Povidone-iodine functionalized nanofibers are prophylactic and protect against dissemination of SARS-CoV-2 infection

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ABSTRACT
The novel personal protection equipment based on a face mask equipped with a nanofiber filter functionalized with povidone iodine has been developed and tested in a clinical trial. This nanofiber filter was characterized with a low flow resistance and, thus, allowed comfortable breathing. The performed study proved that the novel nanofiber filter with incorporated povidone-iodine was characterized with a slow release of iodine which minimized side effects but kept disinfection efficiency. Our clinical study performed on 207 positively tested SARS-CoV-2 patients wearing the PPE for 4–8 hours daily for 1 to 4 days has shown that even the iodine amount as low as 0.00028 ppm was sufficient to significantly decrease the reproduction number and, very importantly, to protect against severe course of disease.

KEYWORDS
SARS-CoV-2 – personal protection equipment – povidone-iodine – nanofiber

SOUHRN
Divín R., Vojáček V., Sopko B., Divín R., Pashchenko A., Varga J., Nečas A., Celer V, Filipejova Z., Urbanová L., Rulc J., Krajníková M., Jarošíková T., Amler E.: Nanovlákna funkcionalizovaná jodovaným povidonem chrání před šířením infekce SARS-CoV-2

Novým osobním ochranným prostředkem je obličejová maska vybavená nanovlákenným filtrem s enkapsulovaným jodovaným povidonem, která byla vyvinuta a otestována při klinické zkoušce. Filtr s funkcionalizovanými nanovlákny se vyznačuje nízkým průtokovým (vdechovým) odporem, umožňuje tudíž pohodlné dýchání a minimalizuje vdechování vzduchu procházejícího mimo filtr. Filtr je díky přítomnosti aktivní látky vhodný i pro aplikaci po nechráněné virové expozici. Uskutečněná zkouška prokázala, že testovaný nanovlákenný filtr s jodovaným povidonem se vyznačuje pomalým uvolňováním jódu, které minimalizuje vedlejší účinky, ale zachovává dezinfekční účinnost. Z výsledků naší klinické zkoušky provedené na 207 pozitivně testovaných pacientech se SARS-CoV-2, kteří nosili osobní ochranné prostředky po dobu 4–8 hodin denně po dobu 1 až 4 dnů, vyplynulo, že i množství jódu pouhých 0,00028 ppm postačovalo k významnému snížení reprodukčního čísla a také k ochraně před závažným průběhem onemocnění.

KLÍČOVÁ SLOVA
SARS-CoV-2 – osobní ochranné prostředky – funkcionalizovaná nanovlákna – jodovaný povidon

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INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) can reside in high concentrations in airways as well as it can remain aerosolized for several hours under [1]. Prevention against viral exposure is primarily focused on Personal Protective Equipment (PPE) [2–4]. However, the governmental measures are less and less effective, namely in connection with decreasing willingness of polyphenylene oxide (PPE) proper application among population. This manuscript deals with an alternative approach: application of nanofiber filters functionalized with povidone iodine (PVPI) in supposing infected environments or shortly after a contact with SARS-CoV-2 virus.

Povidone iodine is broadly used antiseptic because of two metabolites: molecular I2 and hypoiodous acid [5], effective due to oxidation of cell surface receptors prevent the attachment of viruses to cellular receptors [6]. Short exposition of mucosa to povidone iodine is broadly used in topical applications as well as during oral surgery and considered as safe [7, 8093.

Several studies have shown gargling of povidone iodine as effective against similar viruses like against SARS-CoV-1 [9], upper respiratory tract infection [10] or even against common cold and influenza.

