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Quantitation of plasmid DNA deposited on gold particles for particle-mediated epidermal delivery using ICP-MS

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Abstract DNA-plasmid-based vaccines are a promising class of next generation therapeutics. Particle-mediated epidermal delivery is an attractive method for the administration of DNA plasmid vaccines. This technology utilizes minute quantities of DNA plasmid which have been deposited onto the surface of 2–3-µm gold particles, and so the development of this technology requires the use of analytical methods that can accurately quantitate the amount of the DNA on the particle. Spectroscopic methods are generally insufficient for this task due to interference from the gold particle. ICP-MS circumvents this issue while allowing for the sensitive, reproducible, and accurate determination of the quantity of DNA on the particle surface. This report will detail the development and application of such a method.

Keywords ICP-MS · Nucleic acids

Introduction

Vaccines typically function by using a molecular mimicry trick. A vaccine will typically contain an antigen which is

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similar to that of a disease-causing agent. After administration of the vaccine, the body mounts an immune response against the antigen, which primes the immune system to deal with the actual disease-causing agent whether it is bacteria, virus, or even a cancer cell, whenever it would be encountered. With DNA plasmid vaccines, the antigen itself is not directly introduced into the body. Rather, a gene encoding for the antigen is engineered into a self expressing plasmid vector, and the plasmid is introduced into the body. Once inside the body, the body's own cellular protein synthesis machinery expresses the gene coding for the antigen on the plasmid, the antigen is produced, and the body then mounts an immune response against the antigen [1].

DNA vaccines have many potential advantages over more traditional antigen-containing vaccines. One is that a DNA vaccine can generate an antigen that more closely resembles the native disease related epitopes. This is because live attenuated and dead virus vaccines may possess altered protein structure as a result of the way they are manufactured, for example, through formaldehyde inactivation of toxins. The use of DNA plasmids also allows the development of vaccines without using the actual infectious agents, increasing the safety of vaccine development [2]. Also, DNA plasmid vaccines can be produced rapidly, in some instances with antigens that are difficult to produce using other more traditional methods, such as the production of flu antigens in eggs [3, 4]. As plasmids encoding several proteins can be administered simultaneously, multi-valent vaccines can be developed to improve the efficacy of the vaccine [5–9]. DNA vaccines can also be rapidly manufactured in large scale compared to traditional vaccines giving these vaccines the ability to be available more quickly in epidemics. All of these features make DNA very attractive as a vaccine technology platform.

Despite these advantages, one challenge that still remains for DNA vaccines is the delivery of the plasmid into body in a way that provides a sufficient immune response. Such a response requires that the plasmids ultimately become localized in the nuclei of cells, since the nucleus is where plasmids are expressed in eukaryotic cells. Plasmids are large molecules, and a cell's cytoplasmic and nuclear membranes represent barriers to delivery of plasmids to the nuclei. One promising mechanism to deliver DNA vaccines is the use of particle-mediated epidermal delivery (PMED) [10–13]. In PMED, the DNA plasmid is deposited onto a gold particle, and the gold particles are then accelerated through the skin using a compressed gas. Some of the particles come to rest in the nuclei of cells, and the plasmids then elute off the particles and are expressed. The advantage of PMED for delivering plasmid DNA vaccines is evident in that microgram quantities of plasmid can achieve strong immune responses, whereas much larger quantities of plasmid are typically needed to elicit a similar immune response using intramuscular injection [14–18]. PMED also has the advantage of being a rapid and needle-less delivery method [19, 20].

The use of plasmid DNA-based vaccines for pharmaceutical therapies requires effective and robust methodologies for analyzing both the plasmid and the content of DNA on the gold particle. Detection and quantitation of DNA tends to focus on spectroscopic techniques like UV–vis absorbance and fluorescence [21, 22]. Typically, for pure samples of DNA plasmids, UV absorbance provides an accurate determination of concentration. Fluorescent probes and labels are typically used for detection in extremely complex environments or when multiple sequences are present such as in serum or inside living cells [23, 24]. While gold particle surfaces are much simpler in comparison, the spectral properties of gold particles make any spectroscopic quantitation method for adsorbed macromolecules or excipients exceedingly difficult. Gold particles have broad absorbance spectra and large extinction coefficients [25] that obscure UV absorbance measurements for DNA. In addition, the excellent quenching efficiency of gold particles inhibits fluorescence measurements on their surface [26]. The solution to these issues would be to remove the gold particle through centrifugation after eluting off the DNA plasmid. This method requires the complete recovery of the DNA from gold particle surface which is difficult to achieve reproducibly while keeping the plasmid intact for UV-based measurements. Therefore a method that can quantitate DNA in the presence of the gold particle and is compatible with degraded DNA from a harsh elution would allow for a more sensitive, accurate, and reproducible method for DNA quantitation.

