture; two samples for each time point and the type of the material were used. After each time point, the culture medium was removed and 80 μ L of the MTS reagent and 400 μ L of the fresh medium were added and incubated for 4 h at 37 °C, a humidity of 90%, and CO₂ concentration at 5%. Next, 100 μ L of the reaction solution from each well was transferred to the new 96-well plate in triplicates and the absorbance was measured at 490 nm. After 1, 3, and 7 days of cell culture, samples were transferred to the new 24-well plate and rinsed three times with PBS. Next, they were fixed with 2.5% formaldehyde solution for 2 h at 4 °C. The solution was removed and samples were again rinsed with PBS solution and then with deionized water; membranes were left to dry under the hood.

2.5.3. Tomographic Microscopy. Tomographic microscopy was used for live imaging of NIH 3T3 cells on PVB fibers. Prior to fibroblast seeding, LM_w and HM_w PVB was electrospun onto 14 mm glass cover slides for 10 s with the process parameters mentioned above. Slides with fibers were placed into 35 mm Ibidi glass-bottom μ -Dish dishes (Ibidi GmbH, Germany) sterilized with UV light, and NIH 3T3 cells were seeded at 5 × 10³ cells/dish and cultured for 5 days. Dishes were then placed on a holotomographic microscope (NanoLive 3D Cell Explorer Fluo, Switzerland), and images were taken at 60× magnification. Images were analyzed using STEVE FULL software (ver. 1.6.3496, Switzerland) to obtain a refractive index-based z—stack; one slice of the z—stack was used in results for each type of fibers.

2.6. Surface Tension and Viscosity of Oils. In this study, the following oils were used: evening primrose (Oenothera biennis, OlVita, Poland), black cumin seed (Nigella sativa, Your Natural Side, Poland), and borage (Borago officinalis, Etja, Poland). All of them were coldpressed. The surface tension of three oils was determined in air by the pendant drop method using an optical tensiometer, Attension theta model (Biolin Scientific, Sweden). A 1 mL Hamilton precision syringe with a needle (22 G) was used to generate the oil droplet. Images of the droplet were successively taken through a high-resolution CCD camera. Temperature was monitored during measurement and it was between 20 and 22 °C. Surface tension analysis [OneAttension Version 3.0 (r5808), Finland] applied the axisymmetric drop shape analysis method along with the Laplace equation to successive droplets imaged. The mean value was calculated as the average from six measurements. Surface tension of evening primrose, black cumin seed, and borage oil was measured at the temperature of 20–22 $^{\circ}$ C.

The rheological measurements were carried out with a Physica MCR 501 rotational rheometer (Anton Paar, Austria) equipped with concentric cylinders (26.6/28.9 mm inner/outer diameters). The oils were measured in steady shear modes at different temperatures, 20, 27.5, 35, and 40 °C, three times per sample and at each temperature. The mean value was calculated as the average from three measurements.

2.7. Cryo-SEM. The three different oils evening primrose, black cumin seed, and borage were mixed in 2:1 ratio with water in 2.0 mL Eppendorf tubes that were placed in an ultrasonic bath (Langford Sonomatic 375TT, UK) for 5 min to ensure even mixing. Water was introduced to enable stable atomization of oils. Solutions were then

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Figure 1. Scheme showing electrospun PVB fibers using LM_w and HM_w PVB obtaining nano- and microfibers, respectively. A macroscopic picture of (A) nanofiber mat and (B) microfibers. (C,D) SEM micrograph representative images of water droplets on membranes, respectively, for nanoand microfibers. (E) 3D reconstruction of random PVB nanofibers interconnected in the membrane, with a voxel size of $12 \times 12 \times 48$ nm. (F) Representative stress–strain curves from tensile tests of nano- and microfiber patches. (G) 3D reconstruction of random PVB microfibers, with a voxel size of $44 \times 44 \times 147$ nm.

deposited using a commercial atomizer (Superdrug, UK) from the distance of approximately 20 cm onto electrospun nanofiber mats attached to Al stubs and flash frozen using a liquid nitrogen bath (Gatan, UK). Then, the procedure was followed as it was previously described for 3D microfiber reconstruction.