Similar expectations of PVPI effectivity could be hypothesized also against SARS-CoV-2 virus. Indeed, the recent study has shown that oronasal application of PVPI may serve as a prophylaxis measure in the era of SARS-CoV-2 pandemic [1]. The reservoir for SARS-CoV-2 shedding is in the nasopharynx and nasal and oral cavities and, thus, the application of viricidal agents to these surfaces may reduce virus burden. The recent study developed a prophylactic treatment protocol for the application of topical povidone-iodine to the upper aerodigestive tract [1]. Such an approach represented for the medical personnel a low-cost, low-morbidity measure that may reduce the risks associated with aerosol-generating procedures performed commonly in otorhinolaryngology operating rooms. An alternative approach is application of nanofiber membrane as a filter in PPE. This is seemingly an excellent idea, but one largest problem exists: dense nanofiber network could protect against virus penetration through the nanofiber membrane, but it simultaneously increases the pressure lost and, consequently, most breathed air is not filtered but penetrates through leaks in protective mask.

The main aims of the present study were, first, to avoid gargle of povidone iodine and to prepare, instead, an effective personal protective equipment based on a face mask with a nanofiber filter functionalized with povidone iodine and, second, to test this novel PPE in a clinical observation trial.

METHODS

Nanofiber production and functionalization

Povidone-iodine (1-ethenyl-2-pyrrolidone, BETADINE) has been used for functionalization of Poly(vinyl alcohol) (PVA) nanofibers (Figure 1).

PVA 5-88 (MERCK) and PVA 40-88 (MERCK) in ration 1 : 1 was dissolved in 10% povidone-iodine solution to create 6.2% PVA spinning solution with povidone-iodine. Final iodine concentration in nanofibers was 10 mg/mL. Dissolving of PVA in povidone-iodine was provided during continual stirring and heating of the solution to 60 °C. PVA functionalized nanofiber scaffold was prepared by AC electrospinning method by supplying voltage of 40 kV to spinning electrode. Grammage of the prepared PVA functionalized scaffold was 1 g/m^2.

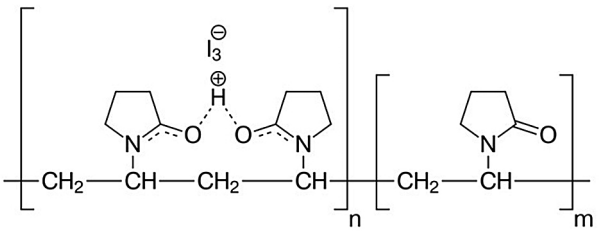

Figure 1. Structure of Povidone-iodine

Nanofiber visualization

Functionalized nanofiber scaffold visualization was provided by scanning electron microscope (SEM) Vega3 SB (TESCAN a. s.). To remove electric charge induced in the scaffold during the scaffold imaging, the nanofiber scaffold was coated by golden conductive layer sputtered by coater Q150R (Quorum) in thickness of 8–12 nm.

Degradation of the functionalized nanofiber scaffold during wearing of the face mask with the functionalized nanofiber scaffold membrane was visualized by SEM images.

Determination of nanofiber degradation

Nanofiber degradation was observed in distilled water, by releasing of Fluorescein Isothiocyanate-Dextran (Sigma-Aldrich) (FSC) from the scaffold into distilled water. For the degradation observation were created samples according to method described in Nanofiber production and functionalization, wherein into PVA spinning solution was added 0.042% FSCfluorescent dye.

Six identical functionalized PVA samples loaded with FSC of weight of 0.280 g ± 0.003 g were put into glass bottles with 177 ml of distilled water. The bottles with samples were located into the shaking incubator with shaking frequency 60 Hz and temperature 37 °C. The water samples of 100 µl from each glass bottle were taken in defined time intervals and analyzed by SYNERGY H1 (BioTeK) on emission frequency 520 nm and with excitation with 490 nm. Degradation of the functionalized PVA loaded with FSC was statistically evaluated according to calibration curve prepared by dissolving of 0.007 g, 0.014g, 0,028 g, 0.056 g, 0.084 g, 0.112 g, 0.14 g, 0.0168 g, 0.196 g, 0.224 g, 0.252 g, 0.28 g of functionalized PVA samples loaded with FSC in 177 ml of distilled water.

