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EFFECTS OF POLYMERIC NANOPARTICLE SURFACE PROPERTIES ON INTERACTION WITH BRAIN TUMOR ENVIRONMENT

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Abstract

Current chemotherapy treatments are limited by poor drug solubility, rapid drug clearance and systemic side effects. Additionally, drug penetration into solid tumors is limited by physical diffusion barriers [e.g., extracellular matrix (ECM)]. Nanoparticle (NP) blood circulation half-life, biodistribution and ability to cross extracellular and cellular barriers will be dictated by NP composition, size, shape and surface functionality. Here, we investigated the effect of surface charge of poly(lactide)-poly(ethylene glycol) NPs on mediating cellular interaction. Polymeric NPs of equal sizes were used that had two different surface functionalities: negatively charged carboxyl (COOH) and neutral charged methoxy (OCH₃). Cellular uptake studies showed significantly higher uptake in human brain cancer cells compared to noncancerous human brain cells, and negatively charged COOH NPs were uptaken more than neutral OCH₃ NPs in 2D culture. NPs were also able to load and control the release of paclitaxel (PTX) over 19 days. Toxicity studies in U-87 glioblastoma cells showed that PTX-loaded NPs were effective drug delivery vehicles. Effect of surface charge on NP interaction with the ECM was investigated using collagen in a 3D cellular uptake model, as collagen content varies with the type of cancer and the stage of the disease compared to normal tissues. Results demonstrated that NPs can effectively diffuse across an ECM barrier and into cells, but NP mobility is dictated by surface charge. In vivo biodistribution of OCH₃ NPs in intracranial tumor xenografts showed that NPs more easily accumulated in tumors with less collagen. These results indicate that a robust understanding of NP interaction with various tumor environments can lead to more effective patient-tailored therapies.

Keywords

Nanoparticle; drug delivery; surface charge; uptake; biodistribution

1. Introduction

Nanoparticle (NP) drug delivery systems are expected to improve the diagnosis and treatment of cancer. In fact, polymeric NPs of PLA-PEG and PLGA-PEG for delivery of anticancer drugs have gained approval outside of the United States, and are in late-phase clinical trials to treat cancer within the United States.^{1–3} Moreover, improvements in NP manufacturing, control over synthesis and functionalization on a molecular level are enabling NPs as a technology that has potential to improve current chemotherapies.^{4,5} It is understood that NP physicochemical properties will mediate their interaction with the biological environment, and research has shown that NP properties such as size, shape, surface charge and surface functionalization determines the ability of NPs to increase circulation time and navigate biological barriers.^{4,6–9}

Tumor tissue is characterized by leaky vasculature, poor lymphatic development, vascular tortuosity and increased interstitial fluid pressure.^{10–12} Moreover, diffusion of macromolecules and NPs into tumors is hindered by high cell density and interaction with the extracellular matrix.^{13,14} Thus, understanding of NP interaction with the biological milieu due to NP physicochemical characteristics may lead to patient-tailored therapies due to tumor characteristics and type. Here we investigated polymeric PLA-PEG NPs with two different surface functionalities: negatively charged carboxyl (COOH) and neutral charged methoxy (OCH₃). PLA-PEG was synthesized using a ring-opening polymerization, and NP surface functionalities were controlled by initiating the polymerization with different heterobifunctional PEG molecules. Changing surface charge resulted in differences in cellular uptake. Moreover, preliminary in vivo biodistribution studies of neutral methoxy NP resulted in differential accumulation of NPs in tumors.

2. Materials and Methods

2.1. Materials

D,L lactide (C₆H₈O₄, PURASORB DL) was supplied by Purac Biomaterials. Glycolide (C₄H₄O₄, >99%), tin(II) 2-ethylhexanoate ([CH₃(CH₂)₃CH(C₂H₅)-CO₂]₂-Sn, ~95%), sodium sulfate (Na₂SO₄; >99%), anhydrous magnesium sulfate (MgSO₄, >99.5%), anhydrous toluene (C₆H₅CH₃, 99.8%), methanol (CH₃OH, >99.9%) and chloroform (CHCl₃, >99.8%) were supplied by Sigma-Aldrich. Methoxypoly(ethylene glycol) was supplied by JenKem Technology USA (M-PEG-OH, Mw 5000). Acetonitrile (ACN, C₂H₃N, 99.9%) was supplied by Fisher Scientific. PrestoBlue Cell Viability Reagent, Collagen, Type I Bovine, Alexa Fluor 647 cadaver-ine, and Alexa Fluor 488-phalloidin were supplied by Life Technologies. Paclitaxel was provided by LC Laboratories. DAPI was provided by Vector Laboratories.