2.8. Oil Spreading Test on Nano- and Microfibers. Three oils with different viscosities were used, borage (18.7 \pm 0.1 mPa·s), black cumin seed (44.3 \pm 0.2 mPa·s), and evening primrose oil (54.6 \pm 0.1 mPa·s), for investigation of oil spreading in nano- and microfibers manufactured via electrospinning. A total of 10 µL of each oil was deposited on electrospun patches in a rectangular shape $(4 \times 6 \text{ cm})$. Immediately after oil deposition, 30 pictures of oil droplet spreading were taken with 2 min intervals using a Canon EOS 700D camera with EF-S 60 mm f/2.8 Macro USM zoom lens, from the top. See the schematics of the experimental setup in Figure S1 in the Supporting Information. The surface area of oil spreading was measured using ImageJ software (version 1.51 s, USA) on nano- and microfiber membranes with three types of oils, three replicates per sample. First, the scale was set for each picture, and then, the circle was marked on the taken images of the fiber area where oil was visible (Figure S2); the surface area was automatically measured using the measure function in ImageJ. The mean area was calculated as the average from three measurements.

2.9. Preliminary Oil Release Test on the Skin. Evening primrose oil was used for preliminary oil release test on the skin. Prior to the test, electrospun nano- and microfiber membranes were cut into rectangles (1.8×2 cm) and weighed (Pioneer PA214CM/1, OHAUS Europe GmbH, Switzerland). Next, 10 μ L (10.6 mg) of oil was deposited on the patches. Pictures, before and after oil application, were taken with 30 min intervals within 1 h using a Canon EOS 700D camera with EF-S 60 mm f/2.8 Macro USM zoom lens. Subsequently, patches were removed from the skin and weighed to calculate percentage of released oil using the following equation

% oil release =
$$\frac{\text{patch weight + oil weight after the test}}{\text{patch weight + oil weight}} \times 100\%$$

2.10. Statistical Analysis. The statistical analyses were performed using OriginPro (ver. 2019 SR2, OriginLab, USA). Analysis of variance (ANOVA) with Tukey test was performed with significance at p < 0.05. For fiber diameter, contact angle measurement, roughness, maximum stress and strain, surface tension, viscosity, and oil spreading errors are based on standard deviation calculation.

3. RESULTS AND DISCUSSION

3.1. Fiber Morphology and Mechanical Properties. PVB membranes were manufactured using two different molecular weights of the polymer, resulting in nano- and microsized fibers. On the macroscopic scale (Figure 1A,B), differences between nano- and microfibers cannot be observed; it seemed to be a smooth material, similar to tissue. When observed under SEM imaging, one can easily see the difference in fiber size; see Figure 1C,D. The average fiber diameter for the nanofibers was 335 ± 86 nm, while for the microfibers, it was close to 1 μ m (966 ± 92 nm); see histograms in the Supporting Information Figure S3A,B. The obtained diameter of microfibers was about three times larger than that for nanofibers through an increase in the molecular weight of the polymer used to prepare the solution for electrospinning. Our results confirmed a similar effect observed in another study on controlled fiber diameter by molecular weight of the polymer.⁴⁹ The wettability tests for both types of fiber membranes showed a hydrophobic character with very similar contact angles, reaching 139 \pm 2.9° for nanofibers and 132 \pm 1.8 for microfibers; see Figure 1C,D. The roughness of PVB membranes increased with fiber diameter from 0.75 \pm 0.06 μ m



Figure 2. Cytotoxicity test after 24 h of fibroblasts cultured with the medium incubated with nanofibers from LM_w and microfibers from HM_w PVB for 1, 3, 7, 14, 21, and 28 days; TCPS was used as a positive control and cells incubated with 1% Triton were used as a negative control (A). Cell culture study on electrospun nanofibers from LM_w and microfibers from HM_w PVB showing (B) LDH cytotoxicity test after 4 h and 1 and 3 days and (C) MTS proliferation assay after 1, 3, and 7 day of cell seeding. *Statistical significance calculated with ANOVA, followed by Tukey's post hoc test, p < 0.05; error bars are based on standard deviation.



