Published in final edited form as:

Small. 2015 October ; 11(38): 5088–5096. doi:10.1002/sml.201500892.

Recovery of Drug Delivery Nanoparticles from Human Plasma using an Electrokinetic Platform Technology

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Abstract

The effect of complex biological fluids on the surface and structure of nanoparticles is a rapidly expanding field of study. One of the challenges holding back this research is the difficulty of recovering therapeutic nanoparticles from biological samples due to their small size, low density, and stealth surface coatings. Here we present the first demonstration of the recovery and analysis of drug delivery nanoparticles from undiluted human plasma samples through the use of a new electrokinetic platform technology. The particles are recovered from plasma through a dielectrophoresis separation force that is created by innate differences in the dielectric properties between the unaltered nanoparticles and the surrounding plasma. We show this can be applied to a wide range of drug delivery nanoparticles of different morphologies and materials, including low density nano-liposomes. These recovered particles can then be analyzed using different methods including scanning electron microscopy to monitor surface and structural changes that result from plasma exposure. We believe that this new recovery technique is broadly applicable to the recovery of nanoparticles from high conductance fluids in a wide range of applications.

Keywords

Drug Delivery Nanoparticles; Plasma; Recovery; Surfaces; dielectrophoresis; nanoparticle surface

1. Introduction

Exposure to complex biological fluids, such as blood, urine, and cerebrospinal fluid, alters the nature of therapeutic nanoparticles both in terms of their structure and their surface characteristics. These alterations can influence the *in-vivo* performance of these particles and can greatly limit their therapeutic efficacy [1]. It is essential to understand these changes so that new particles can be designed to have better *in-vivo* therapeutic properties. These particles need to be isolated directly from biological fluids and tissue extractions in order to study their *in-vivo* state. Currently, model nanoparticles, such as polystyrene beads, are used in place of drug delivery particles to study the effect of biological fluid exposure due to their greater ease of recovery [2–7]. However, these particles differ from actual drug delivery vehicles in their surface characteristics, their nonbiodegradable nature, and lack of drug carrying capacity. Polystyrene beads also have a robust inflexible structure that does not replicate the deformability of liposome or polymersome based particles. The outer membrane of these particles is created by lipids and polymers and can deform in response to external forces which can cause changes in the coverage of a protective surface coating.

Recovering actual drug delivery nanoparticles designed specifically for *in-vivo* use is a major challenge due to their inherently small size, stealth surface coatings (such as polyethylene glycol), and low density. These properties are essential for the proper biological function and long circulation times of these particles [8–11]. However, these properties create significant technical challenges in concentrating and purifying the particles from complex biological samples as they present very little in terms of chemical or physical characteristics that can be used for conventional separation methods. These methods most often involve centrifugation [5, 12, 13], filtration [14], or the use of magnetic fields [14, 15]. Centrifugation is limited to recovery of nanoparticles that have high density. Filtration is limited due to clogging of the filters by contaminants. The application of magnetic separation is limited to nanoparticles that respond to magnetic fields, such as iron oxide nanoparticles. None of these techniques work on a common physical characteristic that is shared between different types of drug delivery nanoparticles.

One of the characteristics that these drug delivery nanoparticles do have in common is that the material contained within the nanoparticle has different dielectric properties from the surrounding media. This inherent difference in dielectric properties opens the door for the application of electrokinetic separation techniques such as dielectrophoresis (DEP). DEP is ideal for nanoparticle recovery because it creates an attraction force between the particles and the electrodes of the DEP microarray that arises due to permittivity and dielectric/conductivity differences between the materials making up the particle and the surrounding fluid [16]. The charges in the particle and in the fluid reorient at different speeds creating a momentary dipole across the particle when a non-uniform AC electric field is applied. By modulating the AC electric field at the correct frequency, a force with constant direction is created that pulls the particle into the DEP high field region. The high conductance of biological samples has historically prevented the application of DEP techniques. However, an enabling electrokinetic platform technology now allows DEP to be carried out in high conductance aqueous solutions (0.2–1.5 S/m) [17–20], opening the door for use with complex biological samples including blood, plasma, and serum [20–22].

