Review on bibliography related to antimicrobials

Antimicrobials

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References on chitosan antimicrobial and derivatives pg 45

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   Peptides in phospholipid monolayers and membranes pg 75
Antimicrobials

In this report, a bibliographic research has been done in the field of antimicrobials. Not all antimicrobials have been included, but those that are being subject of matter in the group GBMI in Terrassa, and others of interest. It includes chitosan and other biopolymers. The effect of nanoparticles is of great interest, and in this sense, the effect of Ag nanoparticles and antibiotic nanoparticles (nanobiotics) has been revised. The report focuses on new publications and the antimicrobial effect of peptides has been considered. In particular, the influence of antimicrobials on membranes has deserved much attention and its study using the Langmuir technique, which is of great utility on biomimetic studies. The building up of antimicrobials systems with new techniques (bottom-up approach), as the Layer-by-Layer technique, can also be found in between the bibliography. It has also been considered the antibiofilm effect, and the new ideas on quorem sensing and quorum quenching.

The first section includes general and specific bibliography on antimicrobials. The second section is devoted to chitosan and derivatives. The third section is focused on peptides and peptides in phospholipid biomembranes. The reader is able to get in deep in the filed trough the references reported in each of the selected references included in this report.
A Review of the Recent Advances in Antimicrobial Coatings for Urinary Catheters
Priyadarshini Singha, Jason Locklin, Hitesh Handa
DOI: http://dx.doi.org/10.1016/j.actbio.2016.11.070
To appear in: Acta Biomaterialia

Abstract
More than 75% of hospital-acquired or nosocomial urinary tract infections are initiated by urinary catheters, which are used during the treatment of 15-25% of hospitalized patients. Among other purposes, urinary catheters are primarily used for draining urine after surgeries and for urinary incontinence. During catheter-associated urinary tract infections, bacteria travel up to the bladder and cause infection. A major cause of catheter-associated urinary tract infection is attributed to the use of non-ideal materials in the fabrication of urinary catheters. Such materials allow for the colonization of microorganisms, leading to bacteriuria and infection, depending on the severity of symptoms. The ideal urinary catheter is made out of materials that are biocompatible, antimicrobial, and antifouling. Although an abundance of research has been conducted over the last forty-five years on the subject, the ideal biomaterial, especially for long-term catheterization of more than a month, has yet to be developed. The aim of this review is to highlight the recent advances (over the past 10 years) in developing antimicrobial materials for urinary catheters and to outline future requirements and prospects that guide catheter materials selection and design.

A versatile plasmonic thermogel for disinfection of antimicrobial resistant bacteria
Mohamed A. Abdou Mohamed, Vahid Raeesi, Patricia V. Turner, Anu Rebbapragada, Kate Banks, Warren C.W. Chan
DOI: 10.1016/j.biomaterials.2016.04.009
Biomaterials 97 (2016) 154-163

The increasing occurrence of antimicrobial resistance among bacteria is a global problem that requires the development of alternative techniques to eradicate these superbugs. Herein, we used a combination of thermosensitive biocompatible polymer and gold nanorods to specifically deliver, preserve and confine heat to the area of interest. Our data demonstrates that this technique can be used to kill both Gram positive and Gram negative antimicrobial resistant bacteria in vitro. Our approach significantly reduces the antimicrobial resistant bacteria load in experimentally infected wounds by 98% without harming the surrounding tissues. More importantly, this polymer-nanocomposite can be prepared easily and applied to the wounds, can generate heat using a hand-held laser device, is safe for the operator, and does not have any adverse effects on the wound tissue and healing process.

Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates
Khursheed Ali, Sourabh Dwivedi, Ameer Azam, Quaiser Saquib, Mansour S. Al-Said, Abdulaziz A. Alkhedhairy, Javed Musarrat
ZnO nanoparticles (ZnONPs) were synthesised through a simple and efficient biogenic synthesis approach, exploiting the reducing and capping potential of Aloe barbadensis Miller (A. vera) leaf extract (ALE). ALE-capped ZnO nanoparticles (ALE-ZnONPs) were characterized using UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM) analyses. XRD analysis provided the average size of ZnONPs as 15 nm. FTIR spectral analysis suggested the role of phenolic compounds, terpenoids and proteins present in ALE, in nucleation and stability of ZnONPs. Flow cytometry and atomic absorption spectrophotometry (AAS) data analyses revealed the surface binding and internalization of ZnONPs in Gram +ve (Staphylococcus aureus) and Gram -ve (Escherichia coli) cells, respectively. Significant antibacterial activity of ALE-ZnONPs was observed against extended spectrum beta lactamases (ESBL) positive E. coli, Pseudomonas aeruginosa, and methicillin resistant S. Aureus (MRSA) clinical isolates exhibiting the MIC and MBC values of 2200, 2400 lg/ml and 2300, 2700 lg/ml, respectively. Substantial inhibitory effects of ALE-ZnONPs on bacterial growth kinetics, exopolysaccharides and biofilm formation, unequivocally suggested the antibiotic and anti-biofilm potential. Overall, the results elucidated a rapid, environmentally benign, cost-effective, and convenient method for ALE-ZnONPs synthesis, for possible applications as nanoantibiotics or drug carriers.

Amino Acid-Based Zwitterionic Polymer Surfaces Highly Resist Long-Term Bacterial Adhesion
Qingsheng Liu, Wenchen Li, Hua Wang, Bi-min Zhang Newby, Fang Cheng, and Lingyun Liu

DOI: 10.1021/acs.langmuir.6b01329
Langmuir 2016, 32, 7866–7874

ABSTRACT: The surfaces or coatings that can effectively suppress bacterial adhesion in the long term are of critical importance for biomedical applications. Herein, a group of amino acid-based zwitterionic polymers (pAAZ) were investigated for their long-term resistance to bacterial adhesion. The polymers were derived from natural amino acids including serine, ornithine, lysine, aspartic acid, and glutamic acid. The pAAZ brushes were grafted on gold via the surface-initiated photoiniferter-mediated polymerization (SI-PIMP). Results show that the pAAZ coatings highly suppressed adsorption from the undiluted human serum and plasma. Long-term bacterial adhesion on these surfaces was investigated, using two kinds of representative bacteria [Gram-positive Staphylococcus epidermidis and Gram-negative Pseudomonas aeruginosa] as the model species. Results demonstrate that the pAAZ surfaces were highly resistant to bacterial adhesion after culturing for 1, 5, 9, or even 14 days, representing at least 95% reduction at all time points compared to the control unmodified surfaces. The bacterial accumulation on the pAAZ surfaces after 9 or 14 days was even lower than on the surfaces grafted with poly[poly(ethyl glycol) methyl ether methacrylate] (pPEGMA), one of the most common antifouling materials known to date. The pAAZ brushes also exhibited excellent structural stability in phosphate-buffered saline after incubation for 4 weeks. The bacterial resistance and stability of pAAZ polymers suggest they have good potential to be used for those applications where longterm suppression to bacterial attachment is desired.

Antibacterial activity of silver nanoparticles: A surface science insight
Benjamin Le Ouay, Francesco Stellacci


Summary Silver nanoparticles constitute a very promising approach for the development of new antimicrobial systems. Nanoparticulate objects can bring significant improvements in the antibacterial activity of this element, through specific effect such as an adsorption at
bacterial surfaces. However, the mechanism of action is essentially driven by the oxidative dissolution of the nanoparticles, as indicated by recent direct observations. The role of Ag+ release in the action mechanism was also indirectly observed in numerous studies, and explains the sensitivity of the antimicrobial activity to the presence of some chemical species, notably halides and sulfides which form insoluble salts with Ag+. As such, surface properties of Ag nanoparticles have a crucial impact on their potency, as they influence both physical (aggregation, affinity for bacterial membrane, etc.) and chemical (dissolution, passivation, etc.) phenomena. Here, we review the main parameters that will affect the surface state of Ag NPs and their influence on antimicrobial efficacy. We also provide an analysis of several works on Ag NPs activity, observed through the scope of an oxidative Ag+ release.

Antimicrobial activity of poly(acrylic acid) block copolymers
Günther Gratzl, Christian Paulik, Sabine Hild, Josef P. Guggenbichler, Maximilian Lackner

Materials Science and Engineering C 38 (2014) 94–100

The increasing number of antibiotic-resistant bacterial strains has developed into a major health problem. In particular, biofilms are the main reason for hospital-acquired infections and diseases. Once formed, biofilms are difficult to remove as they have specific defense mechanisms against antimicrobial agents. Antimicrobial surfaces must therefore kill or repel bacteria before they can settle to form a biofilm. In this study, we describe that poly(acrylic acid) (PAA) containing diblock copolymers can kill bacteria and prevent from biofilm formation. The PAA diblock copolymers with poly(styrene) and poly(methyl methacrylate) were synthesized via anionic polymerization of tert-butyl acrylate with styrene or methyl methacrylate and subsequent acid-catalyzed hydrolysis of the tert-butyl ester. The copolymers were characterized via nuclear magnetic resonance spectroscopy (NMR), size-exclusion chromatography (SEC), Fourier transform infrared spectroscopy (FTIR), elemental analysis, and acid–base titrations. Copolymer films with a variety of acrylic acid contents were produced by solvent casting, characterized by atomic force microscopy (AFM) and tested for their antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The antimicrobial activity of the acidic diblock copolymers increased with increasing acrylic acid content, independent of the copolymer-partner, the chain length and the nanostructure.

Antibacterial carboxymethyl cellulose/Ag nanocomposite hydrogels cross-linked with layered double hydroxides
Mehdi Yadollahi, Hassan Namazi, Mohammad Aghazadeh

dx.doi.org/10.1016/j.ijbiomac.2015.05.002

Abstract This paper deals with the preparation of antibacterial nanocomposite hydrogels through the combination of carboxymethyl cellulose (CMC), layered double hydroxides (LDH), and silver nanoparticles (AgNPs). CMC-LDH hydrogels were prepared by intercalating CMC into different LDHs. Then, Ag/CMC-LDH nanocomposite hydrogels were prepared through in situ formation of AgNPs within the CMC-LDHs. XRD analysis confirmed the intercalating CMC into the LDH sheets and formation of intercalated structures, as well as formation of AgNPs within the CMC-LDHs. SEM and TEM micrographs indicated well-distribution of AgNPs within the Ag/CMC-LDHs. The prepared hydrogels showed a pH sensitive swelling behavior. The Ag/CMC-LDH nanocomposite hydrogels have rather higher swelling in different aqueous solutions in comparison with CMC-LDHs. The antibacterial activity of CMC-LDHs increased considerably after formation of AgNPs and was stable for more than one month.

Antibacterial activity of novel benzopolycyclic amines
Marta Barniol-Xicota, Alex Escandell, Elena Valverde, Esther Julián, Eduard Torrents, Santiago Vázquez

**Antibacterial Activity of Silver Nanoparticle-Loaded Soft Contact Lens Materials: The Effect of Monomer Composition**

Maryam Shayani Rad, Bahman Khameneh, Zahra Sabeti, Seyed Ahmad Mohajeri & Bibi Sedigheh Fazly Bazzaz

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Purpose: In the present work, the effect of monomer composition on silver nanoparticles’ (SNPs) binding capacity of hydrogels was investigated and their antibacterial efficacy was evaluated.

Materials and methods: Three series of poly-hydroxyethyl methacrylate (HEMA) hydrogels were prepared using methacrylic acid (MAA), methacrylamide (MAAM), and 4-vinylpyridine (4VP) as co-monomers, and ethylene glycol dimethacrylate (EGDMA) as cross-linker. SNPs binding capacity of hydrogels was evaluated in different concentrations (2, 10, and 20 ppm). In vitro antibacterial activity of SNP-loaded hydrogels was studied against *Pseudomonas aeruginosa* (*P. aeruginosa*) isolated from patients’ eyes. Then, inhibitory effect of hydrogels in biofilm formation was evaluated in the presence of Staphylococcus epidermidis (*S. epidermidis*) (DSMZ 3270).

Results: Our data indicated that poly(HEMA-co-MAA-co-EGDMA) had superior binding affinity for SNPs in comparison with other hydrogels. All SNP-loaded hydrogels demonstrated excellent antimicrobial effects at all times against *P. aeruginosa* and *S. epidermidis* after soaking in 10 and 20 ppm SNP suspensions. Scanning electron microscope (SEM) images revealed excellent inhibitory effect of SNPs against biofilm formation on the surface of the hydrogels.

Conclusions: This study indicated the effect of monomer compositions in SNP loading capacity of poly(HEMA) hydrogels and antibacterial efficacy of SNP-loaded hydrogels against *P. aeruginosa* and *S. epidermidis*, but further in vivo evaluation is necessary.

**Antimicrobial association with phospholipid nano-assemblies: a comparison between Langmuir-Blodgett films and supported lipid bilayers.**

S Morandi, M. Puggelli, G Caminati

COLSUA 321 (2008) 125-130

Supported layers of a dipalmitoyl phosphatidylglycerol (DPPG) were prepared on solid surfaces by means of two different approaches: by Langmuir–Blodgett (LB) and by direct adsorption of a liposomal dispersion onto gold surfaces (SLB).

The penetration behaviour of an ansamycin antibiotic Rifaximin (Rfx) in the two different supported DPPG layers was monitored by a variety of techniques: quartz crystal microbalance (QCM) with dissipation monitoring, UV–vis absorption spectroscopy and cyclic voltammetry (CV) investigation. Experiments were run as a function of time and of the antibiotic concentration in solution to clarify the kinetics and the mechanism of Rfx association with the phospholipid layer. The results evidenced differences in the extent of Rfx penetration depending on the film fluidity and on the type of outer layer exposed to the water environment and demonstrate that the investigated supported layers can be successfully used as sensing surfaces for the detection of food-contaminants such as Rfx for concentrations lower than the allowed maximum residue limit in food.

Antimicrobial films obtained from latex particles functionalized with quaternized block copolymers
New amphiphilic block copolymers with antimicrobial properties were obtained by atom transfer radical polymerization (ATRP) and copper catalyzed cycloaddition following two approaches, a simultaneous strategy or a two-step synthesis, which were proven to be very effective methods. These copolymers were subsequently quaternized using two alkyl chains, methyl and butyl, to amplify their antimicrobial properties and to investigate the effect of alkyl length. Antimicrobial experiments in solution were performed with three types of bacteria, two gram-positive and one gram-negative, and a fungus. Those copolymers quaternized with methyl iodide showed better selectivities on gram-positive bacteria, Staphylococcus aureus and Staphylococcus epidermidis, against red blood cells, demonstrating the importance of the quaternizing agent chosen. Once the solution studies were performed, we prepared poly(butyl methacrylate) latex particles functionalized with the antimicrobial copolymers by emulsion polymerization of butyl methacrylate using such copolymers as surfactants. The characterization by various techniques served to test their effectiveness as surfactants. Finally, films were prepared from these emulsions, and their antimicrobial activity was studied against the gram-positive bacteria. The results indicate that the antimicrobial efficiency of the films depends not only on the copolymer activity but also on other factors such as the surface segregation of the antimicrobial agent to the interface.

Antioxidant and antimicrobial activities of *Tamarix ramosissima*


*Journal of Ethnopharmacology* 78 (2001) 201–205

The ethylacetate and water–acetone extracts of *Tamarix ramosissima* were screened for their antioxidant, antibacterial, antifungal and DNA damaging activities through in vitro experiments. All fractions as well as precipitates showed significant antioxidant activity. A known compound tamarixetin (1) was isolated which showed significant DNA damaging activity in mutant yeast bioassay. Results revealed that antioxidant and antibacterial activities were associated with the presence of polyphenolic substances.

Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors

Timothy V. Duncan

*Journal of Colloid and Interface Science* 363 (2011) 1–24

In this article, several applications of nanomaterials in food packaging and food safety are reviewed, including: polymer/clay nanocomposites as high barrier packaging materials, silver nanoparticles as potent antimicrobial agents, and nosensors and nanomaterial-based assays for the detection of foodrelevant analytes (gasses, small organic molecules and food-borne pathogens). In addition to covering the technical aspects of these topics, the current commercial status and understanding of health implications of these technologies are also discussed. These applications were chosen because they do not involve direct addition of nanoparticles to consumed foods, and thus are more likely to be marketed to the public in the short term.

Attenuation of thrombosis and bacterial infection using dual function nitric oxide releasing central venous catheters in a 9 day rabbit model

- Elizabeth J. Brisbois, Terry C. Major, Marcus J. Goudie, Mark E. Meyerhoff
- Robert H. Bartlett, Hitesh Handa
- [http://dx.doi.org/10.1016/j.actbio.2016.08.009](http://dx.doi.org/10.1016/j.actbio.2016.08.009)
Abstract

Two major problems with implanted catheters are clotting and infection. Nitric oxide (NO) is an endogenous vasodilator as well as a natural inhibitor of platelet adhesion/activation and an antimicrobial agent, and NO-releasing polymers are expected to have similar properties. Here, NO-releasing central venous catheters (CVCs) are fabricated using Elasteon™ E2As polymer with both diazeniumdiolated dibutylnhexanediamine (DBHD/NONO) and poly(lactic-co-glycolic acid) (PLGA) additives, where the NO release can be modulated and optimized via the hydrolysis rate of the PLGA. It is observed that using a 10% w/w additive of a PLGA with ester end group provides the most controlled NO release from the CVCs over a 14-d period. The optimized DBHD/NONO-based catheters are non-hemolytic (hemolytic index of 0%) and noncytotoxic (grade 0). After 9 d of catheter implantation in the jugular veins of rabbits, the NO-releasing CVCs have a significantly reduced thrombus area (7 times smaller) and a 95% reduction in bacterial adhesion. These results show the promise of DBHD/NONO-based NO releasing materials as a solution to achieve extended NO release for longer term prevention of clotting and infection associated with intravascular catheters.

Bacteria-responsive multilayer coatings comprising polycationic nanospheres for bacteria biofilm prevention on urinary catheters
Antonio Francesko, Margarida M. Fernandes, Kristina Ivanova, Sara Amorim, Rui L. Reis, Iva Pashkuleva, Ernest Mendoza, Annett Pfeifer, Thomas Heinze, Tzanko Tzanov
Acta Biomaterialia 33 (2016) 203–212

This work reports on the development of infection-preventive coatings on silicone urinary catheters that contain in their structure and release on demand antibacterial polycationic nanospheres. Polycationic aminocellulose conjugate was first sonochemically processed into nanospheres to improve its antibacterial potential compared to the bulk conjugate in solution (ACSol). Afterward the processed aminocellulose nanospheres (ACNSs) were combined with the hyaluronic acid (HA) polyanion to build a layer-by-layer construct on silicone surfaces. Although the coating deposition was more effective when HA was coupled with ACSol than with ACNSs, the ACNSs-based coatings were thicker and displayed smoother surfaces due to the embedment of intact nanospheres. The antibacterial effect of ACNSs multilayers was 40% higher compared to ACSol coatings. This fact was further translated into more effective prevention of Pseudomonas aeruginosa biofilm formation. The coatings were stable in the absence of bacteria, whereas their disassembling occurred gradually during incubation with P. aeruginosa, and thus eradicate the biofilm upon release of antibacterial agents. Only 5 bilayers of HA/ACNSs were sufficient to prevent the biofilm formation, in contrast to the 10 bilayers of ACSol required to achieve the same effect. The antibiofilm efficiency of (HA/ACNSs)10 multilayer construct built on a Foley catheter was additionally validated under dynamic conditions using a model of the catheterized bladder in which the biofilm was grown during seven days.

Bioinspired synthesis of polydopamine/Ag nanocomposite particles with antibacterial activities
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http://dx.doi.org/10.1016/j.msec.2015.05.032

Mussel-inspired chemistry (polydopamine) offers great opportunities to develop inexpensive and efficient process for many types of materials with complex shapes and functions in a mild and friendly environment. This paper describes a facile, yet green approach to synthesize polydopamine/silver (PDA/Ag) nanocomposite
particles with a combination use of polydopamine chemistry and electroless metallization of Ag. In this approach, monodisperse spherical polydopamine particles are first synthesized by the oxidation and self-polymerization of dopamine (monomer) in an alkaline water–ethanol solution at room temperature, which are served as the active templates for secondary reactions due to the abundant catechol and amine groups on the surface. Subsequently, the silver precursor-[Ag(NH)2]+ ions introduced are easily absorbed onto the surface of the PDA particles, and are immediately in situ reduced to metallic Ag nanoparticles with the help of these active catechol and amine groups. During the preparation, no additional reductants, toxic reagents and intricate instruments are needed. These as-synthesized PDA/Ag nanocomposite particles are ideal candidates for antibacterial application because they do not show significant cytotoxicity against HEK293T human embryonic kidney cells in the in vitro cytotoxicity assay, whereas demonstrate enhanced antibacterial abilities against Escherichia coli (Gram-negative bacteria) and Staphylococcus aureus (Gram-positive bacteria) in the antibacterial assays. Owing to their excellent cytocompatibilities and antibacterial activities, these PDA/Ag nanocomposite particles can be considered as the promising antibacterial materials for future biomedical applications.

Building an Antifouling Zwitterionic Coating on Urinary Catheters Using an Enzymatically Triggered Bottom-Up Approach
Carlos Diaz Blanco, Andreas Ortner, Radostin Dimitrov, Antonio Navarro, Ernest Mendoza, and Tzanko Tzanov*,
dx.doi.org/10.1021/am501961b | ACS Appl. Mater. Interfaces 2014, 6, 11385–11393

Catheter associated urinary tract infections are common during hospitalization due to the formation of bacterial biofilms on the indwelling device. In this study, we report an innovative biotechnology-based approach for the covalent functionalization of silicone catheters with antifouling zwitterionic moieties to prevent biofilm formation. Our approach combines the potential bioactivity of a natural phenolics layer biocatalytically conjugated to sulfobetaine-acrylic residues in an enzymatically initiated surface radical polymerization with laccase. To ensure sufficient coating stability in urine, the silicone catheter is plasma-activated. In contrast to industrial chemical methods, the methacrylate-containing zwitterionic monomers are polymerized at pH 5 and 50 °C using as an initiator the phenoxy radicals solely generated by laccase on the phenolics-coated catheter surface. The coated catheters are characterized by X-ray photoelectron spectroscopy (XPS), Fourier transformed infrared (FTIR) analysis, atomic force microscopy (AFM), and colorimetrically. Contact angle and protein adsorption measurements, coupled with in vitro tests with the Gram-negative Pseudomonas aeruginosa and Gram-positive Staphylococcus aureus in static and dynamic conditions, mimicking the operational conditions to be faced by the catheters, demonstrate reduced biofilm formation by about 80% when compared to that of unmodified urinary catheters. The zwitterionic coating did not affect the viability of the human fibroblasts (BJ-5ta) over seven days, corresponding to the extended useful life of urinary catheters.

Cellulbiose dehydrogenase functionalized urinary catheter as novel antibiofilm system
Barbara Thallinger, Martin Brandauer, Peter Burger, Christoph Sygmund, Roland Ludwig, Kristina Ivanova, Jutta Kun, Denis Scaini, Michael Burnet, Tzanko Tzanov, Gibson S. Nyanhongo, Georg M. Guebitz

Abstract: Urinary catheters expose patients to a high risk of acquiring nosocomial infections. To prevent this risk of infection, cellulbiose dehydrogenase (CDH), an antimicrobial enzyme able to use various oligosaccharides as electron donors to produce hydrogen peroxide using oxygen as an electron acceptor, was covalently grafted onto plasma-activated urinary polydimethylsiloxane (PDMS) catheter surfaces. Successful immobilization of CDH on PDMS was confirmed by Fourier transformed-infrared spectrometry and production of H2O2. The CDH functionalized PDMS surfaces reduced the amount of viable Staphylococcus aureus by 60%, total biomass deposited on the surface by 30% and 70% of biofilm formation. The immobilized CDH was relatively stable in artificial urine over 16 days, retaining 20% of its initial activity. The CDH-coated PDMS surface did not affect the growth and physiology of HEK 239 and RAW 264.7
mammalian cells. Therefore this new CDH functionalized catheter system shows great potential for solving the current problems associated with urinary catheters.

Characterization of antimicrobial activity against Listeria and cytotoxicity of native melittin and its mutant variants

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Colloids and Surfaces B: Biointerfaces 143 (2016) 194–205

Antimicrobial peptides (AMPs) are relatively short peptides that have the ability to penetrate the cell membrane, form pores leading to cell death. This study compares both antimicrobial activity and cytotoxicity of native melittin and its two mutants, namely, melittin I17K (GIGAVLKLTTGLPALKSWIKRKRQQ) with a higher charge and lower hydrophobicity and mutant G1I (IGAVLKVTTLGLPALISWIKRKRQQ) of higher hydrophobicity. The antimicrobial activity against different strains of Listeria was investigated by bioassay, viability studies, fluorescence and transmission electron microscopy. Cytotoxicity was examined by lactate dehydrogenase (LDH) assay on mammalian Caco-2 cells. The minimum inhibitory concentration of native, mutant I17K, mutant G1I against L. monocytogenes F4244 was 0.315 ± 0.008, 0.814 ± 0.006 and 0.494 ± 0.037 µg/ml respectively, whereas the minimum bactericidal concentration values were 3.263 ± 0.0034, 7.412 ± 0.017 and 5.366 ± 0.019 µg/ml respectively. Lag time for inactivation of L. monocytogenes F4244 was observed at concentrations below 0.20 and 0.78 µg/ml for native and mutant melittin I17K respectively. The antimicrobial activity against L. monocytogenes F4244 was in the order native > G1I > I17K. Native melittin was cytotoxic to mammalian Caco-2 cells above concentration of 2 µg/ml, whereas the two mutants exhibited negligible cytotoxicity up to a concentration of 8 µg/ml. Pore formation in cell wall/membrane was observed by transmission electron microscopy. Molecular dynamics (MD) simulation of native and its mutants indicated that (i) surface native melittin and G1I exhibited higher tendency to penetrate a mimic of bacterial cell membrane and (ii) transmembrane native and I17K formed water channel in mimics of bacterial and mammalian cell membranes.

‘Chocolate’ silver nanoparticles: Synthesis, antibacterial activity and cytotoxicity

Neelika Roy Chowdhury, Melanie MacGregor-Ramiasa, Peter Zilm, Peter Majewski, Krasimir Vasilev

Journal of Colloid and Interface Science 482 (2016) 151-158

Hypothesis: Silver nanoparticles (AgNPs) have emerged as a powerful weapon against antibiotic resistant microorganisms. However, most conventional AgNPs syntheses require the use of hazardous chemicals and generate toxic organic waste. Hence, in recent year’s, plant derived and biomolecule based synthetics have gained much attention. Cacao has been used for years for its medicinal benefits and contains a powerful reducing agent – oxalic acid. We hypothesized that, due to the presence of oxalic acid, cacao extract is capable of reducing silver nitrate (AgNO3) to produce AgNPs.

Experiments: In this study, AgNPs were synthesized by using natural cacao extract as a reducing and stabilizing agent. The reaction temperature, time and reactant molarity were varied to optimize the synthesis yield.

Findings: UV-visible spectroscopy (UV-vis), dynamic light scattering (DLS) and transmission electron microscopy (TEM) characterization demonstrated that the synthesized AgNPs were spherical particles ranging in size from 35 to 42.5 nm. The synthesized AgNPs showed significant antibacterial activity against clinically relevant pathogens such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus epidermidis. Importantly, these green AgNPs are not cytotoxic to human dermal fibroblasts (HDFs) at concentrations below 32 lg/ml. We conclude that cacao-based synthesis is a reproducible and sustainable method for the generation of stable antimicrobial silver nanoparticles with low cytotoxicity to human cells. The AgNPs synthesized in this work have promising properties for applications in the biomedical field.
Controllable \textit{in situ} synthesis of silver nanoparticles on multilayered film-coated silk fibers for antibacterial application

Mei Meng, Huawe He, Jing Xiao, Ping Zhao, Jiale Xie, Zhisong Lu

Journal of Colloid and Interface Science 461 (2016) 369–375

Layer-by-layer (LbL) assembly is a versatile technique for the preparation of multilayered polymeric films. However, fabrication of LbL polymeric film on silk for the \textit{in situ} growth of high-density silver nanoparticles (AgNPs) has not been realized. Herein poly(acrylic acid) (PAA)/poly(dimethyldiallylammonium chloride) (PDDA) multilayers are constructed on silk via the LbL approach, subsequently serving as a 3-dimensional matrix for \textit{in situ} synthesis of AgNPs. After 8 rounds of LbL assembly, the silk is fully covered with a layer of polymeric film. AgNPs with good crystalline structures could be \textit{in situ} generated in the silk-coated multilayers and their amount could be tailored by adjusting the bilayer numbers. The as-prepared silk could effectively kill the existing bacteria and inhibit the bacterial growth, demonstrating the antimicrobial activity. Moreover, the release of Ag+ from the modified silk can last for 120 h, rendering the modified silk sustainable antimicrobial activity. This work may provide a novel method to prepare AgNPs-functionalized antimicrobial silk for potential applications in textile industry.

Review

Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces

Laura Tamayo, Manuel Azócar, Marcelo Kogan, Ana Riveros, Maritza Páez

Materials Science and Engineering C 69 (2016) 1391–1409

The development of polymer nanocomposites with antimicrobial properties has been a key factor for controlling or inhibiting the growth of microorganisms and preventing foodborne diseases and nosocomial infections. Commercially available antibacterial products based on silver-polymer are the most widely used despite the fact that copper is considerably less expensive. The incorporation of copper nanoparticles as antibacterial agents in polymeric matrices to generate copper-polymer nanocomposites have presented excellent results in inhibiting the growth of a broad spectrum of microorganisms. The potential applications in food packaging, medical devices, textiles and pharmaceuticals and water treatment have generated an increasing number of investigations on preparing copper based nanocomposites and alternative polymeric matrices, as potential hosts of nano-modifiers. This review presents a comprehensive compilation of previous published work on the subject, mainly related to the antimicrobial activity of copper polymer nanocomposites. Within all the phenomenology associated to antibacterial effects we highlight the possible mechanisms of action. We discuss the differences in the susceptibility of Gram negative and positive bacteria to the antibacterial activity of nanocomposites, and influencing factors. As well, the main applications of copper polymer-metal nanocomposites are described, considering their physical and chemical characteristics. Finally, some commercially available copper-polymer nanocomposites are described.

Current advances on bacterial pathogenesis inhibition and treatment strategies

K. Ivanova, M. M. Fernandes and T. Tzanov

Microbial pathogens and strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.)

Bacterial pathogenesis is a multi-factorial process that is regulated by the production of virulence factors, enabling bacteria to cause various infectious diseases. For many years, antibiotics have been successfully applied for fast treatment of bacteria-mediated diseases, offering an effective infection transmission control. However, it has become clear in recent years that the overuse of antibiotics leads to an increased emergence and spread of multi-drug resistant microorganisms. As a consequence, bacteria cause life-threatening infections and increased mortality, morbidity, length of hospitalization and health care costs. There is a need to decrease the antibiotic usage and to develop new effective antimicrobial strategies to prevent and treat certain infections. This review summarizes recent advances in attenuation of bacterial virulence and treatment of infections. Prevention strategies that provide minimal evolutionary stress and no resistance development such as interference with bacterial cell-to-cell signalling (quorum sensing) pathways and virulence mechanisms are reviewed.
Moreover, new advances on antimicrobial agents such as antimicrobial peptides, bacteriophages, nanoantibiotics and natural polyphenols as well as a new strategy of resistance genes disruption using the bacterial adaptive immune system as a potential therapeutic tool, are also considered.

**Current applications of nanoparticles in infectious diseases**

Hinojal Zazo, Clara I. Colino, José M. Lanao  
Journal of Controlled Release 224 (2016) 86–102

For decades infections have been treated easily with drugs. However, in the 21st century, they may become lethal again owing to the development of antimicrobial resistance. Pathogens can become resistant by means of different mechanisms, such as increasing the time they spend in the intracellular environment, where drugs are unable to reach therapeutic levels. Moreover, drugs are also subject to certain problems that decrease their efficacy. This requires the use of high doses, and frequent administrations must be implemented, causing adverse side effects or toxicity. The use of nanoparticle systems can help to overcome such problems and increase drug efficacy. Accordingly, there is considerable current interest in their use as antimicrobial agents against different pathogens like bacteria, virus, fungi or parasites, multidrug-resistant strains and biofilms; as targeting vectors towards specific tissues; as vaccines and as theranostic systems. This review begins with an overview of the different types and characteristics of nanoparticles used to deliver drugs to the target, followed by a review of current research and clinical trials addressing the use of nanoparticles within the field of infectious diseases.

