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Polymeric Nanoparticles for Targeted Radiosensitization of Prostate Cancer Cells

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Abstract

One of the many issues of using radiosensitizers in a clinical setting is timing daily radiation treatments to coincide with peak drug concentration in target tissue. To overcome this deficit, we have synthesized a novel nanoparticle system consisting of poly (lactic-co-glycolic acid) (PLGA) nanoparticles conjugated with prostate cancer cell penetrating peptide-R11 and encapsulated with a potent radio-sensitizer 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (NU7441) to allow prostate cancer-specific targeting and sustained delivery over 3 weeks. Preliminary characterization studies showed that the R11-conjugated nanoparticles (R11-NU7441 NPs) had an average size of about 274 ± 80 nm and were stable for up to 5 days in de-ionized water and serum. The nanoparticles were cytocompatible with immortalized prostate cells (PZ-HPV-7). Further, the particles showed a bi-phasic release of encapsulated NU7441 and were taken up by PC3 prostate cancer cells in a dose- and magnetic field-dependent manner while not being taken up in non-prostate cancer cell lines. In addition, R11-NU7441 NPs were effective radiation sensitizers of prostate cancer cell lines *in vitro*. These results thus demonstrate the potential of R11-conjugated PLGA NPs as novel platforms for targeted radiosensitization of prostate cancer cells.

Keywords

Nanoparticles; radiosensitization; prostate cancer; NU7441; targeting

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1. Introduction

Prostate cancer remains one of the leading causes of cancer-related mortality in men with an estimated 233,000 new cases and 29,480 deaths expected by the end of 2014.¹ Conventional treatment methods for this disease include surgery or radiation therapy. Ionizing radiation therapy is an established method of treatment of localized prostate cancer; however, many high-grade tumors display radiation resistance and often recur after therapy. High-grade prostate cancers are often radiation resistant because of their increased DNA double strand break (DSB) repair ability, especially through Non-Homologous End Joining (NHEJ).² Previously, studies have shown that 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (NU7441) is a highly potent compound that can selectively inhibit DNA-dependent protein kinase (DNA-PKcs), a central regulator of NHEJ. As a result of DNA-PKcs inhibition, cells are unable to effectively repair DNA damage and die through various mechanisms.³ Although NU7441 has demonstrated excellent radiosensitization both *in vitro* and *in vivo*, this compound is limited by its poor solubility in water and reduced oral bioavailability.⁴ Therefore there is an urgent need for alternative methods that facilitate localized NU7441 delivery for effective radiosensitization.

In recent years there has been a push for localized therapy in hopes of reducing normal tissue effects as well as limiting side effects of the treatment. Nanoparticles (NPs) have become central players in the localized therapy push. NPs have been used successfully as radiation sensitizers; specifically, several studies have shown that gold can radiosensitize prostate cancer cells by arresting the cell cycle into G2/M.⁵ Similarly, Jin et al.⁶ have synthesized poly lactic-co-glycolic acid (PLGA) NPs encapsulating etanidazole (SR-2508), which successfully radiosensitized hypoxic HeLa cervical cancer and MCF-7 breast cancer cells. However, multifunctional NPs capable of delivering the radiosensitizer, imaging and cancer targeting of the prostate cancer cells have not been studied so far. Also, there have been no studies to the authors' knowledge on the development of NU7441-incorporated NPs for the potent radiosensitization of prostate cancers.

