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# Effect of physiological media on the stability of surface-adsorbed DNA-dendron-gold nanoparticles

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## Abstract

Plasmid DNA was adsorbed onto 87-nm gold nanoparticles to which were adsorbed a layer of novel cationic dendrons. The behaviour of this DNA-dendron-gold system in cell culture media has been described. Adsorption onto the gold nanoparticles of lipophilic cationic dendrons, with either 8 $[(C_{12})_3Lys_7(NH_2)_8]$ or 16 $[(C_{12})_3Lys_{15}(NH_2)_{16}]$ free amino groups on their outer surfaces and incorporating a nuclear localization signal peptide (NLS), resulted in positively charged nanoparticles with a corresponding small increase in particle size. Evidence suggested that the interaction between the gold nanoparticles and the dendron was mediated by hydrophobic forces. With an increase in ionic strength, the apparent particle size of the dendron-stabilized-gold particles increased, but at higher salt concentrations than plain gold sols. Addition of plasmid DNA did not markedly reduce the surface potential of the dendron-gold complex but resulted in an approximately 10–20% increase in hydro-dynamic diameter. Increasing ionic strength increased the apparent size of the DNA-dendron-gold particles, up to a maximum diameter of approximately 900 nm. Importantly, in cell culture media the size of the DNA-dendron-gold nanoparticles increased markedly, as surface potential was reduced. The presence of serum components partially ameliorated these effects, possibly due to steric stabilization of the particles. Release of the DNA from the complex was compromised in cell culture media (compared with water). This, coupled with the flocculation of the carrier, demonstrated the importance of testing delivery systems in the presence of relevant physiologically based fluids before cell culture or in-vivo studies.

## Introduction

The limited success of non-viral DNA delivery systems can be attributed to many factors, which include the poor release of plasmid DNA from the complex and the susceptibility of carrier complexes to changes in the physical properties of biological media. Encapsulation of DNA within carriers such as nanoparticles may render DNA inactive due to the shearing processes involved, or the formation of irreversible adducts during polymerization or encapsulation. In contrast, adsorption of DNA onto particles, such as poly (lactide-co-glycolide) systems via the cationic surfactant cetyl trimethylammonium bromide has demonstrated equivalence of DNA expression compared with encapsulation. In some instances adsorbed systems are superior to 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 target cells, as DNA release is presumed to be rapid. Release of plasmid DNA from drug delivery systems in sufficient concentration near or at the cell surface is a prerequisite for successful transfection. Another important factor for successful gene delivery has been deemed to be the density of the particles (Luo and Saltzman 2000), which might enhance the concentrations of transfecting agent on cell surfaces, especially in cell culture studies. Whether density is important in-vivo is less clear.

The effect of complex biological milieu such as gastrointestinal and serum fluids on nanoparticle-based delivery systems is rarely taken into account, despite its mention over 80 years ago (Searle 1920). Physical instability may in part explain the relatively poor efficiency of "inert" delivery systems vs their viral/bacterial counterparts. It is important to look at physical stability, not only in water, but also in cell culture and relevant

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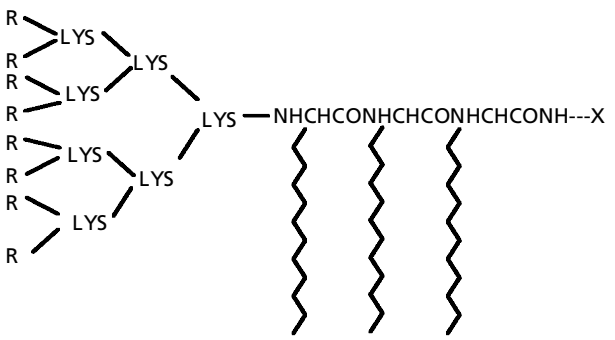
physiological media. Although surface modified gold nanoparticles have shown great diversity regarding potential applications, a common feature has been the use of thiol linkers on the gold surface (Fritzsche 2001; Li and Li 2002; Liu et al 2002). Although gold thiolates used in the treatment of rheumatoid arthritis have been shown to be potent against various tumours (Tiekink 2002), the toxic effect of these gold-thiol conjugates for other applications such as gene delivery must be assessed. An alternative to the much used thiol linker could therefore be useful, and also an assessment of the stability of the gold adducts in buffers such as phosphate-buffered saline, which are widely used.