We hypothesized that this technology can be applied to one of the most challenging applications, the recovery of drug delivery nanoparticles designed for *in-vivo* use from whole human plasma. Plasma has a high protein [23] and salt content making it a high conducting, viscous, and sticky liquid. Diluting the plasma to reduce these properties significantly changes the natural behavior of the proteins [13, 24]. Drug delivery nanoparticles are significantly different from other nanoparticles that might be present in the plasma, such as cell free recirculating DNA, because these synthetic nanoparticle morphologies and material compositions are not naturally found in the body. The development of this new recovery technique and its application to actual drug delivery nanoparticles will enable future studies into *in-vivo* stability of the particles [1].

2. Methods and Materials

2.1 Electrokinetic collection and recovery of the nanoparticles

2.1.1 DEP parameters—The basic drug delivery nanoparticle recovery protocol is outlined in Figure 1. Specifically, 10 μ l of the nanoparticle sample was spiked into 90 μ l of undiluted human plasma and allowed to incubate for 10 min. The formation of a protein corona around particles has been shown to start within 30 seconds of contact with plasma [5]. The 10 min incubation time allowed proteins to adhere to the surface of the spiked particles to determine if this would interfere with particle recovery. The plasma was purchased from Sigma Aldrich (St. Louis, MO, USA). 6 μ l of spiked plasma was introduced into the chip microfluidic chamber. The DEP microfluidic chips and control system were generously donated by the Biological Dynamics Corporation (La Jolla, CA, USA).

The drug delivery nanoparticles have a small net charge, as discussed below, and using DEP parameters that are consistent with the conductivity regime of classical DEP theory is most effective at creating a positive DEP force that pulls these particles down to the electrodes [25]. If the driving frequency is too high then the permittivity regime dominates because the particles are less polar than water. This will not result in the nanoparticles being pulled down to the electrodes. If the driving frequency is too low then the direct current electrophoresis regime begins to dominate and particles being to collect based mostly on charge. All the samples were run with a frequency of 15 KHz which maintained the conductivity regime in our experimental setup and was found to be effective for creating a positive DEP force on nanoparticles that had diameters between 100 and 200 nm. The DEP force exerted on a particle is highly dependent on the radius of the particle [25] and individual plasma proteins, including albumin [26], are below the size range where the DEP field at this frequency would be able to exert a significant force on them. We ran experiments with fluorescently labeled 10–20 nm diameter micelles and short 10 nm long DNA strands in 0.5X PBS buffer and were not able to see any collection at this frequency. Protein aggregates that grow to about 100 nm in diameter could potentially be pulled down in the DEP field along with the drug delivery nanoparticles. However, these large aggregates were removed from the plasma by performing a short centrifugation before the nanoparticles were spiked into the sample.

The magnitude of the DEP force can be increased by increasing the voltage applied to the chip. However there is an upper limit of applied voltage above which electrolysis is likely to occur at the electrodes which forms gas bubbles that greatly hinder the collection of the

particles. The chips were run at a voltage level that allowed efficient collection while lowering the probability of electrolysis. Each drug delivery nanoparticle type required a different field strength to be pulled down effectively. This was due to the differences in the dielectric properties between the different particles based on their structural geometries and their composite materials. The correct field strength can be quickly determined for a new nanoparticle sample by running a series of increasing voltages until particle capture begins to occur over the span of a few minutes. The gel filled nanoliposomes were collected at the electrode surface over a period of 10 min by running the chip electrodes at 12 Vpp. The hollow silica shells were run at 8Vpp for 15 min. The empty nanoliposomes were run at 18Vpp for 11 min. The solid polymer nanoparticles were recovered using 15Vpp for 17.5 min. The length of time that the alternating field was applied to the sample depended on the relative change in fluorescence over time. If the fluorescence level stopped increasing over the period of 1 min then it was determined that the maximum number of particles from the sample had been collected.

The nanoparticles used were labeled with fluorescent fluorescein or DiO dye for visualization purposes. Unlabeled nanoparticles can be recovered once the correct DEP parameters have been established using the fluorescently labeled versions. The presence of the fluorescent dye in the particle does not significantly change the particle's dielectric properties allowing both labeled and unlabeled nanoparticles to be recovered using the same DEP parameters [22].