Therefore in this study, multifunctional PLGA-based NPs were synthesized for sustained release of NU7441 for targeted radiosensitization of prostate cancer cells. The FDA-approved biocompatible and biodegradable PLGA is a commonly used polymer in drug delivery and is known to provide a sustained release of the encapsulated agent over time both by degradation and diffusion.⁷ Iron oxide NPs were incorporated as contrast agents for Magnetic Resonance Imaging (MRI), for magnetic targeting and also to induce hyperthermia in the tumor using an external alternating magnetic field.^{8,9} To increase the target specificity of PLGA particles, the particles were further conjugated with R11-a polyarginine cell permeable peptide with prostate specificity,¹⁰ to form R11-NU7441 NPs. Studies conducted previously have already shown that the R11 peptides have higher specificity and uptake efficiency by prostate cancer cells in comparison with other cell-penetrating peptides such as TAT, KALA, penetratin and L-lysine K11.¹¹ Further, we have also previously demonstrated the significantly higher accumulation of R11-conjugated thermoresponsive NPs in the prostate tumor of NOD SCID mice, compared to other vital organs.¹² In this study, R11-conjugated PLGA-based NPs containing NU7441 were synthesized to selectively enhance the radiation sensitivity of prostate cancer cells.

2. Materials and Methods

2.1. Preparation of R11-conjugated, NU7441-loaded NPs

The PLGA magnetic NPs (PLGA-MNPs) were prepared using a single emulsion technique in which PLGA (Lakeshore Biomaterials, Birmingham, AL), bare iron oxide NPs (Meliorum technologies, Rochester, NY), and NU7441 (Tocris Biosciences, Minneapolis, MN) solution in dichloromethane (DCM) formed the organic/ oil phase. This mixture was then sonicated and added dropwise to 5% (w/v) poly vinyl alcohol (PVA) solution which was the aqueous/ water phase. The resultant solution was further sonicated at 50W for 3 minutes using a high-speed sonicator following which it was allowed to stir overnight to allow evaporation of DCM. This sample was then centrifuged and the pellet containing the NPs was freeze-dried. The supernatant obtained after centrifugation was saved to determine the amount of unloaded drug, against an NU7441 standard curve. This amount was subtracted from the original amount of drug used, to determine the total drug amount loaded in the NPs.

For preparation of R11-NU7441 NPs, 5 mg of the freeze-dried unconjugated NPs were taken and dissolved in 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH ~4.7). Then 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxy succinimide (NHS) was added with 30 minutes incubation following addition of each chemical. Lastly, 380 µg of R11 peptide was added and the solution was kept for shaking at room temperature overnight. The sample was washed and freeze-dried to obtain R11-conjugated NPs.

2.2. Characterization studies

Dynamic light scattering (DLS) (ZetaPals Analyzer, Brookhaven Instruments, Holtsville, NY) was used to study the size, surface charge and zeta potential of unconjugated and R11-NU7441 NPs. Briefly, the NP suspension was added to transparent cuvettes and inserted into the machine where scattering of laser light by the Brownian motion of NPs gave values for physical characterization of the particles. Additionally, a drop of NP suspension was added to a 200-mesh Formvar-coated copper grid (Electron Microscopy Sciences, Hatfield, PA) and air-dried for observation of NP morphology using Transmission Electron Microscopy (TEM, FEI Tecnai G2 Spirit BioTWIN, Hillsboro, OR). Further, Fourier Transform Infrared spectroscopy (FTIR, Bruker VECTOR 22 spectrometer, Madison, WI) was used to determine the successful conjugation of R11 and incorporation of iron oxide within the PLGA NPs. Iron content was determined based on a standard iron assay as described previously.¹³ Briefly, the NP solution was incubated with 30% (v/v) hydrochloric acid at 55°C for 2 h. Then ammonium persulfate (APS, 1mg/ml) and potassium thiocyanate (0.1 M) was added consecutively to the solution followed by 15 mins shaking. The iron concentration in the NPs was determined by obtaining absorbance readings at 520 nm, which was compared against iron oxide NP standards. A superconducting quantum interference device (SQUID, Quantum design, CA) magnetometer was also used to study the superparamagnetic behavior of iron oxide-encapsulated NPs. Briefly, the NPs were embedded in an epoxy gel and mounted on the instrument using a plastic straw. The behavior of the NPs in response to changing magnetic field at room temperature was determined.