Figure 3. SEM micrographs showing fibroblast growth on nanofibers after (A,B) 1st, (C,D) 3rd, and (E,F) 7th days in cell culture and on microfibers after (G,H) 1st, (I,J) 3rd, and (K,L) 7th days in cell culture.

for nanofibers (Figure 1C) to $1.45 \pm 0.15 \ \mu m$ for microfibers (Figure 1D), as it was previously described for various electrospun membranes.⁵⁰ The 3D reconstructions based on slice and view tomography, shown in Figure 1E,G, indicate similar porosity. For nanofibers, the size of the 3D reconstruction is 11.064 \times 5.436 \times 6.912 μ m and 40.086 \times $25.67 \times 20.28 \ \mu m$ for microfibers, with the volume of fibers from each membrane being 30.781 and 576.449 μ m³, respectively. As the total reconstructed volume of the nanofiber membrane and microfiber membrane was 415.715 and 21269.648 μ m³, the porosity values for each membrane are 92.6 and 97.3%, respectively. Mechanical testing showed that both membranes have the average maximum tensile stress at the similar range, 0.66 \pm 0.11 MPa for nanofibers and 0.48 \pm 0.12 MPa for microfibers. However, the average maximum elongation is 59 \pm 9% for nanofibers and 199 \pm 19% for microfibers (Figure 1F and also in the Supporting Information Figure S4A,B). Macroscopic pictures of patches and tensile testing indicate that the material is very robust under normal handling situations (Figure 1A,B). What is more is that manufactured materials exhibit high elongation, which is critical in bandages and skin patch applications.

3.2. Cell Culture Study. To demonstrate the possibility of using PVB membranes in the biomedical field, the cytotoxicity of manufactured materials and proliferation of fibroblasts were investigated. A long-term analysis, up to 28 days, of the release of any toxic substances into the cell culture medium from PVB membranes was also performed. LDH release assay for cells cultured in the medium and incubated with PVB fibers for 1, 3, 7, 14, 21, and 28 days showed no significant difference in the absorbance values of the samples; see Figure 2A. None of the absorbance values for PVB samples reached the level for cells incubated with triton, which was a negative control, as shown in Figure 2A. The LDH assays demonstrated that there was no significant cytotoxicity for up to 28 days of material incubation within the cell culture medium. Standard long-term cytotoxicity assays are based on proliferation tests such as MTS (proliferation assay) using medium extracts⁵¹ and encapsulated cells.⁵² In this study, medium extracts were used for LDH assay. Both cytotoxicity assays, based on LDH and additionally on MTS, showed the great biocompatibility of PVB membranes with fibroblasts. We assessed the direct cytotoxicity along with indirect effects of PVB fibers on fibroblasts, through LDH assays performed for up to 3 days in cell culture, which did not show a significant increase in the absorbance

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reading (Figure 2B). Almost a constant level of absorbance proved that there was no release of cytotoxic substances. Based on the small absorbance difference and previous toxicity test, we can conclude no cytotoxic effect. The attachment of cells on membranes was the same—absorbance did not differ between nanofibers electrospun with LM_w PVB and microfibers electrospun with HM_w PVB after 1 day of cell culture. After 3 days, the absorbance value increased for nanofibers and almost reached the value for TCPS; however, microfibers did not enhance proliferation significantly. After 7 days of fibroblast culture, absorbance for microfibers increased, but it was still slightly lower than that for nanofibers, see Figure 2C.