**CTAB Modified Dellite: A Novel Support for Enzyme Immobilization in Bio-Based Electrochemical Detection and its in vitro Antimicrobial Activity**  
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DOI: http://dx.doi.org/doi:10.1016/j.snb.2016.05.042  

A novel support material for enzyme immobilization based on cetyltrimethylammonium bromide modified Dellite (CTAB-Del) was successfully synthesized and used to manufacture pyranose oxidase (PyOx) biosensors (CTAB-Del/PyOx). The intercalation of CTAB into Dellite was confirmed by FTIR, XRD and TGA techniques. PyOx was immobilized onto the glassy carbon electrode, via glutaraldehyde crosslinking, by using CTAB-Del as a support. In order to test the analytical performance of CTAB-Del/PyOx biosensors, chronoamperometric measurements were carried out using three electrodes configurations, at a constant potential of -0.7V in working buffer, under stirring, with successive addition of glucose. The linear response for CTABDel/PyOx biosensor ranged from 0.01 to 0.50 mM with an equation of $y = 4.42x + 0.004$ (R²=0.998), and the limit of detection for glucose was calculated to be 0.081 μM (S/N=3). In order to confirm its practical use, the CTAB-Del/PyOx biosensor was also applied for glucose measurement in various beverages. In addition, the antimicrobial activities of Del and CTAB-Del were screened in vitro by means of the disc diffusion susceptibility test, selecting a yeast, three different Gram-positive, and five different Gram-negative bacteria. The obtained results showed a moderate antibacterial activity of CTAB-Del against Gram-positive bacteria.

**Development of antibacterial and high light transmittance bulk materials: Incorporation and sustained release of hydrophobic or hydrophilic antibiotics**

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Colloids and Surfaces B: Biointerfaces 141 (2016) 483–490
Infection associated with medical devices is one of the most frequent complications of modern medical biomaterials. Bacteria have a strong ability to attach on solid surfaces, forming colonies and subsequently biofilms. In this work, a novel antibacterial bulk material was prepared through combining poly(dimethylsiloxane) (PDMS) with either hydrophobic or hydrophilic antibiotics (0.1–0.2 wt%). Scanning electron microscopy, water contact angle and UV–vis spectrophotometer were used to measure the changes of surface topography, wettability and optical transmission. For both gentamicin sulfate (GS) and triclosan (TCA), the optical transmission of the PDMS-GS and PDMS-TCA blend films was higher than 90%. Drug release studies showed initial rapid release and later sustained release of GS or TCA under aqueous physiologic conditions. The blend films demonstrated excellent bactericidal and sufficient biofilm inhibition functions against Gram-positive bacteria (Staphylococcus aureus, S. aureus) measured by LIVE/DEAD bacterial viability kit staining method. Kirby-Bauer method showed that there was obvious zone of inhibition (7.5–12.5 mm). Cytocompatibility assessment against human lens epithelial cells (HLECs) revealed that the PDMS-GS blend films had good cytocompatibility. However, the PDMS-TCA blend films showed certain cytotoxicity against HLECs. The PDMS-0.2 wt% GS blend films were compared to native PDMS in the rabbit subcutaneous S. aureus infection model. The blend films yielded a significantly lower degree of infection than native PDMS at day 7. The achievement of the PDMS-drug bulk materials with high light transmittance, excellent bactericidal function and good cytocompatibility can potentially be widely used as bio-optical materials.

Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections
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Infections caused by biofilm-forming bacteria are a major threat to hospitalized patients and the main cause of chronic obstructive pulmonary disease and cystic fibrosis. There is an urgent necessity for novel therapeutic approaches, since current antibiotic delivery fails to eliminate biofilm-protected bacteria. In this study, ciprofloxacin-loaded poly(lactic-co-glycolic acid) nanoparticles, which were functionalized with DNase I, were fabricated using a green-solvent based method and their antibiofilm activity was assessed against Pseudomonas aeruginosa biofilms. Such nanoparticles constitute a paradigm shift in biofilm treatment, since, besides releasing ciprofloxacin in a controlled fashion, they are able to target and disassemble the biofilm by degrading the extracellular DNA that stabilize the biofilm matrix. These carriers were compared with free-soluble ciprofloxacin, and ciprofloxacin encapsulated in untreated and poly(lysine)-coated nanoparticles. DNase I-activated nanoparticles were not only able to prevent biofilm formation from planktonic bacteria, but they also successfully reduced established biofilm mass, size and living cell density, as observed in a dynamic environment in a flow cell biofilm assay. Moreover, repeated administration over three days of DNase I-coated nanoparticles encapsulating ciprofloxacin was able to reduce by 95% and then eradicate more than 99.8% of established biofilm, outperforming all the other nanoparticle formulations and the free-drug tested in this study. These promising results, together with minimal cytotoxicity as tested on J774 macrophages, allow obtaining novel antimicrobial nanoparticles, as well as provide clues to design the next generation of drug delivery devices to treat persistent bacterial infections.

Effect of tetracycline antibiotic on the monolayers of phosphatidylcholines at the air–water interface
K. Kotecka, P. Krysinski

Tetracycline is a broad-spectrum antibiotic belonging to the polyketide class. It is widely used against bacterial infections as well as food additive to a live-stock. Since tetracycline is resistant against degradation, it accumulates in the environment, leading also to antibiotic residues in animal and food products, which is now considered to be an important health risk because of increasing antibiotic resistance of pathogenic microorganisms. In this work we studied the interactions of tetracycline with phospholipid
monolayers at the air/water interface in order to elucidate the mechanism of its action on cell membrane biomimetic system. We selected three phosphatidylcholines, having the same head-group structure, differing with respect to their hydrophobic chain length, the presence of unsaturated bonds between carbon atoms and, consequently, the phase transition temperature. Analysis of the results presented here suggests that tetracycline interacts with the three phosphatidylcholines mainly through the electrostatic interactions with hydrophilic groups of these lipids. On the other hand, the shape of presented pressure-area isotherms and their compressibility moduli show the synergistic effect of hydrophobic interactions that are larger for longer alkyl chains, promoting easier ordering of monolayer molecules with increased surface pressure when drug is present in the subphase, particularly for saturated DPPC. Based on our contact potential data we think that positively charged tertiary amino group of TC is facing negatively charged phosphate groups of phosphatidylcholines, penetrating only the hydrophilic head group region but not the hydrophobic moiety of these monolayers. However, the observed differences in CPD values for the case of DMPC point toward the drug disordered influence also on the organization of the hydrophobic region of this monolayer. This is because the drug penetration disturbs the van der Waals ordering interactions between its shorter hydrophobic chains to a greater extent comparing to DPPC and DOPC molecules.

Efficacy of Antimicrobials against Biofilms of Achromobacter and Pseudomonas
Jaclyn M. L. Chang, David J. McCanna, Lakshman N. Subbaraman, and Lyndon W. Jones

ABSTRACT
Purpose. Achromobacter xylosoxidans and Pseudomonas aeruginosa biofilms can develop in ophthalmic products and accessories such as contact lens cases, leading to the development of ocular infections. This study evaluated the efficacy of the antimicrobials polyaminopropyl biguanide (PAPB) and benzalkonium chloride (BAK) against A. xylosoxidans and P. aeruginosa biofilms.

Methods. Biofilms of A. xylosoxidans and P. aeruginosa used as a comparative control were formed by incubating the bacteria on contact lens cases and on coverslips in phosphate-buffered saline. The biofilms were then exposed to PAPB and BAK for 5 minutes and 4 hours. After exposure, alginate swabs were used to remove the biofilms from the lens cases and the bacteria were plated on tryptic soy agar for determination of survivors. Also, after exposure to these disinfectants, the A. xylosoxidans and P. aeruginosa biofilms were stained with SYTO 9 and propidium iodide. Using a confocal microscope with a 488-nm laser, the number of cells with damaged cell membranes was determined.

Results. After 5 minutes of exposure to BAK or PAPB, A. xylosoxidans biofilms were more resistant to the antimicrobial effects of these disinfectants than P. aeruginosa biofilms. After 4 hours, both organisms were reduced by more than 3 logs after exposure to either BAK or PAPB. Confocal microscopy studies revealed that BAK was more effective at damaging A. xylosoxidans and P. aeruginosa cell membranes than PAPB at the concentrations used in ophthalmic products.

Conclusions. Biofilms of the emerging pathogen A. xylosoxidans were more resistant to the disinfectants PAPB and BAK than biofilms of P. aeruginosa. Because of the emergence of A. xylosoxidans and the demonstrated greater resistance to the common ophthalmic preservatives BAK and PAPB than the standard Gram-negative organism P. aeruginosa, A. xylosoxidans biofilms should be assessed in antimicrobial challenge tests to assure the safety of multiuse ophthalmic products.

Elucidation of innovative antibiotic materials
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DOI: http://dx.doi.org/doi:10.1016/j.colsurfb.2015.08.007
An asymmetric substrate with an outer dense layer and an inner porous structure was obtained from a natural biomaterial via a simple phase-inversion method

- Correlation between the physico-chemical fabrication parameters and the final microstructure was investigated
- The microporous structure was loaded with an antibiofilm protein
- The material qualitatively and quantitatively exhibited antibiofilm activity on preformed bacterial films
- Applications include innovative bioactive dressings for chronic wounds

Abstract: It is known for roughly a decade that bacterial communities (called biofilms) are responsible for significant enhanced antibiotherapy resistance. Biofilms are involved in tissue persistent infection, causing direct or collateral damage leading to chronic wounds development and impairing natural wound healing. In this study, we are interested in the development of supported protein materials which consist of asymmetric membranes as reservoir supports for the incorporation and controlled release of biomolecules capable of dissolving biofilms (or preventing their formation) and their use as wound dressing for chronic wound treatment. In a first step, polyhydroxyalkanoates (PHAs) asymmetric membranes were prepared using wet phase inversion technique. Scanning microscopy (SEM) analysis has showed the influence of different processing parameters. In a second step, the porous side of the membranes were functionalized with a surface treatment and then loaded with the antibiofilm agent (dispersin B). In a third step, the properties and antibiofilm performance of the loaded-membranes were evaluated. Exposure of *Staphylococcus epidermidis* biofilms to such systems weakly inhibited biofilm formation (weak preventive effect) but caused their detachment and disaggregation (strong curative effect). These initial results are promising since they open the way to a new generation of effective tools in the struggle against persistent bacterial infections exhibiting enhanced antibiotherapy resistance, and in particular in the case of infected chronic wounds.

Engineering nanosilver as an antibacterial, biosensor and bioimaging material
Georgios A Sotiriou and Sotiris E Pratsinis

The capacity of nanosilver (Ag nanoparticles) to destroy infectious micro-organisms makes it one of the most powerful antimicrobial agents, an attractive feature against ‘super-bugs’ resistant to antibiotics. Furthermore, its plasmonic properties facilitate its employment as a biosensor or bioimaging agent. Here, the interaction of nanosilver with biological systems including bacteria and mammalian cells is reviewed. The toxicity of nanosilver is discussed focusing on Ag+ ion release in liquid solutions. Biomedical applications of nanosilver are also presented capitalizing on its antimicrobial and plasmonic properties and summarizing its advantages, limitations and challenges. Though a lot needs to be learned about the toxicity of nanosilver, enough is known to safely use it in a spectrum of applications with minimal impact to the environment and human health.
Enzymatic Functionalization of Cork Surface with Antimicrobial Hybrid Biopolymer/Silver Nanoparticles
Antonio Francesko, Lucas Blandón, Mario Vázquez, Petya Petkova, Jordi Morato, Annett Pfeifer, Thomas Heinze, Ernest Mendoza, and Tzanko Tzanov

Abstract
Laccase-assisted assembling of hybrid biopolymer-silver nanoparticles and cork matrices into an antimicrobial material with potential for water remediation is herein described. Amino-functional biopolymers were first used as doping agents to stabilize concentrated colloidal dispersions of silver nanoparticles (AgNPs), additionally providing the particles with functionalities for covalent immobilization onto cork to impart durable antibacterial effect. The solvent-free AgNPs synthesis by chemical reduction was carried out in presence of chitosan (CS) or 6-deoxy-6-(ω-aminoethyl) aminocellulose (AC), leading to simultaneous AgNPs biofunctionalization. This approach resulted in concentrated hybrid NPs dispersion stable to aggregation and with hydrodynamic radius of particles of about 250 nm. Moreover, laccase enabled coupling between the phenolic groups in cork and amino moieties in the biopolymer-doped AgNPs for permanent modification of the material. The antibacterial efficiency of the functionalized cork matrices, aimed as adsorbents for wastewater treatment, was evaluated against Escherichia coli and Staphylococcus aureus during 5 days in conditions mimicking those in constructed wetlands. Both intrinsically antimicrobial CS and AC contributed to the bactericidal effect of the enzymatically-grafted on cork AgNPs. In contrast, unmodified AgNPs were easily washed off from the material, confirming that the biopolymers potentiated a durable antibacterial functionalization of the cork matrices.

Enzyme-assisted formation of hybrid biopolymer hydrogels incorporating active phenolic nanospheres
Petya Petkova Antonio Francesko Tzanko Tzanov
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Advances in development of nanocomposite gels that provide localized delivery of pharmaceuticals for treatment of chronic wounds are being highly pursued. To design such materials, the use of natural polymers is recommendable due to their intrinsic biocompatibility and biodegradability. Moreover, the use of biocatalytic approaches for composite assembling is preferred compared to harsh chemical cross-linking reagents. In this study, HRP catalyzed cross-linking of hydrogels from aqueous solution of thiolated chitosan to in situ incorporated sonochemically synthesized epigallocatechin gallate nanospheres (EGCG NSs). The potential of the generated NSs for chronic wound treatment was evaluated by assessing their antibacterial properties and inhibitory effect on myeloperoxidase and collagenase—major enzymes of inflamed chronic wounds. The EGCG NSs displayed better antibacterial and anti-enzymatic properties compared to the EGCG in solution. Also, the NSs were incorporated into hydrogels without affecting their integrity and were released intact in a sustained manner during 6 days). The cytotoxicity assay confirmed the compatibility of the hybrid material with human fibroblasts that suffered less than 10% decrease in viability during 24 h. Release of functional phenolic NSs and good compatibility of the composite hydrogel with cells suggested its potential application in chronic wound management.
Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens
Monika Smekalova, Virginia Aragon, Ales Panacek, Robert Prucek, Radek Zboril, Libor Kvitěk
The Veterinary Journal 209 (2016) 174-179

Antibiotic resistant bacteria are a serious health risk in both human and veterinary medicine. Several studies have shown that silver nanoparticles (AgNPs) exert a high level of antibacterial activity against antibiotic resistant strains in humans. The aim of this study was to evaluate the antibacterial effects of a combined therapy of AgNPs and antibiotics against veterinary bacteria that show resistance to antibiotics.

A microdilution checkerboard method was used to determine the minimal inhibitory concentrations of both types of antimicrobials, alone and in combination. The fractional inhibitory concentration index was calculated and used to classify observed collective antibacterial activity as synergistic, additive (only the sum of separate effects of drugs), indifferent (no effect) or antagonistic.

From the 40 performed tests, seven were synergistic, 17 additive and 16 indifferent. None of the tested combinations showed an antagonistic effect. The majority of synergistic effects were observed for combinations of AgNPs given together with gentamicin, but the highest enhancement of antibacterial activity was found with combined therapy together with penicillin G against Actinobacillus pleuropneumoniae A. pleuropneumoniae and Pasteurella multocida originally resistant to amoxycillin, gentamicin and colistin were sensitive to these antibiotics when combined with AgNPs. The study shows that AgNPs have potential as adjuvants for the treatment of animal bacterial diseases.

Enzyme multilayer coatings inhibit Pseudomonas aeruginosa biofilm formation on urinary catheters
Kristina Ivanova & Margarida M. Fernandes & Ernest Mendoza & Tzanko Tzanov

Abstract Bacteria use a signaling mechanism called quorum sensing (QS) to form complex communities of surfaceattached cells known as biofilms. This protective mode of growth allows them to resist antibiotic treatment and originates the majority of hospital-acquired infections. Emerging alternatives to control biofilm-associated infections and multidrug resistance development interfere with bacterial QS pathways, exerting less selective pressure on bacterial population. In this study, biologically stable coatings comprising the QS disrupting enzyme acylase were built on silicone urinary catheters using a layer-by-layer technique. This was achieved by the alternate deposition of negatively charged enzyme and positively charged polyethylenimine. The acylase-coated catheters efficiently quenched the QS in the biosensor strain Chromobacterium violaceum CECT 5999, demonstrated by approximately 50 % inhibition of violacein production. These enzyme multilayer coatings significantly reduced the Pseudomonas aeruginosa ATCC 10145 biofilm formation under static and dynamic conditions in an in vitro catheterized bladder model. The quorum quenching enzyme coatings did not affect the viability of the human fibroblasts (BJ-5ta) over 7 days, corresponding to the extended useful life of urinary catheters. Such enzyme-based approach could be an alternative to the conventional antibiotic treatment for prevention of biofilm-associated urinary tract infections.

Evaluation of Magainin I interactions with lipid membranes: An optical and electrochemical study

- Jéssica M. Nascimento, Octávio L. Franco, Maria D.L. Oliveira, César A.S. Andrade
- Chemistry and Physics of Lipids Volume 165, Issue 5, July 2012, Pages 537–544

Most antimicrobial peptides (AMPs) have shown clear activity related to the disruption of lipid bilayers. In order to improve knowledge of this subject, the interaction of Magainin I (Magl)
with phospholipid layers (PLs), uncoated or coated with synperonic (Synp), was studied using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and surface plasmon resonance (SPR) techniques. MagI peptide was immobilized on gold electrode via a self-assembling monolayer obtained from liposomes and liposomes covered by Synp. MagI induces pores in the supported lipid membranes, which are reflected in an increased amperometric response and also a decreased electron-transfer resistance ($R_{CT}$). In addition, MagI showed a significant interaction with the PL-Synp-modified gold electrode, but MagI showed a reliable contact with the PL-modified gold electrode, leading to a decrease in the relative resistance charge transfer value of $-17.38\%$. Our results demonstrated that Synp acts as a membrane sealant after exposure of the lipid membrane to MagI. A parallel reaction model was proposed for the interaction of MagI and a hybrid layer that result in a complex bimolecular interaction. In short, the importance of triblock copolymer to stabilize liposomes for future applications as drug delivery systems for MagI was demonstrated.

Gold–Nanoparticles coated with the antimicrobial peptide Esculentin–1a(1–21)NH₂ as a reliable strategy for antipseudomonal drugs
Bruno Casciaro, Maria Moros, Sara Rivera-Fernandez, Andrea Bellelli, Jesús M de la Fuente, Maria Luisa Mangoni
DOI: http://dx.doi.org/10.1016/j.actbio.2016.09.041

Naturally occurring antimicrobial peptides (AMPs) hold promise as future therapeutics against multidrug resistant microorganisms. Recently, we have discovered that a derivative of the frog skin AMP esculentin-1a, Esc(1-21), is highly potent against both free living and biofilm forms of the bacterial pathogen Pseudomonas aeruginosa. However, bringing AMPs into clinics requires to overcome their low stability, high toxicity and inefficient delivery to the target site at high concentrations. Importantly, peptide conjugation to gold nanoparticles (AuNPs), which are among the most applied inorganic nanocarriers in biomedical sciences, represents a valuable strategy to solve these problems. Here we report that covalent conjugation of Esc(1-21) to soluble AuNPs [AuNPs@Esc(1-21)] via a poly(ethylene glycol) linker increased by ~15-fold the activity of the free peptide against the motile and sessile forms of P. aeruginosa without being toxic to human keratinocytes. Furthermore, AuNPs@Esc(1-21) resulted to be significantly more resistant to proteolytic digestion and to disintegrate the bacterial membrane at very low concentration (5 nM).

Finally, we demonstrated for the first time the capability of peptide-coated AuNPs to display a wound healing activity on a keratinocytes monolayer. Overall, these findings suggest that our engineered AuNPs can serve as attractive novel biological-derived material for topical treatment of epithelial infections and healing of the injured tissue.

Interactions of a Fungistatic antibiotic, griseofulvin, with phospholipid monolayers used as models of biological membranes.


Griseofulvin (GF) is an oral antibiotic for widely occurring superficial mycosis in man and animals caused by dermaphyte fungi; it is also used in agriculture as a fungicide. The mechanism of the biological activity of GF is poorly understood. Here, the interactions of griseofulvin with lipid membranes were studied using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), and 1,2-myristoyl-sn-glycero-3-phosphoethanolamine (DMPE) monolayers spread at the air/water interface. Surface pressure ($\Gamma$), electric surfacepotential ($\bar{\varepsilon}$V), grazing incidence X-ray diffraction (GIXD), and Brewster angle microscopy (BAM) were used for studying pure phospholipid monolayers spread on GF aqueous solutions, as well as mixed phospholipid/GF monolayers spread on
pure water subphase. Moreover, phospholipase A2 (PLA2) activity toward DLPC monolayers and molecular modeling of the GF surface and lipophilic properties were used to get more insight into the mechanisms of GF membrane interactions. The results obtained show that GF has a meaningful impact on the film properties; we propose that nonpolar interactions are by and large responsible for GF retention in the monolayers. The modification of membrane properties can be detected using both physicochemical and enzymatic methods. The results obtained maybe relevant for elaborating GF preparations with increased bioavailability.

Impact of solution chemistry on the properties and bactericidal activity of silver nanoparticles decorated on superabsorbent cryogels
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Journal of Colloid and Interface Science 461 (2016) 104–113

This study investigated the effects of dissolved organic matter (DOM) and various electrolytes commonly found in environmental aqueous matrices on the physicochemical properties and bactericidal efficacy of silver nanoparticles (AgNPs), which are immobilized on cryogels (or PSA/AgNP cryogel). The AgNPs in the PSA/AgNP cryogel that were exposed to different media underwent morphological transformation in terms of particle size and structure. In addition, the presence of DOM and electrolytes increased the release of dissolved Ag. The biological uptake of Ag species (determined as the total Ag in exposed cells) increased in the presence of DOM, but decreased in the presence of electrolytes. The release of electrolytes did not result in any significant reduction in the bactericidal activity. Although an initial increase of the DOM to 2.5 mg-C L\(^{-1}\) attenuated the bactericidal efficacy of the immobilized AgNPs, an increase in the DOM concentration beyond 5 mg-C L\(^{-1}\) enhanced the bactericidal efficacy. This study found that the bactericidal activity of the immobilized AgNPs is less sensitive to the solution chemistry relative to the free AgNPs. This suggests that immobilizing the AgNPs in a supporting material is a good strategy to preserve their efficacy for disinfection in various aqueous matrices.

Insight into membrane selectivity of linear and branched polyethylenimines and their potential as biocides for advanced wound dressings
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Acta Biomaterialia 37 (2016) 155–164

We report here structure-property relationship between linear and branched polyethylene imines by examining their antimicrobial activities against a wide range of pathogens. Both the polymers target the cytoplasmic membrane of bacteria and yeasts, eliciting rapid bactericidal properties. Using multiscale molecular dynamic simulations, we showed that, in both fully or partially protonated forms LPEI discriminates between mammalian and bacterial model membranes whereas BPEI lacks selectivity for both the model membranes. Simulation results suggest that LPEI forms weak complex with the zwitterionic lipids whereas the side chain amino groups of BPEI sequester the zwitterionic lipids by forming tight complex. Consistent with these observations, label-free cell impedance measurements, cell viability assays and high content analysis indicate that BPEI is cytotoxic to human epithelial and fibroblasts cells. Crosslinking of BPEI onto electrospun gelatin mats attenuate the cytotoxicity for fibroblasts while retaining the antimicrobial activity against Gram-positive and yeasts strains. PEI crosslinked gelatin mats elicit bactericidal activity by contact-mediated killing and durable to leaching for 7 days. The potent antimicrobial activity combined with enhanced selectivity of the crosslinked ES gelatin mats would expand the arsenal of biocides in the management of superficial skin infections. The contact-mediated microbial properties may avert antimicrobial resistance and expand the diversity of applications to prevent microbial contamination.

Interaction between a cationic bolaamphiphile and DNA: The routetowards nanovectors for oligonucleotide antimicrobials
Bacterial resistance to antimicrobials is a global threat that requires development of innovative therapeutics that circumvent its onset. The use of Transcription Factor Decoys (TFDs), DNA fragments that act by blocking essential transcription factors in microbes, represents a very promising approach. TFDs require appropriate carriers to protect them from degradation in biological fluids and transfect them through the bacterial cell wall into the cytoplasm, their site of action. Here we report on a bolaform cationic surfactant, [12-bis-THA]Cl2, with proven transfection activity in vivo. By studying the physical-chemical properties of its aqueous solutions with light scattering, cryo-TEM, zeta-potential, absorption and fluorescence spectroscopies, we prove that the bolaamphiphiles associate into transient vesicles which convert into one-dimensional elongated structures over time. These surfactant assemblies complex TFDs with extremely high efficiency, if compared to common cationic amphiphiles. At Z+/Z−= 11, the nanoplexes are stable and have a size of 120 nm, and they form independently of the original morphology of the [12-bis-THA]Cl2 aggregate. DNA is compacted in the nanoplexes, as shown through CD spectroscopy and fluorescence, but is readily released in its native form if sodium taurocholate is added.

Investigational Agents for the Treatment of Gram-Negative Bacterial Infections: A Reality Check
Karen Bush
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ACS Infect. Dis. 2015, 1, 509–511

Antibiotic-resistant Gram-negative bacteria are, arguably, the most difficult organisms to treat, with a limited number of new antibiotics in the development pipeline. Currently 24 new agents in phase 1, phase 2, or phase 3 clinical development were identified for the potential treatment of infections caused by Gram-negative bacteria. Of these agents, most are improved iterations of known antibiotic classes, including new aminoglycosides, β-lactams, β-lactamase inhibitors, quinolones, and tetracyclines with greater potency or a broader spectrum of activity. However, novel structures also appear, with host defense peptide mimetics, boronic acid, and bridged diazabicyclooctane β-lactamase inhibitors and unique bacterial topoisomerase inhibitors. Most of the new agents have received a Qualified Infectious Disease Product (QIDP) designation that may help to accelerate FDA drug approvals. Because resistance will inevitably arise to any antibacterial agent, it will be necessary to continue to identify additional new agents in the future.

In situ synthesis of silver nanoparticles uniformly distributed on polydopamine-coated silk fibers for antibacterial application
Zhisong Lu, Jing Xiao, Ying Wang, Mei Meng
Journal of Colloid and Interface Science 452 (2015) 8-14

Fabrication of silver nanoparticles (AgNPs)-modified silk for antibacterial application is one of the hottest topics in the textile material research. However, the utilization of a polymer as both 3-dimensional matrix and reductant for the in-situ synthesis of AgNPs on silk fibers has not been realized. In this work, a facile, efficient and green approach was developed to in-situ grow AgNPs on the polydopamine (PDA)-functionalized silk. AgNPs with the size of 30–90 nm were uniformly deposited on the silk fiber surface with the PDA coating layer as a reduction reagent. The AgNPs exhibit excellent face-centered cubic crystalline structures. The bacterial growth curve and inhibition zone assays clearly demonstrate the antibacterial properties of the functionalized silk. Both high Ag+ release level and long-time release profile were observed for the as-prepared AgNPs–PDA-coated silk, indicating the high-density loading of AgNPs and the possible long-term antibacterial effects. This work may provide a new method for the preparation
Interactions of oritavancin, a new lipoglycopeptide derived from vancomycin, with phospholipid bilayers: Effect on membrane permeability and nanoscale lipid membrane organization

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Biochimica et Biophysica Acta 1788 (2009) 1832–1840

Antibiotics acting on bacterial membranes are receiving increasing attention because of widespread resistance to agents acting on other targets and of potentially improved bactericidal effects. Oritavancin is an amphiphilic derivative of vancomycin showing fast and extensive killing activities against multi-resistant (including vancomycin insusceptible) Gram-positive organisms with no marked toxicity towards eukaryotic cells. We have undertaken to characterize the interactions of oritavancin with phospholipid bilayers, using liposomes (LUV) and supported bilayers made of cardiolipin (CL) or phosphatidylglycerol (POPG) and phosphatidylethanolamine (POPE), all abundant in Gram-positive organisms. Changes in membrane permeability were followed by the release of calcein entrapped in liposomes at self-quenching concentrations, and changes in nanoscale lipid organization examined by Atomic Force Microscopy (AFM).

Oritavancin caused a fast (b5 min) and complete (N95%) release of calcein from CL:POPE liposomes, and a slower but still substantial (50% in 60 min) release from POPG:POPE liposomes, which was (i) concentration-dependent (0–600 nM; [microbiologically meaningful concentrations]); (ii) enhanced by an increase in POPG:POPE ratio, and decreased when replacing POPG by DPPG. AFM of CL:POPE supported bilayers showed that oritavancin (84 nM) caused a remodeling of the lipid domains combined with a redisposition of the drug and degradation of the borders. In all the above studies, vancomycin was without a significant effect at 5.5 μM. Electrostatic interactions, together with lipid curvature, lipid polymorphism as well of fluidity play a critical role for the permeabilization of lipid bilayer and changes in lipid organization induced by oritavancin.

Layer-by-layer deposition of antimicrobial silver nanoparticles on textile fibres.


Antimicrobial silver nanoparticles were immobilized on nylon and silk fibers by following the layer-by-layer deposition method. The sequential dipping of nylon or silk fibers in dilute solutions of poly(diallyldimethylammonium chloride) (PDADMAC) and silver nanoparticles capped with poly(methacrylic acid) (PMA) led to the formation of a colored thin film possessing antimicrobial properties. The layer-by-layer deposition was monitored by measuring the K/S value, which is the ratio between the sorption coefficient (K) and the scattering (S) of the coated fibers, with a reflectance spectrophotometer. The K/S values for both silk and nylon fibers were found to increase as a function of the number of deposited layers. Although the film growth was observed on both fibers, the K/S value of the nylon fiber was significantly lower than silk fibers. Scanning electron microscopy studies of both fibers confirmed that the layer-by-layer coating on the nylon fibers was not as uniform as on the silk fibers.

Antimicrobial tests against Staphylococcus aureus bacteria were performed and antimicrobial activity was demonstrated for both coated fibers. The deposition of 20 PDADMAC/PMAcapAg layers onto the fibers resulted in 80% bacteria reduction for the silk fiber and 50% for the nylon fiber. Although the film growth was more efficient on the silk fibers, these results suggest that this technique could be used in the design of newsynthetic or natural technical fibers where antimicrobial properties are required.
There are requirements for surfaces with antibacterial properties in various technological fields. UPEO hybrids with antibacterial properties were synthesized by the sol-gel process, incorporating combinations of cerium and silver salts at different silver molar fractions (0, 0.02, 0.05, 0.10, and 1) relative to the total amount of doped cations. The loaded hybrids were characterized by TGA, XRD, and Raman spectroscopy. Release tests were performed using UV–vis spectroscopy, and the antibacterial properties of the hybrids were studied in agar tests and turbidimetry assays. The nanostructural evolution of the hybrids during the release of the antibacterial agents was investigated by in situ SAXS. XRD results showed the presence of the AgCl crystalline phase in the loaded hybrids from a silver molar fraction of 0.05. Raman spectroscopy evidenced the interaction of silver cations with the polymeric part of the hybrid. SAXS results confirmed these interactions and showed that cerium species interacted with both organic and inorganic parts of the hybrids. The loaded U-PEO hybrids were found to release all the incorporated cerium in 1 h, while the hybrid containing 100% of silver released only 78% of the incorporated silver. All the loaded hybrids displayed antibacterial activity against the Pseudomonas aeruginosa bacterium. The antibacterial activity was found to increase with silver molar fraction. Due to its high antibacterial activity and low silver molar fraction, the loaded hybrid with a silver molar fraction of 0.10 seemed to be a good compromise between efficiency, esthetic transparency, and photostability.

Making the hospital a safer place by sonochemical coating of all its textiles with antibacterial nanoparticles
Ilana Perelshtein, Anat Lipovsky, Nina Perkas, Tzanko Tzanov, M. Arguirova, M. Leseva, Aharon Gedanken
Ultrasonics Sonochemistry 25 (2015) 82–88

The ability to scale-up the sonochemical coating of medical textiles with antibacterial nanoparticles is demonstrated in the current paper. A roll-to-roll pilot installation to coat textiles was built taking into consideration the requirements of the sonochemical process. A long-run experiment was conducted in which 2500 m of fabric were coated with antibacterial ZnO nanoparticles (NPs). The metal oxide NPs were deposited from an ethanol:water solution. In this continuous process a uniform concentration of coated NPs over the length/width of the fabric was achieved. The antibacterial efficiency of the sonochemically coated textiles was validated in a hospital environment by a reduction in the occurrence of nosocomial infections. NP-coated bed sheets, patient gowns, pillow cover, and bed covers were used by 21 patients. For comparison 16 patients used regular textiles. The clinical data indicated the reduced occurrence of hospital-acquired infections when using the metal oxide NP-coated textiles. In order to reduce the cost of the coating process and considering safety issues during manufacturing, the solvent (ethanol:water) (9:1 v:v) used for the long-run experiment, was replaced by water. Although lesser amounts of ZnO NPs were deposited on the fabric in the water-based process the antibacterial activity of the textiles was preserved due to the smaller size of the particles.