In order to detect plasmid DNA in the presence of the gold particle, non-spectroscopic methods need to be employed. The technique also needs to possess excellent accuracy, reproducibility, and a low limit of detection in order to be an effective quantitation assay. One technique in particular that has the capability to demonstrate these requirements is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS introduces the sample into the mass analyzer through an ICP torch. The ICP atomizes and ionizes the samples yielding the elemental composition. The use of an internal standard minimizes matrix effects and drift in the samples allowing for the detection of elements with excellent repeatability and sensitivity. Due to these advantages, ICP-MS has been used for many applications of trace metal analysis [27–29]. More recently, ICP-MS has been used in the biological realm by detecting DNA [30–35] and metalloproteins [36, 37]. Applying ICP-MS to DNA-formulated gold particles enables the quantitation of the DNA plasmid with sensitivity, accuracy, and reproducibility not possible with other methods. ICP-MS is effective in this analysis as the phosphorus in the DNA nucleotides is the only source of phosphorus in the samples. Therefore, the phosphorus content is proportional to the amount of plasmid DNA present on the particle. ICP-MS can be used to accurately quantitate the amount of phosphorus present in the sample which can be interpolated back to the amount of DNA present in the sample. Contrary to spectroscopic techniques, the gold particle does not interfere with the phosphorus signal on the ICP-MS allowing for the detection and quantitation of DNA in the presence of the gold particle. The results presented herein will show that the ICP-MS method developed for quantitating plasmid DNA deposited on gold particles demonstrates excellent sensitivity, reproducibility, and accuracy.

Experimental

Formulation of plasmid DNA onto gold particles

Plasmid DNA (5.4 kbp, >90% supercoil) is condensed with a polycation and ethanol precipitated on to 2–3 μm gold particle (from Ferro GMBH, Germany) along with EDTA and trehalose as chelator and stabilizer, respectively [38]. The formulated gold particles are nitrogen dried to yield a gold powder and stored in desiccated pouches at 5 °C. Formulated gold particles with plasmid DNA concentrations ranging from 2 to 12 μg per mg of gold powder were produced using the same excipients at different volumes maintaining the same stoichiometric ratios.

Sample preparation for UV absorbance measurements

For UV–vis samples, gold powder was weighed out and then transferred to a 15 mL conical centrifuge tube. Then, 10 mL of elution buffer consisting of 10 mM EDTA (Sigma-Aldrich, St. Louis, MO) in pH 7.4 phosphate-buffered saline (Gibco,

Invitrogen, Carlsbad, CA) was added to the centrifuge tube. The samples were then vortexed for 30 s and sonicated for 60 s. Next, the samples were incubated in the elution buffer for 4 h followed by further vortexing and sonication. Following these steps, the samples were centrifuged at 4,500 rpm for 10 min. The supernatant was transferred to a 0.7 mL Quartz Cuvette (VWR International) and the absorbance was measured using a Beckman DU800 Spectrophotometer.

Sample preparation for fluorescence measurements

For fluorescence samples, gold powder was weighed and then transferred to a 15 mL conical centrifuge tube. Then, 10 mL of elution buffer consisting of 10 mM EDTA (Sigma-Aldrich, St. Louis, MO) in pH 7.4 phosphate-buffered saline (Gibco, Invitrogen, Carlsbad, CA) was added to the centrifuge tube. The samples were then vortexed for 30 s and sonicated for 60 s. The samples were then incubated in the elution buffer for 4 h followed by another vortex and sonication step. The samples were then centrifuged at 4,500 rpm for 10 min. The 1 mL of supernatant was then removed from the tube and transferred to a microcentrifuge tube. SYBR Gold (Invitrogen, Carlsbad, CA) was then prepared via a 1,000-fold dilution. The SYBR Gold solution was used to label the samples and a calibration curve. The calibration curve was made using 1 mL, 500 μL, 250 μL, 125 μL, 100 μL, and 50 μL of 1.0 mg/mL plasmid DNA diluted to 100 mL with Milli-Q ultrapure water. Fluorescence measurements were made using Varian Cary Eclipse Fluorescence Spectrophotometer using 495 nm for excitation and monitoring 540 nm for emission. Concentrations were interpolated from the calibration curve.

ICP-MS preparation for gold powder samples

For each gold powder sample, 50 mg were weighed in a conical centrifuge tube. 1 mL of ultrapure nitric acid (Fluka TraceSelect Ultra 02650) was then added to each sample followed by vortexing for 30 s. The samples were left on an orbital shaker for 4 h. Following the 4 h, the samples were centrifuged at 4,000 rpm for 15 min and the supernatant was quantitatively transferred to a 100 mL graduated cylinder. After transferring the supernatant, 1 mL of 10 μg/mL scandium internal standard (VWR International 82026-114) was added to the graduated cylinder and Milli-Q water was added to reach 100 mL. The sample was then mixed thoroughly and transferred to 50 mL conical tubes for ICP-MS analysis.

