FABRICATION OF FILTER MEMBRANE OF ORGANIC COMPOUND TO PROTECT THE UPPER RESPIRATORY TRACT FROM VIRAL AND BACTERIAL INFECTIONS, INCLUDING SARS-CoV-2, COMPLIANT WITH FFP2 STANDARD

Natalia Brzezińska[1*], Maciej Pyza[1], Anna Kłeczek[1], Karolina Kulińska[2], Jadwiga Gabor[1], Żaneta Garczyk-Mundała[1], Andrzej Swinarew[1,2]

[1] Faculty of Science and Technology,
University of Silesia,
75 Pułku Piechoty 1A, 41-500 Chorzów, Poland
[2] Institute of Sport Science,
The Jerzy Kukuczka Academy of Physical Education,
Mikołowska 72A, 40-065 Katowice, Poland
*e-mail: natalia.brzezinska9@op.pl

Abstract

Bacteria, viruses, and other pathogens in the surrounding environment are biological threat to human health and life. The development of various diseases in the world, as well as the pandemic caused by the rapid spread of the SARS-CoV-2 virus, have increased the demand for the use of upper respiratory protection devices. Out of concern for the natural environment, the aim of this work was to develop an innovative solution i.e. the FFP3 filtering membrane made of an organic compound. First, preliminary tests of the FFP2 mask were carried out to assess the chemical composition, morphological structure - fibers geometry, thickness, density, and arrangement. The FTIR analysis study was conducted to confirm that the main chemical in the mask was polypropylene (PP) and high-density polyethylene (HDPE). Optical, confocal microscopy and computer microtomography studies showed the fibers structure. They were densely arranged and their thickness was less than 1 den. The fiber structure of the FFP2 mask was also compared before and after immersing in betulin, an organic compound obtained from birch bark via the Soxhlet extraction. In addition, the assessment of microbiological activity was made on the reference strain Escherichia coli ATCC 25922, and the anti-inflammatory activity on normal human skin fibroblasts on polycarbonate with betulin. The studies showed that betulin supported the material antibacterial and anti-inflammatory properties.

Keywords: *face masks, viral and bacterial infections, betulin, filtering membrane, FFP2 standard*

[Engineering of Biomaterials 166 (2022) 2-11]

doi:10.34821/eng.biomat.166.2022.2-11

Submitted: 2022-12-31, Accepted: 2023-02-24, Published: 2023-02-28

Introduction

Bacterial and viral infections pose a real threat to human health and life. In current times, the need for the use of upper respiratory protection devices has increased significantly due to the rapid spread of the SARS-CoV-2 virus around the world which is highly contagious and dangerous. The virus is one of the β-coronaviruses that causes a contagious respiratory disease called Covid-19. It is characterized by a round, near-oval shape and has a diameter of 0.06-0.14 μm. The SARS-CoV-2, like other RNA viruses, can evolve genetically with the development of mutations over time, resulting in variants that may have different characteristics from the original virus strain [1]. During the ongoing pandemic, several cases of SARS-CoV-2 have been singled out, only a few of which have been recognized according to the World Health Organization (WHO) as variants of concern in terms of both the impact on public health and the spread rate. According to the WHO epidemiological data as of 22 June 2021, 4 variants of SARS-CoV-2 have been detected since the pandemic beginning: Alpha, Beta, Gamma, and Delta. As of 22/06/21, according to WHO, the Alpha variant has spread to 170 countries, the Beta variant to 199, the Gamma variant to 71, and the Delta variant to 85. The global case fatality rate for Covid-19 is 2.2%, according to WHO estimates [2]. It should also be mentioned that new mutations of the virus may still appear and therefore differ in the structure and course of the disease [3]. The SARS-CoV-2 virus is transmitted by the droplet route. The infection can occur not only through contact with an infected person, but also through exposure to the environment in which the sick individual has previously lived. This was proven by a study by N. van Doremalen et al. who showed that SARS-CoV-2 virions suspended as an aerosol in an environment with a temperature of 21-23°C and a relative humidity of 65% was still infectious 3 hours after spraying. It also turned out that the SARS-CoV-2 virus was able to survive on different types of surfaces - e.g. on copper for about 4 hours, up to 24 hours on cardboard and up to 72 hours on stainless steel or plastic [4].

To protect the population, it is necessary to maintain an appropriate social distance and use masks with high filtration efficiency. Their function is to trap airborne particles (natural or man-made) as well as biological organisms, e.g. bacteria, viruses, fungi, or other pathogens [5]. Respiratory masks equipped with a suitable filtering membrane can effectively protect the respiratory system [6].

There is a variety of masks available on the medical market. There are simple surgical masks which are designed to protect the wearer from the spread of microorganisms, loosely fitted to the wearer's face as well as FFP3/N95 masks used as protection against inhalation of small airborne particles. Each of these devices is also characterized by different filtration efficiency and performance [7].

The classification divides masks into three FFP (filtering facepiece) safety classes, depending on the degree of protection according to the European standard (EN 149) [8]. The protection classes FFP1, FFP2, and FFP3 provide respiratory protection against various concentrations of contaminants found in the surrounding environment. The FFP1 class has the least protection against biohazards - it allows for the filtration of at least 80% of the particles found in the air which are no larger than 0.6 μm. The FFP2 standard provides protection against solid and liquid dust, smoke and aerosol particles that can have adverse effects on health, especially on the respiratory system. The threshold value for capturing these types of particles from the air is at least 94%, and their size does not exceed 0.6 μm.

The highest degree of respiratory protection against various types of contaminants is provided by the FFP3 class which filters out carcinogenic particles and radioactive substances. It also ensures defence against pathogens, such as viruses, bacteria, and even fungi. It protects against solid and liquid dust that is harmful to the human body and smoke and aerosol particles up to 0.6 μm in size. The filtration value is at least 99% - therefore, it can be considered high and effective protection [9].

Particles found in the surrounding environment have different sizes, shapes and properties, that is why it is so important to develop a mask with a filtering membrane with high performance while maintaining the comfort of use and good breathability [10]. As viruses and bacteria are very small particles that can easily penetrate the layers of the filter, the right material is a key issue. The specifications should focus on the proper chemical composition, porosity, as well as the thickness and density of the fibers arrangement in the material [5].

In order to provide the material with antibacterial and antiviral properties, a well-established method is the use of silver ions. However, from the point of view of clinical microbiology, silver is unfavourable since its ions can sterilize the environment due to the wide spectrum of their activity. Another important factor that also limits the use of nanosilver is the relatively high price [11,12]. Therefore, it is necessary to look for alternative and safer materials with antimicrobial properties.

Current joint solutions are neither economical nor environmentally friendly, which motivates researchers to find an environmentally friendly material that can be used to build the filter membrane in respiratory protective equipment.

The aim of the study was to obtain the biostatic surface on the personal protective face mask. We used betulin, an organic compound easily obtained via extraction with simple alcohol. Large amounts of betulin are found in the outer bark of white birch species (25-30% content). Bark is a readily available raw material as a by-product in paper mills; therefore, betulin can be produced in a low-cost extraction process, even on an industrial scale [13]. Betulin and its easily obtained derivatives show not only antimicrobial and antiviral properties, but also other beneficial biological behaviours, e.g. anticancer, anti-inflammatory, hepatoprotective, and antilithic activity already at very low concentrations, in the absence of toxicity both in vitro and in vivo [14-16]. The anti-inflammatory effect results from the inhibition of cell migration to the inflammation sites, thus it will not cause dermatitis. The antiviral betulin property results from inhibiting the virus life cycle in the infected cell at an early stage, which prevents its further development and infection induction. Betulin has an interesting structure with two hydroxyl groups (FIG. 1) at C-3 and C-28, and an isopropenyl group at C-19. As a biofunctional monomer of natural origin, it provides a convenient starting material for many chemical modifications, including polymer synthesis.

