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Antibacterial and antifungal properties of dendronized silver and gold nanoparticles with cationic carbosilane dendrons

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ABSTRACT

Water soluble silver nanoparticles (AgNPs) capped with cationic carbosilane dendrons have been synthesized by direct reaction in water of dendrons, silver precursor and a reducing agent. These nanoparticles have been characterized by nuclear magnetic resonance (NMR), transmission electron microscopy (TEM), dynamic light scattering (DLS), thermogravimetric analysis (TGA), ultraviolet spectroscopy (UV), elemental analysis, and zeta potential (ZP). The antibacterial and antifungal properties of the cationic dendrons and dendronized AgNPs and AuNPs with these dendrons have been evaluated against Gram-negative and Gram-positive bacterial –including resistant strains- and yeast strains, respectively. The results stand out for the activity of AgNPs covered with first generation dendron compared with this free dendron and corresponding dendronized AuNPs.

1. Introduction

Resistance of microorganisms to traditional antibiotics (Blair et al., 2015) and microorganisms contamination (Campoccia et al., 2013; Clasen, 2009), are of major concerns to public health systems (WHO, 2014). Furthermore, the response of microorganisms to a specific drug can be variable; for example, differences in bacterial cell walls call for different drug treatment. Hence, the search for systems with non-specific activity towards a library of microorganisms is of prime importance. With this goal in mind, metal-based nanoparticles (NPs) (Hajipour et al., 2012) and polycationic macromolecules (Xue et al., 2015) are being investigated.

Within metal NPs, silver nanoparticles (AgNPs) are clearly one of the better developed system (Le Ouay and Stellacci, 2015), as a consequence of the well-known antibacterial properties of silver (Alexander, 2009). They are active against a broad range of microorganisms, even at low doses, with the advantage of reducing toxicity. Release of Ag^+ cations into the surrounding has also been associated with their activity (Xiu et al., 2012). Regarding the mechanism of action, there are different ways. It forms metal-organic complexes and insoluble compounds with sulfhydryl groups. In the cellular membrane silver cations interact with proteins involved in respiration and transport, thereby moderating ATP production and influencing membrane permeability. It also blocks the electron transport chain, interferes with cell wall synthesis, and influences a number of metabolic pathways and DNA replication and transcription (Pandey et al., 2014; Sondi and Salopek-Sondi, 2004). Moreover, even multidrug-resistant strains have being successfully inhibited by silver compounds (Kapoor et al., 1989; Lara et al., 2010). On the other hand, AuNPs have also been explored as antibacterials (Li et al., 2014), particularly because of the inertness and low toxicity of gold (Connor et al.,

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2005), the activity being dependent on ligands attached to NPs surface.

Regarding polycationic systems, cationic multivalency is responsible of their activity, the target being the cytoplasmic membrane in bacteria (Chen and Cooper, 2002; McDonnell and Russell, 1999). Removal of the divalent cations present into its structure destabilizes the membrane leading to bacteria death (Clifton et al., 2015). Dendritic systems are one type of such antibacterial macromolecules (Tülü and Ertürk, 2012), being highly branched monodisperse macromolecules with well-defined shape and structure as consequence of their step- by-step synthesis (Newkome et al., 2001). Two main topologies can be distinguished for dendritic macromolecules: i) spherical dendrimers; ii) dendrons that are cone-shaped with a periphery similar to dendrimers and the focal point at the vertex of the cone. Thus, this latter topology makes it possible for them to be bound to material surfaces or other biomaterials through this moiety, transferring dendron properties to them (Peña-González et al., 2016; Ghosh and Banthia, 2004; Moussodia et al., 2010). A variety of dendritic molecules are known, being the structure and composition of their scaffold their hallmark: polyamidoamine (PAMAM), polypropileneimine (PPI), polyester, phosphorus or silicon (carbosilane) containing dendrimers, and others. These scaffolds can be hydrophilic (PAMAM, PPI, polyester), hydrophobic (phosphorus and silicon containing derivatives), hydrolizable (polyester). In the particular case of carbosilane dendrimers, due to their lypophilic nature, which facilitates interaction with lipidic membranes (Wrobel et al., 2012), and an adequate functionalization they have shown attractive biomedical applications as gene carriers (Bermejo et al., 2007; Sánchez-Nieves et al., 2014; Serramía et al., 2015), bactericides (Ortega et al., 2011; Rasines et al., 2009) or antivirals (Arnáiz et al., 2014; Vacas-Córdoba et al., 2014, 2016), being able also to cross the blood brain barrier (Fuentes-Paniagua et al., 2015; Serramía et al., 2015).

Recently, we have reported the antibacterial (Fuentes-Paniagua et al., 2014, 2016) and antiamebicide (Heredero-Bermejo et al., 2015, 2013) activity of cationic carbosilane dendrimers and dendrons highlighting the wide spectra of lower generation systems against Gram-positive and Gram-negative bacteria, methicillin resistant *S. aureus*, and also against *Acanthameba* trophozoites. The good action of these small systems is consequence of a suitable balance between the hydrophilic and hydrophobic parts of the molecules, the ammonium periphery and the carbosilane framework, respectively (Fuentes-Paniagua et al., 2016). One of these compounds –decorated with $—NH_3^+$ groups- when combined with chlorhexidine digluconate acts synergistically against *A. polyphaga* (Heredero-Bermejo et al., 2016). Other ammonium dendritic structures also have antibacterial activity, although in the case of well-known PAMAM and PPI systems, the best dendrimers were of the fourth and higher generations, which lead to longer synthetic procedures (Chen et al., 2000; Tülü et al., 2009), whilst for viologen-phosphorus dendrimers good activity were found for low generation systems (Ciepluch et al., 2012). On the other hand, it has been reported that combinations of low active dendrimers and dendrons with Ag^+ notably improve their antibacterial behaviour compared with both dendritic systems and $AgNO_3$ (Suleman et al., 2015).

Herein, we report the synthesis of AgNPs capped with cationic carbosilane dendrons with the aim of exploring the combination of two microbicides systems. Their antibacterial and antifungal activity were explored and compared with those observed for free dendrons and also with analogous AuNPs covered with the same type of dendrons (Peña-González et al., 2017). For this purpose, four Gram-positive and two Gram-negative bacterial strains (including two multiresistant strains) and two strains of yeasts were selected.

