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REVIEW

Biotech innovations in the prevention of respiratory infectious diseases

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SUMMARY

Acute respiratory infections place a huge burden on society in terms of disability, premature mortality, and also direct health service costs (drugs prescriptions) and the indirect costs related to lost production. Therefore, prevention of respiratory infections is an important goal for public health interventions.
In this context, silver nanoparticles (AgNPs) represent an interesting perspective for research and development by virtue of their favorable antimicrobial activity against many respiratory pathogens. One of the latest innovations in the biotech field discussed in this review is the creation of a biocompatible, biogel-based nasal filter enriched with AgNPs. Compared to traditional personal protective equipment (PPE), this type of nasal filter has the advantage of combining the antibacterial and antiviral activity of AgNPs with the common filtration capacity shared by other PPEs. This dual mechanism means that AgNP-enriched nasal filters serve to reduce the infecting microbial-load and protect the lower airways, without interfering with the normal respiratory capacity (airflow-resistance <5%).
Given their antimicrobial characteristics and performance, AgNP-enriched nasal filters can meet many community and occupational currently unmet needs in the prevention of airborne infectious diseases, by ensuring an excellent respiratory-comfort and a continuous day-use.

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CLINICAL AND ECONOMIC RELEVANCE OF RESPIRATORY INFECTIONS

Acute lower respiratory infections are the third cause of mortality worldwide, accounting for 2.7 million deaths in 2015, of which 704,000 were children under 5 years of age (12.1% of total deaths in this age group) (WHO 2011, WHO 2011b, WHO 2012, Mortality and Causes of Death 2016). From 2005 to 2015, despite the marked improvement of global hygienic conditions, and the spread of antibiotic therapy, the number of deaths due to lower respiratory tract infections decreased by only 3.25% (Mortality and Causes of Death 2016). At greater risk for severe complications and death are adults over 65, individuals with comorbidities and children, with pneumococcal pneumonia and *H. influenzae* type B still being responsible for about 65% of paediatric deaths worldwide (Mortality and Causes of Death 2016).
Both viral and bacterial lower respiratory infections are associated with exacerbations of chronic obstructive pulmonary disease (COPD) (De Serres *et al.*, 2009, Ko *et al.*, 2016), with COPD expected to be the third leading cause of death worldwide by 2030 (WHO, http://wwwwhoint/respiratory/copd/burden/en/.2015). In turn, lower respiratory infections are often exacerbated by upper respiratory infections, or can arise as secondary superinfections after common (seasonal) viral diseases. For instance, preventable infections such those as by influenza A, influenza B, respiratory syncytial virus, rhinovirus, human coronavirus, parainfluenza virus and adenovirus, among others (Falsey *et al.*, 2013) may lead to bacterial pneumonia pandemics (Rynda-Apple *et al.*, 2015; Bakaletz, 2016) able to cause a significant number of premature deaths, especially in children (Lafond *et al.*, 2016).
Upper and lower respiratory infections thus place significant strain on health systems, with a huge social and economic impact. The total cost for respiratory diseases in the 28 EU countries amounted to more than 380 billion, including costs for direct primary healthcare and hospitals (at least 55 billion euros), those for productivity loss (at least 42 billion euros) and disability-adjusted life years ([DALYs], at least 280 billion euros) (European Respiratory Society, 2013).
Every year in the European Union (EU), respiratory and lung diseases cause at least 6 million hospital admissions, of which hundreds of thousands depend upon preventable infections. Between 1982 and 2012, influenza was associated with 10% of respiratory hospitalizations in children <18 years worldwide, ranging from 5% among children <6 months to 16% among children 5-17 years (Lafond, Nair *et al.*, 2016). In Europe, influenza causes 29,000 (CI: 4,000-210,000) paediatric hospitalizations per year (Lafond *et al.*, 2016), and an average number of 40,000 premature deaths (ECDC, 2015; ECDC, 2016).
A significant number of respiratory infections can be prevented by effective vaccination programmes that, however, still present some flaws. It has been recently highlighted that the inclusion of healthy children and adolescents

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in seasonal influenza vaccination programmes remains far less widespread than for the elderly or people with predisposing conditions (McGuire *et al.*, 2016), and vaccine effectiveness may be reduced by the mismatch between vaccine and circulating strains, as happened in 2014-2015 for H_3N_2 and Yamagata viruses (Pebody *et al.*, 2015, ECDC, 2016).

