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Development of Anti-Bacterial and Anti-Viral Nonwoven Surgical Masks for Medical Applications
Razvoj protibakterijskih in protivirusnih netkanih kirurških mask za medicinske namene

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Abstract

This article aims to investigate the development of surgical masks for medical applications by incorporating biocidal silver nanoparticles. Medical masks were developed in three layers of a nonwoven fabric, where the outer and inner layers were made of a spun-bond polypropylene nonwoven fabric and the middle layer consisted of a melt-blown nonwoven polypropylene fabric. In this study, silver nanoparticles in the concentrations of 1–5% were applied to masks with the pad-dry-cure method. The samples were cured at room temperature and subsequently examined for antimicrobial properties. Scanning electron microscopy, energy dispersive spectroscopy and Fourier transform infrared spectroscopy were used to investigate the morphological characteristics and chemical composition of the samples. Microbial cleanliness, bacterial filtration efficiency, antiviral effect and breathability tests were performed according to standard test protocols. The results revealed that the application of silver nanoparticles to a three-layer mask rendered the end product with outstanding antimicrobial and antiviral properties with poor breathability (air permeability) results.
Keywords: polypropylene, nonwoven fabric, antimicrobial, silver nanoparticles

Izvleček

V članku je predstavljen razvoj kirurških mask za medicinske namene z vgradnjo biocidnih nanodelcev srebra. Medicinske maske so bile izdelane iz treh slojev vlaknovin, pri čemer sta bila zunanja in notranja plast izdelani iz spunbond polipropilenske vlaknovine, srednji sloj pa iz polipropilenske vlaknovine meltblown. Na masko so bili z metodo impregniranja in zamreževanja naneseni srebrovi nanodelci v koncentraciji 1–5 % pri sobni temperaturi ter nato analizirani glede protimikrobnih lastnosti. Za raziskovanje morfoloških značilnosti in kemične sestave vzorcev so bile uporabljene metode rastrske elektronske mikroskopije, energijske disperzivne rentgenske spektroskopije in infrardeče spektroskopije s Fourierjevo transformacijo. Testi mikrobne čistosti, učinkovitosti bakterijske filtracije, protivirusnega učinka in zračne prepustnosti so bili opravljeni po standardnih testnih protokolih. Rezultati so pokazali, da je nanos srebrovih nanodelcev na triplastno masko dal končni izdelek izjemnih protimikrobnih in protivirusnih lastnosti s slabo zračno prepustnostjo. Ključne besede: polipropilen, netkana tekstilija, protimikrobno sredstvo, srebrovi nanodelci

1 Introduction

Medical masks have long been used in hospitals by medical staff and nurses to protect themselves and patients from infectious microorganisms [1, 2]. Today, with the outbreak of the COVID-19 pandemic caused by the novel coronavirus (SARS-CoV-2), the use of medical masks in daily life has become inevitable according to the World Health Organization recommendations [3].

In general, a virus is an infectious genetic unit that has the smallest structure of all bacteria. The specific characteristics of viruses place them on the border between the animate and inanimate nature. They can infect both eukaryotic cells (e.g. animals, insects and plants) and prokaryotes (e.g. bacteria) [4–8]. An epidemic is a disease that affects a large number of people within a community, population or region at a particular time, while a pandemic is the spread of a new disease around the world affecting many people [9–11]. Numerous epidemics and pandemics have occurred throughout human history. Some respiratory viral diseases which turned into an epidemic and global pandemic include the Russian Flu (1889–1890), Spanish Flu (1918–1919), Asian Flu (1957–1958), Hong Kong Flu (1968–1970), Severe Acute Respiratory Syndrome (SARS) (2002–2003), Swine Flu (2009–2010), Middle East Respiratory Syndrome (MERS) (2012–Present), Coronavirus Disease 2019 (COVID-19) (2019–Ongoing) [12–21].

Doctors, nurses and medical staff working in hospitals and health centres have been applying face masks since the late nineteenth century to prevent the entry of various bacteria, viruses and microorganisms into their bodies and that of patients [22]. Face masks can be introduced as part of effective personal care equipment (PCE) when confronting infectious viruses [23]. Nevertheless, several studies revealed that face masks, when contaminated, could be a major source of transmission, especially in case of the SARS-CoV-2 virus [24, 25]. According to the latest information, the risk of transmission of the SARS-CoV-2 virus through surfaces is low; however, the virus can survive on the surface for several days. In contrast, the transmission of the virus through surface is high at the medical masks and gowns used during the treatment of COVID-19 patients in the intensive care unit (ICU) [26].

