

Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties

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Hybrids of silver particles of 1 to 2 nm in size with highly branched amphiphilically modified polyethyleneimines adhere effectively to polar substrates providing environmentally friendly antimicrobial coatings.

An increased general desire for hygiene in everyday life prompts a strong interest in antimicrobial modification of surfaces to prevent the growth of harmful microorganisms. In addition, recent changes in legislation call for environmentally benign replacements of coatings widely used today. Coatings based on a slow-release of toxic agents are very effective and have been employed successfully for a long time. However, due to environmental concerns many such systems are to be abolished.¹

Therefore, systems which show antimicrobial effects toward germs on contact without releasing toxic biocides are of strong current interest.² Silver colloids have been known for a long time to possess antimicrobial properties, and also to be non-toxic and environmentally friendly.³ These often ill-defined silver particles are usually unsuited for coatings owing to their lacking binding properties to surfaces. We now report on the synthesis of hybrids of silver nanoparticles with highly branched amphiphilic macromolecules and their antimicrobial properties on surfaces.

Amphiphilically modified hyperbranched polymers can effectively stabilize solutions of metal nanoparticles in apolar organic solvents, as evidenced *e.g.* by the possibility of repeated recycling of catalytically active palladium particles stabilized by modified polyglycerol by means of ultrafiltration.⁴ With respect to solubilization of metal precursors for nanoparticle synthesis, polymers with a more strongly coordinating polyamine core are more versatile by comparison to polyglycerols.⁵ In contrast to dendrimers with a tediously prepared perfectly branched polyamine scaffold⁶ (degree of branching⁷ DB 100%), amphiphilic polymers based on hyperbranched polyethyleneimine (DB *ca.* 60%) are readily available. Amidation of commercially available polyethyleneimine (PEI) results in the formation of amphiphilic materials, soluble in apolar organic solvents.⁵ Amidations were carried out in the bulk at 120 to 150 °C with palmitic acid or its methyl ester as the amidating agent, or with carbonyl imidazol-activated palmitic acid in THF (routinely on a scale of up to 100 g).⁸ The latter two methods offer the advantage of exclusively non-acidic reagents and by-products (methanol respectively imidazole) which are conveniently removed by ultrafiltration. In the former case, residual unreacted palmitic acid could be removed completely from the polyamine core by ultrafiltration washing with a 10 wt% solution of tributylamine in toluene, as evidenced by the absence of the ¹³C NMR carbonyl peaks of the acid or carboxylates (180–181 ppm).[†] ⁹ The primary amine groups, constituting the ends of branches (Fig. 1), react preferentially, and a complete and selective conversion can be achieved within the limit of NMR detection when a corresponding stoichiometric amount of amidating agent is used (complete disappearance of the CH₂CH₂NH₂ peaks at 41.4 and 39.5 in the ¹³C NMR, degree of amidation 40 to 50% corresponding to

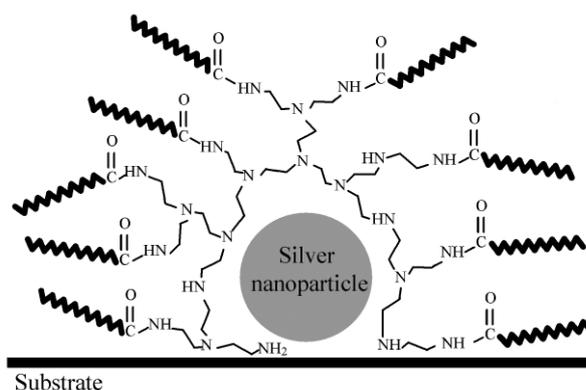


Fig. 1 Proposed structure of polymer-encapsulated silver nanoparticles on a substrate surface.

complete conversion of all NH₂ groups from ¹H NMR and elemental analysis).^{†‡}¹⁰

