Nanoparticle-Based Brachytherapy Spacers for Delivery of Localized Combined Chemoradiation Therapy

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Summary

This work describes the creation of brachytherapy spacers for in situ release of drug eluting nanoparticles to provide tissue residence and sustained drug release with time constants appropriate for permanent brachytherapy. The in vivo behavior of spacers was studied by optical fluorescence imaging of mice implanted with spacers releasing free dye molecules or dye eluting nanoparticles, and demonstrated sustained release for synchronous chemoradiation therapy to enhance the therapeutic ratio.

Purpose: In radiation therapy (RT), brachytherapy-inert source spacers are commonly used in clinical practice to achieve high spatial accuracy. These implanted devices are critical technical components of precise radiation delivery but provide no direct therapeutic benefits.

Methods and Materials: Here we have fabricated implantable nanoplatforms or chemoradiation therapy (INCeRT) spacers loaded with silica nanoparticles (SNPs) conjugated containing a drug, to act as a slow-release drug depot for simultaneous localized chemoradiation therapy. The spacers are made of poly(lactic-co-glycolic) acid (PLGA) as matrix and are physically identical in size to the commercially available brachytherapy spacers (5 mm × 0.8 mm). The silica nanoparticles, 250 nm in diameter, were conjugated with near infrared fluorophore Cy7.5 as a model drug, and the INCeRT spacers were characterized in terms of size, morphology, and composition using different instrumentation techniques. The spacers were further doped with an anticancer drug, docetaxel. We evaluated the in vivo stability, biocompatibility, and biodegradation of these spacers in live mouse tissues.

Results: The electron microscopy studies showed that nanoparticles were distributed throughout the spacers. These INCeRT spacers remained stable and can be tracked by the use of optical fluorescence. In vivo optical imaging studies showed a slow diffusion of nanoparticles from the spacer to the adjacent tissue in contrast to the control Cy7.5-PLGA spacer, which showed rapid disintegration in a few days with a burst release of...
Introduction

With an estimated 233,000 new cases of prostate cancer anticipated in 2014 in the United States alone, prostate cancer is the most frequently diagnosed cancer and second leading cause of cancer death in men (1). The treatment choices are primarily based on the prostate-specific antigen levels, biopsy grade (Gleason score), and clinical stage of the disease (2). Radiation therapy involving brachytherapy may be used as monotherapy for early-stage disease or in conjunction with other therapies for more advanced disease (3-5). Data from 2 randomized trials suggest that increased radiation dose is associated with improved cancer control, but radiation dose escalation is generally limited by toxicity from receipt by the rectal wall and urethra of dose levels beyond tolerance. After initial radiation for localized prostate cancer, between 20% and 30% of men may experience prostate-specific antigen failure (6, 7). For those with local-only recurrence, salvage brachytherapy offers a second chance for cure but leads to a high rate of rectal toxicity, including up to 13% rate of prostate-rectum fistulas requiring colostomy in a prospective phase II study (8, 9). Inert biocompatible spacers (brachytherapy), which are frequently implanted for controlling the spatial distribution and accuracy of radiation to the prostate, are critical technical components for radiation delivery but have zero direct therapeutic benefits (10-13).

Several chemotherapy agents are also radiosensitizers (14, 15). The chemotherapy schedules generally involve weekly intravenous delivery with elimination timescales on the order of 24 hours, implying that less than half the radiation fractions in a week are delivered with agent circulating. Chemotherapy dose is limited by systemic toxicities that usually prevent the use of chemotherapy through the entire course of radiation. A means of delivering focal distributions of the chemotherapy agent to the prostate without systemic delivery could increase the synergistic effect of radiation and chemotherapy agent and improve the therapeutic ratio.

