Published in "Journal of Materials Chemistry 18: 5359-5362, 2008"
which should be cited to refer to this work.

Silver coordination compounds as light-stable, nano-structured and anti-bacterial coatings for dental implant and restorative materials

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Silver coordination polymer chains were deposited on Au(111) as a model surface, as well as on gold alloy and titanium as dental implant and restorative materials. The topography of the surface was analysed on the model substrate and it was found to be a nano-structured crystalline material. *In vitro* investigations in a flow chamber imitating the oral environment prove the anti-bacterial properties of the silver compound.

Introduction

Over the past years, silver compounds[1] and nanoparticles[2] have been studied for their anti-microbial activity as replacements for antibiotics, against which bacteria become more and more resistant. In this context, and working on silver coordination compounds,[3] we became particularly interested in applications for implantable materials, *e.g.* artificial prostheses, and understanding the activity of silver compounds with respect to chemical composition, nano-structuring and anti-microbial activity. While orthopedic operations are generally carried out under sterile conditions, biofilm formation and subsequent inflammatory host responses may occur later, *e.g.* as result of bacterial multiplication (septic failure, hematogenous seeding).[4] Adhesion of bacteria to dental implant components and restorative materials is a prerequisite for the formation of a biofilm that may lead to the development of dental diseases like dental caries, periodontal diseases and peri-implantitis.[5,6] To prevent bacterial adhesion to surfaces, the so far tested silver compounds turned out to be either too soluble, or, as far as nanoparticles are concerned, toxic and both are thus not usable for long term purposes.[7] As an alternative class of silver compounds, we propose coordination polymer networks to be tested as anti-bacterial coatings on implant components. As a special challenge, we chose to work with tooth implant materials, for which the additional condition of light stability is highly required.[8] We report here on a) the nano-structured surface topology, b) the chemical composition and c) the anti-microbial properties obtained for the deposition of a silver coordination polymer compound onto implant titanium, dental gold alloy, and Au(111) as model surface.

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Results and discussion

Coating

As shown earlier, we are able to control the synthesis of different silver coordination polymer compounds based on L = ethanediyl bis(isonicotinate), such as the two polymorphs of $[Ag(L)NO_3]_n$, **1a** and **1b**, $[Ag(L)(NO_3)(H_2O)]_n$, **2**, and $[Ag(L)(NO_3)(H_2O)_2]_n$, **3** (Fig. 1). Compound **1** presents simple chains bridged into sheets *via* weak coordination of the nitrate anions, and the ligand in *anti* conformation, while **2** and **3** form double-chains with short Ag–Ag contacts and *gauche* (**2**) or *anti* (**3**) conformations of L.[9]

In order to choose the appropriate compound for anti-microbial tests, two properties were required: i) light stability, and ii) (in)solubility of our compounds in biological media. For the first purpose, solutions of **1–3** in organic solvents were deposited on filter papers, and subsequently exposed to light (Philips 15 W, from 10 cm distance) for 3.5 h, and compared to

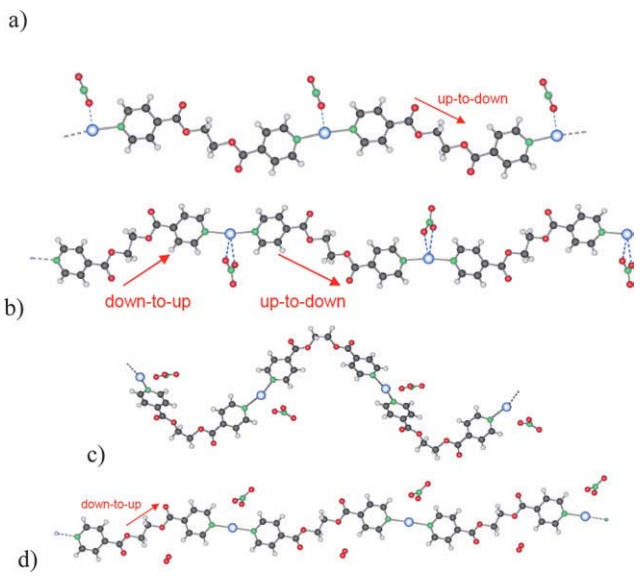


Fig. 1 Structures of $[Ag(L)NO_3]_n$, **1a** (a) and **1b** (b), $[Ag(L)(NO_3)(H_2O)]_n$, **2** (c), and $[Ag(L)(NO_3)(H_2O)_2]_n$, **3** (d); Color code: Ag: blue; O: red; N: green; C: grey; H: white.