Clinical trial and statistics

207 adults (40.6 ± 9.7 years old) indicated as SARS-CoV-2 positive by PCR method have been involved into the clinical observation trial in April and May 2021. All subjects have agreed with participation in the study including all conditions. The participants applied an active nanofiber membrane with incorporated povidone-iodine into their regular face mask and breathed

over the active filter from four to eight hours daily for from one to four days, starting immediately after their positive PCR test. The participants have answered questionnaires with 11 questions (see the Appendix 1) three weeks after their positive PCR test. The data have been analysed and compared with the ÚZIS data (IHIS CR – Institute of Health Information and Statistics of the Czech Republic) as a reference group. Instead of reference group we used the above-mentioned state collected epidemiological data, following the "WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects – 2013" [11]. For statistical analyses the R program [12] with package survey was used [13–15]. The results were stratified using the age structure of the county [22].

RESULTS AND DISCUSSION

SARS-CoV-2 pandemic is a huge medical problem creating, in addition to medical complications, vast social challenges. Vaccination and/or an illness recovery are currently the most promising tools and weapons fighting spreading epidemy by generating antibodies in patients. However, an antibody level, necessary for efficient protection against illness, and half-time of their disappearance is highly volatile and unpredictable. Despite of undoubtedly overweighting positive effects of vaccination, neither vaccination nor recovery do not fully guarantee protection against illness. Application of disinfection of airways mucosa, thus, emerges as a highly desirable supplement or alternative.

