HYGIENIC COATINGS

Antimicrobic Low Surface-Free Energy Nanocomposite Coatings

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Antimicrobic low surface-free energy nanocomposite coatings for medical applications

Abstract

A new type of silver nanoparticles containing coating based on inorganic-organic hybrid materials has been developed by the sol-gel technique to achieve antimicrobic properties. The formation of an inactive biofilm layer, which can block the antimicrobic effect, was reduced by decreasing the surface-free energy of the coating. Ag^+ ions as effective oligodynamic components are generated by Ag nanoparticles with a size between 5-10 nm. The Ag nanoparticles are produced during the drying and curing process. The investigation of the long-time release behavior of Ag^+ ions show that the coating has oligodynamic properties for at least several month. The antimicrobic effect of the coating has been proved by inoculation with Gram negative bacterium E.coli. A low surface-free energy of about 19,5 mN/m could be achieved by using side chain perfluorinated organo alkoxy silanes, which upconcentrate at the coating surface by thermodynamical reasons. Compared with uncoated samples the low surface-free energy of the coating lead to a reduction of biofilm adhesion of about 95 %.

Introduction and state of the art

The adhesion of microbes on surfaces from medical instruments or conditioning systems represents a high risk of infections for human. The development of new materials with antimicrobic properties shows a wide field in the material research.

Since the oligodynamic effect of silver ions are known [1] some research was carried out to modify materials with these components. First investigations were done by introducing soluble silver compounds like $AgNO_3$ into polymers for medical applications. The antimicrobic property of these modified polymers showed no long-term stability, because the silver components were soluble in water and were washed out fast [2]. Another possibility is the incorporation of metallic silver particles into polymers. Thereby the generation of the antimicrobic effective Ag^+ ions from elementary silver results from the interaction of water and oxygen. To achieve a sufficient concentration of released Ag^+ ions the particles must show a high surface, as it can be obtained by submicron particles. Colloidal Ag particles were used to modify polymers like polyurethane for medical applications, e.g. shunts or cannulas. After absorption of a thin layer of colloidal silver on the polymer surface the products are produces by extruding or kneading of the modified polymer [3]. Colloidal Ag particles in coatings for medical applications can be achieved by thermal or UV light activation of silver salt solutions [4, 5, 6].

The aim of this work was the development of new coating materials which combine antimicrobic properties and low surface-free energies to avoid biofilm building. The coating material, which is produced by the sol-gel technique, offers the possibility of an application on polymers, glass or metal surfaces by using commonly industrial application and curing techniques.

Experimental

Synthesis and application of the coating material

The inorganic-organic hybrid material was synthesized by the hydrolysis and condensation of methacryloxypropyltrimethoxysilane in presence of deionized water with nanoscaled AlO(OH) particles. After adding of an alcoholic $AgNO_3$-solution and N-(2-aminoethyl-3-aminopropyl)trimethoxysilane the mixture was stirred during 24 h. Then the side chain perfluorinated organo alkoxy silane, an organic comonomer and the UV-initiator Benzophenon were added.

The coating material was applicated on PC-plates by spin coating with a remaining coating thickness between 5 and 7 µm and UV-cured by Hg high pressure lamps.

Characterization

To analyze the micro structure with a high resolution electron microscope (FEG, type 6400F, JOEL), the coating-material was scarped of from the substrate and was pulverized in the present of ethanol. The determination of the concentration of the released Ag^+-ions was carried out by atomic absorption spectroscopy. For this reason coated PC (A: 4,5 cm^2) was placed in 10 ml physiologic solution at 37 °C and after 24 h the Ag-concentration was measured. The contaminated solution was replaced after every experiment. The surface free energy of the coating was calculated according to Wu by the measurement of the contact-angle of liquids with different polar and apolar surface tensions (water, glycerin, 1-octanole and hexadecane) using a goniometer (Krüss, type G2/DAS2) [7].

The biological experiments were executed with Gram negative bacterium *E. coli* 498 , cultivated in M 1 medium (5 g/L peptone, 3 g/L meat extract, 15 g/L agar if necessary, pH = 7 at 30°C). The antimicrobic properties of sterilised coated and uncoated PC-plates (5*5 cm, sterilised with 76 % ethanol) were examined by placing the substrates in the centre of petri dishes. M 1 agar was poured on the substrates (50-60 mg/cm^2) and inoculated with 100 µl E. coli (diluted 1:10.000 fold in M 1 medium). The number of colony forming units were determined after incubation at 30°C (three days). Additionally, the agar covering the samples was detached from the samples and weighed.

