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# Improved Radiosensitive Liquid-Core Microcapsules for Targeting of Chemotherapeutic Agents

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**Abstract.** Microcapsules comprising alginate and hyaluronic acid that can be decomposed using radiation are currently under development. Previously, we had observed that such microcapsules supplemented with yttrium (Y) could be efficiently decomposed using radiation. In this study, we report the development of microcapsules that were considerably improved by calcium- (Ca-) and Y-induced polymerization. Moreover, we determined the amount of core contents released from the microcapsules and the frequencies of microcapsule decompositions by using colorimetric analysis and a micro particle-induced x-ray emission (PIXE) camera, respectively. A solution of 0.1% (w/v) hyaluronic acid was mixed with 0.2% alginate solution. To this mixture, carboplatin (l mg) and indocyanine green (12.5 μg) were added, and the resulting material was used for capsule preparation. The capsules were prepared by spraying the material into mixtures containing 4.34% $CaCl_2$ solution supplemented with 0–0.01% Y. These capsules were irradiated with a single dose of 0.5, 1.0, 1.5, or 2 Gy $^{60}$Co γ-rays. Immediately after irradiation, the frequencies of the decomposed microcapsules were determined using a micro PIXE camera, and the amount of core contents released was measured using colorimetric analysis with 0.25% indocyanine green. The frequency of decomposed microcapsules determined by the micro PIXE camera strongly correlated with the amount of core contents released, as determined by colorimetric analysis. Microcapsules that were polymerized using 4.34% $CaCl_2$ solution supplemented with $5.0 \times 10^{-3}$% Y demonstrated the maximum decomposition, and the release of core contents occurred after 2-Gy irradiation. The percentage of decomposition was 83.4% ± 0.4%, and that of core content release was 4.2% ± 4.4%. Our liquid-core microcapsules suggest a new potential use for radiation: the targeted delivery of chemotherapeutic agents or radiosensitizers.

**Keywords:** Microcapsule, radiation, drug delivery system

## INTRODUCTION

The recently developed conformal external beam radiation therapy enables the focusing of the radiation dose into a malignant tumor while restricting the field of radiation to the target tumor[1, 2]. If liquid-core microcapsules that release their core contents in response to radiation can be developed, focusing radiation onto the microcapsules could result in the targeted delivery of their core contents to tumors [3]. Moreover, if the core contains an anticancer drug that acts synergistically with radiation therapy, a type of therapy that combines the effects of conformal radiation and of the targeted anticancer drug can be developed. This could produce an enhanced antitumor effect[4] and reduce the side effects of both chemotherapy and radiation therapy, particularly drug-induced

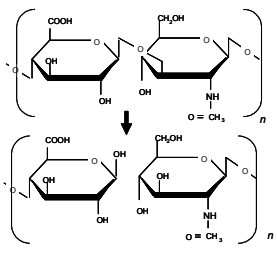


Fig.-1. Chemical change in hyaluronic acid induced by irradiation.

diarrhea, nausea, and alopecia.
We have developed microcapsules that release their liquid-core contents in response to radiation, based on the following phenomena: (1) alginate forms microcapsules by Ca-induced polymerization and (2) hyaluronic acid can be decomposed by radiation-induced superoxides (Fig. 1) [5, 6]. These microcapsules are generated by spraying a mixture of alginate and hyaluronic acid into $CaCl_2$ solution for more than 30 min. However, the use of Ca results in the hardening of the alginate, and the long polymerization time of more than 30 min results in the formation of microcapsules with thick outer shells. These factors prevent the efficient release of core contents from microcapsules[7].
During microcapsule development, we observed that yttrium (Y) prevents the regular polymerization of alginate by calcium (Ca), thereby allowing easy rupture of the microcapsules. Moreover, sonication of a mixture of alginate and hyaluronic acid results in shorter polymerization time that in turn results in the formation of microcapsules with thin outer shells[8]. These methods increased the frequency of microcapsule rupture. In this study, we report a procedure for the quantitative analysis of the amount of liquid core released after γ-radiation.

