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Research Paper

Regional Radiochemotherapy Using *In Situ* Hydrogel

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Purpose. To evaluate the feasibility of regional radiochemotherapy of mammary tumors using *in situ* hydrogel loaded with cisplatin (CDDP) and rhenium-188 (^{188}Re).
Methods. Sodium alginate (SA) and calcium chloride were used to create a hydrogel for delivery of CDDP and ^{188}Re. *In vitro* studies were performed to evaluate cytotoxic effects of ^{188}Re-hydrogel and sustained-release ability of the CDDP-hydrogel. Tumor-bearing rats were injected with ^{188}Re-hydrogel (0.5–1 mCi/rat), ^{188}Re-perrhenate (0.5–1 mCi/rat, intratumoral, I.T.), CDDP-hydrogel (3 mg/kg), and ^{188}Re-hydrogel loaded with CDDP (3 mg/kg body weight, 0.5–1 mCi/rat), respectively, and groups receiving ^{188}Re were imaged at 24 and 48 h postinjection. Tumor volume, body weight, imaging, and kidney function were assessed as required for each group.
Results. Successful formation of the hydrogel was demonstrated by cytotoxic effects of ^{188}Re-hydrogel and slow release of CDDP-hydrogel *in vitro*. Tumor volume measurements showed significant delay in tumor growth in treated vs. control groups with minimal variation in normal kidney function for the CDDP-hydrogel group. Scintigraphic images indicated localization of ^{188}Re-hydrogel in the tumor site up to 48 h postinjection.
Conclusions. Our data demonstrate the feasibility of using hydrogel for delivery of chemotherapeutics and radiation locally. This technique may have applications involving other contrast modalities as well as treatment in cases where tumors are inoperable.

KEY WORDS: chemotherapy; hydrogel; radiotherapy; Rhenium-188.

INTRODUCTION

Radiation therapy (XRT) is a form of cancer treatment that uses ionizing radiation to destroy cancer cells. Roughly 60% of all cancer patients receive XRT for multiple cancers including lung, breast, prostate, and head and neck. XRT can be delivered via external beams (photon, proton, neutron, boron-neutron capture therapy) or internally using brachytherapy seeds. Conventional external beam radiation plays a limited role in the management of patients with certain nonresectable tumors because the maximum tumor dose is limited by the normal tissue tolerance (1). Internal radiation therapy (IRT) with iodine-125 (^{125}I) (150 keV β-emission, $t_{1/2}$ = 59.4 days) brachytherapy seeds are the preferred method for localized prostate cancer treatment. However, several drawbacks exist with such a procedure. First, brachytherapy seeds can only be used once, and the procedure cannot be repeated within a patient. Second, the long half-life of ^{125}I may result in radiation-induced tissue damage to surrounding tissues (2). Also, due to the inability of the low-energy gamma rays of ^{125}I to escape the body as well as the geometry of the seeds, radiation dosimetry estimation is difficult to perform. Additionally, brachytherapy seed preparation involves labeling with ^{125}I using copper or silver wires in a method that has low yield (3) and more potential radiation exposure, thus a simple and efficient IRT method is needed. Such factors have led investigators to explore a multitude of therapeutic isotopes that may be suitable for IRT.

Nuclear medicine is experiencing a resurgence of interest in therapeutic procedures as imaging scientists and radiochemists are looking to different radionuclides possessing suitable characteristics for IRT (4–8). Yttrium-90 (^{90}Y) (2.28 MeV β^- emission, $t_{1/2}$ = 64 h) labeled ibritumomab tiuxetan (Zevalin) is the first radioimmunotherapy to receive FDA approval and is used for the treatment of relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin lymphoma (NHL). Although extensive clinical and preclinical studies have been undertaken with different ^{90}Y-molecules, one major drawback exists: it is a pure beta emitter and must therefore use In-111 as a "matching pair" surrogate for imaging, biodistribution, and assessing dosimetry (9–11). Many assumptions are made using this technique, therefore, much attention has been directed toward practical application of therapeutic radionuclides that have imaging capabilities for more accurate dosimetry calculations (12–18).

Rhenium-188 (^{188}Re) is in the same family as technetium-99m (^{99m}Tc), the most prevalent diagnostic radionuclide and workhorse of clinical nuclear medicine, and shares certain similarities in its chemical properties. The attractiveness

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ABBREVIATIONS: CDDP, cisplatin; IRT, internal radiation therapy; SA, sodium alginate; XRT, radiation therapy.

of ^{188}Re lies in its accessibility and radioactive characteristics. It can be eluted from a ^{188}W/^{188}Re generator in high specific activity and has a 16.9 h half-life. ^{188}Re has a beta emission of 2.1 MeV, which has a tissue penetration of 3.5 mm. Furthermore, it possesses a 15% gamma emission, which can allow for accurate dosimetry and imaging of ^{188}Re-complexes. The ability to directly image and assess the biodistribution and dosimetry of therapeutic radionuclides *in vivo* will assist in determining target specificity as well as validating the localization of dose over time. Currently, ^{188}Re-complexes have been used in bone pain palliation, stem cell transplantation, prevention of restenosis, treatment of fungal infections, and multiple cancers (19–24).