2.2. Statistical analysis

All statistical analysis was performed using a two-tailed t-test with at least three repeats each. Statistical significance was set at $p < 0.05$. Error bars on graphs represent the standard deviation from the mean.

2.3. Cell culture

U-87 glioblastoma cells (American Type Culture Collection, ATCC), U138-MG glioblastoma cells (ATCC), NCI-H460 nonsmall lung cancer cells (ATCC), MCF-7 breast adenocarcinoma cells (ATCC), human brain microvascular endothelial cells (HBMEC, ScienCell Research Laboratories) and human umbilical vein endothelial cells (HUVEC, Lonza) were used for studies. All cells were of human origin and were grown in monolayer cultures at 37° C and 5% of CO₂. U-87 and U-138 glioblastoma cells were cultured using Eagle's Minimum Essential Media (EMEM, ATCC) supplemented with 10% fetal bovine serum (Atlanta Biologics) and 1% penicillin-streptomycin-amphotericin (Media-Tech, Inc.). NCI cells were cultured using RPMI-1640 medium (ATCC) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-amphotericin. MCF-7 cells were cultured with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.5 mg/mL insulin. HBMECs were cultured in Endothelial Cell Medium (Scien-Cell Research Laboratories). HUVECs were cultured with Endothelial Cell Media (Lonza).

To assemble cellular spheroids, equal volumes of solutions containing suspended collagen and cells in cell culture media were combined and dispensed using a hanging drop method. Samples were immediately inverted and incubated at 37° C with 5% CO₂ for three days to allow for spheroid assembly and collagen gelation. All spheroids were assembled using U-87 glioblastoma cells with 20 000 cells per spheroid and varying collagen content.

2.4. Histology

A multiple organ tumor tissue array slide was purchased from US Biomax, Inc. Tumor tissue arrays contained 5- μ m thick samples of various tissues at various stages of cancer development. Slides were deparaffinized via xylene and ethanol prior to staining with Gomori's Trichrome (Poly Scientific) to qualitatively analyze collagen content.

2.5. NP synthesis and characterization

Poly(lactide)-poly(ethylene glycol) block copolymers were synthesized with a ring-opening polymerization. D,L lactide (17.4 mmol) and methoxypoly(ethylene glycol)-hydroxyl (0.133 mmol) were placed in a round-bottom flask with sodium sulfate (2.19 mmol) and dried overnight under vacuum (32 in Hg). Next, the reaction components were dissolved in 10 mL anhydrous toluene at 120° C under a N₂ blanket with reflux. Stannous octoate (0.016 mmol) was added to the solution, with the reaction vessel purged under N₂. The solution was stirred for 12 h. Next, the reaction vessel was removed from heat and allowed to come to room temperature. The reaction product was washed with chloroform and water. Products were dried over magnesium sulfate, filtered, and concentrated using a rotary evaporator. Polymer was then precipitated in cold (–80° C) methanol overnight. Product was collected via centrifugation and lyophilization. Polymers were characterized with nuclear magnetic

resonance spectroscopy (Bruker 300 MHz) and with Fourier transform infrared spectroscopy (Thermo-Nicolet Magna 550).

Polymeric NPs were assembled using a solvent evaporation technique. Briefly, PLA-PEG-R block copolymer was dissolved in acetonitrile (5 mg/mL). NPs were synthesized by mixing the polymer solution at a 1:2 ratio in water and stirring for 2 h. Solutions were collected and washed in 100 kD molecular weight cut-off centrifugal filter units (3500 rpm, 5 min) twice in water and once in phosphate buffered saline (PBS). NPs were resuspended in cell culture media at the desired concentration per application.