Many different dendrimers have been developed as new particulate delivery agents. Their unique structure and properties with large numbers of controllable peripheral functionalities makes it possible to modify and optimize the dendrimers according to need. In this paper we report on the physicochemical behaviour of plasmid DNA adsorbed onto gold nanoparticles, rendered positively charged by the prior adsorption of cationic dendrons (partial dendrimers). We have examined the effect of media commonly employed in cell culture studies on the properties of the DNA-dendron-gold adduct.

## Materials and Methods

### Materials

Lipophilic cationic dendrons with either 8 [$(C_{12})_3Lys_7(NH_2)_8$] or 16 [$(C_{12})_3Lys_{15}(NH_2)_{16}$] free amino groups on their outer surfaces and incorporating nuclear localization signals (NLS) were synthesized (Figure 1). These are referred to as dendrons I and II, respectively (Sakthivel et al 1998). Colloidal gold particles 100 nm ($5.6 \times 10^9$ gold particles $mL^{-1}$) were purchased from British Biocell (Cardiff, UK). Plasmid DNA encoding for red fluorescent protein (RFP; Clontech, Palo Alto, CA) was purified after growth in Luria Broth using Wizard Maxiprep kit (Promega, Madison, WI). The cell culture media was 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% foetal bovine serum (FBS; Life Technologies, Paisley, UK). The bicinchoninic acid protein assay (Perbio Sciences, Cheshire, UK) was used to determine the amount of dendron bound to the colloidal gold particles, whilst the Picogreen assay (Molecular Probes, Eugene, OR) determined the amount of DNA bound to the particles.




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

**Figure 1** Chemical structures of poly-lysine dendrons with lipidic chains and nuclear localization sequences. Dendrons $(C_{12})_3Lys_7(NH_2)_8$ and $(C_{12})_3Lys_{15}(NH_2)_{16}$ are referred to as I and II, respectively. The nuclear localization signal (NLS) (pro-lys-lys-arg-lys-val) is shown as X.

### Statistical analysis

Data presented are mean values ± standard deviation (s.d.). Results were compared using the $z$ test for two independent samples, as the magnitude of the differences between two means was under investigation. The 95% confidence intervals were calculated although sample data were compared using the values (± s.d). The critical $z$ value for $\alpha = 0.05$ was 4.3 as $n = 3$ for all samples. Values above 4.3 indicated a significant difference between results.

### Preparation of gold adducts

A standard solution (5, 10, 50, 70, 500, or 1000 $\mu$g) of the water-soluble dendrons (5 mg $mL^{-1}$ solution) was incubated with 1 mL 100-nm gold nanoparticles for 1 h, with gentle shaking, at room temperature. Gold nanoparticles were suspended in distilled water. The optimal concentration for surface adsorbed dendrons (100 $\mu$g $mL^{-1}$) was found, and complexes were incubated with 5, 50, 150 or 500 mM NaCl and 5 or 50 mM $CaCl_2$.

The gold-dendron complexes (1 mL gold and 100 $\mu$g $mL^{-1}$ dendrons) were incubated with various amounts (5, 15, 30 or 60 $\mu$g $mL^{-1}$) of plasmid DNA encoding for RFP (2.25 $\mu$g $\mu L^{-1}$) in distilled water for a further 1 h at 25 °C. The optimal concentration (10 $\mu$g $mL^{-1}$) was then used and incubated with 5, 50, 150 or 500 mM NaCl. The effect of the sequential addition of dendrons and plasmid DNA on the charge and size of the gold nanoparticles was measured using a Zetasizer 3000 (Malvern Instruments, UK).

### Cell culture media studies

Varying amounts of water-soluble dendrons in a total volume of 100 $\mu$L (1 mg $mL^{-1}$ solution) were incubated with 1 mL 100-nm gold nanoparticles for 1 h, with gentle shaking, at room temperature. Gold nanoparticles were suspended in either distilled water or HBSS with or without 10% FBS. Dendron-labelled gold nanoparticles, suspended in water, were incubated with 10 $\mu$L plasmid DNA (1 $\mu$g $\mu L^{-1}$), encoding for RFP, for 1 h at 25 °C. The DNA-dendron-gold nanoparticles were suspended in HBSS with or without 10% FBS.