Silver (Ag) nanoparticles (NPs) were first identified as an antibacterial agent and subsequently, their antiviral effect was reported [27, 28]. Ag salts have been known to have antibacterial properties since ancient ages [29], and they are still used to monitor bacterial growth in a number of uses, incl. dental work, catheters and burn wounds [30, 31]. Indeed, Ag ions and Ag-based compounds are considered to be extremely toxic to microorganisms, with significant biocidal effects on bacteria species, incl. *Escherichia coli* [32]. Ag NPs are the most commonly used metal nanoparticles in nanotechnology for several medical applications. Silver molecules, individually or in the presence of antibiotics, have antimicrobial activity against a wide range of pathogens, incl. *Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Micrococcus luteus*, *Enterococcus faecium*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Staphylococcus epidermidis* [33–35].

This paper reports for the first time on a novel approach towards the application of silver nanoparticles onto nonwoven three-layer masks with the pad-dry-cure method. The effect of various concentrations of silver nanoparticles on the antimicrobial and antiviral activity, and breathability were established. The surface morphology of treated and non-treated nonwoven masks were analysed with SEM and the chemical composition with FT-IR.

2 Experimental

Three-layer disposable face masks were developed from 100% polypropylene (PP) melt-blown and spun-bond nonwoven fabrics (cf. Figure 1) purchased from Akinal Textile, Turkey and Gulsan Synthetics, Turkey. Rudolf's RUCO-BAC AGP product, which is the reaction mass of titanium dioxide and silver chloride, was applied in the face-mask treatments as a biocidal finish.
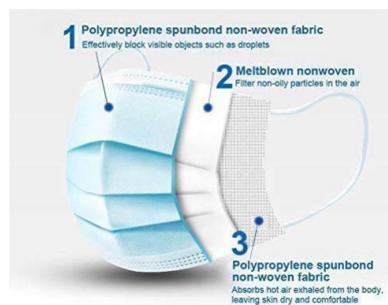

Figure 1: Three-layer nonwoven mask showing spun-bond and melt-blown polypropylene sheets

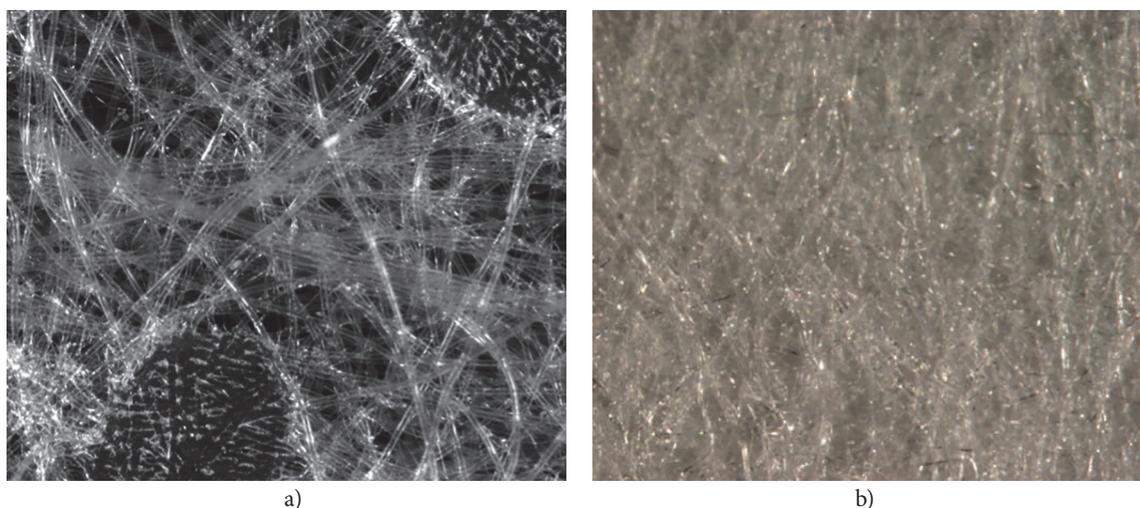| | a) | | b) |

Figure 2: Original polypropylene fabric stereo microscope micrograph (100×): a) spun-bond, b) melt-blown

A stereo microscope micrograph (100×) of a spun-bond nonwoven fabric prepared for the fabrication of surgical masks is shown in Figure 2.

Technical specifications of three-layer nonwoven fabrics are summarised in Table 1. Face-mask wires and elastic string bands (stretchable earloops) were purchased from Serhat Lastik, Turkey. Biocidal silver nanoparticles were supplied by Rudolf Chemicals Co., Turkey and were used as received without further purification. A PROWHITE vertical foulard machine (Model Y001, Turkey) was used in the finishing process to apply the finish onto the fabrics.

Table 1: Technical specifications of nonwoven fabrics used in mask development

Substrate type	Fabric type	Density (g/m²)
Outer layer	PP spun-bond nonwoven	30
Middle layer	PP melt-blown nonwoven	25
Inner layer	PP spun-bond nonwoven	20

2.1 Production of masks

A fully automatic surgical mask production line (cf. Figure 3) was used for the manufacturing of the masks with the dimensions of 170 mm × 95 mm. The main production process flow was coil material feeding, nose bridge tendon feeding, folding and pressing, mask cutting and shaping, ear band feeding, welding and unloading of the finished product. Nose wires and ear bands of the masks were automatically cut and welded by ultrasonic welding.