## METHODS AND MATERIALS

### Preparation of Microcapsules with Different Concentrations of Ca and Y concentration.

A 0.2% (w/v) alginate solution and 0.1% (w/v) hyaluronic acid were mixed in an agar pestle; these percentages were determined in our previous experiment[7]. To this mixture, 0.2 mmol carboplatin (a platinum (Pt)-containing anticancer drug)[9] and 2.5 mg/l indocyanine green were added. A droplet of this mixture was sonicated using an ultrasound disintegrator and atomized to yield a 4.34% solution of $CaCl_2$ that was supplemented with Y at concentrations ranging from 0% to 0.01%. Polymerization was completed within 5 min to yield microcapsules[8]. The microcapsules thus generated were purified using a Nalgene disposable filter kit (8-0301-84 DP591) and resuspended in 0.1 mmol tris hydroxymethyl aminomethane (THAM) buffer. Finally, the concentration of the capsules was adjusted to $1.0 \times 10^8$ capsules/ml, and they were suspended in 10 ml THAM.

### Irradiation and Target Preparation

A piece of a 0.1-μm thick Mylar film was stretched over a glass ring frame (diameter: 2 cm). The glass ring with the mounted Mylar film was placed in the center of a petri dish (diameter: 3 cm), and 10 ml of the microcapsule-containing suspension was poured onto the Mylar film. The petri dish was placed in the center of a $10 \times 10$ cm$^2$ radiation field and then irradiated with single doses of 0.5, 1.0, 1.5, or 2 Gy $^{60}$Co γ-radiation. After irradiation, the suspension was rapidly frozen using liquid nitrogen. The frozen suspensions were dried in vacuum at $1 \times 10^{-3}$ Torr and used as targets for microprobe imaging. Prior to target preparation, optical microscopy revealed morphological changes in the microcapsules. The mean interval time between the completion of irradiation and observation was 3.3 ± 0.1 min.

### Microprobe Imaging

Each target was irradiated with a 2-MeV proton beam (diameter: 2 μm), and the induced X-rays were recorded using a Si (Li) detector. The targets were scanned by using fields of 2 sizes, namely, $100 \times 100$ μm and $25 \times 25$ μm. The software Transform (ver. 3.0, Fortner Software Inc.) was used to generate and analyze the images of the characteristic X-rays detected.

### Colorimetric Assay

Colorimetric assay for the quantization of the released core contents was performed using indocyanine green at a wavelength of 890 nm using a spectrophotometer (Hitachi, UI 5600). Before radiation, the indocyanine green content in the microcapsule-containing suspension was measured. After irradiation, the unruptured microcapsules and the outer shells of the ruptured ones were removed using a Nalgene disposable filter kit (8-0301-84 DP591). The indocyanine green content in the supernatant was measured. Next, the ratio of indocyanine green content in the supernatant to that in the microcapsule-containing suspension was calculated; this was considered to be the percentage of the released liquid-core content.

### Statistical Analysis

All statistical analyses were performed using analysis of variance (ANOVA). Results were considered significant at $P = 0.05$.

## RESULTS

### Generated Microcapsules

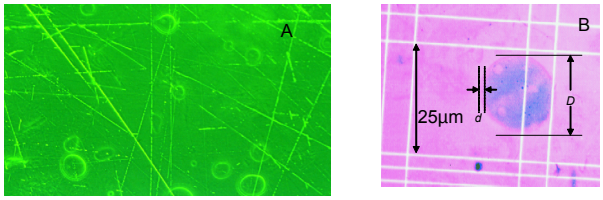


Fig-2. Generated microcapsule prior to radiation A: Microcapsules floated in THAM buffer B: Microcapsules viewed on hemocytometer. The generated microcapsules were spherical with smooth contours. Their diameter (D) and thickness of the outer shell (d) were measured using the scale of hemocytometer.

Figs. 2A and B show images of the generated microcapsules that were floated in the THAM buffer. As described in our previous report, the microcapsules consisted of 2 components: (1) a thin outer shell prepared from alginate and hyaluronic acid and (2) a liquid core containing carboplatin and indocyanine green (Fig. 2A and B). The mean diameter of the microcapsules was 23.3 ± 3.2 μm (D in Fig. 2B), and the thickness of the outer shell was 1.3 ± 0.5 μm (d in Fig. 2B).

## Formation of Microcapsules with Y Addition

The numbers of generated microcapsules were plotted against the final concentration of the added Y (Fig. 3). By using a method similar to the one used in our previous study, Y was added until a final concentration of 5.5 $\times 10^{-3}$% was attained; there were no significant changes in the number of microcapsules generated (Fig.3). When the concentration of the added Y ranged from 6.0 $\times$ $10^{-3}$% to 7.5 $\times$ $10^{-3}$%, the number of microcapsules began to significantly and drastically decrease (Fig. 3). When the concentration of the added Y exceeded 8.0 $\times$ $10^{-3}$%, no microcapsules were generated (Fig. 3).

