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Effects of dendrimer oil dispersants on *Dictyostelium discoideum*

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Chemical dispersion is a frequently used yet controversial strategy in an attempt to minimize the environmental and economic impact of large-scale oil spills. Previously, cationic poly(amidoamine) (PAMAM) dendrimers have been proposed as novel, biocompatible oil dispersants. Here, we investigate the effect of such dendrimer oil dispersants on the soil amoeba *Dictyostelium discoideum*, using phenanthrene as a model polyaromatic hydrocarbon. In examining cell culture proliferation and changes in membrane potential, we find that low concentrations of cationic generation-4 PAMAM dendrimers were non-toxic to *Dictyostelium discoideum.* In comparison, the same low concentration of the primary surfactant in the COREXIT oil dispersant, Tween 80, did display limited acute cytotoxicity. Higher concentrations of dendrimer oil dispersant elicited cytotoxicity due to significant depolarization of the cell membrane resulting from the uptake of highly cationic PAMAM dendrimers. Cellular uptake of cationic PAMAM dendrimers was confirmed by a cellular association experiment conducted at room temperature and 2 °C, using fluorescence imaging. The uptake and corresponding membrane depolarization was found to be significantly inhibited by the presence of phenanthrene within and around the periphery of the dendrimers. This study offers new insight into the environmental implications of oil dispersants.

Introduction

For decades, the use of oil dispersants has been a frequent yet controversial response to marine oil spills.[1] The purpose of such practice is to break up persistent oil slicks and plumes in an attempt to mitigate the impact on shorelines and near-surface life while simultaneously making the oil more accessible for biodegradation by oil-eating microbes.[2,3] The first oil dispersants developed were highly toxic to marine life. Modern dispersants, such as the COREXIT dispersant used during the 2010 Deepwater Horizon disaster, are considerably less toxic yet studies by the US EPA and others show that even these current options are equally or even more toxic than crude oil alone.[4–7] In addition, because they are not recovered after deployment, dispersants are free to diffuse through the water column and even to shorelines to impact aquatic and soil organisms in the ecological sphere. Some oil dispersant formulations are designed to be applied directly to shorelines, as well.[8]

Dictyostelium discoideum is a soil amoeba commonly used to assess the health of a soil environment and the impact of toxins or pollutants on that environment, and also as a model organism in toxicity studies.[9,10] They are also applicable to aquatic environments as well as soil/aquatic environment interfaces (such as wetlands), as many amoebas can transition from soil to aquatic environments and there are generally no fundamental physiological differences between aquatic and soil amoebae.[11] Over the course of its developmental cycle, this eukaryotic species transitions from single celled amoebae to multicellular slugs and fruiting bodies and is prevalent in the soil of forests, within degrading organic matter, or in nutrient-rich sediment. Because they lack the cell walls possessed by bacteria or plants, *D. discoideum* are particularly sensitive to the threat imposed by pollutants. *D. discoideum* also form a crucial link in the local food chain, feeding on bacteria and being consumed by nematodes and other small organisms.[12]

Dendrimers are a well-ordered and monodisperse nanomaterial within the class of dendritic polymers. Structurally, dendrimers consist of a central core and several iterative branches emanating from this core, ending in terminal groups.[13] The number of branching iterations determines the size of the dendrimer and is termed the "generation". Poly(amidoamine) (PAMAM) dendrimers possess charged surface groups owing to their primary amines and hydrophobic

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interiors at physiological pH, making them a soluble amphiphilic molecule. For this reason dendrimers are a focus of studies in water purification[14–17] and drug delivery,[18] being used to encapsulate a wide variety of hydrophobic substances (pollutants, drugs, and prodrugs) in the aqueous phase as well as metallic ion water contaminants. In a prior study we showed that generation-4 (G4) PAMAM dendrimers interact strongly with both polyaromatic (phenanthrene) and linear (hexadecane) hydrocarbons, acting effectively as an oil dispersant.[19] The dendrimers accomplish this by partially partitioning hydrocarbons into their hydrophobic interiors, thus suspending them in aqueous solution in quantities far greater than their natural water solubility.