To assemble fluorescently labeled NPs used in cellular uptake studies, PLA polymer was conjugated with AlexaFluor 647 cadaverine using EDC chemistry. Briefly, PLA was dissolved in anhydrous dimethylformamide (DMF) and mixed with 10-fold excess EDC (Pierce). Solution was vortexed and 10-fold excess of AlexaFluor 647 cadaverine was stirred with PLA overnight. This solution was concentrated under rotary evaporator, redissolved in chloroform, and precipitated in cold methanol as previously described. The product was collected via lyophilization. This PLA-AF647 polymer was mixed with PLA-PEG in acetonitrile at a 6:4 (PLAPEG:PLA-647) ratio and assembled using the same solvent evaporation technique previously described.

To assemble paclitaxel-(PTX) loaded NPs used for drug delivery studies, PTX was first dissolved in acetonitrile (1 mg/mL). Next, PLA-PEG was dissolved in PTX-containing acetonitrile at 5 mg/mL and allowed to mix on a rotisserie for 1 h. NPs were prepared with this solution using the same solvent evaporation technique previously described.

2.6. NP uptake

AlexaFluor 647 fluorescently labeled NPs were used to analyze NP uptake into cells. To analyze NP uptake into two-dimensional (2D) cell culture models, cells were plated overnight onto a 96-well plate at 10 000 cells per well. The following day, 100 µg of fluorescent NPs were added to the wells and incubated for 24 h. After incubation, NP-containing media was removed, and the wells were gently washed three times with sterile PBS. Next, the wells were trypsinized, collected and resuspended in 200 µL sterile PBS. Samples were then transferred to a new 96-well plate, with fluorescent measurements (Ex/Em 645/680) recorded for each sample.

To analyze NP uptake into three-dimensional (3D) cell culture models, cellular spheroids were utilized to mimic the native 3D cellular environment. Microcentrifuge tubes (0.65 mL) were filled with fluorescent NP-containing media (500 µg/mL), with an individual cellular spheroid placed into each tube. Samples were placed on a rotisserie and rotated inside an incubator for 24 h. Next, samples were washed three times with sterile PBS, followed by dissociation of cellular spheroids via collagenase and trypsin. Spheroids were incubated with collagenase (80 min), followed by incubation with trypsin (10 min). Spheroids were centrifuged, resuspended in 200 L sterile PBS and physically dissociated. Samples were then transferred to a new 96-well plate, with fluorescent measurements recorded for each sample.

Confocal microscopy was performed to confirm that NPs were internalized within cells. Cells were plated into a coverglass bottom chamber slide and allowed to adhere overnight. Samples were incubated with fluorescent NPs (500 µg/mL) for 24 h, followed by three washes with sterile PBS. Cells were fixed with 4% formaldehyde for 20 min, followed by permeabilization with 0.1% Triton-X for 1 min. Next, actin was stained with Alexa Fluor 488-phalloidin for 60 min. Finally, nuclei were stained with DAPI (3 min), and samples were coverslipped for imaging with a Nikon Eclipse Ti confocal microscope. Samples were washed three times with sterile PBS between each step.

2.7. NP toxicity for drug delivery

Release of PTX from PLA-PEG NPs was measured using high-performance liquid chromatography (HPLC). Paclitaxel (LC Laboratories, Woburn, MA, USA) was encapsulated by dissolving PLAPEG and PTX in ACN and rotating on a rotisserie protected from light. The PLA-PEG/PTX solution was then dropped in HyPure deionized water and PTX was encapsulated via solvent evaporation. Samples were washed three times in HyPure water via centrifugation. PTX-loaded NPs were then re-dispersed in HyPure water and added to the top of a 3.5 kD Slide-a-Lyzer MINI dialysis unit. Each dialysis unit was placed in a microcentrifuge tube with HyPure deionized water. Five repeats were used in this release study and samples were placed in an incubator at 37° C and 5% CO₂ for the duration of the study. At each time point, dialysate was removed and frozen, and fresh HyPure water was replaced. Lyophilized aliquots were analyzed via HPLC on a Waters 1525 Binary HPLC pump with a 2998 photodiode array detector. An Alltima C18 column (Grace, Deerfield, IL, USA) was used that was 4.6 × 25 mm with 5 µm pores. Samples and standards were dissolved in acetonitrile. The mobile phase used was 60% acetonitrile and 40% water. The mobile phase flow rate was 1 mL/min and PTX was detected at a wavelength of 227 nm with an average elution time of 11.5 min.