Cell spreading and proliferation in the PVB nanofibers and microfibers are dependent on surface morphology, fiber diameter, wetting properties, and roughness of membranes. Here, different cell behaviors were influenced by preparing fibers using LM_w and HM_w , resulting in two fiber diameters. The microscopy investigation indicated differences in fibroblast shape and flattening on membranes from PVB nanofibers and microfibers after 1 day of cell culture. Fibroblasts were more spread and attached to the nanofibers (Figure 3A,B), while for microfibers, cells kept their round shape (Figure 3G,H). After 3 days, cells were flattened (Figure 3C,D,I,J) and connected to the fibers by creating a lot of filopodia (Figure 4C,D) on both types of membranes. After 7 days of fibroblast



Figure 4. Snapshots from live imaging of fibroblasts after the 5th day on PVB membranes made of (A) nanofibers and (B) microfibers. SEM micrographs focused on cell-fiber attachment and filopodia formation after the 3rd day of cell culture on (C) nanofibers and (D) microfibers.

culture, almost the entire surface of the PVB nanofiber membrane was covered with cells, extending their filopodia further (Figure 3E,F). In contrast, cells were incorporated into the pores of the microfiber membrane, electrospun from HM_w PVB, rather than spread on the surface (Figure 3K,L). This investigation shows that the cell behavior, morphology, and membrane penetration depend on the fiber diameter. The diameter of fibers correlates to the spaces between fibers in electrospun membranes, as the larger fiber diameter increases the pore size.⁵³ The additional images in Figure 4 indicate the variation in filopodia extension once cells start to form a tissue on electrospun PVB nano- and microfibers. The distances between microfibers allow cell penetration inside the membrane, not limiting them to the top surface, and enhance their filopodia formation and extension.¹⁸ SEM observations of cells spreading on PVB nano- and microfiber membranes combined with results obtained by MTS assay suggest that fibroblasts prefer nanofiber membranes electrospun from LM_w PVB, as a smoother surface with lower roughness.⁵⁴

The biocompatibility of PVB fibers was also assessed with a Nanolive tomographic microscope, allowing us to perform live imaging of fibroblasts seeded onto the PVB fibers; see Figure 4A,B. After 5 days of cell culture, we observed many filopodia formed and protruding from cells for both types of membranes with nano- and microfibers. In Figure 4A,B, we show the snapshots from live observation of fibroblast attachment to the PVB fibers. These live observations help to demonstrate the biocompatibility of PVB fibers, showing cell integration and adhesion with electrospun nano- and microfibers. Similar shapes of filopodia were also visible on SEM micrographs after 3 days of fibroblast culture (Figure 4C,D).

3.3. Viscosity and Surface Tension of Oils. For the oil testing, we selected evening primrose, black cumin seed, and borage oil because of their known beneficial effect on AD.^{18–21} The steady shear viscosity was measured at the temperature range from 20 to 40 °C. In all cases, the Newtonian character of the individual oils was observed; see Figure S5 in the Supporting Information. The average viscosity of oils decreased with the increase in temperature, as it was shown in previous studies.⁵⁵ Surface tension for evening primrose, black cumin seed, and borage oil was very similar; see Table 1.

Table 1. Average Surface Tension and Viscosity at 20 °C for Evening Primrose, Black Cumin Seed, and Borage Oil

oil	evening primrose	black cumin seed	borage
surface tension [mN·m ⁻¹]	32.5 ± 1.1	32.6 ± 0.7	32.3 ± 1.0
viscosity [mPa·s]	54.6 ± 0.1	44.3 ± 0.2	18.7 ± 0.1

3.4. Oil Wetting Behavior on Fibers. The wetting of PVB fibers was investigated with oils using cryo-SEM (Figure 5). Borage oil exhibits different spreading behaviors on nanoand microfibers. For nanofibers, the borage oil penetrated the fiber network more evenly and individual fibers covered in oil were observed (Figure 5A). In the case of microfibers, the oil wetted only the outermost layers of fibers and sharp ends of oil droplets can be observed with no leakage into neighboring fibers; see Figure 5D. These observations indicate the different wetting behaviors caused by fiber diameters ranging from 300 nm to 1 μ m. Black cumin wetting on nano- and microfibers was similar but did not follow the way of spreading like two other oils (Figure 5B,E). Similar to borage oil, vast differences in evening primrose oil wetting on different types of fibers can be observed. For microfibers, we notice strands of fibers branching out from droplets of oil that were wetted and oil crept hundreds of micrometers worth of bundles until it reached other droplets/oil deposits; see Figure 5F. For nanofibers, such behavior was not observed (Figure 5C). A similar behavior has been already observed for polystyrene, where fibers with smaller diameter and lower roughness showed higher oil sorption. It was reported that oil is absorbed



