Design and Preparation of Ethyl Cellulose Microcapsules of Gadopentetate Dimeglumine for Neutron-Capture Therapy Using the Wurster Process

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Microcapsules of hygroscopic, highly water-soluble gadopentetate dimeglumine (Gd-DTPA-DM) for use in preliminary *in vivo* experiments for neutron-capture therapy were designed. They were prepared with such properties as a particle size small enough to be suspended and injected through a syringe, a negligible release of Gd-DTPA-DM, and a high drug content by means of the Wurster process, a spray coating method using a spouted bed with a draft tube. They were composed of lactose cores of 53—63 μm, an undercoat of ethyl cellulose (EC) and polyvinylpyrrolidone (PVP), a drug-layer of Gd-DTPA-DM, EC and PVP, a waterproof coat and a release-sustaining overcoat of EC and cholesterol (1 : 1), and a surface treated with hydrogenated egg lecithin. By curing at 110 °C for 30 min after mixing with 20% pulverized mannitol powder, the 20% overcoating suppressed the release of Gd-DTPA-DM from 75—106 μm microcapsules to less than 10% for the first 20 min, which was the period required to prepare a suspension, inject it and irradiate the neutron. The microcapsules could be used to confirm that the intracellular presence of Gd is not critical in gadolinium neutron-capture therapy.

Keywords neutron-capture therapy; gadopentetate dimeglumine; microcapsule; coating; fluidized bed; ethyl cellulose

The neutron-capture therapy with gadolinium (GNCT) has attracted much attention recently.[1] GNCT differs from the therapy with boron (BNCT) in emitted radiation: electrons, X rays and gamma rays are emitted in the former, and ^{7}Li and alpha particles in the latter. Therefore, in BNCT, boron has to be present intracellularly to induce inactivation due to the short ranges of the released alpha particles. In contrast, the ranges of electrons and photons produced in gadolinium neutron-capture reactions are much longer, allowing speculation that cell inactivation occurs even if gadolinium is present within the vicinity of the cell. The purpose of this study was to prepare gadolinium-containing microcapsules by the Wurster process to evaluate whether, if gadolinium exists extracellularly, cell inactivation still results; therefore, microcapsules were designed and prepared so that they could suppress the release of gadolinium to the greatest extent possible. Further, they had to be small enough to be suspended in an aqueous fluid and injected through a syringe.

The Wurster process[2] is characterized by a fine powder coating. Ethyl cellulose (EC) microcapsules with fine calcium carbonate cores (32—44 μm) and drug (phenacetin) layers were prepared to evaluate the performance of the fine powder coating in the previous study.[2] The effect of additives on particle size distribution, the variation in drug content with particle size and the dissolution properties of the products were evaluated. Among eight additives used, cholesterol (CH) most suppressed the release of phenacetin, resulting in a saving of coating material and time. Sustained release of phenacetin could be achieved with only 6.25% or less coating material relative to core material. In addition, CH remarkably reduced particle agglomeration. The mass median diameter of a product 25% coated with an EC : CH = 2 : 1 mixture was 58 μm. However, the drug content and phenacetin release were strongly dependent on the particle size of the product. This was the result of retardation of particle circulation in the Wurster chamber due to its adhesion to the wall by electrostatic charge. Stearyltrimethylammonium chloride (STAC), a cationic surfactant, reduced the particle adhesion when it was added to EC–CH (2 : 1) in a 1% amount on a dry basis. As a result, the particle size distribution became sharper, and there was higher homogeneity of the physical properties.

In this study, microencapsulation of gadopentetate dimeglumine (Gd-DTPA-DM), a contrast medium for magnetic resonance imaging, with EC was attempted on the basis of the previous study.[2] Gd-DTPA-DM differed from the hydrophobic phenacetin used previously in physicochemical properties: it was extremely water-soluble and hygroscopic. Therefore, the membrane permeation had to be more strongly suppressed.

Experimental

Materials Lactose (DMV 200M) sieved to 53—63 μm was used as a core material. EC (30—50 cps), polyvinylpyrrolidone (PVP, K30), CH (SP reagent grade) and hydrogenated egg lecithin (HEL) were used as purchased from Nacalai Tesque Co., Ltd. without purification. Gd-DTPA-DM (Magnevist®) was supplied by Nihon Schering Co., Ltd. Glass beads (200 μm, GB-02, TOP) were washed with ethanol and distilled water. An aqueous solution of dextran 40 (LMD-L) used as a dissolution fluid was purchased from Otsuka Pharm. Co., Ltd. The viscosity of LMD-L was 3.28 cps at 37 °C.[3] The other reagents were also used as purchased without purification.