A concern when using ^{188}Re, ^{90}Y, or any other therapeutic radionuclides is radiation exposure to whole-body, blood-forming organs (red marrow, spleen), gonads (testes, ovaries), and effective dose equivalent based on clinical doses. Therefore, the delivery method of such radionuclides plays an important role in the feasibility of its use in humans. In general, delivery of therapeutics to target organs can be active or passive. Active targeting involves the use of a "homing device" such as specific ligands, antibodies, or biomarkers that enhance the ability of the therapeutic to localize in the site of interest (25,26), whereas passive targeting is commonly associated with slow-release polymer technologies, particulates and hydrogels among others. A major advantage in using passive delivery is reduced systemic toxicity (27,28). Local administration, coupled with slow-release, has demonstrated the ability to deliver effective doses of chemotherapy while significantly reducing commonly associated systemic toxicities (29). While slow-release has favorable characteristics in chemotherapy, it is not ideal when using radionuclides due to leakage of radioactive material and irradiation of nontarget tissues. Current hydrogel technologies are well established for slow-release chemotherapy (30,31), and are under investigation for radionuclide retention (32–34).

In the current study, the use of a hydrogel to deliver combined therapeutic modalities, radiation and chemotherapy, administered local-regionally in tumor-bearing animal models was evaluated. The hydrogel is composed of two components, sodium alginate (SA) and calcium chloride, delivered separately from a dual-lumen type syringe. SA is a hydrophilic gelling polysaccharide extracted from giant brown seaweed that has previously been described as having satisfactory hemocompatibility. Alginates have not been observed to accumulate in any of the major organs, and some evidence for *in vivo* degradation has even been described (35). Alginate beads or microspheres can be instantly formed by cross-linking with divalent cations such as calcium. The reaction is simple, and no toxic material is produced as described below:

$$2\ \underset{\text{(solution)}}{\text{Sodium alginate}} + CaCl_2 \rightarrow \underset{\text{(cross-linking solid)}}{\text{Calcium alginate}} + 2NaCl$$

Our preliminary studies suggest this methodology is suitable for treatment of tumors in animal models using cisplatin as the chemotherapeutic and ^{188}Re as the radiotherapeutic.

MATERIALS AND METHODS

Chemicals and Preparation of Hydrogel

Cisplatin (5.4 mg) was added to sodium alginate water solution (1 ml, 5% w/w). For *in vitro* release assays, the cisplatin containing solution was added dropwise to calcium chloride water solution (2 ml, 8% w/w). The hydrogel was formed immediately. The aqueous solution was decanted, and the hydrogel were dried by air. Preparation of hydrogel for *in vivo* studies required two injections through a single puncture site in the tumor using a single-lumen syringe. First, sodium alginate was injected intratumorally, followed by injection of calcium chloride. Mild pressure was applied to the injection site to aid in mixing and expedite hydrogel formation. This method was used with and without the therapeutic components, cisplatin and Re-188, as dictated by the study. Preparation of ^{188}Re consisted of elution of radioactivity from a ^{188}W/^{188}Re generator (Oak Ridge National Laboratories, Oak Ridge, TN, USA; 250 mCi) using 5 ml 0.9% sodium chloride solution. The eluant was passed through a wetted alumina SepPak (Waters) to remove any tungsten-188 parent breakthrough, collected in a Pyrex reaction vessel and reduced in the presence of tin (II) chloride (2 mg) and heat