Figure 5. Cryo-SEM micrographs showing oil spreading: borage (A,D), black cumin seed (B,E), and evening primrose (C,F) on nano-(A-C) and microfibers (D,E).



Figure 6. Images showing the oil droplet behavior on PVB nano- and microfibers at different time intervals. (A-F) Borage oil on nano- and microfibers (G-L), black cumin seed on nano- and microfibers (M-S), and evening primrose on nano- and microfibers (J-L). The graphs representing the surface area measurements of oils on PVB nanofiber and microfiber for borage, black cumin seed, and evening primrose oil (T,U)—graph showing the maximum spread of the oil droplet in electrospun PVB nano- and microfibers over a 60 min test.

by trapping on the mesh surface and then incorporated into the material. $^{\rm 22,56}$

3.5. Oil Spreading Test on Nano- and Microfibers. The wetting of PVB fibers with oils was observed throughout the macroscopic investigation; see Figure 6. The membrane



Figure 7. Human skin test of PVB electrospun patches with evening primrose oil release from nano- and microfibers (A) before oil application, (B) immediately after oil application on the patch placed directly on the skin, (C) after 30 min, (D) after 60 min, and (E) skin after removing the patches.

surface area coated with oils was higher for nanofibers for all tested oils (Figure 6T), especially for borage and evening primrose oil, where the area was about three times higher for nanofibers than that for the microfiber membrane. In the case of microfibers, the increase in area was slower and plateaued earlier than that for nanofibers (Figure 6T), whereas black cumin seed oil represented a different behavior, as also cryo-SEM results indicated, for both nano- and microfibers. The increase in surface area was during the first 10 min; then, it had almost a constant value for both of nano- and microfibers (Figure 6T), as proved by the pictures of spread oil (Figure 6H,I,K,L) and also with cryo-SEM micrographs (Figure 5B,E). The surface area of black cumin seed oil was about 3 and 2 cm² on nanofibers and microfibers, respectively. Borage oil was continuously spreading on nanofibers for up to 60 min and reached a surface area of about 9 cm^2 (Figure 6C,T). For microfibers, the surface stopped changing after 15 min and reached 5 cm²; see Figure 6E,F,T. A similar behavior was observed for evening primrose oil (Figure 6M-S), which also reached 9 cm² for nanofibers but only 3 cm² for microfibers. The oil spreading tests confirmed results obtained with cryo-SEM; for nanofibers, oils were diffused into the porous interior, which is visible in Figure 6B,C,N,O, where an outer ring of oil can be seen, whereas for microfibers, they remained on the surface of fiber meshes. We saw the oil divergent behavior related to surface morphology on the basis of fiber diameter and correlated pore size. The obtained results allow us to verify if oil will remain on the fiber surface or diffuse into the porous interior, as shown in 3D reconstruction in Figure 1E,G. In Figure 6U, we compared the maximum spreading area during a 1 h test for all three oils, starting from the highest viscosity, evening primrose and black cumin seed, and the lowest viscosity, borage oil. The spreading area in nanofibers is much larger regardless of the viscosity of the oil. The most striking result to emerge from the data is that the size of electrospun fibers is the main parameter controlling the spread of the oils. The collected data indicate the suitability of electrospun PVB membranes to be used as a carrier for various oils in the form of skin patches, with the controlled fiber diameter giving the possibility to govern oil spreading and release.