Figure 3: Surgical mask production line collected from Otima, Turkey

2.2 Application of biocidal antiviral chemical finishing onto fabrics

The application of biocidal properties to nonwoven fabrics with a biocidal finish was carried out in a vertical Foulard machine (PROWHITE Y001). The wet pick-up was 100% using liquor ratio of 1:10, and the dry add-on was 1% and 5% on the weight of an untreated sample. During the padding process, the Foulard pressure was 2.95 bar at 3.5 m/min speed under ambient temperature. PP nonwoven fabrics were padded with biocidal nanoparticles of different concentrations and coded M-1, M-2, M-3, M-4, M-5, M-6, M-7 and M-8, as shown in Table 2. No chemical treatment was applied on the middle layer in all cases.

The padded nonwoven fabrics were air-dried at room temperature. Treated PP nonwoven fabrics were utilised to fabricate disposable face masks by means of a fully automatic surgical face mask production line as shown in Figure 3.

Table 2: Configuration of masks used in study

Sample no.	Outer layer – treated with silver nanoparticles (g/l)	Inner layer – treated with silver nanoparticles (g/l)
M-1	1	no treatment
M-2	1	1
M-3	2	no treatment
M-4	2	2
M-5	3	no treatment
M-6	3	3
M-7	4	no treatment
M-8	5	no treatment
Reference	no treatment	no treatment

2.3 Characterisation

The surface morphologies of the untreated and treated PP nonwoven fabric in different concentrations of biocidal silver nanoparticles were analysed with scanning electron microscopy (SEM) (HITACHI TM3030 Plus, Japan). In addition, the distribution of nanoparticles on face masks was investigated by using SEM. A small piece of a mask (1 × 1 mm) was cut from the outermost layer of the nanoparticle-coated mask. The sample was mounted onto a coverslip, coated with gold alloy and analysed with scanning electron microscopy. An EDAX AMETEK spectrometer equipped with an octane detector using TEAM™ software was used to record the spectra and for a subsequent EDS spectral analysis of uncoated samples.

Fourier transform infrared spectroscopy (FT-IR) was performed using a Shimadzu FT-IR (Japan) spectrophotometer operated in % transmittance mode at room temperature in the range 600–4000 cm^{-1} and the resolution of 8 cm^{-1}. FTIR works by measuring how much light is absorbed by the bonds of vibrating molecules to create a molecular fingerprint in the infrared region of the electromagnetic spectrum. The infrared spectrum is divided into three parts, i.e. near IR, mid-IR and far IR. Near infrared has the most energy and can penetrate a sample considerably deeper than mid or far infrared; however, it is also less sensitive. When infrared light is absorbed, molecules vibrate and bonds stretch and bend, according to IR principles. It operates by delivering an IR beam across a sample and the sample molecules must experience a dipole moment shift during the vibration in order for an IR observable transition to occur. Absorption happens when the IR frequency matches the vibrational frequency of the bonds, allowing the spectrum to be recorded. Different functional groups absorb heat at different frequencies when using infrared. It is determined by their structure and the functional groups contained in a sample can be determined using a vibrational spectrum. The results of an IR spectrometer analysis are compared to a frequency table to determine which functional groups are present.

2.4 Assessment of microbiological tests on mask

The antibacterial activity of masks was checked according to the standard test protocol, i.e. EN 14683:2019+AC:2019 [36]. The bacterial filtration efficiency (BFE) of the face mask material as a barrier to bacterial penetration was measured using the BFE test method based on EN 14683:2019+AC:2019 Annex B [37]. The test area was 4.9 cm^2 and the tests were repeated 5 times for accuracy. The test samples were conditioned at 21 °C ± 5 °C and (85 ± 5) % relative humidity for 4 hours. *Staphylococcus aureus* (ATCC 6538) was used for the BFE tests. The bacterial concentration was 5×10^5 CFU/ml and incubated for 24 h at 35 °C ± 2 °C. The specimen of the mask material was clamped between an impactor in an aerosol chamber and aerosol of *Staphylococcus aureus* was introduced into the aerosol chamber. The BFE of the mask was given by the number of CFU (colony forming unit) passing through the medical face-mask material and expressed as the percentage of the number of CFU in the aerosol. The test flow rate and the time were 28.3 l/min and 2 minutes, respectively. The mean particle size of samples was 3.0 µm.

For the microbial cleanliness (bioburden) analysis, EN 14683:2019+AC:2019 Annex D [37] standard test method was used and five repetitions were performed for each type of test. The samples were weighed and put in extraction liquid after being shaken for 5 min at 250 rpm and then inoculated on suitable agar plates. The plates were incubated for 3 days at 30 °C ± 1 °C for 72 hours, and 7 days at 20–25 °C for Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) plates, respectively. After the incubation, total microorganism counts were calculated as CFU/g.