To analyze *in vitro* toxicity, U-87 glioblastoma was seeded at 10 000 cells/well in a 96-well plate. NPs were loaded with PTX as previously described and suspended in cell culture media (EMEM). Free drug was prepared by dissolving PTX in dimethyl sulfoxide (DMSO) and diluting in EMEM. The final DMSO concentration was less than 1 v/v%. PTX-loaded NP or free PTX was incubated with cells for 6 h. After initial incubation, cells were washed with sterile PBS and media was exchanged for fresh EMEM. Cells were then incubated for a total of 72 h following initial exposure and viability was measured with a Presto Blue cell viability assay.

2.8. In vivo biodistribution

For *in vivo* biodistribution studies, nude Balb/c mice were implanted with intracranial tumors of U-138 glioblastoma following the protocol of Ozawa and James.¹⁵ Animals were housed at Clemson University's Godley-Snell Research Center. All animal work was done in accordance with Clemson University Institutional Animal Care and Use Committee (IACUC) approved protocols. Briefly, U-138 cells were washed with sterile PBS. Cells were collected and concentrated in serum-free EMEM at 40 000 000 cells/mL. About 100 µL of cell suspension was added to 100 µL of matrigel with or without 0.017 mg/mL collagen I. Athymic nude Balb/c mice were anesthetized with ketamine-xylazine. Skin was disinfected

with chlorohexidine and eyes were lubricated with PuraLube eye ointment. A 1 cm sagittal incision was made across the top of the skull. The skull was sterilized with hydrogen peroxide. A hole was made 2 mm anterior and 1 mm lateral of the bregma using a 25-gauge needle. About 3 μ L of solution was injected 3 mm deep into the brain over a 1-min period. The skull was closed using dental cement and the incision site was stapled shut. Tumors were allowed to grow for 10 days. After 10 days, biodistribution studies were performed.

Fluorescent NPs were prepared as previously described. Samples were washed in sterile H₂O and re-dispersed in sterile PBS at 1 mg/mL. Next, 200 μ L of NPs were administered via a tail vein injection. Fluorescent images of mice were taken at varying time points using IVIS Lumina XR small animal imaging system. Mice were euthanized at 24 h after injection. Fluorescent images were taken using an excitation wavelength of 640 nm and a Cy5 emission filter. Tissues were fixed in 4% formal-dehyde and sectioned at 5 μ m. Tissue sections were stained with hematoxylin and eosin, or mounted with VectaShield fluorescent mounting medium containing DAPI (to stain for nuclei). Confocal microscope images were taken on a Nikon Eclipse Ti.

3. Results and Discussion

It has been shown that NP interaction with cells is mediated by their physicochemical properties.^{4,5,9,16–18} Changes in the cellular uptake of nanomedicines will vary due to surface charge and the type of nanomaterial. While NPs can improve circulation time and biodistribution of payloads, they must be internalized into cells to deliver target payloads and induce desired effects.¹⁹ Therefore, the effect of NP surface charge on cellular internalization was analyzed to understand how NP surface charge mediates the interaction between NPs and brain cancer cells. Using negatively charged carboxyl NPs and neutral charged methoxy NPs, the internalization of NPs into a variety of human cell types, both cancerous and healthy, was analyzed quantitatively and qualitatively after trypsinization to wash off surface-bounded NPs. After incubating NPs with cells for 24 h, negatively charged carboxyl NPs showed the highest uptake per cell compared to neutral charged methoxy NPs for all cell lines [see Fig. 1(a)]. Furthermore, both glioblastoma cell types (U-87 and U-138) showed the highest internalization of both types of NPs compared to all other cell types tested. Nonsmall lung cancer cells (NCI) demonstrated almost no uptake of either NP, an interesting characteristic compared to the other cell types. Finally, confocal microscopy was performed on U-87 glioblastoma cells to ensure that NPs were internalized within cells and not bound to the cellular membrane [see Fig. 1(c)]. Results showed that both NPs were internalized within cells, with an increased amount of internalized carboxyl NPs compared to methoxy NPs, which corresponds to quantitative data described earlier [see Fig. 1(a)]. To further study the interaction between NPs and cells, we expanded internalization studies to work with cellular spheroids that closely mimic the native 3D tissue structure [see Fig. 1(b)].