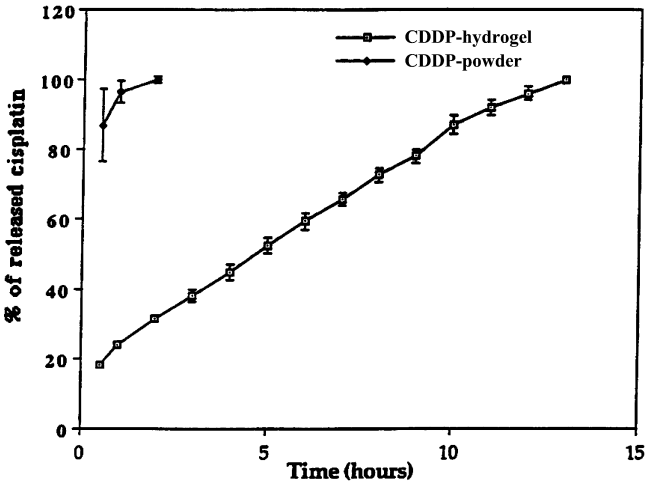

Fig. 1. Slow-release assay of CDDP-hydrogel *vs.* CDDP-powder. Alginate beads were loaded with 5.4 mg of CDDP prior to *in situ* cross-linking with calcium chloride. The sample was placed in a test tube containing PBS and incubated at 37°C for up to 24 h. One hundred microliter aliquots were removed at specified intervals and quantified by determining ultraviolet absorbance. Mean ± SE.

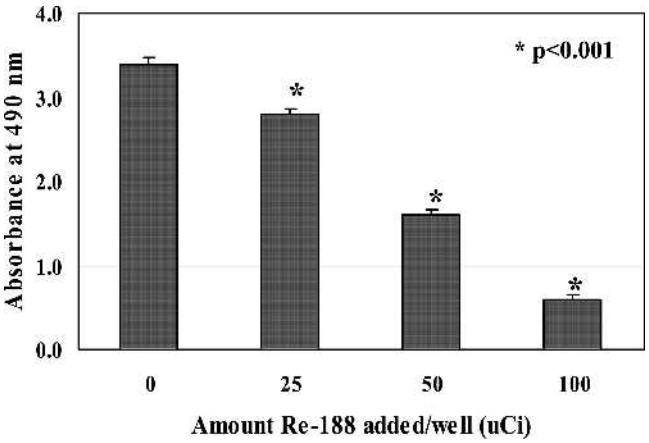

Fig. 2. *In vitro* effect of ^{188}Re-hydrogel on 13762 cells. Increasing radioactivity was administered to each group, and cytotoxicity was assessed using a protein assay kit to determine UV absorbance. *$p < 0.05$, *t* test compared to control group. Mean ± SE, $n = 3$.

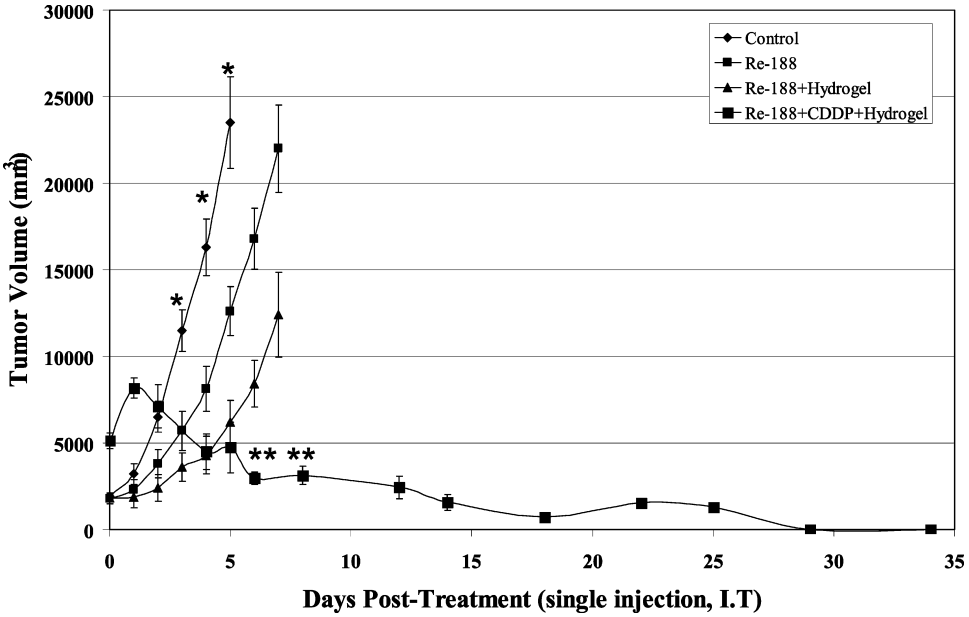

Fig. 3. Mammary tumor-bearing rats were randomized into three groups and injected with ^{188}Re and ^{188}Re-hydrogel (single injection, intratumorally, 0.5 mCi/rat). Tumor volume was measured for 7 days post-treatment and compared to the untreated group. $p < 0.05$, *t* test: *compared to treated groups; **compared to Re-188 and ^{188}Re-hydrogel. Mean ± SE; ♦, n = 3; ■, n = 3; ▲, n = 6; ■, n = 3.

