Moisture Content Impacts the Stability of DNA Adsorbed onto Gold Microparticles

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ABSTRACT: Particle-mediated epidermal delivery (PMED) of small quantities of DNA (0.5–4.0 μg) has been reported to both induce an immune response and protect against disease in human subjects. In order for the PMED of DNA to be a viable technique for vaccination, the adsorbed DNA must be stable during shipping and storage. Here, we report that the storage stability of plasmid DNA adsorbed to 2-μm gold particles is strongly dependent on sample water content. Gold/DNA samples stored at 60°C and 6% relative humidity (RH) maintained supercoil content after 4-month storage, whereas storage at higher RHs facilitated degradation. Storage with desiccants had stabilizing effects on DNA similar to storage at 6% RH. However, storage with "indicating" Drierite and phosphorus pentoxide resulted in enhanced rates of DNA degradation.
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Keywords: microparticles; DNA delivery; stability; vaccine delivery; plasmid DNA; drying; drug delivery systems

INTRODUCTION

Cellular bombardment with accelerated DNA adsorbed onto gold microparticles has been routinely used as a means for creating transgenic plants[1] since Sanford et al. introduced the technique in 1987.[2] The microprojectiles effectively enter live cells delivering foreign DNA. Similarly, this "gene gun" DNA delivery method is used to deliver DNA encoding antigenic or therapeutic material directly into epidermal cells of live mammals. The delivery of antigen-encoding DNA to epidermal cells has been shown to elicit an immune response resulting in both cellular and humoral immunity.[3–5] Microparticle DNA delivery to Langerhans and dendritic cells found in the epidermis[6] are believed to express foreign antigen or uptake foreign antigen expressed by transfected keratinocytes. The antigen presenting cells then migrate to the lymph node to stimulate primary T cells.[7] This strategy has been used to immunize mice against human growth hormone,[8] rabies virus,[9] Ebola virus,[10] and the hantavirus.[11] More recently, human clinical trials delivering DNA encoding foreign antigens of hepatitis B, influenza virus, and malaria virus have resulted in significant cellular and humoral responses.[12–17] Furthermore, vaccination with particle-mediated epidermal delivery (PMED) is well tolerated in human patients with the exception of minimal redness at the site of vaccination.[12–17] Despite these promising results, there has been surprisingly little investigation into the stability of the adsorbed DNA that is critical in eliciting the therapeutic effect.

PMED is a unique drug delivery method capable of avoiding many of the safety concerns associated with live attenuated, and killed viral particles.[18] DNA vaccines are unable to replicate and spread, but retain antigenic specificity. Additionally, PMED has proven advantageous over direct DNA injection as 100- to 500-fold less DNA is needed to elicit an immune response,[12] likely due to PMED's direct delivery of the plasmid to the cytosol as opposed to the extracellular space. Further, DNA vaccines present a low-cost alternative to protein-based vaccines.[8,19,20] However, the ability to express antigen through PMED is dependent on the integrity of the DNA adsorbed to the gold microparticle, and thus the shelf-life of the DNA must be consistent with a commercial pharmaceutical product in order for this technology to be fully exploited.

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PMED typically employs antigen-encoding DNA that is produced as a supercoiled plasmid. The beneficial effects of supercoiled DNA over open circle and linear DNA have been reported in both *in vitro* transfection and *in vivo* DNA vaccination studies; supercoiled structure promotes increased antigen expression and leads to greater immune responses.[21,22] However, the degradation of supercoiled plasmid DNA in pharmaceutical products is not widely studied, especially in dried systems.[23] Solution studies have shown that DNA is sensitive to depyrimidination,[24] deamination,[25] and depurination at low pH,[26] where depurination increases the rate of β-elimination resulting in the cleavage of the phosphodiester backbone and loss of supercoil structure.[27] Analogous studies in dried systems have not been conducted, and the effect of even very basic parameters (e.g., water content) on DNA stability has not been thoroughly investigated. Interestingly, recent studies by Bonnet et al.[28] have demonstrated the remarkable stability of DNA that is fully dehydrated by very high temperatures (>100 °C), suggesting that the presence of water at more conventional storage conditions may play a significant role in DNA degradation.[28] In contrast, observations of DNA stored in the presence of phosphorous pentoxide, a strong desiccation agent, suggest that overdrying can lead to increased vulnerability to oxidative damage.[28,29]

While the above studies highlight the relatively poor understanding of DNA stability in the dried state, the adsorption onto gold microparticles for PMED may have additional stability ramifications that have not been addressed. The only previous study that investigates the stability of DNA adsorbed to gold particles demonstrated that supercoil content is compromised by storage at ambient temperatures, but that complexation with protamine helped protect the adsorbed DNA.[30] The study also demonstrated that the immune response is compromised as adsorbed DNA degrades during storage at ambient temperature.