2. Materials and methods

2.1. General considerations

All reactions were carried out in an inert atmosphere and solvents were purified with appropriate drying agents if necessary. Thiol-ene reactions were carried out with an HPK 125W Mercury Lamp from Heraeus Noblelight with maximum energy at 365 nm in normal glassware under inert atmosphere. ^{1}H NMR spectra were recorded on a Varian Unity VXR-300 (300.13 MHz) or on a Bruker AV400 (400.13 MHz). Chemical shifts (δ) are given in ppm. ^{1}H resonances were measured relative to solvent peaks considering TMS = 0 ppm. Elemental analyses were done on a LECO CHNS-932. UV–vis absorption was measured with a Perkin-Elmer Lambda 18 spectrophotometer. The spectra were recorded by measuring dilute samples in a quartz cell with a path length of 1 cm. The silver content of filtered solutions as determined by ICP (Inductive Coupling Plasma) using an ICP Optical Emission Spectrometer Varian 720-ES at 328.068 nm Each sample was divided in two portions and measured twice. The detection limit is below 10 ppb. $AgNO_3$ and $NaBH_4$ were obtained from commercial sources. Compounds $HSG_n(S\text{-}NMe_3Cl)_m$ and AuNPs (**1Au**-**3Au**) were synthesized as previously published (Peña-González et al., 2017).

2.2. Synthesis of compounds

A description of the synthesis of first generation AgNPs (**1Ag**) follows, the procedures and data of compounds of all compounds and techniques used being found in the Supporting information.

AgNP(SG1(S-NMe3Cl)2) (1Ag)

An aqueous solution of compound $HSG_1(S\text{-}NMe_3Cl)_4$ (**1**) (40 mL, 0.5 mmol, 12.5 mM) was added dropwise to an aqueous solution of $AgNO_3$ (16.3 mL, 0.5 mmol, 30 mM). $NaBH_4$ in water (13.5 mL, 2.7 mmol, 200 mM) was next added dropwise, and the mixture stirred for 4 h. Nanoparticles were purified by dialysis (MWCO 10.000) yielding **1Ag** (108 mg), which were stored in deionized water at 4 °C.

Data for **1Ag**: NMR (D_2O): ^{1}H NMR: δ 0.06 ($SiCH_3$), 0.60 ($SCH_2CH_2CH_2CH_2Si$), 0.90 ($SiCH_2CH_2S$), 1.40 ($SCH_2CH_2CH_2CH_2Si$), 1.78 ($SCH_2CH_2CH_2CH_2Si$), 2.74 ($SiCH_2CH_2S$), 2.97 (SCH_2CH_2N), 3.10 (NCH_3), 3.50 (SCH_2CH_2N). Ag/(l) reactant molar ratio = 1:1. TGA (%): Ag, 46.4; (l), 53.6. Calc. molar ratio Ag/(l) = 3.99:1 in nanoparticle. SPR (UV–vis): 447.8 nm. Zeta Potential: +53.4. DLS (Z-average d. nm) = 11.70 nm. Mean diameter of silver core (TEM): D = 1.70 nm. Number of silver atoms: N_{Ag} = 143; number of dendrons N_d = 36. Molecular formula: $Ag_{143}(C_{19}H_{45}Cl_2N_2S_3Si)_{36}$. Average Mw = 64733309.85 $gmol^{-1}$.

2.3. Antimicrobial activity

Methods used for microbial susceptibility tests in vitro followed instruction M7-A7 of Clinical and Laboratory Standards Institute (CLSI, 2006, 2008). Antimicrobial activity was assayed against four gram positive and two gram-negative bacterial strains and two strains of yeast:

Staphylococcus aureus (ATCC 6538P)–S, susceptible strain recommended for antimicrobial activity testing;

Staphylococcus aureus (ZMF KSK)–R, multiresistant strain from clinical specimen from human (MRSA–methicillin resistant S. aureus);

Staphylococcus hemolyticus R (ZMF SV212), multiresistant strain from clinical specimen from human (MRSH–methicillin resistant S. hemolyticus);

Enterobacter fecalis (ZMF BD156), strain isolated from a clinical specimen;

Escherichia coli (ATCC 8739), susceptible strain recommended for antimicrobial activity testing;

Pseudomonas aeruginosa (ATCC 27853), susceptible strain recommended for antimicrobial activity testing;

Candida albicans (ATCC 10231), yeasts commonly used for such testing;

Candida glabrata (ZMF40), yeasts isolated from a clinical specimen.

Compounds for analysis were suspended in small amount of demineralized sterile water and then in Mueller-Hinton Broth (BioMaxime) for testing of bacteria, and in RPMI-1640 Medium (Sigma) for yeasts. Serial two-fold dilutions were prepared in a microtiter tray in range 500–3.9 mg/L.

Test strains were inoculated into each well of a microtiter plate at 10^6 CFU of bacteria and 10^4 CFU of yeasts per 1 mL. After 24 h incubation at 37 °C for bacteria and 48 h at 25 °C for yeasts, increase in turbidity at 595 nm was measured with microplate reader (MR 680 Bio-Rad). MIC (minimal inhibitory concentration) values were the lowest concentrations where there was no measurable increase in optical density. MBC (minimal bactericidal concentration) was the lowest concentration at which the compound killed all cells, there being no growth in subculture on the surface of appropriate rich agar for each organism in Petri dish after 24 or 28 h of incubation at the appropriate temperature.

2.4. CytotoxicityMTT assay

The human Caucasian embryo skin cell (Detroit 551 (ATCC® CCL-110™)) grown routinely in DMEM (Dulbecco's modified Eagle Medium) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin B (all from Sigma-Aldrich) at 37 °C, 5% CO_2 and 95% of humidity. Cells were seeded in 24-well plates (Nunclon Delta Surface, Thermo Fischer Scientific) as monolayers (*ca.* 8×10^3) and grown for 72 h in complete medium (450 μL).

Solutions of compounds were prepared by diluting a freshly prepared stock solution (in water) of the corresponding compound in aqueous medium (DMEM). Afterward, the intermediate dilutions of the compounds were serially diluted to the appropriate concentration with DMEM (ranging from 0 to 100 μM) and the cells were incubated for another 24 h.