The use of betulin as a modifier in the material is one of the goals of this study, in which we evaluated the betulin-modified polycarbonate for antibacterial and anti-inflammatory activity.

The test was also carried out on samples of KN95 (FFP2) mask layers. The chemical composition of these samples was characterized by the Fourier transform infrared (FTIR) spectroscopy.

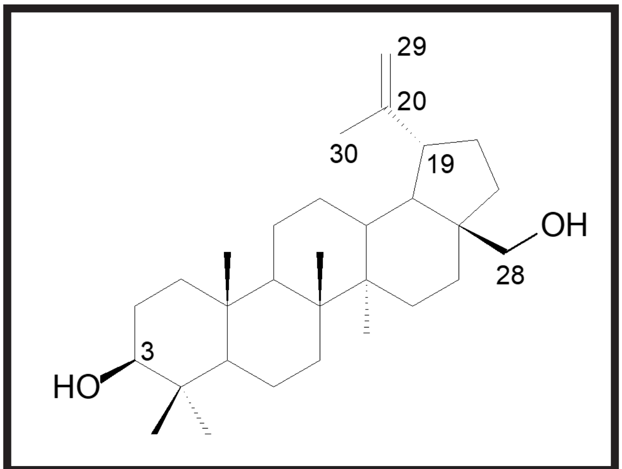

FIG. 1. Chemical formula of betulin.

The geometry of the fibers, their structure, thickness, and arrangement density were assessed using optical and confocal microscopy, as well as computed microtomography. The sample was immersed in a solution of betulin obtained via the Soxhlet extraction, and the results were compared on an optical and confocal microscope to illustrate the differences in the fibers structure.

Materials and Methods

The facemask subjected to preliminary testing regarding the filtration efficiency was a medical KN95 one- equivalent to the FFP2 class (FIG. 2). The tests provided information on the material properties, chemical composition and morphological structure of the mask layers.

The first research step was the FTIR infrared spectroscopy test used to obtain an IR spectrum based on the amount of absorption or IR transmittance in a sample. For accurate testing, the KN95 mask was separated into layers (FIG. 3), and then each layer was subjected to the FTIR analysis to determine the chemical composition of the mask.

The study was carried out on an FTIR spectrophotometer - IRTracer-100, equipped with an ATR accessory (attenuated total reflectance, ATR). The measurements were carried out at 100 scans per sample. The obtained results were compared with the spectra library.

FIG. 2. KN95 mask.

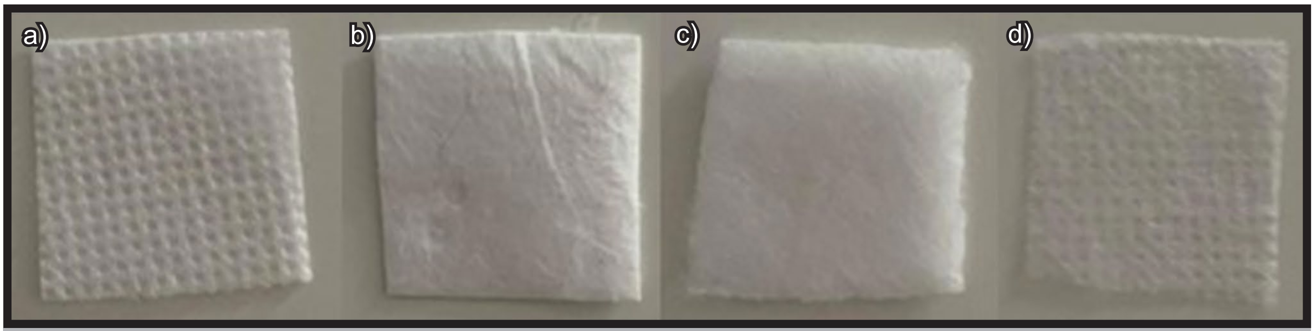

FIG. 3. Layers of the mask filter: a) first layer – outer part, b) first layer – inner part, c) second layer, d) third layer.

To ensure high filtration efficiency against harmful particles, it is important to select the right material with regard to its chemical composition, morphological structure - the diameter, thickness, and density of the fibers arrangement. For this purpose, a test was carried out using an Axiovert 40 MAT optical microscope connected to a PowerShot A640 camera. After placing the sample on the table, the microscope was adjusted to obtain better-quality images. They were taken at different magnifications so as to achieve a detailed analysis of the fibers structure. The surface images were analyzed using ImageJ software.

To obtain the microscopic images, the LEXT OLS4000 scanning confocal microscope was used. The surface images were analyzed using MountainsMap® Premium software.

The next stage of the research was computed microtomography to thoroughly examine the object without interfering with its structure. The technique also made it possible to distinguish areas with different values of the linear X-ray attenuation coefficient, proportional to the density of the tested material. This study was conducted using the GE Phoenix v|tome|x high-resolution x-ray scanner. The parameters of the microtomographic examination were as follows: voxel size (μm^3): 10, number of projections: 1000, image resolution (px): 2024 × 2024, detector type: dxv–250, number of photos to average: 3, number of skips: 0, accelerating voltage (kV): 80, glow current (μA): 130, filters: none, scan time (min): 15.

The result was a three-dimensional density map creating a precise 3D model used in further research [17]. Three-dimensional images of the samples were obtained using the MyVgl2 program. Computed microtomography yielded results on the structure of the material and the pore size.

In addition to the structural studies of the mask, we assessed the microbiological activity of the produced polymer modified with betulin. Betulin was obtained by the Soxhlet extraction using ethanol in the Soxhlet apparatus consisting of three parts: a flask, an extractor, and a reflux condenser. The solid - birch bark - was placed in a thimble made of filter paper. The flask contained the solvent - ethanol which boiled after the flask was heated with a heating mantle. The alcohol vapor passed to the reflux condenser. After condensation, the solvent accumulated in the thimble. The liquid with the extracted substance was poured into the flask through a siphon closure where the solvent was then distilled again. The process was long as the sample was extracted repeatedly, while changing the thimble and its contents, until the betulin appropriate concentration was obtained, namely when its colour changed. The higher the concentration, the more brown the substance in the flask. The proper concentration was achieved due to the closed circulation and distillation of the solvent. FIG. 4a shows the colour of the substance in the flask before heating, FIG. 4b during the boiling process, after the liquid with the extracted substance was transferred to the flask, and FIG. 4c depicts the concentration of betulin after several extractions.

FIG. 4. Process of extracting betulin from birch bark: a) before heating, b) during boiling, c) high concentration of botulin.

The modifier obtained according to patent 235673 - can be applied to the material in several ways, e.g. by spraying a betulin solution onto the finished material by ultrasonic atomization (nebulization). The disadvantage of this technique is the high cost of the equipment. Another option is to immerse the material in a betulin solution to endow it with antibacterial and antiviral properties. However, the easiest approach is to mix a betulin modifier into already available polymeric materials, such as polycarbonate, polylactide, polypropylene, polyvinyl chloride, high pressure polyethylene, and polyamide. According to the patent 235673, the method of obtaining a modified thermoplastic polymer with antimicrobial and anti-inflammatory properties is essential. It consists in introducing a thermoplastic base polymer and its modifier into the reactor. The polymer is in the form of granules, aggregates or meal, betulin with a purity of ≥ 75% is in the form of a purified powder or a suspension in alkoxides, preferably in propylene glycol, in a weight ratio of from 5:1 to 100,000:1, preferably from 20:1 to 100:1. The ingredients are mixed for 10 to 90 min until a uniform coverage of the polymer surface is obtained, and then dried for at least 1 h at a temperature of 10 to 110°C, depending on the technological parameters, i.e. the structure and processing temperature of the polymer used (granulate or aggregate or grits). The resulting mixture of betulin with a suitable polymeric material is a blend. Then, a granulate or a filament of neutral colour is obtained from the blend, following further plastic processing carried out by a selected method.