It has been proposed that the fiducials and brachytherapy spacers offer the opportunity for in situ delivery of drugs as part of minimally invasive radiation therapy procedures that are currently routine (11). Modeling of the drug distributions that could be achieved by transforming the inert fiducials or spacers into drug reservoirs, capable of sustained drug release over periods appropriate to radiation therapy, indicate that significant portions of the prostate could be radiosensitized while a localized drug distribution is maintained. Thus, there exists an opportunity to fabricate drug-loaded spacers in such a way that it improves the therapeutic ratio of the brachytherapy procedure by delivering radiosensitizer to the prostate without systemic toxicity. We previously conducted a theoretical evaluation of prostate brachytherapy spacers containing slow-release polymers that elute the radiosensitizer docetaxel (11). The use of nanoparticles facilitates local delivery of therapeutic in high doses to the tumor, slow and sustained release of the drugs, incorporation of imaging agents, minimal systemic drug toxicities to nontargeted organs, and greater spatial distribution of the drug in the tumor (16-19).

Here we report a novel fabrication approach for the INCeRT spacers in which the biocompatible matrix composed of poly(lactic-co-glycolic) acid (PLGA) polymer forms the backbone of the spacers which contains embedded silica nanoparticles in the matrix as drug/fluorophore depot. These spacers are identical to the routinely used brachytherapy spacers in shape and size but with an additional therapeutic and diagnostic value. We have extensively explored the use of SNPs loaded with near infra-red (NIR) fluorophore Cy7.5 as a model drug that can be visualized in vivo. We have also fabricated the docetaxel-loaded spacers to evaluate the therapeutic response of these INCeRT spacers. The spacers were characterized by optical fluorescence studies, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy dispersive spectroscopy. The preliminary in vivo data obtained with these implanted INCeRT spacers show their great potential in developing novel therapeutic strategies, leading to localized drug delivery to tumor. This would result in preferential radiosensitization of the prostate compared with the normal structures, which can be used in combination with radiation therapy to maximize the therapeutic effect.

Methods and Materials

The spacers were designed to be compatible with a preloaded needle brachytherapy technique. The diameter was chosen to fit within an 18G brachytherapy needle. The composition and length were chosen so the spacer would be rigid during insertion to ensure appropriate spacing of the brachytherapy implant.
sources, and the spacer will degrade over time once implanted into the tissue, releasing the drug locally. The details of materials used and methods for SNPs and spacer synthesis, the physicochemical characterization of the nanoparticles, and INCeRT spacers, imaging setup and analysis, and in vivo studies are included in the supplementary information (available at www.redjournal.org).

Methods

Fabrication of INCeRT spacers

The synthesis of Cy7.5 SNPs was carried out by the oil-in-water microemulsion method, according to a previously reported protocol with several modifications (20, 21). The fabrication of spacers was carried out in 2 steps: (1) extraction of SNPs from the aqueous media and (2) polymer extrusion along with nanoparticles (see supplementary information, available online at www.redjournal.org).

Results

Figure 1a shows the schematic presentation of the structure of INCeRT spacers fabricated with PLGA matrix impregnated with SNPs. These spacers were fabricated with a range of different molecular weights of PLGA polymer with a combination of nonpolar solvent systems. The slurry made with PLGA and SNPs was extruded in a controlled manner in silicon tubing with an internal diameter similar to the diameter of the commercial brachytherapy spacers. These nanoparticles-doped INCeRT spacers were cut into lengths similar to those of commercial spacers (5 mm).

Figure 1b shows the images of the fabricated INCeRT spacers compared with the commercially available spacers. All these spacers were identical in terms of morphology, dimensions, and structural integrity. The dual-release mechanism involves the dissolution of PLGA in biological fluid to release the nanoparticles in extracellular space, after which in a second phase these nanoparticles, upon interaction with cellular fluids, will start releasing the encapsulated drug in a sustained manner at the target site (22). The SNPs platform provides a method for delivery of drugs and imaging agents, with ease of surface modification, covalent conjugation to fluorophores, and size modulation (20). The rationale behind using PLGA as a matrix polymer lies in its biocompatibility, biodegradability, and low glass transition temperature, which have been studied previously as a host matrix for different chemotherapeutics in implants (23, 24). The fluorescence from the conjugated Cy7.5 helped track the nanoparticles, which otherwise was very difficult with drug-loaded nanoparticles (25, 26).