Mechanistic approaches on the antibacterial activity of poly(acrylic acid) copolimers

The availability of polymeric antimicrobially active surfaces, which are mainly based on cationic surf-face effects, is limited. We have previously reported the discovery that, in addition to cationic surfaces, anionic surfaces based on poly(acrylic acid) (PAA) copolymers have a bactericidal effect. In this study, poly(styrene)-poly(acrylic acid)-diblock copolymers (PS-b-PAA) are used to describe the major variables causing the material to have a bactericidal effect on Escherichia coli ATCC 25922 in aqueous suspensions. Upon contact with water, the surface structure of the copolymer changes, the pH value decreases, and the PAA-block migrates toward the surface. Systematically modified antimicrobial tests show that the presence of acid-form PAA provides maximum antimicrobial activity of the material in slightly acidic conditions, and that an ion-exchange effect is the most probable mechanism. Antimicrobially inactive counter-ions inhibit the bactericidal activity of the copolymers, but the material can be regenerated by treatment with acids.
Methyl-Hydroxylamine as an Efficacious Antibacterial Agent That Targets the Ribonucleotide Reductase Enzyme
Esther Julián, Aida Baelo, Joan Gavaldà, Eduard Torrents

PLoS One 10.3 (Mar 2015). e0122049

The emergence of multidrug-resistant bacteria has encouraged vigorous efforts to develop antimicrobial agents with new mechanisms of action. Ribonucleotide reductase (RNR) is a key enzyme in DNA replication that acts by converting ribonucleotides into the corresponding deoxyribonucleotides, which are the building blocks of DNA replication and repair. RNR has been extensively studied as an ideal target for DNA inhibition, and several drugs that are already available on the market are used for anticancer and antiviral activity. However, the high toxicity of these current drugs to eukaryotic cells does not permit their use as antibacterial agents. Here, we present a radical scavenger compound that inhibited bacterial RNR, and the compound's activity as an antibacterial agent together with its toxicity in eukaryotic cells were evaluated. First, the efficacy of N-methyl-hydroxylamine (M-HA) in inhibiting the growth of different Gram-positive and Gram-negative bacteria was demonstrated, and no effect on eukaryotic cells was observed. M-HA showed remarkable efficacy against Mycobacterium bovis BCG and Pseudomonas aeruginosa. Thus, given the M-HA activity against these two bacteria, our results showed that M-HA has intracellular antimycobacterial activity against BCG-infected macrophages, and it is efficacious in partially disassembling and inhibiting the further formation of P. Aeruginosa biofilms. Furthermore, M-HA and ciprofloxacin showed a synergistic effect that caused a massive reduction in a P. aeruginosa biofilm. Overall, our results suggest the vast potential of M-HA as an antibacterial agent, which acts by specifically targeting a bacterial RNR enzyme.

Microbial surfactants: Fundamentals and applicability in the formulation of nano-sized drug delivery vectors
Ligia R. Rodrigues


Microbial surfactants, so-called biosurfactants, comprise a wide variety of structurally distinct amphipathic molecules produced by several microorganisms. Besides exhibiting surface activity at the interfaces, these molecules present powerful characteristics including high biodegradability, low toxicity and special biological activities (e.g. antimicrobial, antiviral, anticancer, among others), that make them an alternative to their chemical counterparts. Several medical-related applications have been suggested for these molecules, including some reports on their potential use in the formulation of nano-sized drug delivery vectors. However, despite their promises, due to the generalized lack of knowledge on microbial surfactants phase behavior and stability under diverse physicochemical conditions, these applications remain largely unexplored, thus representing an exciting field of research. These nano-sized vectors are a powerful approach towards the current medical challenges regarding the development of efficient and targeted treatments for several diseases. In this review, a special emphasis will be given to nanoparticles and microemulsions. Nanoparticles are very auspicious as their size, shape and stability can be manipulated by changing the environmental conditions. On the other hand, the easiness of formulation, as well as the broad possibilities of administration justifies the recent popularity of the microemulsions. Notwithstanding, both vector types still require further developments to overcome some critical limitations related with toxicity and costs, among others. Such developments may include the search for other system components, as the microbial surfactants, that can display improved features.

Nanostructured multilayer polyelectrolyte films with silver nanoparticles as antibacterial Coatings
Tomasz Kruk, Krzysztof Szczepanowicz, Dorota Kręgiel, L. Szyk-Warszyńska, Piotr Warszyński

Colloids and Surfaces B: Biointerfaces 137 (2016) 158–166

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Ultrathin polyelectrolyte films containing silver nanoparticles appear to be a promising material for antimicrobial coatings used in the medical area. The present work is focused on the formation of multi-layer polyelectrolyte films using: polyethyleneimine (PEI) as polycation, Poly(sodium 4-styrenesulfonate)(PSS) as polyanions and negatively charged silver nanoparticles (AgNPs), which led to the polyelectrolyte-silver nanocomposite coatings. The film thickness and mass were measured by ellipsometry and quartz crystal microbalance with dissipation monitoring (QCM-D) and the structure and morphology of films were visualized using scanning electron microscopy (SEM). Systematic increase of the UV–Vis absorption confirmed formation of the consecutive layers of the film. The analysis of bacteria cell adhesion to film surface was done by the luminometry measurement. Three gram-negative bacterial strains with strong adhesive properties were used in this study: Escherichia coli, Aeromonas hydrophila, and Asaia lannenesis. It was found that nanocomposite films have antimicrobial properties, which makes them very interesting for a number of practical applications, e.g. for the prevention of microbial colonization on treated surfaces.

New cationic vesicles prepared with double chain surfactants from arginine: Role of the hydrophobic group on the antimicrobial activity and cytotoxicity
A. Pinazo, V. Petrizelli, M. Bustelo, R. Pons, M.P. Vinardell, M. Mitjans, A. Manresa, L. Perez
Colloids and Surfaces B: Biointerfaces 141 (2016) 19–27
Cationic double chain surfactants have attracted much interest because they can give rise to cationic vesicles that can be used in biomedical applications. Using a simple and economical synthetic approach, we have synthesized four double-chain surfactants with different alkyl chain lengths (LANHC_x). The critical aggregation concentration of the double chain surfactants is at least one order of magnitude lower than the CMC of their corresponding single-chain LAM and the solutions prepared with the LANHC_x contain stable cationic vesicles. Encouragingly, these new arginine derivatives show very low haemolytic activity and weaker cytotoxic effects than conventional dialkyl dimethyl ammonium surfactants. In addition, the surfactant with the shortest alkyl chain exhibits good antimicrobial activity against Gram-positive bacteria. The results show that a rational design applied to cationic double chain surfactants might serve as a promising strategy for the development of safe cationic vesicular systems.

New frontiers for anti-biofilm drug development
Suzana M. Ribeiro, Mário R. Felício, Esther Vilas Boas, Sónia Gonçalves, Fabrício F. Costa, Ramar Perumal Samy, Nuno C. Santos, Octávio L. Franco
Pharmacology & Therapeutics 160 (2016) 133–144
Pathogenic microbial biofilm, a consortium of microbial cells protected by a self-produced polymer matrix, is considered a worldwide challenge due to the inherent antibiotic resistance conferred by its lifestyle. Living, as it does, in a community of microbial organisms in a clinical situation, makes it responsible for severe and dangerous cases of infection. Combating this organisation of cells usually requires high antibiotic doses for a prolonged time, and these approaches often fail, contributing to infection persistence. In addition to therapeutic limitations, biofilms can be a source of infections when they grow in medical devices. The challenge imposed by biofilms has mobilised researchers in the entire world to prospect or develop alternatives to control biofilms. In this context, this review summarises the new frontiers that could be used in clinical circumstances in order to prevent or eliminate pathogenic biofilms.

PEGylated ofloxacin nanoparticles render strong antibacterial activity against many clinically important human pathogens
Gregory Marslin, Ann Mary Revina, Vinoth Kumar Megraj Khandelwal, Krishnamoorthy Balakumar, Caroline J. Sheeba, Gregory Franklin
The rise of bacterial resistance against important drugs threatens their clinical utility. Fluoroquinones, one of the most important classes of contemporary antibiotics has also reported to suffer bacterial resistance. Since the general mechanism of bacterial resistance against fluoroquinone antibiotics (e.g. ofloxacin) consists of target mutations resulting in reduced membrane permeability and increased efflux by the bacteria, strategies that could increase bacterial uptake and reduce efflux of the drug would provide effective treatment. In the present study, we have compared the efficiencies of ofloxacin delivered in the form of free drug (OFX) and as nanoparticles on bacterial uptake and antibacterial activity. Although both poly(lactic-co-glycolic acid) (OFX-PLGA) and methoxy poly(ethylene glycol)-b-poly(lactic-co-glycolic acid) (OFX-mPEG-PLGA) nanoformulations presented improved bacterial uptake and antibacterial activity against all the tested human bacterial pathogens, namely, Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae and Staphylococcus aureus, OFX-mPEG-PLGA showed significantly higher bacterial uptake and antibacterial activity compared to OFX-PLGA. We have also found that mPEG-PLGA nanoencapsulation could significantly inhibit Bacillus subtilis resistance development against OFX.

Perturbation of cellular mechanistic system by silver nanoparticle toxicity: Cytotoxic, genotoxic and epigenetic potentials. Historical perspective

Poornima Dubey, Ishita Maiti, S. Uday Kumar, Abhay Sachdev, Bharat Bhushan, P. Gopinath

Advances in Colloid and Interface Science 221 (2015) 4–21

Currently the applications of silver nanoparticles (Ag NPs) are gaining overwhelming response due to the advancement of nanotechnology. However, only limited information is available with regard to their toxicity mechanism in different species. It is very essential to understand the complete molecular mechanism to explore the functional and long term applications of Ag NPs. Ag NPs could be toxic at cellular, subcellular, biomolecular, and epigenetic levels. Toxicity effects induced by Ag NPs have been evaluated using numerous in vitro and in vivo models, but still there are contradictions in interpretations due to disparity in methodology, test endpoints and several other model parameters which needs to be considered. Thus, this review article focuses on the progressive elucidation of molecular mechanism of toxicity induced by Ag NPs in various in vitro and in vivo models. Apart from these, this review also highlights the various ignored factors which are to be considered during toxicity studies.

Polymer/nanosilver composite coatings for antibacterial applications

Liya Guo, Weiyong Yuan, Zhisong Lu, Chang Ming Li


Nanosilver is regarded as a new generation of antibacterial agents and has great potential to be utilized in antibacterial surface coatings for medical devices, food package and industrial pipes. However, disadvantages such as easy aggregation, uncontrollable release of silver ions and potential cytotoxicity greatly hinder its uses. Recently, polymers possessing unique functions have been employed to fabricate nanocomposite coatings with nanosilver for better biocompatibility and enhanced antibacterial activity. This review starts with progress on antibacterial mechanism and cytotoxic effects of nanosilver. Antibacterial functions of polymers are subsequently discussed. Advances of fabrication of polymer/nanosilver composite coatings for antibacterial applications are surveyed. Finally, conclusions and perspectives, in particular future directions of polymer/nanosilver composite coatings for antibacterial applications are proposed. It is expected that this review is able to provide the updated accomplishments of the polymer/nanosilver composite coatings for antibacterial applications while attracting great interest of research and development in this area.
Preparation and characterization of novel b-chitin/nanosilver composite scaffolds for wound dressing applications
P.T. Sudheesh Kumar, S. Abhilash, K. Manzoor, S.V. Nair, H. Tamura, R. Jayakumar
Carbohydrate Polymers 80 (2010) 761–767

We developed novel b-chitin/nanosilver composite scaffolds for wound healing applications using b-chitin hydrogel with silver nanoparticles. The prepared nanosilver particles and nanocomposite scaffolds were characterized using SEM, FT-IR, XRD and TGA studies. The antibacterial, blood-clotting, swelling, cell attachment and cytotoxicity studies of the prepared composite scaffolds were evaluated. The prepared b-chitin/nanosilver composite scaffolds were bactericidal against Escherichia coli and Staphylococcus aureus and it showed good blood-clotting ability as well. Cell attachment studies using vero (epithelial) cells showed that the cells were well attached on the scaffolds. These results suggested that b-chitin/nanosilver composite scaffold could be a promising candidate for wound dressing applications.

Polysaccharide-based antibiofilm surfaces
Guy-Alain Junter, Pascal Thébault, Laurent Lebrun
DOI: http://dx.doi.org/10.1016/j.actbio.2015.11.010

ABSTRACT
Surface treatment by natural or modified polysaccharide polymers is a promising means to fight against implant-associated biofilm infections. The present review focuses on polysaccharide-based coatings that have been proposed over the last ten years to impede biofilm formation on material surfaces exposed to bacterial contamination. Anti-adhesive and bactericidal coatings are considered. Besides classical hydrophilic coatings based on hyaluronic acid and heparin, the promising anti-adhesive properties of the algal polysaccharide ulvan are underlined. Surface functionalization by antimicrobial chitosan and derivatives is extensively surveyed, in particular chitosan association with other polysaccharides in layer-by-layer assemblies to form both anti-adhesive and bactericidal coatings.

Probing the potential of apigenin liposomes in enhancing bacterial membrane perturbation and integrity loss
Kacoli Banerjee, Shubhadeep Banerjee, Subhayan Das, Mahitoshi Mandal
http://dx.doi.org/10.1016/j.jcis.2015.04.030

abstract
Along with discovery of new antibacterial agents, it is important to develop novel drug delivery systems to effectively deliver drugs within bacterial cells for enhanced therapeutic activity. Liposomes have been extensively investigated as pharmaceutical carriers for improvement of therapeutic index of antimicrobial agents. The aim of this present study was to evaluate the antibacterial activity of free and liposomal formulation of apigenin, a plant based isoflavone and elucidate the mode of action. Distearoylphosphatidylcholine liposomes were prepared having nano-range particle size (104.3 ± 1.8 nm), narrow particle Distribution (0.204) and high encapsulation efficiency of apigenin (89.9 ± 2.31%). Antibacterial activity of apigenin and efficacy of liposome-mediated apigenin delivery were determined from minimum inhibitory concentration values. Interaction studies using electron microscopy revealed adherence and fusion of liposomal apigenin with the bacteria causing membrane perturbation through reactive oxygen species generation which was evaluated by epi-fluorescence microscopy and fluorescence activated cell sorting. The interaction of apigenin liposomes with bacterial membrane increased intracellular drug concentration and thus, can be employed to deliver apigenin within cells to augment its antibacterial activity. Increased efficacy and hemocompatibility of this formulation paves way for future evaluation of underlying molecular mechanisms and in vivo testing for enhanced therapeutic effects.
Quorum Quenching and Matrix Degrading Enzymes in Multilayer Coatings Synergistically Prevent Bacterial Biofilm Formation on Urinary Catheters

Kristina Ivanova, Margarida M. Fernandes, Antonio Francesko, Ernest Mendoza, Jamil Guezguez, Michael Burnet, Tzanko Tzanov

ACS Appl Mater Interfaces 7 (2015) 27066-27077

ABSTRACT

Bacteria often colonize indwelling medical devices and grow as complex biofilm communities of cells embedded in a self-produced extracellular polymeric matrix, which increases their resistance to antibiotics and the host immune system. During the biofilm growth bacterial cells cooperate through specific quorum sensing (QS) signals. Taking advantage on this mechanism of biofilm formation, we hypothesized that interrupting the communication among bacteria and simultaneously degrading the extracellular matrix would inhibit the biofilm growth. To this end, coatings comprising the enzymes acylase and α-amylase, able to degrade bacterial QS molecules and polysaccharides respectively, were built on silicone urinary catheters using a layer-by-layer deposition technique. Multilayer coatings from either acylase or amylase alone suppressed the biofilm formation of correspondingly Gram-negative P. aeruginosa and Gram-positive S. aureus. Further assembling of both enzymes in hybrid nanocoatings resulted in a stronger biofilm inhibition as a function of acylase or amylase position in the layers. Hybrid coatings, with the QS signal degrading acylase as outermost layer, demonstrated 30% higher antibiofilm efficiency against medically relevant Gram-negative bacteria compared to the other assemblies. These nanocoatings significantly reduced the occurrence of single (P. aeruginosa) and mixed (P. aeruginosa and E. coli) species biofilms on silicone catheters at both static and dynamic conditions. Moreover, in an in vivo animal model, the quorum quenching and matrix degrading enzyme assemblies delayed the biofilm growth up to 7 days.

Rapid biological synthesis of silver nanoparticles and their enhanced antibacterial effects against Escherichia fergusonii and Streptococcus mutans

Sangiliyandi Gurunathan


Abstract Emergence of antibiotic resistance has become an increasingly important public health issue. Although several new antibiotics have been developed in the last few decades, none of them show improved activity against multidrug-resistant bacteria. Silver nanoparticles (AgNPs) have long been known for their broad-spectrum antibacterial effects. The development of a rapid, dependable, simple, cost-effective, biocompatible, and environmentally friendly method to synthesize nanoparticles is an essential aspect of current biomedical research. This paper describes the extracellular biochemical synthesis of AgNPs using supernatants from Bacillus cereus cultures and characterization of the synthesized AgNPs, using several analytical techniques. The nanoparticles showed a maximum absorbance at 420 nm in ultraviolet-visible spectra. Particle size analysis by dynamic light scattering and transmission electron microscopy revealed the formation of homogeneous and well-dispersed nanoparticles with an average size of 10 nm. We investigated the dosedependent antibacterial activity of AgNPs against Escherichia fergusonii and Streptococcus mutans. In addition, the efficiency of AgNPs with various broad-spectrum antibiotics against these test strains was evaluated. The results show that the combination of antibiotics with AgNPs has significant
antimicrobial effects. The greatest enhancement was observed with gentamycin and vancomycin against E. fergusonii and S. mutans, respectively. This work supports that AgNPs can be used to enhance the activity of existing antibiotics against Gram-negative and Gram-positive bacteria.

**Responsive and “smart” antibacterial surfaces: Common approaches and new developments (Review)**
Alex Cavallaro, Shima Taheri, and Krasimir Vasilev
Citation: Biointerphases 9, 029005 (2014); doi: 10.1116/1.4866697

Bacterial infections are continuing to pose a significant threat to human health. Coatings with inherent antibacterial properties are becoming increasingly common as an infection preventative measure. The aim of this review is to highlight recent progress in development of “smart” and responsive antibacterial surfaces. The review describes various strategies utilized for generation of such surfaces and the specific stimuli that are used to trigger antibacterial action. It also provides a critical discussion of the advantages and drawbacks of different approaches. The review concludes with a perspective about the future of the field and outlines the challenges and obstacles that need to be overcome in order to make future advances.

The approaches are: bioresponsive surfaces, temperature responsive surfaces, photoactive surfaces, bioelectric surfaces, pH responsive surfaces

**Review of antimicrobial food packaging**
Paola Appendini, Joseph H. Hotchkiss
Innovative Food Science & Emerging Technologies 3 2002, 113_126

Research and development of antimicrobial materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymer materials and antimicrobials. This article reviews the different types of antimicrobial polymers developed for food contact, commercial applications, testing methods, regulations and future trends. Special emphasis will be on the advantages_disadvantages of each technology. _2002 Elsevier Science Ltd. All rights reserved.

**Ribonucleotide Reductase NrdR as a Novel Regulator for Motility and Chemotaxis during Adherent-Invasive Escherichia coli Infection**
Nicolas Dreux, Maria del Mar Cendra, Sébastien Massier, Arlette Darfeuille-Michaud, Nicolas Barnich, Eduard Torrents
April 2015 Volume 83 Number 4 Infection and Immunity iai.asm.org 1305

A critical step in the life cycle of all organisms is the duplication of the genetic material during cell division. Ribonucleotide reductases (RNRs) are essential enzymes for this step because they control the de novo production of the deoxyribonucleotides required for DNA synthesis and repair. Enterobacteriaceae have three functional classes of RNRs (Ia, Ib, and III), which are transcribed from separate operons and encoded by the genes nrdAB, nrdHIEF, and nrdDG, respectively. Here, we investigated the role of RNRs in the virulence of adherent-invasive Escherichia coli (AIEC) isolated from Crohn’s disease (CD) patients. Interestingly, the LF82 strain of AIEC harbors four different RNRs (two class Ia, one class Ib, and one class III). Although the E. coli RNR enzymes have been extensively characterized both biochemically and enzymatically, little is known about their roles during bacterial infection. We found that RNR expression was modified in AIEC LF82 bacteria during cell infection, suggesting that RNRs play an important role in AIEC virulence. Knockout of the nrdR and nrdD genes, which encode a transcriptional regulator of RNRs and class III anaerobic RNR, respectively, decreased
AIEC LF82’s ability to colonize the gut mucosa of transgenic mice that express human CEACAM6 (carcinoembryonic antigen-related cell adhesion molecule 6). Microarray experiments demonstrated that NrdR plays an indirect role in AIEC virulence by interfering with bacterial motility and chemotaxis. Thus, the development of drugs targeting RNR classes, in particular NrdR and NrdD, could be a promising new strategy to control gut colonization by AIEC bacteria in CD patients.

Silver–perfluorodecanethiolate complexes having superhydrophobic, antifouling, antibacterial properties

Jae-Seung Chung, Byoung Gak Kim, Soojin Shim, Seong-Eun Kim, Eun-Ho Sohn, Jeyong Yoon, Jong-Chan Lee

Journal of Colloid and Interface Science 366 (2012) 64–69

Silver-perfluorodecanethiolate complexes having superhydrophobic, antifouling, antibacterial properties were prepared by a reaction of silver nitrate with perfluorodecanethiol. When the silver nitrate to perfluorodecanethiol molar ratio was 1/2, silver-perfluorodecanethiolate complexes having hierarchical micro-/nano-sized wire shapes were obtained, and they showed superhydrophobic and antifouling properties. After UV irradiation, silver nanoparticles were generated on the wires and exhibited antibacterial properties.

Silver nanoparticles: Green synthesis and their antimicrobial activities

Virender K. Sharma, Ria A. Yngard, Yekaterina Lin

Advances in Colloid and Interface Science 145 (2009) 83–96

This review presents an overview of silver nanoparticles (Ag NPs) preparation by green synthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity. Green synthetic methods include mixed-valence polyoxometallates, polysaccharide, Tollens, irradiation, and biological. The mixed-valence polyoxometallates method was carried out in water, an environmentally-friendly solvent. Solutions of AgNO3 containing glucose and starch in water gave starch-protected Ag NPs, which could be integrated into medical applications. Tollens process involves the reduction of Ag(NH3)2+ by saccharides forming Ag NP films with particle sizes from 50–200 nm, Ag hydrogels with particles in the order of 20–50 nm, and Ag colloid particles of different shapes. The reduction of Ag(NH3)2+ by HTAB (n-hexadecyltrimethylammonium bromide) gave Ag NPs of different morphologies: cubes, triangles, wires, and aligned wires. Ag NPs synthesis by irradiation of Ag+ ions does not involve a reducing agent and is an appealing procedure. Eco-friendly bio-organisms in plant extracts contain proteins, which act as both reducing and capping agents forming stable and shape-controlled Ag NPs. The synthetic procedures of polymer-Ag and TiO2–Ag NPs are also given. Both Ag NPs and Ag NPs modified by surfactants or polymers showed high antimicrobial activity against Gram-positive and Gram-negative bacteria. The mechanism of the Ag NP bactericidal activity is discussed in terms of Ag NP interaction with the cell membranes of bacteria. Silver-containing filters are shown to have antibacterial properties in water and air purification. Finally, human and environmental implications of Ag NPs to the ecology of aquatic environment are briefly discussed.

Size and Aging Effects on Antimicrobial Efficiency of Silver Nanoparticles Coated on Polyamide Fabrics Activated by Atmospheric DBD Plasma

Andrea Zille, Margarida M. Fernandes, Antonio Francesko, Tzanko Tzanov, Marta Fernandes, Fernando R. Oliveira, Luis Almeida, Teresa Amorim, Noémia Carneiro, Maria F. Esteves, and António P. Souto

DOI: 10.1021/acsami.5b04340
ACS Appl. Mater. Interfaces, 2015, 7 (25), pp 13731–13744

ABSTRACT: This work studies the surface characteristics, antimicrobial activity, and aging effect of plasma-pretreated polyamide 6,6 (PA66) fabrics coated with silver nanoparticles (AgNPs), aiming to identify the optimum size of nanosilver exhibiting antibacterial properties suitable for the manufacture of hospital textiles. The release of bactericidal Ag+ ions from a 10, 20, 40, 60, and 100 nm AgNPs-coated PA66 surface was a function of the particles’ size, number, and aging. Plasma pretreatment promoted both ionic and covalent
interactions between AgNPs and the formed oxygen species on the fibers, favoring the deposition of smaller-diameter AgNPs that consequently showed better immediate and durable antimicrobial effects against Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus bacteria. Surprisingly, after 30 days of aging, a comparable bacterial growth inhibition was achieved for all of the fibers treated with AgNPs <100 nm in size. The Ag⁺ in the coatings also favored the electrostatic stabilization of the plasma-induced functional groups on the PA66 surface, thereby retarding the aging process. At the same time, the size-related ratio (Ag+/Ag0) of the AgNPs between 40 and 60 nm allowed for the controlled release of Ag⁺ rather than bulk silver. Overall, the results suggest that instead of reducing the size of the AgNPs, which is associated with higher toxicity, similar long-term effects can be achieved with larger NPs (40−60 nm), even in lower concentrations. Because the antimicrobial efficiency of AgNPs larger than 30 nm is mainly ruled by the release of Ag⁺ over time and not by the size and number of the AgNPs, this parameter is crucial for the development of efficient antimicrobial coatings on plasma-treated surfaces and contributes to the safety and durability of clothing used in clinical settings.

Sonochemically Processed Cationic Nanocapsules: Efficient Antimicrobials with Membrane Disturbing Capacity
Margarida M. Fernandes, Antonio Francesko, Juan Torrent-Burgues, F. Javier Carrión-Fité, Thomas Heinze, and Tzanko Tzanov,
dx.doi.org/10.1021/bm4018947 | Biomacromolecules 2014, 15, 1365−1374

ABSTRACT: Bacterial-mediated diseases are a major healthcare concern worldwide due to the rapid spread of antibiotic-resistant bacteria. One strategy to manage the bacterial infections while avoiding the emergence of resistant strains implies specific targeting and disruption of bacteria membranes. This work evaluates the potential of nanostructured biopolymer derivatives, nanocapsules (NCs), to disrupt the bacteria cell walls and effectively kill planktonic microorganisms. Two biopolymers, chitosan and cellulose, were chemically modified to synthesize derivatives with improved cationic character (thiolated chitosan and aminocellulose) prior to their processing into nanocapsules via a one-step sonochemical process. The interactions of NCs, displaying an average size of around 250 nm, with bacteria membrane were evaluated using two membrane models: Langmuir monolayers and liposome bilayers composed of a L-α-phosphatidylglycerol phospholipid extracted from Escherichia coli. NCs possessed improved membrane disturbing capacity in comparison to the nonprocessed biopolymer derivatives, by drastically increasing the monolayer fluidity and inducing more than 50% leakage of a dye inserted in the bilayered liposomes. In addition, membrane disturbance was directly proportional to the NCs cationic charge. Whereas evidence showed that thiolated chitosan and aminocellulose interacted with the bacteria membrane through a “carpet model”, the NCs were found to induce larger surface defects and high local perturbation through a “detergent model”. Importantly, the degree of disruption caused by the biopolymer derivatives and NCs correlated well with the antimicrobial capacity against Escherichia coli, selectively killing bacteria cells without imparting toxicity to human fibroblasts.

Strategies for Silencing Bacterial Communication
Kristina Ivanova, Margarida M. Fernandes, and Tzanko Tzanov


Chapter book on quorum sensing (QS), quorum quenching (QQ).

Surface properties of Vancomycin after interaction with laser beams
This study presents results about UV laser beam interaction with Vancomycin (VCM) solutions in ultra-pure water performed on bulk samples (5 mL). Photoproducts and molecular fragments resulting from the parent VCM and generated by exposure to 266 nm are evidenced by UV–vis absorption and FTIR spectroscopy and liquid chromatography electro spray ionization time-of-flight mass spectrometry (LC/ESI-TOF-MS) measurements. A novel method is reported to characterize surface active compounds produced in VCM solutions during exposure to UV laser radiation, by measuring in real time the dynamic interfacial tension of the irradiated solutions in emerging, constant volume bubble configuration. This shows that amphiphilic photoproducts are generated after the interaction of VCM molecules with laser beam that migrate at the interface air bubble/VCM solution. They accumulate at the interface leading to transition surface effects. In the paper four such amphiphilic photoproducts were identified and their chemical structures were proposed.

Purpose/Aim: Bacterial infections of the ocular surface are commonly treated empirically with broad spectrum antibiotics. Due to concerns over increasing antibiotic resistance, we evaluated current susceptibility patterns of the ocular bacterial pathogens in Europe.

Materials and methods: Non-consecutive ocular isolates of Staphylococcus aureus, coagulase-negative staphylococci (CoNS), Streptococcus pneumoniae, Haemophilus influenzae, and Pseudomonas aeruginosa were collected in 2011 from centers in France, Germany, Italy, Poland, Slovak Republic, Spain, and the United Kingdom. Centers were asked to provide similar numbers of methicillin-susceptible and -resistant staphylococcal isolates. Minimum inhibitory concentrations were determined for fluoroquinolones (besifloxacin, ciprofloxacin, moxifloxacin), aminoglycosides (tobramycin, gentamicin, netilmicin), oxacillin, chloramphenicol and erythromycin. Isolates were categorized as susceptible, intermediate, or resistant according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

Results: A total of 741 ocular isolates were obtained. Antibiotic resistance rates depended not only on the antibiotic and species, but also varied greatly by the country of origin. Resistance to ciprofloxacin, tobramycin, erythromycin, and to a lesser extent, chloramphenicol, was a concern for all staphylococci. Multidrug resistance was common among methicillin-resistant S. aureus (MRSA) and MRCoNS and isolates of S. pneumoniae, H. influenzae, and P. aeruginosa were frequently non-susceptible to erythromycin, beta-lactams, and ciprofloxacin/tobramycin, respectively. Resistance rates showed substantial differences among the seven countries tested. Fluoroquinolones and aminoglycosides showed differences in antibacterial potency and resilience toward the antibiotic resistance mechanisms.

Conclusions: Methicillin-resistant staphylococcal isolates were frequently non-susceptible to a multitude of other antibiotics, making MRSA and MRCoNS a potentially significant concern. The broad range of resistance rates observed across Europe in this study confirms the importance of considering current local resistance patterns when antibacterial agents are chosen for empiric management of ocular infections.

Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium


Carbohydrate Polymers 89 (2012) 1159-1165

A simple one-step rapid synthetic route is described for the preparation of silver nanoparticles by reduction
of silver nitrate (AgNO₃) using aqueous dextran solution which acts as both reducing and capping agent. The formation of silver nanoparticles is assured by characterization with UV–vis spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM) and X-ray diffraction (XRD). The absorbance of the silver nanoparticles is observed at 423 nm. The AFM image clearly shows the Surface morphology of the well-dispersed silver nanoparticles with size range of 10–60 nm. TEM images show that the nanoparticles are spherical in shape with ~5–10 nm dimensions. The crystallinity of Ag nanoparticles is assured by XRD analysis. The antimicrobial activity of as synthesized silver nanoparticles is tested against the bacteria, Bacillus subtilis, Bacillus cereus, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The bacterial growth is inhibited by gradual reduction of the concentration of the silver nanoparticles.

Silver nanoparticles: Green synthesis and their antimicrobial activities
Virender K. Sharma, Ria A. Yngard, Yekaterina Lin

Advances in Colloid and Interface Science 145 (2009) 83–96

This review presents an overview of silver nanoparticles (Ag NPs) preparation by green synthesis approaches that have advantages over conventional methods involving chemical agents associated with environmental toxicity. Green synthetic methods include mixed-valence polyoxometallates, polysaccharide, Tollens, irradiation, and biological. The mixed-valence polyoxometallates method was carried out in water, an environmentally-friendly solvent. Solutions of AgNO₃ containing glucose and starch in water gave starchprotected Ag NPs, which could be integrated into medical applications. Tollens process involves the reduction of Ag(NH₃)₂⁺ by saccharides forming Ag NP films with particle sizes from 50–200 nm. Ag hydrosols with particles in the order of 20–50 nm, and Ag colloid particles of different shapes. The reduction of Ag(NH₃)₂⁺ by HTAB (n-hexadecyltrimethylammonium bromide) gave Ag NPs of different morphologies: cubes, triangles, wires, and aligned wires. Ag NPs synthesis by irradiation of Ag⁺ ions does not involve a reducing agent and is an appealing procedure. Eco-friendly bio-organisms in plant extracts contain proteins, which act as both reducing and capping agents forming stable and shape-controlled Ag NPs. The synthetic procedures of polymer-Ag and TiO₂–Ag NPs are also given. Both Ag NPs and Ag NPs modified by surfactants or polymers showed high antimicrobial activity against Gram-positive and Gram-negative bacteria. The mechanism of the Ag NP bactericidal activity is discussed in terms of Ag NP interaction with the cell membranes of bacteria. Silver-containing filters are shown to have antibacterial properties in water and air purification. Finally, human and environmental implications of Ag NPs to the ecology of aquatic environment are briefly discussed.

Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria
Ivan Sondi and Branka Salopek-Sondi


The antimicrobial activity of silver nanoparticles against E. coli was investigated as a model for Gram-negative bacteria. Bacteriological tests were performed in Luria–Bertani (LB) medium on solid agar plates and in liquid systems supplemented with different concentrations of nanosized silver particles. These particles were shown to be an effective bactericide. Scanning and transmission electron microscopy (SEM and TEM) were used to study the biocidal action of this nanoscale material. The results confirmed that the treated E. coli cells were damaged, showing formation of “pits” in the cell wall of the bacteria, while the silver nanoparticles were found to accumulate in the bacterial membrane. A membrane with such a morphology exhibits a significant increase in permeability, resulting in death of the cell. These nontoxic nanomaterials, which can be prepared in a simple and cost-effective manner, may be suitable for the formulation of new types of bactericidal materials.
Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens

In the present study, biosynthesis of silver nanoparticles and its activity on water borne bacterial pathogens were investigated. Silver nanoparticles were rapidly synthesized using leaf extract of Acalypha indica and the formation of nanoparticles was observed within 30 min. The results recorded from UV–vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles. From high-resolution transmission electron microscopy (HRTEM) analysis, the size of the silver nanoparticles was measured 20–30 nm. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens Viz., Escherichia coli and Vibrio cholerae. Silver nanoparticles 10 g/ml were recorded as the minimal inhibitory concentration (MIC) against E. coli and V. cholerae. Alteration in membrane permeability and respiration of the silver nanoparticle treated bacterial cells were evident from the activity of silver nanoparticles.

Synthesis and characterization of zinc/iron oxide composite nanoparticles and their antibacterial properties
Tamar Gordon, Benny Perlstein, Ofir Houbara, Israel Felner, Ehud Banin, Shlomo Margel

Inorganic metal oxides may serve as effective disinfectants, due to their relatively non-toxic profile, chemical stability and efficient antibacterial activity. Among metal oxide nanoparticles, zinc oxide demonstrates significant bacterial growth inhibition on a broad spectrum of bacteria, mainly by catalysis of reactive oxygen species (ROS) formation from water and oxygen. Aqueous suspensions of ZnO nanoparticles (ZnO nanofluids) are the preferred formulation for using the antibacterial agent in liquid phases and for the incorporation of the nanoparticles in different commercial products. However, ZnO nanoparticles in aqueous media tend to aggregate into large flocculates, due to their hydrophobic nature, and thus do not interact with microorganisms effectively. In this study, zinc oxide was combined with iron oxide to produce magnetic composite nanoparticles with improved colloidal aqueous stability, together with adequate antibacterial activity. For this purpose, the Zn/Fe oxide composite nanoparticles were synthesized by basic hydrolysis of Fe2+ and Zn2+ ions in aqueous continuous phase containing gelatin. The obtained composite nanoparticles were composed of iron oxide, zinc oxide and zinc ferrite phases. The effect of the weight ratio [Zn]/[Fe] of the composite nanoparticles on their properties (composition, size, magnetic behavior and colloidal stability) was elucidated. The antibacterial activity of these nanoparticles was tested against Staphylococcus aureus and Escherichia coli and was found to be dependent on the weight ratio [Zn]/[Fe], i.e., the higher the ratio, the higher the antibacterial activity. In addition, the activity against Staphylococcus aureus was significantly higher than that observed against Escherichia coli.

Sonochemical co-deposition of antibacterial nanoparticles and dyes on textiles
Ilana Perelshtein, Anat Lipovsky, Nina Perkas, Tzanko Tzanov and Aharon Gedanken
The sonochemical technique has already been proven as one of the best coating methods for stable functionalization of substrates over a wide range of applications. Here, we report for the first time on the simultaneous sonochemical dyeing and coating of textiles with antibacterial metal oxide (MO) nanoparticles. In this one-step process the antibacterial nanoparticles are synthesized in situ and deposited together with dye nanoparticles on the fabric surface. It was shown that the antibacterial behavior of the metal oxides was not influenced by the presence of the dyes. Higher K/S values were achieved by sonochemical deposition of the dyes in comparison to a dip-coating (exhaustion) process. The stability of the antibacterial properties and the dye fastness was studied for 72 h in saline solution aiming at medical applications.

Self-Defensive Layer-by-Layer Films with Bacteria-Triggered Antibiotic Release

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ABSTRACT We report on highly efficient, bioresponsive, controlled-release antibacterial coatings constructed by direct assembly of tannic acid (TA) with one of several cationic antibiotics (tobromycin, gentamicin, and polymyxin B) using the layer-by-layer (LbL) technique. These films exhibit a distinct "self-defense" behavior triggered by acidification of the immediate environment by pathogenic bacteria, such as Staphylococcus epidermidis (S. epidermidis) or Escherichia coli (E. coli). Films assembled using spin-assisted and dip-assisted techniques show drastically different morphology, thickness and pH-/bacteria-triggered antibiotic release characteristics. While dip-deposited films have rough surfaces with island-like, granular structures regardless of the film thickness, spin-assisted LbL assemblies demonstrate a transition from linear deposition of uniform 2D films to a highly developed 3D morphology for films thicker than ~45 nm.

Ellipsometry, UV-Vis and mass spectrometry confirm that all coatings do not release antibiotics in phosphate buffered saline at pH 7.4 for as long as onemonth in the absence of bacteria and therefore do not contribute to the development of antibiotic resistance. These films do, however, release antibiotics upon pH lowering. The rate of triggered release can be controlled through the choice of assembled antibiotic and the assembly technique (spin- vs dip-deposition) and by the spinning rate used during deposition, which all affect the strength of TA_antibiotic binding. TA/antibiotic coatings as thin as 40 nm strongly inhibit S. epidermidis and E. coli bacterial growth both at surfaces and in surrounding medium, but support adhesion and proliferation of murine osteoblast cells. These coatings thus present a promising way to incorporate antibacterial agents at surfaces to prevent bacterial colonization of implanted biomedical devices.

Synthesis and characterization of anti-bacterial and anti-fungal citrate-based musselinspired bioadhesives

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Bacterial and fungal infections in the use of surgical devices and medical implants remain a major concern. Traditional bioadhesives fail to incorporate anti-microbial properties, necessitating additional anti-microbial drug injection. Herein, by the introduction of the clinically used and inexpensive anti-fungal agent, 10-undecylenic acid (UA), into our recently developed injectable citrate-based mussel-inspired bioadhesives (iCMBAs), a new family of anti-bacterial and anti-fungal iCMBAs (AbAf iCs) was developed. AbAf iCs not only showed strong wet tissue adhesion strength, but also exhibited excellent in vitro cyto-compatibility, fast degradation, and strong initial and considerable long-term anti-bacterial and anti-fungal ability. For the first time, the biocompatibility and anti-microbial ability of sodium metaperiodate
(PI), an oxidant used as a cross-linking initiator in the AbAf iCs system, was also thoroughly investigated. Our results suggest that the PI-based bioadhesives showed better anti-microbial properties compared to the unstable silver-based bioadhesive materials. In conclusion, AbAf iCs family can serve as excellent anti-bacterial and anti-fungal bioadhesive candidates for tissue/wound closure, wound dressing, and bone regeneration, especially when bacterial or fungal infections are a major concern.

Thiolated-2-methacryloyloxyethyl phosphorylcholine protected silver nanoparticles as novel photo-induced cell-killing agents

Arunee Sangsuwan, Hideya Kawasaki, Yasuhiko Iwasaki

Colloids and Surfaces B: Biointerfaces 140 (2016) 128–134

Silver nanoparticles (AgNPs) have several medical applications as antimicrobial agents such as in drug delivery and cancer therapy. However, AgNPs are of limited use because of their toxicity, which may dam-age the surrounding healthy tissue. In this study, thiolated-2-methacryloyloxyethyl phosphorylcholine (MPC-SH) protected silver nanoparticles (MPC-AgNPs) are prepared as cell-killing agents under UV irradi-ation. MPC-AgNPs are characterized by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and UV–visible spectrophotometry. The surface plasmon resonance (SPR) band of MPC-AgNPs is observed at 404 nm, and the average diameter of the particles is determined at 13.4 ± 2.2 nm through transmis-sion electron microscopy (TEM) and at 18.4 nm (PDI = 0.18) through dynamic light scattering (DLS). Cell viability in contact with MPC-AgNPs is relatively high, and MPC-AgNPs also exhibit a cell-killing effect under UV irradiation.

Toward bioactive yet antibacterial surfaces


The fabrication of antibacterial yet biocompatible and bioactive surfaces is a challenge that biological and biomedical community has faced for many years, while no “dream material” has been developed so far. The primary goal of this study was to establish an optimal range of Ag concentration and its state of agglomeration in bioactive nanocomposite TiCaPCON films which would provide a strong bactericidaleffect without compromising the material biocompatibility and bioactivity. To obtain samples with differ-ent Ag content and redistribution, two different methods were employed: (i) TiCaPCON films deposition by magnetron sputtering of composite TiC0.5–Ca3(PO4)2 target followed by Ag+ ion implantation and (ii) Ag-doped TiCaPCON films obtained by co-sputtering of composite TiC0.5–Ca3(PO4)2 and Ag targets. Inorder to reveal the antibacterial role of Ag nanoparticles and Ag+ ions, both separate and in synergy, part of the samples from the first and second groups was subjected to additional ion etching to remove an Agrich surface layer heavily populated with Ag nanoparticles. All resultant films were characterized with respect to surface morphology, chemical composition, surface roughness, wettability, and Ag+ ion release. The antibacterial and antifungal effects of the Ag-doped TiCaPCON films were evaluated against clinicallyisolated Escherichia coli O78 (E. coli) and Neurospora crassa wt-987 spores. The influence of the surfacechemistry on spreading, proliferation, and early stages of MC3T3-E1 osteoblastic cell differentiation was also studied. Our data demonstrated that under optimal conditions in terms of Ag content and agglomeration, the Ag-doped TiCaPCON films are highly efficient against E. coli bacteria and, at the same time, provide good adhesion, spreading, proliferation and differentiation of osteoblastic cells which reflect highlevel of biocompatibility and bioactivity of the films. The influence of Ag+ ions and nanoparticles on the MC3T3-E1 osteoblastic cells and E. coli bacteria is also discussed.

The Design of Antimicrobial LL37-Modified Collagen-Hyaluronic Acid Detachable Multilayers

Margaret E. Cassin, Andrew J. Ford, Sophia M. Orbach, Scott E. Saverot,
The design of antimicrobial membranes and thin films are critical for the design of biomaterials that can combat bacterial contamination. Since the long-term use of conventional antibiotics can result in bacterial resistance, there is a critical need to incorporate natural antimicrobial peptides (AMPs) that not only prevent a wide range of pathogens from causing infections but can also promote many beneficial outcomes in wounded tissues. We report the design and antimicrobial properties of detachable collagen (COL)/hyaluronic acid (HA) polyelectrolyte multilayers (PEMs) modified with LL-37, a naturally occurring human AMP. LL-37 was physically adsorbed and chemically immobilized on the surface of PEMs. The antimicrobial and cytotoxic properties of PEMs were tested with Gram-negative Escherichia coli (E. coli, strain DH10B) and primary rat hepatocytes, respectively. The ability to prevent bacterial adhesion and to neutralize an E. coli layer was investigated as a function of LL-37 concentration. An interesting trend was that even unmodified PEMs exhibited a 40% reduction in bacterial adhesion. When LL-37 was physically adsorbed on PEMs, bacterial adhesion was significantly lower on the surface of the films as well as in the surrounding broth. Immobilizing LL-37 resulted in less than 3% bacterial adhesion on the surface due to the presence of the peptide. LL-37 modified PEMs did not result in any cytotoxicity up to input concentrations of 16 μM. More importantly, urea and albumin secretion by hepatocytes were unaffected even at high LL-37 concentrations. The COL/HA PEMs can serve as antimicrobial coatings, biological membranes and as in vitro platforms to investigate pathogen-tissue interactions.

Unaffected features of BSA stabilized Ag nanoparticles after storage and reconstitution in biological relevant media
Laura E. Valenti, Carla E. Giacomelli

Abstract Silver-coated orthopedic implants and silver composite materials have been proposed to produce local biocidal activity at low dose to reduce post-surgery infection that remains one of the major contributions to the patient morbidity. This work presents the synthesis combined with the characterization, colloidal stability in biological relevant media, antimicrobial activity and handling properties of silver nanoparticles (Ag-NP) before and after freeze dry and storage. The nanomaterial was synthesized in aqueous solution with simple, reproducible and low-cost strategies using bovine serum albumin (BSA) as the stabilizing agent. Ag-NP were characterized by means of the size distribution and morphology (UV–vis spectra, dynamic light scattering measurements and TEM images), charge as a function of the pH (zeta potential measurements) and colloidal stability in biological relevant media (UV–vis spectra and dynamic light scattering measurements). Further, the interactions between the protein and Ag-NP were evaluated by surface enhanced Raman spectroscopy (SERS) and the antimicrobial activity was tested with two bacteria strains (namely Staphylococcus aureus and Staphylococcus epidermidis) mainly present in the infections caused by implants and prosthesis in orthopedic surgery. Finally, the Ag-NP dispersed in aqueous solution were dried and stored as long-lasting powders that were easily reconstituted without losing their stability and antimicrobial properties. The proposed methods to stabilize Ag-NP not only produce stable dispersions in media of biological relevance but also long-lasting powders with optimal antimicrobial activity in the nanomolar range. This level is much lower than the cytotoxicity determined in vitro on osteoblasts, osteoclasts and osteoarthritic chondrocytes. The synthesized Ag-NP can be incorporated as an additive of biomaterials or pharmaceutical products to confer antimicrobial activity in a powdered form in different formulations, dispersed in aqueous and non-aqueous solutions or coated on the surface of different materials.
Understanding the biocide action of poly(hexamethylene biguanide) using Langmuir monolayers of dipalmitoyl phosphatidylglycerol
Colloids and Surfaces B: Biointerfaces 132 (2015) 117–121
The disinfectant activity of poly(hexamethylene biguanide) (PHMB) has been explored in industrial applications, in agriculture, and in food manipulation, but this biocide action is not completely understood. It is believed to arise from electrostatic interactions between the polyhexanide group and phosphatidylglycerol, which is the main phospholipid on the bacterial membrane. In this study, we investigated the molecular-level interactions between PHMB and dipalmitoyl phosphatidylglycerol (DPPG) in Lang-muir monolayers that served as cell membrane models. PHMB at a concentration of $2 \times 10^{-4}$ g L$^{-1}$ in a Theorell–Stenhagen at pH 3.0 and in a phosphate at pH 7.4 was used as a subphase to prepare the DPPG monolayers. Surface pressure–area isotherms showed that PHMB adsorbs and penetrates into the DPPG monolayers, expanding them and increasing their elasticity under both conditions examined. Results from polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) indicated that PHMB induces disorder in the DPPG chains and dehydrates their C O groups, especially for the physiological medium. Overall, these findings point to hydrophobic interactions and dehydration being as relevant as electrostatic interactions to explain changes in membrane fluidity and permeability, believed to be responsible for the biocide action of PHMB.

Zeta potential study of biodegradable antimicrobial polymers
Kamil Wojciechowski, Ewa Klodzinska
Biodegradable polymers have gained increasing interest in recent years, especially in food packaging industry. The biodegradation process is intrinsically related to adhesion of degradation-promoting bacteria on the surface of a biodegradable packaging. On the other hand, because of a direct contact with food, these materials should be resistant to colonisation by pathogenic bacteria. Thus, successful design of abiodegradable antimicrobial material critically depends on the interplay between the ability to control the strength of bacteria–polymer interactions. The aim of this work was to measure and analyse the effect of three positively charged antibacterial polyhexamethylene guanidine hydrochloride (PHMG) derivatives (sulfanilic acid salt, stearic acid salt, and polyethylene (PE) wax blend) on electrokinetic potential ($\zeta$) of three biodegradable polymers: poly(hydroxybutyric acid) (PHB), polycaprolactone (PCL) and poly(lactic acid) (PLA). For this purpose, the streaming current vs pressure curves were recorded and analysed using the Helmholtz–Smoluchowski theory. The $\zeta$ vs pH dependency provides a simple and useful characterisation of an electrical double layer forming on the surface of biodegradable polymers in contact with anaerobic electrolyte. The undoped polymers displayed the $\zeta$ vs pH curves typical for electrically neutral polymers, with the isoelectric point, IEP = 3.5–4.0. The effect of PHMG on $\zeta$ vs pH curves depends on both the nature of the matrix polymer and the chemical form of PHMG. The stearate salt is shown to affect the curves to the smallest extent, while the most pronounced shifts towards higher pH were observed for PHMG in the form of PE wax (for PCL and PHB), or sulfanilic acid salt (for PLA). The surface of almost all PHMG-doped polymers used in this study (with exception of PLA doped with PHMG stearate) was more positive than the bare biodegradable polymers. From the point of view of electrostatic interactions, the addition of PHMG (especially in the form of PE wax, and especially for PHB) would thus probably enhance electrostatic polymer–bacteria interactions, possibly altering the antimicrobial activity of such polymers.
Related drugs

An efficient electrochemical disinfection of E. coli and S. Aureus in drinking water using ferrocene–PAMAM–multiwalled carbon nanotubes–chitosan nanocomposite modified pyrolytic graphite electrode
Kun Shang & Zhi Qiao & Bing Sun & Xianzhong Fan & Shiyun Ai

Abstract This work reported an efficient electrochemical treatment for drinking water disinfection using a pyrolytic graphite electrode modified with ferrocenyl tethered poly(amidoamine) dendrimers–multiwalled carbon nanotubes–chitosan nanocomposite. The influence parameters of electrochemical disinfection of Escherichia coli and Staphylococcus aureus, such as applied potential and sterilization time, were investigated. Further investigation indicated that almost all (99.99 %) of the initial bacteria were killed after applying a low potential of 0.4 V for 10 min. During the electrochemical disinfection process, the oxidized form of ferrocene was formed on electrode, which played a key role in the disinfection towards E. coli and S. aureus. Hence, the proposed method may provide potential application for the disinfection of drinking water.

Core–shell microcapsules of solid lipid nanoparticles and mesoporous silica for enhanced oral delivery of curcumin
Sanghoon Kim, Roudayna Diab, Olivier Joubert, Nadia Canilho, Andreea Pasc
Colloids and Surfaces B: Biointerfaces 140 (2016) 161–168

Newly designed microcapsules (MC) combining a core of solid lipid nanoparticle (SLN) and a mesoporous silica shell have been developed and explored as oral delivery system of curcumin (CU). CU-loaded MC(MC-CU) are 2 μm sized and have a mesoporous silica shell of 0.3 μm thickness with a wormlike structure as characterized by small angle X-ray scattering (SAXS), nitrogen adsorption/desorption and transmission electron microscopy (TEM) measurements. It was found that SLN acts as reservoir of curcumin while the mesoporous shell insures the protection and the controlled release of the drug. MC-CU displayed a pH-dependent in vitro release profile with marked drug retention at pH 2.8. Neutral red uptake assay together with confocal laser scanning microscopy (CLSM) showed a good cell tolerance to MC-CU at relatively high concentration of inert materials. Besides, the cell-uptake test revealed that fluorescent-MC were well internalized into Caco-2 cells, confirming the possibility to use MC for gut cells targeting. These findings suggest that organic core-silica shell microcapsules are promising drug delivery systems with enhanced bioavailability for poorly soluble drugs.

Cell penetrating peptides from agglutinin protein of Abrus precatorius facilitate the uptake of Imatinib mesylate
Birendra Behera, Devdeep Mukherjee, Tarun Agarwal, Joyjyoti Das, Sudip K. Ghosh, Tapas K. Maiti
Colloids and Surfaces B: Biointerfaces 140 (2016) 169–175

Targeted drug delivery is of paramount importance for cancer patients. Cell penetrating peptides (CPPs) have emerged as potent vehicles for this purpose. Herein, we demonstrate CPP-like properties of two peptides: NH2-SGASDDEIIAR-COOH (SR11) and NH2-ICSSHYEPTVGRIGR-COOH (IR15), derived from the tryptic digest of Abrus precatorius agglutinin. Both IR15 and SR11 were found to be non-toxic at lower doses (up to 50 μg/ml). These two peptides entered into HeLa cells through lipid raft-mediated endocytosis within 15 min and penetrated the nuclear membrane in 60 min of incubation. Co-treatment of peptides (20 μg/ml) and Imatinib (5 μM) in HeLa cells increased uptake of the drug by ~55% and lowered the IC50 value to one-third in comparison to the drug added exclusively. However, co-treatment of TAT Peptide (standard CPP) did not alter the Imatinib uptake significantly. In summary, we have identified two novel CPPs from tryptic digest of Abrus agglutinin which increased the cellular uptake of Imatinib co-administration. Further studies may result in deciphering a novel mode of drug delivery.

Doxorubicin carriers based on Au nanoparticles – effect of shape and gold-drug linker on the carrier toxicity and therapeutic performance
Olga A. Swiech, Lidia J. Opuchlik, Grzegorz Wojciuk, Tomasz M. Stepkowski,
Gold nanoparticles (AuNPs) prepared by the Turkevich method and near-IR absorbing non-spherical anisotropic nanotriangles (AuNTs) prepared by the thiosulfate method were used for doxorubicin binding. The drug was connected to the polyethylene glycol modified gold nanoparticle by covalent peptide or pH-active hydrazone linkers. Their optical properties were studied by UV-vis spectroscopy. The shape and size of the nanoparticles were evaluated by SEM and DLS. Fluorescence studies demonstrated different drug release profiles depending on the pH. An MTT assay performed on two cell lines (A549 and HeLa) revealed that gold nanostructures modified with doxorubicin were more toxic than free doxorubicin. Viability tests and confocal microscopy revealed that the bond between the Au carrier and the drug determined the pathway of cell death – apoptotic in the case of peptide bonding and sudden and necrotic when a hydrazone linker was employed.

Edelfosine disturbs the sphingomyelin–cholesterol model membrane system in a cholesterol-dependent way – The Langmuir monolayer study

Katarzyna Hac-Wydro, Patrycja Dynarowicz-Łatka, Paweł Wydro, Katarzyna Bąk

Colloids and Surfaces B: Biointerfaces 88 (2011) 635 – 640

Synthetic alkyl-lysophospholipids, represented by edelfosine (ED), reveal strong anticancer activity and therefore are promising drugs used in anticancer therapy. Primary target for edelfosine is cellular membrane, which is in contrast to traditional cytostatics affecting DNA. The mechanism of antitumor activity of edelfosine was hypothesized to be related to its accumulation in membrane rafts. Inspired by these findings, we have performed the Langmuir monolayer studies on the influence of edelfosine on systems composed of sphingomyelin (SM) and cholesterol (Chol), being the principal components of membrane rafts. Sphingomyelin–cholesterol proportion in monolayers was varied to reflect the composition of solely membrane rafts (SM/Chol = 2:1) and contain excess of cholesterol (SM/Chol = 1:1 and 1:2). Into these systems, edelfosine was added in various concentrations. The analysis of surface pressure–area isotherms, complemented with films visualization with Brewster angle microscopy (BAM) allowed us to compare the effect of edelfosine on condensation and ordering of SM/Chol monolayers. The results evidenced that the influence of ED on the interactions in model membranes and its fluidizing effect is highly cholesterol-dependent. The strongest decrease of monolayer ordering was observed for model raft system, while the excess of cholesterol present in the remaining mixtures was found to weaken the fluidizing effect of the drug.

Effect of CTAB and CTAB in the presence of hyaluronan on selected human cell types

Marie Kalbáková, Martina Verdánová, Filip Mravec, Tereza Halasová, Miloslav Pekař


The effect of different concentrations of CTAB (in the range of 0.2 mM–2 mM, i.e., including its criticalmicelle concentration) on viability of selected human cells (osteoblasts and keratinocytes) was studied by a variety of methods (immunocytochemical and biochemical), testing the cell viability and metabolism, to get a complex overview. All of the used methods confirmed the cytotoxic effect of CTAB, which could, however, be suppressed by the presence of hyaluronan (molecular weight 806 kDa, in the concentration of 1 g/l) in the case of the lowest CTAB concentration used (0.2 mM) when the fetal bovine serum was also present in the cultivation medium. Thus, it could be concluded that hyaluronan can be used as successful protector of specific cell types against cytotoxic CTAB at low concentrations.
Effects of lidocaine-HCl salt and benzocaine on the expansion of lipid monolayers employed as bio-mimicking cell membrane
S-Y Choi, S-G Oh, J-S Lee, COLSUB 20 (2001) 239-244
Effects of lidocaine–HCl salt and benzocaine on the expansion of lipid monolayers employed as bio-mimicking cell membrane were investigated using Langmuir–Blodgett film balance to figure out the molecular mechanism for anesthesia by these local anesthetics. Lidocaine–HCl salt in subphase expanded the monolayer of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Benzocaine was not mixed with lipids in the monolayer, but the monolayer of lipids on the surface of water saturated with benzocaine was expanded same as the case of lidocaine–HCl salt. Even though this study can not explain the whole molecular mechanism for anesthesia by lidocaine–HCl salt and benzocaine, it can be asserted from the results of this study that the expansion of cell membrane by lidocaine–HCl salt and benzocaine contribute, at least partially, to the generation of anesthesia.

Electrochemical analysis of quorum sensing inhibition
Ohad Bukelman, Neri Amara, Roi Mashiach, Pnina Krief, Michael M. Meijler* and Lital Alfonta*
Chem. Commun., 2009, 2836–2838
We are studying quorum sensing and quorum sensing inhibition by analysis of biofilm forming bacteria on the surface of electrodes. We follow the formation or inhibition of biofilm and measure the generation of redox active virulence factors, by various electrochemical techniques such as: cyclic voltammetry, differential pulse voltammetry and Faradaic impedance spectroscopy.

Hyaluronic acid as a modulator of the cytotoxic effects of catiònic surfactants
Pavla Sauerová, Martina Verdánová, Filip Mravec, Tereza Pilgrová, Tereza Venerová, Marie Hubálek Kalbáˇcová, Miloslav Pekaˇr
CTAB (cetyltrimethylammonium bromide) and Septonex (carbethoxypendecinium bromide) are cationicsurfactants known for harmful effects on different cell types (bacteria, fungi, mammal cells, etc.). Colloidalcomplexes of CTAB or Septonex with oppositely charged hyaluronic acid (HyA), based primarily on elec-strostatic interactions, were prepared with the aim to test potential modulation of surfactants cytotoxiceffects. Complexes were tested for their cytotoxicity on human osteoblasts—the cell metabolic activity was determined after 24 h of treatment. Our data show that CTAB–HyA or Septonex–HyA complexes reduce (in different rate according to the used surfactant and HyA concentrations) cytotoxicity of bothsurfactants in all tested concentrations. In addition, a significant role of fetal bovine serum (importantsupplement of cell culture medium) in cell recovery under the stress conditions like CTAB or Septonexpresence was observed. Taken together, HyA could be a useful modulator of CTAB or Septonex effects oncels at diverse levels. Drug or nucleic acid delivery system, diagnostic dye carriers or cosmetic industryare the possible applications of prepared complexes.

Interaction of curcumin with lipid monolayers and liposomal bilayers
Anna Karewicz, Dorota Bielska, Barbara Gzyl-Malcher, Mariusz Kepczynski, Radosław Lach, Maria Nowakowska
Colloids and Surfaces B: Biointerfaces 88 (2011) 231- 239
Curcumin shows huge potential as an anticancer and anti-inflammatory agent. However, to achieve a satisfactory bioavailability and stability of this compound, its liposomal form is preferable. Our detailed studies on the curcumin interaction with lipid membranes are aimed to obtain better understanding of the mechanism and eventually to improve the efficiency of curcumin delivery to cells. Egg yolk phosphatidylcholine (EYPC) one-component monolayers and bilayers, as well as mixed systems containing additionally dihexadecyl phosphate (DHP) and cholesterol, were studied. Curcumin binding constant to EYPC liposomes was determined based on two different methods: UV/Vis absorption and fluorescence...
measurements to be $4.26 \times 10^4 M^{-1}$ and $3.79 \times 10^4 M^{-1}$, respectively. The fluorescence quenching experiment revealed that curcumin locates in the hydrophobic region of EYPC liposomal bilayer. It was shown that curcumin impacts the size and stability of the liposomal carriers significantly. Loaded into the EYPC/DPH/cholesterol liposomal bilayer curcumin stabilizes the system proportionally to its content, while the EYPC/DPH system is destabilized upon drug loading. The three-component lipid composition of the liposome seems to be the most promising system for curcumin delivery. An interaction of free and liposomal curcumin with EYPC and mixed monolayers was also studied using Langmuir balance measurements. Monolayer systems were treated as a simple model of cell membrane. Condensing effect of curcumin on EYPC and EYPC/DPH monolayers and loosening influence on EYPC/DPH/chol ones were observed. It was also demonstrated that curcumin-loaded EYPC liposomes are more stable upon interaction with the model lipid membrane than the unloaded ones.

**Phosphatidylserine or ganglioside – Which of anionic lipids determines the effect of cationic dextran on lipid membrane?**


In this work the influence of cationic polymer, namely diethylaminoethyl DEAE-dextran on model lipid membranes was investigated. This polymer is of a wide application as a biomaterial and a drug carrier and its cytotoxicity toward various cancer cells was also confirmed. It was suggested that anticancer effect of cationic dextran is connected with the binding of the polymer to the negatively charged sialic acid residues overexpressed in cancer membrane. This fact encouraged us to perform the studies aimed at verifying whether the effect of cationic DEAE-dextran on membrane is determined only by the presence of the negatively charged lipid in the system or the kind of anionic lipid is also important. To reach this goal systematic investigations on the effect of dextran on various one-component lipid monolayers and multicomponent hepatoma cell model membranes differing in the level and the kind of anionic lipids (phosphatidylserine, sialic acid-containing ganglioside GM3 or their mixture) were done. As evidenced the results the effect of DEAE-dextran on the model system is determined by anionic lipid–polymer electrostatic interactions. However, the magnitude of the effect of cationic polymer is strongly dependent on the kind of anionic lipid in the model system. Namely, the packing and ordering of the mixtures containing ganglioside GM3 were more affected by DEAE-dextran than phosphatidylserine-containing monolayers. Although the experiments were done on model systems and therefore further studies are highly needed, the collected data may indicate that ganglioside may be important in the differentiation of the effect of cationic dextran on membranes.

**Polydopamine-assisted synthesis of raspberry-like nanocomposite particles for superhydrophobic and superoleophilic surfaces**

Zhenn Li, Chengjiao Wu, Kai Zhao, Bo Peng, Zwei Deng


Inspired by nature, we combine the easy decoration of polydopamine with the attractive biomimetic silification to develop a facile synthetic route toward raspberry-like nanocomposite particles, and further lead to the superhydrophobic and superoleophilic surfaces by mimicking the lotus leaf surface structures in the usage of these particles. In this approach, monodisperse polystyrene (PS) particles are used as the template particles, and then, follows with a subsequent polydopamine (PDA) coating step through the self-polymerization of dopamine in a weakly alkaline aqueous environment (pH = 8.5). The obtained core–shell PS/PDA particles are used as the active substrates for the biomimetic silification under ambient conditions, allowing a well-controlled synthesis of raspberry-like PS/SiO2 nanocomposite par-ticles with a tunable surface roughness. Upon adjusting the concentration of silica precursor, the surface geometry and the coverage degree of silica nanoparticles of PS/PDA composite particles can be easily tailored. The whole procedure is carried out in a mild environment, no intricate instruments or toxic reagents are involved. In addition, these raspberry-like PS/SiO2 nanocomposite particles self-assemble onto the glass slides driven by capillary force during drying, forming a hierarchical dual-sized rough structure, which is analogous to the surface morphology of lotus leaf in nature. Making full use of this hierarchical surface, superhydrophobic and superoleophilic surfaces can be successfully achieved via rational surface
modification of this lotus leaf-like surface structure. The superhydrophobic performances can be readily adjusted by varying the scale ratio of the micro/nano surface structures.

**Protein corona as a proteome fingerprint: The example of hidden biomarkers for cow mastitis**

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*Colloids and Surfaces B: Biointerfaces 140 (2016) 40–49*

Proteome modifications in a biological fluid can potentially indicate the occurrence of pathologies, even if the identification of a proteome fingerprint correlated to a specific disease represents a very difficult task. When a nanomaterial is introduced into a biological fluid, macromolecules compete to form a protein corona on the nanoparticle surface, and depending on the specific proteome, different patterns of proteins will form the final protein corona shell depending on their affinity for the nanoparticle surface. Novel surface active maghemite nanoparticles (SAMNs) display a remarkable selectivity toward protein corona formation, and they are able to concentrate proteins and peptides presenting high affinities for their surface even if they are present in very low amounts. Thus, SAMNs may confer visibility to hidden biomarkers correlated to the occurrence of a pathology. In the present report, SAMNs were introduced into milk samples from healthy cows and from animals affected by mastitis, and the selectively bound protein corona shell was easily analyzed and quantified by gel electrophoresis and characterized by mass spectrometry. Upon incubation in mastitic milk, SAMNs were able to selectively bind $\beta_2$-casein fragments containing the FALPQYLK sequence, as part of the larger casocidin-1 peptide with strong antibacterial activity, which were not present in healthy samples. Thus, SAMNs can be used as a future candidate for the rapid diagnosis of mastitis in bovine milk. The present report proposes protein competition for SAMN protein corona formation as a means of mirroring proteome modifications. Thus, the selected protein shell on the nanoparticles results in a fingerprint of the specific pathology.

**Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae**

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*Colloids and Surfaces B: Biointerfaces 142 (2016) 392–399*

Bacterial resistance to conventional antibiotics is currently one of the most important healthcare issues, and has serious negative impacts on medical practice. This study presents a potential solution to this problem, using the strong synergistic effects of antibiotics combined with silver nanoparticles (NPs). Sil-ver NPs inhibit bacterial growth via a multilevel mode of antibacterial action at concentrations ranging from a few ppm to tens of ppm. Silver NPs strongly enhanced antibacterial activity against multiresistant, $eta$-lactamase and carbapenemase-producing Enterobacteriaceae when combined with the following antibiotics: cefotaxime, ceftazidime, meropenem, ciprofloxacin and gentamicin. All the antibiotics, when combined with silver NPs, showed enhanced antibacterial activity at concentrations far below the mini-mum inhibitory concentrations (tenths to hundredths of one ppm) of individual antibiotics and silver NPs. The enhanced activity of antibiotics combined with silver NPs, especially meropenem, was weaker against non-resistant bacteria than against resistant bacteria. The double disk synergy test showed that bacteria produced no $eta$-lactamase when treated with antibiotics combined with silver NPs. Low silver concentrations were required for effective enhancement of antibacterial activity against multiresistant bacteria. These low silver concentrations showed no cytotoxic effect towards mammalian cells, an important feature for potential medical applications.

**Stability and softening of a lipid monolayer in the presence of a pain-killer drug**

Uttam Kumar Basak, Alokmay Datta, Dhananjay Bhattacharyya

*Colloids and Surfaces B: Biointerfaces 132 (2015) 34–44*

The aim of this study is to investigate the interaction of a drug (Piroxicam, 4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1 dioxide) with a lipid (DMPC) monolayer used as a membrane-mime in terms of drug-induced changes in stability and compressibility with variation
intemperature, surface-pressure, drug-dose and ionic states of the monolayers. Drug-induced fluidizationis noticed in the _E = A isotherms through increase in phase-transition pressure at constant temperature. The long-term dynamics of the lipid-monolayer is characterized by algebraic decays in surface-energyE with time t, E ~ t−p, with an initial decay exponent p that changes to p2after ~1000 s, and, at high pressures and/or drug-dose, to a third exponent p3after ~3500 s, suggesting structural reorganizations in the monolayer. With increasing drug–lipid ratio (D/L), p1 shows a decrease end-ing at an almost constant value after 0.05, p2 shows an almost negligible lowering while p3 shows a monotonic and considerable increase. The reorganization is summarized by proposing two mechanisms: (a) ’charging–discharging’ where drug-molecules sitting parallel to the interface increase headgroups separations and (b) ’discharging–charging’ where drug-molecules sitting roughly perpendicular to the interface bring headgroups closer. Drug-induced softening of lipid-monolayers is characterized by the compressibilities of pure and mixed lipid monolayers. Compressibility-change (i.e., compressibility difference between drug/lipid and pure lipid monolayer) with pressure is maximum in the LE–LC transition zone and compressibility-change with drug-dose reveals an optimum dose of drug for maximum increase in compressibility. Molecular dynamics simulation shows that the ordering in the different parts of the lipid chains is changed to different extents in the presence of drugs with maximum change near the headgroups and again points to an optimum dose for maximum disorder.

The potential use of a layer-by-layer strategy to develop LDPE antimicrobial films coated with silver nanoparticles for packaging applications
Shafrina Azlin-Hasim, Malco C. Cruz-Romero, Enda Cummins, Joseph P. Kerry, Michael A. Morris
Journal of Colloid and Interface Science 461 (2016) 239–248

Commercial low-density polyethylene (LDPE) films were UV/ozone treated and coated using a layerby-layer (LbL) technique by alternately depositing polyethyleneimine (PEI) and poly(acrylic acid) (PAA) polymer solutions and antimicrobial silver (Ag). The effects of the initial pH of the PEI/PAA polymer solutions alternating layers (pH 10.5/4 or 9/6.5) on the antimicrobial activity of the developed LbL coatings combined with Ag against Gram-negative and Gram-positive bacteria were investigated. The results from fourier transform infrared spectroscopy and toluidine blue O assay showed that LDPE LbL coated using PEI/PAA polymer solutions with initial pH of 10.5/4 significantly increased the presence of carboxylic acid groups and after Ag attachment the coating had higher antimicrobial activity against both Gram-negative and Gram-positive bacteria compared to the LDPE LbL coated using PEI/PAA polymer solutions with initial pH of 9/6.5. The LDPE LbL coated films using non-modified pH PEI/PAA polymer solutions decreased the water contact-angle indicating an increased hydrophilicity of the film, also increased the tensile strength and roughness of LDPE LbL coated films compared to uncoated LbL samples. The LDPE LbL coated films attached with Ag+ were UV/ozone treated for 20 min to oxidise Ag+ to Ag0. The presence of Ag0 (Ag nanoparticles (NPs)) on the LDPE LbL coated films was confirmed by XRD, UV–vis spectrophotometer and colour changes. The overall results demonstrated that the LbL technique has the potential to be used as a coating method containing antimicrobial Ag NPs and that the manufactured films could potentially be applied as antimicrobial packaging.

Transport of stearic acid-based solid lipid nanoparticles (SLNs) into human epithelial cells
Rohan M. Shah, Dhiya Rajasekaran, Mandy Ludford-Menting, Daniel S. Eldridge, Enzo A. Palombo, Ian H. Harding
Colloids and Surfaces B: Biointerfaces 140 (2016) 204–212

Development of drug delivery systems, as much as the drug molecule itself, is an important considerationfor improving drug absorption and bioavailability. The mechanisms by which drug carriers enter target cells can differ depending on their size, surface properties and components. Solid lipid nanoparticles (SLNs) have gained an increased attention in recent years and are the drug carriers of interest in this paper. They are known to breach the cell-membrane barrier and have been actively sought to transport biomolecules. Previous studies by our group, and also other groups, provided an extensive
characterization of SLNs. However, few studies have investigated the uptake of SLNs and these have had limited mechanistic focus. The aim of this work was to investigate the pathway of uptake of SLNs by human epithelial cells i.e., lung A549 and cervical HeLa cells. To the best of our knowledge, this is first study that investigates the cellular uptake of SLNs by human epithelial cells. The mechanism of cellular uptake was deciphered using pharmacologic inhibitors (sucrose, potassium-free buffer, filipin and cytochalasinB). Imaging techniques and flow assisted cell sorting (FACS) were used to assess the cellular uptake of SLNs loaded with rhodamine 123 as a fluorescent probe. This study provided evidence that the cellular uptake of SLNs was energy-dependent, and the endocytosis of SLNs was mainly dependent on clathrin-mediated mechanisms. The establishment of entry mechanism of SLNs is of fundamental importance for future facilitation of SLNs as biological or drug carriers.

Two antibacterial nalidixate calixarene derivatives in cholesterol monolayers: Molecular dynamics and physicochemical effects

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Colloids and Surfaces B: Biointerfaces 145 (2016) 777–784

The interaction of two antibacterial calixarene derivatives with cholesterol, a eukaryotic cell membrane lipid, was investigated with the aim to get more insight in the potential adverse effects on our cells. The derivatives used had one or two nalidixic acid arms grafted on the lower rim of the calixarene aromatic crown. Monomolecular films spread at the air-water interface were used as model lipid membranes. Pure cholesterol and pure calixarene derivatives, as well as binary cholesterol – calixarene derivativemixtures were studied using surface pressure measurements, polarization modulation infrared reflectionabsorption spectroscopy and molecular dynamics simulations. The properties of the mixed monolayers were described quantitatively using thermodynamic models. The analysis of surface pressure–area isotherms of mixed monolayers shows that cholesterol may form homogenous but metastable domains with both nalidixate derivatives. This phenomenon is more clearly observed with mono-substituted calixarene. A detailed modeling analysis indicates that cholesterol favors dehydration of the calixarene polar headgroups and transfer of the derivatives from the aqueous to the gas phase. This effect is more pronounced in the case of the monosubstituted calixarene, can be linked to the hydrophobic interaction with cholesterol. This observation may be useful for developing new calixarene derivatives allowing us to control disease-causing bacteria without harming our own cells.
References on chitosan antimicrobial and derivatives

Application of chitosan microparticles for treatment of metritis and in vivo evaluation of broad spectrum antimicrobial activity in cow uteri
Soo Jin Jeon, Zhengxin Ma, Minyoung Kang, Klifs N. Galvarino, Kwangcheol Casey Jeong
Biomaterials 110 (2016) 71-80

Uterine disease such as metritis is associated with multiple bacterial infections in the uteri after parturition. However, treatment of metritis is challenging due to considerably high antibiotic treatment failure rate with unknown reason. Recently, chitosan microparticles (CM) have been developed to exert broad spectrum antimicrobial activity against bacterial pathogens, including multidrug resistant bacteria, without raising CM resistant mutants. In this study, we tested, using metagenomics analysis, if CM maintain strong antimicrobial activity against pathogenic bacteria such as Fusobacteriaceae and Bacteroidaceae in cow uteri and evaluated CM's potency as an alternative antimicrobial agent to cure metritis in cows. Here, we report that efficacy of CM treatment for metritis was comparable to the antibiotic ceftiofur, and CM greatly altered uterine microbiota of sick animals to healthy uterine microbiota. Among uterine bacteria, CM significantly decreased Fusobacterium necrophorum, which is known pathogenic bacteria within the uterus. Taken together, we observed the broad spectrum antimicrobial activity of CM in vivo with an animal model, and further evaluated treatment efficacy in cows with metritis, providing insights into promising use of CM as an alternative antimicrobial agent for controlling uterine disease.

Bacterial inhibition by chitosan coatings loaded with silver-decorated calcium phosphate microspheres
Jessica Amber Jennings, Diego A. Velasquez Pulgarin, DomLal Kunwar, Jegdish Babu, Sanjay Mishra, Joel Bumgardner

Porous calcium phosphate microspheres have been modified to contain nanoparticles of silver to provide both osteoconductive and antimicrobial components to implant coatings. These microspheres have been mixed with chitosan and bonded to titanium via alkoxysilane reaction. Silver concentration on calcium phosphate microspheres was varied from 0 to 50% and microspheres were loaded at 30 wt.% within chitosan coatings. Increasing concentrations of silver loaded on calcium phosphate microspheres within the chemically bound coating reduces bacterial viability by up to 90% in both anaerobic and aerobic pathogenic microorganisms, including Staphylococcus aureus, Prevotella denticola, and Porphyromonas gingivalis. This novel coating could reduce the incidence of infection in orthopaedic and dental implant applications.

Characterization and bacterial adhesion of chitosan-perfluorinatedacid films
Karina L. Bierbrauer, Roxana V. Alasino, Adrián Múñoz, Dante M. Beltramo, Miriam C. Strumia
Colloids and Surfaces B: Biointerfaces 114 (2014) 201–208

We reported herein the study and characterization of films obtained by casting of chitosan solutions in perfluorinated acids, trifluoroacetic (TFA), perfluoropropionic (PFPA), and perfluorooctanoic (PFOA). The films were characterized by FTIR, solid state 13C NMR, X-ray, AFM, contact angle, thermogravimetric effluent analysis by mass spectrometry, and rheology. The results showed a marked influence of chainlength of the perfluorinated acids on the hydrophobic/hydrophilic ratio of the modified chitosan films which was evidenced by the different characteristics observed. The material that showed greater surface stability was chitosan-PFOA. Chitosan film with the addition of PFOA modifier became more hydrophobic, thus water vapor permeability diminished compared to chitosan films alone, this new material also depicted bacterial adhesion which, together with the features already described, proves its potential in applications for bioreactor coating.

Chitosan as a subphase disturbant of membrane lipid monolayers. The effect of temperature at varying pH: I. DPPG
Temperature-mediated effects of chitosan dissolved in acetate buffer at different pHs, on the structural and thermodynamic characteristics of dipalmitoylphosphatidylglycerol (DPPG) monolayers, employed here as a model of the bacterial cell membrane, were investigated. The investigation was done to improve the understanding if in its antibacterial activity chitosan disturbs the bacterial cell membrane. The Langmuir film technique was employed, where the compression surface pressure–area ($\pi$–$A$) isotherms of DPPG monolayer formed at the air-buffer interface were measured at five temperatures in the range 15–37 °C for three pHs in the range 3.5–6.0, with the chitosan concentration in the subphase varied between 0.002 and 1 mg/mL. Illustrating the extent of DPPG–chitosan interactions, the characteristics of the monolayers on the chitosan-containing solutions were assessed by monitoring differences in the course of the $\pi$–$A$ isotherms relative to pure DPPG films. The characteristics revealed that chitosan interacted with the lipid film not only superficially but also inserted to a certain degree into the film. Increasing temperature enhanced these effects, the more strongly, the lower was the pH. Furthermore, the transition of the monolayer from the liquid-expanded to liquid-condensed phase was found to be an endothermic process accompanied by an increase in disorder. The effects were most pronounced at pH 3.5, and were markedly enhanced by chitosan. Most interestingly, the analysis of the critical temperatures provided evidence that the interaction of chitosan with DPPG monolayers is not only due to electrostatic but also to non-electrostatic contributions. The most effective disturbing effects of chitosan on DPPG monolayer were observed at the highest temperature 37 °C applied at pH 3.5.

Chitosan as a subphase disturbant of membrane lipid monolayers.
The effect of temperature at varying pH: II. DPPC and cholesterol
Barbara Krajewska, Agnieszka Kyziol, Pawel Wydro

In this part of our work aimed at describing temperature-mediated effects of chitosan on membrane lipids in monolayers at different pHs, we studied the monolayers of dipalmitoylphosphatidylcholine (DPPC) and cholesterol. The lipids are typical components of eukaryotic cell membranes. The compression surface pressure–area ($\pi$–$A$) isotherms of the lipid monolayers were recorded in the Langmuir film balance, in the presence of chitosan dissolved in the subphase (0.1–1 mg/mL) at different temperature (15–37 °C) and pH conditions (3.5 and 6.0). Structural and thermodynamic parameters of the monolayers were estimated and compared to those of the pure lipid films. The overall work was performed with the general aim to examine if antibacterial activity of chitosan consists of the loosening of the bacterial membrane structure. It was ascertained in this study that unlike on DPPG monolayer, chitosan produced a minor disturbing effect on DPPC films. The data obtained suggest that the interactions in the system responsible for this weak effect should be primarily non-electrostatic. Further, the effect was found to be only slightly promoted by increasing temperature and importantly, it appeared to be a little stronger at pH 6.0 than at 3.5. This finding is of consequential significance for the possible application of chitosan as an antibacterial agent, which is because in addition to overall weak disturbance of DPPC monolayer compared to DPPG, the preferred condition for its occurrence is pH 6.0 which is opposite to that of DPPG.
at pH 3.5. Finally, the expansions of cholesterol monolayers were ascribed to the insertion of chitosan to the monolayer, an effect possible due to hydrophobic interactions and hydrogen bonding. Although not particularly prominent, these expansions are important in that they prove that hydrophobic interactions and hydrogen bonding play an important role in the cell membrane disturbing activity of chitosan.

Chitosan-triclosan films for potential use as bio-antimicrobial bags in healthcare sector
Aleksandra Nesic, Milan Gordic, Antonije Onjia, Sladjana Davidovic, Miona Miljkovic, Suzana Dimitrijevic-Brankovic
Materials Letters 186 (2017) 368–371

In this work, antimicrobial bioinspired films made from chitosan incorporated with triclosan were investigated. The tensile strength of these films were in the range of 33 and 39 MPa, which presented satisfied mechanical stability comparable to the synthetic-based packages commonly used in industry. The addition of triclosan enhanced thermal stability and antimicrobial activity of chitosan films against Escherichia coli and Staphylococcus aureus. Results obtained in this work demonstrated that chitosan/triclosan films could be potentially used as an eco-sustainable package in healthcare sector to prevent infections/contaminations.

Designing chitosan–silver nanoparticles–graphene oxide nanohybrids with enhanced antibacterial activity against Staphylococcus aureus
Bogdan Marta,a,1 Monica Potara, Maria Ilut, Endre Jakab, Teodora Radu, Florica Imre-Lucaci, Gabriel Katona, Octavian Popescu, Simion Astilean

Designing hybrid nanomaterials that exhibit multiple mechanisms of antibacterial action provides a new paradigm in the fight against resistant bacteria. Herein, we present such a new hybrid nanomaterial which integrates the antibacterial and physico-chemical properties of silver nanoparticles, graphene oxide and chitosan biopolymer. The formation, stability and structure of the integrated three-component chitosan-silver nanoparticles–graphene oxide (chit–AgNPs–GO) nanomaterial is analyzed by UV–vis extinction spectroscopy, transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and zeta potential measurements. The antibacterial activity is evaluated against two representative methicillin-resistant Staphylococcus aureus (MRSA) strains (UCLA 8076 and 1190R) and relative to individual components (GO, chit–GO, AgNPs–GO) by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The three-component nanocomposites (chit–AgNPs–GO) exhibit higher antibacterial activity than most of the antibacterial agents based on AgNPs or AgNPs–GO reported so far and, more interestingly, their activity can be controlled by the ratio between the amount of chitosan and AgNPs–GO. The results presented in this study demonstrate the promising potential of the chit–AgNPs–GO hybrids as effective nanomaterial to fight against the bacterial infections.

Dry powder aerosols of curcumin-chitosan nanoparticle complex prepared by spray freeze drying and their antimicrobial efficacy against common respiratory bacterial pathogens
Hong Yu, The-Thien Tran, Jeanette Teo, Kunn Hadinoto

While the therapeutic benefits of curcumin delivery to the lung to treat various pulmonary disorders have been established, development of inhaled curcumin formulation that can address its inherently low aqueous solubility remains lacking. Although curcumin nanocapsules prepared by conventional encapsulation methods can improve the dissolution rate, their intricate preparation makes them less attractive for widespread implementation. Recently, our group developed a new class of curcumin nanoparticles in the form of curcumin-chitosan nanoparticle
complex (or curcumin nanoplex in short) by a simple, cost-effective, and highly efficient method based on self-assembly drug-polysaccharide complexation. Owing to its nanosize and amorphous state, the curcumin nanoplex possessed high supersaturation generation capability upon dissolution that in turn produced high apparent solubility of curcumin.

Effect of thiol-functionalisation on chitosan antibacterial activity: Interaction with a bacterial membrane model
Margareta M. Fernandes, Antonio Francesko, Juan Torrent-Burgués, Tzanko Tzanov
Reactive & Functional Polymers 73 (2013) 1384–1390

The antibacterial activity of chitosan modified with the thiol-containing 2-iminothiolane HCl (TC-IMI) and N-acetyl-L-cysteine (TC-NAC) was studied by Langmuir film balance technique using a dipalmitoylphosphatidylglycerol (DPPG) monolayer bacterial membrane model. The interactions of the biopolymer with the membrane model were assessed by monitoring the differences in the shape of the compression isotherms recorded in the absence and presence of chitosan and thiolated conjugates in the subphase. A low molecular weight chitosan (15 kDa) shifted the compression isotherms of DPPG monolayers towards larger areas ($A_{0,CS} = 145 \, \text{Å}^2$), confirming its membrane disturbance capacity. Further thiolation induced higher yield of expansion, more pronounced in the case of TC-IMI. The expansion of the monolayer increased significantly ($A_{0,TC_NAC} = 150 \, \text{Å}^2$ vs $A_{0,TC_{IMI}} = 175 \, \text{Å}^2$) and the elasticity at a surface pressure of 30 mN/m, typical for bio-membranes decreased to a greater extent ($C_{1s;30 \, TC_{IMI}} = 120 \, \text{mN/m}$ vs $C_{1s;30 \, TC_{IMI}} = 87 \, \text{mN/m}$) in presence of TC-IMI. Antibacterial tests against a Gram-negative Escherichia coli and a Gram-positive Staphylococcus aureus were in good agreement with these findings, suggesting that chitosan thiolated with 2-iminothiolane HCl acts as a bactericide disrupting the integrity of the bacterial cell membrane.

Extent of shielding by counterions determines the bactericidal activity of N,N,N-trimethyl chitosan salts
Carbohydrate Polymers 137 (2016) 418–425

In this study, we show that the bactericidal activity of quaternized chitosans (TMCs) with sulfate, acetate, and halide counterions against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) correlates with the “availability” of N-quaternized groups [+N(CH3)3] in the TMCs backbones. N,N,N-trimethylchitosan sulfate (TMCS) and N,N,N-trimethyl chitosan acetate (TMCAc) displayed the highest activities, probably due to their delocalized _ system. Among TMCs with halide counterions, activity was higher for N,N,N-trimethyl chitosan chloride (TMCCI), whereas N,N,N-trimethyl chitosan iodide (TMCI) and N,N,N-trimethyl chitosan bromide (TMCBr) exhibited lower, similar values to each other. This is consistent with the shielding of [+N(CH3)3] groups inferred from chemical shifts for halide counterions in 1HNMR spectra. We also demonstrate that TMCs with distinct bactericidal activities can be classified according to their vibrational spectra using principal component analysis. Taken together, these physico-chemical characterization approaches represent a predictive tool for the bactericidal activity of chitosan derivatives.

Hyaluronan/chitosan nanofilms assembled layer-by-layer and their antibacterial effect: A study using Staphylococcus aureus and Pseudomonas aeruginosa
Colloids and Surfaces B: Biointerfaces 141 (2016) 499–506

In the last few years, chitosan-based coatings have been proposed as antibacterial surfaces for biomedical devices in order to prevent nosocomial infections. In that sense, this work reports the optimized synthesis of hyaluronan/chitosan
HA/CHI nanofilms assembled layer-by-layer in order to maximize the antibacterial effect for two important human pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In this assembly, HA forms a soft, highly hydrated, and nontoxic film, whereas CHI shows antimicrobial characteristics. Our HA/CHI nanofilm synthesis optimization was based on changing pH values of the biopolymer stem-solutions and the consequent variation of their ionization degree. Furthermore, the surface density of primary amino groups, which are related to the antibacterial effect, was also enhanced by increasing the number of HA/CHI bilayers. The antibacterial effect of HA/CHI nanofilms was evaluated by the spread plate counting method for both bacteria. These results were correlated with the morphology of nanofilms (characterized using SEM and AFM), as well as with their chemical properties studied by UV–vis, Kelvin Probe Force microscopy and XPS spectroscopy.

**Hybrid chitosan/polyaniline-polypyrrole biomaterial for enhanced adsorption and antimicrobial activity**

- **Rajeev Kumar, Mohammad Oves, Talal Almeelbi, Naief H. Al-Makishah, M.A. Barakat**
- *Journal of Colloid and Interface Science*
- **Volume 490, 15 March 2017, Pages 488–496**

In this work, chitosan (CS) functionalized polyaniline-polypyrrole (Pani-Ppy) copolymer (CS/Pani-Ppy) was synthesized applying a facile one-pot method for the enhanced adsorption of Zn(II) and antimicrobial activity for *E. coli* and *E. agglomerans*. The synthesized materials were characterized using scanning electron microscopy, energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The adsorption of the Zn(II) on the synthesized materials was highly dependent on the pH of the solution, the initial metal ion concentration, and temperature. The adsorption of Zn(II) on the studied materials was as follows: CS/Pani-Ppy > Pani-Ppy > Ppy > Pani > CS. The results reveal that adsorption of Zn(II) follows the Langmuir adsorption isotherm, and that chemisorption occurs through pendant and bridging interactions, with active adsorbent sites. Thermodynamic results show the adsorption is spontaneous and exothermic in nature. The synthesized materials show excellent antimicrobial activity against *E. coli* and *E. agglomerans* bacterial organisms, and an approximately 100% decline in the viability of both strains was observed with CS/Pani-Ppy and Pani-Ppy. The order of antimicrobial activity for the synthesized materials was as follows: CS/Ppy-Pani > Ppy-Pani > Ppy > Pani > CS. The results show that the greater activity of CS/Ppy-Pani resulted from the electrostatic interaction between positively charged amine groups and negatively charged bacteria.

**Incorporation of chitosan nanospheres into thin mineralized collagen coatings for improving the antibacterial effect**

Ziqiang Kong, Mengfei Yu, Kui Cheng, Wenjian Weng, Huiming Wang, Jun Lin, Piyi Du, Gaorong Han

*Colloids and Surfaces B: Biointerfaces* **111** (2013) 536–541

It is desired that the coatings on metallic implants have both excellent biological responses and good loading-release capacities of biological factors or drugs. So far, the challenge still remains, because the morphology and composition of the bioactive coatings are usually not favorable for accommodating drug molecules. In this study, we adopted an approach of incorporating chitosan nanospheres into a thin mineralized collagen coating; this approach is based on the good loading-release behavior of the nanospheres and the good cytocompatibility of the thin coating.
The incorporation of chitosan nanospheres into the mineralized collagen coatings was realized by electrolytic co-deposition. The morphologies and microstructures of the resulting coatings were characterized by SEM, and the phase and chemical compositions of the coatings were measured by XRD and FTIR. The loading–release capacity for vancomycin hydrochloride (VH) was determined by ultraviolet spectrophotometry. MTS assay was used to evaluate cytocompatibility, and in vitro bacterial adhesion was tested for assessing the antibacterial effects of the VH-loaded coatings. The chitosan nanospheres adhered tightly to collagen fibrils. The incorporated coatings facilitated the sustained release of VH, and had a clear antibacterial effect.

The incorporation of chitosan nanospheres into mineralized collagen coatings demonstrates an effective way to improve the drug loading–release capacity for the thin coatings. This formulation had a highly effective biological response.

In Situ Impregnation of Silver Nanoclusters in Microporous Chitosan-PEG Membranes as an Antibacterial and Drug Delivery Percutaneous Device

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Langmuir 2016, 32, 10305–10316

An in situ synthesis method for preparing silver nanoclusters (AgNCs) embedded in chitosan-polyethylene glycol (CS-PEG) membranes is disclosed. The aim is to develop implantable multifunctional devices for biofilm inhibition and drug release to reduce percutaneous device related complications (PDRCs). A multiple array of characterization techniques confirmed the formation of fluorescent AgNCs with sizes of ~3 nm uniformly distributed in CS-PEG matrix and their active role in determining the fraction and interconnectivity of the microporous membranes. The presence and increasing contents of AgNCs enhanced the mechanical stability of membranes and decreased their susceptibility to degradation in the presence of lysozyme and \( \text{H}_2\text{O}_2 \). Moreover, the presence and increasing concentrations of AgNCs hindered biofilm formation against \textit{Escherichia coli} (Gram negative) and \textit{Staphylococcus aureus} (Gram positive) and enabled a sustainable release of an anti-inflammatory drug naproxen in vitro until 24 h. The overall results gathered and reported in this work make the AgNCs impregnated CS-PEG membranes highly promising multifunctional devices combining efficient antibacterial activity and biocompatibility with active local drug delivery.

Layer-by-layer nanocoating of live Bacille-Calmette-Guérin mycobacteria with poly(I:C) and chitosan enhances pro-inflammatory activation and bactericidal capacity in murine macrophages

Martin Tobias Speth, Urska Repnik, Gareth Griffiths

Biomaterials 111 (2016) 1-12

Tuberculosis (TB) is a major disease burden globally causing more than 1.5 million deaths per year. The attenuated live vaccine strain Bacille Calmette-Guérin (BCG), although providing protection against childhood TB, is largely ineffective against adult pulmonary TB. A major aim therefore is to increase the potency of the BCG vaccine to generate stronger and more sustained immunity against TB. Here, we investigated the use of layer-by-layer (LbL) nanocoating of the surface of live BCG with several layers of polyinosinic-polycytidylic acid (poly(I:C)), a strong inducer of cell-mediated immunity, and the biodegradable polysaccharide chitosan to enhance BCG immunogenicity. Nanocoating of live BCG did not affect bacterial viability or growth in vitro but induced killing of the BCG in infected mouse bone marrow-derived macrophages and enhanced macrophage production of pro-inflammatory cytokines and expression of surface co-stimulatory molecules relative to uncoated BCG. In addition, poly(I:C) surfacecoated BCG, but not BCG alone or together with soluble poly(I:C), induced high production of nitric oxide.
(NO) and IL-12. These results argue that BCG and surface absorbed poly(I:C) act in a synergistic manner to elicit pro-inflammatory macrophage activation. In conclusion, nanocoating of live BCG with the immunostimulatory agent poly(I:C) may be an appropriate strategy to enhance and modulate host responses to the BCG vaccine.

Low molecular-weight chitosans are stronger biomembrane model perturbants

Adriana Pavinatto, Felippe J. Pavinatto, Jorge A. de M. Delezuk, Thatyane M. Nobre, Adriano L. Souza, Sergio P. Campana-Filho, Osvaldo N. Oliveira Jr.

Colloids and Surfaces B: Biointerfaces 104 (2013) 48 - 53

The influence from the chitosan molecular weight on its interaction with cell membrane models has been studied. A low molecular weight chitosan (LMWChi) adsorbed from the subphase expanded the surface pressure-area and surface potential–area isotherms of dimyristoyl phosphatidic acid (DMPA) monolayers and decreased the compressional modulus. The expansion in the monolayers and the decrease in the compressional modulus were larger for LMWChi than for a high molecular weight chitosan (Chi). The polymeric nature is still essential for the interaction though, which was demonstrated by measuring negligible changes in the mechanical properties of the DMPA monolayer when the subphase contained glucosamine and acetyl-glucosamine. The results were rationalized in a model through which chitosan interacted with the membrane via electrostatic and hydrophobic interactions, with the smaller chains of LMWChi having less steric hindrance to be accommodated in the membrane. In summary, the activity based on membrane interactions depends on the distribution of molar mass, with lower molecular weight chitosan more likely to have stronger effects.

Modified chitosan encapsulated core-shell Ag Nps for superior antimicrobial and anticancer activity

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This investigation reports a one pot synthesis of silver nanoparticles (Ag Nps) using aqueous solution of chitosan-graft-poly(acrylamide) (Cts-g-PAAm) as a reducing agent and polyethylene glycol (PEG) as a stabilizing agent. The as synthesized Ag Nps was characterized by ultra violet-visible (UV-vis), Fourier transform infrared (FTIR) and X-ray diffraction (XRD) analysis. Field emission scanning electron microscopy (FESEM), dynamic light scattering (DLS) and transmission electron microscopy (TEM) showed that Ag Nps, which were stable upto more than 60 days, were spherical in shape and the particle sizes was in the range of 5–50 nm. Atomic force microscopy (AFM) image also supported the above obtained result. The prepared Ag Nps exhibited strong antimicrobial activity against different gram positive bacteria (Alkaliphilus, Bacillus substilis, Lysinibacillus) and gram negative bacteria (Enterobacter aerogenus, Vibrio vulnificus and Escherichia coli) and haemolytic assay revealed its blood compatible nature. The synthesized Ag Nps showed significant cytotoxicity over human cervical HeLa cancer cells and it was found that the inhibitory concentration for 50% cell death (IC50) was 8 μg/ml.

PEGylated chitosan protected silver nanoparticles as water-borne coating for leather with antibacterial property

- Gongyan Liu, Kaijun Li, Quanqing Luo, Haibo Wang, Zongcai Zhang
- Journal of Colloid and Interface Science
- Volume 490, 15 March 2017, Pages 642–651
Development of eco-labeled and effectively antibacterial coatings for final leather products has been desiderated both by industry and by consumers. Herein, PEGylated chitosan modified silver nanoparticles (PEG-g-CS@AgNPs) were prepared and characterized by UV–vis spectroscopy, transmission electron microscopy and dynamic light scattering. The antimicrobial activity of such silver nanoparticles was investigated against Gram-negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus), exhibiting much lower minimum inhibitory concentration (MIC) than chitosan or PEG-g-CS. Water-borne coating was formed by immobilizing the PEG-g-CS@AgNPs onto the leather surface through the electrostatic interaction between amino groups of chitosan and carboxyl groups of leather collagen. Scanning electron microscopy and water contact angle were employed to study the coating’s morphology and hydrophilicity, respectively. After coating, leather samples showed significantly high bactericidal efficiency with reusability after release of dead cells from the coating by simply water washing. The excellent antibacterial property of PEG-g-CS@AgNPs coating was ascribed to the combination of bacteria-resistance and bacteria-release by PEGylation, and dual bacteria-killing based on chitosan and Ag⁺ release.