Investigations into the effect of particle zeta potential and cellular uptake show that uptake generally varies between cell types. He *et al.*²⁰ showed that murine macrophages showed an increase in the uptake of poly(styrene) NPs 150 nm in diameter as zeta potential decreased (–15 mV, –25 mV and –40 mV). Uptake of NPs also increased with increasing zeta

potential (+15 mV, +25 mV and +35 mV). Thus, with murine macrophages it would appear that the presence of charge is responsible for uptake. With normal human hepatocytes L02 and human hepatocarcinoma SMMC7721 the opposite trend was seen, where decreasing zeta potential (−15 mV, −25 mV and −40 mV) led to a decrease cellular uptake. Sahay *et al.*¹⁹ reviewed mechanisms of NP endocytosis, and it is apparent that NP uptake is mediated by nanomaterial, size, shape, surface properties and initial contact with cells.

Due to the high uptake of both NPs into U-87 glioblastoma cells, we assembled cellular spheroids with varying collagen contents to determine if extracellular matrix (ECM) barriers may hinder NP internalization into cells. The local ECM micro-environment mediates almost all cellular behavior through both direct and indirect mechanisms.^{21–23} Furthermore, the architecture and physical properties of tumor ECM are different than those of normal tissue.^{24,25} Collagen, a key ECM component, can be found below the basal membrane in a dense 3D fibrillar network, with orientation and collagen cross-linking varying based on tissue and tumor type.^{26,27} This rich network forms a dense structural barrier to NPs, with pore sizes estimated to be on the order of 150 nm.²⁸ Therefore, an understanding of how NP surface properties mediate ECM interactions within tumor tissues is needed. This information has potential use as a predictive model for NP diffusion into tumors possessing various ECM compositions. Results of NP uptake in cellular spheroids with varying collagen content showed that the surface charge of NPs mediates its ability to transverse an ECM barrier [see Fig. 1(b)]. NPs exhibited differences in cellular uptake based on ECM content, and negatively charged carboxyl NPs showed high uptake with low collagen content and low uptake with high collagen content. Furthermore, neutral charged methoxy NPs showed the opposite trend. Lieleg *et al.*²⁹ investigated the mobility through a model ECM of poly(styrene) microparticles 1 μm in diameter with negative carboxyl, neutral methoxy and positive amine surface charges. Differently charged liposomes approximately 165 nm in diameter were also investigated. Diffusivity of the neutral charged particles was significantly higher than the charged particles in the model ECM, suggesting that charge on a particle results in increased electrostatic interaction with ECM proteins. These results suggest that it is critical to understand the interactions of NPs with the biological environment and one type of NP will not be useful for every type of cancers and every stage of disease.

Histological analysis of a tumor tissue microarray was performed to gain a qualitative understanding of how collagen content varies based on tissue type and tumor stage (see Fig. 2). Brain and prostate tissues were chosen to emphasize the significant collagen content variations between tissue types, as tissue such as breast tumors have been associated with decreased collagen content through tumor progression.³⁰ Analysis showed that collagen content changes with tumor progression dependent on tissue type. Brain tissue demonstrated an increase in collagen content as the tumor developed from Grade 1 to Grade 3, showing little collagen content at Grade 1. On the contrary, prostate tissue had a high collagen content at Grade 1, but demonstrated very low collagen content at Grade 3. These results are consistent with other studies that have found increased collagen deposition during tumor formation.^{31–33} as well as a decrease in collagen content and deposition within other tumor tissues based on tissue type as well as age.^{30,34,35} These results confirm that collagen

content varies significantly between tumor tissue types, as well as within the stage of tumor development within a singular tissue type.

Paclitaxel (PTX), a common chemotherapy drug, has been previously loaded in PLA-PEG NP and shown to improve therapeutic efficacy.^{3,36,37} In fact, Genexol-PM, a PLA-PEG polymeric micelle formulation of PTX, is in Phase III clinical trials and approved for marketing and use in several countries. PTX-loaded PLA-PEG NP have been shown to increase the maximum tolerated dose (MTD) to 390 mg/m² compared to free PTX at 240 mg/m².^{37,38} Moreover, PLA-PEG with PTX was shown to have a higher MTD compared to Abraxane (300 mg/m²), a clinically approved albumin-bound PTX formulation.³⁹ Therefore, NPs can serve as a drug delivery vehicle to improve the efficiency of PTX such that the PTX payload has less interaction with the biological system until delivered to the intercellular space, thereby avoiding adverse effects on undesired cells. First, the release of PTX from both NP formulations was analyzed to determine their release profiles. Results showed that surface charge of NPs has no significant effect on PTX release from NPs, as both formulations presented a steady release over the course of 19 days in H₂O at 37° C [see Fig. 3(a)].

