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# Antimicrobial Coatings for Implant Surfaces

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§SCS Poster Prize Winner

*Abstract:* Body-foreign materials are used more and more frequently in our lives: joint implants (hips, knees, fingers, *etc*.), catheters, pacemakers, dental and aesthetic implants, *etc.* The increasing numbers of patients requiring such implants also raises the absolute numbers of implant-related infections. Thus, it is known that body-foreign materials are prone to bacterial adhesion and subsequent biofilm formation, either *via* bacterial debris on implant materials, infections during implantation or, later on, *via* haematogenous seeding. Biofilms, once formed, are impossible to treat with antibiotics, and the immune system response leads to implant loosening, requiring total replacement. The strategy is thus to prevent bacterial adhesion to implant materials' surfaces. Different strategies have been tested in this context and will be presented here, together with our own approach, using a combination of different anti-microbial compounds.

**Keywords:** Antibiotics · Bacterial biofilms · Implants · Silver coordination chemistry · Surface coatings

## 1. Introduction

Implant-associated infections are serious complications of medicinal device insertions. At the cellular level, these infections are the result of bacterial adhesion to a biomaterial surface. A very large proportion of all implant-related infections are caused by *Staphylococci*, *S. aureus* and *S. epidermidis*. In particular, it has been found that bacteria, *i.e. S. aureus*, express many surface receptor proteins, which mediate cell anchorage[1–3] to result in a bacterial population encased in a polysaccharide glycocalyx, the so-called 'biofilm'. Any implant surface provides an ideal environment to facilitate bacterial adhesion and proliferation. Indeed, it has been shown that bacteria adhere preferentially to body-foreign materials as compared to *i.e.* bone or enamel.[4] Once a biofilm has formed, it is very difficult to treat clinically because the bacteria within the biofilm are well protected from phagocytosis and antibiotics.[5] Bacteria develop virulence factors (extracellular toxins and extracellular proteases) to evade the host's defence system. Thus, despite aggressive antibiotic treatment, eradication of established implant-associated infections often fails, leading to implant loosening, removal and replacement.

Bacterial infections can arise during implantation due to the contamination and later, from haematogenous sources.[6] Among the potent pathogens, *S. aureus* is considered to be a major, virulent pathogen, found naturally on the skin and in nasopharynx of the human body. Once *S. aureus* attaches to a surface, *e.g.* bone joint and metal biomaterial,[7,8] host cells are unable to dislodge it. Also, *S. epidermidis* is the most frequently isolated coagulase-negative staphylococci (CoNS) from implant-associated infections[9] and is associated with nosocomial (or hospital-acquired) infections. It has been found to be even more resistant to treatments than *S. aureus*.

Aseptic implant loosening may occur in the absence of live bacteria as implant materials are often sterilized by gamma irradiation prior to implantation, and retain bacterial debris on the surface, which are subsequently inserted into the body during implantation. Remaining traces of bacterial polysaccharides on the implant surface will lead, together with microparticles of material debris, to a possible inflammatory response.[10–12]

## 2. Vancomycin: Antibiotic of Last Resort

The majority of antibacterial agents inhibit the synthesis of DNA, RNA, proteins, or peptidoglycan in bacteria. Bacterial peptidoglycan biosynthesis is indeed a good target for antimicrobial chemotherapy, because it is essential for bacterial survival. The enzymes involved are extracellular and are thus accessible to compounds that would not penetrate the bacterial cell membranes.

Until recently, vancomycin has remained active against organisms resistant to other antibiotics, and has therefore been useful as an antibiotic of last resort. Vancomycin was discovered in 1956 and has been approved for human use in many countries. Its general mechanism of action was elucidated in the 1960s[13] and its structural features were determined in 1978, when Sheldrick and Williams determined the structure of a degradation product, Crystalline Degradation Product 1 (CDP-1), through X-ray analysis.[14] Thus

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vancomycin is a member of the glycopeptide family of antibiotics and has served as model system for many mechanistic investigations of glycopeptides. Glycopeptides are natural products elaborated by actinomycete soil bacteria. They inhibit the maturation of the peptidoglycan layer surrounding bacterial cells by binding to D-Ala-D-Ala, a dipeptide found in bacterial peptidoglycan precursors. More specifically, vancomycin inhibits glycan polymerization and/or cross-linking by binding to the substrate of transglycosylases and transpeptidases. The bacterial peptidoglycan is composed of linear chains of β-(1,4)-N-acetyl hexosamine units joined by peptide cross-linking (Fig. 1). This carbohydrate polymer provides the mechanical support necessary to prevent the cells from lysing as the osmotic pressure fluctuates[15] (for bacteria survival, their cell membranes must be able to withstand osmotic pressures in excess of 5–15 atm without rupturing).