Cytotoxicity was determined using the MTT assay (MTT 3-(4,5dimethyl 2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide). After incubation, MTT (5.0 mg/mL solution) was added to the cells and the plates were incubated for a further 3.5 h. Then the culture medium was removed and the purple formazan crystals formed by the mitochondrial dehydrogenase and reductase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 570 nm (background correction at 690 nm) using a multiwell plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells.

The IC_{50} value indicates the concentration needed to inhibit a biological function of the cells by half and is presented as a mean (±SD) from three independent experiments, each comprising three microcultures per concentration level.

3. Results and discussion

3.1. Synthesis of nanoparticles

The nomenclature used for dendrons is of the type $XG_n(S\text{-}Y)_m$, which describes the structure of compounds as follow (see Fig. 1 and S1): X refers to the type of focal point; G_n corresponds with the carbosilane framework and respective dendron generation (n); and $(S\text{-}Y)_m$, which indicates the type of peripheral groups (Y), its number (m), and the presence of a sulfur atom close to the surface due to the preparative method.

Dendronized AgNPs were easily produced in water by the direct reaction of $AgNO_3$, cationic carbosilane dendrons with a thiol moiety at the focal point $HSG_n(S\text{-}NMe_3^+)_m$ (n = 1, m = 2 (**1**); n = 2, m = 4 (**2**); n = 3, m = 8 (**3**)) (Fig. S1) (Peña-González et al., 2017), and $NaBH_4$ acting as reducing agent (Scheme 1, Fig. 1), using a Ag/dendron ratio of 1/1. The cationic nanoparticles $AgNP(SG_n(S\text{-}NMe_3^+)_m)$ (n = 1, m = 2 (**1Ag**); n = 2, m = 4 (**2Ag**); n = 3, m = 8 (**3Ag**)) were isolated in high yield as black solids soluble in water. These systems were characterized by nuclear magnetic resonance (^{1}H NMR), transmission electron microscopy (TEM, Figs. S2, S4 and S6), thermogravimetric analysis (TGA), dynamic light scattering (DLS), ultraviolet spectroscopy (UV), elemental analysis, and zeta potential (ZP) (Table 1). AgNPs **1Ag-3Ag** were stable, maintaining their size and shape for 3 months (Fig. S8), i.e. they are less stable than their AuNPs counterparts that remain unchanged for about 10 months (Peña-González et al., 2017) and than other AuNPs stabilized with cationic systems as viologen dendrimers (Katir et al., 2014). It is important to note that there was no formation of related AgNPs with the cationic monomer $HS(CH_2)_2NMe_3^+$, probably due to the small size of this ligand.

The size of the three AgNPs, measured by TEM, increases with dendron generation (Table 1, Fig. 2), but the obtained Ag/dendron relationship does not follow this order, there being maximum for the second generation derivative **2Ag**. However, the number of dendrons and cationic groups on NPs also increase with dendron generation due to the greater size of NPs. The larger size seen by DLS can be attributed to several factors. For example, DLS discriminates smaller NPs (below 2 nm) as consequence of its detection threshold, and also enhances bigger NPs due to greater light scattering due to their size. Moreover, DLS measures the hydrodynamic size, which comprises also the dendrons on a metallic surface, whereas the TEM data corresponds mostly with the metallic core, and is done after drying the NPs (Cho et al., 2014). The diameter (d_z) and polidispersity (PDI) obtained by DLS can be used to calculate a theoretical d_n value ($Cd_n = d_z/(1+Q)^5$; Q corresponds with PDI) (Cho et al., 2014; Hanus and Ploehn, 1999). This formula gives results for d_n (Cd_n) closer to those measured by TEM (Table 1).

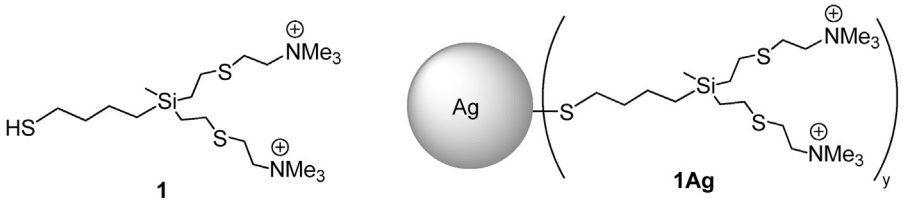
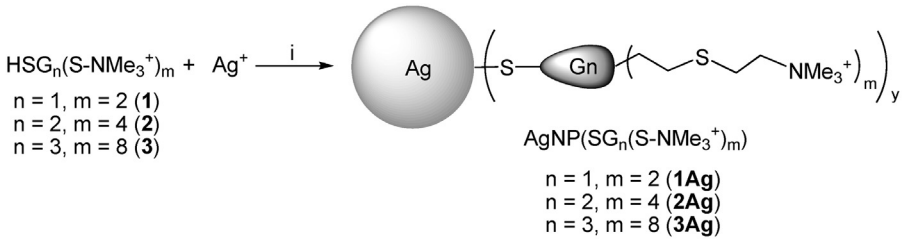


Fig. 1. Drawing of first generation cationic dendron **1** and its corresponding AgNP **1Ag**.



Scheme 1. Synthesis of silver nanoparticles AgNP(SG_n(S-NMe_3^+)$_m$) (n = 1, m = 2 (**1Ag**); n = 2, m = 4 (**2Ag**); n = 3, m = 8 (**3Ag**)). (i) $NaBH_4$.

Table 1
Selected data of dendronized AgNPs and AuNPs with dendrons **1-3**. a) L refers to dendron; b) Diameter (d_n, nm) obtained by TEM, corresponds with the mode value; c) Diameter (d_z, nm) obtained by DLS; d) d_n (nm) calculated ($Cd_n = d_z/(1+Q)^5$; Q corresponds with PDI); (Hanus and Ploehn, 1999); e) Polydispersity index (PDI) obtained by DLS; f) Molecular formula (MF) and weight (MW, $gmol^{-1}$) obtained from TEM and TGA (see Experimental Section); g) Number of $-NMe_3^+$ groups by NP; h) Zeta potential (mV). Data of **1Au-3Au** have been reported elsewhere (Peña-González et al., 2017).