The present study examines the effect of G4 PAMAM dendrimers on the soil amoeba *D. discoideum* when utilized as an oil dispersant. In this way we hope to shed light on the impact of such an oil dispersant on shoreline ecosystems such as wetlands, forests, and estuaries. We employ phenanthrene (PN) as a model polyaromatic hydrocarbon (PAH), known to be among the most ubiquitous and toxic components of petroleum products, often being studied for its impact on aquatic and soil ecosystems.[10,20–22] Our investigation covers the physicochemical aspects of dendrimer–PN complexation and the biophysical and toxicological responses of *D. discoideum* to the exposure of PN-laden dendrimers.

Materials and methods

Preparation of phenanthrene-loaded PAMAM dendrimers

Stock solutions of G4 amine-terminated PAMAM dendrimers (M_w: 14 kDa) were purchased from Dendritech (Midland, MI) and first diluted in 1× phosphate buffered saline (PBS) at pH 7.4 (HyClone, Thermo Fisher Scientific). The capture of PN molecules in a dendrimer solution was performed as described previously.[19] Briefly, an excess of dry PN powder (Sigma-Aldrich) was added to the dendrimer stock solutions, bath sonicated for 15 min, and subsequently rotated overnight. The result was a dendrimer stock solution saturated with PN molecules through hydrophobic interactions between the dendrimer interiors and the hydrocarbon, with excess PN settling out from the solution. Previous studies have indicated that this method results in approximately 5 PN molecules complexed with every G4 dendrimer in solution, as well as free PN at its maximum natural water solubility (1.3 mg L^{-1}).[19] To confirm that PN did not cause dendrimers to aggregate in PBS, thus reducing their bioavailability, we measured the hydrodynamic size of the complexes using dynamic light scattering (DLS). We found that there was no observable aggregation of dendrimer–PN complexes (10 μM dendrimers) and that the hydrodynamic size increased modestly from 4.5 ± 1 nm to 5.5 ± 1 nm upon incubation with PN. This also suggests that some PN were not completely encapsulated and "hidden" by the dendrimers, but instead were partially protruding from the dendrimer core due to steric hindrance. Simulation studies have confirmed the presence of PN near the periphery of the PAMAM dendrimers.[23] Both with and without PN, the zeta potential of the PAMAM dendrimers at neutral pH is 23.5 ± 4.5 mV.

Fluorescent labeling of PAMAM dendrimers

G4 PAMAM dendrimers were fluorescently labeled using sulforhodamine 101 sulfonyl chloride (SSC) (Anaspec, San Jose, CA), an amine-reactive fluorescent dye. Dendrimer stock solutions (50 μM) were prepared in DI water and the pH adjusted to 8.5 in order to slow the hydrolysis of SSC before attaching to dendrimers. This solution was then chilled to 4 °C, and dry SSC was added at a stoichiometric ratio of 2 dye molecules per dendrimer and rotated in the dark at 4 °C for 30 min. In this way, approximately 2 out of the 64 surface primary amines of each dendrimer were fluorescently labeled; such a low labeling ratio ensured the physicochemical properties of the dendrimers remained largely intact. Excess dye not covalently bound to the dendrimers was removed by centrifugation using a 3 kDa cutoff centrifugal filtration unit (Amicon, Billerica, MA). The labeled dendrimers were then diluted in PBS to a final stock concentration of 50 μM and stored in the dark. This stock was then split and half was incubated with PN as described above.

Dictyostelium discoideum cultures

D. discoideum wild-type (Strain Ax2) cells were grown axenically as described elsewhere.[24,25] Briefly, cells were maintained in T25 or T75 cell culture flasks (Corning, Tewksbury, MA), in HL5 medium (per liter: oxoid proteose peptone, 10 g; glucose, 10 g; yeast extract, 5 g; Na_2HPO_4, 0.19 g; KH_2PO_4, 0.35 g; pH 6.6) supplemented with ampicillin (100 μg mL^{-1}), and were subcultured twice per week. For all experiments cells were harvested during log phase.