ICP-MS preparation for DNA plasmid solution samples

For each DNA plasmid solution sample, 100 μL of plasmid DNA were added to a 15 mL conical tube. A 100-μL

volume of ultrapure nitric acid was then added to the tube and vortexed for 30 s. After vortexing the plasmid DNA was incubated with the nitric acid for 4 h. Subsequently, the solution was transferred to a 10-mL graduated cylinder and 100 μL of internal standard (VWR International 82026-114) were added. The solution was then diluted to 10 mL with Milli-Q water and transferred to a 15-mL conical tube for ICP-MS analysis.

ICP-MS analysis

For all ICP-MS samples, an Agilent 7500 ICP-MS with Agilent ASX-500 Series Autosampler was used for analysis. The instrument was run in no-gas mode monitoring m/z 45 for scandium and 31 for phosphorus taking six repeat scans at each m/z. The injector was rinsed for 90 s between samples to minimize carryover. Prior to analyzing samples, the instrument was tuned using 1 PPB tuning solution (Agilent Technologies 5185-5959). The raw data was then exported to Microsoft Excel for analysis.

ICP-MS calibration curve

For all ICP-MS samples, DNA was quantitated using a calibration curve and analyzed in sequence with the test samples. The calibration curve was prepared by incubating 1 mL, 500 μL, 250 μL, 125 μL, 100 μL, and 50 μL of 1.0 mg/mL plasmid DNA solution with 1 mL of concentrated nitric acid for 4 h. After the incubation, 100 μL of internal standard was added and the solution was diluted to 100 mL with Milli-Q ultrapure water. After the samples were analyzed an equation for the calibration curve was generated by Microsoft Excel and used to interpolate the DNA concentration of the other solutions.

Results and discussion

The first experiments performed were to test the feasibility of using ICP-MS to quantitate the amount of DNA on the gold particles. The goal of these experiments was to demonstrate that the phosphorus from the plasmid DNA was capable of yielding a suitably strong phosphorus signal to detect and that the signal emanates from the DNA and not from the other components of the analysis or compound. The DNA can be visualized on the surface of the particle using SEM in which the DNA appears as a darker region on the surface of the particle. Unformulated and formulated gold particles can be seen in the SEM images in Fig. 1a and c, respectively. When the magnification is increased dark patches of DNA can be seen on the formulated gold particle surface while similar patches are absent on the unformulated particles in Fig. 1b and d, respectively. In order to determine

Fig. 1 Scanning Electron
Micrographs of bare gold
particles (**a**, **b**) and gold
particles formulated with
plasmid DNA (**c**, **d**) at two
different magnifications
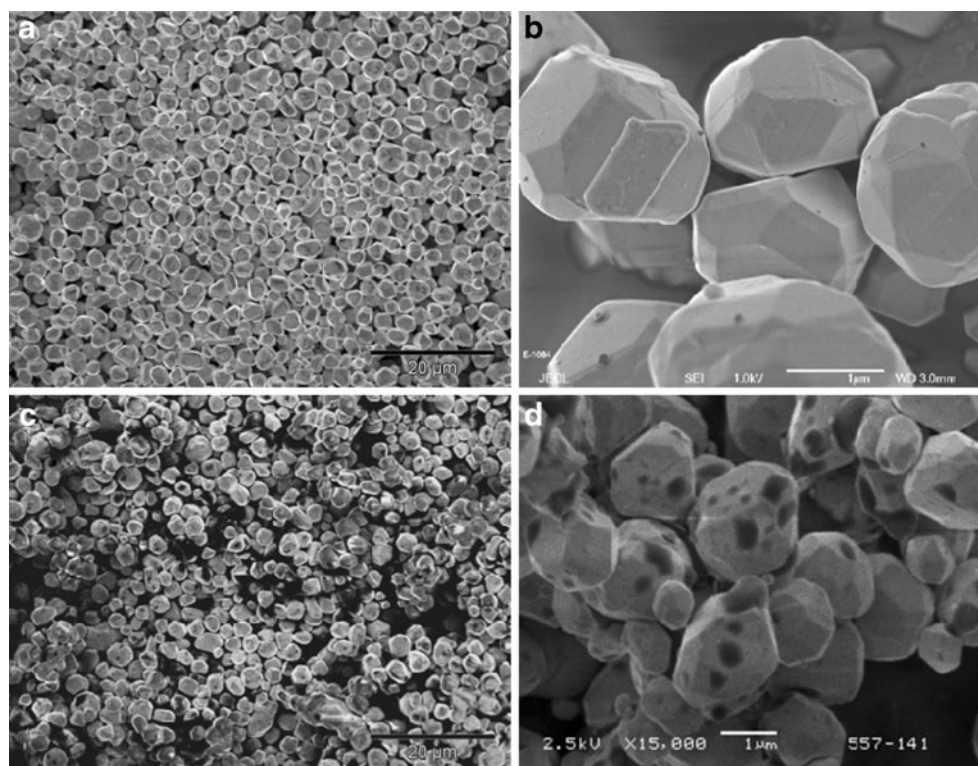
whether ICP-MS could be used to quantitate the DNA, testing involved not only DNA plasmid in solution and DNA-formulated gold powder, but also unformulated and processed gold powder to demonstrate that the phosphorus signal is from the DNA backbone and not from impurities in the gold. In these samples, raw gold refers to the gold particles prior to any steps to deposit DNA on their surface, while the processed gold has gone through all of the deposition procedures with excipients without the addition of the DNA to the matrix. Additionally, Milli-Q water and the ultrapure nitric acid were also analyzed to determine the background levels present from the preparation. For all samples involving gold particles, the gold powder sample preparation was followed. All other samples utilized the liquid plasmid DNA sample preparation, except the Milli-Q water was analyzed in the presence of only the internal standard. Each sample was prepared in triplicate and the phosphorus signals for each sample were normalized against the scandium signal from the internal standard and averaged for each sample. The average ratios and their standard deviations were plotted in Fig. 2.