Coating An NQ-GM spouted bed coater with a draft tube (Fuji Paudal Co., Ltd.) was used. A spray nozzle of 0.8 mm diameter and a bag filter with an opening of about 5 μm were set throughout all experiments.

Particle Size Distribution A sieve analysis was performed using a row-tap shaker (Iida Seisakusho Co., Ltd.). The shaking time was 10 min and the charged weight was 25 g.

Dissolution and Drug Content Dissolution tests were performed by a column method using a high-performance liquid chromatograph (HPLC, Shimadzu LC3A). The prepared microcapsules were dried in a vacuum at room temperature for 12 h. Curing was performed by mixing with 20% pulverized mannitol powder and then by heating at 110 °C for 30 min in an air stream oven. Microcapsules of 36 mg (30 mg with unheated ones) were mixed with glass beads of 120 mg and poured into a stainless column with 4 mm inner diameter and 8.3 mm length for HPLC. The column connected to the HPLC system was immersed in a water bath

TABLE I. Formulations of Spray Solutions and Operating Conditions

	Undercoating	Drug-layering	Waterproofing	Release-sustaining	Surface-treatment
Core (g)	30[a]	30[b]		25[c]	
EC (g)	2.5	17.5	12.5	2.5	
PVP (g)	1.5	6.7			
CH(g)			12.5	2.5	
Gd-DTPA-DM (g)		84.4			
HEL (g)					0.75
Total (ml) Ethanol	100	700	500	100	
1:1 Ethanol–CH_2Cl_2					75
Product (g)/core (g)[d]	1.13	5.22	6.16	7.39	7.58
Inlet air temperature (°C)	30	30	30	30	
Outlet air temperature (°C)	18	20—24	21	23	
Inlet air rate (m^3/min)	0.1	0.10—0.18	0.21	0.22—0.27	
Spray rate (ml/min)	5.4	4.0—4.3	8.1	5.7	
Spray pressure (atm)	1.5	1.6—2.1	2.1	2.1	
Product Weight (g)	31.9	—	132.2	—	28.5
Yield (%)	93.8	—	80.8	—	92.7
Mass median diameter (μm)			114	126	126

a) 53—63 μm lactose. *b*) Undercoated particles. *c*) Coated particles. *d*) This ratio was that to initial lactose cores (30 g). Final ratio was calculated to be 7.58, which should result in Gd-DTPA-DM content of 42.8%.

temperature-regulated at 37 °C. A 0.9% saline solution containing 0.05% sodium dodecyl sulfate (SDS) or the aqueous solution of dextran was used as a dissolution fluid. This fluid was passed through a heat exchanger immersed in the water bath and flowed at 1.0 ml/min. The solution eluted from the column was collected in 1 ml samples. Lactose and Gd-DTPA-DM in the eluted solution samples were determined by phenol–sulfuric acid method[4] and inductively coupled plasma emission spectrography (ICP, STS 1200A, Seiko I & E), respectively. To determine drug content in the microcapsules, their suspension in 30 ml of dissolution fluid was homogenized by a Physcotron (Niti-On) and then centrifuged. The concentrations of lactose and Gd-DTPA-DM in supernatant were similarly determined, and were also used to estimate the value of 100% release in dissolution tests.

Scanning Electron Microscopy (SEM) SEM was performed on a JEOL JSM-5300LV scanning electron microscope.

Results and Discussion

Design and Preparation of Microcapsules The requirements for microcapsules to be prepared in this study were as follows: 1) their dissolution was to be suppressed as much as possible; 2) their size was to be small enough to be easily suspended in an aqueous solution of dextran and to be injected through a syringe; 3) they did not necessarily have to be biodegradable; 4) the content of Gd-DTPA-DM had to be as high as possible. With regard to their size, since Murata *et al.*[5] reported that particles smaller than 200 μm could pass through 20G syringes, microcapsules were designed to be smaller than 200 μm. Incorporation of a larger amount of highly water-soluble materials generally leads to a more rapid release, so a membrane with an unusually low permeability was required to achieve a negligible release. Obviously, simple membrane-thickening for suppressing release leads to enlargement of particle size. The challenge in this study was how to harmonize these incompatible requirements.