2.5 Assessment of antiviral activity on fabric

Antiviral tests were applied to samples with the highest antibacterial activity (M-3, M-5, M-6, M-7 and M-8). For the antiviral activity tests, control fabric samples, which were sterilised and dried in autoclave at 121 °C for 15 minutes, and six test fabric samples at the mass of 0.4 grams were used. Three control fabric samples were used to measure the virus titre immediately after the inoculation, three control fabric samples and three test fabric samples were used for the control test of the effect of the virus-free test sample. 400 μl of the Bovine coronavirus ATCC's reference strain VR-874 was inoculated on the remaining three control fabric samples and three test fabric samples for the main test. At the end of the contact period of 2 hours, 20 ml of Casein Digest-Soy Lecithin Polysorbate (SCDLP) medium was added to the samples. After the virus recovery procedures in the ISO 18184 standard, they were cultivated in MDBK (NBL-1) ATCC (CCL-22) cells with serial dilutions. All recovery and logarithmic reduction calculations were conducted using the Spearman-Karber method, taking into consideration the virus dilutions that create visible cytopathic effects on the control and test samples that were studied simultaneously on an inverted microscope.

2.6 Determination of physical properties (breathability) on mask

Air permeability tests were carried out on untreated and treated PP nonwoven fabrics to investigate their breathability. The differential pressure required to draw air through the face mask was measured at a constant airflow rate using a differential manometer according to the EN 14683:2019+AC:2019 Annex C. The samples were conditioned at 21 °C ± 5 °C temperature and 85% ± 5% humidity for 4 hours. The area of tests was 25 mm in diameter and the airflow was adjusted to 8 l/min.

2.7 Performance of medical face masks

The TS EN 14683:2014 standard defines the features and performance requirements of the product. The performance levels are determined by testing the products according to these requirements. Table 3 shows the test and performance limits used in the classification of the product.

3 Results and discussion

3.1 Characterisation of reference and silver oxide-doped samples

The FT-IR spectra of samples are presented in Figure 4, the aliphatic C-H stretching vibration of spun bond PP is associated with multiple absorption peaks at 2840–3000 cm^{-1}. The in-plane rocking vibration of the CH_2 group is associated with the absorption peak at 840 cm^{-1} and the C-H bending vibrations in the CH_2 group are observed at 1454 and 1377 cm^{-1}.

3.2 Morphological observations

In the scanning electron microscope and stereo microscope micrographs, the presence of silver and titanium nanoparticles on nonwoven fabrics is evident. The morphological results (cf. Figures 5

Table 3: Performance requirements for medical face masks

Test	Type I[a]	Type II	Type IIR
Bacterial filtration efficiency (BFE), (%)	≥ 95	≥ 98	≥ 98
Differential pressure (Pa/cm²)	< 40	< 40	< 60
Splash resistance pressure (kPa)	Not required	Not required	≥ 16
Microbial cleanliness (CFU/g)	≤ 30	≤ 30	≤ 30

[a] Type I medical face masks should only be used for patients and other persons to reduce the risk of spreading infections particularly in epidemic or pandemic situations. Type I masks are not intended for use by healthcare professionals in an operating room or in other medical settings with similar requirements.

and 6) illustrate the clogged pores and corroborate the worse differential pressure (breathability) test results (cf. section 3.6 Determination of physical properties (breathability) on mask).
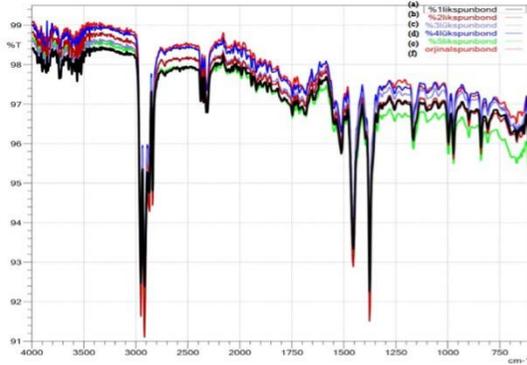
The presence of Ti and Ag in the finishing masks were confirmed by the EDS investigations as mentioned in Figure 7. The weight % of each element observed in the EDS analysis of PP treated with 5% add-on is given in Table 4.