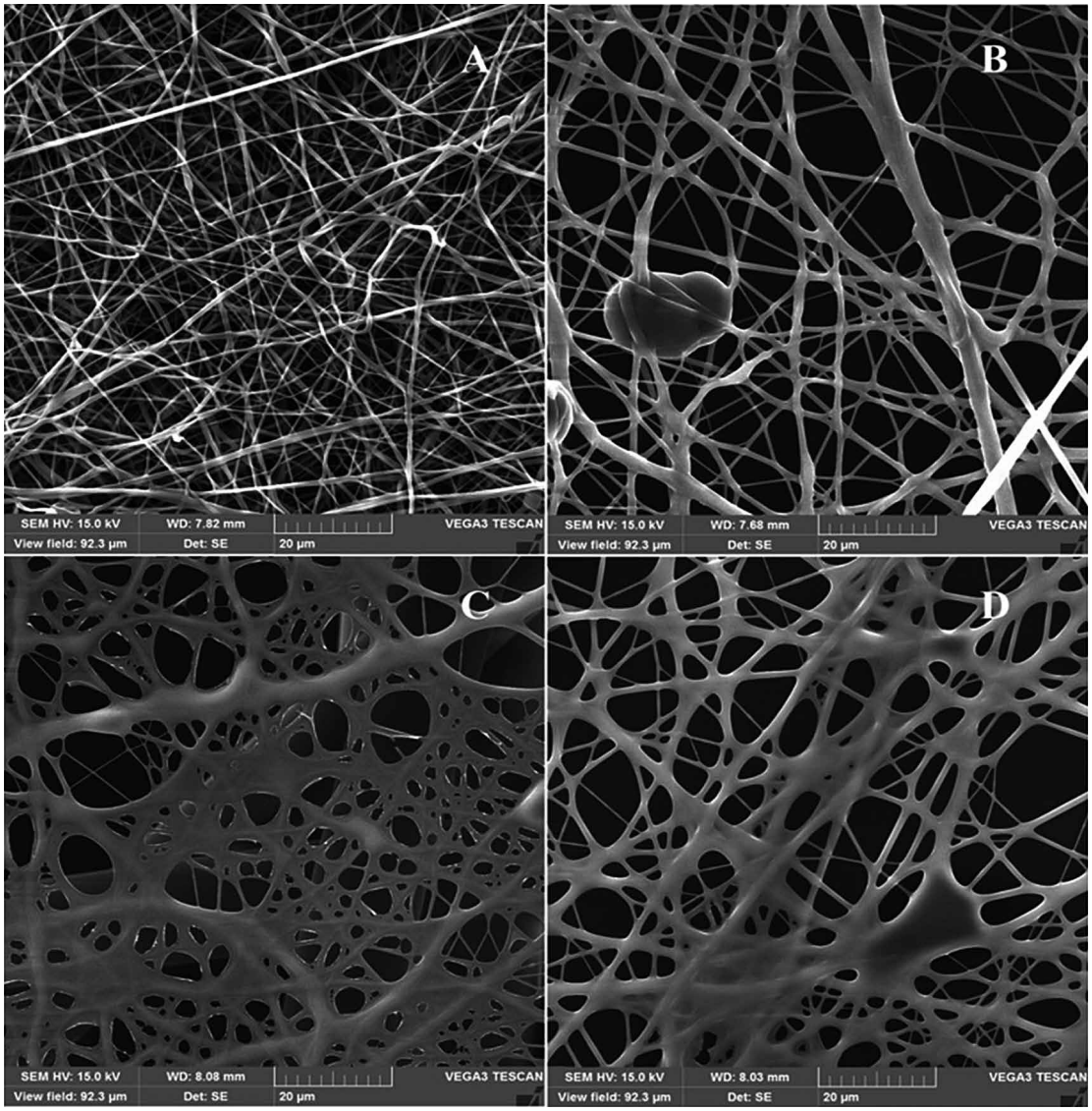

Figure 2. Electron microscopy image of the PVA Povidone-iodine functionalized nanofibers
A) PVA Povidone-iodine functionalized nanofibers at the beginning of the test,
B) 1.5 hours of breathing through the PVA Povidone-iodine functionalized nanofibers,
C) 3 hours of breathing through the PVA Povidone-iodine functionalized nanofibers,
D) 6 hours of breathing through the PVA Povidone-iodine functionalized nanofibers.

Iodine is known for a long time as a bactericide substance, moreover active against viruses, yeasts, moulds, fungi, viruses, and protozoa, as well. Among disadvantages of free iodine, however, belonged namely tissue irritation of its aqueous solvents. This drawback has been overcome by discovery of povidone-iodine, a bound iodine complex, decreasing drastically a free iodine concentration. Clinical studies and already a long-lasting practice clearly proved superiority of the povidone-iodine complex compared to all other iodine preparations with the sensitization dropping to 0.7% only [16]. Povidone-iodine is usually applied in 3–10% w/w concentrations in solutions, sprays or tampons and is available without medical prescription [17]. Free iodine from the complex is slowly released which minimize side effects. Reportedly, low povidone-iodine concentrations could be superior to more concentrated solutions [18]. Nano-structures could lead to extremely large surface compared to volume which could further contribute to increasing the effectivity of iodine molecules and, thus, decreasing of the used concentration. In addition, nanovesicles or fractionalized nanofibers have been reported as highly efficient system for influencing of not only external interactions of nanovesicles with cells but also for modification of cell functions [19]. We can expect a similar phenomenon also for interaction of nanodroplets of povidone-iodin with viruses.

We encapsulated 10% povidone-iodine solution into nanofibers from polyvinyl alcohol (PVA) in 1 : 10 ratio w/w. (Figure 2). Consequently, the final povidone-iodine concentration in nanofiber mesh was 1% w/w.

The nanofiber membrane (with the basis weight about 1 g/m^2) was formed (Figure 3). This nanofiber mesh was very tiny but sufficiently solid for further processing. The mesh was subsequently cut into 100 cm^2 filters, folded and the functionalized filters slipped into an ordinary face mask.