Organic solvent systems were selected to coat Gd-DTPA-DM, because Gd-DTPA-DM was very hygroscopic and consequently no aqueous coating systems seemed to be applicable. An EC–CH organic solution system was selected because its coating performance had been well evaluated in the previous study.[2] EC–CH–STAC (2:1:0.03) of 6.25% or less seemed sufficient to sustain release of hydrophobic phenacetin which was used as a model drug. However, since Gd-DTPA-DM was much more water-soluble than phenacetin, the membrane permeation had to be further suppressed. Hence, STAC was eliminated, since it, even though only slightly, enhanced phenacetin release, and CH was enriched to EC:CH = 1:1 to further suppress drug-release.[2]

Details of the cores, the composition of spray solution and the coating conditions are listed in Table I. Gd-DTPA-DM was available as an aqueous solution, so that water was eliminated by freeze-drying. Since the dried solid (84.4 g) strongly adhered to the glass wall of the reservoir, PVP (6.7 g) had been previously added. By adding 400 ml of ethanol to freeze-dried materials and then homogenizing, Gd-DTPA-DM was suspended in ethanol.

Lactose cores of 53—63 μm were first undercoated with EC–PVP to modify their surface condition. In subsequent layering, Gd-DTPA-DM particles were fixed to the core particles with EC and PVP:EC was added to the spray solution to suppress possible agglomeration due to hygroscopicity of Gd-DTPA-DM (Table I). However, since the drug-layered particles were hygroscopic under room conditions, they could not be discharged from the coating chamber just after drug-layering; a subsequent coating with EC–CH (1:1) for waterproofing was therefore continued without a break. The product, which was coated to the level of 18.0% against drug-layered particles (Table I), became much less hygroscopic under room conditions of RH 50% and 25 °C and was only a little sticky to handle. The product yield was 80.8% (Table I), which was satisfactorily high, and its mass median diameter was 114 μm. Further, overcoating was performed to suppress release of the drug even more. Surface treatment with HEL was finally performed so that the microcapsules could be easily suspended in aqueous fluids. As shown in Table I, when the overcoating level was 20%, weight of the final particles should theoretically be increased to 7.58 times that of the initial lactose cores (58 μm), and when the density of the particles was assumed to be the same as that of lactose (1.54 g/cm^3), diameter should be increased to 1.96 times

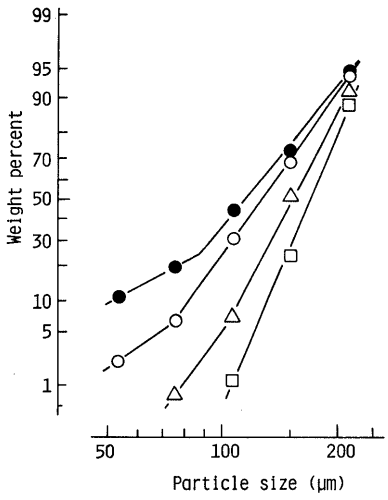

Fig. 1. Cumulative Undersize Distributions of Microcapsules Coated at Various Overcoating Levels

Overcoating (%): ●, 0; ○, 20; △, 40; □, 120.

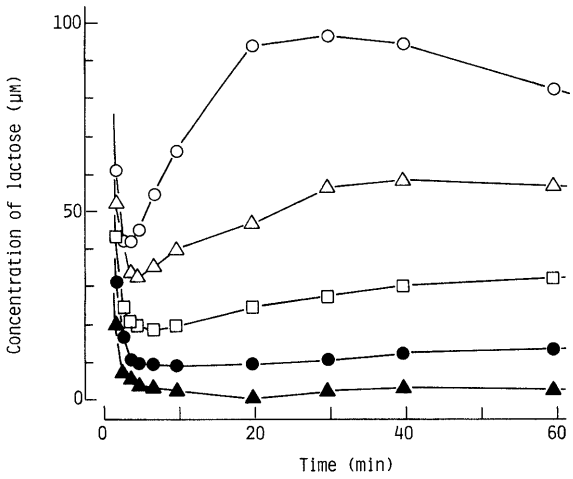

Fig. 2. Effect of Overcoating Level on the Release of Lactose from Gd-DTPA-DM Microcapsules

Microcapsules: cured at 110 °C for 30 min, after being mixed with 20% mannitol. Dissolution fluid: 0.9% saline solution containing 0.05% SDS. Overcoating (%): ○, 0; △, 20; □, 40; ●, 60; ▲, 120.