The release profile of the Cy7.5 from Cy7.5 spacers in buffer showed a gradual increase in fluorescence intensity until day 4 in contrast to the Cy7.5 silica spacer, in which the fluorescence intensity continued to increase during 7 days. Also, the absence of fluorescence in the flow-through indicated covalent conjugation of Cy7.5 to SNPs and thus tracking of intact nanoparticles (Fig. E1, available at www.redjournal.org). Figure 2 shows the time-space profile of the different formulations (free dye, 30-nm Cy7.5 SNPs, and 200-nm Cy7.5 SNPs) in agar phantoms based on diffusion through the agar matrix over time. The result showed a rapid diffusion of free dye within 1 hour, whereas 30-nm and 200-nm SNPs showed size-dependent diffusion through the matrix during 15 days.

The characterization data of the Cy7.5 SNPs showed a typical fluorescence emission peak at $\lambda_{\text{max}}$ 820 nm, with a

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**Fig. 1.** (a) Schematic representation of the modified brachytherapy spacer formulated using poly(lactic-co-glycolic) acid (PLGA) polymer impregnated with Cy7.5-labeled silica nanoparticles (NPs). (b) Physical appearance of synthesized modified brachytherapy spacers with different formulations of silica NPs and comparison with commercial spacers used in clinics.
slight bathochromic shift for conjugated nanoparticles (Fig. E2a, available at www.redjournal.org), which can be attributed to slight aggregation of the dye molecules in the core of Cy7.5 SNPs (27). The TEM image of SNPs showed a spherical morphology with a size of 254 ± 5 nm (average of ~100 nanoparticles) with a unimodal distribution (Fig. E2b, available at www.redjournal.org). The dynamic light scattering (DLS) data also confirmed the size to be 260 ± 8 nm (data not shown). This slight increase in size can be attributed to the hydrodynamic diameter of the SNPs (28). For both the formulations (encapsulated or conjugated Cy7.5), as expected the surface charge remained the same (ie -25 mV).

The pore size for the 250-nm silica nanoparticles was previously reported to be approximately 2.3 nm (29). The size of 250 nm was selected to increase the percentage loading of the drug per nanoparticle; however, we have also tested smaller nanoparticles (30 nm), which have a higher capability to penetrate the tumor matrix.

After incorporation of the SNPs into the PLGA matrix, the INCeRT spacers were analyzed by SEM for surface morphology analysis and the distribution pattern of the SNPs into the PLGA matrix. Figures 3a and 3b show the SEM image of the lateral and transverse surfaces of the fabricated Cy7.5 SNPs spacers. The SEM image showed a solid structure without any air spaces or vacuoles, with a smooth outer surface. At a higher magnification, it can be visualized from the lateral view that the SNPs were embedded in the PLGA matrix in patches all along the spacer length (Fig. 3c). The transverse section showed that nanoparticles accumulated more toward the core of the spacer. To qualitatively differentiate between the electron-dense SNPs (relative to PLGA polymer) from PLGA, we used a back scattered electron imaging technique (Fig. 3d). The increase in contrast between the silica patches in the dark PLGA background confirms the presence of SNPs. To confirm it further, we carried out energy dispersive spectroscopy analysis by selecting various regions of interest on the spacers (bright patches), and the results showed the presence of silicon in the region of interest along with carbon and oxygen (Fig. 3f).

Further, to see the distribution pattern and visualize the nanoparticles inside the PLGA matrix, we used the freeze-fracture technique by flash-freezing the spacers in liquid N₂ and fracturing them. Figure 3e clearly shows that the fractured surface has well-defined spherical grooves. We believe that these grooves are the impressions of SNPs that were shredded or fell off while the spacers were fractured. The average size of the individual grooves was 260 nm, which very well correlates with the TEM size of the SNPs, indicating minimal aggregation of SNPs. Further, to confirm our technique for visualizing nanoparticles in the PLGA matrix by this method, we doped electron-dense gold nanoparticles in the PLGA matrix. The results clearly indicated that although the nanoparticles were present in patches in the PLGA matrix, the aggregation between nanoparticles was minimal (Fig. E3, available at www.redjournal.org).