Following initial characterization, the NPs were further studied for their stability and drug release kinetics. The NPs were suspended in de-ionized (DI) water and 10% Fetal bovine serum (FBS) in RPMI 1640 media for a period of 5 days at 37°C and particle size was measured at every 24 hour-time point using DLS. To study the drug release kinetics, 1 mg/ml NP suspension was added to 1000 Da Molecular weight cut-off (MWCO) dialysis bags (Spectrum laboratories, Rancho Dominguez, CA) and shaken at 37°C for 21 days. At pre-determined time points, 1 ml of the dialysate was removed and 1 ml of fresh DI water was added. The removed dialysate was stored at -20°C for further analysis. The amount of NU7441 released was analyzed against NU7441 standards (0.5, 0.25, 0.0125, 0.0625, 0.03125, 0.0156, 0 mg/ml) at an excitation/emission wavelength of 470/585 nm using a UV spectrofluorometer.

2.3. *In vitro* toxicity study

To study the toxicity of our NPs, a 96-well plate was first seeded with immortalized human prostate epithelial cells, PZ-HPV-7 at a seeding density of 15,625 cells/cm² and incubated at 37°C overnight. Cells were incubated with increasing concentration of R11-NU7441 NPs (0, 250, 500, 1000, 2000 µg/ml) and incubated at 37°C for 24 hours. The cells were washed thrice with 1× PBS and cell viability was assessed using MTS Assays (Promega Corporation, Madison, WI) per the manufacturer's directions. Briefly, 200 µl of media and 20 µl of MTS reagent were added to each well and the wellplate was incubated in the dark at 37°C for about 2 hours. Media in wells containing greater number of cells would be more purplish in color due to the bioreduction of the tetrazolium compound in MTS reagent into purple formazan crystals by the cells. Absorbance values of each sample was obtained at 490 nm using a spectrometer.

2.4. Rate of cellular uptake of NPs

The dose- and magnetic field-dependent uptake of unconjugated NPs and R11-NU7441 NPs by PC3 prostate cancer cells was assessed in the presence and absence of a 1.3 Tesla (T) magnet. The cells were seeded at a density of 12,631 cells/cm² in a 48-well plate and allowed to grow for 24 hours. The PC3 cells were seeded at lower seeding density to ensure that these rapidly dividing cells do not become overconfluent and die due to insufficient growth area, for the duration of the experiment. A 96 wellplate was used for cytotoxicity study above as only one assay (MTS assay) was required to be conducted for this study. For cellular uptake study, a greater volume of cell lysis samples was required as 2 assays (iron assay and BCA protein assay) were to be conducted to determine the amount of NPs and the amount of cell/ total protein per well. Therefore the 48 wellplate was used so that sufficient sample would be available for analysis. Following 24 hour incubation, the PC3 cells were exposed to increasing concentration of NP suspension (0, 100, 200, 300, 500, 1000 µg/ml) in media and incubated for 2 hours. To study magnetic field-dependence of uptake, the wellplate was placed directly above a 1.3 T external magnet to ensure that the cells are uniformly exposed to the magnetic field. These cells were then incubated at 37°C for 2 hours. The following day, the media was aspirated and the cells were washed with phosphate buffered saline (PBS) before being lysed using 1% Triton X-100. The iron oxide present within the cells was quantified using iron assay as explained previously.¹³ A Pierce BCA protein assay

was also performed to determine the amount of cell protein per well for normalization of iron oxide taken up by the cells.

2.5. Colony Formation Assay

Exponentially growing prostate and lung cancer cells were treated with either control or R11-NU7441 NPs for 4 hours in the absence of a magnet. After incubation, cells were washed and then treated with increasing doses of ionizing radiation (IR) (0, 2, 4, 6, and 8 Gy). Cells were then trypsinized and counted using a particle counter (Beckman Coulter, Inc., Brea, CA), diluted serially to appropriate concentrations and plated into 60-mm dish in triplicate. After 7 to 14 days of incubation, the colonies were fixed and stained with 4% formaldehyde in PBS containing 0.05% crystal violet. Colonies containing >50 cells were counted. Surviving fraction was calculated as $(\text{mean colony counts})/[(\text{cells plated}) \times (\text{plating efficiency})]$, in which plating efficiency was defined as $(\text{mean colony counts})/(\text{cells plated})$ for unirradiated controls. The data are presented as the mean $\pm$ SD of at least three independent experiments. The curve $S = e^{-(\alpha D + \beta D^2)}$ was fitted to the experimental data using a least square fit algorithm and the program Sigma Plot (Systat Software, Inc., San Jose, CA). The radiation dose enhancement ratio (DER) was calculated as the dose (Gy) for radiation alone divided by the dose (Gy) for radiation plus drugs (normalized for drug toxicity) for a surviving fraction of 0.25.