Nanoparticles	Molar Ratio M/L[a]		d_n[b]	d_z[c]	Cd_n[d]	PDI[e]	MF[f]	MW[f]	N[g]	ZP[h]
	Theoretic	Obtained								
AgNP(SG_1(S-NMe_3^+)$_2$) (**1Ag**)	1:1	4.0:1	1.7	11.7	1.5	0.5	$Ag_{143}(C_{19}H_{45}Cl_2N_2S_3Si)_{36}$	33309.85	72	+53.4
AgNP(SG_2(S-NMe_3^+)$_4$) (**2Ag**)	1:1	4.8:1	3.0	18.2	4.4	0.3	$Ag_{788}(C_{41}H_{97}Cl_4N_4S_5Si_3)_{165}$	255383.86	660	+41.0
AgNP(SG_3(S-NMe_3^+)$_8$) (**3Ag**)	1:1	3.2:1	3.9	43.8	5.9	0.5	$Ag_{1792}(C_{85}H_{201}Cl_8N_8S_9Si_7)_{557}$	1365420.42	4456	+60.1
AuNP(SG_1(S-NMe_3^+)$_2$) (**1Au**)	1:1	3.1:1	1.8	10.3	2.0	0.4	$Au_{180}(C_{19}H_{45}Cl_2N_2S_3Si)_{59}$	64761.64	118	+50.0
AuNP(SG_2(S-NMe_3^+)$_4$) (**2Au**)	1:1	2.5:1	2.2	15.8	2.5	0.4	$Au_{329}(C_{41}H_{97}Cl_4N_4S_5Si_3)_{132}$	201105.70	528	+63.7
AuNP(SG_3(S-NMe_3^+)$_8$) (**3Au**)	1:1	2.0:1	2.0	22.1	2.0	0.6	$Au_{247}(C_{85}H_{201}Cl_8N_8S_9Si_7)_{123}$	307482.90	984	+59.6

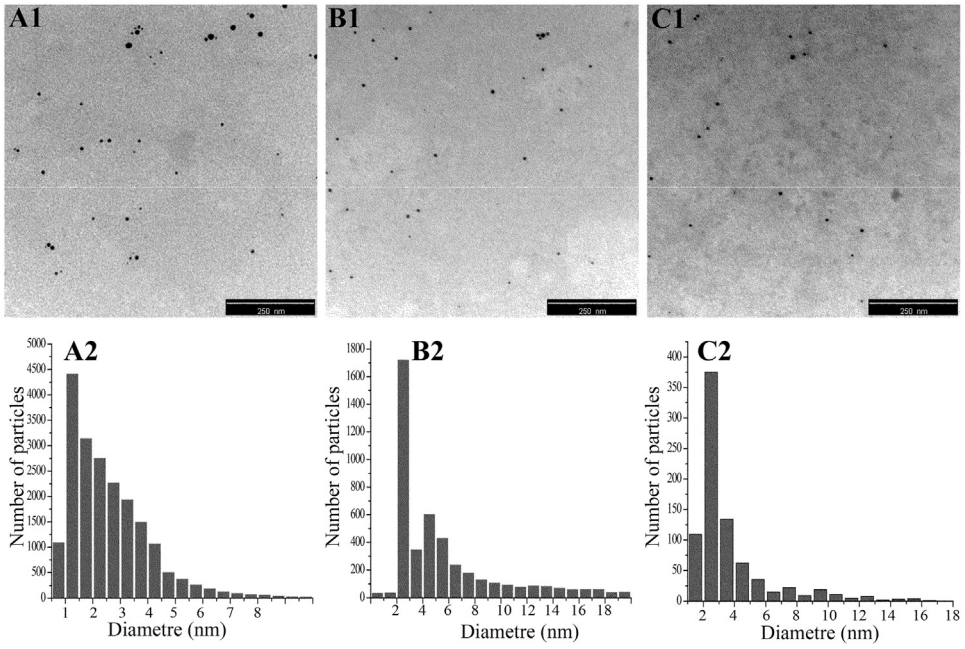


Fig. 2. TEM images (1) and size distribution histograms (2) associate to AgNPs **1Ag** (A), **2Ag** (B), and **3Ag** (C).

UV spectroscopy confirmed formation of NPs by means of the band at about 440 nm, which belongs to surface plasmon resonance. The ^1H NMR spectra of AgNPs showed the same resonances as dendrons, but were clearly broader (Fig. 3). There was no signal from the $—CH_2S$ group at the focal point of dendron (Figs. S3, S5 and S6), in a similar way to other cationic NPs (Peña-González et al., 2017). Finally, the zeta potential measured in aqueous solution at neutral pH attested to the stability of NPs **1Ag-3Ag** at this pH, since values higher than 40 mV were observed for the three systems. This means that the repulsion between the particles under these conditions is strong enough to avoid aggregate formation and precipitation. By this technique, a clearly smaller value was found for **2Ag**, which was the AgNPs at a higher Ag/dendron ratio, and this value was also smaller than those for corresponding AuNPs.

Comparison of AgNPs and AuNPs capped with the same type of dendron and synthesized following the same procedure and starting M/dendron relationship (1/1) (Peña-González et al., 2017), shows smaller sizes for AuNPs but first generation derivatives **1Ag** and **1Au**, which were of similar size. The size histograms also indicate higher dispersity for AgNPs (Fig. 2). Regarding the obtained M/dendron ratio, this was lower for AuNPs.

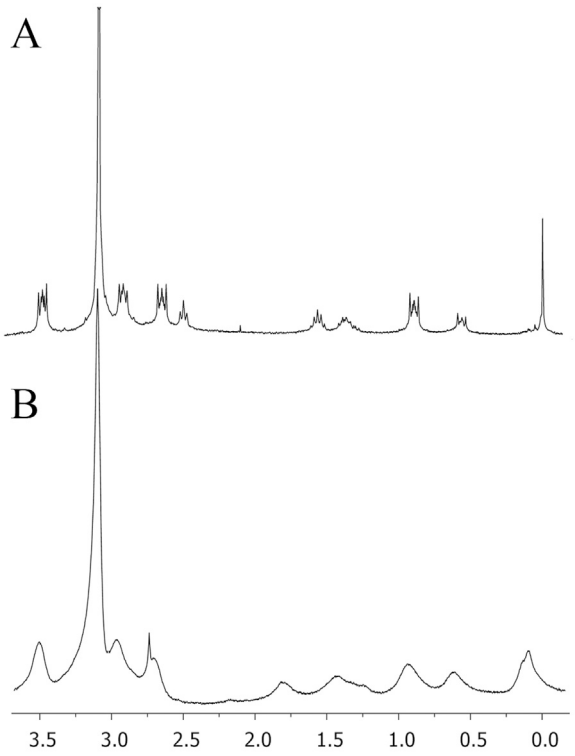


Fig. 3. ^{1}H NMR of first generation dendron **1** (A) and corresponding AgNPs **1Ag** (B) in D_2O.