In our study, the polycarbonate mixed with betulin was obtained as follows: the granulated polycarbonate (1500 g) and the betulin powder (7500 mg) with a purity of ≥98% were introduced into the reactor i.e. a stainless steel mixer with a capacity of 3000 cm^3, equipped with an electric charge discharge system at 25°C. The ingredients were mixed using a Teflon agitator with scraper blades for 30 min at a speed of 50 rpm, after which the mixture was aerostatically dried at 100°C for 24 h. The dry mixture was extruded using a single-screw, four-zone screw extruder with a screw of 32 l/d using the temperatures of heating zones on the head and the next three zones (265°C, 230°C, 210°C and 160°C, respectively) and an extrusion speed of 60 rpm. The resulting material was collected on a conveyor belt, air-cooled over a three-meter section, and then tested for microbiological activity.

As described above, the studied material was obtained according to the patent 235673 - it was the polycarbonate containing betulin in the range of 0.025-0.5%. This evaluation of its antimicrobial activity was performed in accordance with ISO 22196: 2007 (E) “Plastics - Measurement of antibacterial activity on plastics surfaces”. The manufactured material was prepared as a control and test sample in the form of a square with a size and thickness of 50 mm x 50 mm. A reference strain of Escherichia coli ATCC 25922 was used as the reference material, with a bacterial inoculum of 0.4 ml and a concentration of 6 x 10^5 bacteria/ml. The incubation of inoculum samples took place at 35°C, with a humidity of no less than 90%, for 24 h. The neutralization of each sample was carried out in accordance with PN ISO 18593: 2005; PN ISO 14562: 2006. After a series of 10-fold dilutions, the samples were incubated in Petri dishes under the conditions described in the standard. For both the test and control samples, the factor N, the number of live bacteria recovered per cm^2 of the sample, was calculated.

In addition to evaluating the antimicrobial activity, the anti-inflammatory activity of the obtained polymeric materials was also tested in accordance with ISO10993-5:2009(E). The study was carried out using normal human dermal fibroblasts (NHDF cell line, CC-2511; Clonetics, San Diego, CA, USA), cultured for 24 h in the FBM medium (Fibroblast Basal Medium; Lonza, Basel, Switzerland), enriched with hFGF-B (Human Fibroblast Growth Factor-basic), insulin and gentamicin (FGMTMSingleQuotsTM; Lonza, Basel, Switzerland). The anti-inflammatory activity was assessed by analyzing changes in the cell transcriptome, determined by expression microarray, using HG-U133A plates. The validation of the array experiment was performed by the qRT-PCR.

Results and Discussions

The preliminary studies on the structure and layering of the FFP2 mask filter proved that several factors affected its antibacterial and anti-viral efficiency, e.g. the material of the filter, its porosity, and the fibers properties - density, diameter, and thickness of the arrangement. Such material characteristics was studied by the FTIR analysis, optical microscopy and computed microtomography.

The FTIR spectroscopy was chosen because of its speed and the lack of interference with the sample structure. Thus, we could identify the type of material, the structures of matter at the molecular scale, and the additives that may change its properties. The FTIR analysis consists in producing an optical signal with all IR frequencies encoded in it. The next step is decoding the signal using the Fourier transform and then mapping the spectral information [17]. The result is a graph, a spectrum, which in turn is searched in reference libraries to identify a given sample. In our study, after placing the samples (the KN95 mask layers) in the IRTracer-100 spectrophotometer, the following spectra plots were obtained sequentially.

Based on the obtained reports, the main chemical compound was polypropylene (PP) found in the first layer (outer and inner), as well as in the third layer (FIGs 5, 6, 8). It is a polymer that belongs to the polyolefin group and is used in the production of plastics in both industry and in medicine. It is characterized by high chemical resistance, good air permeability and low water vapor permeability. Therefore, it is suitable for use in masks or other upper respiratory protection devices.

The chemical compound found in the second layer was high-density polyethylene, HDPE (high density PE) (FIG. 7). It is a thermoplastic polymer made from ethylene which has high tensile strength and is chemically unreactive. Both polypropylene (PP) and polyethylene (HDPE) are suitable materials for use in upper respiratory protection devices. On the other hand, for an innovative and more environmentally friendly solution, a better material for filters in masks would be cellulose fibers obtained from an organic compound. Natural fibers are not antibacterial or antiviral. It is modifiers, e.g. silver ions, that can provide such antibacterial and antiviral properties. In addition, silver ions also exhibit antifungal properties [18]. However, it should be noted that the silver release can lead to environmental sterilization as a result of its broad action spectrum. Therefore, silver must not be present in the filter layer in direct contact with the user’s face. Moreover, silver ions exhibit many cytotoxic characteristics against human cells [11,19]. Another important factor that inhibits the widespread use of nanosilver is its relatively high cost. For this purpose, we produced a filter membrane with betulin and subjected it to biological activity tests with a polymeric material.

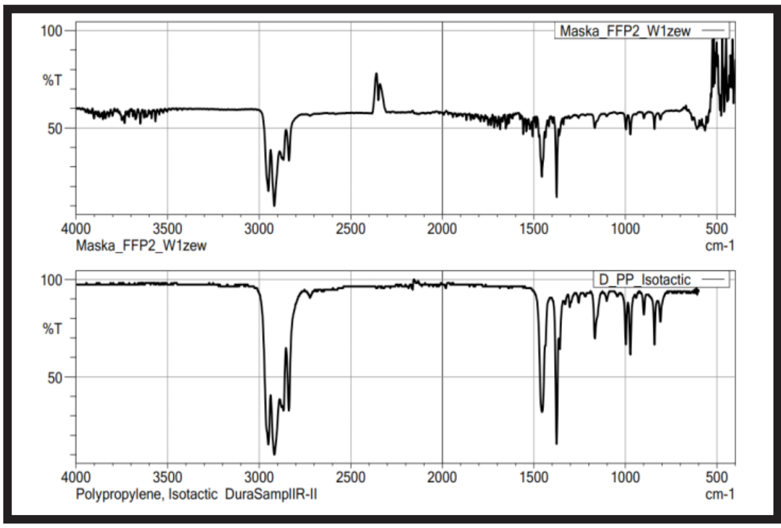

FIG. 5. FTIR analysis plot for the first layer - outer part.

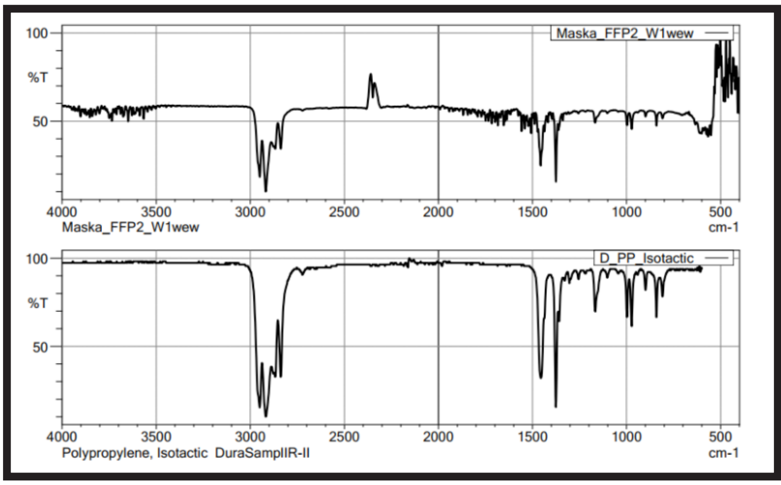

FIG. 6. FTIR analysis plot for the first layer - inner part.