In the present study, we investigate the storage stability of PMED formulations comprised of DNA adsorbed to gold microparticles. To assess recovery, we quantify both the amount of DNA that is extracted into aqueous buffer and its supercoil content. The former measurement allows us to quantify the amount of DNA that could be liberated in the target cell after PMED, whereas the latter measurement provides a direct measure of DNA integrity. Our investigation focuses on the role of sample water content, controlled by storage in various relative humidities (RH), on the storage stability of adsorbed plasmid DNA in PMED formulations.

MATERIAL AND METHODS

Chemicals

Plasmid was purchased from Aldevron (Fargo, North Dakota). Both trehalose and gold particles (2–3 μ in size) were purchased from Ferro (Niagara Falls, New York). Tetra-arginine was purchased from Bachem (King of Prussia, Pennsylvania). Silica gel packets (10 g) were purchased from Multisorb Technologies (Buffalo, New York). Ethidium bromide, 0.5 M EDTA, tris(hydroxymethyl)aminomethane, tris(hydroxymethyl)aminomethane hydrochloride, anhydrous dimethylformamide (DMF), potassium hydrochloride, magnesium chloride were purchased from Sigma (St. Louis, Mousa). Lithium chloride, phosphorous pentoxide, and cesium fluoride were obtained from Acros Organics (Fairlawn, New Jersey). Agarose gel was purchased from BioExpress (Kaysville, Utah). Pyridine-free vessel solution and generator solution used for determining water content were obtained from Photovolt Instruments Incorporation (St. Louis Park, Minnesota). Sodium nitrate was purchased from Honeywell Riedel-de Haen (Morristown, New Jersey). Drierite was purchased from Drierite Co Ltd (Xenia, Ohio).

Gold DNA Complexation

Plasmid DNA was ethanol-precipitated onto 2-μm gold particles in the presence of EDTA, trehalose, and tetra- arginine as previously described by Medley et al.[31] DNA was added at a concentration of 2.6 μg per mg gold. Higher quantities of DNA can be loaded onto gold particles,[32] but this concentration was chosen because it was similar to a potential target for a flu vaccine in development.[15] After formulation, samples were dried for 2 h under nitrogen gas.

Sample Storage

For storage, 100 or 300 mg gold/DNA samples were allocated into 1 mL glass lyophilization vials, left uncapped and placed in desiccators to control sample moisture content (depicted in Fig. 1). The desiccators were then placed into an incubator set at 60°C and stored for up to 6 months.

Controlling Sample Moisture Content

The RH within the desiccators was controlled with different saturated salt solutions and desiccants. Saturated salt solutions consisting of cesium fluoride, potassium hydroxide, lithium chloride, magnesium chloride, ammonium nitrate, and sodium nitrate allowed for sample storage at 2%, 6%, 11%, 30%, 42%, and 60% RHs, respectively, at 60°C.[33–35] Desiccants used included Drierite, silica gel and phosphorous pentoxide. An additional set of gold/DNA samples was

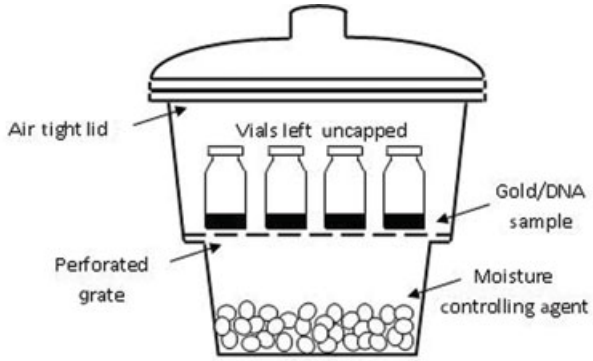

Figure 1. Desiccator with desiccant or saturated salt solutions in the lower compartment. Gold/DNA samples sit above the moisture controlling agent on a perforated grate and stored in unstoppered lyophilization vials for moisture equilibration.

dried by lyophilization to assess the effects of removing residual moisture left after the standard drying procedure with nitrogen gas (described above). Accordingly, samples that had been dried for 2 h under nitrogen gas were subsequently dried at 35°C and 60 mtorr for 10 h in an FTS DuraStop® lyophilizer (Stone Ridge, New York). The chamber pressure was increased to 0.5 atm prior to stoppering. No significant changes in sample consistency or appearance were evident even after prolonged storage at high humidity.