As the results in Fig. 2 show, the DNA plasmid solution, the formulated gold particles, and the P(31) standard all exhibited a strong phosphorus signal. In addition, the raw and processed gold particles did not exhibit a phosphorus signal above the background signal from the nitric acid indicating that they are not a source of the phosphorus measured during this experiment. The Milli-Q water sample possessed the smallest amount of phosphorus as expected

while the nitric acid did possess a small phosphorus signal. This indicates that the background levels of phosphorus in the experiment are primarily from the ultrapure nitric acid; however, the levels are low enough that they do not interfere with the analysis of the DNA plasmid solution or the formulated gold particles. Similarly, isobaric interferences such as $^{15}N^{16}O^+$, $^{14}N^{16}O^1H^+$, or $^{12}C^1H_3^{16}O^+$ present in the nitric acid or processed or raw gold are also at sufficiently low levels as to not affect the results. It is possible that these interferences could be generated during the digestion of the DNA however this possibility will be
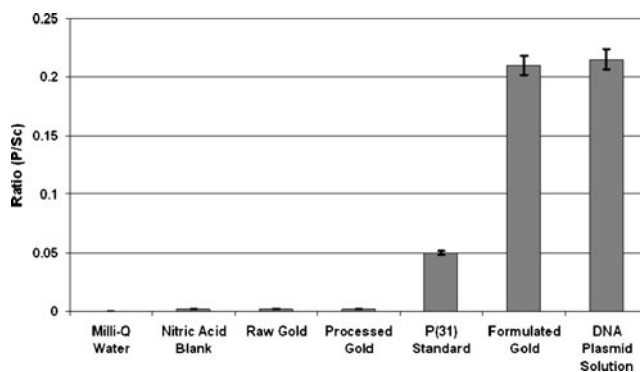

Fig. 2 The phosphorus to scandium ratios for various samples and materials related to the plasmid DNA quantitation assay. Milli-Q water, the nitric acid, unmodified gold particles, and gold particles treated with the formulation excipients all demonstrate a negligible phosphorus signal while the P(31) elemental standard, DNA-formulated gold particles, and the DNA plasmid solution all exhibit a significant phosphorus signal

addressed in the linearity section. Based on these results, the phosphorus signals from the DNA-formulated gold particles and the DNA plasmid solution samples must result from the phosphodiester backbone of the DNA as all other sources have been accounted for in the other samples. The results indicate that ICP-MS is capable of measuring the phosphorus content in the plasmid DNA thereby allowing the detection of DNA in both the plasmid solution and deposited on the surface of gold particles.

Optimization of sample preparation

After optimizing the DNA–nitric acid incubation time and the sample amounts (data not shown), it was critical to evaluate and select the acid used to digest the DNA deposited on the gold particle surface. Two different solutions were used one containing only ultrapure nitric acid and the other containing ultrapure nitric and hydrochloric acids. The mixture of nitric and hydrochloric acids completely dissolves the gold particle in addition to digesting the DNA, while the nitric acid only digests the DNA and a small portion of the gold particle. The complete digestion of the gold particles provides two distinct advantages. First, it eliminates the need to remove the gold particles from the samples and second it reduces the sample preparation time and labor. In addition, one of the major obstacles in using UV–vis to quantitate the DNA amount of the particles is removing all of the DNA from the particles. Completely dissolving the particles in acid ensures all of deposited DNA can be recovered. Despite these advantages, two more practical disadvantages were encountered when preparing samples using this methodology. The first disadvantage was the suppression of the internal standard and phosphorus ions in the analysis. When plotting the response of the internal standard versus the amount of gold powder prepared illuminates this issue. For this experiment, increasing amounts of DNA-formulated gold powder were digested and the scandium (45 amu) and phosphorus (31 amu) signals were analyzed independently versus the amount of gold powder. This data is shown in Fig. 3.