Live animal fluorescence imaging studies were carried out with mice implanted with INCeRT spacers with approved Institutional Animal Care and Use Committee protocols (Fig. 4). The fluorescence intensity of the control spacer (Cy7.5 spacer; upper right) kept decreasing and completely disappeared by day 14. This can be attributed to the rapid release of Cy7.5 incorporated in the PLGA matrix over time, which resulted in a decrease of local Cy7.5 concentration in the spacer. The released Cy7.5 from the spacer, which was distributed around the spacer, was difficult to measure because the intact skin provided a barrier for the excitation light to penetrate and excite such...
low concentrations of Cy7.5. The quantification of the fluorescence intensity at half width maximum for the lower left Cy7.5 SNPs spacer showed a constant increase for the nanoparticles-doped spacer within the same time duration of 14 days (Fig. 4b). From the figure it can be clearly visualized that Cy7.5 fluorescence is very stable and localized around the spacer, suggesting a slow diffusion of SNPs from the PLGA matrix. Also, it is worth noting that for the spacers with Cy7.5 SNPs, we tracked the nanoparticles rather than free dye because the dye molecules were covalently conjugated into the silica core.

The spacers implanted in the upper left flank of the mice showed a result very similar to that observed for the lower left spacer. Despite the minimal diffusion, stable fluorescence intensity suggests that the release of the nanoparticles from the spacers and the degradation of the PLGA were not very rapid, and the silica-doped spacers maintained structural integrity in both the intradermal and the intratumoral environments. The quantitation of the intensity from the figure indicated a constant increase in intensity for Cy7.5 SNPs spacers for 14 days, in contrast to the Cy7.5 spacer, which kept decreasing over the same time frame. We further carried out preliminary size-dependent diffusion of nanoparticles (30-nm and 200-nm SNPs in spacers), which also confirmed minimal diffusion for larger nanoparticles (Fig. E4, available at www.redjournal.org).

Figure 5 shows the fluorescence images of the dissected animal by removing the skin and exposing the implanted

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**Fig. 3.** Scanning electron micrograph images of the spacers doped with Cy7.5 silica nanoparticles (SNPs). (a) Lateral surface. (b) Transverse view. (c) Magnified lateral view. (d) Back-scattered electron image of lateral section. (e) Spacer freeze-fractured by using liquid N2 (magnified area shows SNP impressions). (f) Energy dispersive spectroscopy spectra show the presence of silicon in the region of interest.

**Fig. 4.** (a) In vivo optical fluorescence imaging of live mice implanted with Cy7.5 silica nanoparticles (SNPs) and Cy7.5 spacers. (b) Quantification of fluorescence intensity.
spacers. The lower left spacer (Cy7.5 SNPs spacers) showed localized fluorescence, which can be attributed again to the intact spacer; however, for the upper right flank spacer (Cy7.5 spacer) the fluorescence emission can be observed from a wide area around the spacer. Also, it was interesting to observe that although the left flank spacer maintained its integrity, the upper right flank spacer looked like a white thread and altogether dissociated into small pieces. Also, it was very encouraging to observe the fluorescence emission from the muscles where the lower left spacer was implanted. Because the spacer was still attached to the skin (Fig. 5c) and the area exactly under that showed the emission, it clearly indicates that small populations of nanoparticles were released from the spacer. Also, inasmuch as Cy7.5 was conjugated to nanoparticles, the fluorescence emission has to be from the released nanoparticles. The fluorescence images of tumor showed a bright fluorescence from the location where the spacer was implanted, and when we dissected the tumor laterally (lower panel) along the axis from where the fluorescence was originating, we observed that fluorescence was coming from the spacers, with only very low fluorescence from the surrounding tissue (Fig. 5e).

The pilot in vivo therapeutic studies with docetaxel INCeRT spacers implanted in tumors showed suppression of tumor growth, in contrast with control mice, in which the tumor size kept increasing (Fig. E5, available at www.redjournal.org). The free docetaxel group also showed tumor growth suppression but was associated with weight loss in all mice, reflecting systemic toxicity associated with free drug (Fig. E6, available at www.redjournal.org).