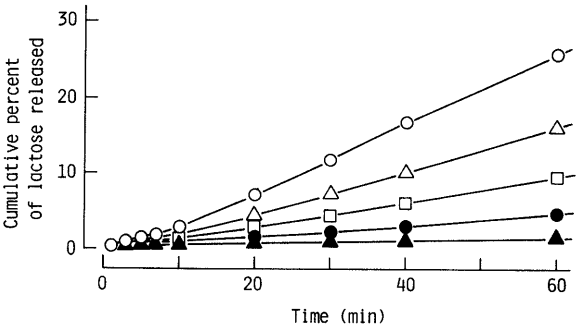

Fig. 3. Effect of Overcoating Level on the Cumulative Release of Lactose from Gd-DTPA-DM Microcapsules

Microcapsules: cured at 110 °C for 30 min, after being mixed with 20% mannitol. Dissolution fluid: 0.9% saline solution containing 0.05% SDS. Overcoating (%): ○, 0; △, 20; □, 40; ●, 60; ▲, 120.

(114 μm). The mass median diameter of 126 μm of the final product suggested that agglomeration could not be avoided during this hard coating process.

Particle Size Distribution The size distributions of microcapsules coated to various overcoating levels are shown in Fig. 1. Although the formulation of 20% overcoating is shown in Table I, the overcoating was performed up to the level of 120% against the particles with a waterproof coat.

The weight of a product at 0% overcoating level should reach 6.16 times that of the lactose cores (Table I). The diameter of the 0% overcoated particles was calculated to be 106 μm under the assumption that the product density was identical to that of lactose. On microscopic observation, the fraction of 75—106 μm contained many agglomerates composed of about two cores. The fraction larger than 106 μm was composed of agglomerates of three or more cores. As seen in Fig. 1, the size distribution of microcapsules at 0% overcoating level had a broken point at 87 μm of particle size and 24% of undersize weight. Microscopic observation appeared to show that particles larger than 87 μm might be agglomerates. This would mean that very frequent agglomeration would occur during drug-layering and/or subsequent coating with EC–CH for waterproofing. The low rate and temperature of inlet air and the low spray pressure during the drug-layering could be the causes of the high degree of agglomeration occuring in spite of the low spray rate used (Table I). Although such mild fluidizing conditions were presumed to be necessary to gain a high layering efficiency, they led to agglomeration due to the hygroscopicity of Gd-DTPA-DM. On the other hand, it was expected from previous findings[2] that agglomeration would seldom occur in subsequent coating with EC–CH (1 : 1) for waterproofing. However, since the spray rate was raised, with the inlet air temperature remaining low (30 °C), and the particles layered with Gd-DTPA-DM remained possibly hygroscopic, some agglomeration might have occurred. The spray rate had to be raised due to the electrostatic charging of particles coated with only EC–CH, since the STAC used to reduce the charging was eliminated in this study.[2] The facts that the theoretical content, 43.8%, of Gd-DTPA-DM in 20% overcoated microcapsules (Table I) decreased to 26.5% of measured content for 75—106 μm, and that dissolution of lactose from these 20% overcoated microcapsules were strongly suppressed suggested that some particles might not be steadily circulated during drug-fixing because of their adherence to the chamber wall, thus leading to the low drug content; they had, however, been steadily fluidized during the coating with EC–CH for waterproofing.

In the next overcoating, the weight fraction of particles smaller than 106 μm was rapidly decreased by agglomeration and/or thickening of the membrane (Fig. 1). Mass median diameter reached 170 μm at 120% overcoating. Particles larger than 106 μm were found too large to be stably suspended owing to the density of Gd-DTPA-DM microcapsules which is far higher than that of the swollen lecithin microcapsules reported by Murata *et al.*,[5] though they could pass through a syringe.

Dissolution of Lactose and Gd-DTPA-DM To evaluate the release of contents from microcapsules, lactose was monitored at first. Effects of overcoating level on the dissolution of lactose from microcapsules cured at 110°C for 30 min are shown in Figs. 2 and 3, which are time courses of the concentration in eluted solution and the cumulative percent released, respectively. The reason microcapsules

were cured at 110 °C are that those with no antiadherent on their surfaces became sticky at 110 °C when heated in an oven. This was related to the fact that the glass transition temperature of cast film of EC–CH (1 : 1) was 115 °C.[2] Pulverized mannitol powder of 20% was mixed with microcapsules before heating to avoid aggregation.