Kinetics of dendrimer–cell association

The cell association of dendrimers with and without PN was performed using SSC-labeled dendrimers. Three subcultures of *D. discoideum* were prepared and the number of cells in each culture was counted immediately before beginning the experiment using an image-based cytometer (Countess, Life Technologies, Carlsbad, CA). The live cell concentrations were then adjusted by adding HL5 nutrient medium to dilute all subcultures to 2.2×10^5 cells per mL. Each of the three subcultures were then split into 12 aliquots of 4 mL in two 6-well plates and allowed to adhere to the plate bottom surface for 2 h, at which time the cultures were gently serial-washed into PBS, resulting in a low final concentration of HL5 (~5%). Both 6-well plates received SSC-labeled dendrimers (with or without PN) in each well, resulting in a final dendrimer concentration of 2.5 μM. One plate was then incubated at 2 °C, and the other at room temperature in the dark for 2 h, approximately corresponding to the time required for culture membrane potential measurements to come to equilibrium. This time scale also allows us to largely ignore the death of cells due to the uptake of dendrimers, which would significantly convolute the results. At each time point, 150 μL was removed from the top layers of each well and set aside in a 96-well plate along with control samples containing no cells. Cell-only controls confirmed that cells and HL5 medium displayed no fluorescence above 530 nm. The fluorescence in each well of the 96-well plate was then read using a Cary

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Eclipse fluorescence spectrophotometer with plate reader (excitation: 575 nm). The fluorescence intensities from 595–615 nm were integrated and normalized to initial values for analysis.

Viability/proliferation assay

Cell viability and proliferation of *D. discoideum* was performed using a DHL assay kit (Anaspec, San Jose, CA). Specifically, on a sterile 96-well plate, 5 portions of 80 μL each of live cells in HL5 nutrient medium were incubated with 20 μL of dendrimer stock solutions in PBS, resulting in 100 μL samples at final dendrimer concentrations of 1 μM, 10 μM, and 50 μM, both with and without PN. Two sets of controls were prepared: one of 80 μL HL5 nutrient medium alone and 20 μL PBS to provide a measurement of background fluorescence, and another with 80 μL cells in nutrient medium and 20 μL PBS without dendrimers. Additional subcultures were incubated with 1, 10, and 50 μM Tween 80 (Sigma-Aldrich, a nonionic surfactant) in PBS in order to compare dendrimers and a primary component of the COREXIT oil dispersant.[26] Each well then received 20 μL of the assay dye. The resulting fluorescence intensities (excitation: 560 nm; emission: 590 nm) were read on a Cary Eclipse fluorescence spectrophotometer with plate reader for 24 h. The first data points are given at 2 h due to the time required to obtain an initial fluorescent signal from metabolized fluorophores. Because of the similarities in baseline fluorescence intensities, fluorescence spectrum characteristics, and intensities at late times for low dendrimer concentration, and in consideration of the similarities between dendrimers and proteins in UV absorption dendrimers should exert no direct impact on the fluorescent assay (excitation: 560 nm). Technically, because the dye must be activated by cellular metabolism within live cells, controls directly examining possible interactions with the fluorescent assay were not feasible.

Membrane potential

The physical integrity of *D. discoideum* membranes was probed using a FLIPR membrane potential assay kit, blue formulation (Molecular Devices, Sunnyvale, CA). This assay kit consists of a buffer solution (HBSS + HEPES buffers, pH = 7.4) and a mixture of fluorescent dyes and quenchers (excitation: 530 nm; emission: 560 nm). Amoeba cultures were first washed by pelleting the cells at 1700 × *g* and re-suspended in PBS in order to avoid the background fluorescence of the HL5 nutrient medium. The washed cells were distributed into a 96 well plate with 100 μL per well. After allowing the cells to adhere to the plate for 2 h, 100 μL of assay dye in buffer solution was added to each well and incubated for 30 min, at which time a baseline fluorescence reading was obtained for each well. We then added 50 μL of dendrimer with or without PN stock solutions and began fluorescence readings immediately, stopping after all signals reached equilibrium (approximately 2 h). The control wells received 50 μL of PBS alone.