**Discussion**

We have demonstrated the feasibility of creating dual-release brachytherapy spacers that have the capacity to deliver drugs to the target without intravenous delivery. This feasibility of using different ways of incorporating chemotherapeutic and imaging agents provides an additional advantage for localized delivery of the chemotherapeutic agent by encapsulation in silica matrix and for optical tracking of drug-loaded nanoparticles through a covalently conjugated fluorophore like Cy7.5 (30, 31).

Based on the results obtained, a detailed in vivo study with spacers fabricated with different sizes of nanoparticles, varying molecular weights of PLGA, and further incorporation of chemotherapeutic drugs is currently under way in a model of tumors in mice. Radiosensitization of the target without simultaneously sensitizing the surrounding normal tissues requires the ability to control both the spatial and the temporal distribution of the drug within the tumor (11). Combined chemoradiation therapy requires synchronization of the 2 modalities to achieve maximum benefit (32, 33). Several drugs are being currently tested as radiosensitizers with external beam radiation therapy (EBRT) in a large ongoing multinational randomized trial (34). However, the systemic administration of these drugs has several drawbacks: high systemic toxicity, fast pharmacokinetics, limited periods of radiosensitization that are not synchronized with the radiation dosage, and poor spatial distribution in tumor (35-38). The use of nanoparticles-doped spacers for localized drug delivery will provide

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**Fig. 5.** Optical fluorescence images of the dissected mice 14 days after spacer implantation. (a, b) Upper flank shows left Cy7.5 silica nanoparticles (SNPs) spacer and right Cy7.5 spacer. (c, d) Hind flank: left, Cy7.5 SNPs spacer implanted intradermally; right, implanted intratumorly. (e) Dissected tumors from mice to locate implanted spacer inside tumor (above) and lateral section to visualize SNPs distribution (below).
several advantages over the conventional systemic delivery as described earlier (39-42).

The 2-stage release platform presented here allows the different time constants for the release of nanoparticles from the spacer and the release of drug from the nanoparticles. The use of nanoparticles provides a tissue residence measured in weeks, making sensitization of permanent brachytherapy implants feasible. Once the nanoparticles are released from the spacer, controlling their size enables optimization of the spatial distribution. The 2-stage process should enable this approach to be used for a variety of radiosensitizing agents, including gold nanoparticles (43). The release profile of the nanoparticles from the spacers can be modulated by varying the molecular weight of the PLGA and by changing the size of the nanoparticles. The presence of inert additives can further enhance the dissolution of PLGA matrix if faster release of the nanoparticles is desired. This work is a significant advancement on earlier nanoparticle-based radiosensitization demonstrating the creation of a drug-releasing spacer appropriate for use in needle-based implantation of brachytherapy sources (22). We are also pursuing this approach for EBRT but with significantly different formulations, which are tailored to match the timescales of EBRT. It was encouraging to see the tumor suppression with the docetaxel-loaded INCeRT spacers implanted in the tumors with minimal observable toxicities, in contrast to control mice, which lays the foundation for a large in vivo therapeutic study involving the docetaxel-doped INCeRT spacers.

In summary, we report the fabrication, characterization, and preliminary in vivo behavior of INCeRT spacers for applications in combined chemoradiation therapy. These INCeRT spacers were doped with fluorescent silica nanoparticles, which were tracked optically in vivo in live animals to monitor the localization of the nanoparticles and their diffusion from the spacers. Given that the same nanoparticles can encapsulate anticancer drugs, localization of nanoparticles by optical imaging will qualitatively determine the extent of drug diffusion into the tumor. The in vivo imaging with the implanted spacers showed a slow degradation to release the nanoparticles from the PLGA matrix with no signs of observable or behavioral toxicologic signs in animals. The preliminary in vivo therapeutic studies with docetaxel-loaded INCeRT spacers showed tumor suppression. Knowing that the feasibility of NIR imaging in real time is difficult, these spacers require further in vivo studies to evaluate their application in prostate cancer. However, these studies provide preliminary answers to some of the key requirements for building the platform for localized drug delivery in conjunction with radiation therapy.

References


