

Nanomaterials as a new opportunity for protecting workers from biological risk

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Abstract: Healthcare-Associated Infections (HAIs) represent a frequent complication for hospitalized patients and more rarely for workers. In recent years, substantial scientific evidence has been reached regarding the role played by the inanimate surfaces, especially those touched in patient-care areas, in the transmission of nosocomial pathogens. Therefore, it is essential to find new collective protective measures to minimize microbial contamination in healthcare facilities, thereby preventing the spread of multi-drug resistant bacteria. We present an overview of the major nano-enabled AntiMicrobial Coatings (AMCs) which may be used as collective protective measures in healthcare setting, discussing also some aspects related to their effectiveness and safety. AMCs may be classified within three groups on base of their mechanism of action: surfaces releasing active compound, contact-killing surfaces and anti-adhesive surfaces. To date, little information is available on the effectiveness of AMCs to reduce the risk of HAIs since the most of studies do not reach conclusive results on their beneficial effects. Moreover, the lack of standard protocols for assessing antimicrobial efficacy and poor data about the interaction between AMCs and disinfectants prevent their placing on the market. Further studies are needed for assessing risks and benefits of AMCs as collective protective measures in healthcare setting.

Key words: Nanomaterials, Antimicrobial coatings, Healthcare-associated infections, Biological risk, Collective protective measures

Introduction

Nano-enabled materials are widespread produced and used in different sectors: from energy to medicine, from automotive to construction, from agriculture and food to personal care¹⁾. Major occupational concerns are related to their potential adverse health effects²⁾ and the related strategies for preventing workers' exposure³⁾.

The novel properties of nanoscale materials may offer also new opportunities to improve the protection of work-

ers exposed to traditional risks through the development of new devices and technologies. As an example, nanotechnologies had several applications in protective clothing and smart textiles for the improvement of personal protective equipment due to their increased thermal, electrical, mechanical and antimicrobial properties^{4–7)}; engineered dressings may reduce the risk of microbial infection⁸⁾; nanocellulose sheets can be used as wound dressing and healing materials in medicine⁹⁾ with promising application for the development of sterile coating for medical applications in surgeries¹⁰⁾.

In this framework and in accordance with European strategies¹¹⁾, the development of nanotechnology has opened up new horizons in the research of “non-traditional”

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antimicrobial compounds that have led to the production of AntiMicrobial Coatings (AMCs).

As known, Healthcare-Associated Infections (HAIs) are the most common complication in hospitalized patients with a significant clinical and economic impact. People who may be at risk of contracting these infections are first of all patients and, with less frequency, healthcare personnel, volunteer assistants, trainees and students¹².

A recent survey on all types of health-associated infections carried out by the European Center for Disease Prevention and Control (ECDC) shows that in Europe 3.2 million patients are affected by at least one HAIs and 37,000 die as direct consequence of these¹³. In particular in Italy, each year about 450–700,000 new cases of HAIs are registered (primarily urinary infections followed by pneumonia and sepsis) almost half of which are preventable¹⁴. Healthcare-associated infections are closely connected with the problem of the antibiotic resistance, increasing global phenomenon. According to the latest report of the Organization for Economic Cooperation and Development (OECD), Italy is the third country with the highest percentage of antibiotic resistant bacteria (33–34% in 2014, doubled compared to 2005 when it was 16–17%), behind only Turkey and Greece¹⁵.

Although, in 20–40% of cases, the source of HAIs is the endogenous flora of the patient, environmental matrices (air, water) and surfaces (patient rooms, medical equipment, etc.) are also involved in the transmission of the microorganisms¹⁶. Substantial scientific evidence has been reached in recent years regarding the role played by the inanimate surfaces in the transmission of nosocomial pathogens, including multidrug-resistant bacteria^{17, 18}.

For this reason it is of the utmost importance to clean and disinfect regularly environmental surfaces with particular attention to those more frequently touched by patient and healthcare personnel (High Touch Surfaces, HTSs) such as bedside tables, switches, push-buttons, computer keyboards, electro-medical devices, blood pressure devices, etc¹⁹. Some studies^{20–22} have however documented the lack of compliance with established guidelines for disinfection and sterilization in healthcare setting.

