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Note

Formulation and stability of surface-tethered DNA–gold–dendron nanoparticles

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Abstract

The formulation of plasmid DNA on 100 nm gold nanoparticles surface-tethered via cationic dendrons, and the behaviour of the complex in cell culture media, is described in this communication. Adsorption of dendrons onto gold nanoparticles in water resulted in the generation of positively charged nanoparticles with a corresponding small increase in particle size. Addition of plasmid DNA did not markedly reduce the surface potential but resulted in a ~10–20% increase in hydrodynamic diameter. More dramatic effects were seen in the presence of cell culture media that, overall, drastically increased the apparent size of the gold–dendron–DNA nanoparticles and reduced the surface potential of the colloids, the presence of serum components partially ameliorating these effects possibly due to steric stabilisation. Release of the surface-tethered DNA was reduced in cell culture media compared to water. This reduced detachment of DNA coupled with the flocculation of the carrier which would likely inhibit endocytosis, demonstrates the importance of testing drug delivery systems with relevant physiologically based fluids prior to their use in vivo studies.

Keywords: Dendron; Nanoparticle; Cell culture; Transfection; Gold; Delivery

The non-realisation of efficient delivery of DNA with non-viral vectors can be attributed to many factors that include the non-decomplexation of plasmid DNA from the carrier, poor in vitro/in vivo correlation and lack of physico-chemical characterisation of formulations. Encapsulation of DNA within carriers such as nanoparticles may render it inactive due to the shearing processes involved or the formation of irreversible adducts during polymerisation/encapsulation. Recently, the innovative step of adsorbing DNA onto particles, such as poly(lactide-co-glycolide) via the cationic surfactant cetyl-trimethylammonium bromide, has demonstrated bioequivalence of DNA expression compared to encapsulation, and which in some instances exceeds that of encapsulated DNA (Singh et al., 2000). Surface-adsorbed DNA is not only resistant to nuclease attack (Romanowski et al., 1991) but can allow delivery of DNA to its target cells, as release is presumably simpler. Release of plasmid DNA from drug delivery systems in sufficient concentration at the cell surface is a perquisite for successful gene transfection. However, the effect of the complex biological milieu such as gastrointestinal and serum fluids on colloidal-based delivery systems is rarely taken into account, despite the rich history in the field (Searle, 1920), and may in part explain the relative

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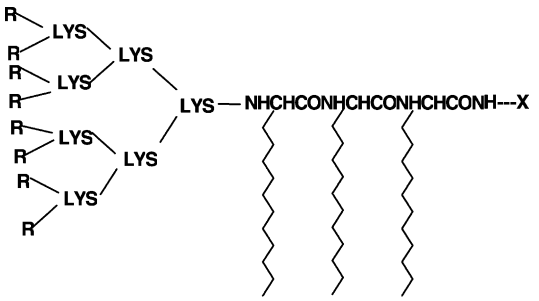

Compound	Amino groups	X	R
I	8	GPKKKRKVG	NH_2
II	16	GPKKKRKVG	$LYS(NH_2)_2$

Fig. 1. Chemical structures of poly-lysine dendrimers with lipidic chains and nuclear localisation sequences. Dendrons $C_{14}Lys_7(NH_2)_8$ and $C_{14}Lys_{15}(NH_2)_{16}$ are referred to as I and II, respectively. X refers to the nuclear localisation signal.

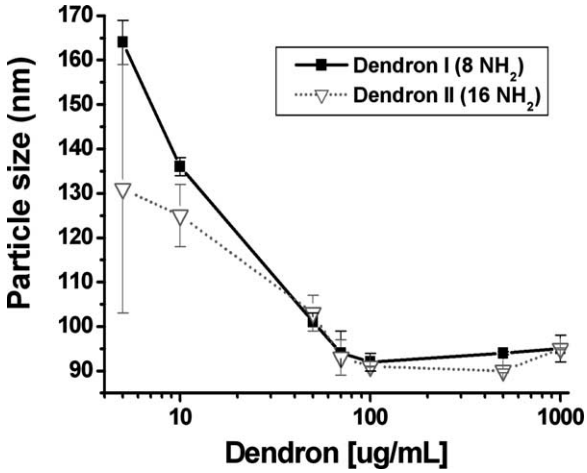

Fig. 2. Adsorption of dendrons with either 8 or 16 free terminal amino groups onto gold nanoparticles in water. Low amounts of both dendrons caused an increase in particle size, resulting in a polydisperse particle population. Increasing the amount to 100 μg for both dendrons alleviated this minor flocculation, and this amount was subsequently employed throughout the studies.