As demonstrated in Fig. 3, the response from the internal standard drops in intensity as increasing amounts of gold powder is used in the analysis. The gold powder is completely dissolved in each sample resulting in an increase of gold ions increases proportionally with the weight of gold powder. The effect on the internal standard signal is likely due to the increase of gold ions in solution suppressing the ionization of the scandium internal standard. The R^2 of 0.9992 for the line generated from the data shows the ion suppression has a linear response versus the gold concentration in solution with an approximately 40% drop in the intensity of the internal standard at the highest gold concentration tested. The response of the phosphorus signal
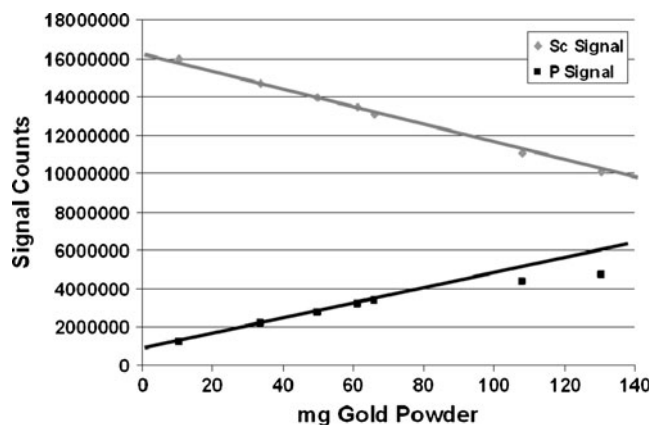

Fig. 3 Response curves of scandium (gray) and phosphorus (black) signal counts versus the amount of gold powder in the sample indicating the effects on increased gold quantities on the analysis. The results indicate the suppression of the analyte ions by the gold ions in solution

also shows a linear relationship with the amount of gold powder digested. Since there is a constant amount of DNA on the formulated particles, the more powder used in the sample, the greater the amount of DNA/phosphorus. Despite the relationship being linear however, the line generated from the data indicates a loss of linearity at the higher amounts of gold powder and an overall R^2 value of only 0.968. When the internal standard is used to normalize the phosphorus response, the resulting response curve has an R^2 of 0.9996. Based on these results and the results from the internal standard, the loss of linearity from the phosphorus signal is likely also due to the increased amount of gold in the sample. While the internal standard corrects for this effect, the overall result is the suppression of the analyte signal that will negatively impact the limit of detection and quantification of the method which is not desirable for a quantitation method.

The other concern with the elevated gold amounts resulting from the gold powder digestion is the irreversible deposition of elemental gold on the skimmer cone of the ICP-MS. While no effect on the instrument or analysis was noted it is likely that continued deposition of gold on the skimmer cone could lead to negative effects. In order to compensate for the gold powder, the hydrochloric acid was omitted and the samples were digested using only nitric acid. In these digestions, the gold powder was no longer dissolved and required centrifugation to remove. This sample preparation route did show greater advantages after implementation. The first advantage was higher intensity from both the scandium internal standard and the phosphorus signals while calculated results between the two methods showed no variation in the amount of the DNA (data not shown). Using nitric acid, it was determined that a smaller amount of acid was required to digest the DNA from the particles. This resulted in a lower noise level for

the blank measurements as the acids were the major source of phosphorus background as mentioned in the previous section. Based on these advantages and the disadvantages from the digesting the gold powder subsequent samples were analyzed without the hydrochloric acid.

Linearity/LOQ

One of the most important properties in a quantitation method is a low limit of quantitation and a response curve that demonstrates linearity over a wide dynamic range. ICP-MS is a well-known technology that has demonstrated the appropriate detection sensitivity and linearity making it an excellent tool for the quantitation of trace elements. In order to utilize ICP-MS for this application, the linearity of DNA in solution needs to be evaluated. This is due to the plasmid DNA solution being used as a standard to precisely quantitate the load of DNA deposited on the gold particles. In addition, the method must also possess excellent linearity with respect to the digested DNA on the gold particles to accurately determine the DNA loaded on the gold particles. In order to thoroughly assess the method, both responses were evaluated.

In order to evaluate the linearity, response curves were prepared for both the liquid DNA plasmid and DNA-formulated gold powder. Aliquots of DNA plasmid solution, 1,000 μL, 500 μL, 250 μL, 150 μL, 100 μL, and 50 μL, were utilized for the determination of linearity. The samples were prepared based on the method in the "Experimental" section. The samples were then analyzed on the ICP-MS with results shown in Fig. 4a. The line generated by the data is visually linear and has an R^2 factor of 0.9999 indicating that there is a strong correlation between the concentration of DNA and the response of the ICP-MS measuring the phosphorus signal in the sample.