simple $AgNO_3$ solutions at different concentrations. The results showed that the compound with the shortest and longest Ag contacts, **1a**, showed the best light stability and also did not degrade when exposed to light for longer time scales (months). Thus, compound **1a** was chosen for further tests on dental implant components, and its solubility was determined. A crop of 43 mg of solid **1a** was exposed to 1 ml of phosphate buffer solution (PBS) (0.1 M pH 7.4) in a microcalorimeter in order to determine its solubility. No detectable heat of dissolution could be observed over a period of 168 h, so the solubility product of **1a** must be very low. The buffer solution was then subjected to atomic absorption spectrometry to determine the content of silver ions in solution to 2–5 ppb.

As substrates for the deposition of compound **1a**, typical metallic dental implant and restorative materials were chosen: titanium (99.9% Tritan, RematitanM, Dentaurum, Ispringen, Germany) (A), and a gold alloy (Au 71%, Pt 3.7%, Ag 12.7%, Cu 10.8%, Zn 1.1%, Ir 0.1%; Neocast 3, Cendres + Metaux SA, Biel-Bienne, Switzerland) (B).[10] Both were used as polished (roughness $R_a = 0.24$ μm) flat plates of 14 × 14 mm, the polishing being similar to that used for real dental applications. As a model surface, an oriented gold monolayer Au(111) on glass (C) was treated in the same way as the two implant material samples. Before deposition, the samples were cleaned with H_2SO_4–H_2O_2 (30%), followed by thorough rinsing with ethanol and distilled water. Several different deposition techniques were used: i) immersion into the mother liquor (EtOH–THF) of a 2 mM solution of **1a** for continuous ongrowth for A, B and C, ii) dip-coating alternating a solution of L and $AgNO_3$ (each 2 mM) for B and C, iii) derivatives of L for grafting to the metal surface (isonicotinic acid for A, a disulfide derivative of our ligand for B and C). The first two methods yielded similar results in XPS, AFM and STM analyses, and will be discussed further. The third method, using an anchor molecule derived from ligand L, leads to the formation of less well ordered deposits in the form of larger crystallites. The latter are easily washed off the surface and were thus not suitable for application.

To identify the coatings obtained with the two first methods, XPS and powder X-ray measurements were used directly on the coated surfaces, and powder X-ray measurements were also performed on material which was cocrystallised from the same solutions as used for the surface coatings. All methods proved that i) a compound was deposited, and ii) that the deposited compound was (poly)crystalline **1a**.

Additionally, AFM and SEM measurements were carried out on a coated surface of Au(111), allowing the investigation of the topology. As the implantable materials A and B were polished prior to deposition of **1a**, the surface of these substrates was too rough for AFM, which was therefore carried out for sample C only. Tapping mode AFM was performed using a PycoLE System, Molecular Imaging, and silicon nitride cantilevers, k = 42 N m^{-1}, at force set point 3.8 V and scan rate 1 line s^{-1}. Different positions of the sample were monitored. All experiments were performed at room temperature. The AFM revealed peak-like structures which were analyzed with respect to their height and width (Fig. 2). The surface pattern corresponds to Ostwald ripening motifs on a two-dimensional surface with a main interpeak distance of 20–30 nm, a distance which is known to be ideal for cell ongrowth.[11] From the mean peak height of 11–14 nm, the average polymer chain length, supposed to be standing upright on the surface, could be determined as an octameric chain [Ag_8L_7]. From the width of the peaks, it can be concluded that more than one chain is attached to the surface to form such a peak. With approximations of the peak width of *ca.* 10–20 nm, and the unit cell of **1a**, one can conclude that there are *ca.* 200–400 chains linked together within one peak. From the profile sequence in Fig. 2, it can be seen that the whole surface, also in between the peaks, is covered with product, even at lower thickness. SEM pictures of several samples (Au(111) and coating types i) and ii)) confirm this analysis as shown in Fig. 3. The Ostwald ripening features on the surface are also clearly seen with this method. They also reveal the size distribution of the