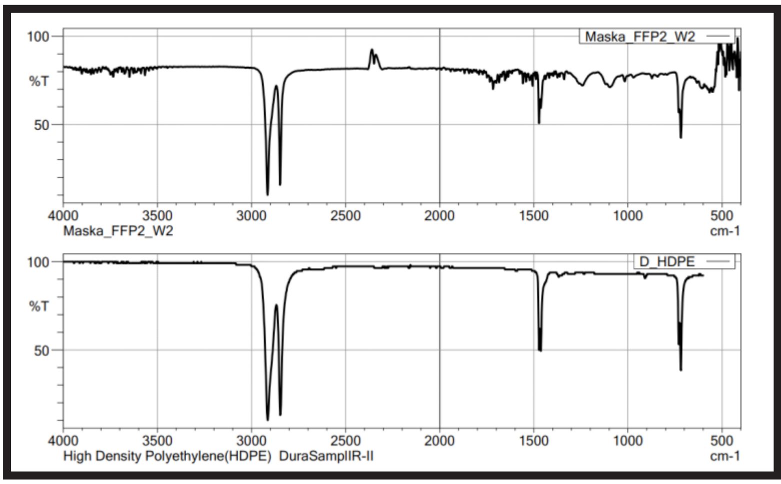

FIG. 7. FTIR analysis plot for the middle layer.

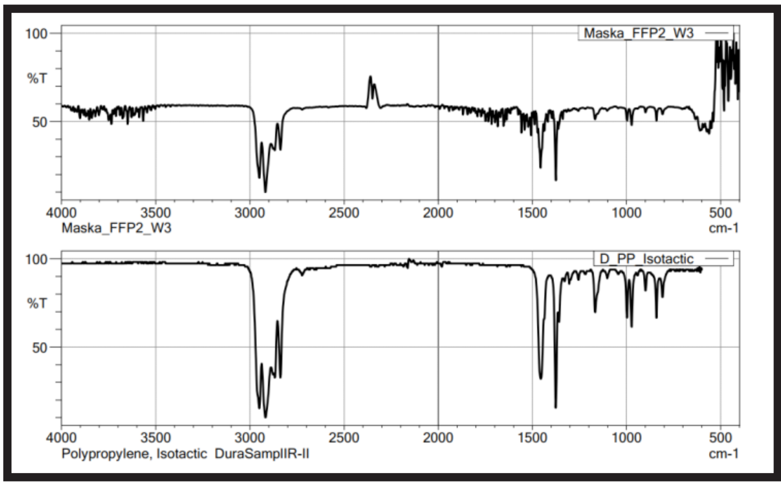

FIG. 8. FTIR analysis plot for the third layer.

Besides betulin, the filter material can also be enriched with electrostatic charges. Filters using electrostatic fields can separate various ionized impurities. After electret treatment, the filter material will receive a positive charge, allowing the filtration efficiency to increase significantly. Biological particles, such as viruses, bacteria and aerosols, are negatively charged and become trapped or blocked by the generated electric field as they are carried by the airflow through the positively charged fibers [20].

Thanks to the optical microscopy images, the geometry (length, thickness) and fibers arrangement of the FFP2 mask was clearly seen before immersing it in the betulin solution (FIGs 9, 12, 15). The LEXT OLS4000 scanning confocal microscope was also used to analyze the mask after the immersion in order to impart antibacterial, antiviral and anti-inflammatory properties (FIGs 10, 11, 13, 14, 16, 17), and to compare the fibers structure.

Using a confocal microscope, changes in the morphology of the mask fibers were observed and compared to the optical microscopy samples that showed smooth fibers. The uneven distribution of betulin particles was clearly observed in the confocal microscope images (FIGs 10, 13, 16). Numerous particles gathered in some areas, while other areas were covered with only a few particles.

The fibers diameter of less than 100 nm defined them as nanofibers, and their thickness was less than 1 denier (unit of linear density of synthetic fibers). The synthetic polypropylene fibers were densely spaced and had characteristic folds, providing a barrier to viruses and bacteria or other airborne contaminants. The shorter the fibers, the more air they are able to hold, and this will allow them to retain more particles and further aid thermal insulation. The appropriate size of the fibers or their arrangement significantly improves effectiveness of filtration, which should be taken into account when choosing the right filter material.

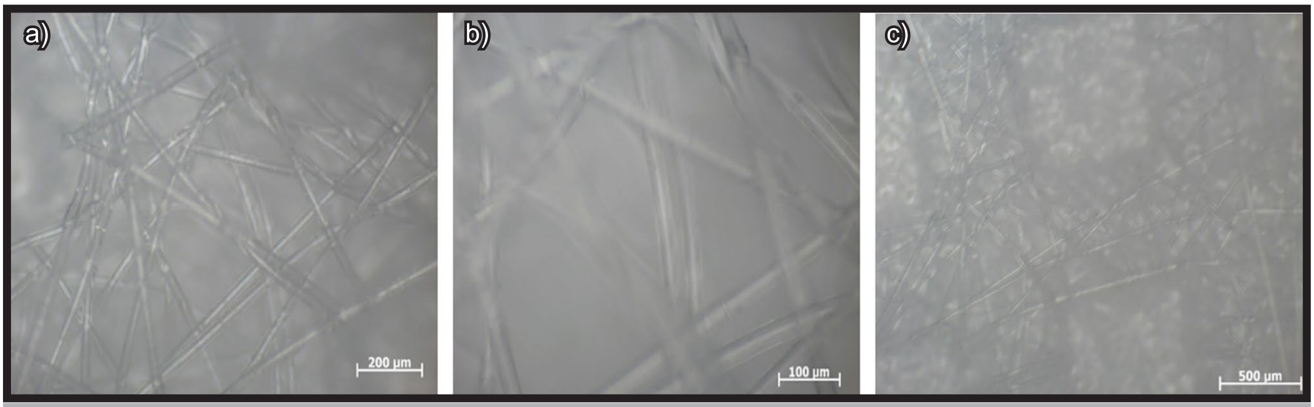

FIG. 9. Optical microscope images of fibers for the first layer (inner and outer).