Reversing the DEP force to push the particles into the bulk buffer required reducing the voltage on the electrodes to 2 Vpp and reducing the frequency to 5Hz for 15 seconds.

2.1.2 Sample Washing—After collection was finished, the chips were then washed with 0.5X PBS buffer at various flow rates depending on the strength of the DEP force holding the particles down. The typical wash speed flowing through the microfluidic chamber was 20 μ l per min for 10 min. Higher speeds could be used if the nanoparticles were seen to remain at the edge of the electrode. Smaller volume chambers could use 10 μ l per min for 10 min.

2.1.3 Particle Recovery—Once the collected particles had been sufficiently washed the reverse DEP field of 2 Vpp voltage at a frequency of 5 KHz was applied to push the particles away from the electrodes and back up into the bulk wash buffer. The entire 60 μ l wash buffer was then collected from the chip and stored at 4 °C for further analysis.

2.2 Scanning Electron Microscopy

The silica shell nanoparticles were studied after recovery from plasma using scanning electron microscopy (SEM) because they were structurally rigid enough to maintain their shape through the drying preparation. A 2 μ l sample of the recovered nanoparticle solution was placed on the surface of a polished silicon wafer and dried overnight. The sample was then sputter coated with iridium and imaged on an FEI XL30 scanning electron microscope. The other nanoparticles were too flexible to yield usable pictures with SEM. Future work will use cryo-TEM to study these more flexible structures.

2.3 Empty Nanoliposome Particle Synthesis

The empty nanoliposomes were synthesized by combining chloroform dissolved samples of cholesterol, DOPE, DSPC, DSPE-mPEG2000, and DiO in a 6:6:6:1:0.5 molar ratio. An argon gas stream was used to evaporate the chloroform. The dried lipid film was hydrated with water and vortexed for 1 minute to remove any adhering lipid film. The sample was sonicated in a bath sonicator (ULTRASONIK 28X) for 1 minute at room temperature to produce multilamellar vesicles. These vesicles were then sonicated with a Ti-probe (Branson 450 sonicator) for 2 minutes to produce unilamellar nanoliposomes, indicated by the formation of a translucent solution. Extrusion through a 100 nm pore size polycarbonate filter (Whatman) was the final stage of a stepwise series of extrusions to reduce nanoliposome size. The final lipid concentration of the purified nanoparticles sample was 3.3 mM. The zeta potential of the particles was -5.52 mV, (SD 9.6 mV) and the average size of the nanoparticles was 109 nm (SD 0.174 nm).

2.4 Solid Polymer Nanoparticle Synthesis

The solid polymer nanoparticles were made through nanoprecipitation of a new chemotherapy prodrug as described in Schutt et al. [27].

2.5 Hollow Silica Shell Synthesis

These hollow silica shell based nanoparticles were designed to have two different levels of porosity, the mesoporous structure of the silica itself and larger pores that are built into the structure using a masking technique. A template particle solution consisting of 50 μ l of 2.5% amino functionalized 200 nm diameter polystyrene beads was mixed with a 4% solution of 60 nm carboxy functionalized polystyrene beads to achieve the desired ratio of particle concentrations. The mixture was shaken overnight. Then 1 ml of anhydrous ethanol was added to the solution. Silica growth was initiated by adding 1 μ l of tetramethoxysilane. The mixture was shaken overnight, and the particles were collected by 5 min of centrifugation at 14000 rpm. The pelleted nanoparticles were washed with deionized water several times and dried under vacuum on a coverslide overnight. The organic compounds were removed by calcination overnight at 450°C by placing the nanoparticle powder on a coverslide on a hot plate. The calcined powder was suspended in 50 μ l water and then dispersed by gentle sonication.

Separately, 5 μ l of 5 mg/ml fluorescein isothiocyanate (FITC) in dimethyl sulfoxide solution was added to 200 μ l of 0.1% poly-L-lysine (molecular weight of 150–300 kDa) in a 1X phosphate buffered saline solution. The mixture was shaken overnight at 4°C.