The release of lactose was prolonged as the overcoating level increased. At the 120% level, the dissolution for 60 min was suppressed to less than 2%. However, the weight fraction of 75—106 μm found applicable to *in vivo* experiments had already been decreased to only 6% at 40% overcoating level (Fig. 1). Hence, 20% overcoated microcapsules were further examined in detail.

Lactose dissolution with three typical size fractions of 20% overcoated microcapsules and the effect of curing at 110 °C for 30 min are shown in Fig. 4. With unheated microcapsules, after lactose dissolution exhibited a little burst, their release rate was increased. The larger particles showed higher release rate and the cumulative percents released for 60 min were calculated as 30.3%, 33.0% and 43.7% with 75—106, 106—150 and 150—212 μm fractions, respectively. By heating at 110 °C for 30 min, lactose release was remarkably suppressed: the percents released for 60 min were lowered to 6.1%, 9.1% and 15.4%, respectively. This size dependency of dissolution tended to agree well with the results in the previous study: the dissolution was slow with single-core microcapsules, but became faster as agglomerates were enlarged.[2] Figure 5 shows photographs of 20% overcoated microcapsules of various sizes. Large particles (Fig. 5b—d) were seen to be agglomerates whose irregular shapes led to uneven film-formation and consequently faster release. Most small particles (Fig. 5a) were also agglomerates, but their membranes were seen to tightly cover the agglomerates composed of only a few cores, leading to more suppressed release. Crystals of CH are observed on the surface (Fig. 5e), as reported earlier.[2]

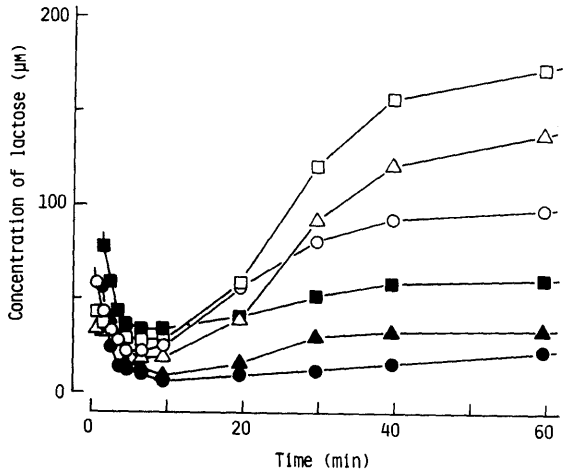

Fig. 4. Release of Lactose with Various Size Fractions of 20% Overcoated and Surface-Treated Microcapsules and Effect of Curing

Dissolution fluid: a 0.9% saline solution containing 0.05% SDS. Size fraction (μm): ○●, 75—106; △▲, 106—150; □■, 150—212. Open symbols, uncured; closed symbols, cured at 110 °C for 30 min, after mixing with 20% mannitol.

In the *in vivo* experiments following this study, the period of neutron irradiation was set at 12 min after administration of microcapsules. It was expected from the low dissolution rate of lactose from 20% overcoated microcapsules of 75—106 μm that the release of the microcapsule water-soluble contents might be sufficiently suppressed during preparation of microcapsule suspension, its injection and neutron irradiation.

The dissolution of Gd-DTPA-DM from 20% overcoated microcapsules of 75—106 μm is shown in Fig. 6. The release rate of Gd-DTPA-DM was remarkably increased during the first 20 min, and then rapidly decreased. The release was reduced in an aqueous dextran solution, though only