## Results and Discussion

### Gold-dendron complexes in water

Adsorption of the dendrons on to 87-nm gold nanoparticles (nominal 100 nm) with a zeta potential of –40 mV resulted in the generation of positively charged nanoparticles with a corresponding small increase in particle size from 87 to ~100 nm (Figure 2A). The optimum ratio of dendron/gold was found to be 100 $\mu$g dendron (I and II) per mL of gold nanoparticles. Rapid particle size increase at the lowest concentrations occurred for both dendrons (dendron I particle size, 164 nm ± 6: dendron II particle size, 131 nm ± 28), indicating that the lowest concentration of dendrons perhaps caused bridging between the particles. A significant difference was observed between mean values of dendron I ($z = 23$) and native gold nanoparticles, but not for dendron II ($z = 2.73$). As the concentration of dendrons increased, the particles were more effectively stabilized and displayed a sharp decrease in apparent particle size. At the higher concentrations of dendrons the increase of particle size was a few nanometers, reasonable given the small size of the dendrons (a few nanometers in diameter). Using 100 $\mu$g dendron (I and II), significant differences between native gold and gold-dendrons were observed ($z = 4.32$ and $z = 9.51$, respectively). The difference in the free terminal amino groups for the dendrons, 8 and 16, respectively, seemed not to cause any significant difference in particle size.

The bridging effect also underlined the amphiphilic nature of the dendrons, as rapid size increase would not be possible otherwise. There appeared to be a relationship between the charge of the resultant particles and the generation of dendron used. Dendron I ($((C_{12})_3Lys_7(NH_2)_8$) yielded nanoparticles with a zeta potential of +20 mV and dendron II ($((C_{12})_3Lys_{15}(NH_2)_{16}$) particles with a zeta potential of +40 mV (Figure 2B). The amount of dendrons bound to the gold differed: 40% (s.d. ± 2) of dendron I and 70% (s.d. ± 10) of dendron II, as determined by the bicinchoninic acid protein assay. An increased density of dendron II on the gold nanoparticles could show particular utility in gene delivery as more DNA could be immobilized on to the nanoparticles.

### DNA-dendron-gold complexes in water

Figure 3 shows the effect of DNA on diameter. An increase in the apparent size of the particles occurred with increasing amounts of DNA. The complexes with dendron I and II resulted in a significant increase ($z = 32.3$ and $z = 11.4$, respectively) in particle size at 60 $\mu$g mL$^{-1}$ (dendron I particle size 350 nm ± 14, dendron II particle size 444 nm ± 53). The critical level of DNA lay between 30–60 $\mu$g mL$^{-1}$. With the increase in particle size a small decrease in zeta potential was apparent, not significant, but enough to indicate the formation of DNA-dendron-gold adducts.

Using 10 $\mu$g DNA/100 $\mu$g dendron (mL gold)$^{-1}$ the Picogreen assay 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 provided some circumstantial evidence that the interaction of the dendron with the lyophobic gold nanoparticles took place via the lipophilic $C_{12}$ chains. Similar cationic dendrons, lacking the NLS, appeared to show no difference in adsorption patterns