The nanoparticle solution was diluted into 1 ml of phosphate buffered saline. A 50 μ l poly-L-lysine/FITC solution was then added to the mixture. TMOS was added to 1 mM HCl in a 74:500 volume ratio and mixed for several minutes to make a silicic acid solution. 25 μ l of the silicic acid solution was then added to the above solution immediately after dilution. The mixture was then shaken for 1 hour to generate the final silica-coated nanoparticles. These suspended nanoparticles were pelleted with 5 min of centrifugation at 14000 rpm and washed several times with water and finally 1X PBS.

The silica nanoparticles were finally coated with a polyethylene glycol (PEG) brush layer as follows. The nanoparticles were concentrated to $\sim 4 \times 10^{12}$ particles/ml and were mixed with an equal volume of 100 mg/ml PEG-silane in aqueous solution. The mixture was shaken overnight at room temperature. The suspended nanoparticles were pelleted by 5 min centrifugation at 14000 rpm and washed several times with water. The particles were then diluted in 0.5X PBS to a concentration of $\sim 4 \times 10^{12}$ particles/ml.

2.6 Gel filled Liposome Nanoparticle Synthesis

The liposome formulation combined chloroform dissolved samples of cholesterol, DOPE, DSPC, DSPE-(PEO)4-cRGDfK, DSPE-mPEG2000, and DiO (6:6:6:1:1:0.5 molar ratio). The chloroform was evaporated under argon gas and the dried lipid film was hydrated with a solution containing 10 mg of human serum albumin in 1 ml phosphate buffer pH 7.4 for a minimum of 30 minutes. The solution was vortexed for 1 minute to remove any adhering lipid film and sonicated in a bath sonicator (ULTRASONIK 28X) for 1 minute at room temperature to produce multilamellar vesicles. These vesicles were then sonicated with a Ti-probe (Branson 450 sonifier) for 2 minutes to produce small unilamellar nanoliposomes filled with the cross-linked human serum albumin gel as indicated by the formation of a translucent solution. To reduce the size of the filled nanoliposomes, stepwise extrusion was carried out with the final step being extrusion through a polycarbonate filter with 200-nm pore size (Whatman). The nanoliposomes are then purified by size exclusion chromatography on sepharose CL-4B columns to remove free albumin. The final concentration, size distribution, and zeta potentials were the same as the empty nanoliposomes described in section 2.3.

2.7 Image Analysis

A custom written MatLab program was used to create the fluorescence intensity graphs for the different stages of nanoparticle collection and recovery shown in Figure 4. The image contrast and brightness were increased in the last two fluorescent images taken after the washing process in each of these figures to allow the fluorescent ring to be easily visible. However, the raw image data was used to create the fluorescence intensity graphs shown below to allow for accurate comparisons. These fluorescence intensity graphs show the absolute fluorescence intensity and are not influenced by the brightness and contrast enhancement.

2.8 UV/visible light absorption data

The samples that were recovered from the DEP chip were analyzed for protein content using a NanoDrop ND-1000 spectrophotometer (Wilmington, DE). A 2 μ l sample of the recovered solution was used for the absorption measurements. The plasma control sample was diluted 12.5 times because the spectrophotometer was saturated by the absorption of the undiluted sample. Three different samples from each category were averaged together to produce the graphs. The error bars represent the standard deviation between the samples.

3. Results

3.1 Electrokinetic collection and recovery of the nanoparticles

3.1.1 Nanoparticle Recovery Protocol—The nanoparticle recovery protocol is shown in Figure 1. The platform technology consists of a microfluidic chamber with a multi-layered DEP microelectrode array at the bottom (Figure 1a, 2a). The DEP chip was fabricated on a silicon base. The platinum electrodes were 60 μm in diameter and were insulated by a SiO_2 layer (Figure 1a). The inter-electrode center distance was 218 μm and electrodes of opposing polarity were arranged at 30 degrees from one another (Figure 2b). The entire surface of the chip was coated with a 150 nm thick hydrogel layer consisting of porous poly(2-hydroxyethyl methacrylate) (poly-HEMA). A 5% poly-HEMA solution in ethanol (PolySciences, Inc.) was spun coated onto the surface of the chip using a commercial spin coater (Brewer Science) at 6000 rpm for 30 s. The coated chips were then heated in air at 60 $^{\circ}\text{C}$ for 45 min to cure the hydrogel. The hydrogel allows the electric field to penetrate into the plasma sample while preventing direct physical contact between the plasma and the electrode. The chip has over 1000 electrodes and their relative geometry creates a field gradient where the highest field region is concentrated in a ring at the edge of each electrode. When the electric field is alternated at the correct frequency (Figure 1d, e, f), the dielectric force pulls the particles towards the edge of the electrodes (Figure 1g, h, i).