PTX-loaded NP were tested *in vitro* with U-87 cells to determine their therapeutic efficacy compared to free drug. First, studies were performed to confirm that unloaded NPs induced no adverse effects on cell viability (500 µg/mL, 24 h, Supplementary Fig. S1). Next, NPs were incubated with either free PTX or PTX-loaded NP for 6 h. Cell viability was measured via a Presto Blue assay at 72 h after initial exposure. With 6 h incubation, both COOH and OCH₃ NPs at doses of 25 µg/mL and 50 µg/mL were shown to be more effective than free PTX at 140 nM [IC-50, Fig. 3(b)]. No statistically significant differences were seen between different charges. Although *in vitro* uptake studies showed difference in NP internalization, these differences may not be significant enough to affect the therapeutic efficacy of NP delivered PTX.

NPs have been shown to passively accumulate in tumors due to fenestrated vasculature, reduced lymphatic development and decreased blood flow rate.^{40–42} We investigated whether NPs would passively accumulate in U-138 glioblastoma intracranial xenografts containing differing amounts of collagen (see Fig. 4). Mice were implanted with intracranial xenografts of U-138 glioblastoma suspended in Matrigel with or without 0.017 mg/mL collagen. Since glioblastomas have been shown to increase in collagen content at later stages, elevated collagen content was integrated into tumors to investigate how neutral charged NPs behave *in vivo* with tumors with varying collagen contents. After 10 days, fluorescently labeled OCH₃ NPs were administered via tail vein injection and NP biodistribution was determined using an IVIS Lumina XR small animal imaging system. Fluorescent images were taken at 1 h, 6 h and 24 h post injection. At 24 h, mice were euthanized and organs were explanted. Figure 4(a) shows the time course bio-distribution of mice that received saline or fluorescent NPs. In Fig. 4(a), mice either had matrigel tumors or matrigel with collagen. NPs were shown to accumulate in brain tumors, and at 24 h there is clear fluorescent signal from the brain. Qualitatively, OCH₃ NPs showed a stronger fluorescent signal than tumors that contained collagen. Explanted organs from mice with only Matrigel tumors [see Fig. 4(b)], and mice with Matrigel and collagen tumors [see Fig.

4(c)] showed strong fluorescent signal in the liver and spleen, indicating clearance via the reticuloendothelial system. Explanted brain tissue showed strong luminescent signal due to NP accumulating in tumors [see Fig. 4(d)]. OCH₃ NPs were shown to accumulate more in tumors containing only Matrigel, compared to tumors with collagen. This indicates that collagen may act as a physical barrier to the diffusion of NP into solid tumors. Histological analysis of mice that received fluorescent OCH₃ NP is shown in Fig. 5. Images were taken on the side containing the tumor, and the healthy contralateral side of the same specimen as a control [see Fig. 5(a)]. Imaging on the tumor side of the brain show a dense cell population in the hematoxylin and eosin stain, and confocal microscopy of tissue stained with DAPI (cell nucleus) show dense cell aggregates that also have fluorescent signal from the fluorescent NPs (red). Thus, *in vivo* biodistribution studies verify the accumulation of OCH₃ NPs in tumors.