Figure 4: FT-IR spectra of PP fabrics treated with silver nanoparticles: a) 1 g/l, b) 2 g/l, c) 3 g/l, d) 4 g/l, e) 5 g/l and f) untreated

Table 4: EDS investigation of finished masks with 5% dry add-on

Element	Weight (%)	Error (σ) (%)
C	74.3	1.1
O	15.0	1.0
Ti	6.8	0.6
Ag	3.1	0.6
Cl	0.9	0.2

3.3 Bacterial filtration efficiency test (BFE)

Bacteria filtration efficiency is a standard that prevents the release of small particles from mouth while speaking and prevents bacteria from entering our respiratory tract. The rise of the bacteria
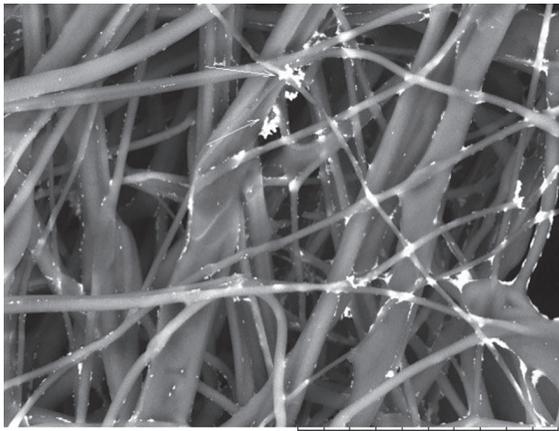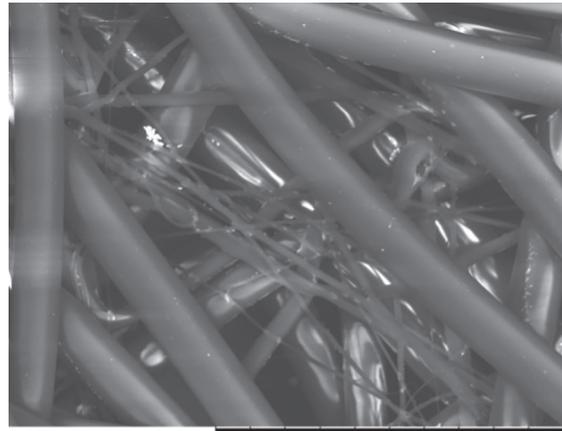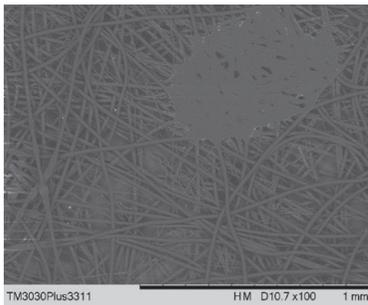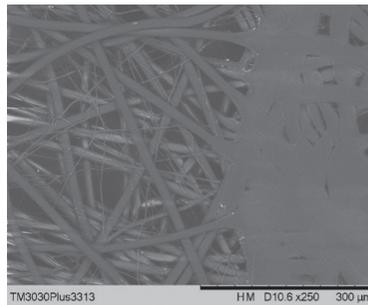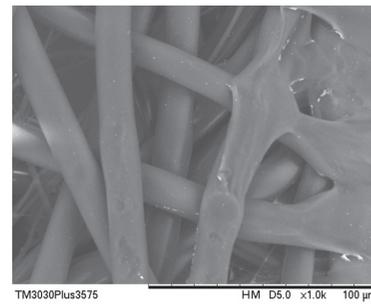

a)   b)

Figure 5: Scanning electron micrographs of silver nanoparticles on nonwoven mask.
After treatment with nanoparticles, white agglomerations are seen in three layers of mask;
melt-blown (a), spun-bond (b); magnification of (a) is 1,500×, magnification of (b) is 1,000×