(75°C, 20 min). This yielded reduced ^{188}Re particles. The ^{188}Re-containing solution was added dropwise to calcium chloride water solution (2 ml, 5% w/w) and the hydrogel was formed. For *in vivo* studies, hydrogel loaded with CDDP and ^{188}Re was prepared *in situ* through a dual-lumen syringe.

Slow-Release Assays

Cisplatin-loaded hydrogel (SA-CDDP, 5.4 mg) was incubated in phosphate-buffered saline (PBS, 1 ml) in tubes at 37°C. Samples were collected in 100 μl aliquots at various time intervals for 15 h, and the amount of cisplatin released from the hydrogel was determined by ultraviolet analysis (λ_{max} = 230 nm). Cisplatin powder was used as a control.

Cell Viability Assays

Breast tumor cells were obtained from RBA CRL-1747 rat breast cancer cell line [American Type Culture Collection (ATCC), Rockville, MD, USA). This cell line was derived from a tumor induced in Fischer-344 rats by giving an oral dose of 7, 12 dimethylbenz[a]anthracene. The cells were cultured in Eagle MEM with Earle's BSS (90%) and fetal bovine serum (10%). Fifty thousand cells were plated into each well

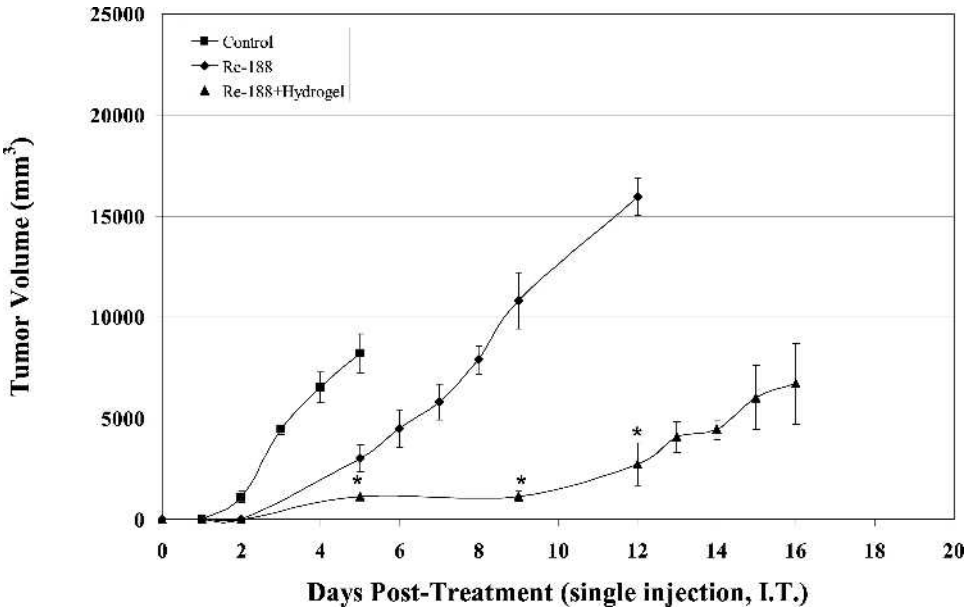

Fig. 4. Mammary tumor-bearing rats were treated with ^{188}Re and ^{188}Re-hydrogel (single injection, intratumorally, 1 mCi/rat), and tumor volume was monitored for up to16 days post-treatment. *$p < 0.05$, *t* test compared to Re-188. Mean ± SE; ■, n = 3; ♦, n = 3; ▲, n = 6.