4. Conclusions

In conclusion, the data presented shows clear differences in NP biological interactions due to surface properties. *In vitro* uptake studies showed that, across numerous cell lines, negatively charged COOH NPs were uptaken more readily than neutral charged OCH₃ NPs. These results were further validated in a 3D cellular spheroid model with U-87 glioblastoma. Uptake into U-87 was qualitatively shown with 2D confocal microscopy for OCH₃ and COOH NPs. Polymeric NPs were able to sustain the release of PTX over a 19-day period, and altering surface properties did not change drug release kinetics. Furthermore, with shorter incubation times *in vitro*, NPs were shown to be more effective against U-87 compared to free PTX. Penetration of NPs into tumors is influenced by interaction with systemic, extracellular and cellular barriers. NPs must navigate the ECM to penetrate solid tumors, and ECM composition varies between cancer type and stage. We showed that OCH₃ NPs more easily diffuse across ECM compared to negatively charged COOH. Finally, we showed that OCH₃ may be passively targeted to intracranial tumor xenografts via the enhanced permeability and retention (EPR) effect. The data described here begin to elucidate the complex problem of tailoring NP therapies that are able to navigate systemic, extracellular and cellular barriers. The results suggest that one NP formulation might not be suitable for all cancers and every disease stages.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Effect of NP surface charge on cellular uptake. The effect of NP surface charge on cellular interaction was determined in both 2D and 3D models. (a) Using both cancerous and healthy cells, results showed that the uptake of NPs into cells was dependent on both surface charge and cell type. Overall, negatively charged carboxyl NPs demonstrated the highest uptake in all cell lines compared to neutral charged methoxy NPs ($*p < 0.05$). Furthermore, both glioblastoma (U-87 and U-138) cell types demonstrated the highest uptake of all cell lines tested, while nonsmall lung cancer (NCI) demonstrated the least uptake. (b) Furthermore, the ability of NPs to transverse an ECM barrier was investigated as the ECM varies within tissues and tumors based on type and stage of progression. Using U-87 cellular spheroids with varying collagen contents, results showed that surface charge plays a large role in the ability of NPs to navigate an ECM barrier. Negatively charged carboxyl NPs showed high uptake with low collagen content and low uptake with high collagen content, while neutral charged methoxy NPs showed the opposite trend. (c) Finally, using U-87 cells, confocal microscopy was performed on samples to confirm that NPs were internalized within cells and not bound to extracellular membranes. Results showed that both types of NPs were internalized within cells membranes. Scale bar = 10 μ m.

Fig. 2.

Effect of tissue and tumor type on tissue collagen content. Analysis of collagen content within various tissue types at different stages of tumor progression was performed via histological analysis with Gomori's Trichrome (blue/green = collagen). Results showed that collagen content changes with tumor progression dependent on tissue type, with brain and prostate tissue shown as examples. Visual analysis shows that brain tissue exhibits low collagen content initially, but demonstrated increased collagen content as tumor developed from Grade 1 to 3. On the contrary, prostate tissue exhibited high collagen content initially, but demonstrated a gradual decrease in collagen content with tumor progression from Grade 1 to 3. Scale bar = 100 μ m.

Fig. 3.

Effect of NP surface charge on NP drug delivery. (a) NPs were successfully loaded with PTX and demonstrate controlled release from NPs with both surface charges over the course of 19 days. (b) Next, using PTX-loaded NPs, the effect of NP surface charge on NP drug delivery was determined using U-87 cells. Results showed no significant differences between NP surface charge, however NP formulations were more effective than free PTX.

Fig. 4. Biodistribution of NPs with intracranial tumor xenografts. (a) Time course bioluminescence images with intracranial tumor xenografts that contained only Matrigel (center) or Matrigel with collagen (right). Control mice had a Matrigel tumor and received a saline injection. OCH₃ NPs were administered via tail vein injection, and accumulation of NPs in tumor can be seen over 24 h. Explanted organs for the Matrigel only tumor (b) or the Matrigel with collagen tumor (c) showed accumulation of the NP in the liver and spleen, indicating clearance through the reticuloendothelial system. Organs shown are: (i) fat, (ii) spleen, (iii) liver, (iv) kidneys, (v) heart, (vi) lung, (vii) brain. (d) Fluorescent OCH₃ NPs were shown to slightly accumulate more in mice that only had Matrigel tumors, compared to Matrigel with collagen. Fluorescent signal was normalized and presented as a fold increase over the fluorescent intensity of mice that only had a saline injection.

Fig. 5. Histological analysis of NP accumulation in tumor xenografts. (a) Schematic representation of histological images that were acquired. Tumors were implanted approximately 2 mm anterior and 1 mm lateral of the bregma. Images were taken on the tumor side of the brain, and on the contralateral side as a control. (b) Hemotoxylin and eosin staining (left) showed that the tumor side of the brain had a dense population of cells not seen on the control side. Confocal microscopy of brain tissue stained with DAPI (cell nuclei in blue) showed the accumulation of red fluorescence from NP in the densely populated tumor tissue. Scale bars 1 100 μm .