PREVENTION OF RESPIRATORY INFECTIONS BY PERSONAL PROTECTIVE EQUIPMENT

About half of hospitalizations for respiratory diseases are due to acute infections related to professional or domestic exposure to infectious agents and air pollutants. Effective prevention of such exposure would thus lead to a major reduction of mortality due to respiratory diseases, and save a considerable amount of money in EU countries.

Microorganisms usually reach the tracheobronchial tree by inhalation and/or aspiration. Afterwards, the development of a symptomatic acute infection depends greatly on the "dose" of pathogen, the duration of the exposure, and air pollution. The literature provides evidence for the correlation between exposure to air pollution and respiratory illnesses, with indoor air constituents such as nicotine, particulate matter (PM 2.5), nitrogen dioxide and carbon monoxide implicated in increasing the risk of respiratory diseases due to bacterial and viral infections in both children and women (Po *et al.*, 2011; Gehring *et al.*, 2013; MacIntyre *et al.*, 2014).

Remedial measures to exposure to biological and particulate air pollutants include, among others, the use of respiratory personal protective equipment (PPE). PPE, if used properly, can reduce the penetration of pathogens by reducing the "microbial load" in inhaled air, substantially decreasing the risk of many respiratory diseases consequent to occupational or community exposure to infectious agents (European Respiratory Society, 2013). In addition, indoor air constituents involved in exacerbation or promotion of respiratory infections can be successfully trapped.

Classical hygienic and surgical masks along with filtering face respirators approved as PPE in Italy (*Table 1*) reduce the inhaled microbial load through mere filtration processes. Such devices trap bacteria and viruses only at the expense of a strong reduction of the inhaled airflow, decreasing respiratory capacity to a level often incompatible with common daily activities.

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

Silver has long been known for its anti-microbial effect (Lara *et al.*, 2011), and silver nanoparticles (AgNPs) are one of the nanomaterials with the most powerful antibacterial and antiviral activity (Kim *et al.*, 2007; Agnihotri *et al.*, 2013; Besinis *et al.*, 2014; Chen *et al.*, 2016; Zhang *et al.*, 2016). AgNPs are effective bactericides (Sondi and Salopek-Sondi, 2004; Li *et al.*, 2010; Agnihotri *et al.*, 2013) with a demonstrated ability in inhibiting biofilm formation and reducing microbial concentration and development of antibiotic resistance (Kalishwaralal *et al.*, 2010). In addition, they can enhance the activity of antibiotics themselves when combined (i.e. penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin) (Shahverdi *et al.*, 2007).

Even at low concentrations, AgNPs are able to completely inhibit the growth of Gram-negative bacteria such as *E. Coli*, and to strongly reduce that of Gram-positive bacteria, including *S. Aureus* (Kim *et al.*, 2007). In the case of Gram-negative bacteria, the antibacterial activity of AgNPs is permitted by their accumulation within the cell wall, and by the formation of pores and gaps, that ultimately result in permeability alterations and cell death (Sondi and Salopek-Sondi, 2004; Li *et al.*, 2010).

AgNPs are also effective antiviral agents, active against a growing number of viruses, including HIV (Elechiguerra *et al.*, 2005; Lara *et al.*, 2010; Xiang *et al.*, 2011), HBV (Lu, Sun *et al.*, 2008), herpes simplex virus type 1 and 2 (Baram-Pinto *et al.*, 2009; Gaikwad *et al.*, 2013), respiratory syncytial virus (Yang *et al.*, 2016), adenovirus type 3 (Chen *et al.*, 2013), and influenza virus H_1N_1 (Xiang, Chen *et al.*, 2011, Mori, Ono *et al.*, 2013).

As the specific antiviral activity of AgNPs is still largely unknown, several studies have demonstrated their ability to strongly reduce viral viability and viral titre, without toxic effects on infected cells (Trefry and Wooley 2013). In mice infected with influenza virus H_3N_2, the intranasal administration of AgNPs significantly reduced the viral titre in lungs, decreased the incidence of lung tissue lesions and substantially increased survival (Xiang *et al.*, 2013), suggesting their potential use as antiviral agents against respiratory infections.