The last column in Figure 1 (panels c, f, i, l, o) shows images from the actual DEP microarray chip containing an undiluted plasma sample spiked with empty fluorescent liposome nanoparticles. The DEP force is strong enough to hold the particles down at the electrode edges while the bulk plasma was gently washed away (Figure 1j, k, l). A 0.5X phosphate buffered saline (PBS) wash was used to mimic the salt concentration of whole blood. Once the plasma was sufficiently washed away, the frequency of the alternating electric field was reduced to create a force that pushed the nanoparticles away from the edge of the electrode. This removed the fluorescent ring around each electrode as the particles moved into the bulk wash buffer making it diffusely fluorescent (Figure 1m, n, o). The wash fluid containing the nanoparticles can be collected from the chip to recover the particles for analysis. The bulk plasma protein level in the recovered nanoparticle sample was below the limit of detection using light absorption techniques after a wash of 10 $\mu\text{l}/\text{min}$ for 10 min (Figure 3). The actual particle recovery time can vary depending on the wash rate and DEP collection time. In this case, the empty liposome particles were recovered from the chip 30 min after introducing the spiked plasma sample.

3.1.2 Collection and Recovery of Different Classes of Nanoparticles—We further hypothesized that this recovery technique could be applied to nanoparticles designed for *in-vivo* use with material compositions and physical properties that were different from those of empty liposomes. Gel filled nanoliposomes have different the dielectric properties from that of empty liposomes along with increased rigidity. Empty silica shell nanoparticles are significantly more dense and rigid than liposomes. Nanoprecipitated prodrug polymer nanoparticles consist of the same material throughout the volume of the particle. Together, these four types of drug delivery nanoparticles represent major subclasses of particles currently under development [28].

All these drug delivery nanoparticle subclasses were successfully recovered by modifying the DEP parameters. The fluorescent images taken at the different stages of DEP collection and recovery for the different nanoparticles were analyzed for fluorescence intensity as shown in Figure 4. The X and Y axes label the pixels of the 2 dimensional image. The z-axis shows the pixel fluorescence intensity level. Before the DEP field is applied, the fluorescence intensity is high across all of the pixels because the fluorescent nanoparticles are distributed throughout the plasma sample. After 7 min of DEP collection the background fluorescence intensity has been reduced as nanoparticles are collected at the edge of the electrode forming a fluorescent ring. After washing away the bulk plasma the fluorescence intensity drops across the entire field of view because the nanoparticles that were not drawn to the electrodes were washed away. The DEP field does not extend all the way from the electrodes to the top of the microfluidic chamber so not all the nanoparticles in the sample will be influenced by the DEP field. The nanoparticles that were collected at the electrode edges remained in place throughout the washing. After reversing the DEP field, the fluorescent ring disappeared and the background fluorescence level increased slightly, (0.2 a.u for the empty liposomes and the prodrug polymer nanoparticles) as those particles became diffusely distributed in the bulk wash.

The bulk of the silica particle collection appeared to occur during the washing stage which was different from the other three particle types. The silica nanoparticles were much denser and settled quickly on the hydrogel layer by themselves. The DEP force was not strong enough in this orientation to pull the silica particles along the surface of the hydrogel to concentrate them at the edge of the electrodes. However, once the 0.5X PBS wash buffer was introduced, the DEP force increased in strength due to changes in the dielectric properties of fluid surrounding the nanoparticles. The particles were pushed by the fluid flow across the chip surface towards the outlet but were captured by the stronger DEP force and were able to collect in the high field regions around the electrode edge. The particles remained at the edge of the electrodes through the rest of the wash.