The results of the DNA plasmid solution response curve also validate the use of the plasmid calibration curve for quantitating the amount of DNA on the gold particle surface. In selecting the standard used in calibration curve, there were two possibilities, one being a previously characterized plasmid sample or a commercially available phosphorus elemental standard. The advantages of the phosphorus elemental standard are that it is commercially available and the concentration is well established. However, the standard is not likely to be in an equivalent matrix to the actual samples. For the DNA plasmid solution calibration curve, the plasmid has gone through the same process as the gold samples and both have a relatively similar matrix of the nitric acid digested DNA. In addition, the DNA plasmid solution shows an excellent response versus the concentration of the plasmid further indicating it is suitable for use in a calibration curve. Based on the linearity results and similar matrix, the DNA plasmid solution was used as the standard for all subsequent experiments.
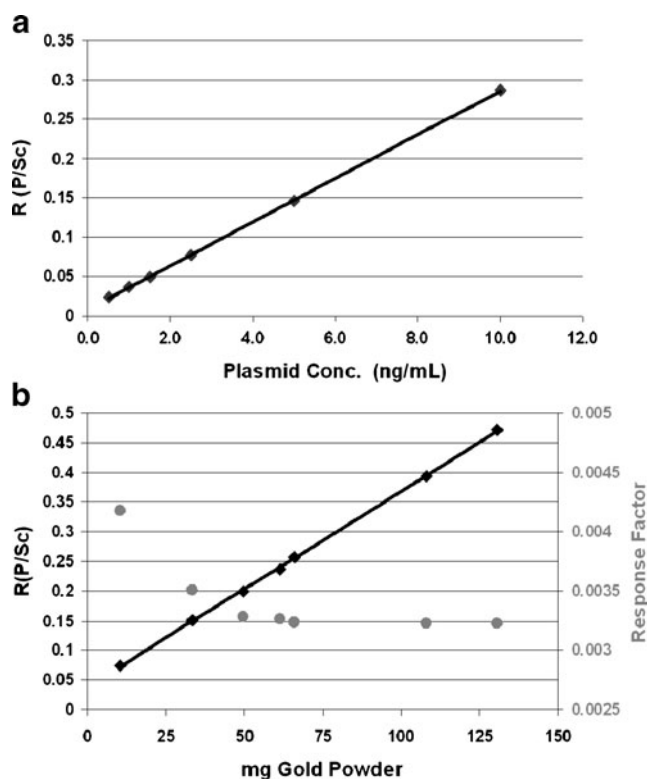

Fig. 4 **a** Calibration curve showing the linear response in the phosphorus to scandium ratio for increasing concentrations of liquid plasmid. **b** calibration curve showing the linear response in the phosphorus to scandium ratio for increasing amounts of gold particles loaded with plasmid DNA (*black*) and the response factors associated with each measurement (*gray*)

The method was also evaluated in terms of the linearity for the amount of gold powder digested in the sample. Various amounts of gold powder from 10 to 135 mg were weighed and digested following the gold powder sample preparation protocol. The phosphorus signals were ratioed against the scandium internal standard and plotted against the weight of gold powder in the sample. The results from the experiment are shown in Fig. 4b (black). The response on the ICP-MS was visually linear and the R^2 of the line generated from the data was 0.9999. Based on these results, the instrument response is linear in response to the amount of DNA digested from the gold powder. To determine whether the concentration of gold particles and ions have an effect on the assay, the response factors for the plasmid DNA versus the amount of gold powder was also plotted in Fig. 4b (gray). The results demonstrate that the response factor of the DNA plasmid stays relative constant until the amount of gold powder is below 50 mg at which the background signals begin to affect the response factor. These results also indicate the nitric acid digestion does not produce isobaric interferences such as $^{15}N^{16}O^+$, $^{14}N^{16}O^1H^+$, or $^{12}C^1H_3^{16}O^+$ at unacceptable levels. If additional interferences were produced during the digestion,

the samples with higher amount of DNA would produce a higher amount of interferences since the nitric acid was in excess in the experiments. If this was occurring, the response factors would be skewed as the higher concentrations would have higher response factors as the response factor would incorporate both the higher amount of phosphorus and the additional generating of the isobaric interferences. Subsequent experiments indicate the protocol can be scaled down proportionally to analyze samples less 50 mg if necessary (data not shown). Based on these results, the ICP-MS based method demonstrates excellent linearity that is more than appropriate for a quantitation assay.

Reproducibility

Reproducibility is an extremely important aspect in an analysis for therapeutic molecules. This is due to the necessity of verifying patients get a specific dosage of a drug and making sure the content uniformity between different vials and different manufactured lots of material are consistent. This is extremely critical in DNA-based therapeutics since the doses can be as low as 0.2–1 μg. In order to demonstrate the reproducibility of the method, six replicate preparations of the same material were analyzed. In addition, one sample was repeatedly injected six times to account for the instrument variation. The % Relative Standard Deviation (%RSD) for the six replicate preparations was calculated to be 1.02% while the %RSD for the six repeated injections was calculated to be 0.80% (data not shown). The %RSD is low due to the use of the scandium internal standard. The internal standard normalizes the effects of instrument and matrix variations allowing for excellent reproducibility.