We chose vancomycin as antibiotic in our studies because it is the most important drug used worldwide to treat Gram-positive bacterial infections, the most common causes of nosocomial and haematogenous periprosthetic infections.

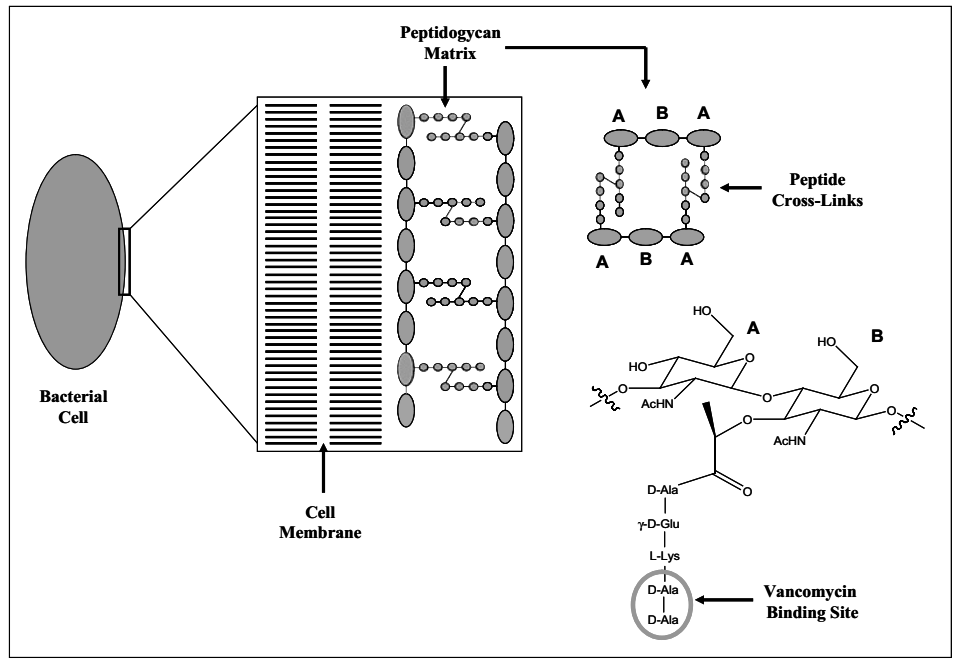


Fig. 1. Schematic representation of the peptidoglycan matrix at the surface of the bacterial cell membrane, showing the potential binding site for the antibiotic vancomycin

## 3. Bactericidal Activity of Silver Ions

The antimicrobial activity of the silver ions was first identified in the 19th century and has found a variety of applications because its toxicity to human cells is considerably lower than to bacteria.[16] Silver nitrate was introduced in therapeutic applications in 1884 for the prevention of *ophthalmia neonatorum*, a few drops of a 1% $AgNO_3$ solution being instilled into the infant's eyes immediately after birth. Because of the discovery of penicillin in the 1920s and other antibiotics, silver has been used less in medicine for many years. Nevertheless, for the management of burn patients in the 1960s, silver compounds began to be used again in the form of 0.5% $AgNO_3$ solutions.[17,18] In 1968, $AgNO_3$ was combined with a sulphonamide antibiotic to produce silver sulfadiazine (SSD) cream, which created a broader spectrum silver-based antibacterial agent that continued to be prescribed mostly for the management of burn wounds.[19]

Concentrations of 0.1 to 100 mg/l of silver ions are sufficient to ensure antimicrobial action. For instance, results from two studies that explored MIC (Minimum Inhibitory Concentration) values for *Staphylococcus aureus* range from 8 to 80 mg/l.[20,21] For bacteria (and probably yeast), three possible mechanisms of inhibition have been proposed for aqueous silver(I) ions:

i) interaction with the bacterial cell membrane;
ii) interference with electron transport, and
iii) binding to DNA.