Such an ordinary face mask was equipped with low-density nanofiber mesh functionalized with encapsulated PVPI. Such a PPE was found as a very comfortable for application (wearing). First, this relatively low nanofiber density allowed a very comfortable breathing since the filter's flow resistance was low. This is important since classical nanofiber filters (often used in PPE) can protect against virus penetration in laboratory conditions. Their effect in PPE, nevertheless, is highly questionable. Clearly, a higher basis weight (density) naturally protects more efficiently against virus penetration since the average pore diameters decreases. Simultaneously, however, the smaller pores also significantly increase air flow resistance. Consequently, major part of breathing air flows into nose via unprotected space instead of through the nanofiber filter. Highest filter porosity of the novel PPE increased the amount of air flowing through the filter due to the overall lower filter air resistance. On the other hand, PVPI functionalization of nanofibers created an active barrier and viruses were not only mechanically trapped, but also inactivated due to the interaction of the virus with the PVPI which is explicitly recommended by WHO as disinfection against SARS-CoV-2. This additional function more than compensated the lower efficiency of mechanical filtration. Naturally, this function works not only for air inhalation but also for air exhalation. Consequently, the novel PPE can protect neighbourhoods of infected persons. Another important property of the nanofiber filter was its controlled degradation and very slow release of iodine molecules due to that a low amount of iodine is released for disinfection of airways. Mixture of povidone-iodine with PVA results in nanofibers with further slowing down of iodine release from povidone-iodine complex. Notably, the release half-time strongly depends on air humidity and it can also be regulated (Figure 4).

Figure 3. Macroscopic image of Povidone-iodine functionalized nanofibers

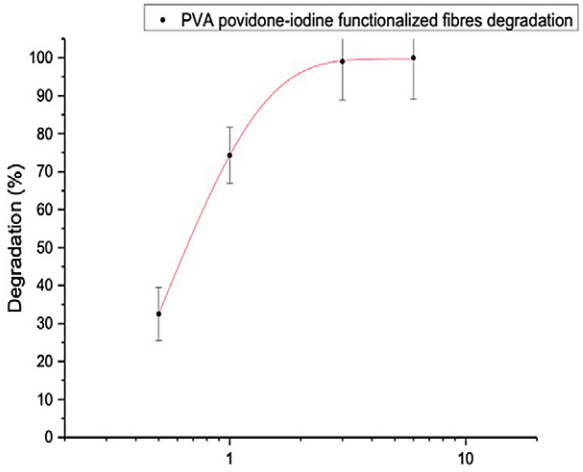

Figure 4. Degradation of PVA povidone-iodine
The red line is the spline connection for easier orientation

We have adjusted nanofiber mesh functionalized with povidone-iodine with majority of its degradation after 8 hours of breathing under normal physical activities in offices or households. We have simply calculated the amount of iodine from nanofibers mass with encapsulated PVPI (1% PVPI solution and 1g/m^2 nanofiber weight) as 100 µg iodine in the filter (size 100 cm^2). The total amount of iodine in the filter is 0.4×10^{-7} mol (0.01 g nanofibers/0.1 iodine concentration in nanofibers/ 257 g per mol – molar mass of iodine). Supposing 16 breath-in and 16 breath-out per minute, each of 2L, the consumed molar gas volume is about 1.378 mol per 8 h (or 689 mol per 4 h), the resulting ratio is 0.00028 ppm in case of 8 hours [23].This is 33 times less than the permissible exposure limit (PEL) 0.0095 ppm. Moreover, the Maximum Allowable Concentration (NPK-P in Czech legislative) is 1 ppm, which cannot be exceed under these conditions. Despite of this extremely low amount, the filter was effective as the study shows (see below). We hypothesize that this is a way how the active filter minimizes the health consequences and why the patients undertook only a mild course of the illness. The nanofiber scaffold can filter particles of nanometric scale, wherein SEM image (Figure 5) illustrates restrain capability of the scaffold for the particles of size from 70 nm because of nanofiber surface chemistry and high fiber density.