2.6. DNA Double Strand Break Repair Assay

DSB repair assay was performed by counting phospho- γ H2AX foci after IR, NP and IR+NP treatment. These cells were plated on poly-lysine-coated coverslips, were allowed to attach overnight and then treated with either control or R11 conjugated NU7441 NPs for 4 hours in the absence of a magnet, followed by washing with PBS and irradiated at 2 Gy. Cells were fixed in 4% formaldehyde/PBS for 30 minutes, permeabilized in 0.5% Triton X-100 in PBS for 1 hour, and blocked in 5% bovine serum albumin and 1% normal goat serum for 1 hour at room temperature. Then, cells were incubated with the primary antibody, anti-phospho-Histone γ H2AX (Ser139; 1:2,000) for 1 hour. Rhodamine red-conjugated goat anti-mouse was used as secondary antibodies. Cells were mounted in a Vectashield mounting medium containing 4', 6-diamidino-2-phenylindole (DAPI). Phospho- γ H2AX foci were examined using a fluorescence microscope (CRG Precision Electronics, Houston, TX). The number of phospho- γ H2AX foci was determined at each time point (average of 100 nuclei), and the percentage foci remaining was plotted against time to obtain DSB repair kinetics. Data is represented as mean $\pm$ SEM.

3. Results

3.1. Characterization of R11-NU7441 NPs

An average diameter of 265.5 ± 64.0 and 274.13 ± 79.96 nm was observed for the unconjugated NPs and R11-conjugated NU7441 NPs respectively. The polydispersity values in the range of 0.12-0.16 indicated that the particles are uniformly dispersed with minimal size variation. Additionally, high zeta potential values of -17.39 ± 0.58 and -24.52 ± 0.54 indicated that both the conjugated and non-conjugated NPs are very stable and do not aggregate. The TEM images concurred with DLS results and indicate that our NPs are

spherical in shape with iron oxide presented in the particles (Fig. 1A). Iron assay results indicated the presence of 8.44% iron content within our R11-NU7441 NPs, which could be attracted towards an external magnet (1.3T) (Fig. 1B).

The NPs were further characterized for the presence of functional groups that can confirm peptide conjugation and inclusion of iron oxide, using Fourier Transform Infrared spectroscopy (FTIR). The peak at $\sim 590\text{ cm}^{-1}$ confirms presence of Fe-O group. The NH_2 peak at 1420 cm^{-1} and NH peak at 1500 cm^{-1} is characteristic of Arginine present in R11 peptide indicating successful R11-conjugation. The CH_2 (2850 cm^{-1}) and C=O peaks (1740 cm^{-1}) are characteristic of PLGA. The C=O peak can also represent O=C-N-H bond formed during EDC-NHS chemistry for R11 conjugation (Fig. 1C). Further, analysis of SQUID measurements indicated that our particles maintained their superparamagnetic behavior although a decrease in saturation magnetization to 48.64 emu/g was observed (Fig. 1D). A remanence and coercivity of 6.21 (Mr/Ms) and 85.009 Oe respectively was observed for the NPs in comparison to 6.7 (Mr/Ms) remanence and 75.556 Oe coercivity of bare iron oxide NPs.

Results from the stability studies showed that there was no statistically significant change in particle size of the R11-NU7441 NPs in DI water and 10% FBS over a period of 5 days (Fig. 2). This indicates that our particles are relatively stable and are not expected to aggregate under physiological conditions.