Determination of Moisture Content

Gold/DNA samples were immediately capped with a rubber stopper after removal from storage to prevent any changes in water content. After removal from storage, 1 mL DMF was added with a syringe through the rubber stopper to each 300 mg gold/DNA sample to extract bound water. Following sonication, DMF and dissolved water were removed for analysis by Karl Fisher titration using a Mettler DL37 coulometric moisture analyzer (Hightstown, New Jersey) containing pyridine-free vessel solutions (Photovolt Instruments, Inc., St. Louis Park, Minnesota). Blank "dummy vials" were used to determine the background water content in the DMF and headspace gas; this value was subtracted from the readings to determine the moisture contents as reported here. More detailed information on Karl Fischer titration can be found in Ruiz 2001.[36]

DNA Extraction from Gold/DNA Samples

To assess DNA recovery, each 100 mg gold/DNA sample was rehydrated with 150 μL of 10 mM Tris-HCl buffer (pH 7.8). Samples were placed in a 37°C water bath for 30 min, and vortexed at 10-min intervals. Samples were then centrifuged at 1500 RPM for 1 min. The concentration of DNA in the supernatant was determined by absorbance at 260 nm on a UV spectrophotometer (Agilent 8453 UV-visible Spectroscopy System). Increased rehydration buffer volumes and rehydration times did not affect the amount of DNA recovered. Further, multiple washes failed to remove additional DNA, suggesting that our procedure is sufficient to extract DNA that is reversibly associated with the gold microparticles.

Determining Percent Initial Supercoil Content

Supercoil content was analyzed by agarose gel electrophoresis. The agarose gel (0.9%) contained EtBr for DNA detection. To each lane, 200 ng sample DNA or control DNA was added. The control (initial) DNA was stored at −80°C prior to use, and possessed a supercoil content of approximately 80%. The agarose gel electrophoresis was run at 60V for 3 h, and subsequently fluorescently imaged on a Molecular Imager® Gel Doc™ XR+ Imaging System from BioRad, and the staining intensity was used to quantify supercoil content. Because a constant amount of DNA (200 ng) was loaded onto each lane, percent initial supercoil content was determined by comparing the supercoil band intensity of samples to control (initial) supercoil band intensity.

RESULTS

We initially observed changes in supercoil content during storage and suspected that free radicals formed during storage may contribute to the observed loss of DNA integrity. Studies have shown that free radicals are capable of promoting DNA single and double strand breaks, likely generated by contaminating transition metals (e.g. Fe^{2+} and Cu^{2+}) found in the formulation.[37–41] Our previous studies have demonstrated the ability of alpha tocopherol to reduce free radical-mediated DNA damage during storage,[42] and thus we attempted to incorporate alpha tocopherol into the PMED formulations. This approach proved to be of minimal benefit, suggesting that free radicals are not significantly contributing to the loss of DNA supercoil content under our conditions (data not shown). Additionally, we investigated the role of molecular oxygen in the stability of PMED formulations to determine if reactive oxygen species (ROS) might be involved in the degradation observed during storage. For these studies, dried samples were stored in sealed vials in which the headspace gas was replaced by nitrogen or argon prior to storage. As with the samples formulated with alpha tocopherol, removal of molecular oxygen had no effect on storage stability (data not shown).

Previous studies with dried DNA, proteins, and vaccines have demonstrated the ability of residual moisture to modulate stability during drying and storage.[43–46] This approach is especially desirable

because it does not involve the use of additional excipients that further complicate the formulation. To study the effects of moisture content on gold/DNA preparations, samples were stored uncapped in desiccators as depicted in Figure 1. Desiccators contained desiccant or saturated salt solutions to control sample moisture content during storage. Saturated salt solutions maintain the RH in the desiccator within a predictable, narrow range,[34] whereas desiccants reduce the RH to undetectable levels (< 3%).

The ability of the different moisture controlling agents to affect the moisture content of samples was analyzed during storage. Preliminary experiments determined that water contents stabilize after approximately 2 weeks under our conditions (data not shown). As shown in Figure 2, water contents after the initial drying procedure (day 0) were comparable to samples stored for 6 months at 30% RH. As would be expected, samples stored at lower RH (11%) exhibited reduced water contents after storage, and samples stored at higher RHs contained greater levels of moisture that further increased after 2 weeks (Fig. 2).

To assess the ability of formulations to release DNA from the gold particles, samples were incubated at 37°C in 10 mM Tris-HCl buffer, pH 7.8 as described in the methods section. As can be seen in Figure 3a, the extent of DNA removal is significantly affected by RH during storage. Greater quantities of DNA were extractable from samples stored at higher RHs, and extraction of samples stored at 60% RH yielded quantities of DNA comparable to the total amount of DNA used to prepare the samples. This indicates that in

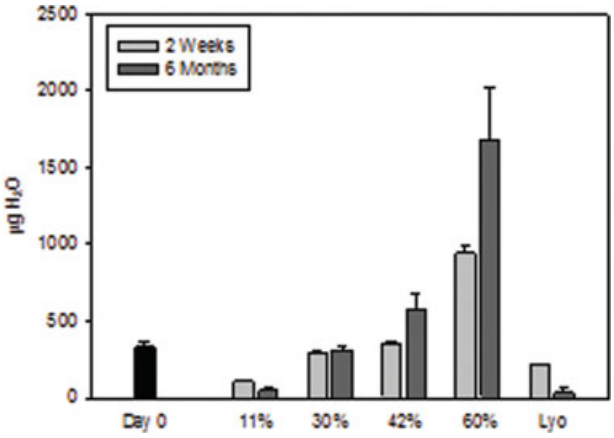

Figure 2. Water contents of 300 mg gold/DNA samples stored at 60°C, analyzed at 2 weeks and 6 months. Samples were stored at 11%, 30%, 42%, and 60% relative humidities within desiccators. A set of samples (Lyo) was prepared identically to the other samples but lyophilized before being stoppered and stored. Day 0 represents moisture content prior to storage. Each bar represents the mean ± 1 SE of triplicate formulations.