The face mask equipped with the functionalized filter was used for the clinical observation study. This filter, however, is characterized with a typical odour and there was easy to recognize it. Consequently, employment of the reference group equipped with a filter without povidone iodine, was fully impropriate and against good practice. Instead of reference group the Czech state collected epidemiological data have been used as a reference group. Altogether, 207 adults indicated as SARS-CoV-2 positive by PCR method have been involved into the clinical observation trial in April and May 2021. The participants applied an active nanofiber membrane with incorporated povidone-iodine into their regular face mask and breathed over the active filter minimum 4 hours, maximally 8 hours daily for a period from one to four days. We have found a clear positive epidemiologic effect since:

We have found that the reproduction number R0 was about 1.1 on the day two and three (Table 1). This fits very well with the number R0 at the Czech Republic in April and May 2021. However, the reproduction number R0 significantly decreased on the day four and five. Notably, respondents of our observational study haven't infected any other person since the 4. day from the beginning of application of the povidone-iodine filter and, consequently, the study the reproduction number R0 decreased to zero (Table 1). This clearly reflects a significant virus transmission in connection with application of the active filter. Importantly, there was neither mortality nor even one hospitalization. In addition, all patients declared only minor symptoms of the disease.

Interestingly, we have not observed dependence on the length of application, which indicated that already 4 h exposition is sufficient for protection.

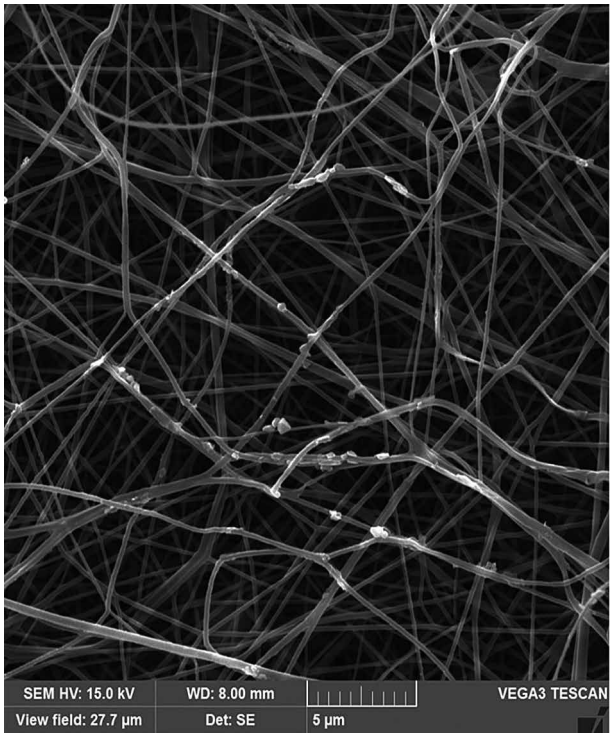

Figure 5. Electron microscopic image of dust on nanofibers

CONCLUSIONS

First, the clinical observation study showed that application of the povidone-iodine filter in the face mask can decrease a risk of transmission of SARS-CoV-2 and protect the neighbourhood of infected persons. This conclusion is clearly reflected by the drop of the reproduction number from the Day 4 from the beginning of application of the povidone-iodine filter. We suppose this observation as the most important as this can significantly contribute to decrease the society burden and explosiveness of the illness. This finding in combination with additional restrictions can also contribute to keep running of points with a higher concentration of people.

Second, the obtained data also indicate that application of the active filter can contribute to a moderate illness course of the patient as no one from 207 has a serious illness course. This conclusion is fully in agreement of previously performed study [1]. Unfortunately, we did not have the access to relevant ÚZIS data (reference sample of patients) to analyse more deeply this conclusion.