Microcapsules were formed when the final concentration of the added Y ranged from 0% to 6.0 $\times$ $10^{-3}$%. Next, we observed the release of the microcapsular core contents when the abovementioned concentrations of Y were added.

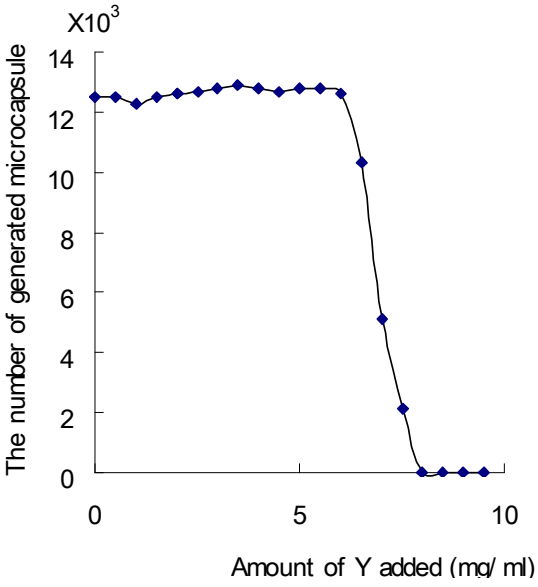


Fig. 3 Formation of microcapsules with Yttrium (Y) addition. The numbers of generated microcapsules were plotted against the final concentration of the added Y.

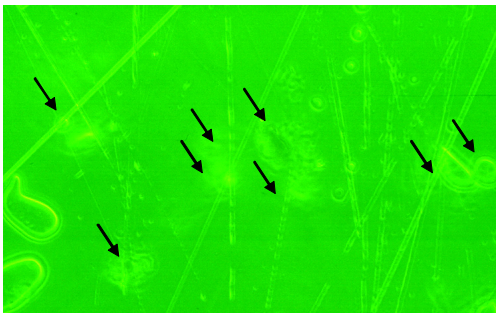

Fig. 4: Representative microcapsules after irradiation (arrows)

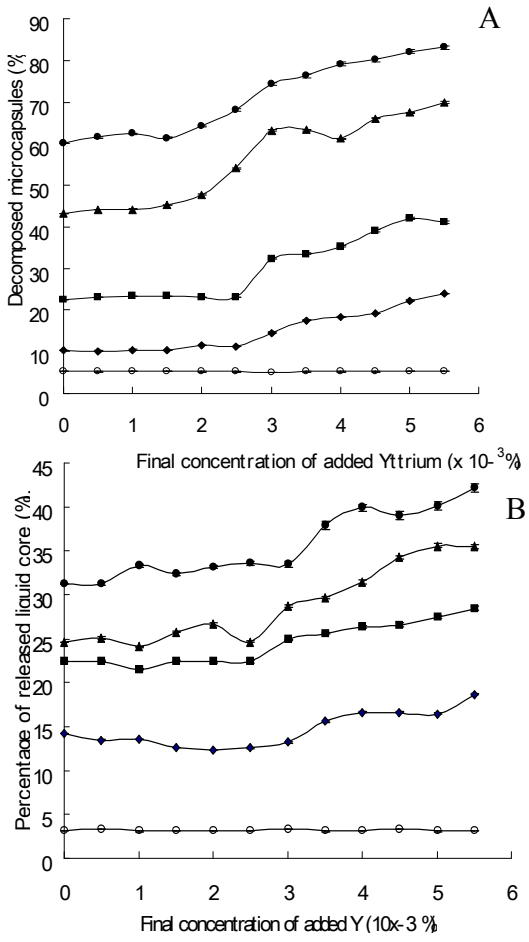


Fig. 5: Frequency of microcapsule decomposition following the addition of Y observed using an optical microscope and micro PIXE camera.
A: Observation by optical microscope. B: Observation by micro PIXE camera
The percentage of microcapsules that was decomposed by radiation was plotted against the final concentration of Y. (O) non-irradiated, (◆) 0.5 Gy, (□) 1.0 Gy, (▲) 1.5 Gy, (●) 2.0 Gy.