3.1.3 Nanoparticle Surface Analysis—As shown in Figure 3 the washing step reduced the background level of plasma proteins below the light absorption detection limit. This allowed the nanoparticles to be recovered with a high degree of purity allowing their surfaces to be analyzed using SEM. The particles were collected from the DEP chip and a sample was spread onto a silicon wafer chip and allowed to dry overnight. The drying process caused aggregation of the nanoparticles, but individual spherical particles can be seen in the group as shown for the hollow silica shell nanoparticles in Figure 5. The silicon chip surface the particles are sitting on shows very little debris collection from the drying process. Incomplete washing of the sample shows a thick layer of residual plasma proteins.

The particles that were incubated in the plasma had very little structure or surface topology changes compared to those recovered from 0.5X PBS as pointed out in the magnified view insets shown in Figure 6. This also shows that the DEP collection process did not cause damage to the nanoparticles themselves.

4. Discussion

The DEP separation and recovery methods developed here were successfully applied to recover a wide range of different drug delivery nanoparticles from undiluted human plasma samples. These non-magnetic particles were designed specifically for *in-vivo* use and had stealth surface coatings that lacked any characteristics for use in affinity binding recovery techniques. Three of these nanoparticle types were liposome and polymer based and could not be recovered using standard centrifugation techniques. This method was also used to successfully recover hollow silica shell nanoparticles that were dense and quickly settled to the bottom of the DEP chip. Each of these particle types contained materials with different dielectric properties showing the wide applicability of this technique. The separation force is based on the inherent difference in the dielectric properties between the particles and the surrounding media. This force was strong enough to retain the collected nanoparticles at the edges of the electrodes through the washing process that removed the bulk plasma sample. These entrapped nanoparticles were released and recovered for SEM surface and structural analysis. This analysis can provide new insights into how plasma exposure effects nanoparticle surface and structural properties and is essential for the continued development of drug delivery nanoparticles with the goal of improving their *in-vivo* properties.

This DEP technique of recovering nanoparticles from plasma can be adapted for other applications as well. In the future, this technique can be expanded to recover nanoparticles from additional high conductance complex biological fluids including urine, synovial fluid, cerebrospinal fluid, and pleural effusion [29]. The DEP technique we describe here can be useful for isolation of a wide variety of drug delivery and other nanoparticles from undiluted clinical and biological samples.

Future applications of this technique could also include recovering nanoparticle contaminants from environmental samples. There are growing concerns about the effects on public health from nanoparticle accumulation in the environment [29] and this technique could be adapted to recover nanoparticles from ocean and river water (both of which are high conductance complex fluids) to better understand these effects. This process could also be applied to separate nanoparticles in industrial applications including separating them from starting materials and aggregates during synthesis to increase yield and open new manufacturing techniques. Expensive nanoparticles could also be recovered for recycling purposes to reduce the financial costs of research and industrial applications.

5. Conclusion

We have shown for the first time the successful use of high conductance DEP for the recovery and analysis of a wide array of different drug delivery nanoparticles from undiluted human plasma. These particles were specifically designed for *in-vivo* use and had stealth surface coatings, low density, and were non-magnetic making them a challenge to recover using traditional methods. The bulk plasma components were washed away leaving the nanoparticles trapped on the DEP chip surface. These nanoparticles were released and recovered allowing their surfaces to be analyzed using scanning electron microscopy to study any changes that occurred as a result of plasma exposure.

Acknowledgments

The authors would like to thank Biological Dynamics for the donation of their DEP chips and to Raj Krishnan, David Charlot, Eugene Tu, and Juan Pablo Hinestrosa for their advice and suggestions on the data collection. The authors also want to thank James McCanna for his advice and insights into the DEP theory and Taeseok Oh for help establishing DEP effectiveness on particle size. Support was provided by Grant Number T32 CA121938 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

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Figure 1.

Schematic and experimental images of the collection and recovery of empty nanoliposomes designed for *in-vivo* use from whole plasma. **a, b, and c.** The plasma sample spiked with the nanoparticle drug delivery vehicles is introduced to the chip. **d, e, and f.** The alternating electric field is applied creating a dielectrophoretic force on the particles but not the free plasma proteins. **g, h, and i.** The particles are attracted to the high field region at the electrode edges forming a fluorescent ring over 7 min. **j, k, and l.** A fluidic wash is run

through the chip. The fluorescent ring remains. **m, n, and o.** The DEP force holding the particles is reversed pushing the particles off the chip surface for collection.