Probing the Modes of Antibacterial Activity of Chitosan. Effects of pH and Molecular Weight on Chitosan Interactions with Membrane Lipids in Langmuir Films
Barbara Krajewska, Paweł Wydro, and Agnieszka Janiczky
dx.doi.org/10.1021/bm2012295 | Biomacromolecules 2011, 12, 4144−4152

Chitosan, a cationic biopolymer derived from chitin, has been described as having antibacterial activity. The modes of this activity, however, have not been established. One mode proposed is that chitosan perturbs bacterial cell membranes. To validate this proposal, in this study we investigated chitosan interactions with lipids in Langmuir monolayers as model membranes. The interactions were assessed by monitoring differences in the shape of the compression isotherms measured in the absence and presence of chitosan in the subphase (acetate buffer). To appraise the contribution of electrostatic interactions versus hydrogen bonding and hydrophobic interactions, three membrane lipids differing in charge were studied: anionic dipalmitoylphosphatidylglycerol (DPPG), zwitterionic dipalmitoylphosphatidylcholine (DPPC), and neutral cholesterol. The pH of the subphase was varied between 3.5 and 6.0. In addition, the impact of the molecular weight of chitosan on the interactions was assessed at pH 3.5. It was found that while chitosan had a negligible effect on DPPC monolayers over the pH range studied, it distinctly affected DPPG and cholesterol monolayers. The effect on DPPG was found to decrease with increasing pH, that at pH 3.5 being ascribed to the charge- mediating action of chitosan on the local ionic environment and that at higher pHs to the intercalation of chitosan to the monolayers. Practically independent of pH, the effect of chitosan on cholesterol was accounted for by the formation of cholesterol−chitosan hydrogen bonds. Chitosan of lower molecular weight facilitated the interactions with all the three lipids studied. The results obtained may be helpful in identifying the mode of antibacterial activity of chitosan versus other modes that involve the disturbance of cell life cycles.

Silver/chitosan/cellulose fibers foam composites: from synthesis to antibacterial properties
Eric Guibal, Simon Cambe, Sandrine Bayle, Jean-Marie Taulemesse, Thierry Vincent
DOI: http://dx.doi.org/10.1016/j.jcis.2012.10.057
To appear in: Journal of Colloid and Interface Science 393 (2013) 411-420

Chitosan, associated with cellulose fibers, can be used for elaborating sponge-like structures (membranes, foams) for the binding of silver ions. The composite material has
very promising antibacterial properties versus Pseudomonas aeruginosa (Gram-) >> Escherichia coli (Gram-) > Staphylococcus hominis (Gram+) >> Staphylococcus aureus (Gram+). The amount of silver required for bactericidal effect is quite low (below 0.1 mg per disc, this means less than 6 mgAg g⁻¹) in antibiogram-type test but also for the treatment of water suspensions (in dynamic mode with water recycling). The presence of cellulose fibers improves the efficiency of metal binding, due to chitosan dispersion and enhancement of the availability and accessibility of amine groups. Silver nanoparticles (about 100 nm) were observed by scanning electron microscopy. The photo-reduction (exposure to sun light or UV lamp) led to the partial aggregation of silver nanoparticles: metal ions that were released tended to aggregate at the surface of the material.

Single-step electrochemical deposition of antimicrobial orthopaedic coatings based on a bioactive glass/chitosan/nano-silver composite system


Acta Biomaterialia 9 (2013) 7469–7479

Composite orthopaedic coatings with antibacterial capability containing chitosan, Bioglass_ particles (9.8 lm) and silver nanoparticles (Ag-np) were fabricated using a single-step electrophoretic deposition (EPD) technique, and their structural and preliminary in vitro bactericidal and cellular properties were investigated. Stainless steel 316 was used as a standard metallic orthopaedic substrate. The coatings were compared with EPD coatings of chitosan and chitosan/Bioglass_. The ability of chitosan as both a complexing and stabilizing agent was utilized to form uniformly deposited Ag-np. Due to the presence of Bioglass_ particles, the coatings were bioactive in terms of forming carbonated hydroxyapatite in simulated body fluid (SBF). Less than 7 wt.% of the incorporated silver was released over the course of 28 days in SBF and the possibility of manipulating the release rate by varying the deposition order of coating layers was shown. The low released concentration of Ag ions (<2.5 ppm) was efficiently antibacterial against Staphylococcus aureus up to 10 days. Although chitosan and chitosan/Bioglass_ coating supported proliferation of MG-63 osteoblast-like cells up to 7 days of culture, chitosan/Bioglass_/Ag-np coatings containing 342 lg of Ag-np showed cytotoxic effects. This was attributed to the relatively high concentration of Ag-np incorporated in the coatings.

Sonochemical Coating of Textiles with Hybrid ZnO/Chitosan Antimicrobial Nanoparticles

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ABSTRACT: Textiles are good substrates for growth of microorganisms especially under moisture and temperature conditions found in hospitals. Microbial shedding from the body occurs continuously at contact of the patient with textile materials used in medical practices, contributing to the occurrence of hospital acquired infections. Thus, the use of efficient antimicrobial textiles is necessary to prevent the transfer of pathogens and the infection incidence. In this work, hybrid antimicrobial coatings were generated on cotton fabrics by means of a one-step simultaneous sonochemical deposition of ZnO nanoparticles (NPs) and chitosan. The process was further optimized in terms of reagents concentration and processing time in order to improve the antibacterial properties of the fabric and ensure their biocompatibility. The highest antibacterial activity of the fabrics against two medically relevant bacterial species was achieved in a 30 min sonochemical coating process using 2 mM ZnO NPs suspension. When chitosan was simultaneously deposited with the same amount of ZnO, the obtained hybrid NPs coating displayed higher by 48 and 17% antibacterial activity against Staphylococcus aureus and Escherichia coli, respectively. The presence of biopolymer also improved the durability of the antimicrobial effect of the coatings by 21% for Staphylococcus aureus and 40% for Escherichia coli, evaluated after applying multiple washing cycles at hospital laundering regimes. Finally, 87% biocompatibility improvement supported by
fibroblast viability was observed for the hybrid ZnO/chitosan coating compared to the steady decrease of cells viability over one week in contact with the fabrics coated with ZnO alone.

Synthesis, characterization, and antibacterial activity of N,O-quaternary ammonium chitosan
Tao Xu, Meihua Xin, Mingchun Li, Huili Huang, Shengquan Zhou, Juezhao Liu
Carbohydrate Research 346 (2011) 2445–2450
N,N,N-Trimethyl O-(2-hydroxy-3-trimethylammonium propyl) chitosans (TMHTMAPC) with different degrees of O-substitution were synthesized by reacting O-methyl-free N,N,N-trimethyl chitosan (TMC) with 3-chloro-2-hydroxy-propyl trimethyl ammonium chloride (CHPTMAC). The products were characterized by 1H NMR, FTIR and TGA, and investigated for antibacterial activity against Staphylococcus aureus and Escherichia coli under weakly acidic (pH 5.5) and weakly basic (pH 7.2) conditions. TMHTMAPC exhibited enhanced antibacterial activity compared with TMC, and the activity of TMHTMAPC increased with an increase in the degree of substitution. Divalent cations (Ba2+ and Ca2+) strongly reduced the antibacterial activity of chitosan, O-carboxymethyl chitosan and N,N,N-trimethyl-O-carboxymethyl chitosan, but the repression on the antibacterial activity of TMC and TMHTMAPC was weaker. This indicates that the free amino group on chitosan backbone is the main functional group interacting with divalent cations. The existence of 100 mM Na+ slightly reduced the antibacterial activity of both chitosan and its derivatives.
References on Peptides and peptides in monolayers

Peptides

2012- Luminiscence


A study of HIV-1 FP inhibition by GBV-C peptides using lipid nano-assemblies

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abstract

Langmuir–Blodgett films (LBs) and supported lipid bilayers (SLBs) of dimyristoylphosphatidylcholine(DMPC)–dimyristoylphosphatidylserine (DMPS) (3:2) were used to investigate the way that a GBV-Cpeptide (P45) inhibits the immunodeficiency human virus fusion peptide (HIV-1 FP) action at membranelevel. Supported lipid bilayers (SLBs) were prepared by direct adsorption of a liposomal dispersion on solid mica slides and Langmuir–Blodgett films (LBs) by a deposition of a monolayer onto mica solid surface at different compression pressures. The behaviour of P45, HIV-1 FP and a mixture of P45 and HIV-1 FP(1:1) were monitored in the two phospholipid membrane models by fluorescence microscopy (FM) and atomic force microscopy (AFM). Experiments with SLBs confirmed that P45 inhibited HIV-1 FP action in vitro. LBs obtained at 10 and 25 mN m−1 confirmed different lipid interactions for DMPC/DMPS (3:2)in combination with either P45 (8:2), HIV-1 FP (8:2), or P45 and HIV-1 FP (8:1:1). The P45 peptide was confirmed to modulate the action of HIV-1 FP. Furthermore, FM and AFM images showed that HIV-1 FP had a pressure-independent membrane-level behaviour when compared with P45 and the P45 + HIV-1 FP (1:1) mixture. This mixture also had dramatic effects on the appearance of liquid expanded (LE)–liquid condensed (LC) phase coexistence as shown by FM and AFM.

Antimicrobial peptides and induced membrane curvature: Geometry, coordination chemistry, and molecular engineering

Nathan W. Schmidt, Gerard C.L. Wong


Short cationic, amphipathic antimicrobial peptides are multi-functional molecules that have roles in host defense as direct microbicides and modulators of the immune response. While a general mechanism of microbialicidal activity involves the selective disruption and permeabilization of cell membranes, the relationships between peptide sequence and membrane activity are still under investigation. Here, we review
Antimicrobial peptides: premises and promises
K.V.R. Reddy, R.D. Yedery, C. Aranha


Antimicrobial peptides (AMPs) are an important component of the natural defences of most living organisms against invading pathogens. These are relatively small (<10 kDa), cationic and amphipathic peptides of variable length, sequence and structure. During the past two decades several AMPs have been isolated from a wide variety of animals, both vertebrates and invertebrates, and plants as well as from bacteria and fungi. Most of these peptides are obtained from different sources like macrophages, neutrophils, epithelial cells, haemocytes, fat body, reproductive tract, etc. These peptides exhibit broad-spectrum activity against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, protozoa, yeast, fungi and viruses. A few peptides have also been found to be cytotoxic to sperm and tumour cells. AMPs are classified based on the three dimensional structural studies carried out with the help of NMR. The peptides are broadly classified into five major groups namely (a) peptides that form _-helical structures, (b) peptides rich in cysteine residues, (c) peptides that form _-sheet, (d) peptides rich in regular amino acids namely histatin, arginine and proline and (e) peptides composed of rare and modified amino acids. Most of these peptides are believed to act by disrupting the plasma membrane leading to the lysis of the cell. AMPs have been found to be excellent candidates for developing novel antimicrobial agents and a few of these peptides show antimicrobial activity against pathogens causing sexually transmitted infection (STI), including HIV/HSV. Peptides, namely magainin and nisin have been shown to demonstrate contraceptive properties in vitro and in vivo. A few peptides have already entered clinical trials for the treatment of impetigo, diabetic foot ulcers and gastric helicobacter infections. In this review, we discuss the source, structures and mode of action with special reference to therapeutic considerations of various AMPs.

Amino acid–based surfactants: New antimicrobial agents. Historical perspective
A. Pinazo, M.A. Manresa, A.M. Marques, M. Bustelo, M.J. Espuny, L. Perez

Advances in Colloid and Interface Science 228 (2016) 17–39

The rapid increase of drug resistant bacteriamakes necessary the development of newantimicrobial agents. Synthetic amino acid-based surfactants constitute a promising alternative to conventional antimicrobial compounds given that they can be prepared from renewable raw materials. In this review, we discuss the structural features that promote antimicrobial activity of amino acid-based surfactants. Monocatenary, dicatenary and gemini surfactants that contain different amino acids on the polar head and show activity against bacteria are revised. The synthesis and basic physico-chemical properties have also been included.

Antimicrobial Activity and Toxicity of the Major Lipopeptide Components of Polymyxin B and Colistin: Last-Line Antibiotics against Multidrug-Resistant Gram-Negative Bacteria
Kade D. Roberts, Mohammad A. K. Azad, Jiping Wang, Andrew S. Horne, Philip E. Thompson, Roger L. Nation, Tony Velkov, and Jian Li

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ACS Infect. Dis. 2015, 1, 568–575

Polymyxin B and colistin are currently used as a “last-line” treatment for multidrug-resistant Gram-negative bacteria. However, very little is known about the pharmacological differences between polymyxin B1, polymyxin B2, colistin A, and colistin.
B, the major cyclic lipopeptide components present in polymyxin B and colistin products. Here, we report on the in vitro and in vivo antimicrobial activity and toxicity of these major lipopeptide components. All four lipopeptides had comparable minimum inhibitory concentrations (MICs) (<0.125–4 mg/L) against a panel of clinical Gram-negative isolates. They also had comparable in vivo antimicrobial activity (Δlog10 colony-forming units (CFU)/mL > −3) and nephrotoxicity (mild to moderate histological damage) in mouse models. However, polymyxin B1 and colistin A showed significantly higher (>3-fold) in vitro apoptotic effect on human kidney proximal tubular HK-2 cells than polymyxin B2 and colistin B, respectively. Compared to the commercial polymyxin and colistin products, the individual lipopeptide components had slightly more in vivo antimicrobial activity. Our results highlight the need to reassess pharmacopoeial standards for polymyxin B and colistin and to standardize the composition of the different commercial products of polymyxin antibiotics.

Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria
Giovanna Batoni, Giuseppantonio Maisetta, Semih Esin
Biochimica et Biophysica Acta 1858 (2016) 1044–1060

Biofilm-associated infections represent one of the major threats of modern medicine. Biofilm-forming bacteria are encased in a complex mixture of extracellular polymeric substances (EPS) and acquire properties that render them highly tolerant to conventional antibiotics and host immune response. Therefore, there is a pressing demand of new drugs active against microbial biofilms. In this regard, antimicrobial peptides (AMPs) represent an option taken increasingly in consideration. After dissecting the peculiar biofilm features that may greatly affect the development of new anti-biofilm drugs, the present article provides a general overview of the rationale behind the use of AMPs against biofilms of medically relevant bacteria and on the possible mechanisms of AMPantibiofilm activity. An analysis of the interactions of AMPs with biofilm components, especially those constituting the EPS, and the obstacles and/or opportunities that may arise from such interactions in the development of new AMP-based antibiofilm strategies is also presented and discussed. This article is part of a Special Issue entitled: Antimicrobial Peptides edited by Karl Lohner and Kai Hilpert.

ANTIMICROBIAL PEPTIDES: PORE FORMERS OR METABOLIC INHIBITORS IN BACTERIA?
Kim A. Brogden
Article in Nature Reviews Microbiology · April 2005
DOI: 10.1038/nrmicro1098 · Source: PubMed
238 | MARCH 2005 | VOLUME 3 www.nature.com/reviews/micro

Abstract | Antimicrobial peptides are an abundant and diverse group of molecules that are produced by many tissues and cell types in a variety of invertebrate, plant and animal species. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers to form pores by ‘barrel-stave’, ‘carpet’ or ‘toroidal-pore’ mechanisms. Although these models are helpful for defining mechanisms of antimicrobial peptide activity, their relevance to how peptides damage and kill microorganisms still need to be clarified.
Recently, there has been speculation that transmembrane pore formation is not the only mechanism of microbial killing. In fact several observations suggest that translocated peptides can alter cytoplasmic membrane septum formation, inhibit cell-wall synthesis, inhibit nucleic-acid synthesis, inhibit protein synthesis or inhibit enzymatic activity. In this review the different models of antimicrobial-peptide-induced pore formation and cell killing are presented.

Antimicrobial functionalization of wool: assessment of the effect of Cecropin-B and [Ala5]-Tritrp7 antimicrobial peptides
Cláudia Mouro and Isabel C. Gouveia
This investigation provides a new strategy to impart antimicrobial properties into wool-based materials using Cecropin-B and [Ala5]-Tritrp7 antimicrobial peptides (AMPs). The process was conducted using exhaustion method at 40 °C for 1–3 h. The presence of the AMPs in the modified-wool samples was confirmed by colorimetric assay of Bradford reagent and possible changes in the morphology of the fibers and damage to its surface were analyzed by scanning electron microscopy. Results showed that 1 h were long enough for the functionalization to occur effectively and that the morphology of the fibers was not influenced by the functionalization process. Furthermore, the antimicrobial activity of the AMPs applied on wool was assessment by JIS L 1902-2002 against Staphylococcus aureus (ATCC 6538) and Klebsiella pneumoniae (ATCC 4352). The results showed that both AMPs have a high reduction in bacterial growth (Cecropin-B resulting in 71.67% reduction against S. aureus and 85.95% against K. pneumoniae. While [Ala5]-Tritrp7 resulting in 66.74% reduction against S. aureus and 88.65% against K. pneumoniae).

Azithromycin Synergizes with Cationic Antimicrobial Peptides to Exert Bactericidal and Therapeutic Activity Against Highly Multidrug-Resistant Gram-Negative Bacterial Pathogens
Leo Lin, Poochit Nonejuie, Jason Munguia, Andrew Hollands, Joshua Olson, Quang Dam, Monika Kumaraswamy, Heriberto Rivera Jr., Ross Corriden, Manfred Rohde, Mary E. Hensler, Michael D. Burkart, Joe Pogliano, George Sakoulas, Victor Nizet
EBioMedicine 2 (2015) 690–698

Antibiotic resistance poses an increasingly grave threat to the public health. Of pressing concern, rapid spread of carbapenem-resistance among multidrug-resistant (MDR) Gram-negative rods (GNR) is associated with few treatment options and high mortality rates. Current antibiotic susceptibility testing guiding patient management is performed in a standardized manner, identifying minimum inhibitory concentrations (MIC) in bacteriologic media, but ignoring host immune factors. Lacking activity in standard MIC testing, azithromycin (AZM), the most commonly prescribed antibiotic in the U.S., is never recommended for MDR GNR infection. Here we report a potent bactericidal action of AZM against MDR carbapenem-resistant isolates of Pseudomonas aeruginosa, Klebsiella pneumoniae, and Acinetobacter baumannii. This pharmaceutical activity is associated with enhanced AZM cell penetration in eukaryotic tissue culture media and striking multi-fold synergies with host cathelicidin antimicrobial peptide LL-37 or the last line antibiotic colistin. Finally, AZM monotherapy exerts clear therapeutic effects in murine models of MDR GNR infections. Our results suggest that AZM, currently ignored as a treatment option, could benefit patients with MDR GNR infections, especially in combination with colistin.

Bacteria-targeted biomaterials: Glycan-coated microspheres to bind Helicobacter pylori
Inês C. Gonçalves, Ana Magalhães, Ana M. S. Costa, José R. Oliveira, Patrícia C. Henriques, Paula Gomes, Celso A. Reis, M. Cristina L. Martins
Acta Biomaterialia 33 (2016) 40–50

Gastric cancer is the third leading cause of cancer-related deaths worldwide and Helicobacter pylori (H. pylori) persistent infection has been pointed as a causative agent of this disease. Current antibiotic based treatments to eradicate this bacterium fail in 20% of the patients, potentially leaving 140 million people in the world without alternative therapy. It is herein proposed the use of azide–alkyne coupling (“click chemistry”) to produce glycan-coated mucodhesive microspheres that bind and remove the H. pylori adherent to the gastric mucosa through specific bacterial adhesin–glycan interactions. Glycan immobilization is performed via chitosan’s primary alcohol group, rather than the more reactive primary amines in order to preserve the amine groups that confer chitosan its mucodhesiveness. It is shown that chitosan microspheres decorated with Lewis b glycans (Leb-Mic) bind specifically to H. pylori strains expressing the BabA adhesin (strains recognized as highly pathogenic) (≤230 bacteria/microsphere), are non-cytotoxic, are retained in the stomach of C57BL/6 mice for around 1.5 h. Also, these Leb-Mic are able to prevent and remove H. pylori adhesion to gastric mucosa expressing the same glycan, in tissue sections from mice and hamangula gastric mucosa (in vitro) and in fresh mice stomachs (ex vivo). These results provide proof-of-concept on the potential of glycandecorated microspheres as an innovative therapeutic strategy against H. pylori and highlight the prospective of using targeted biomaterials to fight gastrointestinal infection.
Broad Activity against Porcine Bacterial Pathogens Displayed by Two Insect Antimicrobial Peptides Moricin and Cecropin B
Han Hu, Chunmei Wang, Xiaozhen Guo, Wentao Li, Yang Wang, and Qigai He
DOI/10.1007/s10059-013-2132-0
In response to infection, insects produce a variety of antimicrobial peptides (AMPs) to kill the invading pathogens.
To study their physicochemical properties and bioactivities for clinical and commercial use in the porcine industry, we chemically synthesized the mature peptides Bombyx mori moricin and Hyalophora cecropia cecropin B. In this paper, we described the antimicrobial activity of the two AMPs. Moricin exhibited antimicrobial activity on eight strains tested with minimal inhibitory concentration values (MICs) ranging between 8 and 128 μg/ml, while cecropin B mainly showed antimicrobial activity against the Gram-negative strains with MICs ranging from 0.5 to 16 μg/ml.
Compared to the potent antimicrobial activity these two AMPs displayed against most of the bacterial pathogens tested, they exhibited limited hemolytic activity against porcine red blood cells. The activities of moricin and cecropin B against Haemophilus parasuis SH 0165 were studied in further detail. Transmission electron microscopy (TEM) of moricin and cecropin B treated H. Parasuis SH 0165 indicated extensive damage to the membranes of the bacteria. Insights into the probable mechanism utilized by moricin and cecropin B to eliminate pathogens are also presented. The observations from this study are important for the future application of AMPs in the porcine industry.

Collagen Tethering of Synthetic Human Antimicrobial Peptides Cathelicidin LL37 and its Effects on Antimicrobial Activity and Cytotoxicity

- Lindsay D. Lozeau, Jonian Grosha, Denis Kole, Fioleda Prifti, Tanja Dominko
- Terri A. Camesano, Marsha W. Rolle
- Acta Biomaterialia in press
- Available online 23 December 2016
- http://dx.doi.org/10.1016/j.actbio.2016.12.047

Wound infections, particularly of chronic wounds, pose a substantial challenge for designing antimicrobial dressings that are both effective against pathogens, and do not interfere with wound healing. Due to their broad-spectrum antimicrobial and immunomodulatory activities, naturally-occurring antimicrobial peptides (AMPs) are promising alternative treatments. However, their cytotoxicity at high concentrations and poor stability hinders their clinical use. To mitigate these undesirable properties, we investigated the effects of tethering human AMP cathelicidin LL37 to collagen, one of the main extracellular matrix proteins in wound sites, secreted by fibroblasts, and in commercially-available wound dressings. The active domain of human AMP cathelicidin, LL37, and two chimeric peptides containing LL37 fused to collagen binding domains (derived from collagenase – cCBD-LL37 or fibronectin - fCBD-LL37) were synthesized and adsorbed to PURACOL® type I collagen scaffolds. After 14 days, 73%, 81% and 99% of LL37, cCBD-LL37 and fCBD-LL37, respectively, was retained on the scaffolds and demonstrated undiminished antimicrobial activity when challenged with both Gram-positive and Gram-negative bacterial strains. Loaded scaffolds were not cytotoxic to fibroblasts despite retaining peptides at concentrations 24 times higher than the reported cytotoxic concentrations in solution. These findings indicate that biopolymer-tethered AMPs may represent a viable alternative for preventing and treating wound infection while also supporting tissue repair.
Design and surface immobilization of short anti-biofilm peptides
Biswajit Mishra, Tamara Lushnikova, Radha M. Golla, Xiuqing Wang, Guangshun Wang
DOI: http://dx.doi.org/10.1016/j.actbio.2016.11.061
To appear in: Acta Biomaterialia 49 (2017) 316-328

ABSTRACT
Short antimicrobial peptides are essential to keep us healthy and their lasting potency can inspire the design of new types of antibiotics. This study reports the design of a family of eight-residue tryptophan-rich peptides (TetraF2W) obtained by converting the four phenylalanines in temporin-SHf to tryptophans. The temporin-SHf template was identified from the antimicrobial peptide database (http://aps.unmc.edu/AP). Remarkably, the double arginine variant (TetraF2W-RR) was more effective in killing methicillin-resistant Staphylococcus aureus (MRSA) USA300, but less cytotoxic to human skin HaCat and kidney HEK293 cells, than the lysine-containing dibasic combinations (KR, RK and KK). Killing kinetics and fluorescence spectroscopy suggest membrane targeting of TetraF2W-RR, making it more difficult for bacteria to develop resistance. Because established biofilms on medical devices are difficult to remove, we chose to covalently immobilize TetraF2W-RR onto the polyethylene terephthalate (PET) surface to prevent biofilm formation. The successful surface coating of the peptide is supported by FT-IR and XPS spectroscopies, chemical quantification, and antibacterial assays. This peptide-coated surface indeed prevented S. aureus biofilm formation with no cytotoxicity to human cells. In conclusion, TetraF2W-RR is a short Trp-rich peptide with demonstrated antimicrobial and anti-biofilm potency against MRSA in both the free and immobilized forms. Because these short peptides can be synthesized cost effectively, they may be developed into new antimicrobial agents or used as surface coating compounds.

Highly Efficient Macromolecule-Sized Poration of Lipid Bilayers by a Synthetically Evolved Peptide
Gregory Wiedman, Taylor Fuselier, Jing He, Peter C. Searson, Kalina Hristova, and William C. Wimley
dx.doi.org/10.1021/ja500462s | J. Am. Chem. Soc. 2014, 136, 4724–4731

ABSTRACT: Peptides that self-assemble, at low concentration, into bilayer-spanning pores which allow the passage of macromolecules would be beneficial in multiple areas of biotechnology. However, there are few, if any, natural or designed peptides that have this property. Here we show that the 26-residue peptide “MelP5”, a synthetically evolved gain-of-function variant of the bee venom lytic peptide melittin identified in a high-throughput screen for small molecule leakage, enables the passage of macromolecules across bilayers under conditions where melittin and other pore-forming peptides do not. In surface-supported bilayers, MelP5 forms unusually high conductance, equilibrium pores at peptide:lipid ratios as low as 1:25000. The increase in bilayer conductance due to MelP5 is dramatically higher, per peptide, than the increase due to the parent sequence of melittin or other peptide pore formers. Here we also develop two novel assays for macromolecule leakage from vesicles, and we use them to characterize MelP5 pores in bilayers. We show that MelP5 allows the passage of macromolecules across vesicle
membranes at peptide:lipid ratios as low as 1:500, and under conditions where neither osmotic lysis nor gross vesicle destabilization occur. The macromolecule-sized, equilibrium pores formed by MelP5 are unique as neither melittin nor other pore-forming peptides release macromolecules significantly under the same conditions. MelP5 thus appears to belong to a novel functional class of peptide that could form the foundation of multiple potential biotechnological applications.

High-Resolution Structures and Orientations of Antimicrobial Peptides Piscidin 1 and Piscidin 3 in Fluid Bilayers Reveal Tilting, Kinking, and Bilayer Immersion
B. Scott Perrin, Jr., Ye Tian, Riqiang Fu, Christopher V. Grant, Eduard Y. Chekmenev, William E. Wieczorek, Alexander É. Dao, Robert M. Hayden, Caitlin M. Burzynski, Richard M. Venable, Mukesh Sharma, Stanley J. Opella, Richard W. Pastor, and Myriam L. Cotten

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ABSTRACT: While antimicrobial peptides (AMPs) have been widely investigated as potential therapeutics, high-resolution structures obtained under biologically relevant conditions are lacking. Here, the high-resolution structures of the homologous 22-residue long AMPs piscidin 1 (p1) and piscidin 3 (p3) are determined in fluid-phase 3:1 phosphati- dylcholine/phosphatidylglycerol (PC/PG) and 1:1 phosphatidylcholine/dipalmitoylphosphatidylglycerol (PE/PG) bilayers to identify molecular features important for membrane destabilization in bacterial cell membrane mimics. Structural refinement of 1H−15N dipolar couplings and 15N chemical shifts measured by oriented sample solid-state NMR and all-atom molecular dynamics (MD) simulations provide structural and orientational information of high precision and accuracy about these interfacially bound α-helical peptides. The tilt of the helical axis, τ, is between 83° and 93° with respect to the bilayer normal for all systems and analysis methods. The average azimuthal rotation, ρ, is 235°, which results in burial of hydrophobic residues in the bilayer. The refined NMR and MD structures reveal a slight kink at G13 that delineates two helical segments characterized by a small difference in their τ angles (<10°) and significant difference in their ρ angles (~25°). Remarkably, the kink, at the end of a G(X)4G motif highly conserved among members of the piscidin family, allows p1 and p3 to adopt ρ angles that maximize their hydrophobic moments. Two structural features differentiate the more potent p1 from p3: p1 has a larger ρ angle and less N-terminal fraying. The peptides have comparable depths of insertion in PC/PG, but p3 is 1.2 Å more deeply inserted than p1 in PE/PG. In contrast to the ideal α-helical structures typically assumed in mechanistic models of AMPs, p1 and p3 adopt disrupted α-helical backbones that correct for differences in the amphipathicity of their N- and C-ends, and their centers of mass lie ~1.2−3.6 Å below the plane defined by the C2 atoms of the lipid acyl chains.

How Changes in the Sequence of the Peptide CLPFFD-NH2 Can Modify the Conjugation and Stability of Gold Nanoparticles and Their Affinity for -Amyloid Fibrils
Ivonne Olmedo, Eyleen Araya, Fausto Sanz, Elias Medina, Jordi Arbiol, Pedro Toledo, Alejandro A’lvarez-Lueje, Ernest Giralt, and Marcelo J. Kogan

In a previous work, we studied the interaction of -amyloid fibrils (A_) with gold nanoparticles (AuNP) conjugated with the peptide CLPFFD-NH2. Here, we studied the effect of changing the residue sequence of the peptide CLPFFD-NH2 on the efficiency of conjugation to AuNP, the stability of the conjugates, and the affinity of the conjugates to the A__fibrils. We conjugated the AuNP with CLPFFD-NH2 isomeric peptides (CDLPFF-NH2 and CLPDFF-NH2) and characterized the resulting conjugates with different techniques including UV-Vis, TEM, EELS, XPS, analysis of amino acids, agarose gel electrophoresis, and CD. In addition, we determined the proportion of AuNP bonded to the A__fibrils by ICP-MS. AuNP-CLPFFD-NH2 was the most stable of the conjugates and presented more affinity for A__fibrils with
respect to the other conjugates and bare AuNP. These findings help to better understand the way peptide sequences affect conjugation and stability of AuNP and their interaction with Aβ fibrils. The peptide sequence, the steric effects, and the charge and disposition of hydrophilic and hydrophobic residues are crucial parameters when considering the design of AuNP peptide conjugates for biomedical applications.

High-density antimicrobial peptide coating with broad activity and low cytotoxicity against human cells

Akhilesh Rai, Sandra Pinto, Marta B. Evangelista, Helena Gil, Silvar Kallip, Mario G.S. Ferreira, Lino Ferreira

Acta Biomaterialia 33 (2016) 64–77

Medical device-associated infections are a multi-billion dollar burden for the worldwide healthcare systems. The modification of medical devices with non-leaching coatings capable of killing microorganisms on contact is one of the strategies being investigated to prevent microorganism colonization. Here we developed a robust antimicrobial coating based on the chemical immobilization of the antimicrobial peptide (AMP), cecropin-melittin (CM), on gold nanoparticles coated surfaces. The concentration of AMP immobilized (110 lg/cm²) was higher than most of the studies reported so far (<10 lg/cm²). This translated onto a coating with high antimicrobial activity against Gram positive and negative bacteria sp., as well as multi-drug resistant bacteria. Studies with E. coli reporter bacteria showed that these coatings induced the permeability of the outer membrane of bacteria in less than 5 min and the inner membrane in approximately 20 min. Importantly, the antimicrobial properties of the coating are maintained in the presence of 20% (v/v) human serum, and have low probability to induce bacteria resistance. We further show that coatings have low toxicity against human endothelial and fibroblast cells and is hemocompatible since it does not induce platelet and complement activation. The antimicrobial coating described here may be promising to prevent medical device-associated infections.

Implications of lipid monolayer charge characteristics on their selective interactions with a short antimicrobial peptide


Colloids and Surfaces B: Biointerfaces 150 (2017) 308–316

Many antimicrobial peptides (AMPs) target bacterial membranes and they kill bacteria by causing structural disruptions. One of the fundamental issues however lies in the selective responses of AMPs to different cell membranes as a lack of selectivity can elicit toxic side effects to mammalian host cells. A key difference between the outer surfaces of bacterial and mammalian cells is the charge characteristics. We report a careful study of the binding of one of the representative AMPs, with the general sequence G(IKK)4I-NH2(G4), to the spread lipid monolayers of DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and DPPG (1,2-dipalmitoyl-sn-glycero-3-phospho-(1-_rac-glycerol) (sodiumsalt)) mimicking the charge difference between them, using the combined measurements from Langmuir trough, Brewster angle microscopy (BAM) and neutron reflection (NR). The difference in pressure riseup peptide addition into the subphase clearly demonstrated the different interactions arising from different lipid charge features. Morphological changes from the BAM imaging confirmed the association of the peptide into the lipid monolayers, but there was little difference between them. However, NR studies revealed that the peptide bound 4 times more onto the DPPG monolayer than onto the DPPC mono-layer. Importantly, whilst the peptide could only be associated with the head groups of DPPC it was wellpenetrated into the entire DPPG monolayer, showing that the electrostatic interaction strengthened the hydrophobic interaction and that the combined molecular interactive processes increased the power of G4 in disrupting the charged membranes. The results are discussed in the context of general antibacterial actions as observed from other AMPs and membrane lytic actions.