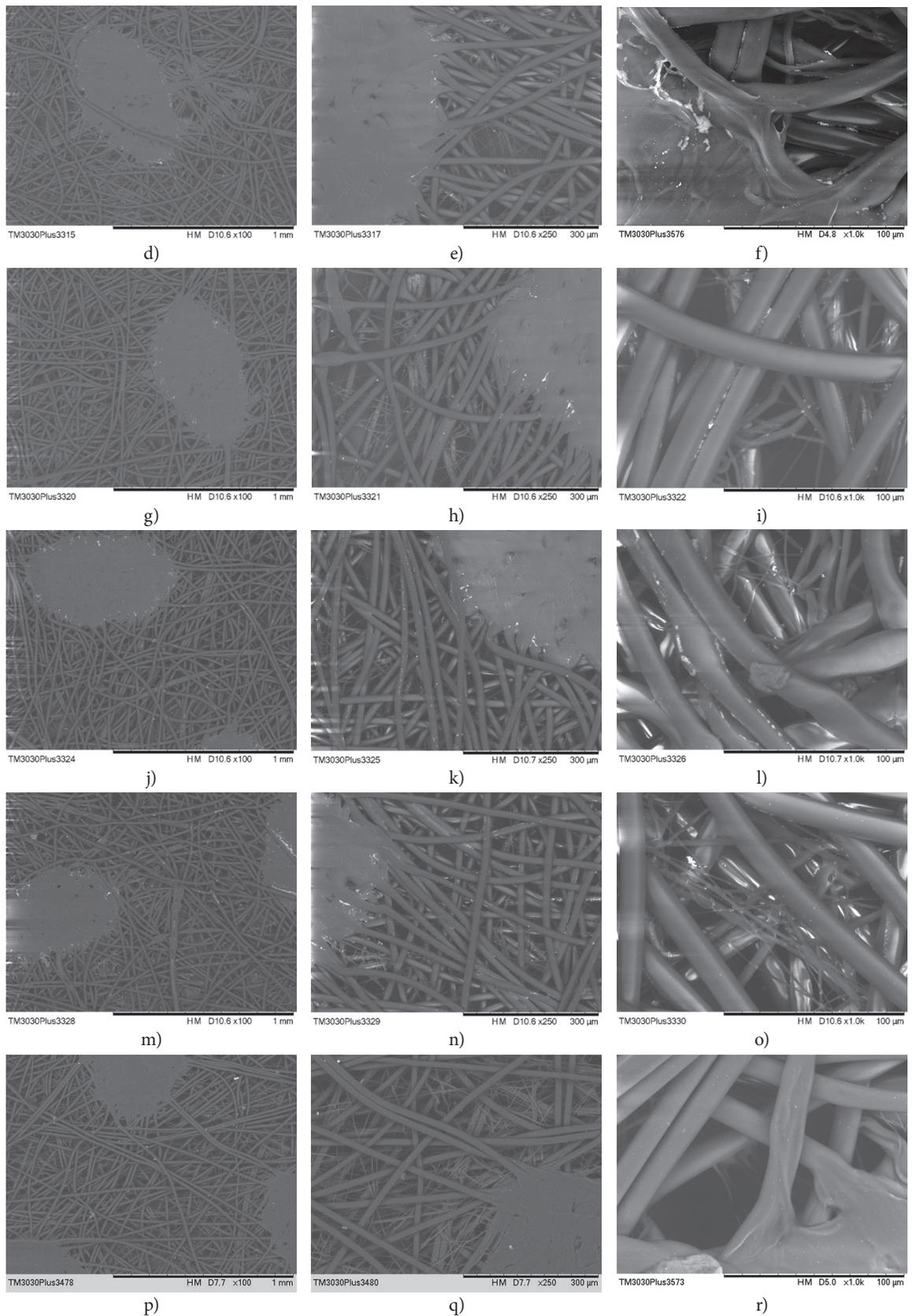a)   b)   c)

Figure 6: Scanning electron micrographs of silver nanoparticles on nonwoven mask (a–c: 1 g/l; d–f: 2 g/l; g–i: 3 g/l; j–l: 4 g/l; m–o: 5 g/l; p–r: untreated). Magnifications are 100×, 250× and 1000×, respectively.
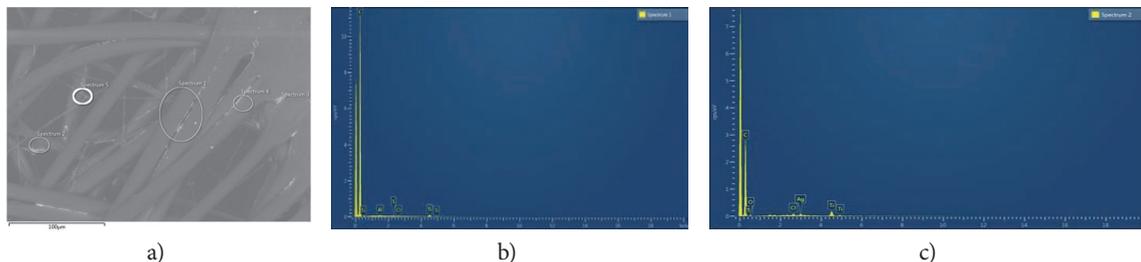
*Figure 7: SEM (a) and EDS (b and c) investigations of finished samples
(5% add-on), confirming presence of Ti and Ag in masks*

filtration efficiency rate means that the protection of masks increases. According to this information, when surgical masks are classified, Type II and Type IIR provide the same amount of protection, while Type I shows less protection than the previous two. Due to the BFE results, all samples are categorised as Type IIR, except for the samples 9 and Reference, which is categorised as Type I (cf. Figure 8).

3.4 Microbial cleanliness (bioburden) (CFU/g)

Regarding microbial cleaning, the biological load of a mask should be evaluated according to Annex D of the ISO 14683 standard or according to the EN ISO 11737-1 standard. This value should be at most 30 CFU/g for Type I, Type II and Type IIR. Due to the obtained results, all samples passed the microbial cleanliness test successfully.

3.5 Antiviral efficiency test

Figure 9 shows a comparison of viral reduction percentage results of samples M-3, M-5, M-6, M-7

and M-8. Comparing the M-3 test fabric virus titres with the control fabric virus titres, it was found that at 25 °C, 5-minute contact time caused at least 0.17 log10 (32.39%) reduction against the Bovine coronavirus in all experimental conditions.