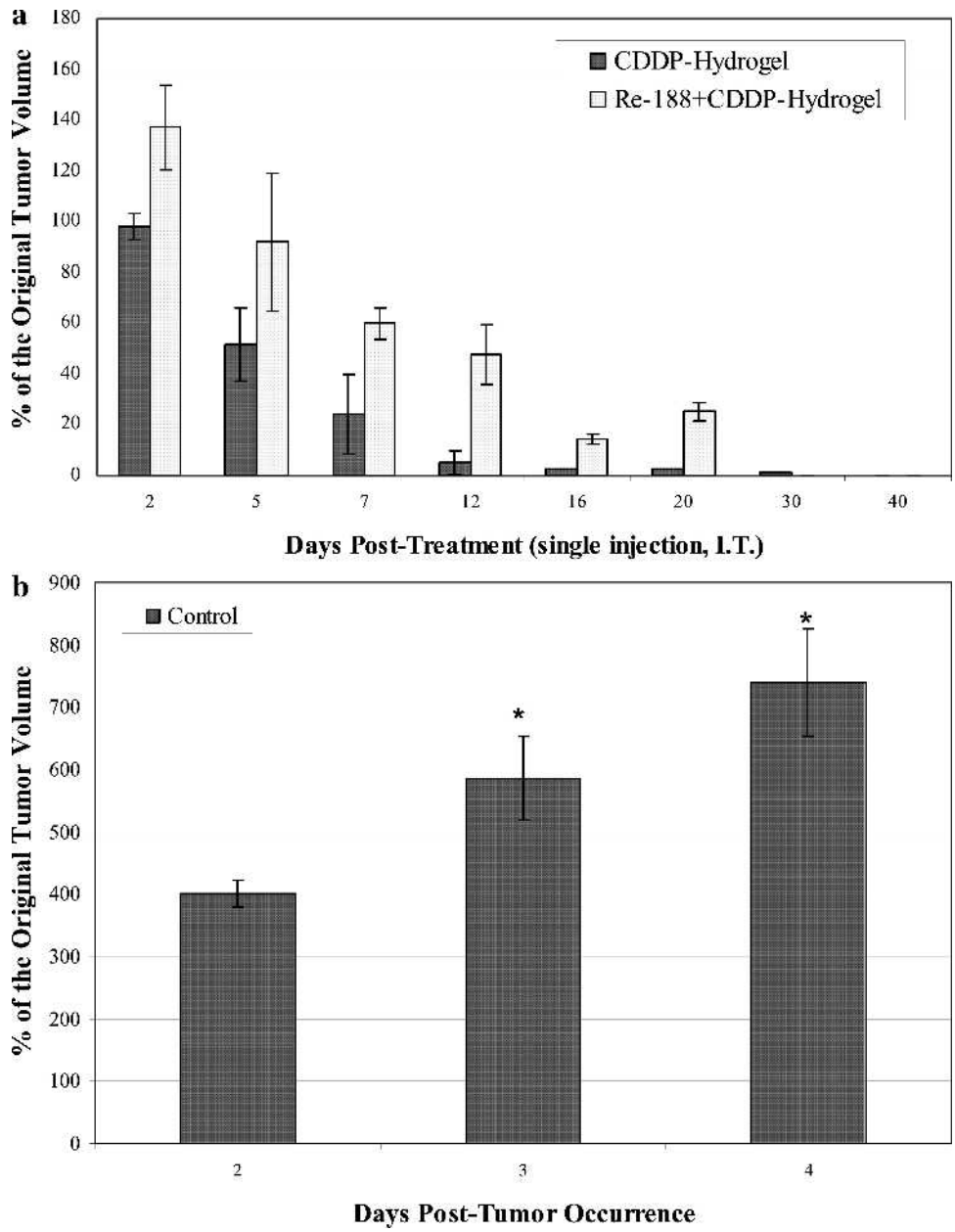

Fig. 5. (a) Mammary tumor-bearing rats were treated with CDDP-hydrogel (single injection, intratumorally, 3 mg/kg, n = 5) and ^{188}Re-CDDP-hydrogel (single injection, intratumorally, 0.5 mCi, n = 3), and tumor volume was monitored until no longer palpable. (b) Tumor volume of untreated mammary tumor-bearing rats was monitored until tumor burden became too large and animals were euthanized (n = 3). *$p < 0.05$, *t* test compared to day 2. Mean ± SE.

of 12-well plates and incubated at 37°C for 48 h to form monolayers. In order to prepare various radioactivity levels for the study, a fixed amount of hydrogel was determined and reacted with varying amounts of ^{188}Re-solution stock solution in test tubes to allow formation of ^{188}Re-hydrogel. ^{188}Re-hydrogel was then added to the cells with varying radioactivity ranging from 25 to 100 μCi/well to determine the relationship between cell viability and increased radiation dose. After 2 h incubation, the cells were harvested and prepared for a protein assay using a modified Lowry's protein assay kit (Sigma, St. Louis, MO, USA). Protein concentration was then determined for each sample using a dual-wavelength spectrophotometer (36,37). Hydrogel without ^{188}Re was used as the control group. Each data point represents an average of three measurements.

Treatment and Imaging in Tumor-Bearing Animal Models

The animals were housed in The University of Texas M. D. Anderson Cancer Center facility. All protocols involving animals are approved by the M. D. Anderson Animal Use and Care Committee and adhered to the principles of laboratory animal care. Fischer-344 Rats (150 ± 25 g) (Harlan Sprague-Dawley, Indianapolis, IN, USA) were inoculated subcutaneously with rat breast adenocarcinoma cells (10^6 cells/rodent) into the lumber region in legs using 25-gauge needles. The studies were performed 10 to 12 days after in-