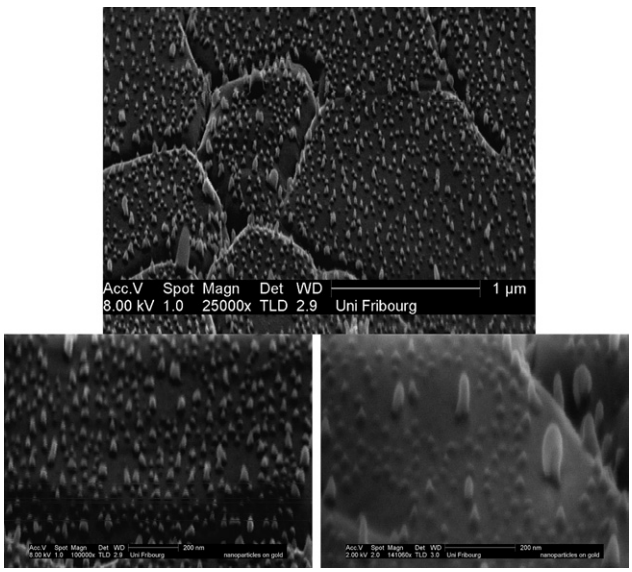


Fig. 3 SEM pictures of the deposit of **1a** on Au(111).

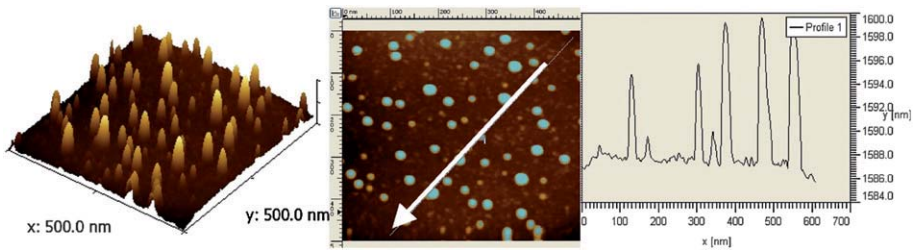


Fig. 2 AFM pictures (left, middle) and profile of the deposit of **1a** on Au(111) along the white arrow (right).

peaks on the surface. Several pictures were taken at different scales, and the peaks measured. Most, about 80%, of the peaks seen by SEM are *ca.* 10–20 nm in height and have a mean diameter of 10–20 nm. The unit cell volume of **1a** being *ca.* 777 Å^3, this leads to roughly 25000 units of **1a** within one such peak. About 10% of the surface peaks are 50–80 nm in height and have a mean diameter of 20 nm (roughly 60000 repeat units of **1a**), whereas another 10% of the peaks are >100 nm in height and have a mean diameter of *ca.* 50 nm (*ca.* 500000 repeat units of **1a**). The coating can thus be estimated to be roughly 0.0125 g m^{-2} or 3×10^{-11} mol cm^{-2}. The surface coating was thus impossible to detect with an analytical balance.

From these data, it can be concluded that a well-defined, nano-structured topology is formed on the substrate surface, and that with high probability, similar structural features are obtained on the polished substrates of the titanium A and gold alloy B.

Anti-bacterial testing

The so prepared samples A and B were then transferred into a flow chamber (Scheme 1) for anti-microbial testing on dental materials.[12]

In a reservoir, a single bacterial strain, *Streptococcus sanguinis* DSM 20068 (German Collection of Microorganisms and Tissue Culture Cells, Braunschweig, Germany), suspended in sterilized human saliva, was added. *S. sanguinis* was selected as one of the known early dental colonizers.[13] The saliva was collected and processed as described in a previous study.[14] The treated samples, together with non-treated blanks, were exposed during 60 min at room temperature to a flow rate of the bacteria saliva suspension of 0.8 ml min^{-1}, which corresponds roughly to physiological oral conditions of low shear.[15] The system was placed on a shaker adjusted to 260 impulses min^{-1} in order to maintain the homogeneity of the bacterial suspension.[14] Thereafter, the test specimens were removed and analyzed microscopically. The vitality of adhered bacteria was evaluated by applying a dual fluorescent staining (Live/Dead BacLight Bacterial Viability Kit; MoBiTec, Luzern, Switzerland) according to Decker *et al.*,[16] which allows differentiation between vital and dead bacterial cells (Fig. 4).