Fluorescence imaging

Fluorescence imaging was performed on a Nikon TI Eclipse fluorescence microscope (Nikon Instruments, Melville, NY). Cells were first washed in PBS as described above and incubated with SSC-labeled dendrimer solutions at a final concentration of 10 μM for 2 h. The cells were then washed twice more in PBS to remove any labeled dendrimers which were not cell associated and then wet mounted in PBS on glass microscope slides for imaging. The cover slips of the sample slides were completely sealed with lacquer to prevent water evaporation. Differential interference contrast (DIC) bright-field and wide-field fluorescence images were obtained (40×, oil-immersion objective, NA = 1.3) for amoeba exposed to the dendrimers and overlaid during analysis. Images were obtained using a Coolsnap HQ2 high-sensitivity quantitative monochrome camera (Photometrics, Tucson, AZ), and the fluorescence images were pseudocolorized red before overlaying with DIC images to aid visualization.

Results and discussion

Cell proliferation and viability

As an initial assessment of *D. discoideum*'s response to cationic dendrimers and PN, we examined the proliferation of amoeba cells incubated with varying concentrations of G4-PAMAM with and without PN over a 24 h period. The fluorescent indicator dye was activated by mitochondrial dehydrogenase enzymes in cellular metabolism and was thus an indicator of total metabolism that occurred in the sample. At early times this was strongly correlated to the total number of viable cells from the initial subculture population and thus a decrease in fluorescence intensity relative to the control served as an indication of acute toxicity. At later times it was more accurately an indication of total metabolism and proliferation of the cell culture, and a decrease in these intensities relative to the control were indicative of long-term toxicity or inhibition of culture proliferation.

Fig. 1(a) shows the fluorescence signal every 2 h for 18 h from each subculture as a fraction of the control culture at each time point, which contained no dendrimers or PN. Therefore, a normalized fluorescence intensity of 1 corresponds to a culture with a metabolic rate identical to the control. Early time points for the 1 μM subculture both with and without PN, as well as the 10 μM subcultures with PN, all show no significant deviations from the control, defined as lying outside the 95% confidence interval. This indicates no acute toxicity in those subcultures. At 10 μM dendrimers without PN, however, the metabolism indicator decreased to 82% of the control, suggesting some acute toxicity due to the dendrimers alone. Similar short-term toxicity is seen for both samples at 50 μM concentrations.

Fig. 1(b) shows the total metabolism in the subcultures after 24 h. At each concentration, we note that there is no statistically significant difference (95% confidence) between samples with (red) and without (blue) PN as well as with Tween 80 (gray) (n = 5). A dendrimer concentration of 1 μM caused no statistically significant deviation from the control, indicating no long-term toxicity or inhibition of proliferation due to dendrimers at that concentration. We do observe, however, that Tween 80 caused a statistically significant decrease in viability at 24 h, with approximately 80% total metabolism compared to the control. Upon increasing the Tween 80 concentration there was no

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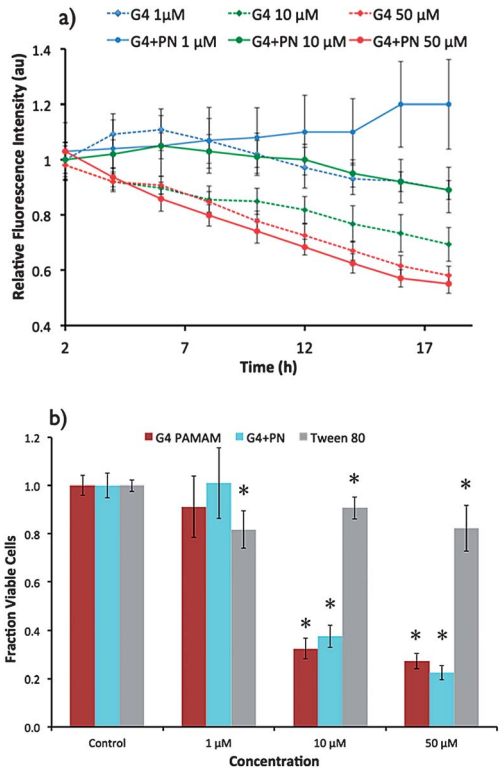