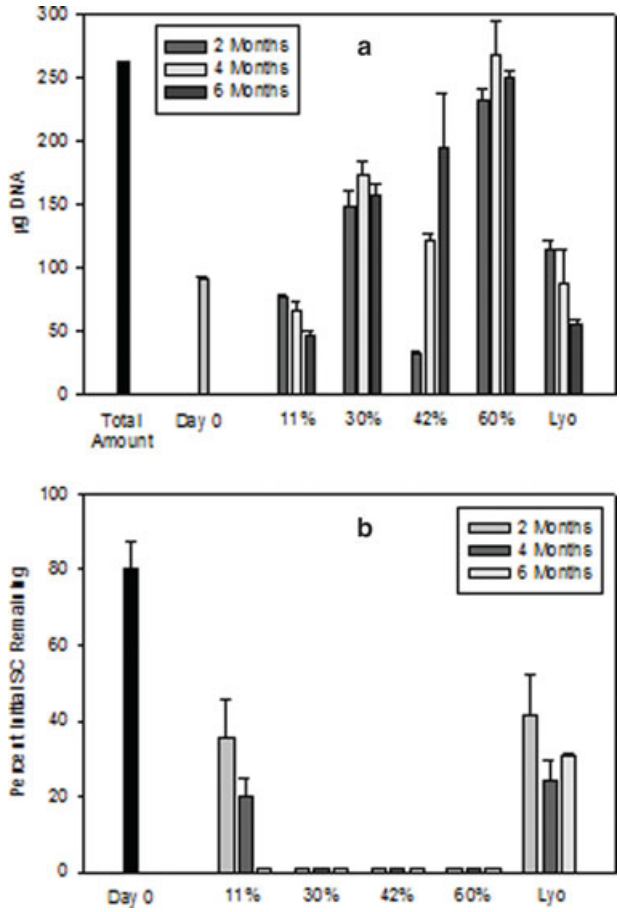

Figure 3. Gold/DNA samples (100 mg) were stored at 60°C at varying relative humidities (11%, 30%, 42%, and 60%) within desiccators. A set of samples (Lyo) was lyophilized before being stoppered and stored. Samples were analyzed at 2, 4, and 6 months. (a) Effect of sample storage condition on DNA extraction. Day 0 represents extraction prior to storage. The total amount of DNA is based on the amount of DNA added during sample preparation. (b) Effect of relative humidity during storage on DNA integrity. Day 0 represents supercoil content prior to storage. Each bar represents the mean ± 1 SE of triplicate formulations.

samples stored at high RHs, the majority of the added DNA dissociates from the gold surface upon exposure to an aqueous environment. Samples stored at 42%, 30%, and 11% RHs had progressively reduced DNA recovery, with 11% yielding the lowest DNA recovery. Interestingly, the amount of DNA extracted from lyophilized samples and samples stored at 11% RH was comparable to day 0 samples (Fig. 3a).

The supercoil content of the extracted DNA also exhibited dramatic differences depending on the moisture content of the samples during storage (Fig. 3b). Sample storage at high RHs (30%, 42%, and 60%) allowed for considerable DNA recovery (Fig. 3a); however, the DNA extracted from these samples was completely degraded (no supercoiled DNA remained)

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after 2 months storage. In contrast, supercoiled DNA was present in preparations stored at 11% RH after 4 months, and lyophilized samples had considerable quantities of supercoiled DNA remaining after 6 months storage (Fig. 3b).

The results above suggest that there is a tradeoff between the amount of DNA that is liberated in an aqueous environment, and the quality of the extracted DNA. Ideally, a PMED formulation would release large quantities of fully intact DNA, but our results show a negative correlation between these two parameters. With the goal of preserving DNA integrity, we further investigated the stabilizing effect of low moisture content by conducting additional experiments at 2%, 6%, and 11% RHs. As seen in Figure 4a, the quantity of DNA that is extracted from formulations stored at low RHs was very consistent both between and across samples. Unlike samples stored at 11% RH, appreciable supercoil content is found in both 2% and 6% samples after 4 months storage demonstrating the beneficial effect of low moisture content on DNA stability (Fig. 4b).

