ANTIMICROBIAL HYBRID NANOLAYERS PREPARED BY SOL-GEL METHOD

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Abstract

One of the most promising type of hybrid nanolayers prepared by sol-gel method are antimicrobial nanolayers based on TMSPM (3-(Trimethoxysilyl)propyl methacrylate) containing ionically bonded cations of silver, copper and zinc. Antimicrobial layer with long-term activity may be applied to the surfaces of almost all materials (metals, glass, ceramics, fabrics, plastics except Teflon). Similar type of nanolayers can be potentially used for different applications, such as organic agent immobilization (drugs, enzymes, etc.) and inert or conversely active interlayer.

The final antimicrobial nanolayer shows significant antimicrobial (against *Staphylococcus aureus, MRSA, Escherichia coli, Proteus vulgaris, Acinetobacter baumanii, P. aeruginosa* and other pathogenic bacterial strains), antiviral (HIV, H1N1, Dengue, HSV1, hepatitis viruses) and antimycotic (*Candida albicans, Candida glabrata*) activity. The basic function - keeping the microbial purity of the spaces is active in normal fluorescent light as well as in the dark. When treated surfaces are irradiated with UV-A radiation (sunlight, UV-A fluorescent lamp), the antimicrobial activity increases significantly. Elimination of almost all bacteria and viruses on the surface is achieved in a few tens of minutes.

The technology is applicable in medical institutions (operating theaters, intensive care units, transplant and neonatal units, offices, waiting rooms, hospices etc.), in the social sphere (schools, cafeterias, senior homes and other types of social institutions) or food (food processing plants, warehouses and stores). The long-term activity is also demonstrated by the preservation of antimicrobial activity on the fabrics after 50 washing cycles.

Developed antibacterial surface treatment technology is patented in the Czech Republic as well as abroad.

Keywords: Antimicrobial nanolayer, sol-gel method, MRSA

1. INTRODUCTION

A big problem in the health sector is the spread of infections in the premises of hospitals and other medical institutions. Many patients who come to hospital with noninfectious health problem (often a banal problem) infect the so-called nosocomial infections during the stay in a hospital. The bases of these infections are highly resistant microorganisms resistant to standard antibiotics. The most common are MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin resistant enterococci). Their incidence is increasing and the treatment is very difficult and expensive. The primary task is to prevent the growth and proliferation of these bacterial strains. The number of highly-resistant bacteria on regularly-used antibiotics is increasing. We can mention the once highly effective penicillin as an example. For all new types of antibiotics, it is only a matter of time until pathogens will develop resistance to them.

A very important finding is that the microorganisms have failed to develop resistance cations of some heavy metals. The best known is a silver cation. Intensive antibacterial activity shows also copper cation or zinc cation (especially against fungi). It is important to note, that the silver cation deactivates bacteria by several mechanisms [1], which are all dependent on the fact that the silver is in the form of highly mobile silver cation (similar to the properties of alkali metal sodium and potassium cations). The antibacterial activity of widely used and promoted silver nanoparticles will therefore depend on their ability to release silver cations into the surroundings. The silver nanoparticles are practically stationary and unable to deactivate bacteria by mentioned mechanisms.

Hybrid antimicrobial nanolayers prepared by sol-gel method are potential solutions to ensure local incidence of silver cation (and other active copper and zinc cations) on the surfaces with high antimicrobial activity requirement. Conventional sol-gel methods for obtaining tightly adhering oxide nanolayers require relatively high temperatures (around 500 °C) and they are unsuitable for applications in medical institutions in principle. Organic-inorganic hybrid nanolayers based on TMSPM (3-(trimethoxysilyl) propylmethacrylate) formed with interconnected polymer 3D networks of silica (partially substituted by titanium atoms instead of silicon atoms) and polymethyl methacrylate networks containing a silver, copper and zinc cations (ionically bonded to silica) prepared by sol-gel method are very promising for this purpose [2]. Nanolayers of similar type (without the bonded heavy metal cations) can be potentially applied also for other purposes, for example for organic agents immobilization (drugs, enzymes etc.) or as an inert or conversely active interlayer in biomedicine.