Indeed, silver ions in low concentration may penetrate a bacterial cell and cause structural damage to the cell envelope. Silver ions form insoluble compounds with sulfhydryl groups, which are essential components of enzymes responsible for the transmembranous energy metabolism and electrolyte transport of bacteria. Furthermore, silver ions block the respiratory chain of bacteria in the cytochrome oxydase and NADH-succinate-deshydrogenase region.[22] Silver cations also displace other metal ions that are essential to bacterial cell survival, including $Zn^{2+}$ and $Ca^{2+}$.

There have been only a few studies of antimicrobial activities of silver(I) complexes with organic ligands, as compared with those of aqueous silver(I) ions. The mode of action and the molecular mechanism of the silver(I) complexes antimicrobial activity have not yet been clarified. It has however been found that most silver(I) complexes having the same or similar core structures (consisting of the same coordinating donor atoms) possess a very similar spectrum of the antimicrobial activities.[23,24] In this context, the antimicrobial activity of Ag(I) complexes has been studied in solution for compounds with Ag–N, Ag–S, Ag–O and Ag–P bonds.[23,25] Thus, it has been speculated that the bond properties of Ag–X, X = N[23] and O,[24] respectively, might play an important role in the width of the antimicrobial spectrum, rather than the solubility, charge, chirality, or degree of polymerization of the coordination complexes.[23–25] Thus, the factor of the coordination donor atom, *i.e.* the ease of ligand replacement of silver(I) complexes, has been attributed to the fact that further ligand replacement with biological ligands is possible.

## 4. Antibiotics as Coatings on Implant Materials

One way to prevent, or at least diminish the bacterial adhesion to implants is to render their surface bactericidal, creating a stable environment with an antimicrobial spectrum similar to the one of soluble antibiotics.[26] The surface of an implant determines on one hand its ability to integrate into surrounding tissue, allowing cell adhesion, and is, on the other hand, prone to bacterial adhesion. Implant-related infections due to bacterial adhesion may be diminished by modifying the topography and/or surface chemistry of biomaterials. Thus, bacterial adhesion can be influenced by

i) the surface chemistry, and
ii) the surface topography.

One approach to make biomaterials surfaces less interactive with bacterial biomolecules is to polish the material surface, combining the micro- and nano-scale.[27,28] Furthermore, to reduce bacterial attachment and thus biofilm formation, implant surfaces can be coated with proteins, *e.g.* heparin or albumin,[29] hydrophilic chains[30] and poly(ethylene glycol) PEG-based compounds. This influences the chemical composition, hydrophilicity and polarity of the coated surface. Also, various antibiotic compounds, including gentamicin,[31] ciprofloxacin[32] and vancomycin,[33] have been used as coatings on implantable materials, *e.g.* titanium. Titanium and its alloys are well estab-

lished as primary metallic biomaterials used to fabricate orthopaedic implants. They have excellent biocompatibility, excellent corrosion resistance, good mechanical properties and lightness compared with other metals.[34] To create bactericidal surfaces, organosilane covalent bonds at the Ti–OH surface of the metal are used to tether antibiotics to the surface. Vancomycin has been attached to titanium in this way, still showing antibiotic activity. However, the main concern with the purely antibiotic coatings is the development of bacterial resistance.[35]

Due to the possibility of developing antibiotic-resistant bacteria by using only antibiotics, we propose the combination of two active species, which would provide additive, synergic activity against most microorganisms, and thus the desired protection. We chose the silver ions and the well-studied antibiotic vancomycin as targets to be bonded in combinations to the surface of implant materials.

## 5. Results and Strategy

### 5.1. *Silver Compounds*

There is an increasing interest in the coordination chemistry of silver(I) ions in the context of coordination polymer networks and their polymorphs, as well as in their biological and pharmaceutical activity. One attractive class of ligands comprises (iso)nicotinic acid and its derivatives (Fig. 2).[36–41] Indeed, our group has considerably contributed to the investigation of coordination polymer compounds of Ag(I) obtained with such ligands and a variety of anions. The main ligand used is the ethanediyl bis(isonicotinate) ligand (**L**) (Fig. 2b) because

i) it has a flexible backbone;
ii) it contains O- and N-donor atoms to allow coordination to a 'soft' cation *via* the nitrogen atoms, and a hard cation *via* the oxygen atoms, and, most importantly in this context here,
iii) it is biocompatible.