The data presented above clearly demonstrate that reduced sample moisture content confers significant sample stability with regards to the maintenance of DNA integrity. Therefore, it is reasonable to assume that storage at the very low RHs, to achieve low moisture contents, would have beneficial effects on DNA stability. Not surprisingly, samples stored in the presence of Drierite obtained very low moisture contents within 2 weeks (Fig. 5a), however, both the amount of DNA extracted and its integrity was severely compromised under these conditions after only 1 week of storage (Figure 5b and 5c). After 2 months, no supercoiled DNA was observed, and no DNA was extractable from these very dry samples after 4 months. It is noteworthy that these experiments included some "indicating" Drierite (2% $CoCl_2$) that changes from blue to pink as water is absorbed; no color change was detected despite the measured decrease in sample water content due to the small amount of sample relative to that of Drierite.

Previous studies have also reported decreased stability in the presence of desiccant[29] which could be attributed to the effect of overdrying.[43,44,47,48] Alternatively, one report suggested that a physical interaction (i.e., "dusting") with the desiccant may contribute to the decreased stability observed under these conditions.[28] To distinguish between these two possibilities, samples were stored with Drierite ("nonindicating") packaged within impermeable bags (coated crepe paper) to prevent particulates from interacting with the dried gold/DNA samples. In addition to bagged Drierite, we also tested the effect of storing samples with other desiccants (silica gel, bagged silica gel, and phosphorus pentoxide). In contrast to the negative effects of desiccant observed in

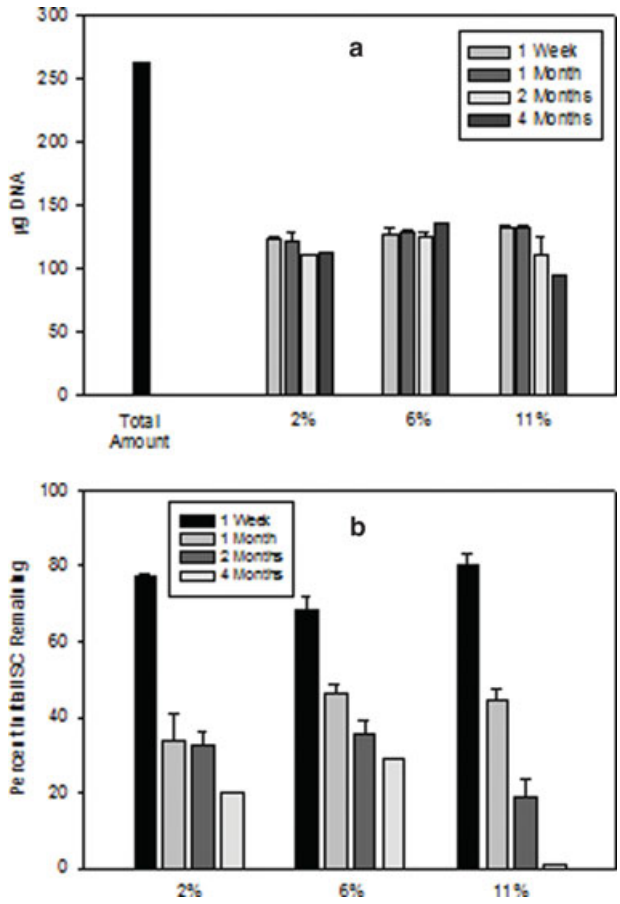

Figure 4. Gold/DNA samples (100 mg) were stored at 60°C at varying relative humidities (2%, 6%, and 11%) within desiccators. Samples were stored and analyzed at 1 week and 1, 2, and 4 months. (a) Effect of low relative humidity storage on DNA extraction. The total amount of DNA is based on the amount of DNA added during sample preparation. (b) Effect of low relative humidity storage on supercoil content. Each bar represents the mean ± 1 SE of triplicate formulations with the exception of the 4-month data that are an average of 2 with no more than 6% difference between duplicate samples.

Figure 5, levels of extractable DNA (Fig. 6a) and supercoil content (Fig. 6b) were comparable to that observed in samples stored at 6% RH. Taken together, the data in Figures 6a and 6b suggest that the use of very low RHs (as experienced under desiccant) does not compromise the stability of these samples. Instead, these results suggest that there may be something specific about the storage conditions used in the experiment depicted in Figure 5 (i.e., "indicating" Drierite not contained within a bag) that contributes to the reduced stability observed under these conditions.