Table 1 - *Protective efficacy of facial masks available on the market.*

Mask	*Respiratory protection*	*Types available*
Hygienic masks	No respiratory protection	
Surgical masks	May confer protection against bacteria, but not against viruses. After 2-3 hours of use they should be replaced as they loose effectiveness with moisture.	4 types available: I, IR, II and IIR. Those of type II (three layers) and IIR (four layers) offer a greater bacterial filtration efficiency (≥98%). In addition, the IIR is also splash resistant.
Facial masks with dust filter	These are the only masks that guarantee respiratory tract protection from bacteria and influenza virus. This type of mask must be replaced after 8 hours of use. The effectiveness of this mask is mainly linked to its adherence to the face. For this reason, it has to be worn without a beard and moustache, and after hand washing. They offer poor comfort and can be dangerous for people with respiratory difficulties.	*FFP1:* Facial filter with low efficacy against solid particles (minimum 78% filter efficiency). It allows the penetration of 20% of environmental dust. *FFP2:* Facial filter with an average efficacy against solid and liquid particles (minimum 92% filter efficiency). It allows the penetration of 6% of environmental dust *FFP3:* Facial filter with high efficacy against solid and liquid particles (minimum 98% filter efficiency). It allows 0.05% penetration of environmental dust.

BIOTECH APPLICATIONS OF SILVER NANOPARTICLES FOR THE PREVENTION OF RESPIRATORY DISEASES

Compared to AgNP solutions, biomaterials with immobilized AgNPs show an enhanced antibacterial activity since the homogeneous diffusion of small AgNPs in a pH-controlled environment favours the contact with bacterial structures, and the consequent bactericidal action (Sanpui *et al.*, 2008; Agnihotri *et al.*, 2013). The development of biocompatible materials enriched with AgNPs is currently an important research area, especially for respiratory devices, thanks to the antimicrobial activity of AgNPs against common respiratory pathogens.

One of the latest innovations in the biotech field, discussed in this review, is the creation of a biocompatible biogel nasal filter containing a proportion of Teflon particles enriched with AgNPs. This device has the advantage of combining the antibacterial and antiviral activities of AgNPs with the common filtration processes shared by other PPEs, thus providing a dual mechanism to reduce microbial load and protect the lower airways, without determining an increased resistance to airflow (<5%) and preserving normal respiratory comfort.

FILTERING CAPACITY OF BIOGEL-AGNPS NASAL FILTERS

The certification procedure of biogel-AgNPs nasal filters as PPE has demonstrated a 52% filtration efficiency of particular matter (PM), and a 42%-82% reduction of SiO2 particle penetration (0-8 microns), calculated on 8 working hours and with a flow-speed of 47.5 l/m. The composition of the PM trapped by nasal filters comprises particles of SiO2 from <100 nm onwards, with very good qualitative efficiency above 200 nm (0.2 µm, thus including PM2.5), primarily due to the adhesive nature of the gel's walls (*Figure 1*). Electron microscopy confirmed effective trapping of particles <2.5 µm, and even <1 µm (*Figure 2*). The proportion of environmental pollutants PM1.0 increased from 10% in the tested powder, to 94% in the filter dust, probably thanks to the air turbulence generated by the coil, able to increase the chances of contact between volatile particles and the adhesive walls of the device.

The ability to filter extremely small particles (0.2-1 µm) can allow the nasal filters to trap effectively also microorganisms with the same size, supported by the intrinsic adherence ability of bacteria and viruses (e.g. adhesins, fibrils, capsular polysaccharides, pili, envelope).

ANTIBACTERIAL PROPERTIES OF AGNP-BIOGEL NASAL FILTERS

The AgNPs included in the composition of the biogel have demonstrated an excellent antibacterial activity against *E. coli*, even at very low concentrations. *Figure 3* shows the dose-dependent efficacy in reducing, until completely inhibiting, bacterial replication *in vitro* as a result of the contact between the AgNP-biogel and an *E. coli* solution. The inhibition of bacterial growth around the sample analyzed is optimal starting from AgNP concentration of 7.5%.

Antiviral action of AgNP-biogel nasal filters

The biogel containing AgNPs is able to cause a marked reduction of viral titre (from 1 to >2 $\log_{10}$, meaning from 10