3.2. Drug loading and release kinetics

The release of NU7441 from our NPs was monitored over a period of 21 days. The particles showed a loading efficiency of 51.89% for NU7441. A burst release of about 41% of the encapsulated drug was observed within 1 day of the study, followed by sustained release of 90.48% of the encapsulated NU7441 from the NPs within 3 weeks (Fig. 3).

3.3. *In vitro* cell studies on R11-NU7441 NPs

Cytocompatibility studies showed greater than 80% PZ-HPV-7 viability up to an NP concentration of $1000\text{ }\mu\text{g/ml}$ (Fig. 4A). Viability at $2000\text{ }\mu\text{g/ml}$ concentration was significantly less than the control group, although $\sim 80\%$ of the cells were still viable. This demonstrates that our R11-NU7441 NPs are cytocompatible with no toxic effects observed even at a high concentration of $1000\text{ }\mu\text{g/ml}$.

Further, cellular uptake of unconjugated NPs by PC3 cells in the presence of a magnet was found to be significantly lower than uptake of R11-NU7441 NPs both in the presence and absence of a magnet. All particles were taken up by PC3 cells in a dose-dependent manner; however, the highest uptake was observed for R11-conjugated NPs in the presence of an external magnetic field (1.3T) (Fig. 4B). This indicates that although R11 conjugation significantly improves NP uptake by PC3 cells, an external magnetic field can facilitate greater cellular uptake of the R11-NU7441 NPs.

3.4. DNA DSB Repair Kinetics

To test both the efficacy and specificity of R11-NU7441 NPs, both lung and prostate cancers were treated with NPs to determine the specificity of R11 targeting and the efficacy of NU7441 release. In H460, a non-small cell lung cancer cell line, treatment with R11 conjugated NPs and radiation did not result in decreased DNA DSB repair kinetics (Fig. 5A). However, in PC3, a prostate cancer cell line, treatment with R11 conjugated NPs and radiation resulted in significantly slower DNA repair kinetics (Fig. 5B). The results of this experiment imply that R11-NU7441 NPs will specifically target prostate cancer cells and also radiosensitize these cells.

3.5. Colony Formation Assay

To further explore the radiosensitizing ability of R11-NU7441 NPs, lung and prostate cancer cells were treated with radiation and a dummy particle containing no NU7441 or radiation and R11-NU7441 NPs. As with the DNA DSB break repair assay H460 cells show no radiation sensitization (Fig. 6A), while PC3 cells show a prominent radiation sensitizing effect (Fig. 6B).

4. Discussion

Recent years have shown a growing interest and demand for multifunctional “theranostic” NPs capable of simultaneous diagnosis and treatment of the disease of interest. In this study, we have synthesized novel multifunctional R11-conjugated PLGA NPs encapsulating MNPs and NU7441 for targeted delivery, imaging and radiation sensitization of prostate cancer cells. PLGA NPs encapsulating various payloads have often been used for numerous cancer-based applications. For example, curcumin-loaded PLGA NPs (81 nm) have been successfully used to inhibit cellular proliferation in DU145 prostate cancer cells *in vitro* and have shown greater bioavailability than free curcumin when administered *in vivo*.¹⁴ Lipid-coated PLGA NPs (~ 207 nm) containing KIF11 small interfering RNA (siRNA) could successfully reduce cell viability of PC3, LNCaP and DU145 prostate cancer cells *in vitro*.¹⁵ Also as mentioned earlier, PLGA NPs have played key roles in radiation sensitization of cervical and breast cancer cells using a combination of drugs.^{6,16}