In the case of the test fabric M-5, it was observed that the log10 reduction factor was 0.25 log10 with a 43.76% reduction against the Bovine coronavirus. Regarding the M-7 test fabric, it was determined that it caused at least a 0.08 log10 (16.82%) reduction against the same virus. By comparing the M-6 test fabric virus titres with the control virus titres, it was found that at 25 °C, 5-minute contact time caused at least a 0.33 log10 (53.22%) reduction against the Bovine coronavirus in all experimental conditions. In the case of the M-8 test fabric, it was found that at 25 °C, 5-minute contact time caused at least a 0.17 log10 (32.39%) reduction against the Bovine coronavirus in all experimental conditions. Table 5 shows a detailed comparison of all results of the antiviral efficiency tests.
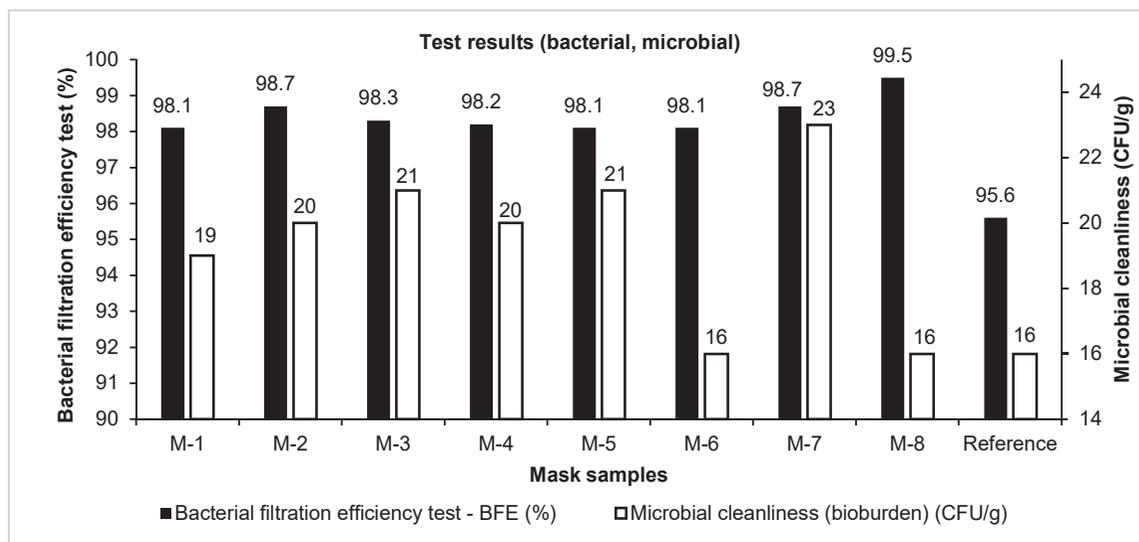

*Figure 8: Bacterial filtration efficiency, microbial cleanliness
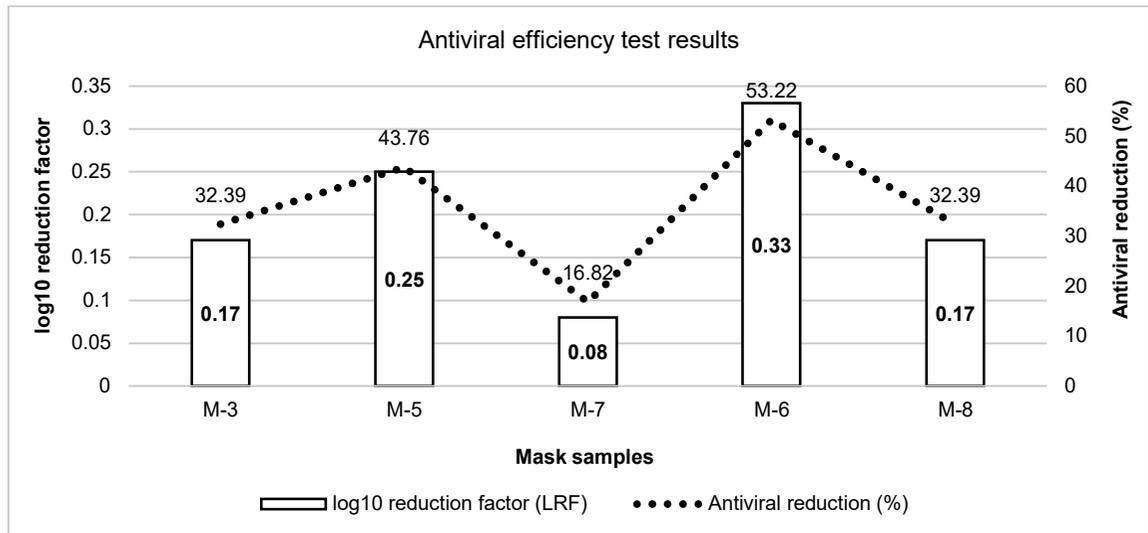and differential pressure results of treated and untreated nonwoven masks*