For antimicrobial action of the nanolayers, the most important is silver cation. Generally, the silver cation is easily reducible to atomic silver by organic substances or radiation (the principle of photography). The atomic silver is then aggregating into silver nanoparticles with significantly lower antimicrobial activity. For long-term antimicrobial action, there is therefore necessary to ensure a permanent presence of silver cations or re-oxidation of reduced forms of silver to the cation. There was used the principle of photochromic glasses with the removed particles AgCl + Cu_2Cl_2 for this purpose [3]. There is provided the re-oxidation of silver by cupric cations:

$$2\ AgCl + Cu_2Cl_2\ (darkness) \leftrightarrow 2\ Ag + 2\ CuCl_2\ (light)$$

There is presented the copper together with the silver in the antimicrobial hybrid layers. The copper has the ability to oxidize from cuprous cation form (Cu^{+}) to the cupric cation form (Cu^{2+}) under the action of atmospheric oxygen. The cupric cation oxidized the atomic silver back to silver cations. This is long-acting mechanism to maintain silver as an active antimicrobial cation:

$$Ag^0 + Cu^{2+} \leftrightarrow Ag^+ + Cu^+$$

$$4\ Cu^+ + O_2 \leftrightarrow 4\ Cu^{2+} + 2\ O^{2-}$$

The zinc cation do not involve in these redox equilibriums (due to its significantly diverging oxidation-reductive potential), but it expands the effectiveness of antimicrobial nanolayer (mainly to fungi). All these heavy metals cations are bonded to the silica part of the hybrid polymer by weakly acidic groups (Si-OH). The conductivity of the silica skeleton (required for electron transfer between atoms of silver and copper during the redox action) is increased by the presence of titanium atoms in the silica skeleton. The entire hybrid system is a polymer, but it is not the nanocomposite in the base form. The nanocomposite can be prepared by adding photoactive nanoparticles of titanium dioxide. These nanoparticles further increase the efficiency in the specific areas.

2. EXPERIMENTAL PART

2.1. Preparation of the nanolayers

The initial sol was prepared by using the sol-gel method from TMSPM (3-(trimethoxysilyl)propyl methacrylate), TEOS (tetraethyl orthosilicate) and IPTI (titanium(IV) isopropoxide) with an addition of the nitrate salts of silver, copper and zinc. Isopropylalcohol (IPA) was used as a solvent. The application of the sol can be carried out by spraying or dipping. The nanolayer was stabilized by using a heat-initiated polymerization at 80 °C to 150 °C or photo initiated polymerization after applying the sol to substrates and solvent evaporation. Description of the procedure can be found in patents [4, 5].

2.2. Antimicrobial tests

For antibacterial tests, bacterial strains normally colonizing the human skin were used. These bacterial strains usually do not cause any problems to healthy person. However when the barrier is disturbed, these bacterial strains represent a major complication for hard healing wounds. *Escherichia coli* (CCM 2024), *Staphylococcus aureus* (CCM 226), *Pseudomonas aeruginosa* (CCM 1959), *Proteus vulgaris* (CCM 1956), *Proteus mirabilis* (CCM 1944), *Klebsiella pneumoniae* (CCM 2318), *Acinetobacter baumanii* (CCM 2265) - (Czech collection of Microorganisms, Masaryk University of Brno) were used for the tests. Bacterial strain of MRSA (methicillin resistant *Staphylococcus aureus*, CCM 4223) was chosen as a representative of nosocomial infection. The antiviral activity was tested against HIV, H1N1, Dengue, HSV1 and hepatitis viruses; the antimycotic activity was tested against *Candida albicans* and *Candida glabrata*.

Bacterial inoculum in a concentration of 10^5 CFU/ml was spotted onto a given substrate and in specified intervals, a certain amount of the inoculum was drawn. Cultivation was carried out on agar medium (blood agar - Biorad Ltd. Praha). Experiments were carried out in daylight and exposure to UV-A radiation.

The samples of textile material with antimicrobial nanolayer were tested according to standards EN ISO 20645, AATCC 147 and AATCC 100.