For examination of the biofilm formation, 20 ml *E. coli* suspension was added to 10 ml M1 liquid medium in sterile tubes. Sterilised coated and uncoated PC plates (1,5*7,0 cm) were added to the inoculated medium. The medium covered about 70 % of the vertically placed substrates. After incubation for seven days at 25°C, the substrates were removed from the medium and the remained biofilm was washed up in three steps in each 45 ml salt solution (Isotone II, Coulter). In the first step loosely bound bacteria were removed by dipping the substrates in salt solution. In the second step the substrates were given into the salt solution and the biofilm was mobilized (1' vortex, 3' ultra-sonication, 1' vortex). In the third step the remaining biofilm was removed by scraping in the salt solution. The number of bacteria in 100 µm solute was volumetrically determined in each wash solution directly with a Coulter-Counter (Multisizer 3, Beckmann Coulter, 30 µm capillary). The second washing solution of the uncoated PC plate showed fast sedimentation of the flocculated biofilm. The number of bacteria was determined by weight of dry biomass. Therefore the solution was washed twice in centrifugation tubes with distilled water and the biomass was weighted after drying under vacuum (50 °C, 20 mbar). Dry biomass weight and particle concentration were correlated using 25 ml *E. coli* in M1 medium taken from the culture in which the bio film was developed.

Results

The nanoscaled silver particles are produced by reducing Ag^+ ions ($AgNO_3$) during the curing process, where the reduction to Ag^0 under UV radiation takes place [8, 9]. To get accurate informations about the size of the silver particles and their distribution in the inorganic-organic hybrid matrix, the coating material was examined with a high resolution electron microscope (fig. 1).

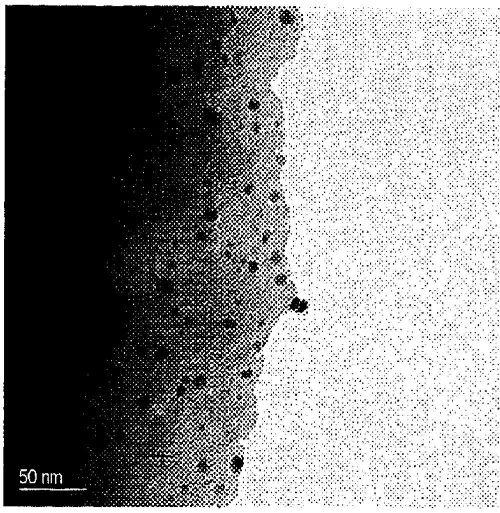


fig. 1: TEM picture of a UV-cured inorganic-organic hybrid coating with nanoscaled silver particles; sample L6 (Ag concentration: 0,87 wt.%)

The picture in fig. 1 shows a homogeneous distribution of the embedded silver particles in the matrix with a particle size between 5-10 nm.
The silver ions are generated by oxidation of nanoscaled silver particles by the interaction of oxygen and water in the coating material. To examine the continuous release of the generated Ag^+ ions out of the coating over a long period, which can assure the long-time stability of the antimicrobic properties, the coated substrate was placed in a physiologic solution at 37 °C. The solution was replaced every 24 h and the Ag^+ ion concentration was measured by AAS, as shown in fig. 2.

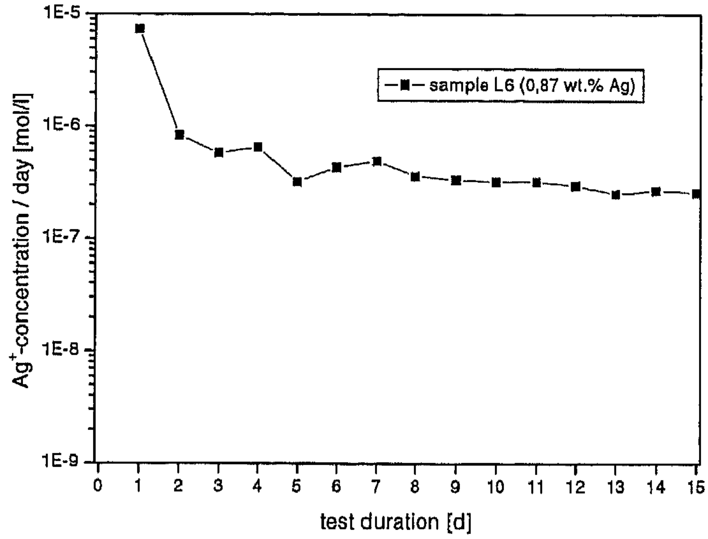


fig. 2: time dependent release of Ag^+ ions in a physiologic solution; UV-curable coating of inorganic-organic hybrid material with a Ag concentration of 0,87 wt.% (sample L6); Coating on PC plate (4,5 cm^2); every measurement represents the Ag^+ concentration after 24 h