Accuracy

Accuracy is an important characteristic of a quantitation assay particularly for the analysis of a therapeutic entity. An accurate determination of the amount of plasmid is important for verifying controlled and uniform dosing and for determining the quality and reproducibility of the manufactured product. In addition to the quality of the product, a method that accurately determines the amount of plasmid on the gold particles is also critical in assessing the amount of delivered during an injection of the gold particles. Since the gold particles significantly interfere with UV–vis and fluorescence detection methods for plasmid DNA, obtaining accurate assay results is a major concern. Utilizing ICP-MS in conjunction with an internal standard should yield a suitably accurate assay in which to determine the amount of DNA on the gold particles.

In order to evaluate the accuracy of the ICP-MS assay different plasmid loads of DNA on gold particles were analyzed using ICP-MS. The targeted amount of plasmid

DNA in the samples was originally determined through mass balance to yield the expected plasmid load on the particles. Plasmid loads targeting 2, 4, 6, 8, 10, and 12 μg/mg were analyzed with results for the ICP-MS are shown in Fig. 5. The ICP-MS analyzed samples demonstrated close agreement with the anticipated values regardless of the targeted plasmid load on the particles. The ability to accurately quantitate various plasmid DNA loads is important as the most efficient way to vary the dose to the patient is to load a different amount of DNA onto the particles as opposed to changing the delivery device to introduce varied amounts of the particles. The reproducibility between the lots analyzed was also very good with %RSDs measuring less than 5% between the samples analyzed. It should be noted that the different plasmid load preparations of the formulated plasmid gold particles also contribute to the 5% value as each plasmid load preparation has some inherent variation associated with it. Based on these results, the ICP-MS assay yields accurate results that correspond to the expected values loaded onto to the gold particles.

Method comparison

While the ICP-MS method has shown a lot of promise for quantitating the amount of DNA on a gold particle surface, other methods exist for quantitating the amount of DNA in solution. Typically, the concentration of plasmids can be determined by measuring their absorbance and using a well established extinction coefficient for DNA. Alternatively, a fluorescence labeling method can be employed for more
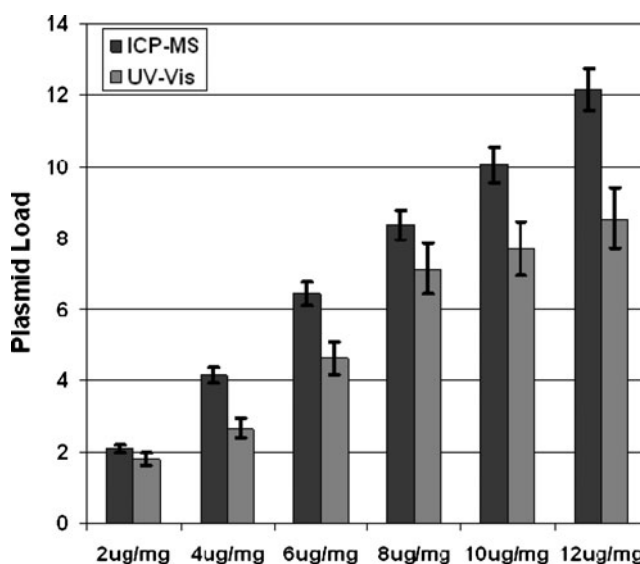

Fig. 5 Bar graph showing the measured plasmid load of various DNA-formulated gold samples with increasing amount of gold deposited onto the particle surface analyzed with ICP-MS- (*dark gray*) and UV–vis (*gray*)-based methods

sensitive analysis. However, the addition of the gold particle in the sample impedes obtaining both UV–vis absorbance and fluorescence measurements. For the DNA plasmid deposited on the gold particle, the absorbance of the gold particle dominates the measurement making the determination of DNA content impossible. In addition, the gold particle acts as an extremely efficient quencher of fluorescence intensity. The solution to this problem would be to extract the DNA from the gold particle and measure the absorbance or fluorescence. Therefore, this approach was used and the results for UV–vis will be compared to the results for ICP-MS determination of the DNA content on the gold particle surface. In order to compare ICP-MS and UV–vis, samples with plasmid loads of 2, 4, 6, 8, 10, and 12 µg/mg were analyzed with UV–vis with the results shown in Fig. 5. The same samples were analyzed using ICP-MS and UV–vis in order to directly compare the assay performance of each.