Fig. 1 (a) Cell metabolism indicated by fluorescence assay, relative to the control containing no dendrimers. Blue, green, and red indicate 1, 10, and 50 μM, respectively, either without (dashed) or with (solid) PN. (b) Total cell proliferation indicated by metabolism after 24 h, relative to the control containing no dendrimers. PN-free shown in red, PN-loaded shown in blue, and Tween 80 in gray. All error bars indicate standard deviation ($n = 5$). * indicates statistically significant difference from the control.

increase in toxicity observed after 24 h of incubation. This is because at 10 μM we reached the critical micelle concentration (CMC) for Tween 80, thus significantly reducing the bioavailability of Tween 80.[26] We note that this is a concentration considerably higher than one would encounter in the field when COREXIT is applied as a dispersant. Higher dendrimer concentrations, in sharp contrast, display marked decreases in total metabolism with approximately 35% as many live cells at 10 μM and 25% at 50 μM compared to the control. Because of the similarity in the long-term toxicity profiles, we conclude that the inhibition of culture proliferation was due to the presence of dendrimers and not PN. The differences in acute toxicity suggest that toxicity was due to interactions with dendrimers and not PN and also that there might be some variation in cellular uptake and response to dendrimers which have encapsulated PN, thus reducing acute toxicity.

Cell-association kinetics

To verify any difference in the ability of *D. discoideum* to uptake PN-loaded dendrimers, we next performed a cell-association study which included both cellular uptake and adsorption to the cell membrane. Fig. 2(a) compares the cell association of SSC-labeled dendrimers (red squares) to those loaded with PN (blue diamonds). Though rates of dendrimer depletion are similar in the two cases, the depletion of PN-loaded dendrimers appears to saturate after 19% of the dendrimers have been removed from solution, while dendrimers alone fall by 30% without clear saturation behavior. This indicates a more efficient uptake of unloaded dendrimers by the amoeba cells, likely through strong electrostatic interactions between the cationic dendrimers and the negatively charged cell membranes as well as endocytosis elicited by the cells.[27]

Fig. 2(b) compares uptake kinetics by cells at room temperature to cells held at 2 °C, at which temperature the energy-dependent endocytotic processes are shut down without compromising the cultures. This low temperature will also cause the membranes to be completely in gel phase, thus limiting passive diffusion of dendrimers across the lipid bilayer. While this low temperature will also slow the diffusion of dendrimers, by ~10% based on the Stokes–Einstein equation, the rapid diffusion of dendrimers and small sample volume should readily compensate for this difference for the timescale of the present experiment. We again note that the depletion of dendrimers alone at room temperature is the most efficient, while dendrimers at 2 °C fall by just 16% in 2 h. Interestingly, the kinetics observed for loaded and unloaded dendrimers at 2 °C are almost identical, implying a common mechanism for interaction in both cases. Because uptake by cells at 2 °C is minimal, this observation implies that the difference seen in Fig. 2(a) is due to a much greater internalization of dendrimers compared to PN-loaded dendrimers. Once cellular uptake is halted, this difference disappears.