Scattering experiments (SANS) on polymer solutions in benzene reveal structures with *R_g* *ca.* 3 nm. This indicates that no large polymer aggregates are involved, and that possibly inverted unimolecular micelles are present.¹¹ Such partially amidated PEIs (PEI-am) with a core molecular weight of *M_w* 5,000 g mol⁻¹ were used for the following experiments. Silver nitrate is solubilized by a dry toluene solution of PEI-am, with loadings of up to 0.5 Ag⁺/N-atom. Reduction with Li[HBEt₃] afforded clear yellow colloidal silver solutions, displaying the characteristic plasmon resonance band (428 nm) in their UV/vis-spectra.[§] ¹² A complete reduction of Ag^I to Ag⁰ is evidenced by extraction of the colloid with an aqueous solution of Na₂S₂O₃ and subsequent addition of Na₂S to probe for any remaining Ag⁺.[¶] Transmission electron microscopy (TEM) reveals silver nanoparticles of 1 to 2 nm in size and reasonably narrow particle size distributions (Fig. 2).

Glass slides were coated^{||} with the polymer–nanoparticle hybrid by evaporating a drop of solution. As environmentally benign release-free systems are of particular interest, general

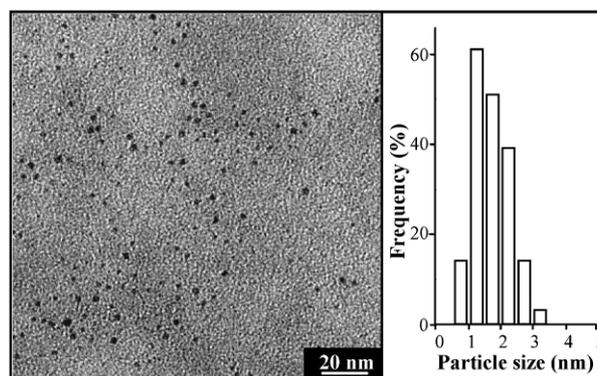


Fig. 2 TEM micrograph and histogram of PEI-am stabilized silver nanoparticles.

applications conditions were simulated by subsequently washing the slides with a PBS buffer solution (pH 7.0) for two hours (the absorbance of the film due to the plasmon resonance of the silver nanoparticles was similar before and after washing proving that the silver particles are not removed). Cells of the ubiquitous bacterium *Escherichia coli* were then applied by aerosol spraying and cultivated under growth agar overnight.* The number of grown bacterial colonies on the silver nanoparticle-coated substrates were found to be at least 98% less than in the surrounding uncoated parts of the substrate (Fig. 3). In order to confirm that antimicrobial activity is exclusively due to the nanoparticles and to exclude any conceivable effect of hydrolysis products of the reducing agent Li[HBET₃], unreacted Ag⁺ ions, or the polymer itself, the following experiments were performed: surfaces were coated with PEI-am–AgNO₃, PEI-am–Li[HBET₃], and PEI-am, respectively, using the same amounts as in the case of PEI-am–silver nanoparticle hybrids. After washing for two hours with the PBS buffer, spraying *E. coli*, and incubating overnight, the number of grown bacterial colonies did not differ from that of the control in any case (adherence of the amphiphilic polymer to the substrate during this entire procedure is evident by the wetting properties of the coated area by comparison to the non-coated substrate). This shows that only the silver nanoparticles and not conceivable impurities are responsible for the antimicrobial effect of the coatings.

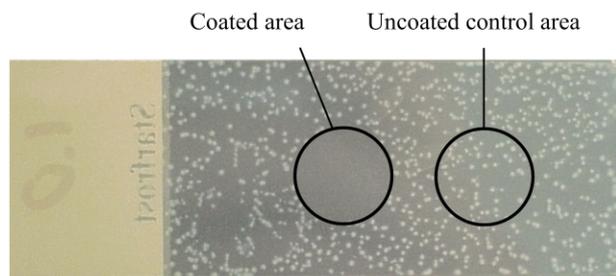


Fig. 3 Bacteria growth on glass slide partially coated with silver nanoparticle–PEI-am hybrid.