To elucidate the detrimental effect of "indicating" Drierite, gold/DNA samples were stored in the presence of unbagged Drierite and "indicating" Drierite. In addition, "indicating" Drierite was packaged in

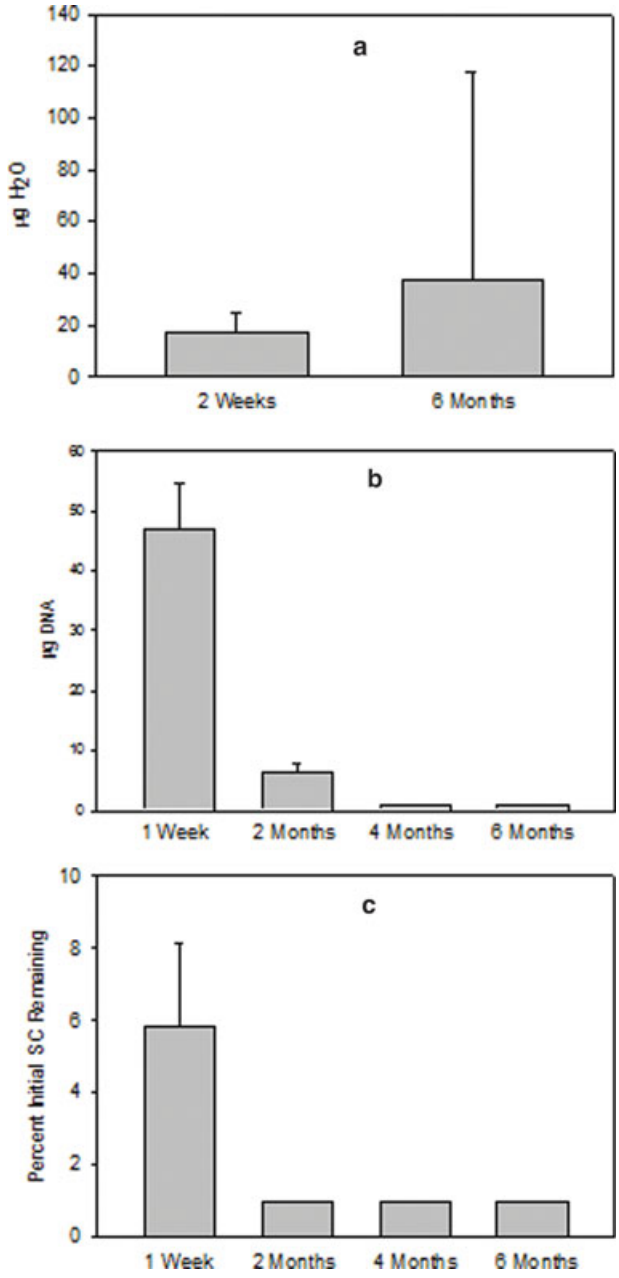

Figure 5. Effect of storage in the presence of "indicating" Drierite at 60°C. (a) Water contents of 300 mg gold/DNA samples analyzed at 2 weeks and 6 months. (b) DNA extracted from gold/DNA samples (100 mg) analyzed at 1 week and 2, 4, and 6 months. (C) Percent initial supercoil content of gold/DNA samples (100 mg) analyzed at 1 week and 2, 4, and 6 months. Each bar represents the mean ± 1 SE of triplicate formulations.

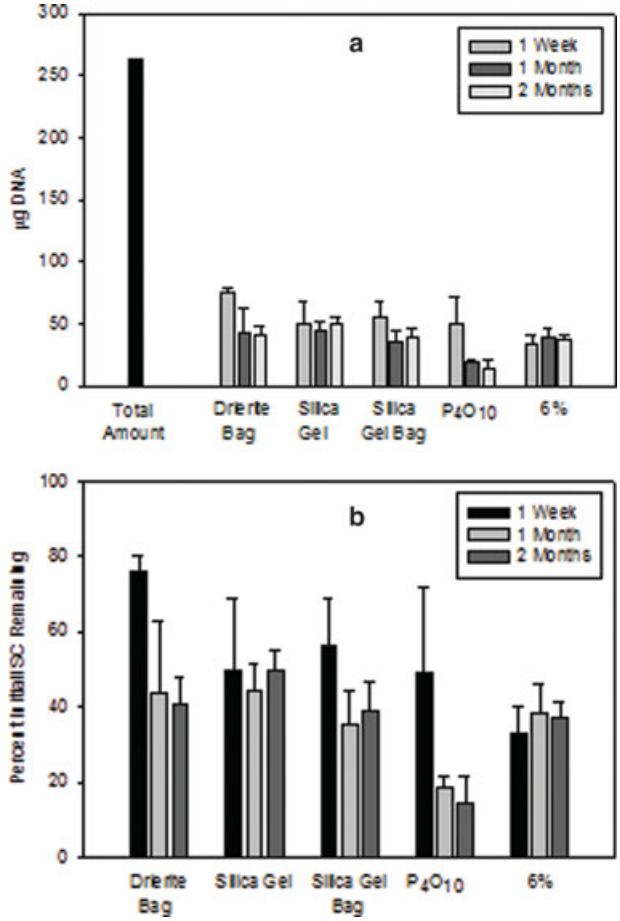

Figure 6. Gold/DNA (100 mg) samples were stored in the presence of bagged Drierite (no indicating $CoCl_2$), silica gel, bagged silica gel, and P_4O_{10} at 60°C. One set of samples was stored at 6% relative humidity for comparison to prior experiments. (a) Effect of sample storage with bagged and "free" desiccants on DNA recovery. The total amount of DNA is based on the total amount of DNA added during sample preparation. (b) Effect of sample storage with bagged and "free" desiccants on sample supercoil content. Each bar represents the mean ± 1 SE of triplicate formulations.