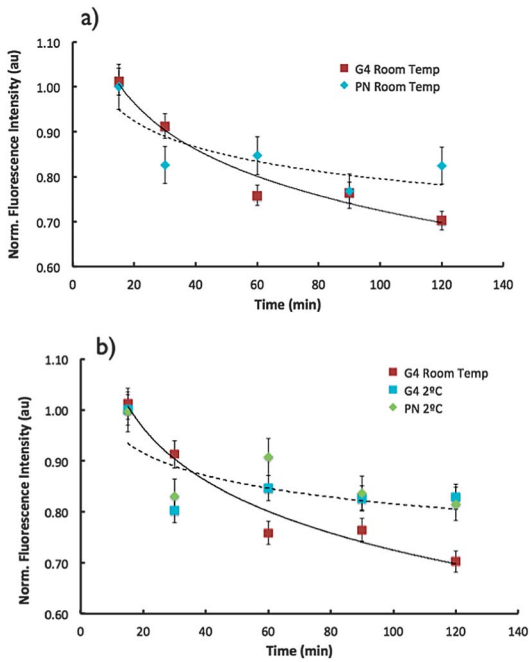

Fig. 2 (a) Uptake kinetics for SSC-labeled dendrimers (red squares) and PN-loaded dendrimers (blue diamonds) at room temperature. (b) Uptake kinetics for SSC-labeled dendrimers at room temperature (red squares), dendrimers at 2 °C (blue squares), and PN-loaded dendrimers at 2 °C (green diamonds). All samples normalized to initial fluorescence intensities. Trend lines added to aide visualization. Error bars indicate standard deviation ($n = 3$).

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By comparing the dendrimer depletion rates at room temperature and 2 °C, we can deduce the rates for dendrimer uptake and adsorption with amoeba cells. To accomplish this, we assume that dendrimers in cultures held at 2 °C are depleted from solution by adsorbing to the exterior of the membranes alone, while at room temperature depletion is due to adsorption as well as cellular uptake. By subtracting the depletion rates of dendrimers alone at room and cold temperatures, we find that uptake alone contributes 0.31% per min to dendrimer depletion, which corresponds to 1.9×10^{13} dendrimers per min. Taking into account of the total number of 0.88 million cells per well, we deduce that each cell internalized approximately 20 million dendrimers every minute over the first hour of incubation. In contrast, dendrimers loaded with PN at room temperature were internalized at a much smaller rate of 1 million per cell per min. This rapid uptake of dendrimers is a likely cause of the acute toxicity observed for dendrimers that have not been PN-loaded.[28]

Observing the saturation points of cultures held at 2 °C also allows the approximation of the coverage of dendrimers adsorbed onto the membrane surface. By noting cellular morphology in bright-field images, we approximate the exposed surface area (approximated as a half sphere, $2\pi r^2$) of each cell to be 400 μm^2. Using a saturation point of adsorption of 16% depleted (Fig. 2(b)), the known initial dendrimer concentration, and assuming an even distribution of the dendrimers across only the exposed cell surface, we find that there were approximately 3–6 nm between adjacent dendrimers. Recall that the measured hydrodynamic diameter of these G4 dendrimers is 4.5 nm. This implies a tightly packed (saturated) monolayer of dendrimers across the entire exposed membrane surface, which leads to a halting of the adsorption of more dendrimers onto the cellular membrane due to the electric repulsions between the dendrimers.

Membrane potential

In order to elucidate the mechanisms of any observed dendrimer toxicity to amoebas, we performed a membrane potential assay. The fluorescent dyes in this assay are membrane permeable; their penetration into the cell increases upon membrane depolarization and decreases upon membrane hyperpolarization. The assay quenchers, however, are not membrane permeable. Therefore, any dyes in the extracellular space are quenched while those that have penetrated into the cell are free to fluoresce with an excitation at 530 nm (emission at 560 nm). Following this scheme, an increase in fluorescence intensity indicates membrane depolarization, while a decrease indicates hyperpolarization.