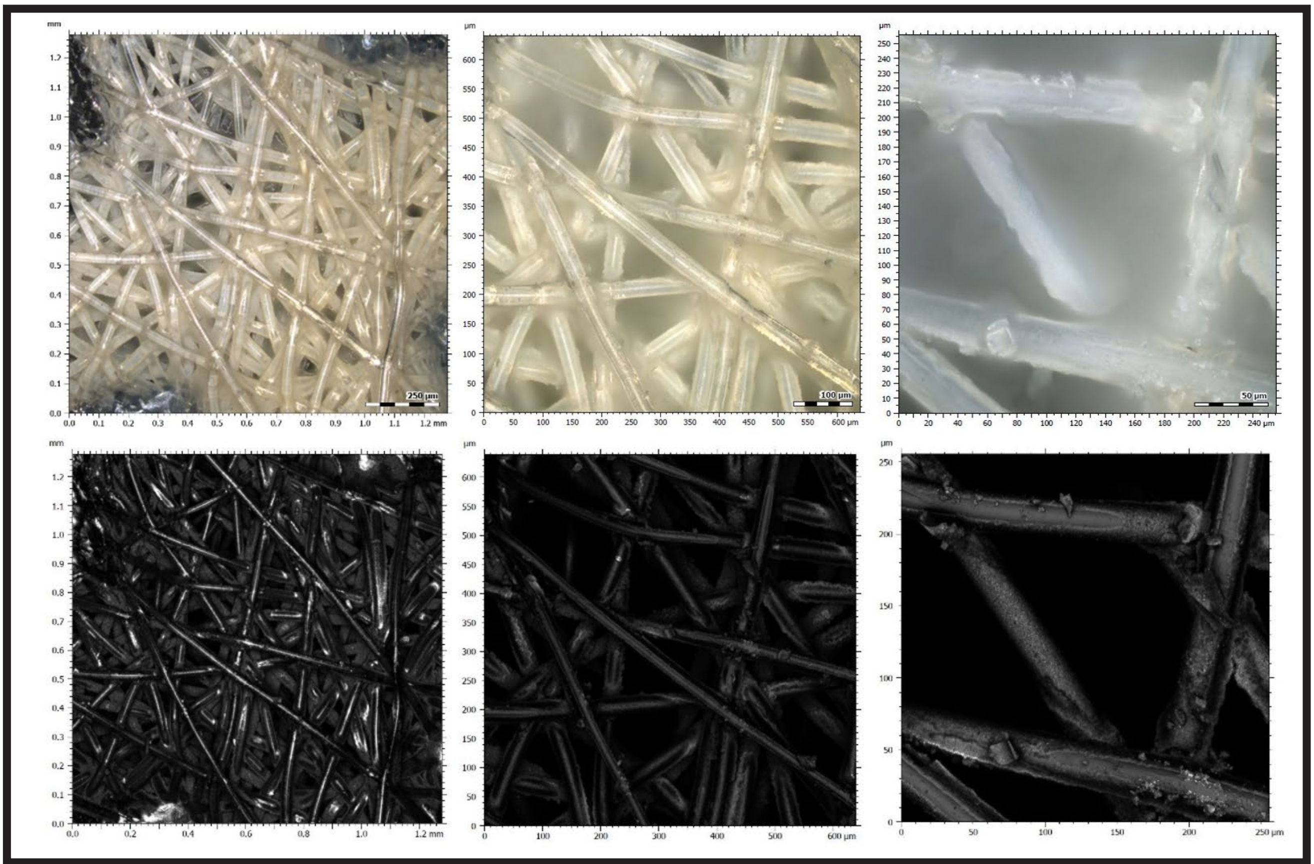

FIG. 10. Confocal microscope images of fibers for the first layer (inner and outer).

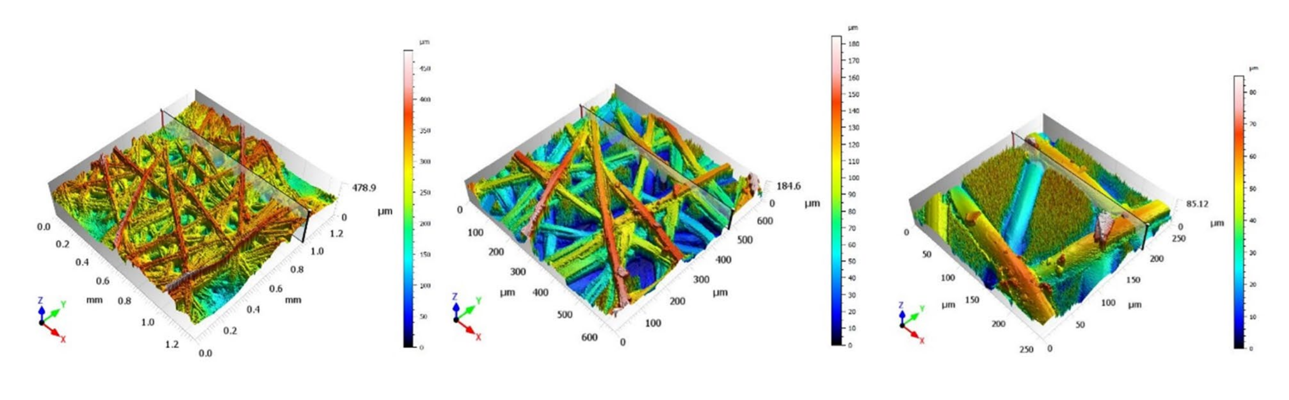

FIG. 11. 3-D view of fibers for the first layer (inner and outer).

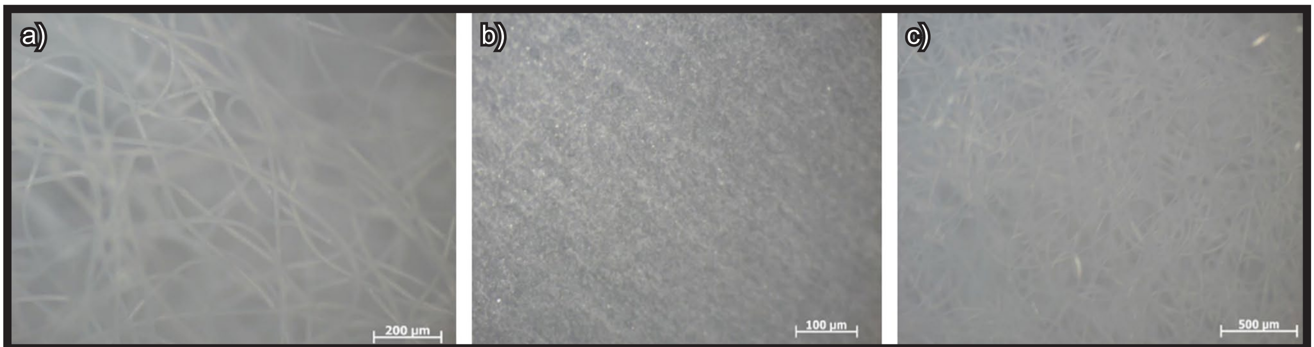

FIG. 12. Optical microscope images of fibers for the second layer.