Before oil application in the preliminary oil release test on humans, both patches were attached to the skin; see Figure 7A. Evening primrose spreading was noticed immediately after oil deposition (Figure 7B), helping us to stick the patch to the skin. After 30 min (Figure 7C), the nanofiber patch was almost invisible because of the oil spreading between fibers. The microfiber patch was less sticky to the skin after the oil deposition as its spreading is smaller, also indicated by the previous results (Figure 6). The 1 h test on the skin showed in Figure 7 indicates the applicability of easy-to-handle electrospun PVB patches. The percentage oil release for nanofibers was 10.1%, while for microfiber patches, it was 12.9%. These preliminary results show the possibility to control oil release through the fiber diameter and also design long-time release, keeping the skin moisturized.

4. CONCLUSIONS

PVB electrospun patches with nano- and microfibers showed high biocompatibility in direct contact with fibroblasts by live imaging observation, proliferation testing, and SEM observations. The indirect long-term storage of PVB fibers in cell culture medium confirmed the noncytotoxic effects of membranes. The diameter of fibers influenced fibroblast proliferation, with smaller fiber diameters and higher membrane roughness of nanofibers enhancing cell development. Also, mechanical properties depend on fiber size; higher elongation was obtained for bigger fibers. PVB patches were tested with the selected oils for atopic skin treatment, borage, black cumin, and evening primrose, which showed Newtonian fluid character in the viscosity tests. The highest viscosity oil was evening primrose, then black cumin seed and borage oil, which decreased with higher temperature T = 40 °C. Surface tension was the same for all tested oils. The obtained nanoand microfiber membranes are hydrophobic surfaces, so lowsurface tension liquids, such as oils, can rapidly spread.⁵⁷ The small fiber diameter of nano- and microfibers $(335 \pm 86 \text{ nm})$ and 966 \pm 92 nm) and high porosity of the meshes (92.6 and 97.3%) have an impact on sorption and favor the adhesion of oils.²² Both cryo-SEM and oil spreading tests showed that nanofibers are better carriers for low-viscosity oils, as they penetrate the structure, extending the spreading further in comparison to microfibers, on which oil remains on the membrane surface. The single most striking observation to emerge from the data comparison was the importance of fiber diameter in the patches. By manipulating the diameter of fibers, we are able to adjust the roughness of the PVB membrane, which affects the wetting with oils. Electrospun membranes are easy to handle, showing great potential for application as bandages or patches because of the high surface area-to-volume ratio, good mechanical properties, and permeability as required.¹³ Our PVB meshes all fulfill all these requirements, especially with their flexibility and high elongation, additionally being an excellent oil carrier. The PVB patches are able to deliver natural oils to skin over a desired period of time to keep it moisturized. The reported results are the first steps in the design of patches for atopic skin treatment using oils.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.0c00854.

Scheme of experimental setup for oil spreading; method of area measurement in the ImageJ software; scheme of fiber diameter distribution and roughness analysis; graph with stress-strain curves; and relative viscosity in dependence on shear rate for all tested oils (PDF)

AUTHOR INFORMATION

Corresponding Author

Urszula Stachewicz – International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland; orcid.org/0000-0001-5102-8685; Phone: +48 12 617 5230; Email: ustachew@agh.edu.pl

Authors

- Zuzanna J. Krysiak International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland
- **Łukasz Kaniuk** International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland
- Sara Metwally International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland
- Piotr K. Szewczyk International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland; orcid.org/0000-0003-1441-7387
- **Ewa A. Sroczyk** International Center of Electron Microscopy for Material Science, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland
- **Petra Peer** Institute of Hydrodynamics of the Czech Academy of Sciences, Prague 16612, Czech Republic; © orcid.org/0000-0002-1426-212X
- Paulina Lisiecka-Graca Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow 30-059, Poland; orcid.org/0000-0002-7861-6847
- **Russell J. Bailey** School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS, U.K.
- Emiliano Bilotti School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS, U.K.; orcid.org/0000-0003-3952-1148

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.0c00854

Notes

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