Fig. 3 (right panel) shows assay fluorescence signal relative to the control with no cells. We observed a statistically significant, concentration dependent depolarization of the cell membrane at all concentrations of dendrimers without PN (red) with a signal increase of 23%, 330%, and 430% at 1 μM, 10 μM, and 50 μM, respectively. Because amine-terminated PAMAM dendrimers are positively charged at neutral pH, this indicates that the internalization of the dendrimers led to a depolarization of

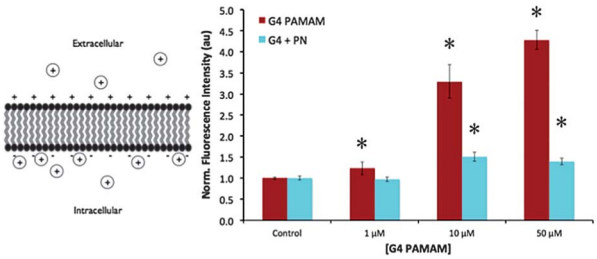

Fig. 3 Schematic of cellular membrane depolarization by cationic dendrimers. The extracellular space is at a higher electric potential than the intracellular space. The internalization of cationic dendrimers (positive circles) causes an increase in the electric potential of the intracellular space, thus depolarizing the membrane. Membrane potential of amoebae incubated with dendrimers (red) and PN-loaded dendrimers (blue). Error bars indicate standard deviation ($n = 5$). * indicates statistically significant difference from the control.

the cell membranes as depicted by the schematic in Fig. 3 (left panel). The exterior of the cell membrane is known to be at a higher net electric potential than the interior due to the peripheral proteins present at the inner membranes. The internalization of highly cationic dendrimers (positive circles) raises the electric potential of the inner membrane through charge neutralization, thus leading to depolarization.

We noted a markedly different response for dendrimers with PN (blue). The lowest concentration, 1 μM, showed no statistically significant variation from the control. Both 10 μM and 50 μM did display membrane depolarization (a 51% and 40% increase relative to the control), though at levels a factor of 2.2 and 3.1 times less than dendrimers alone, respectively. Also we noted that the depolarization due to dendrimers with PN did not increase in a concentration-dependent manner. These results suggest that the cellular uptake and response to dendrimers with PN is inhibited compared to dendrimers alone, causing there to be fewer cationic dendrimers inside of the cell to trigger membrane depolarization. This is consistent with our uptake kinetics experiment, in which the uptake rates of PN-loaded dendrimers were severely inhibited. This may be because PN, though it promotes membrane adhesion due to hydrophobic interactions, should also inhibit membrane translocation of the dendrimer–PN complexes. PN cannot be completely encapsulated by G4 PAMAM dendrimers as shown in our earlier simulation study,[23] and as a result dendrimer–PN complexes were significantly more hydrophobic than the exterior of the dendrimers alone. Therefore, they may slow or prevent dendrimers from fully crossing the hydrophobic interiors of the cell membrane due to partitioning with the fatty acyl tails of the lipids. They may also be more easily encapsulated in the amoeba's vacuoles and lysosomes by the same reasoning. We note that there was no change in membrane potential upon incubation with Tween 80 at concentrations of 1, 10, or 50 μM, which is not surprising, as Tween 80 carries zero net charge (data not shown).

Fluorescence imaging

In another approach to confirm any difference in uptake of dendrimers with and without PN by amoebae, we performed a

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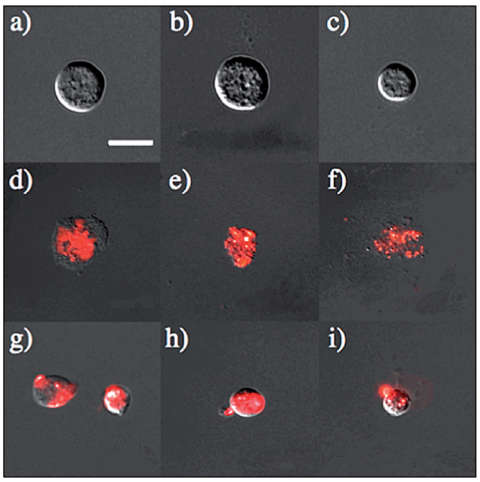

Fig. 4 DIC and fluorescence overlaid images of amoebae. Controls (a–c) with no dendrimers, cells incubated with 10 μM SCC-labeled dendrimers alone (d–f) and cells incubated with 10 μM PN-loaded SCC-labeled dendrimers (g–i). Scale bar: 10 μm.

combination of bright-field DIC and fluorescence imaging (Fig. 4). The 600 nm fluorescence of SSC-labeled dendrimers was overlaid with the DIC images to allow for localization of dendrimers within the amoeba cells. The red coloration in the images indicates the presence of SSC-labeled dendrimers.