In comparative experiments, aqueous solutions of silver nanoparticles (5 nm average diameter) stabilized by unmodified PEI were prepared.†† Whereas glass slides coated with these particles were antimicrobially active prior to washing (>98% reduction of bacterial growth), the aforementioned washing procedure resulted in complete removal of the water soluble polymer–nanoparticle hybrids and consequently in total antimicrobial inactivity. Thus, the amphiphilic nature of the modified PEI-am is essential for effective adherence to the substrate. The function of the polar PEI core is not only solubilization of precursors for particle synthesis and stabilization of the nanoparticles, moreover it appears to be responsible for adherence to the substrate, while at the same time the hydrophobic alkyl chains prevent dissolution and wash-out with water (Fig. 1).

In summary, well-defined hybrids of silver nanoparticles with highly branched amphiphilic polyethyleneimine can be prepared in a simple procedure, based on readily available precursors. Such hybrids afford very effective and environmentally friendly antimicrobial surface coatings.

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Notes and references

† Key analytical data of a partially amidated PEI-am: ¹³C NMR (CDCl₃, 75 MHz): 174.0 (C=O), 51–55 (br, R₂NCH₂CH₂NHR, R₂NCH₂CH₂NR₂,

RHNCH₂CH₂NH(C=O), R₂NCH₂CH₂NH(C=O) with R = CH₂CH₂N), 49.0 (br, RHNCH₂CH₂NHR), 47.5 (br, R₂NCH₂CH₂NHR), 37.7 and 33.5 (br, tentative assignment CH₂NH(C=O)), 36.8 (NH(C=O)CH₂), 26.2 (NH(C=O)CH₂CH₂), 29.9 ((CH₂)₁₀(CH₂)₂CH₃), 32.1 (CH₂CH₂CH₃), 22.9 (CH₂CH₃), 14.3 (CH₃). IR: 1645 and 1545 cm⁻¹ (sec. amide). Elemental anal. Found C 71.59, H 13.20, N 11.25. Calc. for degree of amidation‡ 47%: C 71.9, H 12.3, N 11.5%. From ¹H NMR: degree of amidation 49%. DSC: thermal transition at 34 and 62 °C.

‡ 100% degree of amidation corresponding to complete conversion of R₂NH and RNH₂ to R₂NC(=O)C₁₅H₃₁ and RNHC(=O)C₁₅H₃₁, respectively (R = polymer scaffold). The PEI utilized contains a ratio of tertiary amine (branching, dendritic units): secondary amine (linear units): primary units (ends of branches) of ca. 30:40:30 according to ¹³C NMR, in accordance with literature reports.¹⁰

§ Typical conditions of silver colloid synthesis: 7.3 μmol AgNO₃ dissolved completely upon stirring overnight with a solution of 3.6 mg PEI-am (29.2 μmol of N-atom ≡ 19.3 μmol of amine + 9.9 μmol of amide) in 4 mL toluene. Reduction: 1 mL toluene solution of 36.5 μmol Li[HBET₃] added, stirred 2 h.

¶ Addition of Na₂S afforded no noticeable precipitate of Ag₂S, whereas in a comparative experiment extracting Ag⁺ from a PEI-am toluene solution (5% of the Ag⁺ employed in a colloid synthesis) a precipitation was observed.

|| 5 μl of a silver nanoparticle–PEI-am solution in toluene (1.2 g L⁻¹) were distributed on ca. 1 cm² of a glass slide and air-dried, corresponding to 1 μg Ag cm⁻².

** *E. coli* cells (ATCC 25922, 10⁷ cells per mL distilled water) were sprayed onto the substrate using a chromatography sprayer, dried for 2 min, and then growth agar (0.7% in culture medium) was added.

†† Aqueous silver colloid synthesis: 7.3 μmol AgNO₃ and 1.26 mg PEI solution in 4 mL water stirred overnight. Reduction: 1 mL aqueous solution of 36.5 μmol NaBH₄ added, stirred 2 h.

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