Table 2
Antibacterial and antifungal activity and IC50 in fibroblasts of the best compounds for each type of system: $HSG_2(S\text{-}NMe_3^+)_4$ (**2**) for dendrons, $AgNP(SG_1(S\text{-}NMe_3^+)_2)$ (**1Ag**) for AgNPs and $AuNP(SG_3(S\text{-}NMe_3^+)_8)$ (**3Au**) for AuNPs. Data are in ppm (mg mL^{-1}). MIC (minimal inhibitory concentration); MBC (minimal bactericidal or fungicidal concentration).

	$AgNO_3$		**2**		**1Ag**		**3Au**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus S	15.6	15.6	3.9	15.6	0.9	1.8	17.9	35.8
S. aureus R	7.8	15.6	3.9	3.9	0.9	0.9	14.3	28.6
S. haemolyticus R	3.9	7.8	7.8	15.6	0.4	7.0	17.9	35.8
E. faecalis	3.9	7.8	7.8	15.6	1.8	7.0	35.8	71.4
P. aeruginosa	7.8	15.6	62.5	62.5	1.8	140	57.1	57.1
E. coli	3.9	3.9	7.8	7.8	0.9	7.0	35.8	35.8
C. albicans	3.9	7.8	31.3	250	1.8	1.8	47.6	190
C. glabrata	2.0	2.0	31.3	62.5	1.8	140	47.6	190
IC50	3.19 ± 0.01		8.91 ± 1.12		9.02 ± 0.34		13.51 ± 0.32	

3.2. Antibacterial and antifungal activity

Cationic dendrons $HSG_n(S\text{-}NMe_3^+)_m$ (**1-3**) and dendronized NPs with the dendrons **1Ag-3Ag** and **1Au-3Au** were tested for their antibacterial and antifungal properties (Tables 2 and S1-S3). Analysis of antibacterial activity for each family of compounds (MIC (minimal inhibitory concentration) and MBC (minimal bactericidal or fungicidal concentration)) showed that the second generation derivative **2** was the best dendron (Table 2 and S1), in agreement with previous results obtained for cationic carbosilane dendrons (Fuentes-Paniagua et al., 2016), that **1Ag** were the best AgNPs (Table 2 and S2), and finally that **3Au** were best AuNPs (Tables 2 and S3). Hence, dendronization of these NPs was very effective for **1Ag**, since for all the other NPs dendrons of the same generation were generally more active as microbicides than dendronized NPs. Thus, of the nine systems studied, AgNP **1Ag** and dendron $HSG_2(S\text{-}NMe_3^+)_4$ (**2**) were the best (Table 2). The data obtained for **1Ag** are of special relevance since these NPs improve clearly the values of the first generation dendron, i. e. the dendron easier and cheaper to synthesize, the least toxic, and, in general, improve the values obtained for $AgNO_3$. Regarding the type of bacteria, both dendron **1** and AgNPs **1Ag** act as broad spectrum bactericides, being active even against methicillin resistant *S. aureus* and *S. hemolyticus*. The MBC for *P. aeruginosa* of **1Ag** is the only value surpassed by the other dendrons, except **1**, or NPs, except **1Au** (see comments below).

Regarding yeasts, AgNPs **1Ag** were very active against *C. albicans* (MBC = 1.75 ppm) although the MBC for *C. glabrata* was high (140 ppm, see comments below). This value was better for the other two AgNPs and dendrons **2** and **3** and $AgNO_3$. For the other type of systems, dendrons and AuNPs, the activity is clearly dependent on the number of ammonium groups, increasing with their number.

It is also important to note that toxicity (IC50 in fibroblasts, Tables 2 and S4) was over activity (MIC) only for **1Ag** (bacteria and fungi) and **2Au** (not for fungi). Moreover, MBC of **1Ag** was higher than IC50 only for *P. aeruginosa* and *C. glabrata*, but not even for resistant strains. Regarding $AgNO_3$, this compound were more cytotoxic than active but for *C. glabrata*.

There are more examples showing that *P. aeruginosa* and *C. glabrata* are more resistant to drugs. For some microorganisms, the presence of drugs (in our case possibly dendrons and NPs with high MBC) makes bacterial cells aggregate, with those inside them staying alive. In the case of both these species, drug resistance is due to an active efflux mechanism, this becoming more effective when aggregates are formed (Cushnie et al., 2007; Linares et al., 2005). *C. glabrata* can also upregulate the rate of drug efflux without losing the ability to maintain colonization (Bennett et al., 2004; Vallabhaneni et al., 2015).

Scanning Electron Microscopy (SEM) of *E. coli* and *S. aureus* cells treated with $HSG_2(S\text{-}NMe_3^+)_4$ (**2**) and AgNPs **1Ag** at MIC is shown in Fig. 4. *S. aureus* undergoes changes in morphology and size in the presence of silver nanoparticles; there are cocci cells that are twice the diameter of the controls (Fig. 4b). No significant changes were seen in the presence of dendron **2** at MIC. In a similar study, *E. coli* cells under both treatments become aberrant with alteration in their length (Fig. 4e and f), which can be 5 or 6 times the normal length, possibly caused by alterations in septum formation and/or lack of separation of the daughter cells during the division cycle.

The activity profile of each type of systems is related with their structure and composition, but taking into account that all of them contains peripheral NMe_3^+ groups. For dendrons, the results are determined by an adequate hydrophilic/hydrophobic balance (Fuentes-Paniagua et al., 2014, 2016). On the other hand, for NPs the exposure of the carbosilane framework (hydrophobic moiety) is hampered by its anchorage to the metal core and, therefore, the differences observed for AgNPs and AuNPs can be ascribed to differences in the behaviour of the metal core, since silver is considered an active metal whereas gold is considered innocuous for bacteria. The bactericide properties of AgNPs is due to their ability to release silver cations in contact with air (Xiu et al., 2012), this process being responsible of the high activity observed for **1Ag**, covered with the smallest dendron in comparison with the other AgNPs. On the other hand, for AuNPs the increase on activity should be related with the higher ability of bigger dendrons on nanoparticles surface to interact with bacterial membrane (Peña-González et al., 2017; Tian and Ma, 2012).