In this context, is essential to find new collective protective measures to minimize microbiological contamination on frequently touched surfaces in healthcare facilities, thereby preventing the growth and spread of microorganisms, especially multidrug-resistant bacteria. In this paper, we intend to provide a representative overview of the major AMCs that can be used as collective protective measures to safeguard the health of patients and workers.

We also discuss aspects related to the efficacy, long-term stability and safety of antimicrobial coatings in healthcare facilities.

Methods

Search strategy

We reviewed the international literature including peer-reviewed journal articles and studies extrapolated from technical reviews, books and reports. In order to identify relevant publications, a literature search was performed in PubMed and Scopus databases using a search strategy adapted to each database structure. We used the following search terms ‘antimicrobial’, ‘antibacterial’, ‘microorganism’, ‘healthcare-associated infections’, ‘biological risk’ coupled with the keywords ‘coating’ OR ‘surfaces’ AND ‘nano’. Papers and documents in English language published in the last 15 yr have been examined in this study.

Results

Antimicrobial coatings classification

Different antimicrobial surfaces are described in literature, but these can be classified in three main groups according to their mechanism of action: ‘surfaces releasing the active compound’, ‘contact-killing surfaces’ and ‘anti-adhesive surfaces²³’.

Surfaces releasing the active compound

Currently, the majority of AMCs releases the active compound. They are commonly produced by combining of a porous substrate with the antimicrobial agent; the latter can be either deposited directly on the surface or inside polymeric matrix²⁴. Self-disinfecting surfaces containing silver, copper or zinc nanoparticles or titanium dioxide are widely documented in literature. Their antimicrobial action takes place through various mechanisms of action; among these, the most common are oxidative stress, metal ion release and non-oxidative mechanisms^{25, 26}. As is well known, different nanoparticles (NPs) may generate distinctive reactive oxygen species (ROS), such as superoxide (O_2^-) or hydroxyl radical (OH), hydrogen peroxide (H_2O_2) which are able to pass through the bacterial membrane and cause cell death²⁷. However, as regards the metal ion release, it has been shown that copper oxide (CuO) NPs can interact with functional groups of proteins altering the normal physiological processes in the bacterial cell. About non-oxidative mechanisms, these involve interaction of the NPs with bacterial membrane or cell wall. NPs present

some features which make them better suited to combat infectious agents, such as their functionalization with different (bio) molecules (Ag, Au, Al, Cu, Zn, etc.), controlled time-release and especially mechanisms of action different from those of the antibiotics²⁸).

Silver-based nanoparticles (AgNPs), widely used for decades, show broad-spectrum antimicrobial activities. However, they have limits related to high costs and low durability, as they tend to oxidize and lose their effectiveness in releasing silver ions. In addition, the active compound may gradually become inactive and therefore it may induce the formation of resistant bacterial strains²⁹. Not only AgNPs are considered to be very effective against bacteria but also other metallic nanoparticles (CuONPs, TiONPs, AuNPs, and Fe₃O₂NPs) have shown bactericidal effects^{30–32} because of their interaction with functional groups of proteins and nucleic acids, amino and carboxyl groups²⁷. The most important limit of the surfaces releasing active compounds is their potential toxicity; the active compound can be released from coatings into the environment where it may have adverse effects against eukaryotic cells, especially against aquatic organisms^{33, 34}. In a recent study³⁵, the synergic bactericidal effects of reduced graphene oxide (rGO) and AgNPs were responsible for the increase in antibacterial activity of rGO-nAg nanocomposite with very promising results against several clinically relevant pathogens.