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Figure 2.

Picture of the electrode chip and schematic of the microfluidic channels. **a.** The actual electrodes without the microfluidics are shown next to a dime for size comparison. There are over 1000 electrodes on the chip. **b.** Magnified view of the electrode array. The circular electrodes are highlighted by a ring of collected fluorescent liposome nanoparticles. **c.** Schematic showing the microfluidic system that fits over the electrodes from the top view. The clear optical window allows for visualization of the fluidic channel and the electrodes at the bottom. **d.** Schematic showing the microfluidic channel and the electrodes in a cross-sectional view. The electrodes and the rest of the chip dimensions are not to scale.

Figure 3.

UV/visible light absorption data for the recovered nanoparticles. The average absorption spectra for three different samples of the wash buffer, plasma diluted 12.5X in the wash buffer, and nanoliposomes are compared to the average absorption spectra for three different recovered nanoliposome samples. The error bars show standard deviation. The washed recovered nanoliposome samples do not show the characteristic plasma protein peak at 280nm. The wash was efficient enough to remove the background bulk plasma protein content so that the absorption level was below the detection limit.

Figure 4.

Image analysis at different stages of the DEP collection process for four different drug delivery nanoparticles that were composed of materials with different dielectric properties. Each type of particle was successfully collected and purified using the DEP chip. A schematic representation of the composition and geometry of each particle is shown on the left of each frame. All the particles were fluorescently labeled so they could be tracked on the DEP chip. The top row shows fluorescent images of an electrode on the DEP chip at different stages during the collection process. The fluorescent intensity of each pixel in the image was quantified and plotted on the graph directly below the fluorescent image allowing the direct comparison of different images. The particles were pushed back up into the bulk wash buffer by reversing the DEP force. The last two images after the wash had the brightness and contrast enhanced to better show the rings. This image enhancement does not affect the graphs showing the intensity analysis **a**. Empty liposome drug delivery nanoparticles. **b**. Liposome drug delivery nanoparticles that were filled with a gel consisting of cross-linked human serum albumin. **c**. Hollow silica shell nanoparticles. **d**. Solid nanoparticles made entirely from prodrug monomers.

Figure 5. Scanning electron microscopy images of recovered hollow silica shell nanoparticles showing the effect of washing on the sample. **a.** The silica nanoparticles were incubated in 0.5X PBS and recovered on the DEP chip. There is no visible background protein contamination as shown by the clean silicon substrate that is below the clustered nanoparticles. **b.** The nanoparticle sample recovered from plasma and washed on the chip. The majority of the plasma proteins have been removed and show a clean silicon substrate underneath the clustered nanoparticles. **c.** Not washing the particles sufficiently left behind a significant protein content that buried the nanoparticles as the sample dried for SEM analysis. A crack in the protein layer is shown.

Figure 6.

Scanning electron microscopy images of hollow silica shell nanoparticles recovered from 10 min of plasma incubation. The surfaces of these nanoparticles naturally have a bumpy texture resulting from the deposition of multiple layers of silica. **a.** The control nanoparticles were spiked into a 0.5X PBS buffer and recovered on the chip. The particles aggregated during the drying process for SEM analysis but the surface of individual spherical particles are visible. **b and c.** These two particle samples were recovered from plasma after a 10 min incubation. The nanoparticles that were recovered from plasma do not show any significant changes in overall diameter compared to those recovered from 0.5X PBS (significance determined using a nonparametric Wilcoxon Rank Sum Test comparing nanoparticles recovered from 0.5X PBS ($n=7$) to nanoparticles recovered from plasma sample 1 ($n=8$) ($p=0.135$) and to plasma sample 2 ($n=11$) ($p=0.728$)). The particles retained their bumpy surface texture after exposure to plasma. However the surface features in panel c are larger than the surface features in panel a or b which might be an effect of protein accumulation due to the plasma exposure.