In the test duration the measured Ag^+ ion concentration ranges from $7*10^{-6}$ to $3*10^{-7}$ mol/l per 24 h. The decrease of the Ag^+ ion concentration from $7*10^{-6}$ to $8*10^{-7}$ mol/l after one day can be attributed to a high concentration of unreduced $AgNO_3$ at the coating surface, which is washed away after the first replacement. As known from biological experiences an effective oligodynamic effect is given with Ag^+ concentrations till 10^{-9} mol/l [10, 11]. Based on the experimental data of the release behavior of sample 6 one can say that the

release of Ag^+ ions is high enough to show antimicrobic properties for at least several months.
The antimicrobic effect of the Ag-containing coating was investigated by inoculation the sample surfaces of uncoated and coated PC sheets with the Gram negative bacterium E.coli (fig. 3).

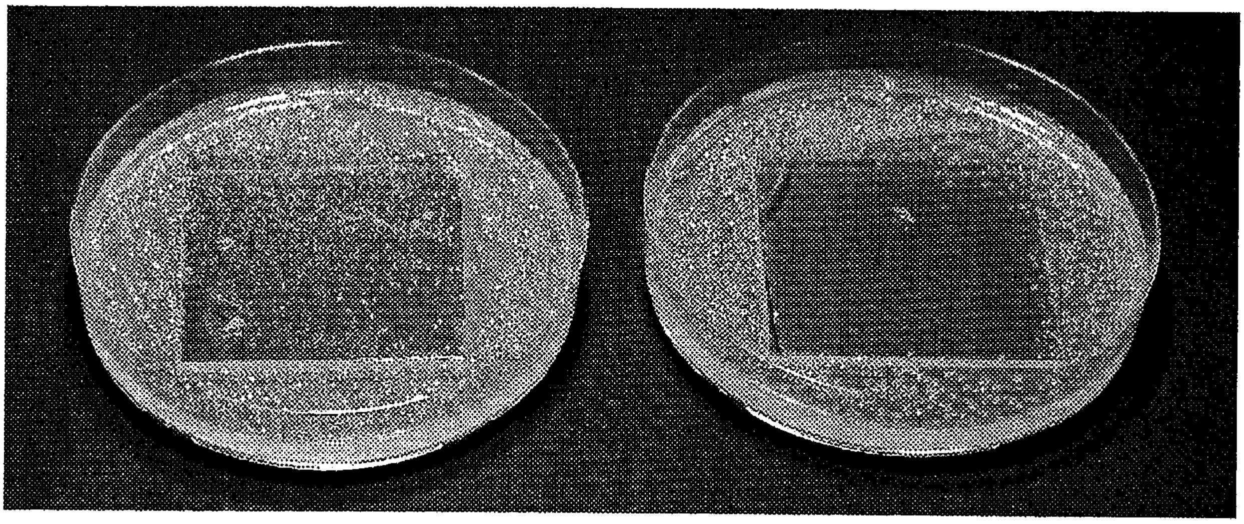
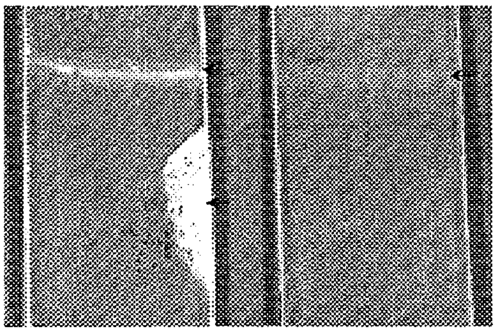


fig. 3: Colonies of the Gram negative bacterium *E. coli* on agar poured over uncoated (left) and coated (right) PC-samples; 100 µl of 10.000fold diluted *E. coli* in standard medium M1 was spread over the standard medium agar surface and incubated at 30°C for 3 days. The amount of agar covering the surfaces are 49 and 62 mg/cm², respectively; coating: UV-curable inorganic-organic hybrid material with 0,87 wt.% Ag (sample L6); coating on PC (5-7µm)