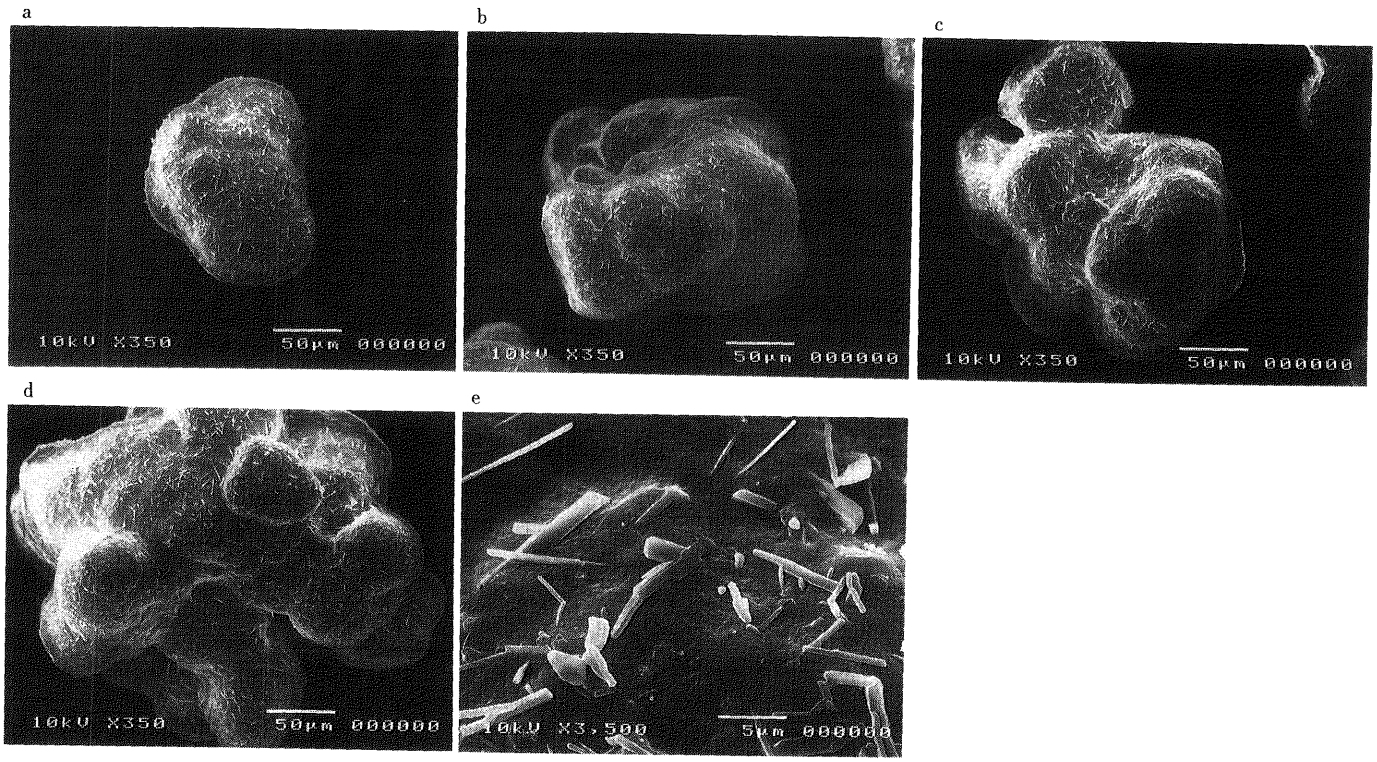

Fig. 5. Photographs of 20% Overcoated Microcapsules of Various Sizes (a—d) and Their Surface (e)

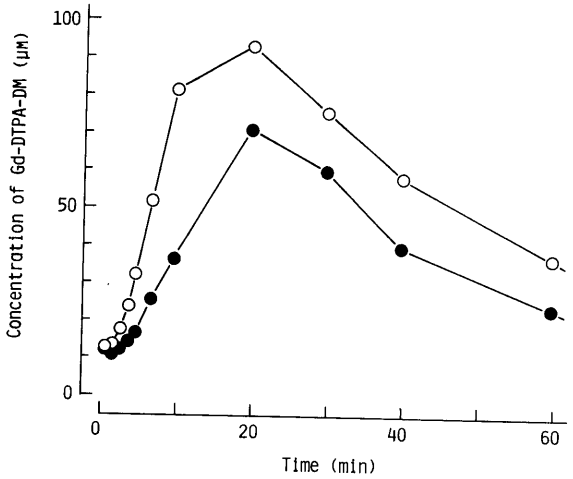

Fig. 6. Release of Gd-DTPA-DM from 20% Overcoated and Surface-Treated Microcapsules of 75—106 μm

Microcapsules: cured at 110°C for 30 min, after mixing with 20% mannitol. Dissolution fluid: ○, 0.9% saline solution containing 0.05% SDS; ●, the aqueous dextran solution.