Contact-killing surfaces

In these surfaces, the active compound is covalently anchored to the coating and interacts with bacterial cell through direct contact. The biocide (e.g. quaternary ammonium polymers or peptides) may become active upon contact with bacterial cell or after activation by light as in the case of titanium dioxide (TiO₂) or photosensitizers. Several biocides are known, such as quaternary phosphoniums compounds (QPCs), carbon nanotubes, antibacterial peptides, quaternary ammonium compounds (QACs) and N-chloramines, but the last two are the most studied³⁶.

In the case of antibacterial surfaces containing ammonium salts or quaternary ammonium compounds (QACs), the positively charged nitrogen in the ammonium group interacts with the negatively charged of the phospholipids in the bacterial membrane, causing the disruption of Gram-positive and Gram-negative cells^{37, 38}. Otherwise, N-chloramines are formed by chlorination of amine, amide or imide groups and contain one or multidrug-resistant N-Cl bonds in which Cl is partially positively charged. The mode of action of surface bound N-chloramines has

been hypothesised to be based on active chlorine transfer from surface N-chloramines to the external protein matrix of bacteria³⁹. Other cationic agents, such as polymers and polysaccharide chitosan act by damaging the cell membrane and cause cell death. In addition, antimicrobial peptides (AMPs) have been successfully used for their broad-spectrum antimicrobial activities⁴⁰. Their mechanisms of action have been widely studied, including ‘polymeric spacer effect’, ‘ion-exchange mechanism’ and ‘phospholipid sponge effect’; anyway, the surface positive charge density seems to be a key parameter to define antibacterial efficacy⁴¹.

Anti-adhesive surfaces

Anti-adhesive surfaces aim to repel microbes or decrease their surface attachment. For this purpose, chemical composition, hydrophilicity, hydrophobicity and topography are modified in order to reduce bacterial adhesion during the initial stage of the biofilm formation process^{38, 42}. As it is known, biofilm is a thick layer of bacteria aggregated to each other on surfaces within the extracellular matrix produced by themselves. Biofilm protects them from adverse environmental conditions, also inhibiting the penetration of antibiotics thus promoting the antibiotic resistance^{43, 44}.

Recent studies have shown^{45–49} that some nanoparticles (AuNPs, ZnONPs, CuNPs, GONPs) are able to hinder biofilm formation by interacting with the extracellular matrix and the bacterial communication—quorum sensing (QS). This latter plays an important role on the bacterial communication through the production of signal molecules able to synchronize the expression of genes which bacteria use to respond to changes in the environment⁵⁰.

Safe By Design (SbD) approach

Whatever technology is used for the production of antimicrobial surfaces, it must however take into account the potential health and safety risks associated with the final product. For this purpose, some European research teams have recently proposed a Safe-By-Design (SbD) approach in Antimicrobial Coatings development aimed to obtain safe products and compliant with all European regulations²³. To develop innovative but at the same time safe antimicrobial coatings, during the early design phase, it is necessary to consider various aspects including their efficacy and long-term stability, but also the potential release of the active compound from the coating into the environment and its toxicity.

In this regard, some European research programs

(NanoFase, SafeNano, ProSafe, NaNo-Reg, NaNo-Reg2, Euro-NanoTox) have examined different issues related to the toxicity and fate of nanomaterials into environment in order to set toxicological measurements and establish international standards²⁴). Silver-resistant bacterial strains have been found in hospital sewage systems⁵¹), while in the study of Pal *et al.*⁵²), resistance genes to metals and biocides have been found in different environments, including those not influenced by large-scale human use of antimicrobials⁵³). As these genes were found together with antibiotic resistance genes on mobile genetic elements such as plasmids or transposable elements⁵⁴), it is clear that these resistance mechanisms are aimed to protect bacteria both from toxic effects of antibiotics and antibacterial compound, contributing to the maintenance and spread of multi-drug resistance strains in the environment. In this regard, particular attention should be focused on cleaning procedures in healthcare facilities, because during these operations, small amounts of biocides from AMC's could be likely removed. These released into the environment may be very harmful to human or animal health. Hence, it is needed that hospital wastewater and cleaning effluent containing potential biocides or multidrug-resistant bacteria are properly treated⁵⁵).