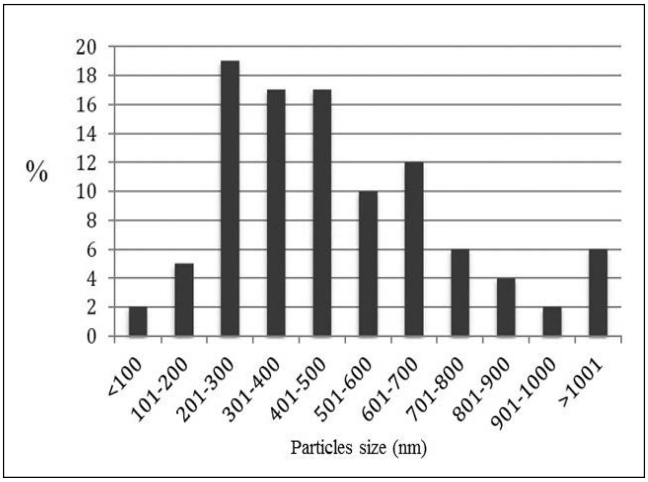

Figure 1 - *Distribution curve of percentages of SiO_2 particles filtered from the helical portion of the biogel nasal filter, divided according size. Nm, nanometres.*

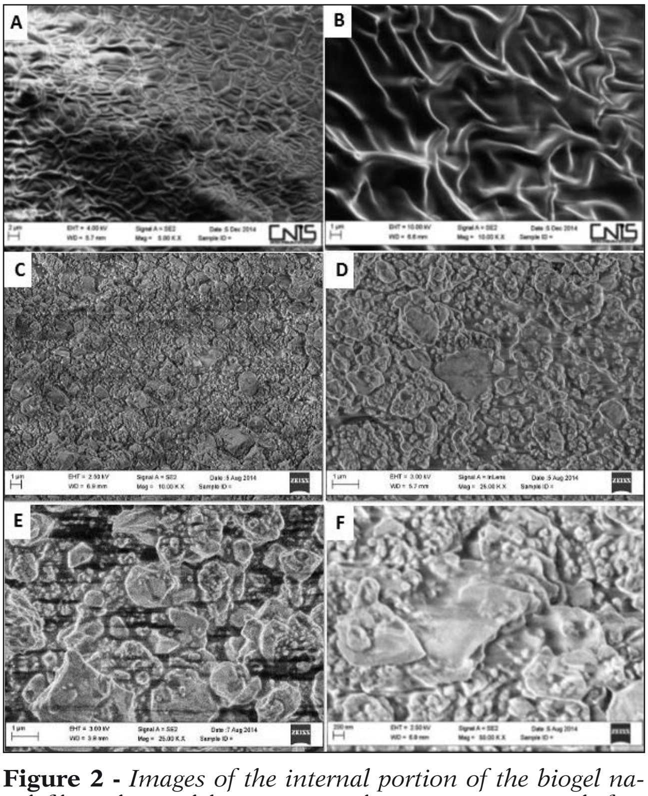

Figure 2 - *Images of the internal portion of the biogel nasal filter obtained by scanning electron microscope before (A-B) and after (C-F) the SiO_2 filtering tests (at increasing magnification). Zeiss Auriga Cross-Beam workstation mod 405, combining a FESEM (Field-Emission Scanning Electron Microscopy) column and a FIB (Focused Ion Beam) column (Cobra by Orsay Physics), and equipped with an EDX (Energy Dispersive X-Ray Spectrometer) Quanta System by Bruker was used. It is a characterization platform at the state-of-the-art, with a spatial resolution better than 1 nm at an acceleration voltage of 15 kV. The particle size distribution was measured with the support of suitable software, combining manual and automated measurements. EDX was used as a checking tool to verify that the observed and measured particles were SiO_2 (testing powder) and not undesired contaminants.*