"nondusting" bags to eliminate any potential airborne desiccant particles from contacting the samples. The results in Figures 7a and 7b clearly demonstrate the detrimental impact of "indicating" Drierite, presumably due to the presence of $CoCl_2$. Containment of the "indicating" Drierite within bags improved both the amount of extractable DNA and its supercoil content, but the detrimental effect of $CoCl_2$ is still evident when compared with samples stored with "free" Drierite lacking the indicating dye. Moreover, samples stored with "free" Drierite lacking $CoCl_2$ maintained greater than 70% supercoil content after 1 month storage at 60°C (Fig. 7b).

DISCUSSION

As mentioned above, PMED formulations deliver DNA directly to the cytoplasm, and therefore the ability of the cell to express the encoded antigens will depend on the release of DNA from the gold particle which allows nuclear entry and ultimately gene expression. In addition, the integrity of the DNA is critical, and supercoil content is the standard

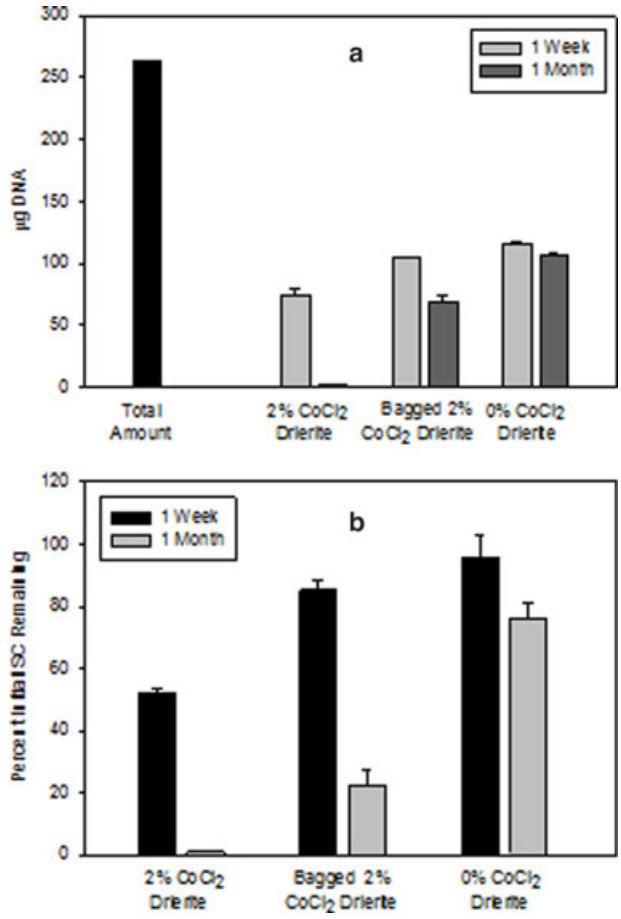

Figure 7. Gold/DNA samples (100 mg) were stored in the presence of "indicating" Drierite (containing 2% $CoCl_2$), bagged "indicating" Drierite, and Drierite lacking $CoCl_2$ at 60°C. (a) Effect of storage with "indicating" Drierite on DNA recovery. The total amount of DNA is based on the amount of DNA added during sample preparation. (b) Effect of storage with "indicating" Drierite on DNA supercoil content. Each bar represents the mean ± 1 SE of triplicate formulations.

measure of plasmid quality. The importance of maintaining supercoil content is clear from *in vitro* transfection experiments demonstrating the superior ability of supercoiled DNA to transfect cultured cells in comparison to open circle and linear DNA.[21] More recently, a DNA-based rabies vaccine in cats produced detectable antibodies and a specific Th-1 response when at least 70% of the DNA was in the supercoil conformation. Contrastingly, vaccination with 20% of the DNA in the supercoiled form only provided partial protection and failed to produce a specific immune response.[22] A similar correlation is found with lyophilized lipoplexes, where supercoil loss negatively affects transfection efficiency.[37,49,50] These studies demonstrate that loss of the supercoiled structure during processing and/or storage may have negative impacts on the ability of PMED of DNA to elicit an immune response.