The UV–vis samples showed a lower amount of DNA plasmid compared to the expected plasmid load and ICP-MS results. In addition, the UV–vis results had a higher % RSD at 10% versus 5.2% for the ICP-MS measurements of the same samples. The explanation for the disparity between the measurements is likely due to incomplete dissolution of the plasmid DNA from the particle surface. Any DNA remaining on the particle surface would be removed from the solution during the centrifugation step to remove the particles. Therefore, any DNA left on the particles would not be accounted for in the UV–vis method. This is also the probable source of the larger %RSD in the UV–vis as the extent of the dissolution is likely not reproducible. In addition, since the larger plasmid amounts demonstrate a lower relative concentration, the amount of DNA in the solution could be beyond the linear range of the method. However, subsequent dilution and retesting gave comparable results indicating that the amount of DNA is still within the linear range of the UV–vis method. The strong acid used to dissociate the DNA in the ICP-MS method allows for the complete recovery of the DNA. However, incubating DNA plasmid solution in the nitric acid results in the UV–vis signal from the sample decreasing significantly with samples incubated for 30 min losing 25% of the UV–vis absorbance signal (data not shown). While the plasmid does not remain intact, the amount of phosphorus is conserved allowing for the accurate quantitation of the plasmid deposited onto the particle using ICP-MS.

If the major limitation is incomplete dissolution of DNA from the particle, other methods for DNA detection like fluorescent labeling would likely produce similar results as they would also require removing the gold particle from the system prior to analysis. In order to verify that the fluorescence methods of detection were also performed

and compared to the UV–vis and ICP-MS assays. For each sample analyzed with UV–vis and fluorescence, around 50 mg of gold powder formulated with 2 µg/mg plasmid DNA was weighed out, followed by the addition of 10 mL of elution buffer. The samples were vortexed for 30 s followed by sonication for 60 min. The samples were allowed to equilibrate for 18 h followed by 30 s of vortexing and 60 min of sonication. The samples were then centrifuged at 4000 rpm and the supernatant was used for both UV–vis and fluorescence testing. UV–vis analysis was conducted directly on the supernatant of the samples while the fluorescence analysis involved a labeling step followed by fluorescence detection. The same gold powder sample was analyzed using ICP-MS following the protocol in the experimental section. The results for all of the samples are shown in Fig. 6.

The UV–vis and fluorescence measurements yielded similar results in terms of the amount of DNA per mg of gold powder however both results were 15–20% lower than the expected result of 2.0 µg/mg (DNA/gold). The ICP-MS measurement however yielded a result that was highly consistent with the expected amount. In addition to yielding more accurate results compared to the spectroscopic methods, the precision of the different methods was also calculated for these samples. For this determination, the % RSD was calculated for the samples and used for comparison. For these samples, the ICP-MS had a %RSD of 0.5% while the UV–vis and fluorescence measurements had %RSD of 3.4% and 3.6%, respectively. These results indicate that ICP-MS yields a more precise and accurate result than UV–vis and fluorescence-based methods.

Conclusion

In summary, ICP-MS in pharmaceutical applications is typically used for trace metal analysis as opposed to strength or quantitation assays. This is typically due to the effectiveness of UV–vis assays and the lack of elements that can be used for detection that are not present in the

Fig. 6 Bar graph illustrating the results of three preparations of the same sample analyzed using ICP-MS, UV–vis, and fluorescence techniques for quantitating the DNA load on the gold particles
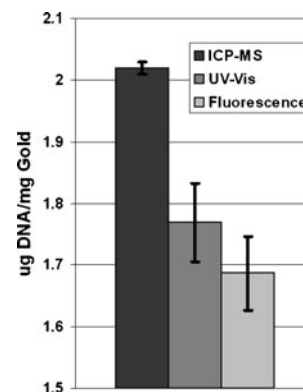
matrix for many molecules. However, the excellent sensitivity, accuracy, and reproducibility of ICP-MS methods make it ideally suited for quantitation assays for applicable samples. While DNA has an effective chromaphore and is easily detected using UV–vis spectroscopy, once the plasmid is desorbed onto to the gold particle spectroscopic methods no longer yield accurate results. The ICP-MS analysis however can overcome these limitations. Plasmid DNA contains one phosphorus atom per base that is detectable by ICP-MS while the DNA can be removed from the particle rapidly and thoroughly since the phosphorus in the plasmid is always conserved despite the final condition of the DNA itself or its structural isoforms.

Utilizing ICP-MS to quantitate the amount of plasmid DNA loaded onto a gold particle imparts several advantages. ICP-MS can detect and quantitate the phosphorus present in the plasmid DNA allowing for excellent selectivity as the only source of phosphorus in the sample is from the DNA plasmid. ICP-MS also enables the assay to have an excellent linear dynamic range allowing for a range of different plasmid loads to be analyzed with a single method preparation. The large dynamic range also enables a low limit of quantitation. The use of scandium as an internal standard also enables the analysis to be highly reproducible from different sample preparations and different lots. All of these characteristics come together to produce a technique that has excellent accuracy as well.

Based on these results, using ICP-MS for DNA quantitation has proven to be an effective route when more typical spectroscopic techniques are insufficient or in some way hindered by the sample. ICP-MS will also be a useful tool in the future for other compounds delivered through PMED as long as unique elements exist for ICP-MS detection. We also anticipate ICP-MS being applied to other samples that do not incorporate gold particles but that other spectroscopic techniques fail to adequately address including DNA, RNA, and even protein-based therapeutics.

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