poor efficiency of inert delivery systems versus their viral/bacterial counterparts. In this communication we present some findings on the physico-chemical behaviour of plasmid DNA adsorbed onto model gold nanoparticles, rendered positively charged by the prior adsorption of cationic dendrons. We have also examined the effect of media commonly employed in cell culture studies on gold–dendron–DNA adducts.

Lipophilic cationic dendrons with either 8 [$C_{14}Lys_7(NH_2)_8$] or 16 [$C_{14}Lys_{15}(NH_2)_{16}$] free amino groups on their outer surfaces and incorporating nuclear localisation signals (NLS) were synthesised (Fig. 1) and are referred to as dendrons I and II, respectively throughout the text (Sakthivel et al., 1998). Varying amounts of water-soluble dendrons in a total volume of 100 μl (1 mg/ml solution) were incubated with 1 ml of (nominal) 100 nm gold nanoparticles (British Biocell, Cardiff, UK) for 1 h at room temperature with gentle shaking. Gold nanoparticles were suspended in either distilled water or Hank's balanced salt solution (HBSS; a mixture of monovalent and divalent salts, predominately NaCl, $CaCl_2$, KCl and $MgSO_4$) with or without 10% fetal bovine serum (FBS; Life Technologies, Paisley, UK). Plasmid DNA encoding for red fluorescent protein (RFP; Clontech, Palo alto, USA) was purified after growth in Luria Broth using Wizard® Maxiprep kit (Promega, Madison, USA). Ten microlitre of DNA (1 μg/μl) in water was then incubated with the dendron-labelled gold nanoparticles for another hour at 25 °C. The effect of the sequential additions of dendrons and plasmid DNA on the charge and size of the gold nanoparticles was measured using a Zetasizer 3000 model (Malvern Instruments, UK).

Adsorption of dendrons on to gold nanoparticles (zeta potential = −27 mV) in water resulted in the generation of positively charged particles with a corresponding small increase in particle size from 87 to ∼100 nm (Fig. 2). These preliminary adsorption studies also determined the optimum ratio of dendron/gold to be employed in later studies, namely 100 μg dendron/ml gold nanoparticles. There appeared to be a stoichiometric relationship between the charge of the resultant particles and the generation of dendron employed; thus $C_{14}Lys_7(NH_2)_8$ (8 free amino groups) yielded +20 mV charged nanoparticles and $C_{14}Lys_{15}(NH_2)_{16}$ (16 free amino groups) registered a charge of +40 mV, respectively, reflecting the amount of dendrons bound to the gold namely 40% (S.D. ± 2) for the former and 70% (S.D. ± 10) for the latter as determined by the Bicinchonic acid Protein assay (Perbio Sciences, Cheshire, UK).

Adsorption of more than 20 μg of plasmid DNA resulted in visible, positively charged flocculates ir-

respective of the dendron or media used to suspend the gold–dendron nanoparticles. Hence 10 μg DNA/100 μg dendron/ml gold was employed for more detailed cell culture media studies that corresponded to a molar charge ratio of dendron:DNA (+/−) of 14:1 and 17:1 for $C_{14}Lys_7(NH_2)_8$ and $C_{14}Lys_{15}(NH_2)_{16}$, respectively, assuming that all the positive charges were available for the interaction/condensation of the added DNA. Picogreen assay (Molecular Probes, Oregon, USA) revealed that ~85% of the added DNA was attached to the nanoparticles, this figure falling to 60% if the order of addition of dendrons and plasmid DNA was reversed. This reduced association of the dendron–DNA complexes (dendriplexes) provides some circumstantial evidence that the interaction of the dendron with the lyophobic gold sols is probably via the lipophilic C_{12} chains rather than electrostatic interaction from the amine groups. Direct evidence of this type of association can only be ascertained by the use of dendrons without lipophilic moieties and calculation of adsorption isotherms. However, cationic dendrons, lacking the NLS, appear to show no difference in adsorption patterns when anionic or neutral liposomes are employed as model colloidal carriers, suggesting a lipophilic interaction between the lipid chains of the dendron and the lipidic surfaces of the liposomes (Purohit et al., 2001).