Several silver(I) coordination compounds of the composition $[Ag\mathbf{L}](NO_3)(H_2O)_n$, n = 0, 1, 2 have been obtained,[37,39] featuring different structures. The solubility effect in different solvents, the co-crystallization of solvent molecules and the counter ion effects were studied so far with respect on the influence on the final solid state structure. Thus, the solubility of $AgNO_3$ in different solvents plays an important role in the reaction between $AgNO_3$ and the ligand **L**, controlling the final Ag–O($NO_3^-$) distances in the product's crystal structure. Without water in the crystal structure (n = 0), simple one-dimensional chains are obtained in which the nitrate anions bridge the silver cations using all three oxygen atoms, whereas co-crystallizing water molecules may lead to the formation of double-chains with short

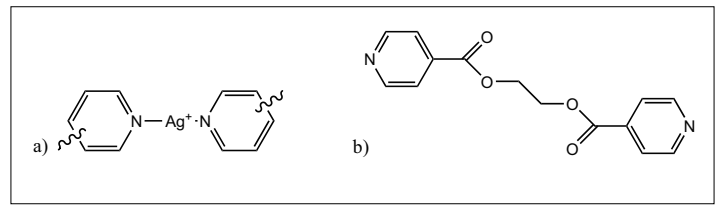


Fig. 2. a) Ligation of nicotinic acid derivatives to silver ions; b) ethanediyl bis(isonicotinate) (**L**)

Ag–Ag contacts. It was also shown that the placement of the silver ions in the solid state plays a role in the light stability of the compounds. Another silver(I) coordination compound, bis(isonicotinamide)silver(I)nitrate $[(Ag(INA)_2(\mu\text{-}NO_3)]_2$[42] also combines light stability in the solid state with ready solubility and with the effective formation of free, unligated $Ag^+$ in solution as does $AgNO_3$. It should, therefore, offer potential as a stabilizing formulation of solid $AgNO_3$.

A preliminary study of silver(I) coordination polymer compounds $[Ag\mathbf{L}](NO_3)$ deposited on metal surfaces as nano-structured coating, characterized by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM), showed good *in vitro* performances of antimicrobial activity against *S. sanguinis* and *S. aureus*.[43]

### 5.2. *Silver–Antibiotic Compounds*

For our present study, we propose to study the binding properties of vancomycin to silver ions and to synthesize different derivatives of vancomycin that are tailored to interact with silver ions and/or bind to a metal surface. As it is known that the vancomycin C-terminal is not necessary for its activity,[44,45] the carboxylic acid group is suitable for covalent coupling to the pyridinyl group *via* flexible hydrophilic bis(ethylene glycol) spacers (Fig. 3), obtaining compounds which may possess useful coordination sites to bind silver ions similar to our ligands used so far. The vancomycin derivatives **1**, **2** and **3** (Fig. 3) were prepared by a HATU-mediated coupling of the carboxylic acid group of vancomycin with the flexible hydrophilic linker and the NH group of 4-aminopyridine or tris-(2-aminoethyl)-amine. Derivatives of vancomycin were then isolated by reverse-phase HPLC and characterized by electrospray (ES) mass spectrometry as well as high field $^1$H-NMR. The soft nature of electrospray ionization makes ESI-MS a powerful tool for studying noncovalent interations including ligand–metal coordination. We therefore used this tool to determine silver ion complexation by vancomycin alone. The obtained mass spectra from different solvents and Ag(I) to vancomycin ratios usually contain multiple peaks that arise from ionizations of free ligands, metal complexes and related molecular fragments. In all cases of ratio Van:Ag (1:1, 1:2, 1:3, 1:4), it can