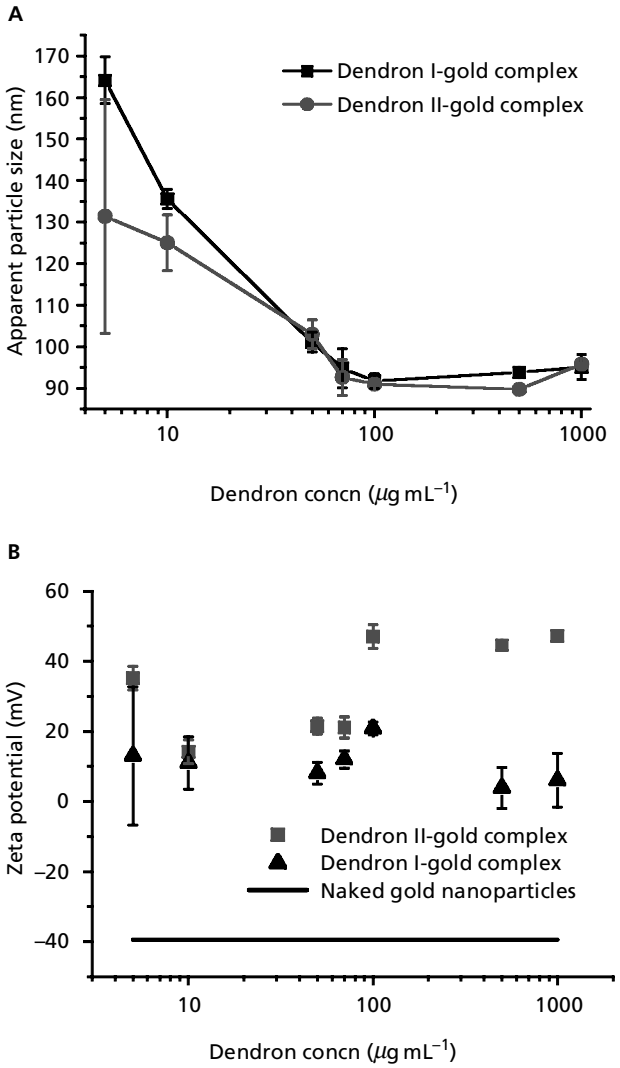



**Figure 2** A. The change in apparent size of the nanoparticles as a function of dendron concentration. Adsorption of low amount of dendrons, with either 8 or 16 free terminal amino groups, onto gold nanoparticles in water caused an increase in particle size, resulting in a polydisperse particle population. B. Relationship between the zeta potential of the system and added dendron concentration. A low amount of both dendrons rendered the surface charge positive but with a high polydispersity. Increasing the dendron concentration resulted in no significant increase in particle charge, but a decrease in polydispersity. Increasing the amount to 100 $\mu$g for both dendrons reduced this minor flocculation, so this amount was subsequently employed throughout the work. The zeta potential values for naked gold nanoparticles (−39.5 mV) without surface adsorbed dendron is shown.

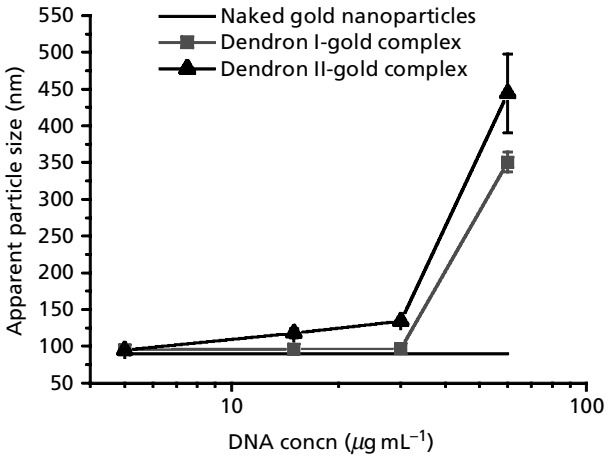



**Figure 3** The change in apparent size of the gold-dendron nanoparticles as a function of pDNA concentration. Adsorption of plasmid DNA onto dendrons with either 8 or 16 free terminal amino groups in water resulted in an increase in apparent size of the particles, with the larger dendron showing a comparatively larger increase in size. Adsorption of more than 30 $\mu$g mL$^{-1}$ plasmid DNA resulted in visible flocculates and therefore a concentration of 10 $\mu$g mL$^{-1}$ plasmid DNA was chosen for the further studies. The values for naked gold nanoparticles (−39.5 mV) without surface adsorbed dendron is shown.

when cationic or neutral liposomes were used as model colloidal carriers, which suggested a lipophilic interaction between the lipidic chains of the dendron and the lipidic surfaces of liposomes (Purohit et al 2001).