oculation when tumors were palpable and could be measured. Treatment groups were designated as control (n = 6), CDDP (n = 5, 5.4 mg/ml, 3 mg/kg body weight), CDDP-hydrogel (n = 5, CDDP equivalent 3 mg/kg body weight), $Na^{188}ReO_4$ (n = 6, 0.5, and 1 mCi/rat), ^{188}Re-hydrogel (n = 12, 0.5, and 1 mCi/rat), and ^{188}Re-hydrogel loaded with CDDP (n = 3, 5.4 mg/ml, 3 mg/kg body weight, 0.5–1 mCi/rat). Each animal received two injections: sodium alginate (with or without CDDP) followed by calcium chloride (with or without $Na^{188}ReO_4$). All injections were intratumoral and administered through the same puncture site. Slight pressure was applied to the injection site to aid in mixing of the injected chemicals and hydrogel formation. Tumor volume and body weight were recorded daily for sixty days. Tumor volumes were measured as [length (l) × width (w) × thickness (h)]/2. Loss of body weight of 15% was considered a chemical-induced toxic effect. Blood chemical assays [blood urea nitrogen (BUN) and serum creatinine, SGOT, SGPT] were performed to detect hydrogel-induced renal or hepatotoxicity. A student *t* test was used to compare whether there is a significant difference between groups.

Assessment of Tumor Growth by Planar Scintigraphy

Scintigraphic images were obtained using an M-camera from Siemens Medical Systems (Hoffman Estates, IL, USA). The camera was equipped with a medium-energy parallel-hole collimator. The field of view is 53.3 cm × 38.7 cm. The intrinsic spatial resolution is 3.2 mm and the pixel size is 19.18 mm (32 × 32, zoom = 1) to 0.187 mm (1024 × 1024, zoom = 3.2).

Animals with injections containing ^{188}Re were imaged at 24 and 48 h post-injection. To determine tumor growth rate post-hydrogel therapy, ^{99m}Tc-EC-deoxyglucose (^{99m}Tc-EC-DG) was selected for tumor localization. We previously have reported that ^{99m}Tc-EC-DG is a marker for tumor growth assessment (38). One rat was selected from each treatment group and was imaged with ^{99m}Tc-EC-DG (300 μCi/rat, i.v., 1 h post-injection) on day 9 post-treatment to visualize tumor mass. Tumors were excised and measurements expressed as tumor size (mm) and tumor volume (mm^3).

RESULTS

Slow-Release Assays

UV analysis of 100 μl aliquots showed complete release from the hydrogel within 15 h. The integrity of the hydrogel matrix was maintained through the endpoint of the study. No physical deterioration or degradation was observed. In the absence of hydrogel use, rapid release of CDDP-powder was observed and complete release was achieved within 2.5 h. (Fig. 1).

Cell Viability Assays

After incubation with different doses of ^{188}Re-hydrogel, protein concentration was determined and showed that cell viability decreased as a function of increasing dose of the radionuclide. The data showed a significant decrease in absorbance in the 50 μCi group as compared to controls (Fig. 2). The 100 μCi group had >80% decrease in absorbance and may serve as a marker for the dose needed to kill tumor cells *in vivo*.

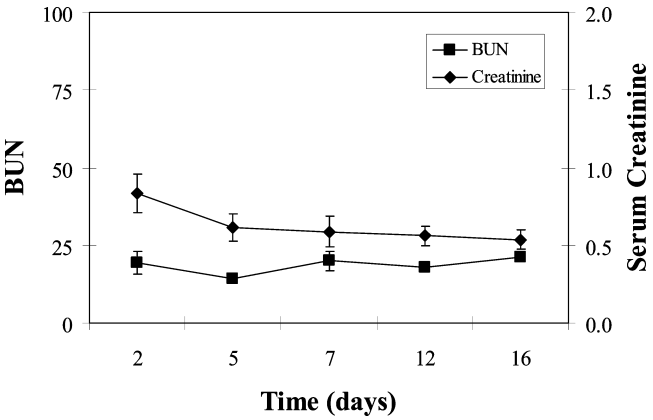

Fig. 6. Effect of CDDP-hydrogel on renal function of treated rats was assessed by monitoring BUN and serum creatinine levels for 16 days post-treatment. Mean ± SE, n = 5.