The control amoebae (Fig. 4a–c) all exhibited round morphology, smooth edges, and clearly defined intra- and extracellular space. The cells incubated with dendrimers alone, in contrast, possessed rough edges and less sharply defined cell membranes. The dendrimers (appearing red) generally filled the intracellular space, sometimes also being localized within bright cellular vesicles (Fig. 4e). Those cells incubated with dendrimers plus PN displayed morphologies more closely resembling that of control cells, strengthening the notion that dendrimers with PN caused less membrane disruption than did dendrimers alone. These amoebas with dendrimers plus PN also appear to be smaller, on average, than control amoeba cultures. This may be due to the negative effect of PN on cell metabolism, or due to the partitioning of PN which compromised the cell fluidity; the latter case is similar to our earlier study of cell contraction induced by gallic acid-suspended fullerene.[29] We also noted that dendrimers with PN filled the intracellular space much less evenly than did dendrimers alone, and were often localized at the membranes (Fig. 4g) or in vesicles (Fig. 4g–i). These observations clearly support our hypothesis that the PN protruding from these dendrimers inhibited the translocation of dendrimers across cell membranes and the lipid bilayers of vesicles, thus reducing their overall uptake and any resulting membrane disruption and cytotoxicity.

It was also observed that some cells incubated with dendrimers plus PN were able to remove the PN-containing dendrimers from themselves (Fig. 4h and i), likely through the exocytosis of vesicles containing these complexes. Fig. 4h also suggests that a "pinching-off" reaction may be occurring, during which the amoeba closed off and removed a contaminated or damaged area of itself in order to preserve the rest of the cell.[12,30,31] Such active transport and segregation of dendrimers alone is much less likely since PAMAM dendrimers are very similar to simple globular proteins in structure and elemental composition, and thus are likely not "recognized" by the cell as a foreign or toxic substance.

Conclusions

We have examined in this study the interactions between soil amoeba *D. discoideum* and G4 PAMAM dendrimers when utilized as an oil dispersant, using PN as a model PAH. We found that the dendrimers alone were taken up by the amoebae, which at concentrations of at least 10 μM resulted in significant toxicity. The mechanism of this toxicity is presumed to be the adsorption and uptake of highly cationic dendrimers, which resulted in the depolarization of the cellular membranes. This uptake of dendrimers was confirmed by cell association experiments at room temperature and 2 °C. At lower concentrations (1 μM), however, we saw no acute toxicity or proliferation inhibition towards these amoebae. We also observed that the ability of amoebae to take up dendrimers loaded with PN was significantly decreased, likely due to increased membrane retention as a result of the increased hydrophobicity of the complexes. Consequently, the presence of cationic dendrimers in intracellular space was reduced, giving rise to reduced membrane depolarization and reduced toxicity. This finding that the dispersant is in fact *less* toxic to *D. discoideum* upon capturing PN is noteworthy and in stark contrast to other dispersant studies, in which oil-dispersant complexes are typically equally or more toxic than either the dispersant or oil alone.[1,6,7]

Our prior work has shown that G4 PAMAM dendrimers were capable of solubilizing both polyaromatic and linear hydrocarbons across a wide range of concentrations.[19] This alone presents a significant advantage over surfactant-based oil dispersants, which must act collectively to be effective. By utilizing dendrimers in low concentrations, we are presented with a nonlinear oil dispersant which is effective not only in hosting capacity but also non-toxic to soil amoebae upon contact with the shoreline. It is not difficult to extend this conclusion to marine amoebae, which would be more immediately impacted by the deployment of any oil dispersant.

Acknowledgements

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