Subsequently, 10 $\mu$g DNA/100 $\mu$g dendron (mL gold)$^{-1}$ was used for more detailed cell culture media studies. This corresponded to a molar charge ratio of dendron:DNA (+/−) of 5:1 and 12:1 for dendron I and dendron II, respectively, assuming that all the positive charges were available for interaction with the added DNA. The molar charge ratio was calculated for the different concentrations of DNA (Table 1). The calculation showed that increasing the amount of pDNA decreased the charge ratio (+/−) but still rendered the overall charge positive. The overall positive charge of the adduct was important when trying to understand the influence of HBSS with or without serum (see below). Steric hindrance of charges, which would change the effective charge ratios, was not taken into account.

**Table 1** Dendron:pDNA charge ratios show the relation between charge ratios and the amount of DNA. With the increasing amount of DNA in the system, the overall charge remained positive, but was decreasing or neutralizing, thus electrostatic stability/repulsion was being compromised. The charge ratios have been calculated taking into account the amount of dendron bound (40 and 70% for dendron I and II, respectively, with 85% DNA binding).

| DNA ($\mu$g) | Dendron I | Dendron II |
|---|---|---|
| 5 | 14 | 28 |
| 15 | 5 | 9 |
| 30 | 2 | 5 |
| 60 | 1 | 2 |

## Effect of ionic strength on dendron-gold and DNA-dendron-gold complexes

A better understanding of gold adducts and their potential behaviour in cell culture media was obtained by studying the effect of a variety of ions at various ionic strengths.

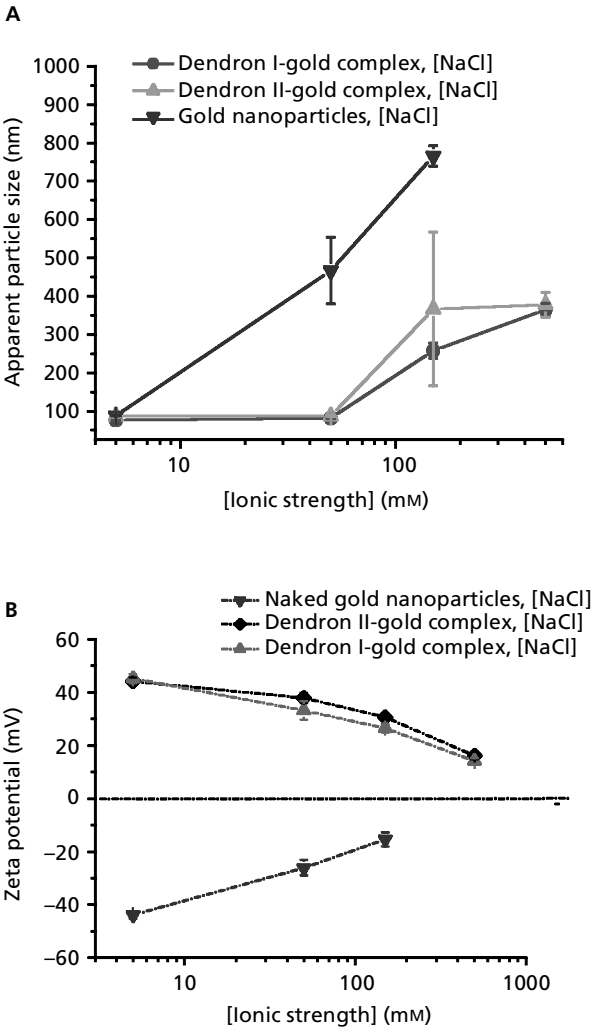



**Figure 4** A. The effect of NaCl ionic strength on apparent particle size of gold-dendron complexes is shown. Increasing the ionic strength led to an increase in apparent size of the complexes. In comparison with naked gold nanoparticles, these adsorbed dendrons appeared to stabilize the adducts with increasing ionic strength as the increase in apparent size of the gold nanoparticles appeared at a higher salt concentration when dendrons where adsorbed. Gold nanoparticles without surface adsorbed dendron is shown. B. The effect of ionic strength on zeta potential. The effect of the counter ions was also apparent when zeta potential was assessed. The gold-dendron complexes showed a decrease in particle charge whereas gold nanoparticles showed an increase in particle charge.