In an attempt to evaluate the release of silver cations from **1Ag-3Ag**, these NPs were stirred in an open stirred cell for 24 h (see SI), the solutions were ultrafiltered (MWCO = 3000 Da) and then the silver content analyzed by ICP. The data from this experiment

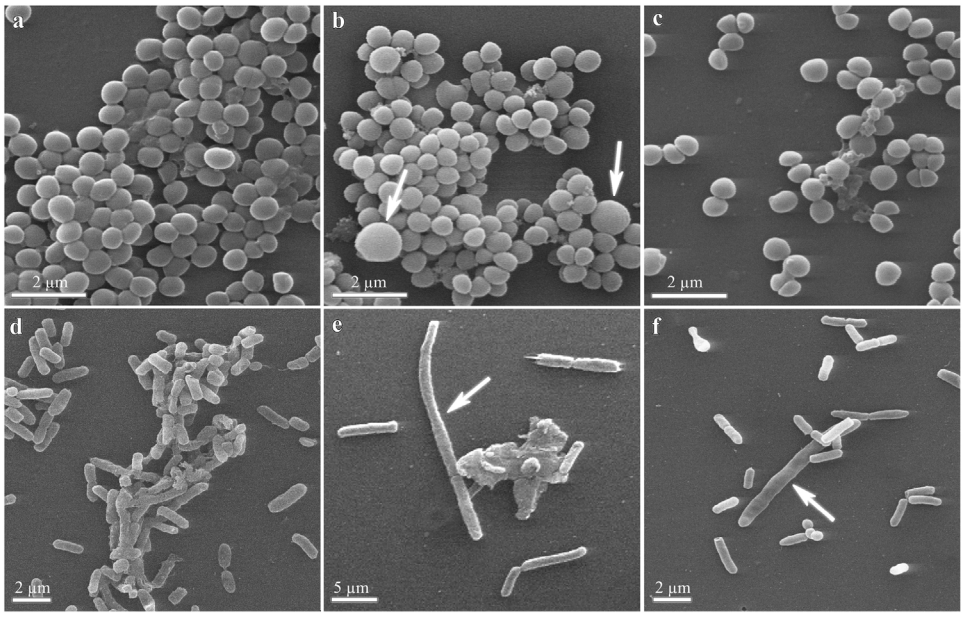


Fig. 4. SEM images of *S. aureus* and *E. coli*. (a) *S.aureus* control; (b) *S. aureus* treated with AgNPs **1Ag**; (c) *S. aureus* treated with $HSG_2(S\text{-}NMe_3^+)_4$ (**2**); (d) *E. coli* control; (e) *E. coli* treated with AgNPs **1Ag**; (f) *E. coli* treated with $HSG_2(S\text{-}NMe_3^+)_4$ (**2**). Scale bar is 2 μm, except in (e) where it is 5 μm.

showed higher values for solution coming from **1Ag** (153.24 ppb) than from **2Ag** (42.97 ppb) and **3Ag** (45.95 ppb). However, these values are probably distorted because formation of a black solid was observed during the stirring, for **1Ag** within few hours, indicating higher instability of this last system, probably because faster release of silver ions is produced due to the covering of these NPs with the smallest dendron.

4. Conclusions

Cationic silver nanoparticles can be straightforward prepared by reaction of cationic carbosilane dendrons with a —SH function at the focal point, $AgNO_3$ and the reducing agent ($NaBH_4$) in water, being stable for up to three months. A comparative study of antibacterial and antifungal behaviour of cationic dendrons, and dendronized AgNPs and AuNPs with these dendrons indicates the relevance of AgNPs dendronization with first generation dendron (**1Ag**). Although this dendron is scarcely active, the corresponding AgNPs are highly active, even against resistant strains. These AgNPs only were not effective as bactericide against gram-negative *P. aeruginosa* and as fungicide against *C. glabrata*, probably due to formation of aggregates protecting bacteria from drugs. For these microorganisms, better data were found with the second generation dendron, which was the other system with microbicide properties close enough to AgNPs **1Ag**. Finally, the activity of these systems are related to different factors, as hydrophobic/hydrophilic balance for dendrons, release of silver cations for AgNPs, and size of dendrons in AuNPs. The activity, mode of action and toxicity of the best system here employed, that is **1Ag**, could be proposed as dressing component for skin wounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.ijpharm.2017.05.067.

References

Alexander, J.W., 2009. History of the medical use of silver. Surg. Infect. 10, 289.
Arnáiz, E., Vacas-Córdoba, E., Galán, M., Pion, M., Gómez, R., Muñoz-Fernández, M. Á., de la Mata, F.J., 2014. Synthesis of anionic carbosilane dendrimers via click chemistry and their antiviral properties against HIV. J. Polym. Sci. Part A: Polym. Chem. 52, 1099.
Bennett, J.E., Izumikawa, K., Marr, K.A., 2004. Mechanism of increased fluconazole resistance in Candida glabrata during prophylaxis. Antimicrob. Agents Chemother. 48, 1773.
Bermejo, J.F., Ortega, P., Chonco, L., Eritja, R., Samaniego, R., Mullner, M., de Jesús, E., de la Mata, F.J., Flores, J.C., Gómez, R., Muñoz-Fernández, A., 2007. Water-soluble carbosilane dendrimers: synthesis biocompatibility and complexation with oligonucleotides; Evaluation for medical applications. Chem. Eur. J. 13, 483.
Blair, J.M.A., Webber, M.A., Baylay, A.J., Ogbolu, D.O., Piddock, L.J.V., 2015. Molecular mechanisms of antibiotic resistance. Nat. Rev. Microb. 13, 42.
CLSI, 2006. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards; Seventh Edition M7-A7 26-2.
CLSI, 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing in Yeasts; Approved standard; Third Edition M27A3 28-14.
Campoccia, D., Montanaro, L., Arciola, C.R., 2013. A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34, 8533.
Chen, C.Z., Cooper, S.L., 2002. Interactions between dendrimer biocides and bacterial membranes. Biomaterials 23, 3359.
Chen, C.Z.S., Beck-Tan, N.C., Dhurjati, P., van Dyk, T.K., LaRossa, R.A., Cooper, S.L., 2000. Quaternary ammonium functionalized poly(propylene imine) dendrimers as effective antimicrobials: structure-activity studies. Biomacromolecules 1, 473.
Cho, T.J., MacCuspie, R.I., Gigault, J., Gorham, J.M., Elliott, J.T., Hackley, V.A., 2014. Highly stable positively charged dendron-encapsulated gold nanoparticles. Langmuir 30, 3883–3893.
Ciepluch, K., Katir, N., Kadib, A.E., Felczak, A., Zawadzka, K., Weber, M., Klajnert, B., Lisowska, K., Caminade, A.-M., Bousmina, M., Bryszewska, M., Majoral, J.P., 2012. Biological properties of new viologen-phosphorus dendrimers. Mol. Pharm. 9, 448.
Clasen, T.F., 2009. Scaling Up Household Water Treatment Among Low-Income Populations Public Health and Environment Water, Sanitation, Hygiene and Health. World Health Organization.