The coated sample (right sample) shows a distinct effect on the growth of bacteria as shown on the petri dishes in fig. 3. On the left side, an uncoated PC-substrate covered with 49 mg / cm² of agar shows a strong layer of *E. coli* colonies, whereas the functionalised surface covered with 62 mg/cm² agar only shows some colonies after incubation for 3 days at 30 °C.
The building of biofilms on antimicrobic active substrate surfaces can lead to an increase of bacteria colonies because the oligodynamic effect is blocked by the inactive layer. To increase the efficient antimicrobic behaviour of the new coating material a surface active component was used to decrease the surface-free energy. The functionalising of the coating leads to a reduced sticking of microorganism in a biofilm and keeps the surface easy to clean. As active component a side chain perfluorinated organo alkoxy silane was hydrolysed and condensated with the inorganic part of the matrix. Due to thermodynamical reasons the active component is upconcentrated at the surface of the coating. The calculating of the surface-free energies by measurements of contact angles of liquids with different polar and apolar surface tensions according to Wu [7] leads to values for the uncoated PC-sample of 31,4 mN/m and for the coated sample (sample 6) of 19,5 mN/m. These results show that the use of fluorinated components in Ag colloid containing inorganic-organic hybrid material leads to reduced surface-free energies which can be compared with PTFE.
The influence of the surface functionality of Ag-containing coatings to the building of biofilms is shown in fig. 4.



uncoated PC   coated PC (sample 6)

fig. 4: Picture of biofilm formation on uncoated and coated PC samples after incubation with E. coli for 7 d at 25 °C; UV-cured inorganic-organic hybrid coating with nanoscaled silver particles, sample L6 (Ag concentration:0,87 wt.%)

After an incubation with E. coli for 7 days at 25 °C the uncoated PC substrate shows a crumbled biofilm slime at the side. Pronounced biofilm formation at the liquid/air interface is seen, whereas at the coated sample only a slight biofilm is visible.
More detailed informations about the kind of sticking of biofilms could give the analysis of the washing solutions. As seen in fig. 5 the number of cells loosely bound to the surfaces, determined after gently dipping the samples in particle free solution, is similar whether the PC-samples were coated or not. Only small amount of biofilm remained on the surfaces after mobilizing the attached cells with ultrasonification and vortex treatment. The main part of the cells was mobilized in this step. As clearly visible in the balance, the sticking of biofilm was reduced by 95 % when the sample surface is treated with a antimicrobic and low surface-free energy functionalised coating.

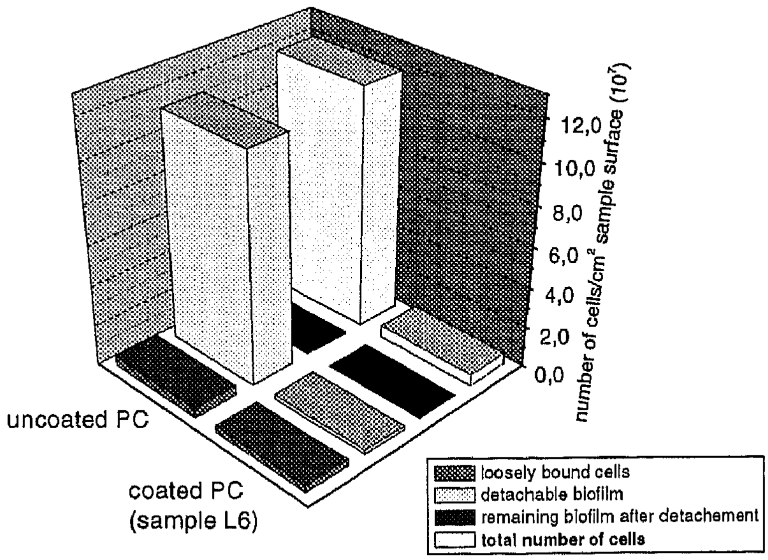


fig. 5: Number of cells determined in three washing solutions applied subsequently for biofilms which developed on polycarbonate substrates after inoculation with *E. coli* for 7 days at 25°C; loosely bound cells were determined after gently dipping the substrates in salt solution; detachable biofilms were mobilized by vortex treatment and sonification; the number of remaining cells was determined after scraping them from the surfaces.

Conclusion and Outlook

The insitu producing of nanoscaled Ag particles and the use of perfluorinated organo alkoxy silanes in an inorganic-organic hybrid material, synthesized by the sol-gel technique, offers a new field of coating materials with antimicrobic properties. The long term stability of the antimicrobic properties by releasing of generated Ag^+ ions was proved and the reducing of biofilm building by increasing the low surface-free energy could be shown.
This new coating system offers the possibility to equip subsequent objects for e.g medical sector or air conditioning systems with antimicrobic properties. Due to the composition of the inorganic-organic hybrid material further more properties like abrasive and scratch resistance or stability against organic solvents and cleaning agents are given.

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