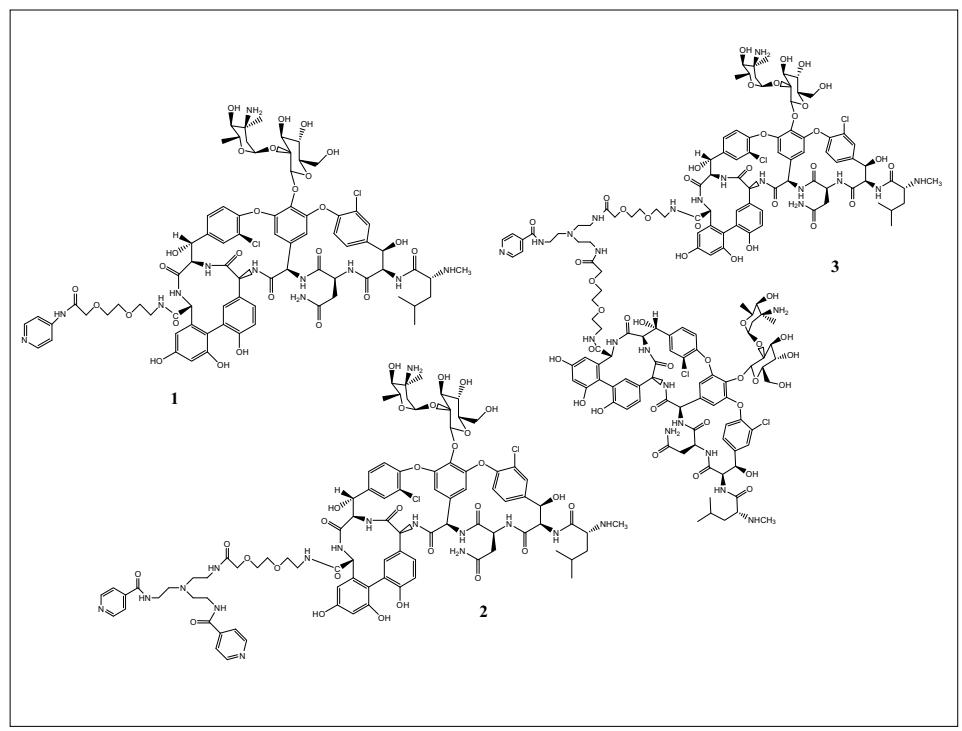


Fig. 3: Derivatives of vancomycin with potential linkers to bind either to a metal surface or to Ag(I) ions

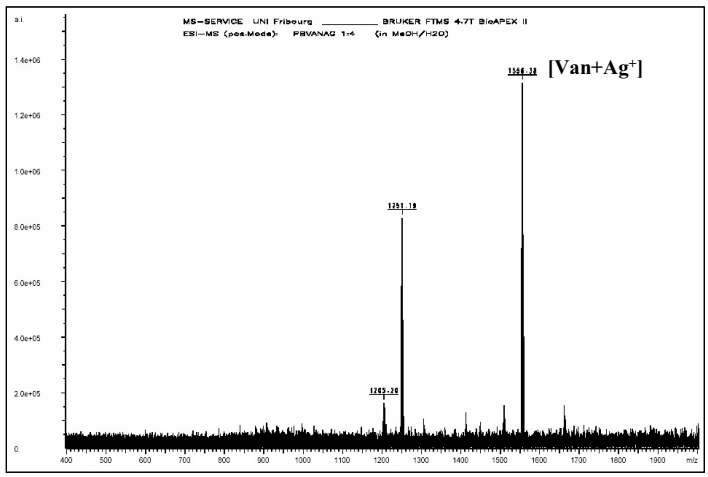


Fig. 4. ESI/MS spectrum of silver ion complexation by vancomycin alone

however be concluded that the most frequent complexes formed are $[VanAg]^+$ (Fig. 4).

We are currently exploring the structural features of these complexes, the complexation of silver ions by the vancomycin derivatives **1**, **2** and **3**, their coating onto implant materials and their antimicrobial properties.

## 6. Conclusion

Direct chemical modification of implant surfaces with bactericidal agents could provide a new generation of self-protective implants. This may be an efficient solution conquering bacterial adhesion and biofilm formation and should be an important step in the fight against infections. Silver coordination polymer compounds combined with antibiotic derivatives are currently being testing in micro-biological studies for their biocompatibility leading to soft- and hard-tissue integration and vascularization.

## 7. Experimental

### 7.1. *Vancomycin Derivatives*

The vancomycin derivative **1**, **2** and **3** are synthesized in solution-phase HATU-mediated coupling protocol. Vancomycin (1.6 g, 1.11 mmol) was reacted with Fmoc-8-amino-3,6-dioxaoctanoate (aminoethoxyethoxyacetate; AEEA, 422 mg, 1.11 mmol) linker and 4-aminopyridine (30 mg, 0.32 mmol) or tris-(2-aminoethyl)-amine (2,54 g, 17,37 mmol) in DMF (10 ml). Compounds are purified by semi-preparative reverse phase HPLC and lyophilized to afford vancomycin derivatives **1**, **2** and **3** in a yield of 10% **1**, 5% **2** and 10% **3** and identified by mass spectrometry and NMR.

### 7.2. *Ligand L*

The synthesis of **L** has been reported previously.[37]

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