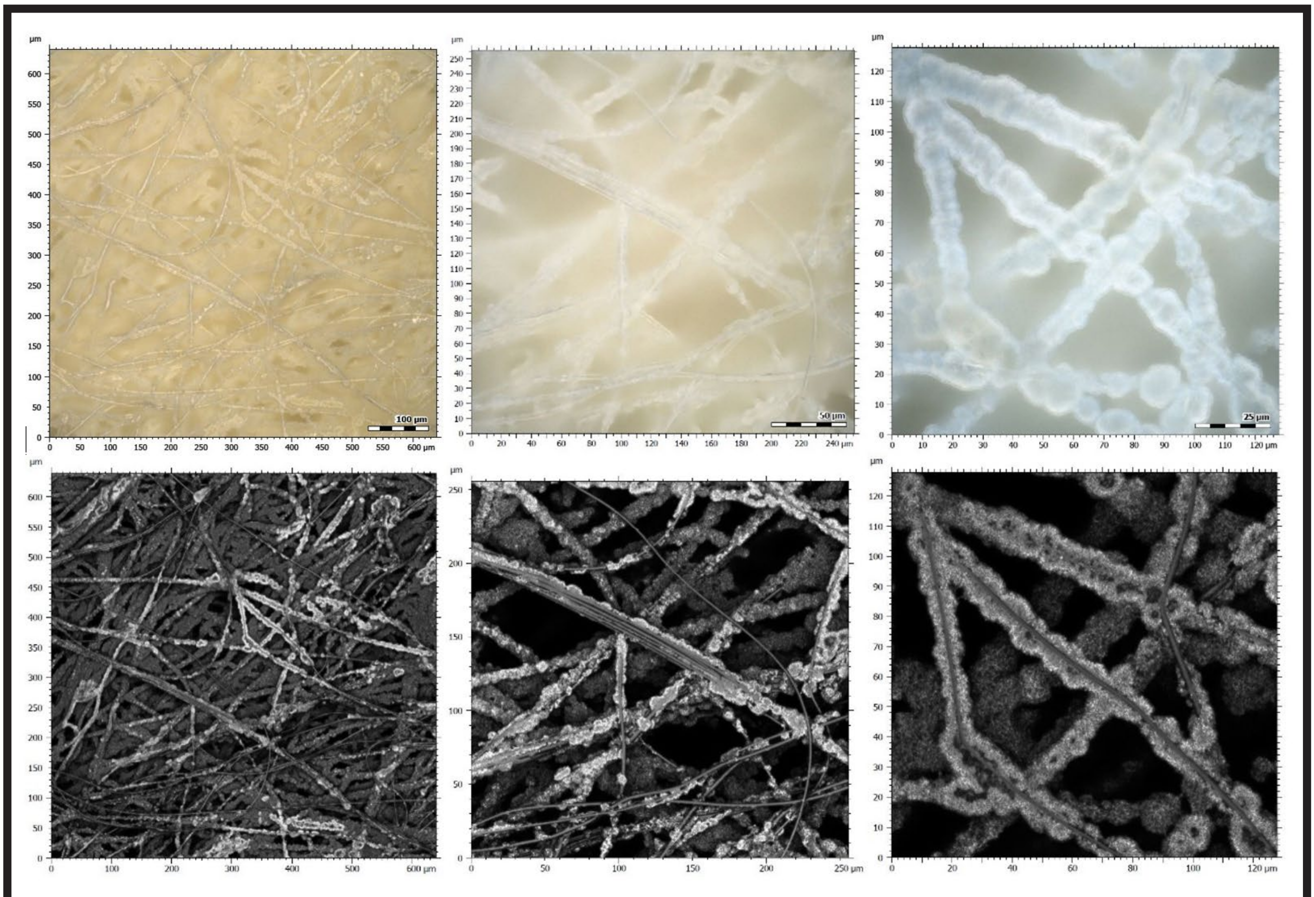

FIG. 13. Confocal microscope images of fibers for the second layer.

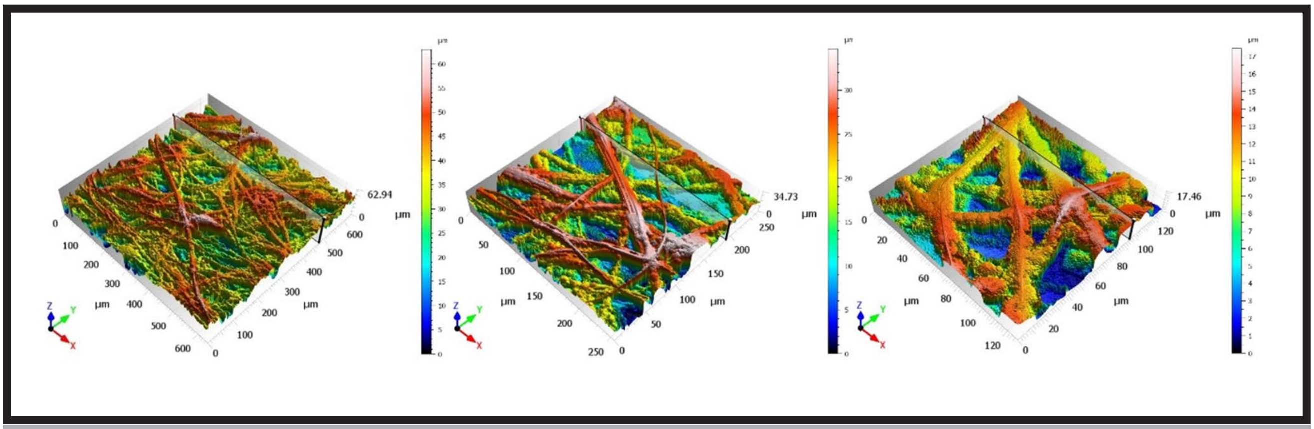

FIG. 14. 3-D view of fibers for the second layer.

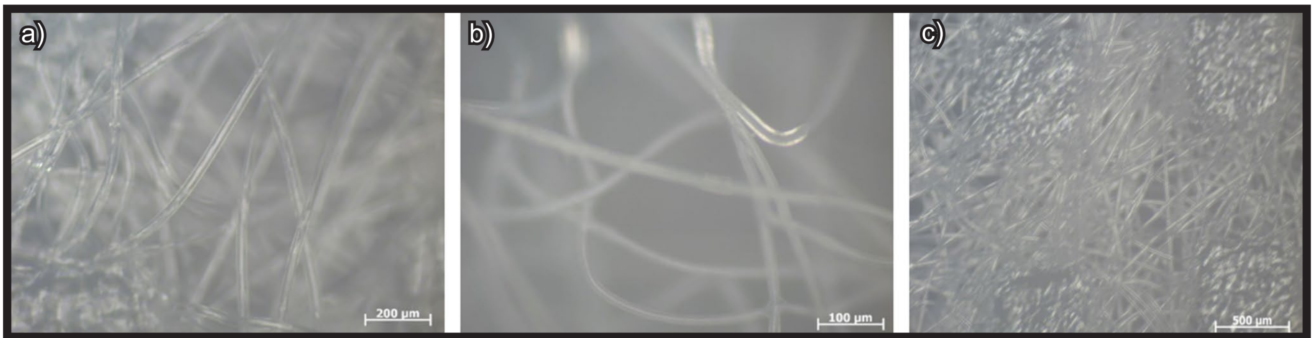

FIG. 15. Optical microscope images of fibers for the third layer.