AMC's effectiveness and long-term stability

In literature, little information is available on the effectiveness of AMC's in healthcare setting since the most of studies do not reach conclusive results on beneficial effects and furthermore poor data are available on the long-term duration of antimicrobial effects. In a recent paper, Muller *et al.*⁵⁶) have carried out a systematic review on the use of self-disinfecting surfaces in patient rooms in order to assess whether these were able to reduce the degree of microbial contamination when compared with standard surfaces. The results show that only 11 out of a total 6,011 studies were eligible under criteria fixed since the most studies was not randomized or, in other cases, confounding factors had not been taken in account. Eleven studies that passed the evaluation criteria concerned mostly copper (n=7), while few papers were found on the impact of non-copper antimicrobial surfaces on microbial contamination in healthcare setting: silver (n=1), metal-alloy (n=1) and organo-silane (n=1). This is partly due to the lack of standardized methods, universally recognized by the scientific community, essential for evaluating antimicrobial efficacy against tested microorganisms and the long-term stability of AMC's. Some industrial standard tests are usually used for assessing the antimicrobial efficacy on surfaces but

they are not suitable for testing products to use in healthcare facilities since they do not provide data regarding the toxicity or the potential release of the active compound in the environment²³) and thus they do not meet SbD criteria. Moreover, many industrial standard tests have been modified over the past years adapting them to specific context, since AMC's based on diverse mechanisms of action (active compound releasing, contact-killing and antiadhesive surfaces) require different *in vitro* tests.

For example, ASTM E2149 is the most commonly used method for evaluating biocide-releasing surfaces⁵⁷) and less suited for non-leaching (immobilized and not water-soluble) antimicrobial products. Adhesion-based methods, on the other hand, are suited for evaluating effectiveness of contact-killing surfaces⁵⁸). In a recent study, van de Lage-maat *et al.*⁵⁹) found that 'Petrifilm system' and 'Japanese Industrial Standard (JIS Z 2801)' were the best methods to assess the antimicrobial activity of contact-killing surfaces, if they were used with a complementary assay (zone of inhibition on agar) to exclude bacterial death due to the release of active compound in the medium. Although the JIS Z 2801 has also been adopted as an International Organization for Standardization procedure (ISO 22196:2011), it is a questionable method, in certain respects. Under experimental conditions, indeed, the microbial inoculum is spread over a wide surface covered with a thin sterile film to ensure close contact with the antimicrobial surface at an incubation temperature of 35°C and in humid chamber, for a period of 24 h. In indoor environments, on the contrary, the microbial contaminants dry quickly onto surfaces where they usually form cell aggregates loosely grouped together, not in direct contact with the surface. Consequently, it is very likely that under these latter conditions, antimicrobial efficacy is lower other than obtained under optimal experimental conditions.

Conclusions

Despite the efforts to prevent and control HAIs, these remain a frequent complication for hospitalized patients and a big challenge for the health system. Nano-enabled antimicrobial coatings on inanimate surfaces, work equipment, personal protective equipment (gloves, face mask, etc.) might make an important contribution to the fight against nosocomial infections, also preventing the spread of antibiotic-resistant bacteria, concomitantly with the adoption of additional prevention and protection measures.

However, as regards the self-disinfecting surfaces, their potential use as measures of collective protection against