More drastic changes in the surface potential and hydrodynamic diameter were seen when the water medium was replaced with Hank balanced salt solution (HBSS) with and without serum (Table 1). As the gold–dendron–DNA complex is a multi-component system, the behaviour of naked gold, gold–dendron (carrier) and gold–dendron–DNA adducts were examined separately to dissect the changes occurring in the different media. More explanation is given with Fig. 3, using conventional theory (Overbeek, 1952) but overall there are a few tentative conclusions that can be drawn. Firstly, zeta potential measurement alone is not sufficient indicator for colloidal stability without taking into account the complement reading of particle sizes. Thus zeta potential measurements of gold–dendron–DNA adducts in HBSS yield +4 and +19 mV for dendrons I and II, respectively, suggesting that the latter would exhibit increased colloidal stability. Yet drastic flocculation occurred with the dendron II (1025 nm) that had more terminal amino groups compared to dendron I (423 nm).

Table 1

Nanoparticle	Zeta potential (mV)	Size (nm)
Gold/water	−27 ± 2.4	87
Gold/HBSS	−24 ± 3.7	640 ± 43
Gold/HBSS + serum	−4 ± 0.8	119 ± 1
I. $C_{14}Lys_7(NH_2)_8$		
Gold/I + HBSS	+2 ± 1	160 ± 8
Gold/I + HBSS + serum	−4 ± 0.4	155 ± 2
Gold/I/DNA + HBSS	4 ± 3	423 ± 33
Gold/I/DNA + HBSS + serum	−4 ± 4	200 ± 27
II. $C_{14}Lys_{15}(NH_2)_{16}$		
Gold/II + HBSS	0 ±1	213 ± 2
Gold/II + HBSS + serum	−3	163 ± 4
Gold/II/DNA + HBSS	19 ± 0.4	1025 ± 141
Gold/II/DNA + HBSS + serum	−14 ± 1.3	204 ± 33

Zeta potentials of gold, gold–dendron and gold–dendron–DNA nanoparticles in the presence of HBSS with and without serum. Dendrons $C_{14}Lys_7(NH_2)_8$ and $C_{14}Lys_{15}(NH_2)_{16}$ are referred to as I and II, respectively. Measurements were conducted using three independent preparations. In this system the zeta potential measurements are not indicative of the stability of the conjugates when comparing their resistance to flocculation.

Secondly, the inclusion of 10% serum in HBSS reversed the zeta potential of both gold–dendron–DNA adducts but stabilised particle agglomeration down to ~200 nm, indicative of some component of the serum mediating a sterically stabilising effect. This is also evident when comparing naked gold nanoparticles suspended in HBSS with (199 nm; zeta potential = −4 mV) and without serum (640 nm; zeta potential = −24 mV), yet the Zeta potential measurements predict the opposite. Thirdly, the dendrons themselves are a source of stabilising entities in the HBSS media when one compares the hydrodynamic diameters of naked gold (640 nm), gold–dendron I (160 nm) and gold–dendron II (213 nm) nanoparticles. It thus appears that the presence of plasmid DNA, a large negatively charged polyelectrolyte, in both the gold–dendron systems suspended in HBSS is responsible for the destabilisation of colloidal stability, and the de-coupling observed between the zeta potential and hydrodynamic measurements (Fig. 3).