Our initial experiments focused on preventing oxidation reactions that may contribute to the loss of DNA integrity observed during storage. However, neither incorporating alpha tocopherol nor storage in the absence of molecular oxygen had significant effects on stability, suggesting that oxidation reactions on the surface of the gold particle are not primarily responsible for DNA degradation. Since water content in the dried cake can affect the stability of dried pharmaceuticals,[44] we investigated the effect of residual moisture on DNA recovery and integrity. Not only does water act as a chemical reagent, it facilitates molecular motion by acting as a plasticizer. Moisture removal combined with decreases in temperature significantly decrease molecular mobility[51] which is thought to attenuate chemical reactions and enhance preservation.[52] Furthermore, increases in the water content of amorphous pharmaceutical samples have been shown to decrease sample stability, induce aggregation,[53–55] and lower the Tg of the formulation.[56]

It is important to realize that the determination of water content in terms of a percent (g H_2O/100 g dry weight) is complicated in PMED formulations because the vast majority (> 99%) of the sample weight is due to the gold particles. Therefore, one must assume that any moisture in the sample is adsorbed on the surface, and involved in the hydration of the adsorbed material (i.e., DNA, EDTA, trehalose, and tetra-arginine). It follows that even though the calculated moisture content for the entire sample is well below 1%, the relevant water content experienced by the surface-adsorbed DNA may be as high as 20%. Because of the difficulty in determining the mass of the adsorbed layer, our water contents are expressed as μg H_2O per 300 mg sample (Fig. 2).

In order for DNA delivered by PMED to elicit an immune response, it must dissociate from the gold microparticle after delivery into the cell.[18,32,57] Our data indicate that water adsorbed to the surface of gold particles not only facilitates DNA extraction, but also aids in DNA degradative processes. The increased water content of the formulations stored in high RHs would be expected to increase the hydration of the adsorbed DNA, at least to some extent. Thus, our data suggest that increased DNA hydration may facilitate dissociation from the particle and degradation (Fig. 3). It is unclear whether degraded DNA is more readily dissociated from the particle and/or if DNA that readily dissociates is more susceptible to degradation. When water is removed from gold/DNA samples by storage at low RHs (<6%) or with desiccant, DNA extraction is poor, yet high initial supercoiled content remains (Figure 4 and 6).

The increased stability of samples stored in low moisture conditions suggests that samples are not deleteriously affected by overdrying. It has been

suggested that overdrying leads to oxidative damage due to the removal of a protective water layer believed to exclude oxygen molecules from coming into contact with the surface of macromolecules.[44,47,48] The fact that we do not observe enhanced damage at very low RHs is consistent with our initial studies showing that oxidative damage does not seriously impact the DNA stability in PMED formulations. Furthermore, the incomplete DNA recovery that we observed is attributed to DNA remaining adsorbed to gold during extraction and failing to dissolve into the elution buffer. Similarly, Medley et al. using a comparable gold/DNA formulation found incomplete DNA extraction, although to a lesser extent.[31] The cause of the poor recovery is not clear, however, poor recovery may impact the clinical efficacy of the PMED formulation.

Storage of formulations over desiccant (a common practice) proved problematic when "indicating" Drierite was used, and we observed minimal DNA extraction and a complete loss of supercoil content under these conditions (Fig. 5). The detrimental effects of "indicating" Drierite were partially alleviated when the desiccant was confined within bags, suggesting that "dusting" from the "indicating" Drierite may contribute to DNA degradation. A similar observation was recently reported by Bonnet et al.[28] who concluded that rapid degradation in the presence of phosphorus pentoxide was due to "dust."[28] In contrast, samples stored with free "nonindicating" Drierite or silica gel had substantial DNA recovery and supercoil content after 1 month storage at 60°C (Fig. 6). A direct comparison of "indicating" (free and bagged) with "nonindicating" Drierite suggests that the indicating dye, $CoCl_2$, may accelerate degradation during storage regardless of any effects of dusting (Fig. 7).[49] Although transition metals (e.g., Co, Fe) are known to promote DNA degradation through free radical-mediated reduction of oxygen[58,59]; further experiments would be required to confirm any direct role of $CoCl_2$ in the observed degradation. Similarly, we report that storage with phosphorous pentoxide results in loss of DNA recovery and supercoil content during storage, although to a lesser extent than is observed with "indicating" Drierite. Although dusting may contribute to degradation in our experiments with phosphorus pentoxide, Lindahl proposed that phosphorus pentoxide removes water molecules required for DNA to maintain its double helical conformation, and suggested that the loss of double helical structure could promote oxidation reactions and lead to poor stability during storage.[29]

In conclusion, our data indicate that the storage stability of PMED formulations consisting of DNA adsorbed to gold particles can be dramatically enhanced by reducing the water content of samples to the lowest possible level. We believe that the presence of water on the gold surface acts as a plasticizer to facilitate chemical reactions, and potentially may directly participate in hydrolytic reactions within the adsorbed layer. These results suggest that formulations for PMED should be stored as dry as possible, while avoiding use of the "indicating" Drierite and phosphorous pentoxide.

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