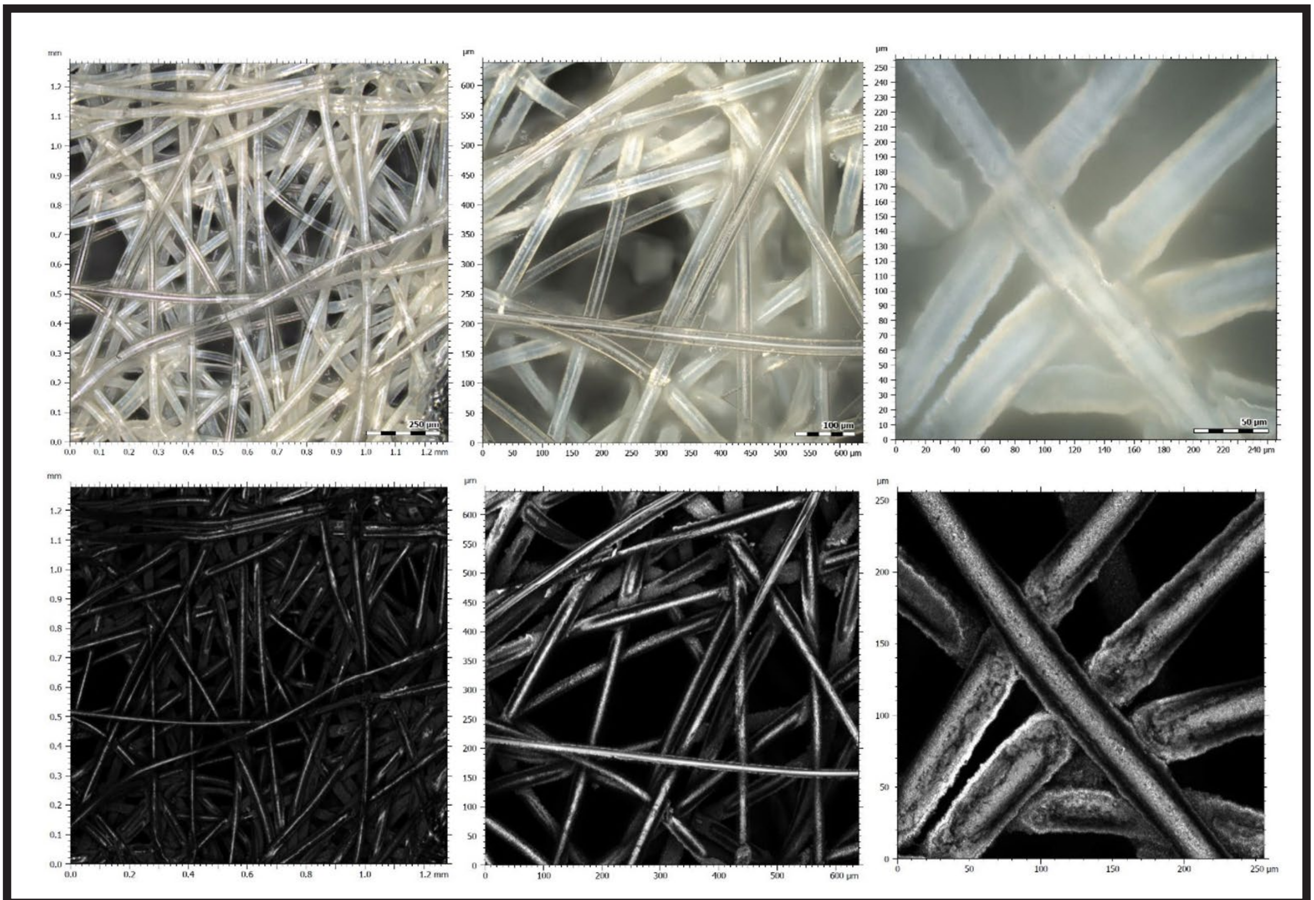

FIG. 16. Confocal microscope images of fibers for the third layer.

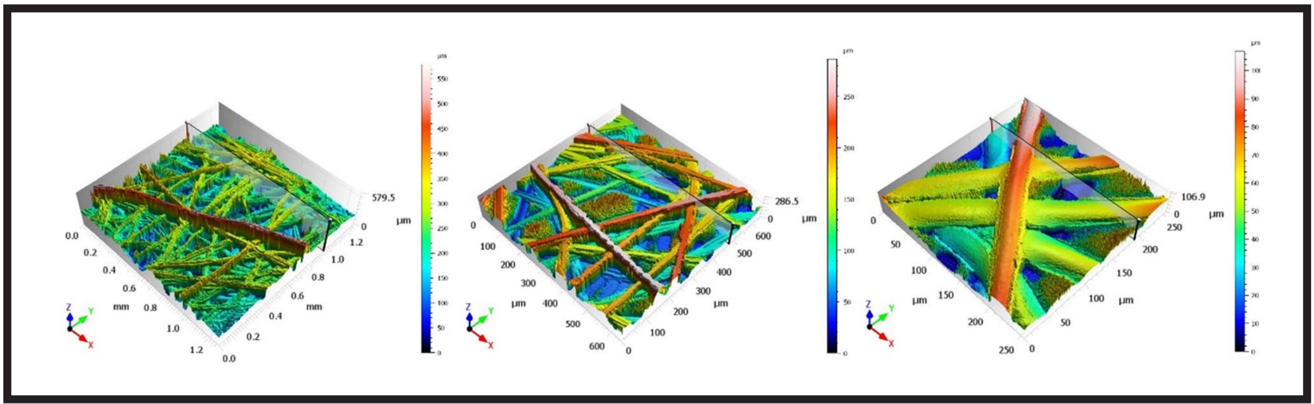

FIG. 17. 3-D view of fibers for the third layer.

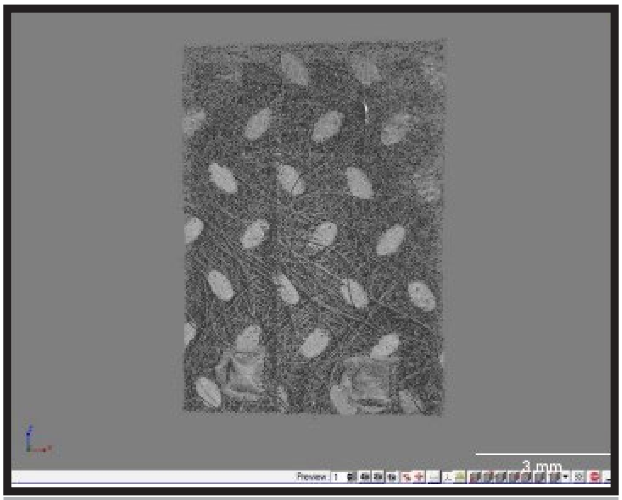

FIG. 18. Image of FFP2 mask from computed microtomography with visible pores.

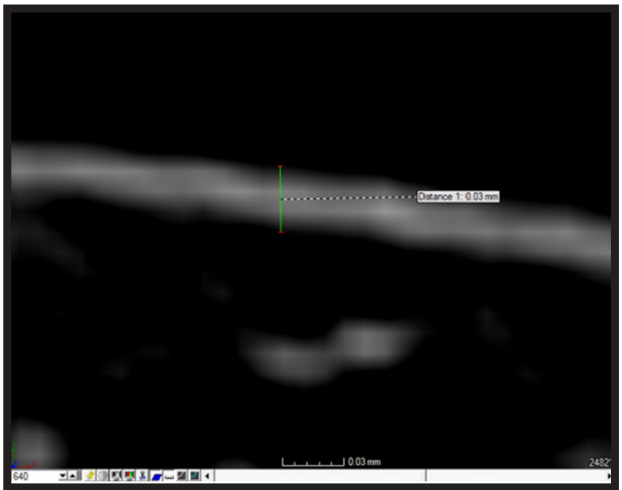

FIG. 19. Fiber thickness.

The difference in the fibers arrangement and density was studied via a non-invasive method of computed microtomography. The obtained images revealed how specific layers were separated. Each layer had a different fiber density. The most dense layer was the middle one, due to HDPE which is a high-density thermoplastic polymer. Pores were seen (FIG. 18), which is very important in terms of the protective filter. The appropriate sized pores retain and "trap" very small particles so that they do not reach the mask user's face. Microtomography revealed the density of the fibers arrangement (FIG. 18) and the fibers thickness, which was about 0.03 mm (FIG. 19).

The tests led to the evaluation of biological activity. Based on the results, the R antimicrobial activity of the 0.025-0.5% betulin polymer plates was found to be in the range (R = 1.45 - 2.0) compared to the starting material without betulin. The average antibacterial activity (R) for the tested material, in this case, was 1.85, according to the following values - *Escherichia coli*: U_0 – 4.21, U_t – 4.91 and A_t – 3.46:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t$$

where:

U_0 - mean of the decimal logarithm of the number of live bacteria, number of cells/cm^2, recovered from the untreated samples after culture,

U_t - mean of the decimal logarithm of the number of viable bacteria, number of cells/cm^2, recovered from the untreated samples after 24 h,

A_t - mean of the decimal logarithm of the number of live bacteria, number of cells/cm^2, recovered from the samples treated after 24 h.

