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Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2016) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Antimicrobial silver-filled silica nanorattles with low immunotoxicity in dendritic cells

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Received 21 September 2015; accepted 1 August 2016

Abstract

The progression in the use of orthopedic implants has led to an increase in the absolute number of implant infections, triggering a search for more effective antibacterial coatings. Nanorattles have recently gained interest in biomedical applications such as drug delivery, as encapsulation of the cargo inside the hollow structure provides a physical protection from the surrounding environment. Here, silver-containing silica nanorattles ($Ag@SiO_2$) were evaluated for their antimicrobial potential and for their impact on cells of the immune system. We show that $Ag@SiO_2$ nanorattles exhibited a clear antibacterial effect against *Escherichia coli* as well as *Staphylococcus aureus* found in post-operative infections. Immunotoxicological analyses showed that the particles were taken up through an active phagocytic process by dendritic cells of the immune system and did not affect their viability nor induce unwanted immunological effects. Silver-containing silica nanorattles thus fulfill several prerequisites for an antibacterial coating on surgical implants.

Key words: Silver nanoparticles; Silica nanocontainers; Nanorattles; Dendritic cells; Immunoresponse; Antimicrobial properties

Abbreviations: AgNPs, silver nanoparticles; $Ag@SiO_2$, silver-containing nanorattles; $Ag@FITC\text{-}SiO_2$, silver-containing fluorescent nanorattles; TEM, transmission electron microscope; FTIR, Fourier transform infrared spectroscopy; DLS, dynamic light scattering; FITC, fluorescein; BMDC, bone marrow-derived dendritic cells.

Funding Sources: The authors would like to thank the University of Fribourg, the Fribourg Center for Nanomaterials (FriMat), the Swiss National Science Foundation (SNSF) (projects 200020-130431 and 406240-126055 to K.F, projects 138284, 156372 and 156871 to C.B.), the National Centre of Competence in Research (NCCR) "Bio-Inspired Materials", the Swiss Cancer Research Foundation (grant KFS-2910-02-2012 to C.B.), the "Fonds de Recherche UNIFR", and the "Fonds d'Encouragement de la Faculté" for generous funding of the project. This project was performed within The National Research Programme NRP-62 "Smart Materials".

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Each year, a large number of patients undergo joint replacement surgery. Postoperative prosthetic joint infection is a severe complication after arthroplasty that affects 1% to 3% of patients,[1] leading initially to surgical and antibiotic treatment and frequently ending with a need for replacement of the implant.[2,3] It is thus of great importance to develop intelligent nanomaterials which prevent infections and are biocompatible at the same time.[4]

With the emergence of bacterial resistance to conventional antibiotics, silver-based compounds and silver nanoparticles again enjoy rising popularity[5–12] as antimicrobial and healing agents.[13,14] Undoubtedly, the greatest advantage of silver originates from its multidirectional mode of action against microbes. In contrast to single-target antibiotics, the development of resistance is thus more difficult and requires several sequential mutations in the bacterial cell.[14,15] At concentrations within a therapeutic window, silver does not exhibit adverse effects toward mammalian cells while at the same time preventing bacterial survival.[14]

http://dx.doi.org/10.1016/j.nano.2016.08.002
1549-9634/

Ag(I)-containing coordination polymers demonstrate good biocompatibility as well as antimicrobial activity, making them promising candidates to fight biomaterial-related infections.[16–18] However, despite their excellent properties, silver is released rapidly during the first weeks after synthesis, leading to a short-term efficacy. For a better controlled, long-term release of silver cations, we propose to use encapsulated silver nanoparticles (AgNPs) as a source of silver cations[19,20] in an inorganic nanocarrier. Nanorattles composed of silica shells may serve as alternatives for silver-based drug delivery because of the possibility of drug loading in their cavity.[21–24] These nanorattles present a strong advantage as they provide a physical barrier for drug protection against the biological environment.[25,26]

Nanoparticles can exhibit immunological effects: particulate material can induce the secretion of proinflammatory cytokines by cells of the immune system[27] or on the contrary suppress immune functions.[28] It is furthermore well established that following arthroplasty, nano-sized debris detach from the implant through wear mechanisms.[29] These debris can interact both locally and systemically with immune cells and induce the release of pro-inflammatory cytokines such as IL-6. In the case of NP designed for biomedical applications such as implant coating, it is therefore critical to carefully assess the impact of particles on the activity of immune cells.

Despite the exceptional antimicrobial properties of AgNPs against bacteria,[30] fungi[31] and viruses,[32] few studies on the preparation of antibacterial nanorattles have been reported so far. Wei et al[33] described $Ag@Fe_2O_3$ nanorattles with conjugated glucose. This system serves as an example of multifunctional nanorattles in which glucose captures the bacteria, silver kills them and the magnetic shell allows the efficient removal of the nanorattles from the contaminated drinking water.