Treatment and Imaging in Tumor-Bearing Animal Models

Animals were successfully injected with sodium alginate and $CaCl_2$ (in the presence of CDDP or ^{188}Re) using the same injection site for each component. A resulting subcutaneous gel-like ball was formed near the injection site and designated that cross-linking had occurred and was also used as a marker to demonstrate that the hydrogel was intact. Repeated measurements of tumor volume showed a clear delay in tumor growth in the ^{188}Re-hydrogel group as compared to ^{188}Re and control groups (Fig. 3), prolonging survival more than 2 times that of untreated rats. Low-dose ^{188}Re-hydrogel (0.5 mCi) loaded with CDDP showed more anticancer effect than ^{188}Re-hydrogel alone (Fig. 3). Rats given higher doses (1 mCi/rat) of ^{188}Re/^{188}Re-hydrogel showed increased delay in tumor growth (Fig. 4), and those treated with CDDP-hydrogel demonstrated complete remission of tumors (Fig. 5a), while the untreated group experience rapid increase in tumor size within 4 days, leading to euthanization due to excessive tumor burden (Fig. 5b). No marked difference in tumor volume was observed between ^{188}Re-hydrogel (0.5 mCi) loaded with CDDP and CDDP-hydrogel (Fig. 5a). This effect might be

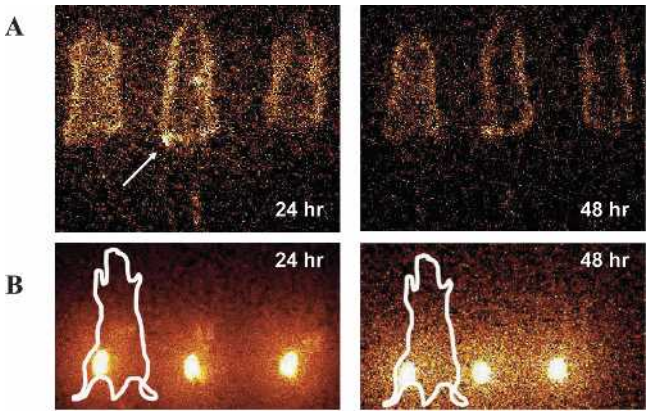

Fig. 7. Three rats were injected with $Na^{188}ReO4$ (A) and ^{188}Re-hydrogel (B) (1 mCi/rat, i.t.) in the hind limb and imaged at 24 and 48 h postinjection. Medium energy collimator was used, and the images demonstrate that ^{188}Re-hydrogel provides local regional radionuclide therapy and prevents whole-body distribution. Injection site shown by arrow.

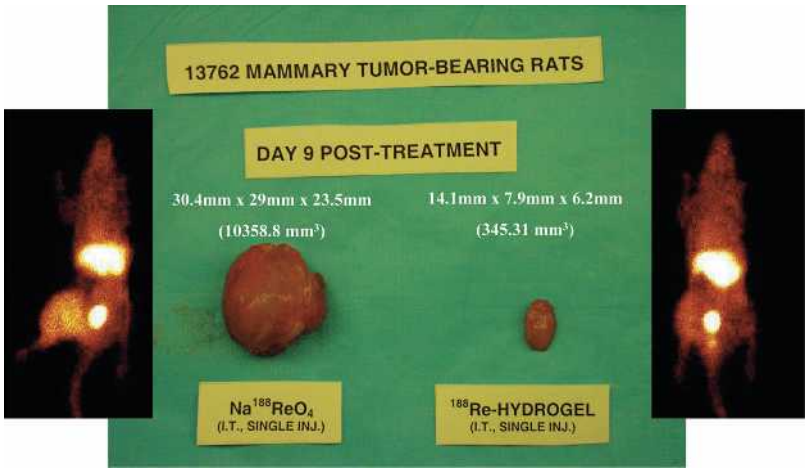

Fig. 8. Mammary tumor-bearing rats treated with $Na^{188}ReO4$ (left) and ^{188}Re-hydrogel (right) were imaged with ^{99m}Tc-EC-DG (at 1 h) on day 9 post-treatment to visualize tumor mass. Tumors were excised, and measurements are shown as tumor size (mm) and tumor volume (mm^3).

due to the dose of ^{188}Re used. Assessment of renal toxicity in CDDP-hydrogel treated rats was shown to be minimal as demonstrated by minor variations in BUN and serum creatinine levels following treatment (Fig. 6).

One of the groups treated with ^{188}Re-hydrogel was monitored with planar scintigraphic imaging (Fig. 7). Images taken at 24 and 48 h postinjection of ^{188}Re showed very poor retention of the radionuclide at the injection site, with diffusion throughout the whole-body and localization in the stomach of one rat. Imaging of the ^{188}Re-hydrogel group demonstrated that the hydrogel can effectively trap the radionuclide at 48 h postinjection. No thyroid or stomach uptake was observed, signifying the absence of free ^{188}Re from systemic circulation. Prior to sacrificing of the animals, imaging with ^{99m}Tc-EC-DG allowed for clear visualization of tumors and qualitatively demonstrated significant differences in tumor size of ^{188}Re and ^{188}Re-hydrogel samples, respectively, based on count density in the tumor region (Fig. 8). Excision of the tumors and measurement of tumor volume showed correlation between count density and tumor volume.