Figure 4A shows the effect of NaCl ionic strength on gold and dendron-gold complexes. The critical coagulation concentration (24 mM NaCl) for native gold nanoparticles with the increase in salt concentration was as predicted (Overbeek 1952). Increasing the ionic strength resulted in a significant increase in the apparent particle size of the gold adducts for both dendrons (500 mM: dendron I particle size, $365 \pm 17$ nm ($z = 28.3$): dendron II particle size $377 \pm 33$ nm ($z = 15.0$)). Dendrons, however, appeared to buffer the coagulation of gold nanoparticles by significantly raising the critical ionic concentration.

Figure 4B shows the effect of ionic strength on the system and to an extent explains the difference between their flocculation tendencies. The gold-dendron complexes showed a significant decrease in ionic strength at 500 mM NaCl (dendron I zeta potential $14 \pm 1$ mV ($z = 32.1$): dendron II zeta potential $16 \pm 1.5$ mV ($z = 26.0$)), showing the effect of the counter ions on the nanoparticles.

With an increase in ionic strength, the DNA-dendron-gold complexes also flocculated (Figure 5) but again at concentrations higher than native gold. At 50 mM NaCl a significant increase in apparent size was observed for both DNA-dendron I-gold and DNA-dendron II-gold complexes (dendron I particle size, $210 \pm 6$ nm ($z = 31.6$): dendron II particle size, $199 \pm 1$ nm ($z = 163.5$)).

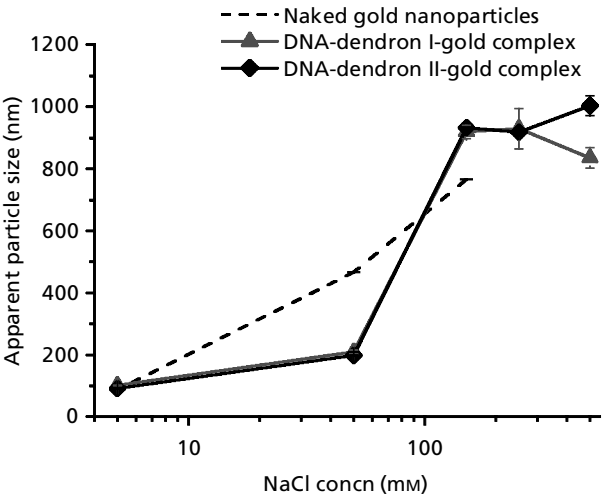



**Figure 5** The effect of ionic strength on apparent size of gold-dendron-DNA nanoparticles. Stability of gold-dendron-DNA particles showed an increase in apparent size with an increase in ionic strength but the crucial flocculation concentration was higher than the value of gold particles shown in Figure 4a.

## Suspension in cell culture media

More drastic changes in the surface potential and hydrodynamic diameter were seen when water was replaced as the solvent with Hank's balanced salt solution (HBSS) with and without serum (Table 2). The behaviour of naked gold, dendron-gold (carrier) and DNA-dendron-gold adducts was examined separately.

Gold nanoparticles suspended in HBSS ($640 \pm 43$ nm; zeta potential $-24 \pm 3.7$ mV) showed a significant increase in apparent particle size. The same was evident for the dendron I and II-gold complex ($160 \pm 8$ nm, zeta potential $2 \pm 1$ mV; and $213 \pm 2$ nm, zeta potential $0 \pm 1$ mV, respectively) and the DNA-dendron I and II-gold complex (423 nm, zeta potential 4 mV; and 1025 nm; zeta potential 19 mV, respectively). The dendrons showed a stabilizing effect on the gold particles, whereas DNA, a large negative polyelectrolyte, seemed in part responsible for the destabilization of the complexes in the media. No direct correlation was found between increase in apparent particle size and zeta potential of the complexes, suggesting that steric factors were also in play.

The inclusion of 10% serum in HBSS reversed the surface potential of the dendron-gold and DNA-dendron-gold complexes, and limited particle agglomeration significantly for the complexes gold-dendron II ($163 \pm 4$ nm), and DNA-gold-dendron I and II ($200 \pm 27$ nm and

**Table 2** Zeta potentials of gold, gold-dendron and gold-dendron-DNA nanoparticles in the presence of HBSS with and without serum.