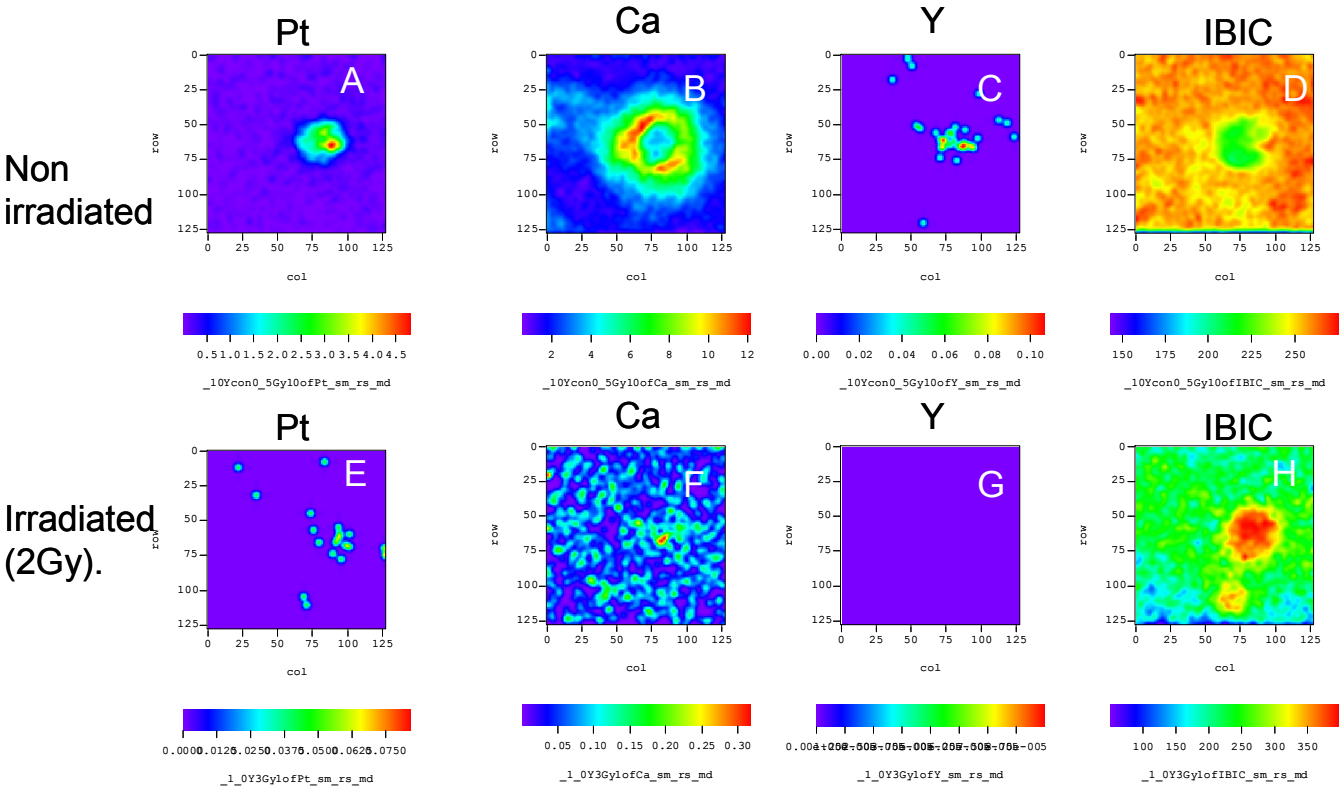


Fig. 6: Distribution of Pt, Ca, Y, and IBIC. A-D: non-irradiated. E-H: irradiated

**Changes in the Release of Core Contents of Microcapsules following the Addition of Y**

By using optical microscopy, we observed the changes in the release of microcapsular core contents when the final concentration of the added Y ranged from 0% to 5.5 × $10^{-3}$%; our observations are shown in Figs. 4 and 5A. After a single dose of $^{60}$Co γ-radiation, the size of some microcapsules decreased, and their liquid cores could not be detected (Fig 4, arrows). The microcapsules that underwent these morphological changes were considered to have decomposed.

The increment in the frequencies of microcapsule decomposition was dependent on the final concentrations of the added Y (Fig. 5A). Greater radiation doses increased the frequencies of microcapsule decomposition (Fig. 5A).

Until the final concentration of the added Y reached 3.0 × $10^{-3}$%, no significant increase in the frequency of microcapsule decomposition was noted (Fig. 5A). When the concentration of the added Y exceeded 3.0 × $10^{-3}$%, the frequency of decomposition increased significantly (Fig. 5A). Significant increases in these frequencies were also observed when the radiation dose exceeded 1.0 Gy (Fig. 5A). The maximum decomposition frequency was observed when the final concentration of the added Y was 5.5 × $10^{-3}$% with 2.0-Gy irradiation.