Here, we report the synthesis of silica nanorattles filled with silver ($Ag@SiO_2$) made by a microemulsion approach.[34] A first screen to assess whether the $Ag@SiO_2$ particles fulfill a set of requirements in order to be considered for the coating of orthopedic implants was carried out. Antibacterial properties of $Ag@SiO_2$ were tested against both gram-negative and gram-positive bacteria, while the cytotoxicity and the proinflammatory activity were determined with primary immune cells. Additionally, we generated for the first time AgNPs encapsulated inside fluorescent silica shells ($Ag@FITC\text{-}SiO_2$) to determine cellular uptake.

Methods

Synthesis of nanocontainers and nanorattles

Non-fluorescent and fluorescent nanorattles were prepared under argon according to the compositions shown in Table S1. First, 1.4 mL of water or $AgNO_3$ (AppliChem, Darmstadt, Germany) aqueous solution (0.01 or 0.05 or 0.1 M) was slowly injected under vigorous stirring into a mixture consisting of 29.6 g of cyclohexane (Sigma-Aldrich, Buchs, Switzerland) and 3.5 mL of Igepal CO-520 (Sigma-Aldrich, Buchs, Switzerland) at 28 °C. After 2 hours, 75 μL of hydrazine monohydrate (Fluka, Buchs, Switzerland) was added and left for 2 hours. Then, silica precursors were added dropwise: first, the non-fluorescent ones (200 μL of tetraethyl orthosilicate (Sigma-Aldrich, Buchs, Switzerland), 50 μL of 12.5 v/v% ethanolic solution of APTMS) and after 1 hour, the fluorescent one (50 μL of ethanolic solution of FITC-APTMS). After another hour, 500 μL of 28-30% aqueous NH_3 was slowly injected and the reaction was stirred for 36 hours. The microemulsion was destabilized by addition of 25 mL ethanol. The resulting precipitate was collected under centrifugation (15,000 rpm, 30 min, room temperature), washed twice with 25 mL ethanol and 25 mL ultrapure water (15,000 rpm, 15 min, room temperature). Final washing with 20 mL warm ultrapure water (60 °C, 40 min, stirring) followed by centrifugation (15,000 rpm, 15 min, room temperature) resulted in the formation of the void in the silica spheres. Calcination of non-fluorescent NPs was performed during TGA analysis (SDTA/TGA 851°, Mettler Toledo AG, Greifensee, Switzerland) using aluminum crucibles (40 μL). The measurement was conducted in the presence of nitrogen gas and air to provide combustion of organic residues. The temperature ranged from 25 to 600 °C, with a heating rate of 10 K min^{-1}.

Characterization of nanocontainers and nanorattles

Morphology of the samples is characterized by transmission electron microscopy (TEM) (CM-100 Biotwin Transmission Electron Microscope, FEI/Philips, Hillsboro, Oregon, USA) at the operating voltage of 80 kV, in bright field mode. Sample preparation included a sonication of NPs in ultra-pure water. A drop of diluted suspension was deposited on the TEM grid (Electron Microscopy Sciences, CF 300-Cu, Carbon Film on 300 Square Mesh Copper Grids) and let to dry.

UV–Vis spectra of nanoparticle suspensions were recorded with UV/Vis Spectrometer (Lambda40, Perkin Elmer, Schwerzenbach, Switzerland) at wavelengths ranging from 250 to 800 nm. The fluorescence spectra were measured by using a Luminescence Spectrometer LS 50B (Perkin Elmer, Schwerzenbach, Switzerland) and the Software FL Win Lab. The samples were excited at λ = 492 nm and the spectra were measured within λ = 400-600 nm (Ex. Slit = 5.0, Scan Speed = 100).

Silver loading and release

The amount of silver for each type of nanorattle was determined by ICP after resuspension in nitric acid (32.5%) with ultrasonication. For the silver loading 2-4 mg of $Ag@SiO_2$ nanorattles were suspended in concentrated nitric acid to reach 0.3 mg mL^{-1} and sonicated for about 3 hours before ICP measurements. For silver release analysis 10 mg of $Ag@SiO_2$ nanorattles were resuspended in 5 mL water by vortexing and sonication (30 min). After dilution to a concentration of 0.2 mg mL^{-1} the suspension was split into samples of 1 mL. Those were shaken at 37 °C (200 rpm) in the dark for the required time periods. After centrifugation (2 h, 17,000 rpm) 0.5 mL of the supernatant was taken for ICP analysis. Each time point was analyzed in triplicate.

Spread plate method

$Ag@SiO_2$-3 nanorattles were resuspended in filtrated distilled water and sonicated for 30 minutes. In general, first 100 μL of $Ag@SiO_2$ suspension and then 10^3 to 10^6 cfu mL^{-1} of