Clifton, L.A., Skoda, M.W.A., Brun, A.P.L., Ciesielski, F., Kuzmenko, I., Holt, S.A., Lakey, J.H., 2015. Effect of divalent cation removal on the structure of gram-negative bacterial outer membrane models. Langmuir 31, 404–412.
Connor, E.E.M.J., Gole, A., Murphy, C.J., Wyatt, M.D., 2005. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small 1, 325.
Cushnie, T.P.T., Hamilton, V.E.S., Chapman, D.G., Taylor, P.W., Lamb, A.J., 2007. Aggregation of Staphylococcus aureus following treatment with the antibacterial flavonol galangin. J. Appl. Microbiol. 103, 1562.
Fuentes-Paniagua, E., Hernández-Ros, J.M., Sánchez-Milla, M., Camero, M.A., Maly, M., Pérez-Serrano, J., Copa-Patiño, J.L., Sánchez-Nieves, J., Soliveri, J., Gómez, R., de la Mata, F.J., 2014. Carbosilane cationic dendrimers synthesized by thiol–ene click chemistry and their use as antibacterial agents. RSC Adv. 4, 1256.
Fuentes-Paniagua, E., Serramía, M.J., Sánchez-Nieves, J., Álvarez, S., Muñoz-Fernández, M.A., Gómez, R., de la Mata, F.J., 2015. Fluorescein labelled cationic carbosilane dendritic systems for biological studies. Eur. Polym. J. 71, 61.
Fuentes-Paniagua, E., Sánchez-Nieves, J., Hernández-Ros, J.M., Soliveri, J., Copa-Patiño, J.L., Gómez, R., de la Mata, F.J., 2016. Structure-activity relationship study of cationic carbosilane dendritic systems as antibacterial agents. RSC Adv. 6, 7022.
Ghosh, S., Banthia, A.K., 2004. Biocompatibility and antibacterial activity studies of polyamidoamine (PAMAM) dendron, side chain dendritic oligourethane (SCDOU). J. Biomed. Mater. Res. A 71A, 1.
Hajipour, M.J., Fromm, K.M., Ashkarran, A.A., Jiménez de Aberasturi, D., Ruiz de Larramendi, I., Rojo, T., Serpooshan, V., Parak, W.J., Mahmoudi, M., 2012. Antibacterial properties of nanoparticles. Trends Biotechnol. 30, 499.
Hanus, L.H., Ploehn, H.J., 1999. Conversion of intensity-averaged photon correlation spectroscopy measurements to number-Averaged particle size distributions. 1. Theoretical development. Langmuir 15, 3091.
Heredero-Bermejo, I., Copa-Patiño, J.L., Soliveri, J., García-Gallego, S., Rasines, B., Gómez, R., de la Mata, F.J., Pérez-Serrano, J., 2013. In vitro evaluation of the effectiveness of new water-stable cationic carbosilane dendrimers against Acanthamoeba castellanii UAH-T17c3 trophozoites. Parasitol. Res. 112, 961.
Heredero-Bermejo, I., Copa-Patiño, J.L., Soliveri, J., Fuentes-Paniagua, E., de la Mata, F.J., Gómez, R., Pérez-Serrano, J., 2015. Evaluation of the activity of carbosilane dendrimers on trophozoites and cysts of Acanthamoeba polyphaga. Parasitol. Res. 114, 473.
Heredero-Bermejo, I., Sánchez-Nieves, J., Copa-Patiño, J.L., Soliveri, J., Gómez, R., de la Mata, F.J., Pérez-Serrano, J., 2016. In vitro anti-Acanthamoeba polyphaga synergistic effect of chlorhexidine and cationic carbosilane dendrimers against both trophozoites and cysts forms. Int. J. Pharm. 509, 1.
Kapoor, N., Chhibber, S., Vadehra, D.V., 1989. Susceptibility of multidrug-resistant isolates of Klebsiella pneumoniae to silver nitrate. Folia Microbiol. (Praha) 34, 94.
Katir, N., El-Kadib, A., Collière, V., Majoral, J.P., Bousmina, M., 2014. Viologen-based dendritic macromolecular asterisks: synthesis and interplay with gold nanoparticles. Chem. Commun. 50, 6981.
Lara, H.H., Ayala-Núñez, N.V., Ixtepan Turrent, L.d.C., Rodríguez-Padilla, C., 2010. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J. Microbiol. Biotechnol. 26, 615.
Le Ouay, B., Stellacci, F., 2015. Antibacterial activity of silver nanoparticles: a surface science insight. Nano Today 10, 339.
Li, X., Robinson, S.M., Gupta, A., Saha, K., Jiang, Z., Moyano, D.F., Sahar, A., Riley, M.A., Rotello, V.M., 2014. Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. ACS Nano 8, 10682.
Linares, J.F., López, J.A., Camafeita, E., Albar, J.P., Rojo, F., Martínez, J.L., 2005. Overexpression of the multidrug efflux pumps MexCD-OprJ and MexEF-OprN is associated with a reduction of type III secretion in Pseudomonas aeruginosa. J. Bacteriol. 187, 1384.
McDonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev. 12, 147.
Moussodia, R.O., Balan, L., Merlin, C., Mustin, C., Schneider, R., 2010. Biocompatible and stable ZnO quantum dots generated by functionalization with siloxane-core PAMAM dendrons. J. Mat. Chem. 20, 1147.
Newkome, G.R., Moorefield, C.N., Vögtle, F., 2001. Dendrimers and Dendrons: Concepts, Syntheses, Applications. Dendrimers and Dendrons: Concepts, Syntheses, Applications. Wiley-VCH, Weinheim, Germany.