Inhibition of HIV infection by caerin1 antimicrobial peptides

Scott VanCompernolle, Patricia B. Smith, John H. Bowie, Michael J. Tyler, Derya Unutmaz, Louise A. Rollins-Smith

Peptides 71 (2015) 296–303
The major mode of transmission of the human immunodeficiency virus (HIV) is by sexual intercourse. In the effort to halt the spread of HIV, one measure that holds great promise is the development of effective microbicidal compounds that can prevent transmission. Previously, we showed that several amphibian antimicrobial peptides (AMPs) completely inhibit HIV infection of T cells while maintaining good viability of the T cell targets. These peptides also inhibited the transfer of HIV by dendritic cells (DCs) to T cells when added up to 8 h after virus exposure. Here, we report on the anti-HIV activity of 18 additional structurally related caerin 1 family peptides in comparison with our previous best candidate caerin 1.9. Nine peptides were equally effective or more effective in the inhibition of T cell infection and disruption of the HIV envelope compared to caerin 1.9. Of those, three peptides (caerin 1.2, caerin 1.10, and caerin 1.20) exhibited excellent inhibition at low concentrations (12–25 μM) and limited toxicity against target T cells and endocervical epithelial cells. There was a direct correlation between the effectiveness of the peptides in inhibition of the viral envelope and their capacity to inhibit infection. Thus, several additional caerin 1 family peptides inhibit HIV infection and would be good candidates for inclusion in microbicidal formulations.

Influence of the Bilayer Composition on the Binding and Membrane Disrupting Effect of Polybia-MP1, an Antimicrobial Mastoparan Peptide with Leukemic T-Lymphocyte Cell Selectivity
Marcia Perez dos Santos Cabrera, Manoel Arcisio-Miranda, Renata Gorjão, Natália Bueno Leite, Bibiana Monson de Souza, Rui Curi, Joaquim Procopio, João Ruggiero Neto, and Mario Sérgio Palma

This study shows that MP-1, a peptide from the venom of the Polybia paulista wasp, is more toxic to human leukemic T lymphocytes than to human primary lymphocytes. By using model membranes and electrophysiology measurements to investigate the molecular mechanisms underlying this selective action, the pore-like activity of MP-1 was identified with several bilayer compositions. The highest average conductance was found in bilayers formed by phosphatidylcholine or a mixture of phosphatidylcholine and phosphatidylserine (70:30). The presence of cholesterol or cardiolipin substantially decreases the MP-1 pore activity, suggesting that the membrane fluidity influences the mechanism of selective toxicity. The determination of partition coefficients from the anisotropy of Trp indicated higher coefficients for the anionic bilayers. The partition coefficients were found to be 1 order of magnitude smaller when the bilayers contain cholesterol or a mixture of cholesterol and sphingomyelin. The blue shift fluorescence, anisotropy values, and Stern–Volmer constants are indications of a deeper penetration of MP-1 into anionic bilayers than into zwitterionic bilayers. Our results indicate that MP-1 prefers to target leukemic cell membranes, and its toxicity is probably related to the induction of necrosis and not to DNA fragmentation. This mode of action can be interpreted considering a number of bilayer properties like fluidity, lipid charge, and domain formation. Cholesterol-containing bilayers are less fluid and less charged and have a tendency to form domains. In comparison to healthy cells, leukemic T lymphocyte membranes are deprived of this lipid, resulting in decreased peptide binding and lower conductance. We showed that the higher content of anionic lipids increases the level of binding of the peptide to bilayers. Additionally, the absence of cholesterol resulted in enhanced pore activity. These findings may drive the selective toxicity of MP-1 to Jurkat cells.

Integration of antimicrobial peptides with gold nanoparticles as unique non-viral vectors for gene delivery to mesenchymal stem cells with antibacterial activity
Li-Hua Peng, Yan-Fen Huang, Chen-Zhen Zhang, Jie Niu, Ying Chen, Yang Chu, Zhi-Hong Jiang, Jian-Qing Gao, Zheng-Wei Mao

Gold nanoparticles (AuNPs) have emerged as attractive non-viral gene vectors. However, their application in regenerative medicine is still limited partially due to a lack of an intrinsic capacity to transfect difficult-to-transfect cell types. In this study, we evaluated the potential of integrative AuNP delivery of antimicrobial peptides for the treatment of infections in mesenchymal stem cells (MSCs). The C. difficile antimicrobial peptide (AMP) was covalently conjugated to AuNPs to form the conjugate AuNPAMP. These AuNPAMP conjugates were able to deliver the AMP into MSCs in a dose-dependent manner. Furthermore, AuNPAMP conjugates were able to deliver the AMP into MSCs in a dose-dependent manner. Additionally, the absence of cholesterol resulted in enhanced pore activity. These findings may drive the selective toxicity of MP-1 to Jurkat cells.
transfect cells such as primary cells or stem cells. In current study, we report the synthesis of antimicrobial peptide conjugated cationic AuNPs (AuNPs@PEP) as highly efficient carriers for gene delivery to stem cells with antibacterial ability. The AuNPs@PEP integrate the advantages of cationic AuNPs and antibacterial peptides: the presence of cationic AuNPs can effectively condense DNA and the antimicrobial peptides are essential for the cellular & nucleus entry enhancement to achieve high transfection efficiency and antibacterial ability. As a result, antimicrobial peptides conjugated AuNPs significantly promoted the gene transfection efficiency in rat mesenchymal stem cells than pristine AuNPs, with a similar extent to those expressed by TAT (a well-known cell-penetrating peptide) modified AuNPs. More interestingly, the combinational system has better antibacterial ability than free antimicrobial peptides in vitro and in vivo, possibly due to the high density of peptides on the surface of AuNPs. Finally we present the concept-proving results that AuNPs@PEP can be used as a carrier for in vivo gene activation in tissue regeneration, suggesting its potential as a multifunctional system with both gene delivery and antibacterial abilities in clinic.

Interaction between antimicrobial peptides and mycobacteria
Thomas Gutsmann
Biochimica et Biophysica Acta 1858 (2016) 1034–1043
Mycobacteria can cause different severe health problems, including tuberculosis (TB). The treatment of TB with conventional antibiotics is successful, however, the number of multi-drug and extensively-drug resistant Mycobacterium tuberculosis strains increases. Moreover, many classical antimycobacterial antibiotics have severe side effects. Therefore, antimicrobial peptides (AMPs) seem to be good candidates for new therapeutic strategies. On the one hand AMPs can be used as a single drug or in combination with conventional antibiotics to directly kill mycobacteria, or on the other hand to act as immunostimulatory agents. This review summarizes the findings on the role of endogenous human AMPs being involved in TB, the antimycobacterial activity of various AMPs, and the molecular modes of action. Most active AMPs interact with the mycobacterial cell envelope and in particular with the mycomembrane and the plasma membrane. The mycomembrane is a very rigid membrane probably leading to a lower activity of the AMPs against mycobacteria as compared to other Gram-negative or Gram-positive bacteria. For some AMPs also other targets have been identified. Because of the complex environment of intracellular mycobacteria being trapped in the phagosome, within the macrophage, within the granuloma, within the lung, the external administration of AMPs in the latent phase of TB is a challenge. However, in the acute phase the AMPs can attack mycobacteria in a direct way. This article is part of a Special Issue entitled: Antimicrobial peptides edited by Karl Lohner and Kai Hilpert.

Interaction of cyclic and linear Labaditin peptides with anionic and zwitterionic micelles
S.C. Barbosa, E.M. Cilli, L.G. Dias, C.A. Fuzo, L. Degrève, R.G. Stabeli, R. Itri, P. Ciancaglini
Conformational changes of the cyclic (Lo) peptide Labaditin (VWTVWGTIAG) and its linear analogue (L1) promoted by presence of anionic sodium dodecyl sulfate (SDS) and zwitterionic L-α-Lysophosphatidylcholine (LPC) micelles were investigated. Results from max blue-shift of tryptophan fluorescence emission combined with Stern-Volmer constants values and molecular dynamics (MD) simulations indicated that Lo interacts with SDS micelles to a higher extent than does Lo. Further, the MD simulation demonstrated that both Lo and L1 interact similarly with LPC micelles, being preferentially located at the micelle/water interface. The peptide–micelle interaction elicits conformational changes in the peptides. Lo undergoes limited modifications and presents unordered structure in both LPC and SDS micelles. On the other hand, L1 displays a random-coil structure in aqueous medium, pH 7.0, and it acquires a b-structure upon interaction with SDS and LPC, albeit with structural differences in each medium.

Labaditin, a cyclic peptide with rich biotechnological potential: preliminary toxicological studies and structural changes in water and lipid membrane environment
S. C. Barbosa · E. M. Cilli · Luis G. Dias · Rodrigo G. Stabeli · P. Ciancaglini
Amino Acids (2011) 40:135–144
DOI 10.1007/s00726-010-0648-6
Cyclic peptides isolated from the plants of the Euphorbiaceae family have been largely studied due to
their rigid conformation, which is considered significant for biologic activity. The peptide Labaditin (L₀) and its open chain analogs (L₁) were synthesized by the solid-phase peptide synthesis technique (Fmoc/tBu), and purified to elucidate its interaction with membrane models. A shift in kₘₐₓ emission and Stern–Volmer constants values indicate that both tryptophans migrate to a more apolar environment, with L₁ decreasing less than L₀. A circular dichroism (CD) study revealed that L₀ was kept unstructured in aqueous media as much as in the presence of dipalmitoilphosphatidylcholine liposomes. The thermodynamic studies by differential calorimetry (DSC) show a Dₕ increase (50 and 18 kcal/mol, for L₀ and L₁, respectively) with peptide concentrations, which is indicative of lipids associating with peptides, resulting in the inability of the lipids to participate in the main transition. Therefore, all CD, DSC, and fluorescence data suggest a greater L₀ membrane insertion. A probable mechanism for Labaditin interaction is based initially on the hydrophobic interaction of the peptide with the lipid membrane, conformational change, peptide adsorption on the lipid surface, and internalization process. Peptide’s antibacterial effect was also evaluated and revealed that only L₀ showed reduction in viability in Gram-positive bacteria while no effects to the Gram-negative.

Liposome-based Intranasal Delivery of Lipopeptide Vaccine Candidates Against Group A Streptococcus
Khairunnisa Abdul Ghaffar, Nirmal Marasini, Ashwini Kumar Giddam, Michael Batzloff, Michael Good, Mariusz Skwarczynski, Istvan Toth
DOI: http://dx.doi.org/10.1016/j.actbio.2016.04.012

Group A Streptococcus (GAS), an exclusively human pathogen, causes a wide range of diseases ranging from trivial to life threatening. Treatment of infection is often ineffective following entry of bacteria into the bloodstream. To date, there is no vaccine available against GAS. In this study, cationic liposomes encapsulating lipopeptide-based vaccine candidates against GAS have been employed for intranasal vaccine delivery. Cationic liposomes were prepared with dimethyldioctadecylammonium bromide (DDAB) using the film hydration method. Female Swiss mice were immunized intranasally with the liposomes. In contrast to unmodified peptides, lipopeptides entrapped by liposomes induced both mucosal and systemic immunity, IgA and IgG (IgG1 and IgG2a) production in mice, respectively. High levels of antibody (IgA and IgG) titres were detected even five months post immunization. Thus, the combination of lipopeptides and liposomes generates a very promising delivery system for intranasal vaccines.

(Lipo)polysaccharide interactions of antimicrobial peptides
Artur Schmidtchen, Martin Malmsten
http://dx.doi.org/10.1016/j.jcis.2014.11.024

abstract
Due to rapidly increasing resistance development against conventional antibiotics, as well as problems associated with diseases either triggered or deteriorated by infection, antimicrobial and anti-inflammatory peptides have attracted considerable interest during the last few years. While there is an emerging understanding of the direct antimicrobial function of such peptides
through bacterial membrane destabilization, the mechanisms of their anti-inflammatory function are less clear. We here summarize some recent results obtained from our own research on anti-inflammatory peptides, with focus on peptide-(lipo)polysaccharide interactions.

Mechanisms of Antimicrobial Peptide Action and Resistance

MICHAEL R. YEAMAN AND NANNETTE Y. YOUNT

Antimicrobial peptides have been isolated and characterized from tissues and organisms representing virtually every kingdom and phylum, ranging from prokaryotes to humans. Yet, recurrent structural and functional themes in mechanisms of action and resistance are observed among peptides of widely diverse source and composition. Biochemical distinctions among the peptides themselves, target versus host cells, and the microenvironments in which these counterparts convene, likely provide for varying degrees of selective toxicity among diverse antimicrobial peptide types. Moreover, many antimicrobial peptides employ sophisticated and dynamic mechanisms of action to effect rapid and potent activities consistent with their likely roles in antimicrobial host defense.

In balance, successful microbial pathogens have evolved multifaceted and effective countermeasures to avoid exposure to and subvert mechanisms of antimicrobial peptides. A clearer recognition of these opposing themes will significantly advance our understanding of how antimicrobial peptides function in defense against infection. Furthermore, this understanding may provide new models and strategies for developing novel antimicrobial agents, that may also augment immunity, restore potency or amplify the mechanisms of conventional antibiotics, and minimize antimicrobial resistance mechanisms among pathogens. From these perspectives, the intention of this review is to illustrate the contemporary structural and functional themes among mechanisms of antimicrobial peptide action and resistance.

Membrane defects enhance the interaction of antimicrobial peptides, aurein 1.2 versus caerin 1.1

David I. Fernandez, Marc-Antoine Sani, Andrew J. Miles, B.A. Wallace, Frances Separovic

Biochimica et Biophysica Acta 1828 (2013) 1863–1872

The membrane interactions of the antimicrobial peptides aurein 1.2 and caerin 1.1 were observed by 31P and 2H solid-state NMR and circular dichroism spectroscopy. Both peptides were relatively unstructured in water. In the presence of dimyristoylphosphatidylcholine (DMPC) and mixed DMPC and dimyristoylphosphatidylglycerol (DMPG) vesicles, both peptides displayed a considerable increase in helical content with the shorter aurein peptide having a higher α-helix content in both lipid systems. In fluid phase DMPC vesicles, the peptides displayed differential interactions: aurein 1.2 interacted primarily with the bilayer surface, while the longer caerin 1.1 was able to penetrate into the bilayer interior. Both peptides displayed a preferential interaction with the DMPG component in DMPC/DMPG bilayers, with aurein 1.2 limited to interaction with the surface and caerin 1.1 able to penetrate into the bilayer and promote formation of amixiture of lipid phases or domains. In gel phase DMPC vesicles, aurein 1.2 disrupted the bilayer apparently through a carpet mechanism, while no additional interaction was seen with caerin 1.1. Although a lamellar bilayer was retained with the mixed DMPC/DMPG vesicles below the phase transition, both caerin 1.1 and aurein 1.2 promoted disruption of the bilayer and formation of an isotropic phase. The peptide interaction was enhanced relative to the fluid phase and was likely driven by co-existence of membrane defects. This study thus demonstrates that the effects of the lipid phase and domains need to be considered when studying membrane interactions of antimicrobial peptides.

Microbial growth inhibition caused by Zn/Ag-Y zeolite materials withdifferent amounts of silver

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Colloids and Surfaces B: Biointerfaces 142 (2016) 141–147

Different loadings of silver exchanged on bimetallic Zn/Ag-NaY zeolite materials were studied for antimicrobial properties against four reference microorganisms. The sensitive indicator strains used were two bacteria (Escherichia coli and Bacillus subtilis) and two yeast species (Saccharomyces cerevisiae and Candida albicans). The bimetallic materials were compared with the monometallic materials prepared with the same concentrations of silver. A synergistic effect between the two metals, zinc and silver, was evidenced on the antimicrobial activity of the materials.
All mono and bimetallic materials showed strong efficacy against bacteria and yeasts, although the later overall displayed lower MIC values. The results of X-ray photoelectron spectroscopy (XPS) confirm the presence of silver and zinc as ions, not homogeneously distributed throughout the zeolite framework, which implies that the metal ions are located in different sites of the faujasite structure.

One-step synthesis of high-density peptide-conjugated gold nanoparticles with antimicrobial efficacy in a systemic infection model
Akhilesh Rai, Sandra Pinto, Tiago Velho, André F. Ferreira, Catarina Moita, Urvish Trivedi, Marta Evangelista, Michela Comune, Kendra P. Rumbaugh, Pedro N. Simões, Luís Moita, Lino Ferreira
DOI: 10.1016/j.biomaterials.2016.01.051
Biomaterials 85 (2016) 99-110
The increase in antibiotic drug resistance and the low number of new antibacterial drugs approved in the last few decades requires the development of new antimicrobial strategies. Antimicrobial peptides (AMPs) are very promising molecules to fight microbial infection since they kill quickly bacteria and, in some cases, target bacterial membrane. Although some AMPs may be stable against proteolytic degradation by chemical modification, in general, low AMP activity and stability in the presence of serum and proteolytic enzymes as well as their cytotoxicity have impaired their clinical translation. Here, we describe a one-step methodology to generate AMP-conjugated gold nanoparticles (Au NPs), with a high concentration of AMPs (CM-SH) (~ 240 AMPs per NP), controlled size (14 nm) and low polydispersity. AMP-conjugated Au NPs demonstrated higher antimicrobial activity and stability in serum and in the presence of nonphysiological concentrations of proteolytic enzymes than soluble AMP, as well as low cytotoxicity against human cells. Moreover, the NPs demonstrated high antimicrobial activity after in vivo administration in a chronic wound and in an animal model of systemic infection.

Overcoming barriers in Pseudomonas aeruginosa lung infections: Engineered nanoparticles for local delivery of a cationic antimicrobial peptide
Ivana d’Angelo, Bruno Casciaro, Agnese Miro, Fabiana Quaglia, Maria Luisa Mangoni, Francesca Ungaro
Cationic antimicrobial peptides (CAMPs) are very promising in the treatment of multi-drug resistant Pseudomonas aeruginosa lung infections experienced by cystic fibrosis (CF) patients. Nevertheless, there is an urgent need of inhalable formulations able to deliver the intact CAMP in conductive airways and to shield its interactions with airway mucus/bacterial biofilm, thus enhancing CAMP/bacteria interactions. Along these lines, the aim of this work was the design and development of nano-embedded microparticles (NEM) for sustained delivery of CAMPs in the lung. To this purpose, nanoparticles (NPs) made of poly(lactide-co-glycolide) (PLGA) containing a model CAMP, colistin (Col), were produced by emulsion/solvent diffusion technique. Engineering NPs with chitosan (CS) and poly(vinyl alcohol) (PVA) allowed to modulate surface properties and, in so doing, to improve NP transport through artificial CF mucus. In order to achieve a long-term stable dosage form useful for NP inhalation, NPs were spray-dried in different carriers (lactose or mannitol), thus producing NEM. The most promising NEM formulations were selected on the basis of bulk and flow properties, distribution of NPs in the carrier and aerosolization performance upon delivery through a breath-actuated dry powder.
inhaled. Of note, selected Col-loadedNEM were found to kill P. aeruginosa biofilm and to display a prolonged efficacy in biofilm eradication compared to the free Col. This effect was likely ascribable to the ability of NPs to penetrate into bacterial biofilm, as demonstrated by confocal analysis, and to sustain Col release inside it. Taken all together, our results indicate that adequate engineering of PLGA NPs represents an enticing technological approach to harness novel antimicrobials for P. aeruginosa lung infection, such as CAMPs, especially in CF.

Peptide-Induced Hierarchical Long-Range Order and Photocatalytic Activity of Porphyrin Assemblies
Kai Liu, Ruirui Xing, Chengjun Chen, Guizhi Shen, Linyin Yan, Qianli Zou, Guanghui Ma, Helmut Mçhwald, and Xuehai Yan
DOI: 10.1002/anie.201409149

Abstract: Long-range structural order and alignment over different scales are of key importance for the regulation of structure and functionality in biology. However, it remains a great challenge to engineer and assemble such complex functional synthetic systems with order over different length scales from simple biologically relevant molecules, such as peptides and porphyrins. Herein we describe the successful introduction of hierarchical long-range order in dipeptide-adjusted porphyrin self-assembly by a thermodynamically driven self-orienting assembly pathway associated with multiple weak interactions. The long-range order and alignment of fiber bundles induced new properties, including anisotropic birefringence, a large Stokes shift, amplified chirality, and excellent photostability as well as sustainable photocatalytic activity. We also demonstrate that the aligned fiber bundles are able to induce the epitaxially oriented growth of Pt nanowires in a photocatalytic reaction.

Prevention of Staphylococcus aureus biomaterial-associated infections using a polymer-lipid coating containing the antimicrobial peptide OP-145
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Journal of Controlled Release 222 (2016) 1-8

The scarcity of current antibiotic-based strategies to prevent biomaterial-associated infections (BAI) and their risk of resistance development prompted us to develop a novel antimicrobial implant-coating to prevent Staphylococcus aureus-induced BAI. We incorporated the antimicrobial peptide OP-145 into a Polymer-Lipid Encapsulation MatriX (PLEX)-coating to obtain high peptide levels for prolonged periods at the implant-tissue interphase. We first confirmed that OP-145 was highly effective in killing S. aureus and inhibiting biofilm formation in vitro. OP-145 injected along S. aureus-inoculated implants in mice significantly reduced the number of culture-positive implants. OP-145 was released from the PLEX coating in a controlled zero-order kinetic rate after an initial 55%-burst release and displayed bactericidal activity in vitro. In a rabbit intramedullary nail-related infection model, 67% of rabbits with PLEX-OP-145-coated nails had culture-negative nails after 28 days compared to 29% of rabbits with uncoated nails. In rabbits with PLEX-OP-145-coated nails, bone and soft tissue samples were culture-negative in 67% and 80%, respectively, whereas all bone samples and 71% of the soft tissue samples of rabbits with uncoated nails were infected. Together, PLEX-OP-145 coatings, of which both compounds have already been found safe in man, can prevent implant colonization and S. aureus-induced BAI.
Promising antimicrobial agents designed from natural peptide templates
J. Michael Henderson, Ka Yee C. Lee
Current Opinion in Solid State and Materials Science 17 (2013) 175-192

Treatment of infectious diseases is a paramount healthcare issue as the number of multidrug resistant pathogens rise rendering our aging small-molecule antibiotics ineffective. Innovation and discovery in new molecular species that are active against novel targets is vital to meet the challenges of resistance development. The ability of host-defense, or antimicrobial, peptides (AMPs) to selectively target the harmful microbial membrane over that of a host’s is a unique characteristic making these innate immune effectors promising candidates to fill the growing therapeutic void. Despite nearly two decades of active research into their selective mechanism against pathogens, few peptides have been found suitable for pharmaceutical applications. Fundamental structure–activity principles underlying the physiochemical properties of AMPs have guided the development and design of synthetic alternatives to peptide-based drugs. Here we first review work in understanding the mechanism and membrane selectivity of AMPs as it provides a good basis for the interpretation of other membrane-active agents as the same physical and chemical driving forces are at work. Recent advances in the rational design of synthetic mimics of antimicrobial peptides (SMAMPs) will also be discussed. Emphasis is placed on the paradigm shift that a rigid secondary structure is not required for the membrane-disruptive ability of SMAMPs.

Probing membrane permeabilization by the antimicrobial peptide distinctin in mercury-supported biomimetic membranes
Lucia Becucci, Martina Papini, Daniel Mullen, Andrea Scaloni, Gianluigi Veglia, Rolando Guidelli
Biochimica et Biophysica Acta 1808 (2011) 2745–2752

The mechanism of membrane permeabilization by the antimicrobial peptide distinctin was investigated by using two different mercury-supported biomimetic membranes, namely a lipid self-assembled monolayer and a lipid bilayer tethered to the mercury surface through a hydrophilic spacer (tethered bilayer lipid membrane: tBLM). Incorporation of distinctin into a lipid monolayer from its aqueous solution yields rapidly ion channels selective toward inorganic cations, such as Tl+ and Cd2+. Conversely, its incorporation in a tBLM allows the formation of ion channels permeable to potassium ions only at non-physiological transmembrane potentials, more negative than ~340 mV. These channels, once formed, are unstable at less negative transmembrane potentials. The kinetics of their formation is consistent with the disruption of distinctin clusters adsorbed on top of the lipid bilayer, incorporation of the resulting monomers and their aggregation into hydrophilic pores by a mechanism of nucleation and growth. Comparing the behavior of distinctin in tBLMs with that in conventional black lipid membranes strongly suggests that distinctin channel formation in lipid bilayer requires the partitioning of distinctin molecules between the two sides of the lipid bilayer. We can tentatively hypothesize that an ion channel is formed when one distinctin cluster on one side of the lipid bilayer matches another one on the opposite side.

Role of lipids in the interaction of antimicrobial peptides with membranes
Vitor Teixeira, Maria J. Feio, Margarida Bastos
Progress in Lipid Research 51 (2012) 149–177

Antimicrobial peptides (AMPs) take part in the immune system by mounting a first line of defense against pathogens. Recurrent structural and functional aspects are observed among peptides from different sources, particularly the net cationicity and amphipathicity. However, the membrane seems to be the key determinant of their action, either as the main target of the peptide action or by forming a barrier that must be crossed by peptides to target core metabolic pathways. More importantly, the specificity exhibited by antimicrobial peptides relies on the different lipid composition between pathogen and host cells, likely contributing to their spectrum of activity. Several mechanisms of action have been reported, which may involve membrane permeabilization through the formation of pores, membrane thinning or micellization in a detergent-like way. AMPs may also target intracellular components, such as DNA, enzymes and even organelles. More recently, these peptides have been shown to produce membrane perturbation by formation of specific lipid–peptide domains, lateral phase segregation of zwitterionic from anionic phospholipids and even the formation of non-lamellar lipid phases. To countermeasure their activity, some pathogens were successful in
developing effective mechanisms of resistance to decrease their susceptibility to AMPs. The functional and integral knowledge of such interactions and the clarification of the complex interplay between molecular determinants of peptides, the pathogen versus host cells dichotomy and the specific microenvironment in which all these elements convene will contribute to an understanding of some elusive aspects of their action and to rationally design novel therapeutic agents to overcome the current antibiotic resistance issue.

Role of amphipathicity and hydrophobicity in the balance between hemolysis and peptide–membrane interactions of three related antimicrobial peptides
Axel Hollmann, Melina Martínez, Martín E. Noguera, Marcelo T. Augusto, Anibal Disalvo, Nuno C. Santos, Liliana Semorile, Paulo C. Maffia
Colloids and Surfaces B: Biointerfaces 141 (2016) 528–536
Cationic antimicrobial peptides (CAMPs) represent important self defense molecules in many organisms, including humans. These peptides have a broad spectrum of activities, killing or neutralizing many Gram-negative and Gram-positive bacteria. The emergence of multidrug resistant microbes has stimulated research on the development of alternative antibiotics. In the search for new antibiotics, cationic antimicrobial peptides (CAMPs) offer a viable alternative to conventional antibiotics, as they physically disrupt the bacterial membranes, leading to lysis of microbial membranes and eventually cell death. In particular, the group of linear _-helical cationic peptides has attracted increasing interest from clinical as well as basic research during the last decade. In this work, we studied the biophysical and microbiological characteristics of three new designed CAMPs. We modified a previously studied CAMP sequence, in order to increase or diminish the hydrophobic face, changing the position of two lysines or replacing three leucines, respectively. These mutations modified the hydrophobic moment of the resulting peptides and allowed us to study the importance of this parameter in the membrane interactions of the peptides. The structural properties of the peptides were also correlated with their membrane-disruptive abilities, antimicrobial activities and hemolysis of human red blood cells.

Self-assembly and interactions of short antimicrobial cationic lipopeptides with membrane lipids: ITC, FTIR and molecular dynamics studies
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http://dx.doi.org/10.1016/j.bbamem.2014.06.016
In this work, the self-organization and the behavior of the surfactant-like peptides in the presence of biological membrane models were studied. The studies were focused on synthetic palmitic acid-containing lipopeptides, C16–KK–NH2 (I), C16–KGK–NH2 (II) and C16–KKKK–NH2 (III). The self-assembly was explored by molecular dynamics simulations using a coarse-grained force field. The critical micellar concentration was estimated by the surface tension measurements. The thermodynamics of the peptides binding to the anionic and zwitterionic lipids were established using isothermal titration calorimetry (ITC). The influence of the peptides on the lipid acyl chain ordering was determined using FTIR spectroscopy. The compounds studied showed surface-active properties with a distinct CMC over the millimolar range. An increase in the steric and electrostatic repulsion between polar head groups shifts the CMC toward higher values and reduces the aggregation number. An analysis of the peptide–membrane binding revealed a unique interplay between the initial electrostatic and the subsequent hydrophobic interactions enabling the lipopeptides to interact with the lipid bilayer. In the case of C16–KKKK–NH2 (III), compensation of the electrostatic and hydrophobic interactions upon binding to the anionic membrane has been suggested and consequently no overall binding effects were noticed in ITC thermograms and FTIR spectra.

Short, multiple-stranded b-hairpin peptides have antimicrobial potency with high selectivity and salt resistance
Shuli Chou, Changxuan Shao, Jiajun Wang, Anshan Shan, Lin Xu, Na Dong, Zhongyu Li
The b-hairpin structure has been proposed to exhibit potent antimicrobial properties with low cytotoxicity, thus, multiple b-hairpin structures have been proved to be highly stable in structures containing tightly packed hydrophobic cores. The aim of this study was to develop peptide-based synthetic strategies for generating short, but effective AMPs as inexpensive antimicrobial agents. Multiple-stranded b-hairpin peptides with the same b-hairpin unit, (WRXxRW)n where n = 1, 2, 3, or 4 and Xx represent the turn sequence, were synthesized, and their potential as antimicrobial agents was evaluated. Owning to the tightly packed hydrophobic core and paired Trp of this multiple-stranded b-hairpin structure, all the 12-residues peptides exhibited high cell selectivity towards bacterial cells over human red blood cells (hRBCs), and the peptide W2 exhibited stronger antimicrobial activities with the MIC values of 2–8 μM against various tested bacteria. Not only that, but W2 also showed obvious synergy with streptomycin and chloramphenicol against Escherichia coli, and displayed synergy with ciprofloxacin against Staphylococcus aureus with the FICI values 60.5. Fluorescence spectroscopy and electron microscopy analyses indicated that W2 kills microbial cells by permeabilizing the cell membrane and damaging membrane integrity. Collectively, based on the multiple b-hairpin peptides, the ability to develop libraries of short and effective peptides will be a powerful approach to the discovery of novel antimicrobial agents.

Statement of significance
We successfully screened a peptide W2 ((WRPGRW)2) from a series of multiple-stranded b-hairpin antimicrobial peptides based on the “S-shaped” motif that induced the formation of a globular structure, and Trp zipper was used to replace the disulfide bonds to reduce the cost of production. This novel structure applied to AMPs improved cell selectivity and salt stability. The findings of this study will promote the development of peptide-based antimicrobial biomaterials. Further exploration of these AMPs will allow for diverse biotechnological and clinical applications such as biomedical coating, food storing, and animal feeding.

Structural and thermodynamic properties of water–membrane interphases: Significance for peptide/membrane interactions
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http://dx.doi.org/10.1016/j.cis.2014.05.002

Water appears as a common intermediary in the mechanisms of interaction of proteins and polypeptides with membranes of different lipid composition. In this review, how water modulates the interaction of peptides and proteins with lipid membranes is discussed by correlating the thermodynamic response and the structural changes of water at the membrane interphases. The thermodynamic properties of the lipid–protein interaction are governed by changes in the water activity of monolayers of different lipid composition according to the lateral surface pressure. In this context, different water populations can be characterized below and above the phase transition temperature in relation to the CH2 conformers’ states in the acyl chains. According to water species present at the interphase, lipid membrane acts as a water state regulator, which determines the interfacial water domains in the surface. It is proposed that those domains are formed by the contact between lipids themselves and between lipids and the water phase, which are needed to trigger adsorption–insertion processes. The water domains are essential to maintain functional dynamical properties and are formed by water beyond the hydration shell of the lipid head groups. These confined water domains probably carries information in local units in relation to the lipid composition thus accounting for the link between lipidomics and aquaomics. The analysis of these results contributes to a new insight of the lipid bilayer as a non-autonomous, responsive (reactive) structure that correlates with the dynamical properties of a living system.