Table 1. Clinical trial

		Questionnaire	Census data	Source
	Number	**207**	**29357**	**SZU**
Illnes rating	Light	(59.9 ± 6.4) %	67.57 %	IHIS CR (ÚZIS ČR)
	Asymptomatic	(33.3 ± 4.1) %		
	Medium serious	(6.8 ± 3.3) %	22.3 %	IHIS CR (ÚZIS ČR)
	Very serious	0	8.1 %	IHIS CR (ÚZIS ČR)
	Death	0	1.03 %	IHIS CR (ÚZIS ČR)
Symptoms	Fatigue	(71.0 ± 7.6) %	69.6 %	[20]
	Fever	(20.3 ± 4.4) %	98.6 %	[20]
	Anorexia	(22.2 ± 3.6) %	39.9 %	[20]
	Cough	(31.4 ± 2.7) %	59.4 %	[20]
	Loss of smell or taste	(45.4 ± 3.6) %	39.6 %	[21]
	Headache	(50.2 ± 5.9) %	6.5 %	[20]
	Diarrhoea	(5.3 ± 4.9) %	10.1 %	[20]
	Rhinitis	(7.2 ± 3.7) %	26.8 %	[20]
	Myalgia	(17.4± 2.6) %	34.8 %	[20]
	Vomiting	(5.8 ± 1.8) %	10.1 %	[20]
	Dyspnea	(8.2 ± 2.0) %	31.2 %	[20]
Out of work	14 days or less	(88.9 ± 7.0) %		
	15 to 21 days	(5.3 ± 2.1) %		
	22 to 28 days	(4.8 ± 3.6) %	22 %	
	More than 28 days	(1.0 ± 2.9) %		
Recovery	Yes	(90.3 ± 7.3) %		
	Almost	(4.4 ± 1.3) %	8.5 %	IHIS CR (ÚZIS ČR)
	Not	(5.3 ± 1.6) %		
Number of infected people	After three days	0		
R	R	(0.0 ± 0.2) %	1.46	IHIS CR (ÚZIS ČR)

The resulting was stratified using the age structure of the county [22].

Third, a very tiny amount of released iodine (0.00028 ppm) was sufficient for the above-mentioned positive effects. Interestingly, we also did not observe dependence on the length of the active filter application when mask was worn a shorter time (4 h daily) or a longer time (8 h daily). This indicated that 4 h daily exposition was sufficient for protection. We hypothesize that this effect is related to extremely small droplets (nanostructures) released from the degrading nanofiber-based filter. Consequently, such a small system has much larger effective surface (one hundred times smaller droplet in diameter has 10,000 times larger surface) and, thus, it could be significantly more effective on airway mucosa.

Appendix
QUESTIONNAIRE

QUESTIONNAIRE

I. Information on the use of the Nanoiodine active filter

a) How many days did you wear a Nanuntio face mask?

4 or more days

3 days

2 days

1 day

I didn't wear it at all other:

b) How many hours a day?

8 hours (and more)

4–8 hours

2–4 hours

less than 2 hours a day

c) Mark between 1 and 5 how have you been instructed about the nanofiber mask? (1 not at all, 5 fully informed)

II. The course and symptoms COVID-19?

a) How would you describe your course of COVID-19 disease?

(Asymptomatic course, Easy course, Moderate course, Difficult course requiring hospitalization)

b) What symptoms did you suffer from?

Multiple options can be selected higher body temperature fatigue anorexia headache joint or spine pain loss of taste or smell vomiting diarrhea cold cough difficult to breathe any of the above other:

c) How many days have you been out of work?

d) Do you already feel healthy when completing this questionnaire?

III. Epidemiological data

a) Please, indicate the date when positively tested on COVID-19

b) How many people from your (family members, co - workers in direct contact, etc.) were tested on the day as you?

c) How many people from your closest surrounding have been positively tested on THE FOLLOWING DAYS?

d) If someone from your closest surrounding has tested positive LATER, after how many days?

Date of completion of this questionnaire:

At the same time, please send the completed Questionnaire to the address below: electronically evzen.amler@lfmotol.cuni.cz or by post Prof. Evžen Amler, Department of Medical Biophysics, 2nd Faculty of Medicine, Charles University, V Úvalu 84, 150 06 Prague 5. An electronic version of this questionnaire can be found at www.nanuntio.com

By sending the questionnaire, the respondent gives his / her explicit consent to the use of information about his / her health condition and all related information provided in this clinical observation survey, for purposes lying in the field of medical research and public health protection. The respondent may acknowledge in this connection that the information provided by him will not be used for any other purpose and that the full anonymity of his answers is guaranteed at the same time.

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