The release of bound plasmid DNA from gold–dendron (I)–DNA adducts, incubated either in water or HBSS/serum, was assessed. As optimal transfection

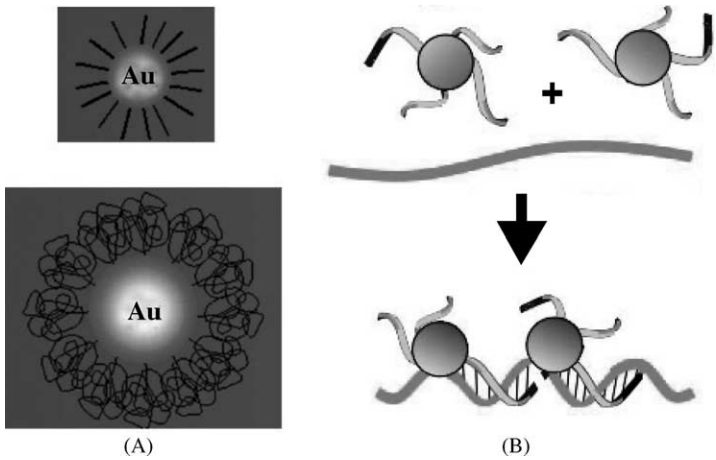

Fig. 3. Diagrammatic representation of the sequential adsorption of dendron and DNA onto gold nanoparticles in water (A), and the flocculation due to bridging of plasmid DNA in the presence of HBSS media (B). Gold nanoparticles are lyophobic sols that display an apparent negative zeta potential in water due to the association of hydroxyl ions. This weak interaction can be displaced by adsorption of dendrons, imparting an actual (positive) charge, thus displacing the double-diffuse layer outside of the adsorbed dendron in the presence of salts such as HBSS. In this instance gold–dendron nanoparticles are stabilised against flocculation. Compared to naked gold nanoparticles suspended in HBSS, where the electric double diffuse layer shrinks closer to the colloid surface, nanoparticles can approach each other, the overlapping of the double-diffuse layer promoting coagulation (Overbeek, 1952). Addition of plasmid DNA in water to gold–dendron nanoparticles in water allows adsorption onto isolated nanoparticles whereas in HBSS the DNA may associate with many nanoparticles, or those that are coagulated.

typically involves the incubation of the transfectant with the cells for a fixed period of time (amongst other parameters such as cell density, cell type, volume of transfectant, etc.), samples were withdrawn at several timepoints, centrifuged and the supernatants quantified with Picogreen dye. Fig. 4 depicts a highly unusual decreasing release profile of tethered DNA in cell culture media (containing serum) with time. The apparent maximal release of DNA was less than 2% at 24 h. In contrast, an opposite but conventional release profile was seen for gold–dendron–DNA nanoparticles suspended in the original medium, water, reaching some 5% of the bound DNA at 24 h. This suggests that the desorbing DNA from the surface of the gold–dendron (I)–DNA adducts in cell culture media is re-absorbed rapidly either by some serum component or by the adducts which show drastic flocculation with time (∼750 nm in cell culture media compared to 110 nm in water after 24 h). A simpler explanation may be that there is a receding population of monodisperse gold–dendron–DNA adducts, as a result of flocculation, capable of releasing tethered DNA. The fact that earlier timepoints (e.g. 30 min) yield almost identical DNA release in both media, coupled with the fact that Picogreen amplifies the signal of *added* DNA in presence of gold or HBSS/serum controls (data not shown) indicates that the latter premise, i.e. poor escape of DNA from the flocculates, may be responsi-

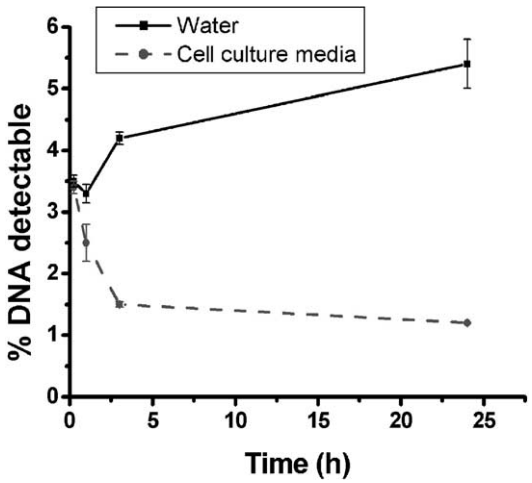

Fig. 4. Release profiles of surface-tethered DNA in water and cell culture media (containing 10% serum).

ble for the atypical release profile. In conclusion, the reduced de-attachment of DNA coupled with the flocculation of the carrier which would prevent its endocytosis, demonstrates the importance of testing drug delivery systems with relevant physiological fluids prior to in vivo studies.

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