Ortega, P., Fuentes-Paniagua, E., Sánchez-Nieves, J., Hernández-Ros, J.M., Copa-Patiño, J.L., Soliveri, J., Muñoz-Fernández, M.A., Gómez, R., de la Mata, F.J., 2011. Hyperbranched polymers versus dendrimers containing a carbosilane framework and terminal ammonium groups as antimicrobial agents. Org. Biomol. Chem. 9, 5238.
Pandey, J.K., Swarnkar, R.K., Soumya, K.K., Dwivedi, P., Singh, M.K., Sundaram, S., Gopal, R., 2014. Silver nanoparticles synthesized by pulsed laser ablation: as a potent antibacterial agent for human enteropathogenic Gram-positive and Gram-negative bacterial strains. Appl. Biochem. Biotechnol. 174, 1021.
Peña-González, Cornelia E., García-Broncano, P., Ottaviani, M.F., Cangiotti, M., Fattori, A., Hierro-Oliva, M., González-Martín, M.L., Pérez-Serrano, J., Gómez, R., Muñoz-Fernández, M.Á., Sánchez-Nieves, J., de la Mata, F.J., 2016. Dendronized anionic gold nanoparticles: synthesis, characterization, and antiviral activity. Chem. Eur. J. 22, 2987.
Peña-González, C.E., Pedziwiatr-Werbicka, E., Shcharbin, D., Guerrero-Beltrán, C., Abashkin, V., Loznikova, S., Jiménez, J.L., Muñoz-Fernández, M.Á., Bryszewska, M., Gómez, R., Sánchez-Nieves, J., de la Mata, F.J., 2017. Gold nanoparticles stabilized by cationic carbosilane dendrons: synthesis and biological properties. Dalton Trans. doi:http://dx.doi.org/10.1039/c6dt03791g.
Rasines, B., Hernández-Ros, J.M., de las Cuevas, N., Copa-Patiño, J.L., Soliveri, J., Muñoz-Fernández, M.A., Gómez, R., de la Mata, F.J., 2009. Water-stable ammonium-terminated carbosilane dendrimers as efficient antibacterial agents. Dalton Trans. 8704.
Sánchez-Nieves, J., Fransen, P., Pulido, D., Lorente, R., Muñoz-Fernández, M.Á., Albericio, F., Royo, M., Gómez, R., de la Mata, F.J., 2014. Amphiphilic cationic carbosilane-PEG dendrimers: synthesis and applications in gene therapy. Eur. J. Med. Chem. 76, 43.
Serramía, M.J., Álvarez, S., Fuentes-Paniagua, E., Clemente, M.I., Sánchez-Nieves, J., Gómez, R., de la Mata, F.J., Muñoz-Fernández, M.A., 2015. In vivo delivery of siRNA to the brain by carbosilane dendrimer. J. Control. Release 200, 60.
Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275, 177.
Suleman, N., Kalhapure, R.S., Mocktar, C., Rambharose, S., Singh, M., Govender, T., 2015. Silver salts of carboxylic acid terminated generation 1 poly (propyl ether imine) (PETIM) dendron and dendrimers as antimicrobial agents against S. aureus and MRSA. RSC Adv. 5, 34967.
Tülü, M., Ertürk, A.S., 2012. Dendrimers as Antibacterial Agents. A Search for Antibacterial Agents. InTech, pp. 89.
Tülü, M., Aghatabay, N.M., Senel, M., Dizman, C., Parali, T., Dulger, B., 2009. Synthesis, characterization and antimicrobial activity of water soluble dendritic macromolecules. Eur. J. Med. Chem. 44, 1093.
Tian, W.-D., Ma, Y.-Q., 2012. pH-responsive dendrimers interacting with lipid membranes. Soft Matter 8, 2627.
Vacas-Córdoba, E., Galán, M., de la Mata, F.J., Gómez, R., Pion, M., Muñoz-Fernández, M.Á., 2014. Enhanced activity of carbosilane dendrimers against HhIV when combined with reverse transcriptase inhibitor drugs: searching for more potent microbicides. Int. J. Nanomed. 9, 3591.
Vacas-Córdoba, E., Maly, M., de la Mata, F.J., Gómez, R., Pion, M., Muñoz-Fernández, M.Á., 2016. Antiviral mechanism of polyanionic carbosilane dendrimers against HIV-1. Int. J. Nanomed. 11, 1281.
Vallabhaneni, S., Cleveland, A.A., Farley, M.M., Harrison, L.H., Schaffner, W., Beldavs, Z.G., Derado, G., Pham, C.D., Lockhart, S.R., Smith, R.M., 2015. Epidemiology and risk factors for echinocandin nonsusceptible candida glabrata bloodstream infections: data from a large multisite population-Based candidemia surveillance program, 2008–2014. Open Forum Infect. Diseases 2 doi:http://dx.doi.org/10.1093/ofid/ofv163.
WHO, 2014. Antimicrobial Resistance: Global Report on Surveillance. Antimicrobial Resistance: Global Report on Surveillance. Geneva.
Wrobel, D., Klys, A., Ionov, M., Vitovic, P., Waczulikowa, I., Hianik, T., Gómez-Ramírez, R., de la Mata, J., Klajnert, B., Bryszewska, M., 2012. Cationic carbosilane dendrimers-lipid membrane interactions. Chem. Phys Lipids 165, 401.
Xiu, Z.-M., Zhang, Q.-B., Puppala, H.L., Colvin, V.L., Álvarez, P.J.J., 2012. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett. 12, 4271–4275.
Xue, Y., Xiao, H., Zhang, Y., 2015. Antimicrobial polymeric materials with quaternary ammonium and phosphonium salts. Int. J. Mol. Sci. 16, 3626.