The test specimens were removed from the flow chamber, carefully dipped into distilled water in order to eliminate planktonic and loosely attached cells, and covered with 7.5 µl of staining solution for 15 min at room temperature in the dark. The cells were analyzed by epifluorescence microscopy (PROVIS AX70, Olympus AG, Volketswil, Switzerland). A total of twelve digital images (ColorView, Olympus AG, Volketswil, Switzerland) using two filters [blue excitation at 450–490 nm (FITC) and green excitation at 546 nm (rhodamine)] were obtained for each sample and the percentage of dead adherent cells was calculated. Each material was tested in at least four independent experiments. From the analysis of dead and alive bacteria on the treated metal surfaces, it can be concluded that compound **1a** has definitely an anti-bacterial effect on *S. sanguinis* cells under the given conditions, by a reduction of adherent live bacteria of more than 90% for gold alloy and for titanium as compared to the corresponding non-treated samples. Together with the light stability and the low solubility, these results are encouraging for further studies in the field of silver-containing, anti-microbial surface coatings. Such experiments, as well as investigations on the mechanism of the anti-bacterial activity, are currently being carried out in our group, and are concerned with different Ag coordination polymer structures, as well as different bacterial strains. Thus, **1a** and **1b** show promising *in situ* activity against *S. epidermidis* as well.

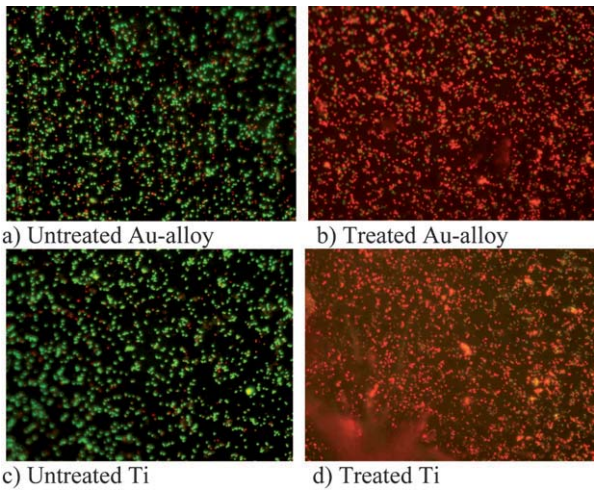
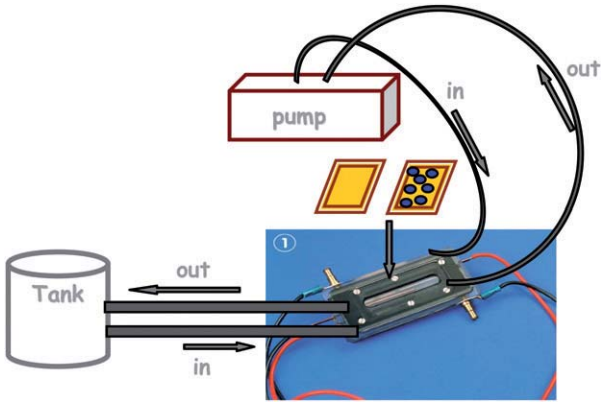


Fig. 4 Representative sector of the dental materials. Many bacteria attached to the untreated materials were stained green because they were still alive. Most on the treated samples were stained red indicating that they were dead at the time of staining.



Scheme 1 Flow chamber for anti-bacterial test with *S. sanguinis*.

Conclusions

We have presented here an easily accessible coating method for dental implant and restorative materials with a silver coordination polymer compound, analysed the surface structure and were able to show effective antimicrobial properties against *S. sanguinis*.

Acknowledgements

T. V. S. and K. M. F. thank the Swiss National Science Foundation for funding *via* the NCCR Nano, Basel, and for an interdisciplinary project. The authors thank Olivier Casse for AFM measurements, Teresa de los Arcos for XPS measurements, Elisabeth Filipuzzi for technical support, and Jürg Meyer

for helpful discussions and support, and the Velux Foundation for financial support.

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