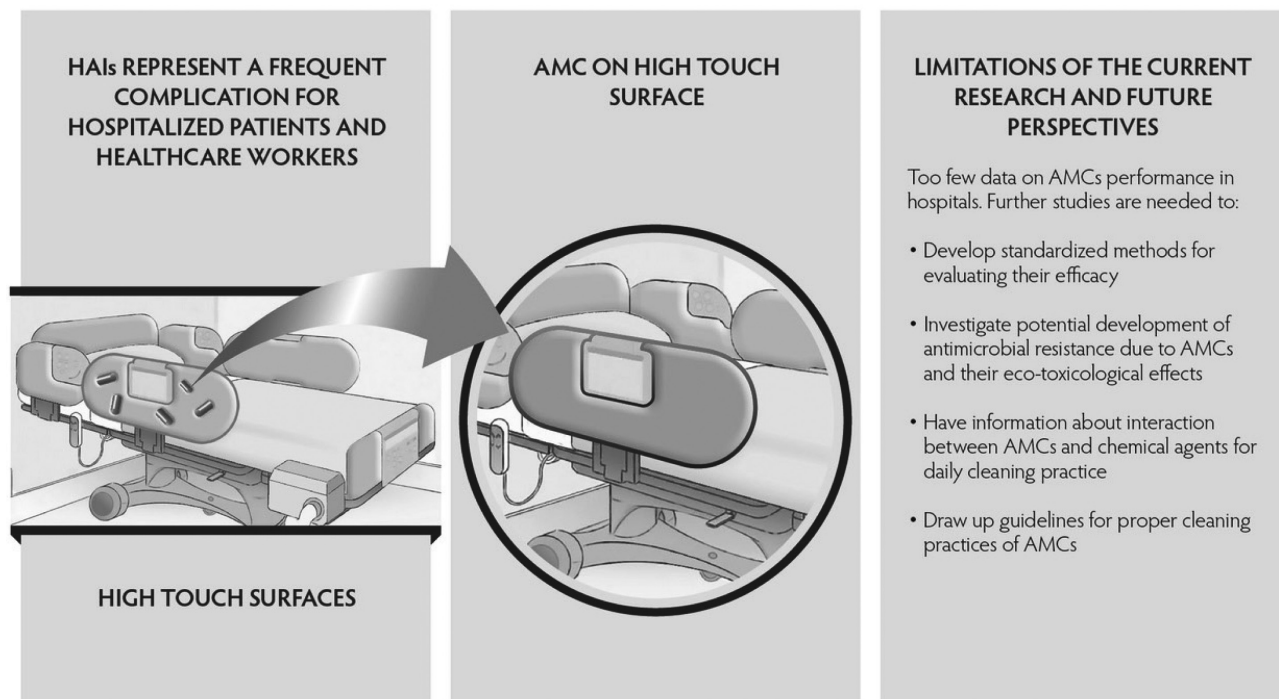


Fig. 1. Nano-enabled antimicrobial coatings (AMCs) as collective protection measures against healthcare-associated infections (HAIs).

the infectious risk is still very limited because, to date, few studies have shown the effectiveness of antimicrobial coatings, under conditions of common use in patients' rooms, in reducing the risk of healthcare-associated infections.

Another issue that needs to consider is that, in the most of cases, the data available in literature on the effectiveness of new AMCs are not sufficiently complete in respect of certain aspects concerning the safety such as the potential induction of antimicrobial resistance and/or eco-toxicological effects. Our findings confirm the need to develop innovative but at the same time safe antimicrobial coatings, therefore the development and implementation of SbD approach is a key point for the design of self-disinfecting surfaces for healthcare environments.

New standardized protocols have also to be developed and widely accepted by the scientific community because those currently in use are mostly inappropriate, as they do not describe the real performance of coatings under micro-climatic conditions as those commonly present in indoor environments.

In addition, the lack of information about interaction between chemical agents for daily cleaning practice and AMCs is one of the main impediments to their commercialization and placing in hospitals in which, various studies^{20, 21)} have documented the lack of compliance with established rules for the disinfection. Therefore, the

development of guidelines for proper cleaning practices of AMCs represents a great challenge for all scientific community. At the same time, however, it is important to search innovative methods for surface disinfection in addition to those currently available, because the healthcare workers are exposed to complex mixtures of disinfectants with alarming acute and chronic effects (skin irritation, chronic bronchitis and asthma, etc.). In Fig. 1 the main topics discussed in this review are summarized.

In conclusion, further studies are needed for assessing risks and benefits deriving from the use of nano-enabled AMCs as collective protective measures and/or personal protective equipment in order to safeguard patients and workers from biological risk and verifying their effectiveness directly in workplace environments, such as hospital wards, surgeries and patients' rooms.

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