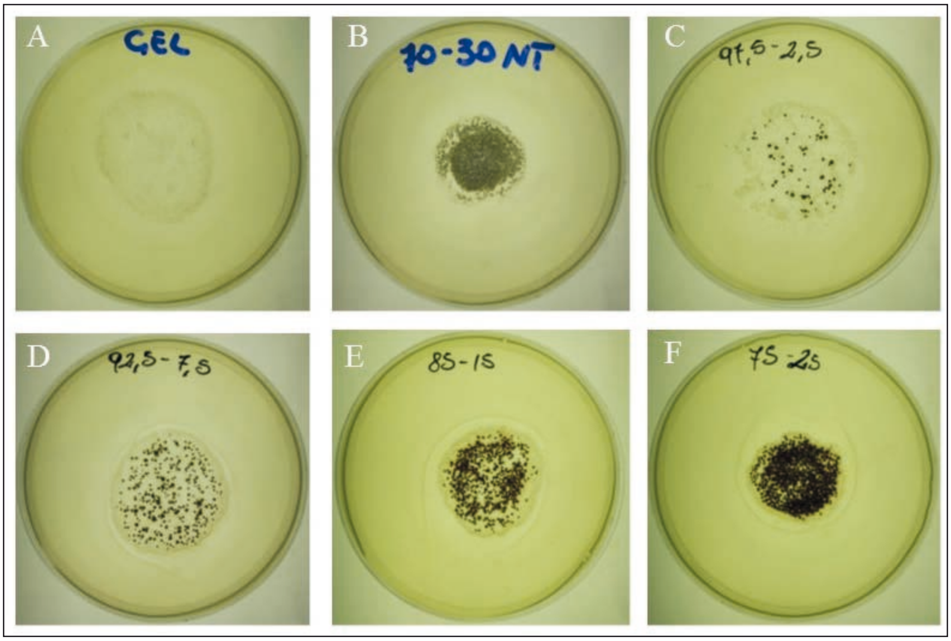

Figure 3 - *Antibacterial efficacy of the biogel blend enriched with increasing concentrations of polytetrafluoroethylene (PTFE) particles treated with silver nanoparticles. (A) 100% standard untreated gel; (B) 70% gel and 30% untreated PTFE particles; (C) 97.5% gel and 2.5% treated PTFE particles; (D) 92.5% gel and 7.5% treated PTFE particles; (E) 85% gel and 15% treated PTFE particles; (F) 75% gel and 25% treated PTFE particles. Microbiological tests were performed in accordance with the Swiss Standard 'SNV 195920-1992', using Escherichia coli as tester microorganism. According to this standard, the sample is placed in contact with a bacterial suspension on an agar plate, and incubated at 37°C for 24 hours. After incubation, the inhibitory effect of the sample on microbial growth and proliferation is evaluated according to the size of the bacterial growth inhibition halo around the sample, and in correspondence with its contact surface. If the zone of bacterial growth inhibition is larger than 1 mm, the antibacterial ability is defined as "good"; if the sample is completely repopulated by bacteria, antibacterial capacity is defined as "not sufficient." The standard also provides intermediate levels of antibacterial efficacy.*

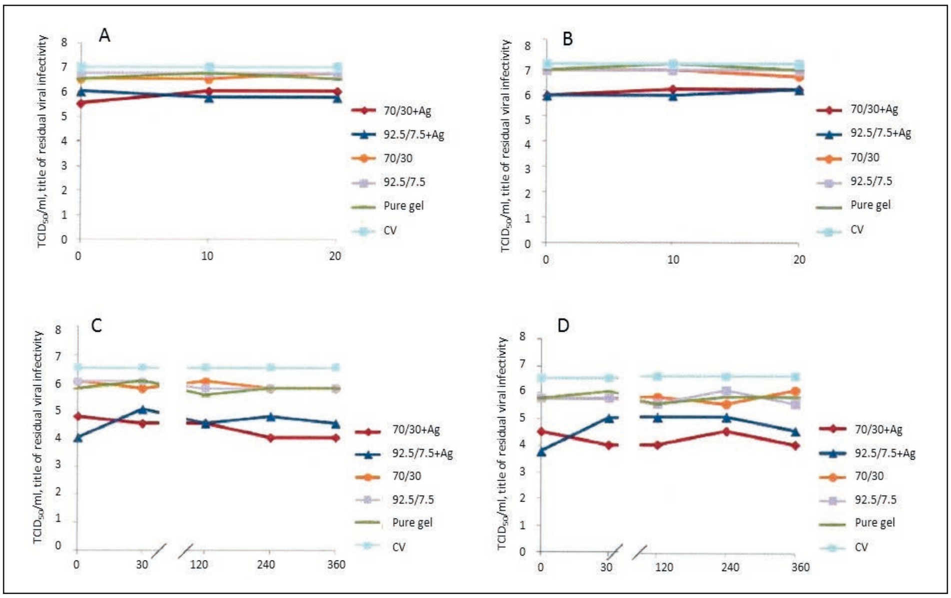

Figure 4 - *Reduction of viral infectivity by increasing concentrations of polytetrafluoroethylene (PTFE) particles treated with silver nanoparticles (AgNPs). A suspension of influenza virus A/PR/8/34 with known titre was homogenized with an equal volume of gel matrices containing varying percentages of polytetrafluoroethylene (PTFE) particles, treated or not with AgNPs. Aliquots of the homogenate were then taken, diluted and used in the infection of MDCK cells for the determination of the titre of residual viral infectivity. The removal of the supernatant for determination of residual viral infectivity was carried out after 0, l0, 20 minutes in experiment 1 (A, B), and after 0, 30, 120, 240 and 360 minutes in the experiment 2 (C, D). For each experiment, two independent samples of 5 matrices were analyzed. $TCID_{50}$/ml, titre of residual viral infectivity; CV, control virus.*