Structure–activity relationship of mastoparan analogs: Effects of the number and positioning of Lys residues on secondary structure, interaction with membrane-mimetic systems and biological activity
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In this study, a series of mastoparan analogs were engineered based on the strategies of Ala and Lys scanning in relation to the sequences of classical mastoparans. Ten analog mastoparans, presenting
from zero to six Lys residues in their sequences were synthesized and assayed for some typical biological activities for this group of peptide: mast cell degranulation, hemolysis, and antibiosis. In relation to mast cell degranulation, the apparent structural requirement to optimize this activity was the existence of one or two Lys residues at positions 8 and/or 9. In relation to hemolysis, one structural feature that strongly correlated with the potency of this activity was the number of amino acid residues from the C-terminus of each peptide continuously embedded into the zwitterionic membrane of erythrocytes-mimicking liposomes, probably due to the contribution of this structural feature to the membrane perturbation. The antibiotoxicity of mastoparan analogs was directly dependent on the apparent extension of their hydrophilic surface, i.e., their molecules must have from four to six Lys residues between positions 4 and 11 of the peptide chain to achieve activities comparable to or higher than the reference antibiotic compounds. The optimization of the antibacterial activity of the mastoparans must consider Lys residues at the positions 4, 5, 7, 8, 9, and 11 of the tetradecapeptide chain, with the other positions occupied by hydrophobic residues, and with the C-terminal residue in the amidated form. These requirements resulted in highly active AMPs with greatly reduced (or no) hemolytic and mast cell degranulating activities.
Antimicrobial resistance has reached alarming levels in many countries, thus leading to a search for new classes of antibiotics, such as antimicrobial peptides whose activity is exerted by interacting specifically with the microorganism membrane. In this study, we investigate the molecular-level mechanism of action for Labaditin (Lo), a 10-amino acid residue cyclic peptide from Jatropha multifida with known bactericidal activity against Streptococcus mutans. We show that Lo is also effective against Staphylococcus aureus (S. aureus) but this does not apply to its linear analogue (L1). Using polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS), we observed that the secondary structure of Lowas preserved upon interacting with Langmuir monolayers from a phospholipid mixture mimicking S.aureus membrane, in contrast to L1. This structure preservation for the rigid, cyclic Lo is key for the self-assembly of peptide nanotubes that induce pore formation in large unilamellar vesicles (LUVs), according to permeability assays and dynamic light scattering measurements. In summary, the comparison between Labaditin (Lo) and its linear analogue L1 allowed us to infer that the bactericidal activity of Lo is more related to its interaction with the membrane. It does not require specific metabolic targets, which makes cyclic peptides promising for antibiotics without bacteria resistance.

The Molecular Basis for Antimicrobial Activity of Pore-Forming Cyclic Peptides

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Biophysical Journal Volume 100 May 2011 2422–2431

ABSTRACT The mechanism of action of antimicrobial peptides is, to our knowledge, still poorly understood. To probe the biophysical characteristics that confer activity, we present here a molecular-dynamics and biophysical study of a cyclic antimicrobial peptide and its inactive linear analog. In the simulations, the cyclic peptide caused large perturbations in the bilayer and cooperatively opened a disordered toroidal pore, 1–2 nm in diameter. Electrophysiology measurements confirm discrete poration events of comparable size. We also show that lysine residues aligning parallel to each other in the cyclic but not linear peptide are crucial for function. By employing dual-color fluorescence burst analysis, we show that both peptides are able to fuse/aggregate liposomes but only the cyclic peptide is able to porate them. The results provide detailed insight on the molecular basis of activity of cyclic antimicrobial peptides.

Tryptophan-containing lipopeptide antibiotics derived from polymyxin B with activity against Gram positive and Gram negative bacteria

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BBAMEM 1858 (2016) 333-343

ABSTRACT Resistance to all known antibiotics is a growing concern worldwide, and has renewed the interest in antimicrobial peptides, a structurally diverse class of amphipathic molecules that essentially act on the bacterial membrane. Propelled by the antimicrobial potential of this compound class, we have designed three new lipopeptides derived from polymyxin B, sp-34, sp-96 and sp-100, with potent antimicrobial activity against both Gram positive and Gram negative bacteria. The three peptides bind with high affinity to lipopolysaccharide as demonstrated by monolayer penetration and dansyl-displacement. The interaction with the cytoplasmic membrane has been elucidated by biophysical experiments with model membranes of POPG or POPE/POPG (6:4), mimicking the Gram positive and Gram negative bacterial membrane. Trp-based fluorescence experiments including steady-state, quenching, anisotropy and FRET, reveal selectivity for anionic phospholipids and deep insertion into the membrane. All three lipopeptides induce membrane fusion and leakage from anionic vesicles, a process that is favored by the presence of POPE. The molecules bind to zwitterionic POPC vesicles, a model of the eukaryotic membrane, but in a different way, with lower affinity, less penetration into the bilayer and no fusion or permeabilization of the membrane. Results in model membranes are consistent with flow cytometry experiments in Escherichia coli and Staphylococcus aureus using a membrane potential sensitive dye (bis-oxonol) and a nucleic acid dye (propidium iodide), suggesting that the mechanism of action is based on membrane binding and collapse of membrane integrity by depolarization and permeabilization.
The expanding scope of antimicrobial peptide structures and their modes of action
Leonard T. Nguyen, Evan F. Haney and Hans J. Vogel

Antimicrobial peptides (AMPs) are an integral part of the innate immune system that protect a host from invading pathogenic bacteria. To help overcome the problem of antimicrobial resistance, cationic AMPs are currently being considered as potential alternatives for antibiotics. Although extremely variable in length, amino acid composition and secondary structure, all peptides can adopt a distinct membrane-bound amphipathic conformation. Recent studies demonstrate that they achieve their antimicrobial activity by disrupting various key cellular processes. Some peptides can even use multiple mechanisms. Moreover, several intact proteins or protein fragments are now being shown to have inherent antimicrobial activity. A better understanding of the structure–activity relationships of AMPs is required to facilitate the rational design of novel antimicrobial agents.

The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver
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Research has clarified the properties required for polymers that resist bacterial colonisation for use in medical devices. The increase in antibiotic-resistant microorganisms has prompted interest in the use of silver as an antimicrobial agent. Silver-based polymers can protect the inner and outer surfaces of devices against the attachment of microorganisms. Thus, this review focuses on the mechanisms of various silver forms as antimicrobial agents against different microorganisms and biofilms as well as the dissociation of silver ions and the resulting reduction in antimicrobial efficacy for medical devices. This work suggests that the characteristics of released silver ions depend on the nature of the silver antimicrobial used and the polymer matrix. In addition, the elementary silver, silver zeolite and silver nanoparticles, used in polymers or as coatings could be used as antimicrobial biomaterials for a variety of promising applications.

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E1(145-162), a peptide corresponding to the structural protein E1 of the GB virus C, has been shown earlier to bind at pH 7.4 to vesicles containing 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol)] (DMPG) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) phospholipids. To deepen the understanding of the interaction of E1(145-162) with the lipid membrane, in this paper, we report a detailed study of the surface properties of peptide, miscibility properties, and behavior of mixed monomolecular films of it and three phospholipids DMPG, DMPC, and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPG). These studies were performed using the Langmuir balance by means of surface adsorption studies, surface pressure-meanmolecular area compression isotherms, and penetration kinetics. The Brewster angle microscopy (BAM) was used to study the morphological properties of pure peptide and the mixed monolayers. The results show that the peptide showed surface activity concentration dependent when injected beneath a buffered solution (HEPES/NaCl, pH 7.4). This tendency to accumulate into the air/water interface confirms its potential capacity to interact with membranes; the higher penetration of peptide into phospholipids is attained when the monolayers are in the liquid expanded state and the lipids are charged negatively maybe due to its negative electric charge that interacts with the positive global charge of the peptide sequence. The area per molecule values obtained suggested that the main arrangement structure for E1(145-162) peptide is the R-helical at the air-water interface that agreed with computational prediction calculations. Miscibility studies indicated that mixtures become thermodynamically favored at low peptide molar fraction.

A study on the interactions of Aurein 2.5 with bacterial membranes

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Colloids and Surfaces B: Biointerfaces 68 (2009) 225-230

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abstract

Aurein 2.5 (GLFDIVKVVGAFSL-NH2) is an uncharacterised antimicrobial peptide. At an air/water interface, it exhibited strong surface activity (maximal surface pressure 25mNm−1) and molecular areas consistent with the adoption of _helical structure orientated either perpendicular (1.72 nm2 molecule−1) or parallel (3.6nm2 molecule−1) to the interface. Aurein 2.5 was strongly antibacterial, exhibiting a minimum inhibitory concentration (MIC) of 30_M against Bacillus subtilis and Escherichia coli. The peptide induced maximal surface pressure changes of 9mNm−1 and 5mNm−1, respectively, in monolayers mimicking membranes of these organisms whilst compression isotherm analysis of these monolayers showed _GMix > 0, indicating destabilisation by Aurein 2.5. These combined data suggested that toxicity of the peptide to these organisms may involve membrane invasion via the use of oblique orientated _helical structure. The peptide induced strong, comparable maximal surface changes in monolayers of DOPG (7.5mNm−1) and DOPE monolayers (6mNm−1) suggesting that the membrane interactions of Aurein 2.5 were driven by amphiphilicity rather than electrostatic interaction. Based on these data, it was suggested that the differing ability of Aurein 2.5 to insert into membranes of B. subtilis and E. coli was probably related to membrane-based factors such as differences in lipid packing characteristics. The peptide was active against both sessile E. coli and Staphylococcus aureus with an MIC of 125_M. The
broad-spectrum antibacterial activity and non-specific modes of membrane action used by Aurein 2.5 suggested use as an anti-biofilm agent such as in the decontamination of medical devices.

All-or-none membrane permeabilization by fengycin-type lipopeptides from Bacillus subtilis QST713
Hiren Patel, Clemens Tscheka, Katarina Edwards, Göran Karlsson, Heiko Heerklotz
http://dx.doi.org/10.1016/j.bbamem.2011.04.008


The fungicidal activity of Bacillus subtilis QST713 has been utilized for the highly effective and environmentally safe protection of crops against a variety of pathogens. It is based mainly on the production of cyclic lipopeptides of the fengycin (FEs), surfactin, and iturin families. The mixed population of native FEs forms micelles which solubilize individual FEs such as agrastatin 1 (AS1) that are otherwise rather insoluble on their own. Fluorescence lifetime-based calcein efflux measurements and cryo transmission electron microscopy show that these FEs show a unique scenario of membrane permeabilization. Poor miscibility of FEs with lipid probably promotes the formation of pores in 10% of the vesicles at only ∼1 μM free FE and in 15% of the vesicles at 10 μM. We explain why this limited, all-or-none leakage could nevertheless account for the killing of virtually all fungi whereas the same extent of graded vesicle leakage may be biologically irrelevant. Then, crystalization of AS1 and micellization of plipastatins cause a cut-off in leakage at 15% that might regulate the biological activity of FEs, protecting Bacillus and plant membranes. The fact that FE micelles solubilize only about 10 mol-% fluid lipid resembles the behavior of detergent resistance.

Designing potent antimicrobial peptides by disulphide linked dimerization and N-terminal lipidation to increase antimicrobial activity and membrane perturbation: Structural insights into lipopolysaccharide binding
Aritreyee Datta, Pullob Kundu, Anirban Bhunia
Journal of Colloid and Interface Science 461 (2016) 335-345

Hypothesis: The remarkable rise in multi-drug resistant Gram-negative bacterial pathogens is a major concern to the well being of humans as well as susceptible plants. In recent years, diseases associated with inflammation and septicemia have already become a global health issue. Therefore, there is a rising demand for the development of novel ‘super’ antibiotics. In this context, antimicrobial peptides offer an attractive, alternate therapeutic solution to conventional antibiotics.

Experiments: Microbroth dilution assay was performed to investigate the antimicrobial activities of the two designed peptides against Gram negative bacterial pathogens. Fluorescence studies including NPN dye uptake assay, Calcein entrapped vesicle leakage assay, quenching and anisotropy in presence of lipopolysaccharide (LPS) were performed to elucidate binding interactions and enhanced membrane permeabilisation. Hemolytic assay and endotoxin/LPS neutralisation assay were performed to study the hemolytic effects and LPS scavenging abilities of the peptides. High resolution NMR studies were performed to obtain insights into LPS-peptide interaction at the molecular level.

Findings: Here, we report more potent analogues of previously designed peptide VG16KRKP, designed through dimerization via Cys-Cys disulphide linkage and N-terminal lipidation. Similar to the parent peptide, VG16KRKP, the modified analogue peptides are non hemolytic in nature, but possessed, 20-fold increase in antibacterial activities against E. coli, human pathogen Pseudomonas aeruginosa and the devastating plant pathogen, Xanthomonas campestris pv. campestris as well as membrane permeabilization, and endotoxin neutralization. LPS bound solution structure of both analogues, as determined by NMR spectroscopy, reveal that the conserved hydrophobic triad motif, formed by Trp6, Leu11 and Phe12 is compactly organized and stabilized either by the acyl chain or disulfide bond. This structural constraint accounts for the separation of polar face from the hydrophobic face of the peptides. Our novel peptides designed through Cys-Cys dimerization and N-terminal lipitation, will serve as a template to develop more potent antimicrobials in future, to control plant and human diseases.

Effect of E1(64–81) hepatitis G peptide on the in vitro interaction of HIV-1 fusion peptide with membrane models
Maria Jesús Sánchez-Martín, M. Antònia Busquets, Victoria Girona, Isabel Haro,
One way to gain information about the fusogenic potential of virus-derived synthetic peptides is to examine their interfacial properties and subsequently to study them in monolayers and bilayers. Here, we characterize the physicochemical surface properties of the peptide E1(64–81), whose sequence is AQLVGEILGSLYPLSVSA. This peptide is derived from the E1 structural protein of GBV-C/HGV which was previously shown to inhibit leakage of vesicular contents caused by the HIV-1 fusion peptide (HIV-1 FP). Mixed isotherms of E1(64–81) and HIV-1 FP were obtained and their Brewster angle microscopy (BAM) and atomic force microscopy (AFM) images showed that the peptide mixture forms a different structure that is not present in the pure peptide images. Studies with lipid monolayers (1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DMPG) and 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DPPG)) show that both peptides interact with all the lipids assayed but the effect that HIV-1 FP has on the monolayers is reduced in the presence of E1(64–81). Moreover, differential scanning calorimetry (DSC) experiments show the capacity of HIV-1 FP to modify the properties of the bilayer structure and the capacity of E1(64–81) to inhibit these modifications. Our results indicate that E1(64–81) interacts with HIV-1 FP to form a new structure, and that this may be the cause of the previously observed inhibition of the activity of HIV-1 FP by E1(64–81).

Fluorescence study of the dynamic interaction between E1 (145–162) sequence of hepatitis GB virus C and liposomes
Maria Jesús Sánchez-Martín & José Manuel Amigo &Montserrat Pujol & Isabel Haro & M. Asunción Alsina & M. Antonia Busquets
DOI 10.1007/s00216-008-2593-8

Abstract The physicochemical characterization of the pepptide sequence E1(145–162) corresponding to the structural protein E1 of the hepatitis G virus was done by studying its interaction with model membranes. Small unilamellar vesicles (SUVs) of dimyristoylphosphatidylglycerol or dimyristoylphosphatidylcholine were chosen as mimetic membranes. Peptide incorporation and location in the phospholipid bilayer was investigated by fluorescence anisotropy with SUVs labeled with diphenylhexatriene (DPH) or trimethylammonium-DPH. The addition of the peptide E1 (145–162) showed significant changes in the anisotropy values of the probe located at the air/water interface. These results indicate that the peptide E1(145–162) preferably interacts with the lipid surface without penetrating inside the bilayer. A series of fluorescence experiments based on tryptophan peptide fluorescence were modeled by means of multivariate curve resolution-alternating least squares (MCRALS) algorithm to further study the peptide interaction with bilayers at different temperatures. The preliminary results obtained with MCR-ALS showed how the peptide concentration decay is directly linked to the appearance of a new specie, which corresponds to the lipid–peptide binding. These results provide useful information for the design of synthetic immunopeptides that can be incorporated into a liposomal system with potential to promote a direct delivery of the membrane-incorporated immunogen to the immunocompetent cells, thus increasing the immuno response from the host.

Interactions of daunorubicin with Langmuir–Blodgett thiolipid monolayers
Dorota Matyszewska, Renata Bilewicz

Interactions of daunorubicin (DNR) with thiolipid membranes composed of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol (DPPTE) formed both at the air–water interface and transferred onto gold electrodes were investigated. The drug incorporates into the DPPTE layers during their formation increasing the area per molecule and causing the fluidization of the layers. The interactions of DNR with preformed layers depend on the membrane organization and therefore the dominating type of the driving forces. For less organized layers both electrostatic and hydrophobic interactions take place, while for more condensed layers precompressed to higher surface pressures, electrostatic interactions seem to be prevailing. The drug adsorbs at the layer formed at the air–water interface, which prevents from its further penetration. The DPPTE layers were transferred onto gold electrodes by means of Langmuir–Blodgett and self-assembly method. Cyclic voltammetry experiments revealed that DNR is
more easily incorporated into LB layers supported on solid substrate than into SAMs, since the former tend to be less compact and hydrophobic interactions between the acyl chains of the thiolipid and hydrophobic anthraquinone part of DNR is facilitated. The influence of pH changes of the supporting electrolyte on the electrode processes of DNR incorporated into the layers was also investigated in order to verify if the lipid environment affects the mechanism of electron transfer.

**Interactions of bioactive molecules & nanomaterials with Langmuir monolayers as cell membrane models**

**Critical review**

Thatyane M. Nobre, Felippe J. Pavinatto, Luciano Caseli, Ana Barros-Timmons, Patrycja Dynarowicz-Łątka, Osvaldo N. Oliveira Jr


Langmuir monolayers at the air/water interface have been used for decades to mimic cell membranes in attempts to determine the mechanisms behind the physiological action of biologically-relevant molecules. In this review, we analyze the vast literature in the area, with the contents organized according to the type of molecules and materials, including peptides, proteins, polysaccharides, a variety of pharmaceuticals, and nanomaterials. The focus is placed on the correlation between the effects induced on the monolayers and the biological activity of the molecules and nanomaterials. Effects observed from these interactions can be coupling or adsorption and penetration of the molecules into the monolayer, which can be expanded, condensed or even disrupted. Changes in monolayer mechanical properties, for example, may be crucial for the biological activity. Whenever possible, we try to identify the forces prevailing in the interaction, which has been made possible with a combination of experimental techniques, including surface-specific spectroscopies, microscopies and rheological techniques, in addition to the traditional surface pressure and surface potential measurements. Overall, the mechanisms are governed by ionic electrostatic forces and hydrophobic interactions. Correlation may be straightforward, as in the cases of positively charged peptides and polymers whose antimicrobial activity is ascribed to electrostatic attraction with the negatively charged microbial membranes. Also general is the importance of hydrophobic interactions for the penetration into the membrane, which can be required for the biological action of, for example, polysaccharides. In other cases, correlation between monolayer properties and the physiological activity cannot be established precisely, as the latter may depend on a multitude of parameters that have not been possible to simulate with a simplified model such as that of a Langmuir monolayer. For nanomaterials, the emphasis is in relating interaction with the monolayers and their possible toxicity. Owing to the relevance of electrostatic and hydrophobic interactions, the effects on monolayers (and indeed toxicity) are found to depend largely on the coating or functionalization of the nanomaterials.

**Investigations of antimicrobial peptides in planar film systems**

**Review**

Roman Volinsky, Sofiya Kolusheva, Amir Berman, Raz Jelinek

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Abstract

Planar systems – monolayers and films – constitute a useful platform for studying membrane-active peptides. Here, we summarize varied approaches for studying peptide organization and peptide–lipid interactions at the air/water interface, and focus on three representative antimicrobial membrane-associated peptides—alamethicin, gramicidin, and valinomycin. Experimental data, specifically surface pressure/area isotherms and Brewster angle microscopy images, provided information on peptide association and the effects of the lipid monolayers on peptides surface organization. In general, film analysis emphasized the effects of lipid layers in promoting peptide association and aggregation at the air/water interface. Importantly, the data demonstrated that in many cases peptide domains are phase-separated within the phospholipid monolayers, suggesting that this behavior contributes to the biological actions of membrane-active antimicrobial peptides.

**Langmuir monolayers as models to study processes at membrane surfaces**

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Advances in Colloid and Interface Science 208 (2014) 197–213
The use of new sophisticated and highly surface sensitive techniques as synchrotron based X-ray scattering techniques and in-house infrared reflection absorption spectroscopy (IRRAS) has revolutionized the monolayer research. Not only the determination of monolayer structures but also interactions between amphiphilic monolayers at the soft air/liquid interface and molecules dissolved in the subphase are important for many areas in material and life sciences. Monolayers are convenient quasi-two-dimensional model systems. This review focuses on interactions between amphiphilic molecules in binary and ternary mixtures as well as on interfacial interactions with interesting biomolecules dissolved in the subphase. The phase state of monolayers can be easily triggered at constant temperature by increasing the packing density of the lipids by compression. Simultaneously the monolayer structure changes are followed in situ by grazing incidence X-ray diffraction or IRRAS. The interactions can be indirectly determined by the observed structure changes. Additionally, the yield of enzymatic reaction can be quantitatively determined, secondary structures of peptides and proteins can be measured and compared with those observed in bulk. In this way, the influence of a confinement on the structural properties of biomolecules can be determined. The adsorption of DNA can be quantified as well as the competing adsorption of ions at charged interfaces. The influence of modified nanoparticles on model membranes can be clearly determined. In this review, the relevance and utility of Langmuir monolayers as suitable models to study physical and chemical interactions at membrane surfaces are clearly demonstrated.

Preparation and properties of pH-responsive, self-assembled colloidal nanoparticles from guanidine-containing polypeptide and chitosan for antibiotic delivery


Amoxicillin is a traditional antibiotic used to treat Helicobacter pylori (H. pylori). However, the clinical applicability was limited by low local concentrations of amoxicillin that are reached at the site of H. pylori infection. In this study, a pH-sensitive, guanidine-containing polypeptide composed of poly(γ-glutamic acid) (γPGA) and arginine (Arg) were synthesized. The γPGA-γArg polypeptide can self-assemble into colloidal nanoparticles at pH lower than 3.0, and the morphological changes are reversibly switched by elevating the pH of the colloidal suspension. The chemical properties of the γPGA-γArg polypeptide were investigated by proton nuclear magnetic resonance (1H NMR), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The γPGA-γArg colloidal nanoparticles were modified with a guanidinylated polymer, the chitosan (CS)-arginine (Arg) conjugate (CS-Arg). The effect of electrostatic complexation between γPGA-γArg polypeptide and CS-N-Arg conjugate extends the stable range of the self-assembled nanoparticles to a higher pH (pH > 6.0), and the surface charge density changes from negative to positive. The morphological changes of the CS-N-Arg/γPGA-γArg complex nanoparticles in response to environmental pH were investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Amoxicillin release from the CS-N-Arg/γPGA-γArg NPs was reduced at pH 2.5 (gastric fluid, fasted state) and 4.5 (the gastric mucosal surface), but the antibiotic released rapidly from the nanoparticles at pH 7.0 (the sites of H. pylori infection). The amoxicillin-loaded CS-N-Arg/γPGA-γArg complex nanoparticles showed a superior antibacterial activity against the growth of H. pylori.

Primary Amphipathic Cell-Penetrating Peptides: Structural Requirements and Interactions with Model Membranes†

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10.1021/bi049298m

ABSTRACT: To identify rules for the design of efficient cell-penetrating peptides that deliver therapeutic agents into subcellular compartments, we compared the properties of two closely related primary amphipathic peptides that mainly differ by their conformational state. On the basis of a peptide Pβ that is nonstructured in water and that promotes efficient cellular uptake of nucleic acids through noncovalent association, we have designed a peptide [Pβ] that is predicted to adopt a helical conformation. We show that [Pβ] undergoes a lipid-induced conformational transition into a sheet structure, while [Pβ] remains helical. Penetration experiments show that both peptides can spontaneously insert into phospholipid membranes. Analysis of compression isotherms indicates that both peptides interact with phospholipids in the liquid expanded and liquid condensed states. AFM observations reveal that the peptides strongly disrupt the lipid organization of the monolayers and that the conformational state can influence the uptake by model membranes.
Study of the inhibition capacity of an 18-mer peptide domain of GBV-C virus on gp41-FP HIV-1 activity
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The peptide sequence (175–192) RFPFHRCGAGPKLTKDLE (P59) of the E2 envelope protein of GBV-C virus has been proved to decrease cellular membrane fusion and interfere with the HIV-1 infectivity in a dose-dependent manner. Based on these previous results, the main objective of this study was to deepen in the physicochemical aspects involved in this interaction. First, we analyzed the surface activity of P59 at the air–water interface as well as its interaction with zwitterionic or negatively charged lipid monolayers. Then we performed the same experiments with mixtures of P59/gp41-FP. Studies on lipid monolayers helped us to understand the lipid–peptide interaction and the influence of phospholipids on peptide penetration into lipid media. On another hand, studies with lipid bilayers showed that P59 decreased gp41-FP binding to anionic Large Unilamellar Vesicles. Results can be attributed to the differences in morphology of the peptides, as observed by Atomic Force Microscopy. When P59 and gp41-FP were incubated together, annular structures of about 200 nm in diameter appeared on the mica surface, thus indicating a peptide–peptide interaction. All these results confirm the gp41-FP–P59 interaction and thus support the hypothesis that gp41-FP is inhibited by P59.

Surface Active Properties of Amphiphilic Sequential Isopeptides: Comparison Between a-Helical and β-Sheet Conformations
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Abstract: Poly(Leu–Lys–Lys–Leu) and poly(Leu–Lys) are sequential amphiphilic peptide isomers that adopt respectively an α-helical conformation and a β-sheet structure in saline solutions and at the air/water interface. The surface active properties of LKKL and LK sequential isopeptides containing 16, 20, and n residues have been compared in order to evaluate the contributions of the α-helical and β-sheet conformations. Both have a natural tendency to spread at the surface of a saline solution and the values of the equilibrium spreading pressure $p_e$ lie in the same range. When dissolved in a saline solution, α-helical peptides diffuse faster and adsorb faster at the interface than the β-sheet isomers. From the compression isotherms of LKKL and LK peptide monolayers it is possible to extract parameters that characterize the behavior of α-helical and β-sheet conformations: β-sheet peptide monolayers are more stable and less compressible than the monolayers formed with the α-helical isomers. The LK peptides differ also by their high degree of selfassociation at the air/water interface.

Surface behaviour and peptide–lipid interactions of the antibiotic peptides, Maculatin and Citropin
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Abstract
Surface behaviour of Maculatin 1.1 and Citropin 1.1 antibiotic peptides have been studied using the Langmuir monolayer technique in order to understand the peptide–membrane interaction proposed as critical for cellular lysis. Both peptides have a spontaneous adsorption at the air–water interface, reaching surface potentials similar to those obtained by direct spreading. Collapse pressures ($P_c$, stability to lateral compression), molecular areas at maximal packing and surface potentials ($D/V$) obtained from compression isotherms of both pure peptidemonolayers are characteristic of peptides adopting mainly α-helical structure at the interface. The stability of Maculatin monolayers depended on the subphase and increased when pH was raised. In an alkaline environment, Maculatin exhibits a molecular reorganization showing a reproducible discontinuity in the P–A compression isotherm. Both peptides in lipid films with the
zwitterionic palmitoyl-oleylphosphatidylcholine (POPC) showed an immiscible behaviour at all lipid–peptide proportions studied. By contrast, in films with the anionic palmitoyl-oleyl-phosphatidylglycerol (POPG), the peptides showed miscible behaviour when the peptides represented less than 50% of total surface area. Additional penetration experiments also demonstrated that both peptides better interact with POPG compared with POPC monolayers. This lipid preference is discussed as a possible explanation of their antibiotic properties.

The importance of bacterial membrane composition in the structure and function of aurein 2.2 and selected variants

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For cationic antimicrobial peptides to become useful therapeutic agents, it is important to understand their mechanism of action. To obtain high resolution data, this involves studying the structure and membrane interaction of these peptides in tractable model bacterial membranes rather than directly utilizing more complex bacterial surfaces. A number of lipid mixtures have been used as bacterial mimetics, including a range of lipid headgroups, and different ratios of neutral to negatively charged headgroups. Here we examine how the structure and membrane interaction of aurein 2.2 and some of its variants depend on the choice of lipids, and how these models correlate with activity data in intact bacteria (MICs, membrane depolarization). Specifically, we investigated the structure and membrane interaction of aurein 2.2 and aurein 2.3 in 1:1 cardiolipin/1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (CL/POPG) (mol/mol), as an alternative to 1:1 palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/POPG and a potential model for Gram positive bacteria such as S. aureus. The structure and membrane interaction of aurein 2.2, aurein 2.3, and five variants of aurein 2.2 were also investigated in 1:1 palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE)/POPG (mol/mol) lipids as a possible model for other Gram positive bacteria, such as Bacillus cereus. Solution circular dichroism (CD) results demonstrated that the aurein peptides adopted α-helical structure in all lipid membranes examined, but demonstrated a greater helical content in the presence of POPE/POPG membranes. Oriented CD and 31P NMR results showed that the aurein peptides had similar membrane insertion profiles and headgroup disordering effects on POPC/POPG and CL/POPG bilayers, but demonstrated reduced membrane insertion and decreased headgroup disordering on mixing with POPE/POPG bilayers at low peptide concentrations. Since the aurein peptides behaved very differently in POPE/POPG membrane, minimal inhibitory concentrations (MICs) of the aurein peptides in B. cereus strain C737 were determined. The MIC results indicated that all aurein peptides are significantly less active against B. cereus than against S. aureus and S. epidermidis. Overall, the data suggest that it is important to use a relevant model for bacterial membranes to gain insight into the mode of action of a given antimicrobial peptide in specific bacteria.

The molecular area characteristics of the HIV-1 gp41-fusion peptide at the airwater interface. Effect of pH

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Biochimica et Biophysica Acta 1326 _1997. 257–264

Abstract
The putative fusion peptide of HIV-1 is a highly surface active substance. Relevant measurements with the Langmuir monolayer technique have been carried out for a broad range of the pH in the aqueous subphase. The data are processed towards a quantitative analysis of the partitioning equilibrium between the interfacial and aqueous moieties. Our results reveal a pronounced decrease of the surface area per peptide molecule upon monolayer compression. This phenomenon could be interpreted in terms of an orientational transition experienced by an α-helical peptide structure. The area requirements at any fixed lateral pressure pass through a distinct minimum at a pH of 5.5 ~0.5,. Such an apparent isoelectric point was confirmed by isoelectric focusing of peptide aggregates. Accordingly a drastic drop of the pK-values of the two basic amino acid residues in comparison with an aqueous medium is indicated. It can be readily explained based on an inherent decrease of the effective dielectric constant. The observed low pH in favor of an enhanced surface affinity of the peptide may be a significant factor concerning its function as a fusion promoting agent.
The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes

Review
Re´gine Maget-Dana

Abstract
Erudites of the antiquity already knew the calming effect of oil films on the sea waves. But one had to wait until 1774 to read the first scientific report on oil films from B. Franklin and again 1878 to learn the thermodynamic analysis on adsorption developed by J. Gibbs. Then, in 1891, Agnes Pockels described a technique to manipulate oil films by using barriers. Finally, in 1917, I. Langmuir introduced the experimental and theoretical modern concepts on insoluble monolayers. Since that time, and because it has been found to provide invaluable information at the molecular scale, the monolayer technique has been more and more extensively used, and, during the past decade, an explosive increase in the number of publications has occurred. Over the same period, considerable and ever-increasing interest in the antimicrobial peptides of various plants, bacteria, insects, amphibians and mammals has grown. Because many of these antimicrobial peptides act at the cell membrane level, the monolayer technique is entirely suitable for studying their physicochemical and biological properties. This review describes monolayer experiments performed with some of these antimicrobial peptides, especially gramicidin A, melittin, cardiotoxins and defensin A. After giving a few basic notions of surface chemistry, the surface-active properties of these peptides and their behavior when they are arranged in monomolecular films are reported and discussed in relation to their three-dimensional structure and their amphipathic character. The penetration of these antimicrobial peptides into phospholipid monolayer model membranes, as well as their interactions with lipids in mixed films, are also emphasized.

The interfacial properties of the peptide Polybia-MP1 and its interaction with DPPC are modulated by lateral electrostatic attractions

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Polybia-MP1 (IDWKKLLDAAKQIL-NH2), extracted from the Brazilian wasp Polybia paulista, exhibits a broadspectrum bactericidal activity without being hemolytic and cytotoxic. In the present study, we analyzed the surface properties of the peptide and its interaction with DPPC in Langmuir monolayers. Polybia-MP1 formed stable monolayers, with lateral areas and surface potential values suggesting a mostly α-helical structure oriented near perpendicular to the membrane plane. In DPPC–peptide mixed monolayers, MP1 co-crystallized with the lipid forming branched domains only when the subphase was pure water. On subphases with high salt concentrations or at acidic or basic conditions, the peptide formed less densely packed films and was excluded from the domains, indicating the presence of attractive electrostatic interactions between peptides, which allow them to get closer to each other and to interact with DPPC probably as a consequence of a particular peptide arrangement. The residues responsible for the peptide–peptide attraction are suggested to be the anionic aspartic acids and the cationic lysines, which form salt bridges, leading to oriented interactions in the crystal and thereby to branched domains. For this peptide, the balance between total attractive and repulsive interactions may be finely tuned by the aqueous ionic strength and pH, and since this effect is related with lysines and aspartic acids, similar effects may also occur in other peptides containing these residues in their sequences.