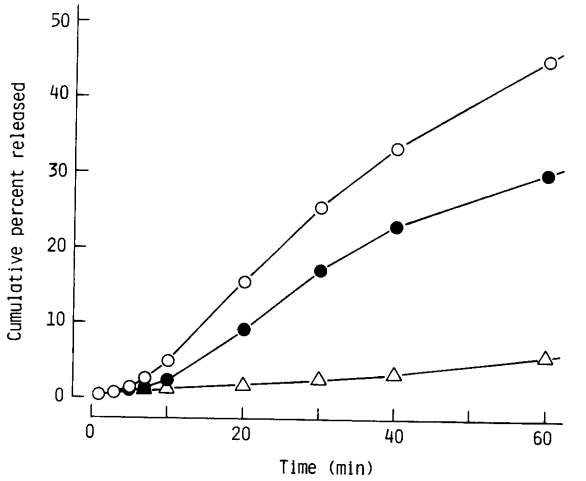

Fig. 7. Cumulative Release of Gd-DTPA-DM and Lactose from 20% Overcoated and Surface-Treated Microcapsules of 75—106 μm

Microcapsules: cured at 110°C for 30 min, after mixing with 20% mannitol. Dissolution fluid: open symbols, 0.9% saline solution containing 0.05% SDS; closed, the aqueous dextran solution. ○, ●, Gd-DTPA-DM released; △, lactose released.

slightly. The dextran solution was used as a dissolution medium, since it was intended to be used as a suspension medium of microcapsules in *in vivo* experiments. The surface-treatment with HEL exhibited no significant effect on the release.

The cumulative percents of Gd-DTPA-DM and lactose released from 20% overcoated microcapsules of 75—106 μm are shown in Fig. 7. Although release of Gd-DTPA-DM was much faster than that of lactose, the dissolution in aqueous solution of dextran was suppressed to 2.6% at 10 min and 9.3% at 20 min. This showed these microcapsules to be applicable to *in vivo* experiments. The final products whose surfaces are layered with mannitol powder as an antiadherent are shown in Fig. 8.

The gadolinium-containing microcapsules prepared in this study were evaluated as an agent for gadolinium neutron-capture therapy. When mice were inoculated intraperitoneally with 10^7 Ehrlich ascites tumor cells and gadolinium microcapsules and exposed to thermal neutrons for 12 min (1.86×10^{12} neutron/cm^2), significantly more

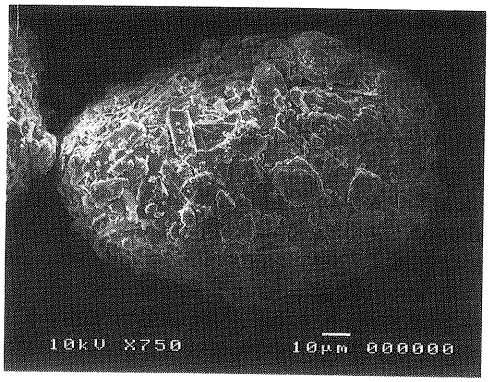

Fig. 8. Typical Photograph of Gd-DTPA-DM Microcapsules in the 75—106 μm Fraction

Microcapsules were 20% overcoated with EC–CH, surface-treated with HEL and mixed with 20% mannitol.

mice given gadolinium microcapsules than those given placebo microcapsules or control survived for 60 d and considerably longer ($p < 0.0001$). Details are reported elsewhere.[6]

Conclusion

Microcapsules of Gd-DTPA-DM applicable to *in vivo* experiments for neutron-capture therapy were designed and prepared. They were composed of lactose cores, an undercoat of EC and PVP, a drug-layer of Gd-DTPA-DM, EC and PVP, a waterproof coat and release-sustaining overcoat of EC and CH, and a surface treated with HEL. The 20% level overcoating suppressed the release of Gd-DTPA-DM from 75—106 μm microcapsules to less than 10% for the first 20 min.

Development of GNCT is now in progress. Microcapsules prepared in this study are to be used to immediately evaluate whether the electrons and photons from gadolinium which exists extracellularly still allow cell inactivation. For this purpose, the above 75—106 μm microcapsules with 20% overcoat seem sufficient. However, the next step of studies on the neutron-capture therapy will require smaller microcapsules to even more strongly suppress the release of gadolinium. Development of such microcapsules is now in progress.

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