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

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.

204 ± 33 nm, respectively), indicative of some component of the serum stabilizing the complex.

Flocculation of DNA-gold-dendron nanoparticles was also assessed in either cell culture media or water as a function of time (Figure 6). The apparent particle size of the complex in water did not change significantly over 24 h, in contrast to a drastic change seen in cell culture media after 24 h (763 ± 36 nm). In assessing the release of DNA in either of these media (Figure 7A), levels of free DNA significantly fell in cell culture medium as a function of time. This suggested that flocculation of the DNA-gold-dendron complexes as seen in Figure 6 bound or entrapped the released, free DNA (Figure 7B). An alternative hypothesis is that as DNA release could only be effected from monodisperse, single particles, the competing force of flocculation reduced the amount of single nanoparticles, thus reducing the DNA release potential thereby yielding a highly atypical release profile. Maximal release of DNA was less than 2% after 24 h, in contrast to gold-dendron-DNA nanoparticles suspended in water that released 5% of the bound DNA after 24 h.

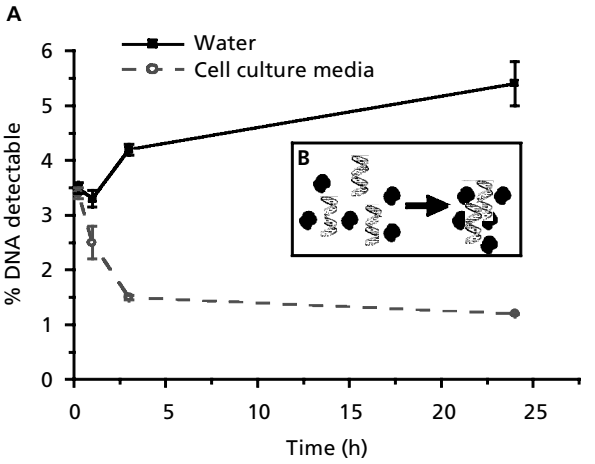



**Figure 7** A. Release profiles of surface adsorbed DNA in water and cell culture media (containing 10% serum) determined by the Picogreen assay. In comparison a lower concentration of DNA was detectable with time in cell culture media. B. Schematic presentation showing the effect of cell culture media on the gold-dendron-DNA complexes.

## Conclusions

The mode of attraction between dendrons and gold nanoparticles seemed to be mediated by hydrophobic interaction. Whilst gold-dendron-DNA nanoparticles in water maintained their original state in term of size and surface potential there was a rapid and a drastic alteration in size of the adducts when high concentrations of salt or cell culture media were used. Aggregation may explain the low rate of release of DNA as flocculation increased with time. This reduced de-attachment of DNA from the carrier complex, coupled with the flocculation of the carrier, would decrease the efficiency of the system. These results demonstrated the importance of characterizing drug delivery systems in the presence of relevant physiological fluids and media before cell culture or in-vivo studies, and may be one reason that in-vivo and in-vitro correlations in gene transfection studies are so limited.

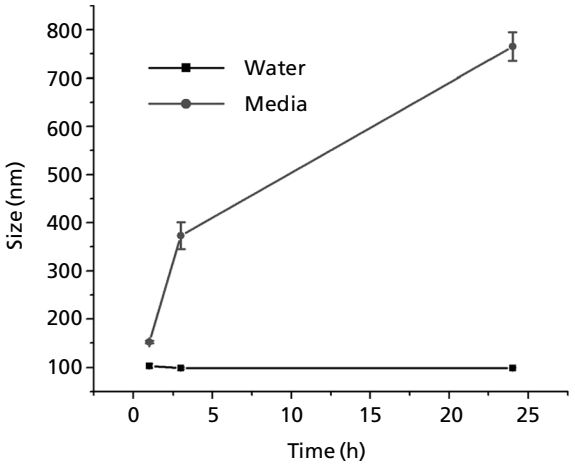



**Figure 6** The effect of either cell culture media and water on the apparent particle size of DNA-dendron I-gold nanoparticles was assessed as a function of time. The figure shows that in cell culture media flocculation occurs steadily over 24 h. In contrast the nanoparticles in water showed no significant increase in apparent particle size.

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