Although the average diameter of our R11-NU7441 NPs is >200 nm, particles in this size range have been used previously by other groups for cancer therapy. For example, Alendronate-coated PLGA-iron oxide NPs (235 nm) were synthesized by Thamake et al.¹⁷ for specific targeting of bone metastasis of breast cancer. *In vivo* studies on athymic female nude mice demonstrated that these particles showed high localization to the bone and could significantly reduce tumor-induced bone resorption as well as tumor growth rate. Our lab has also previously synthesized core-shell NPs of 296 nm diameter for melanoma targeted therapy. These particles showed significantly higher localization than unconjugated NPs, in the tumor of B16F10 melanoma orthotopic mouse model indicating that they can be used for targeted melanoma therapy.¹⁸ Our particles also maintained the superparamagnetic behavior of iron oxide, although a slight decrease in saturation magnetization (48.64 emu/g) is noted in R11-NU7441 NPs. This result is expected due to the presence of the polymer which tends to generate a diamagnetic moment in response to the applied magnetic field.^{18,19} However,

NPs having similar or lower magnetization values have been used previously for various drug delivery applications with some degree of success,^{20,21} indicating that our particles remain within the range suitable for targeted drug delivery. Our stability study results concur with previous studies on PLGA NPs showing good stability and minimal aggregation over a period of 5 days.²² Also, the drug release kinetics of our particles showed a sustained release similar to that obtained by Jia et al.²³ In their study, doxorubicin-loaded PLGA-MNPs showed a burst release for the initial first day followed by sustained release for 7 days. This characteristic bi-phasic release was also observed in curcumin-loaded Alendronate-coated PLGA NPs for active targeting of breast cancer.¹⁷

Following the physical and chemical characterization of our R11-NU7441 NPs, *in vitro* studies were carried out to study cytocompatibility and cellular uptake in the presence or absence of a magnet. Greater than 80% PZ-HPV-7 viability was observed when incubated with all NP concentrations, which is in agreement with previous studies demonstrating the cytocompatibility of PLGA NPs with LAG mouse fibroblast connective tissue²⁴ and 3T3 fibroblasts.²⁵ Additionally, dose-dependent cellular uptake of our NPs by PC3 cells was observed, similar to observations by Akbarzadeh et al.²⁶ and Davda et al.²⁷ using Folate-PLGA-PEG NPs. The significantly higher magnetic-field dependent uptake of our particles by PC3 cells agrees with results obtained by Wadajkar et al.,²⁸ where their biodegradable photoluminescent polymer (BPLP)-coated iron oxide NPs were also significantly uptaken by PC3 in the presence of a 1.3T magnet. The R11 conjugation onto the surface of our NPs also played a key role in active targeting and uptake of NU7441-loaded particles by prostate cancer cells. Our results concur with studies by Patel et al.²⁹ which showed higher dose-dependent uptake of R11-conjugated PLGA NPs by PC3 cells when compared to NPs conjugated with folic acid and RGD-4C.

Previously, we have demonstrated preferential uptake of R11 peptide by the prostate cancer cells and prostate tissues.^{30,31} In addition, Hao et al.³⁰ performed a comparative PET-CT imaging experiment by using prostate (PC3) and lung (H2009) tumor-bearing mouse models and confirmed that the absolute uptake of ⁶⁴Cu-DOTA-NHGR11 in the PC3 tumors was significantly higher than in the lung (H2009) tumors throughout the imaging study. Using the colony formation assays, we show that R11-NU7441 NPs radiosensitize only prostate cancer cell lines. Furthermore, this enhanced radiation sensitivity in prostate cancer cells can be attributed to slower rate in DNA DSB break repair process due to the preferential uptake of R11-NU7441 NPs in prostate cancer cells.

In addition to the targeted delivery of radiosensitizers to prostate cancer cells presented in this work, NPs encapsulating radiosensitizers have also been used for other various cancer cells. For example, HeLa and MCF-7 have been successfully radiosensitized using PLGA NPs containing the hypoxic radiosensitizer etanidazole (SR-2508).⁶ Similarly, PLGA NPs loaded with paclitaxel, which acts as both an anti-cancer drug as well as a radiosensitizer, has been effectively used *in vitro* against MCF-7 cells.³² Also PLGA NPs containing curcumin have been developed and effectively used for chemoradiation sensitization of A2780CP ovarian cells.³³ However to the authors' knowledge, there have been no attempts so far to synthesize NPs for prostate cancer-specific active targeting and imaging *in vivo* as well as for providing a sustained release of a potent radiosensitizer for treatment of localized