2.3. Resistance to washing

Mixed textile material (40% cotton + 60% polyester), which is normally used for the fabrication of protective equipment in health care (jacket, pants, blouses) was used as tested fabrics. The first wash cycle was carried out in industrially produced washer and dryer Miele PROFESSIONAL with detergent Dodecil - Sodium dodecyl sulfate Ultra at 60 °C, the drying program Outdoor. The second to fifty wash cycles were carried out in laboratory equipment Ahiba Nuance from Datacolor also at 60 °C with the same detergent. The samples were dried at 70 °C in a laboratory dryer.

Quantitative analysis of heavy metal content in the textile material with the antimicrobial nanolayer after repeated washing cycles was performed on the Elvax Light, the device performing an energy dispersive X-ray fluorescence analysis. The samples were analyzed in the same location as the original samples (non-treated).

3. RESULTS AND DISCUSSION

3.1. Basic properties of the nanolayers

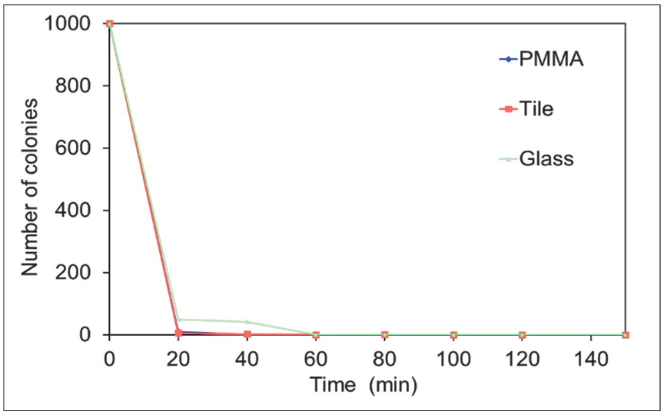

Fig. 1 Results of antibacterial tests: different substrates with antibacterial nanolayer, bacterial strain MRSA, concentration of 10^5 CFU/ml, UV-A radiation

The thickness of the antimicrobial nanolayers is in the range from 80 to 300 nm (150 nm diameter). Chemical resistance of the nanolayers is very good, it is resistant to all organic solvents (including toluene and acetone) and diluted inorganic acids (sulfuric acid, nitric acid and hydrochloric acid). The nanolayers do not resist only hydrofluoric acid and concentrated sodium hydroxide. The nanolayer is heat resistant to 200 °C and its antimicrobial activity was not reduced even after repeated sterilizations (120 °C / 1 h). How was experimentally verified, the nanolayer has no significant cytotoxic effects on VERO and HeLa cells. Total low content of heavy metals is also significant in the nanolayers. When expressed per 1 m^2 of the substrate, there is only about 0.03 g of Ag, Cu and Zn on a flat surface. In comparsion, the textile material has around 0.15 g of Ag, Cu and Zn on the same unit. The release of heavy metals is very slow, as evidenced by their loss during washing (chapter 3.3). The nanolayer is heat resistant to 200 °C and its antimicrobial activity was not reduced even after repeated sterilizations (120 °C / 1 h).

3.2. Antibacterial tests

How the results show (**Figs 1-3**), the rate of bacteria inhibition is faster when exposed to UV-A radiation. However, the daylight tests show very high efficacy of the applied layer, 100% inhibition of the bacterial strain MRSA occurs around 60 minutes from the start of the test. Very similar results were obtained with other above mentioned bacterial strains. The same result was also attempted when the test was carried out with mixture of 8 bacterial strains in the same concentration. It is evident from tests results that antibacterial activity of the nanolayer is not reduced even with repeated sterilization of the samples (**Figs. 2, 3**).

3.3. Resistance to washing

Fig. 4 shows the relative loss of heavy metals during washing depending on the number of washing cycles. The final microbiological inspection (after 50 washing cycles) confirmed persistent antimicrobial activity of the treated fabrics.