The polymers modified with betulin showed significant antimicrobial and anti-inflammatory activity. The observed changes in the expression profile of genes involved in inflammatory processes indicated the anti-inflammatory effect of the obtained polymeric materials, as compared to the starting material without betulin. Due to the achieved properties, the betulin-enhanced materials can be advantageously used in medicine and biotechnology.

Conclusions

In order for the filter to effectively protect the respiratory tract, it is necessary to develop appropriate materials that will contribute to the antibacterial and antiviral functions. Materials with this type of properties are becoming more and more popular on the medical market, especially during the current pandemic, where the demand for antimicrobial activity has increased. That is why we decided to test the innovative solution - a filtering membrane of organic compound - to protect the upper respiratory tract from viral and bacterial infections, compliant with the FFP2 standard.

In our study, betulin was isolated from the outer bark of the birch via the Soxhlet extraction. The evaluation of the microbiological and anti-inflammatory activity of the betulin polymeric material proved it highly useful. The FTIR analysis showed that the main compounds in the FFP2 mask were polymers - polypropylene and high-density polyurethane (HDPE), the surface of which was easily modified with betulin, both by applying it as an aerosol and by immersion via the layer-by-layer method. The described materials are also suitable for modification at the synthesis stage, i.e. in bulk. In the optical and confocal microscope images, the morphological changes of the mask fibers were observed, as compared to the optical microscopy samples showing smooth fibers. The betulin particles were clearly visible in the confocal microscope images. Despite the uneven betulin distribution, the bacteriostatic properties were confirmed and maintained at a satisfactory level. In the future, the immersion method should be replaced with the spray method, due to the formation of low-energy agglomerates. Many particles gathered in some areas, while other areas were covered with only a few particles. The presence of pores and the structure of the fibers were also examined using a non-invasive test - computer microtomography.

In summary, our study proved the applicability of the proposed solution.

Acknowledgements

The work was carried out as part of the statutory research of the Institute of Biomedical Engineering, University of Silesia.

ORCID iD

N. Brzezińska: https://orcid.org/0000-0002-8648-2498
M. Pyza: https://orcid.org/0000-0002-7904-9303
A. Kłeczek: https://orcid.org/0000-0001-6066-3349
K. Kulińska: https://orcid.org/0000-0002-6676-1502
J. Gabor: https://orcid.org/0000-0003-4850-1608
Ż. Garczyk-Mundała: https://orcid.org/0000-0003-4345-5315
A. Swinarew: https://orcid.org/0000-0001-6116-9510

References

[1] Regli A., Sommerfield A., Ungern-Sternberg B.S.: The role of fit testing N95/FFP2/FFP3 masks: a narrative review. Anaesthesia 76(1) (2021) 91-100, doi: 10.1111/anae.15261
[2] Cascella M., Rajnik M., Aleem A., et al.: Features, Evaluation, and Treatment of Coronavirus (COVID-19). StatPearls Publishing (2021)
[3] Nowakowska E., Michalak S.S.: Covid-19 – Choroba wywołana zakażeniem wirusem SARS-Cov-2 globalnym zagrożeniem dla zdrowia publicznego. Postępy Mikrobiologii – Advancements of Microbiology 59(3) (2020) 227-236, doi: 10.21307/pm-2020.59.3.16
[4] Pawlik L., Śpiołek E., Fichna J., Tarasiuk A.: Charakterystyka wirusa SARS-CoV-2 i potencjalne farmakologiczne sposoby leczenia. Postępy Biochemii: Zakład Biochemii, Katedra Chemii i Biochemii Medycznej, Uniwersytet Medyczny w Łodzi 66(2) (2020), doi: 10.18388/pb.2020_321
[5] Fenner F.: Epidemiology and Evolution. In: Baron S., editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston (1996)
[6] Tcharkhtchi A., Abbasnezhad N., Zarbini Seydan M. et al.: An overview of filtration efficiency through the masks: Mechanisms of the aerosols penetration. Bioactive Materials 6(1) (2021) 106-122, doi: 10.1016/j.bioactmat.2020.08.002
[7] Ippolito M., Vitale F., Accurso G. et al.: Medical masks and Respirators for the Protection of Healthcare Workers from SARS-CoV-2 and other viruses. Pulmonology 26(4) (2020) 204-212, doi: 10.1016/j.pulmoe.2020.04.009
[8] Smereka J., Ruetzler K. et al: Role of Mask/Respirator Protection Against SARS-CoV-2. Anesthesia and analgesia 131(1) (2020) e33-e34, doi:10.1213/ANE.0000000000004873
[9] Zhou J., Hu Z., Zabihi F. et al.: Progress and Perspective of Antiviral Protective Material. Advanced Fiber Materials 2 (2020) 123-139, doi: 10.1007/s42765-020-00047-7
[10] Li K.K.W., Joussen A.M., Kwan J.K.C. et al.: FFP3, FFP2, N95, surgical masks and respirators: what should we be wearing for ophthalmic surgery in the COVID-19 pandemic? Graefes Arch Clin Exp Ophthalmol. 258(8) (2020) 1587-1589, doi: 10.1007/s00417-020-04751-3
[11] Palaniappan P., Sathishkumar G., Sankar R.: Fabrication of nano-silver particles using Cymodocea serrulata and its cytotoxicity effect against human lung cancer A549 cells line, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 138 (2015) 885-890 doi: https://doi.org/10.1016/j.saa.2014.10.072.
[12] Massarsky A., Abraham R., Nguyen K.C. et al.: Nanosilver cytotoxicity in rainbow trout (Oncorhynchus mykiss) erythrocytes and hepatocytes. Comp. Biochem. Physiol. Pt. C. 159 (2014) 10-21.
[13] Swinarew A., Boryczka S., Mazurek U. et al.: Modyfikowany polimer termoplastyczny o właściwościach przeciwbakteryjnych i przeciwzapalnych oraz sposób jego otrzymywania, Polska, 422092 B1, 03.07.2017
[14] Tolstikova T.G., Sorokina I.V., Tolstikov G.A. et al.: Biological activity and pharmacological prospects of lupane terpenoids: II. Semisynthetic lupane derivatives. Chem. 32 (2006) 37-49.
[15] Huang T., Li D., Elc M., Water repellency improvement of cellulosic textile fibers by betulin and a betulin-based copolymer. Cellulose 25, 2115-2128 (2018), doi: https://doi.org/10.1007/s10570-018-1695-5.
[16] Alakurtti S., Mäkelä T., Koskimies S., et al.: Pharmacological properties of the ubiquitous natural product betulin. Eur J Pharm Sci. 29(1) (2006) 1-13.
[17] Kowalczuk D., Pitucha M.: Application of FTIR Method for the Assessment of Immobilization of Active Substances in the Matrix of Biomedical Materials. Materials (Basel) 12(18) (2019) 2972, doi: 10.3390/ma12182972;
[18] Pulit J., Banach M., Kowalski Z.: Właściwości nanocząsteczek miedzi, platyny, srebra, złota i palladu. Czasopismo techniczne, Wydawnictwo Politechniki Krakowskiej 10 (2011)
[19] Massarsky A., Abraham R., Nguyen K.C. et al.: Nanosilver cytotoxicity in rainbow trout (Oncorhynchus mykiss) erythrocytes and hepatocytes. Comp. Biochem. Physiol. Pt. C. 159 (2014) 10-21.
[20] Brown R.C.: Effect of electric charge in filter materials. Filtration & Separation 26(1) (1989) 46-51, doi: 10.1016/S0015-1882(89)80057-6.