DISCUSSION

Clinical use of ionizing radiation commonly involves dealing with external sources such as X-ray, proton, and neutron beams. Internal use of radiation is performed using brachytherapy, which takes advantage of the close proximity of the radioactive seeds to the tumor and delivers a predetermined radioactive dose for treatment.

Brachytherapy is an attractive treatment option for prostate cancer because the seeds are deposited intratumorally and spare surrounding healthy tissues from damage. ^{125}I (half-life = 59.4 days) seeds are the preferred source but palladium-103 (^{103}Pd, half-life = 16.9 days) seeds are in use as well. Researchers are now seeking to expand the use of brachytherapy to multiple tumor types such as head and neck, breast and lung among others. Some drawbacks, however, exist when using brachytherapy for treatment. First, brachytherapy seeds are non-biodegradable and will remain in the area which they are injected permanently. Therefore, a patient that already received a seed implant is very unlikely to receive another one and must seek alternative treatment should tumor recurrence occur. Second, although these seeds are radioactive, they lack the ability to incorporate chemotherapy into their existing application.

Hydrogel		Brachytherapy
High	Loading dose	Low
+	Biodegradable	-
+	Possible Chemo R_x	-
More flex.	Dosimetry	Less flex.

Fig. 9. Comparison of some key features of hydrogel and brachytherapy.

Although it has been shown that combined chemoradiation therapy has synergistic effects in various tumor types, such applications are not clinically available with brachytherapy but may be feasible as suggested by the hydrogel findings presented in this paper. Figure 9 shows a comparison of some key differences between hydrogel and brachytherapy. The hydrogel is composed of a biodegradable, hemocompatible polymer which is virtually nontoxic. This polymer, once cross-linked with calcium chloride, forms with a "gel-like" consistency and can be prepared in multiple configurations (thin tracks, globular, etc.). It must be noted that administration of the gel into the tumor will have an adverse effect on tumor volume initially due to an increase in interstitial pressure and general enlargement of the area, followed by reduction in volume resulting from the therapeutic modality delivered (Fig. 5a). Though our *in vivo* imaging studies have not reached the necessary degradation time for alginate hydrogel, the data indicates that ^{188}Re-gel is stable and localized at least up to four days (data not shown). Others have reported that 40% of the gel weight dissolved after 15 weeks, suggesting that degradation can occur in time frames ranging from 3 weeks to more than 4 months by simply varying the number of covalent and ionic cross-links (39). The longevity with which the hydrogel remains intact *in vivo* coincides well with the selection of ^{188}Re because, generally, >99% of a radionuclide is decayed after 10 half-lives, which is 7.1 days in the case of ^{188}Re. This ensures that by the time breakdown of

the hydrogel occurs, no radioactivity remains, and toxicity to surrounding tissue will be negligible. Additionally, the loading dose of the hydrogel can be varied based on the needs of the patient, shape and location of tumor, susceptibility of surrounding healthy tissue to radiation and planned number of treatments. Increasing amounts of hydrogel and accompanying radioactivity can be delivered to certain sites as required based on delivery from a dual-lumen syringe, which can be administered under computed tomography (CT) or ultrasound (US)- guidance using an automated step-motor to ensure accurate and reproducible dose distribution. Ongoing studies have shown promising data using radiochromic film (not shown) for determining dosimetry and absorbed dose spatially and may serve as a powerful tool in treatment planning.

In an attempt to incorporate well-known slow release methodologies for drug delivery with radiotherapy, we developed a hydrogel which plays two roles in cancer treatment: 1) slow release of chemotherapeutic agents and 2) trapping of therapeutic radionuclides. Although many hydrogels exist with similar polymeric composition and drug delivery capability, the uniqueness of this hydrogel lies in the chemical properties associated with the cross-linking agent calcium chloride. Calcium chloride acts bifunctionally in this system: forming a complex with ^{188}Re, and cross-linking with sodium alginate. This leads to gel formation and slow-release of CDDP coupled with trapping of ^{188}Re at the site of injection, which can be monitored by imaging. ^{188}Re was chosen for this particular study because of its therapeutic potential, however, different contrast materials such at gadolinium-containing compounds for magnetic resonance imaging (MRI), or iodine containing-compounds for CT could also be incorporated into the hydrogel framework and serve as a high-resolution guide to demonstrate where the therapy is actually being delivered, as well as how long the hydrogel is able to maintain its integrity *in vivo*.

In summary, we have demonstrated that *in situ* hydrogel can be used for local-regional delivery of chemotherapy and radiotherapy while reducing associated toxicities found with traditional delivery methods. The findings demonstrate the potential uses of image-guided therapy in cancer treatment.

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