The distribution of the microcapsular core contents (carboplatin) was imaged using a micro PIXE camera that detected platinum (Pt) signals (Fig. 6A–H). The contours of the microcapsules were identified by detecting the distribution of Ca and ion beam-induced charge (IBIC) that were incorporated into the outer shell during the Ca- and Y-induced polymerization of alginate (Fig. 6B, D, F, and H). Prior to irradiation, the microcapsule contours were clearly detected, and Pt was observed to be distributed within the capsules (Fig. 6A, B, and D). After irradiation, microcapsules with unclear Ca or Y distribution and with heterogeneous distribution of Pt (Fig. 6 A–C and E–G) were observed. We determined that these types of microcapsules had released their core contents upon irradiation. Optical microscopy revealed an increase in the decomposition of microcapsules following

irradiation. The increase in the frequencies of release of microcapsular core contents depended on the final concentration of the added Y (Fig. 5B). Maximum decomposition was observed when the final concentration of Y was $5.5 \times 10^{-3}$% with 2.0-Gy irradiation (Fig. 5B). In microcapsules irradiated with a 0.5-Gy dose, the addition of Y or further irradiation caused no significant increase in the decomposition frequency (Fig. 5 B)

## Amount of Released Liquid-Core Contents Measured Using Colorimetric Assay

By using colorimetric assay with indocyanine green, we observed changes in the amount of microcapsular core contents released under the same range of the final Y concentration; our observations are shown in Fig. 7. Changes in the amount of released liquid-core contents were similar to those in the decomposition frequencies of the microcapsules detected using optical microscopy and the micro PIXE camera. The frequency of microcapsular decomposition increased following irradiation. This increase in frequency depended on the final concentration of the added Y (Fig. 7). Maximum decomposition was observed when the final concentration of Y was $5.5 \times 10^{-3}$% with 2.0-Gy irradiation (Fig. 5B).

## DISCUSSION

Many potential cancer therapies that synergistically combine radiation and chemotherapy have been proposed1, [9]. It is believed that only treatment with strong antitumor agents can improve cancer treatment. However, patients suffer from severe general adverse effects when such treatments are administered. Chemotherapy causes nausea, alopecia, vertigo, severe fatigue, etc. Radiation therapy causes dermatitis, mucitis, and pain in the treated area, even when exposure is limited by the 3D-conformal technique[1]. These adverse effects often exceed the patients' tolerances and are sufficient to warrant cessation of the therapy and may decrease the patients' life spans. We believe that a cancer therapy that combines the following 2 criteria can be developed. (1) The anticancer effect should be enhanced by the synergistic effects of radiation and anticancer drugs and (2) the delivery of the anticancer drug should be limited or increased within the radiation field to minimize the generalized adverse effects. In order to realize these 2 criteria, we have been developing microcapsules that release anticancer drugs in response to radiation. Previously, we had reported that microcapsules comprising hyaluronic acid and alginate can release their core contents when alginate and hyaluronic acid are conjugated under the influence of Ca and Y[3, 4, 7, 8]. However, this study was based on the percentage of ruptured microcapsules, and we did not measure the amount of liquid-core content that was released[3, 4, 7, 8]. In this study, in addition to the procedures of our previous study, colorimetric assay with indocyanine green was included in order to measure the amount of the released liquid-core contents. However, before the inclusion of this assay, we had to ensure that the addition of indocyanine green to the mixture comprising alginate and hyaluronic acid did not influence microcapsular rupture. Therefore, it was necessary to repeat the same experiment regarding radiation-induced microcapsular decomposition with the addition of indocyanine green, as shown in our previous report[8]. Indocyanine green addition to microcapsules did not significantly influence their decomposition, and only slightly decreased their rupture in response to radiation when the final concentration of the added Y ranged from 0% to $5.5 \times 10^{-3}$%. The increase in the frequency of microcapsular decomposition was dependent on the final concentration of the added Y (Fig. 5A)[8]. Greater radiation doses increased the frequency of microcapsular decomposition (Fig. 5A). The percentage of the amount of released liquid-core contents was dependent on the final concentration of the added Y and the radiation doses. The maximum release percentage was 42.1% ± 4.4%. We believe this is the first step toward the development of an ideal cancer treatment with increased antitumor effects and decreased adverse effects.

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