to >100 times) compared to the untreated control, when placed in contact with a suspension containing a known concentration of influenza virus A/PR/8/34 (*Figure 4*). The reduction of infective capacity does not seem to be time dependent, as being already massive since the start of observation (90-99%). The simple passage through the AgNP-biogel can thus greatly reduce the viral titre, an antiviral effect further potentiated by a prolonged contact with the filter's walls (*Figure 4*).

OCCUPATIONAL, NOSOCOMIAL AND COMMUNITY USE OF NASAL FILTERS

In light of the data presented and discussed, biogel-AgNP nasal filters are believed to be efficient PPEs against infectious agents most commonly involved in airway and lung diseases.

Compared to occupational respiratory disease caused by exposure to gases, vapours and dusts, respiratory infections acquired with work received little attention until the outbreak of severe acute respiratory syndrome (SARS) in 2003, which affected more than 8000 individuals, a fifth of whom were healthcare workers (Ho *et al.*, 2007). Common viral infections or, more rarely, bacterial infections can affect those who work in crowded environments, schools, hospitals and other communities. Biohazard is high for operators in the health sector or research centres, animal professionals, biotechnologists, operators of food and organic farming sectors, the pharmaceutical industry and leather workers. Respiratory PPEs, including biogel-AgNP nasal filters, can thus reduce the risk of several occupational respiratory diseases, and today they constitute the "third line of defence" after technical and organizational measures (Ho *et al.*, 2007).

Given the increased incidence of lower respiratory tract infections in children and the elderly, as well as in immunocompromised subjects, biogel-AgNP nasal filters are adaptable to primary prevention in various clinical and community settings (i.e. bone marrow transplant, solid organs transplant, HIV or AIDS, haematological diseases, chronic lung diseases, heart diseases, diabetes, intake of steroids, chemotherapy or other immunosuppressants). In all these clinical circumstances, it becomes absolutely necessary to reduce to a minimum the microbial load inhaled without affecting the respiratory capacity, often already compromised in such clinical situations. The characteristics of the biogel-AgNP nasal filters seem to be fully in line with this *"unmet clinical need"*.

CONCLUSIONS

Infectious respiratory diseases are a significant problem for society, primarily in terms of disability and premature mortality, but also for the costs directly applied to the health service, costs for the prescription of drugs and indirect costs caused by decreased productivity. Therefore, their prevention is a major public health goal.

In this context, given their favourable antimicrobial activity against many bacterial and viral respiratory pathogens, AgNPs represent an interesting perspective of research and development. A recent innovation in the biotech field has seen the creation of a biogel biocompatible nasal filter, containing a portion of polytetrafluoroethylene enriched by AgNPs. Compared to currently available PPEs in Italy, this type of device has the advantage of combining the antibacterial and antiviral action of AgNPs with common filtration processes shared by other PPEs. The dual mechanism of biogel nasal filters enriched with AgNPs thus serves to reduce microbial infectivity and protect the lower airways.

Experimental studies positively reported the ability of biogel-AgNP nasal filters to trap and inactivate bacteria and viruses, making them a promising PPE to be used both in occupational and community settings. In the nosocomial healthcare environment, biogel-AgNP nasal filter use may be aimed at preventing the transmission of microbial agents both to patients and to healthcare personnel. Their use can also be extended to all other occupational areas wherever there is a high density of crowding (schools, universities, offices), possible contamination by biological agents, and/or risk of microbial transmission to humans (farming, agriculture, timber processing, markets, etc.).

In the general population, the biogel-AgNP nasal filters can make a significant contribution in reducing the transmission of microbial agents through air. This applies both to the domestic environment, often contaminated by biological pathogens, and to the outdoor environment, whenever contaminated by a high concentration of microbes and/or allergens.

The biogel-AgNP nasal filters can thus respond to numerous health issues in the prevention of air-transmitted infectious diseases. The antimicrobial potential of this type of biogel nasal filter does not affect respiratory functions, guaranteeing the possibility of a continuous use for a long period of time in conditions of perfect breathing comfort, allowing day long protection.

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