4. CONCLUSION

This research presents novel hybrid nanolayer with significant antibacterial activity. Antimicrobial tests showed high antibacterial, antiviral and antimycotic activity of hybrid nanolayers prepared by sol-gel method in normal fluorescent light, but also when treated surfaces are irradiated with UV-A radiation (sunlight, UV-A fluorescent lamp). This novel antimicrobial surface treatment technology is potentially applicable in medical institutions (operating theaters, intensive care units, transplant and neonatal units, offices, waiting rooms, hospices etc.), in the social sphere (schools, cafeterias, senior homes and other types of social institutions) or food (food processing plants, warehouses and stores).

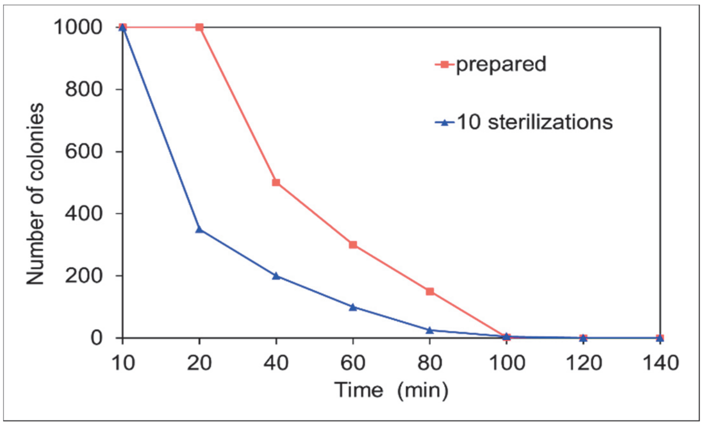

Fig. 2 Results of antibacterial tests: glass slides with the antimicrobial nanolayer performed immediately after preparation and after ten sterilization cycles (120 °C / 1 h), bacterial strain MRSA, concentration of 10^5 CFU/ml, daylight

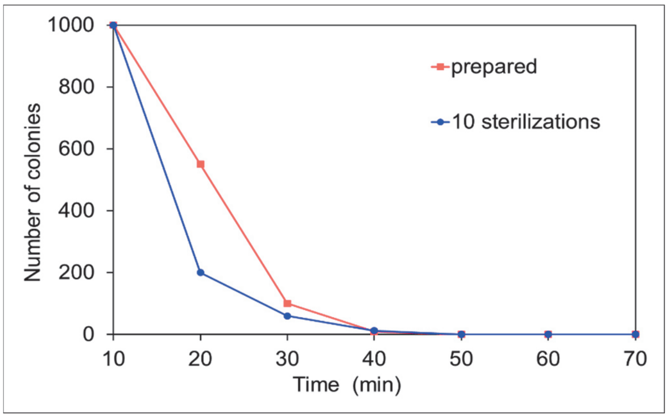

Fig. 3 Results of antibacterial tests: glass slides with the antimicrobial nanolayer performed immediately after preparation and after ten sterilization cycles (120 °C / 1 h), bacterial strain MRSA, concentration of 10^5 CFU/ml, UV-A radiation

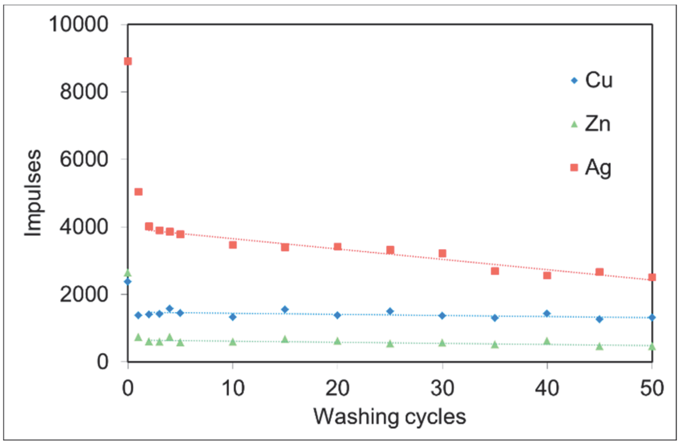

Fig. 4 The content of heavy metals in the textile material with the antibacterial nanolayer after repeated washing cycles (X-ray fluorescence analysis)

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