prostate cancer. This paper demonstrates that R11- conjugation on our NPs provides localized therapy. The R11 specifically targets the laminin receptor on prostate cancer cells resulting in a comparatively higher uptake of the NPs by these cells, compared to other cells in the body. The sustained release of encapsulated NU7441 from the PLGA NPs over time prevents DSB repair within the prostate cancer cells thus sensitizing them to radiation. Incorporation of imaging agents such as iron oxide assists in NP tracking and imaging using MRI as well as magnetic targeting to the cancer site using an external magnet. Therefore, the combination of PLGA NPs conjugated with R11 peptides and containing NU7441 radiosensitizer along with a suitable imaging agent would be beneficial in comparatively accurate tracking, targeting and imaging of the NPs as well as in effective radiosensitization of prostate cancer cells.

5. Conclusions

In this work, we have synthesized R11-conjugated PLGA-based NPs encapsulating NU7441 for targeted radiation sensitization of prostate cancer cells. The particles were stable and cytocompatible with a sustained NU7441 release profile for a period of 21 days. Additionally, the PC3 prostate cancer cells showed dose- and magnetic-dependent cellular uptake of our particles. The particles also showed good targeting capabilities *in vitro* by specifically inhibiting DNA DSB repair kinetics of PC3 cells while not affecting the H460 cells. While these particles require further testing, they have demonstrated a great potential for clinical application of radiation sensitizing strategy by sustained release of the NU7441 over time thus improving its solubility and bioavailability. Future studies will involve testing the radiosensitizing capabilities of the R11-NU7441 NPs *in vivo* in prostate tumor-bearing animals.

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Fig. 1.

(A) TEM image representing spherical PLGA-iron oxide nanoparticles (~ 260 nm) with iron oxide present on the core and surface of the particles. (B) Photographs demonstrating the recruitment of the R11-NU7441 NPs towards a 1.3T external magnet. (C) FTIR spectra indicating successful conjugation of R11 and incorporation of iron oxide onto the NPs. (D) Comparison of hysteresis loops of R11-NU7441 NPs and bare MNPs demonstrating the existence of superparamagnetic behavior of both in the presence of a magnetic field.

Fig. 2.

Comparison of stability of R11-NU7441 NPs in DI water and 10% FBS showing no significant change in particle size for 5 days at 37°C thus demonstrating that the NPs are stable and do not aggregate in these solutions (n=4).

Fig. 3. NU7441 release profile from the NPs demonstrating a bi-phasic release which includes initial burst release of 41% of NU7441 within 1 day followed by sustained release for 3 weeks (n=4).

Fig. 4.

(A) Cell viability study showed that the R11-NU7441 NPs were cytocompatible up to a concentration of 1000 µg/ml with more than 80% cell viability noticed at all concentrations (n=4, *p<0.05 w.r.t. control). (B) Cellular uptake of unconjugated NPs in the presence of a 1.3T external magnet. Uptake of R11-conjugated NPs in the presence (1.3T) and absence (0 T) of an external magnet and at varying NP concentrations showing dose- and magnetic field-dependence of cellular uptake of these NPs (n=4, *p <0.05 w.r.t. R11-NU7441 NP uptake, #p <0.05 w.r.t. uptake of unconjugated NPs).

Fig. 5.

DNA DSB repair kinetics in **(A)** lung cancer (H460) and **(B)** prostate (WT PC3) cells using radiation therapy (RT) (Blue Bar), R11-NU7441 NPs (Red Bar) and R11 NPs (control particle, Green Bar). Cells were treated as indicated and washed before RT (2 Gy). Samples were collected at the indicated time points stained with H2AX foci. Foci counts represent the average of at least 100 nuclei.

Fig. 6. Surviving fraction analysis using R11-NU7441 NPs. (A) H460 cells as well as (B) PC3 cells were treated with increasing dosages of NPs followed by treatment with radiation as indicated.