Figure 9: Comparison of log10 reduction factor and antiviral reduction percentage of different samples at 25 °C and 5-minute contact time

3.6 Determination of physical properties (breathability) on mask

A face mask should protect the wearer's nose, mouth and chin. It should be worn tightly that the sides of the mask fit to the face. At the same time, it should allow the user to breathe comfortably during a long-term use. Since the mouth and nose part are closed during the use of the mask, the exhaled carbon dioxide gas cannot go out and is inhaled back. The breathability rate shows how much of the exhaled carbon dioxide goes outside through the mask. Due to differential pressure (breathability) test results based on the EN 14683:2019+AC:2019 Annex C standard, none of the treated samples passed the limit defined by the standard, i.e. below 40 Pa/cm^2, apart from the reference (untreated) sample (cf. Figure 10). The uneven distribution of silver nanoparticles probably led to pore clogging in spun-bond nonwoven fabric breathability zones, which can also be confirmed by SEM observations.
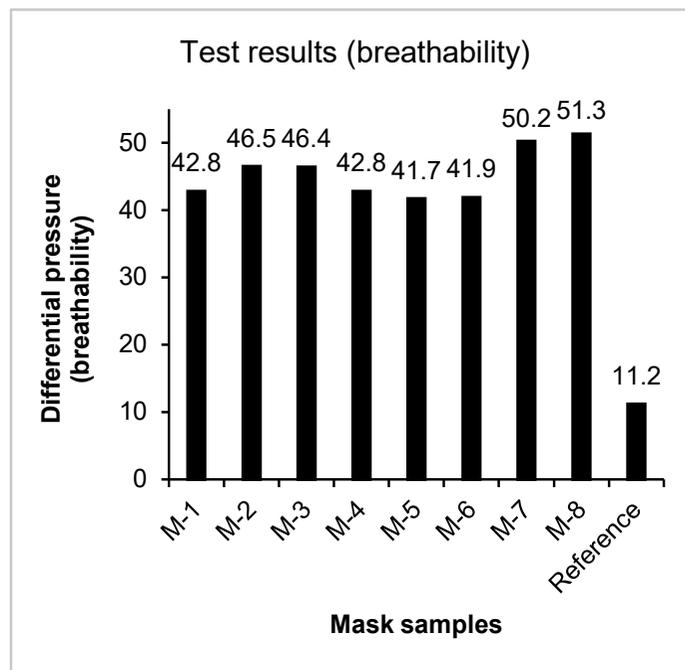

Figure 10: Differential pressure (breathability) results of treated and untreated nonwoven masks

Table 5: Antiviral efficiency test results of different samples against Bovine coronavirus

Sample No.	Sample specifications	Contact time (min)	Log10 reduction factor	Antiviral reduction (%)
M-3	Control fabric 1	0	–	–
	Control fabric 1	5	0.08	16.8
	Test fabric 1	5	0.17	32.39
	Control fabric 1	30	–	–
	Test fabric 1	30	0.2 ≤ 0.5 log10	–
M-5	Control fabric 1	0	–	–
	Control fabric 1	5	0.41	61.09
	Test fabric 2	5	0.25	43.76
	Control fabric 2	30	–	–
	Test fabric 2	30	0.2 ≤ 0.5 log10	–
M-7	Control fabric 1	0	–	–
	Control fabric 1	5	0.33	53.22
	Test fabric 2	5	0.08	16.82
	Control fabric 2	30	–	–
	Test fabric 2	30	0.2 ≤ 0.5 log10	–
M-6	Control fabric 1	0	–	–
	Control fabric 1	5	0.5	68.37
	Test fabric 3	5	0.33	53.22
	Control fabric 3	30	–	–
	Test fabric 3	30	0.2 ≤ 0.5 log10	–
M-8	Control fabric 1	0	–	–
	Control fabric 1	5	0.33	53.22
	Test fabric 4	5	0.17	32.39
	Control fabric 4	30	–	–
	Test fabric 4	30	0.2 ≤ 0.5 log10	–

4 Conclusion

This study successfully developed antibacterial and antiviral surgical masks for medical applications. The fabrication method (pad-dry-cure) was proven feasible and scalable for a large-scale production. The biocidal silver nanoparticles, which have the potential to be applied to surgical face masks, delivered better resistance against bacterial and viral transmission as well as penetration. The adhesion of silver and titanium nanoparticles to nonwoven polypropylene was confirmed by SEM and EDS results. However, the addition of silver nanoparticles resulted in pore clogging of the spun-bond nonwoven polypropylene fabric, which finally resulted in poor differential pressure (breathability) results. The bacterial filtration efficiency, micro-bial cleanliness and antiviral efficiency test results were satisfactory. The increasing amount of biocidal agent in the formulation led to an increase in the antiviral activity, meaning that the developed surgical masks provided better resistance against microbes and viruses; however, for better breathability, lower concentrations of silver nanoparticles are recommended.

Conflict of interests

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