Expert Opinion

Application of drug delivery system to boron neutron capture therapy for cancer

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Background: Tumor cell destruction in boron neutron capture therapy (BNCT) is due to the nuclear reaction between ^{10}B and thermal neutrons ($^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He$ (α) + 2.31 MeV (93.7 %)/2.79 MeV (6.3 %)). The resulting lithium ions and αparticles are high linear energy transfer (LET) particles which give a high biological effect. Their short range in tissue (5 – 9 μm) restricts radiation damage to those cells in which boron atoms are located at the time of neutron irradiation. BNCT has been applied clinically for the treatment of malignant brain tumors, malignant melanoma, head and neck cancer and hepatoma. Sodium mercaptoundecahydro-dodecaborate ($Na_2{}^{10}B_{12}H_{11}SH$: BSH) and borono-phenylalanine (^{10}BPA) are currently being used in clinical treatments. These low molecule compounds are easily cleared from cancer cells and blood, so high accumulation and selective delivery of boron compounds into tumor tissues and cancer cells are most important to achieve effective BNCT and to avoid damage to adjacent healthy cells. *Objective*: In order to achieve the selective delivery of boron atoms to cancer cells, a drug delivery system (DDS) is an attractive intelligent technology for targeting and controlled release of drugs. *Methods*: We performed literature searches related to boron delivery systems *in vitro* and *in vivo Results*: We describe several DDS technologies for boron delivery to cancer tissues and cancer cells from the past to current status. We are convinced that it will be possible to use liposomes, monoclonal antibodies and WOW emulsions as boron delivery systems for BNCT clinically in accordance with the preparation of good commercial product (GCP) grade materials.

Keywords: boron, boron neutron capture therapy, drug delivery system, emulsion, gadolinium, ligand, liposome, monoclonal antibody, porphyrin, targeting

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1. Introduction

Boron neutron capture therapy (BNCT) is based on the nuclear capture and fission reactions that occur when non-radioactive boron-10 is irradiated with low energy thermal neutrons to yield high linear energy transfer (LET) alpha particles (^{4}He) and recoiling lithium-7(^{7}Li) nuclei.

The successful treatment of cancer by BNCT requires the selective delivery of a sufficient number of ^{10}B atoms (approximately 10^9 atoms/cell) to constituent cells within a tumor (**Figure 1**).

BNCT has primarily been used to treat patients with brain tumors, malignant melanoma and, more recently, those with head and neck cancer [1-10]. Two low molecular weight boron delivery agents are currently being used clinically, Sodium mercaptoundecahydro-dodecaborate ($Na_2{}^{10}B_{12}H_{11}SH$: BSH) and borono-phenylalanine (^{10}BPA) (**Figure 1**).

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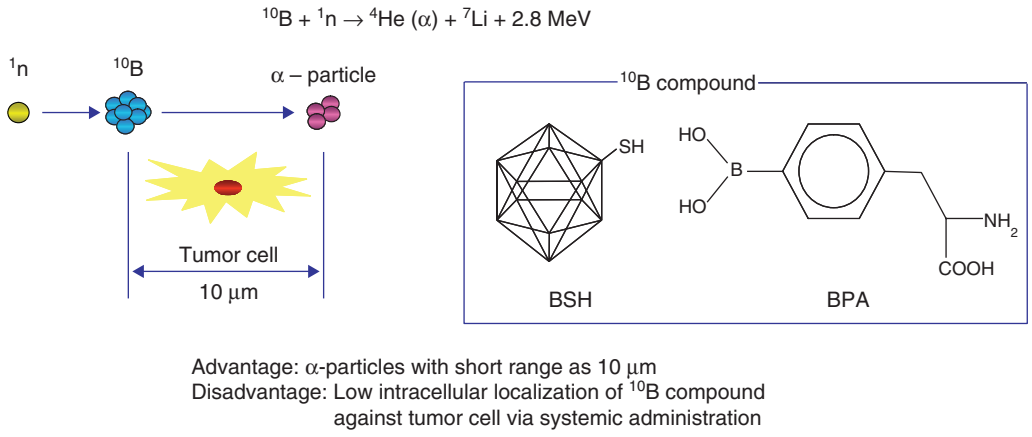


Figure 1. Schema of concept of BNCT. Tumor cell destruction in boron neutron capture therapy (BNCT) is due to the nuclear reaction between ^{10}B and thermal neutrons ($^{10}B + {}^{1}n \rightarrow {}^{7}Li + {}^{4}He\ (\alpha) + 2.31$ MeV (93.7 %)/2.79 MeV (6.3 %)). The resulting lithium ions and αparticles are high linear energy transfer (LET) particles which give a high biological effect. Their short range in tissue (5 – 9 μm) restricts radiation damage to those cells in which boron atoms are located at the time of neutron irradiation. Sodium mercaptoundecahydrododecaborate ($Na_2{}^{10}B_{12}H_{11}SH$;BSH) and borono-phenylalanine (^{10}BPA) are used in clinical treatments.

The number of receptors varies from small to large and the uptake of large amounts of boron for each receptor interaction is necessary in order to deliver sufficient amounts of boron; therefore, each targeting moiety must deliver a large number of boron atoms. One possible way to meet these requirements would be to use drug delivery systems (DDS) containing a large number of boron atoms, including receptor-targeting ligand carriers.

Nanoparticles, such as liposomes, polymeric micelles, lipoplexes and polyplexes have been extensively studied as targeted drug carrier systems over the past three decades (**Table 1**). A wide variety of active agents can be incorporated into or complexed with these particles, varying from low molecular weight drug molecules to macromolecules such as proteins and nucleic acids. An important requirement of the systemic intravenous use of this targeted nanomedicine approach is the ability of the nanoparticles to circulate in the bloodstream for a prolonged period of time. To achieve this, poly(ethylene glycol) (PEG) is often used as a coating material. As a result, the PEG coating reduces uptake by macrophages of the mononuclear phagocyte system (MPS) and provides relatively long plasma residence times. The prolonged circulation time is needed to enable extravasation at sites with increased vascular permeability such as tumors and inflamed sites (enhanced permeability and retention (EPR) effect). The polymer coating, however, may hinder drug release and target cell interaction and can therefore be an obstacle in the realization of the therapeutic response. Attempts have been made to enhance the therapeutic efficacy of sterically stabilized nanoparticles by means of shedding, i.e., losing the coating after arrival at the target site, and/or targeting using ligands [11].

Careful consideration of the composition of the encapsulation medium not only improves drug solubility, but may also facilitate targeted and regulated drug delivery through controlling the rate of drug release, and hence the absorption of the drug *in vivo*. A material that has recently found application as an encapsulation medium is stearic acid, which is inert, non-toxic, cheap and listed as an acceptable excipient in the United States Pharmacopeia (USP). Certain factors are known to affect drug release measurements; these include the pH, temperature and ionic strength of the dissolution medium and the dissolution method chosen [12].

Liposomes have long been used as carrier systems for the delivery of vaccines, therapeutic drugs and hormones because of their easy preparation, good biocompatibility and biodegradability, low toxicity and commercial availability. The efficient functioning of enzymes inside liposomes opens up new possibilities of applications in biocatalysis and bioanalytical tools [13]. A demerit is reported that some PEGylated liposomes lose their long-circulating characteristic upon repeated injection at certain intervals in the same animal (referred to as the 'accelerated blood clearance phenomenon') by immune responses [14]. Molecular modifications can add special features to liposomes in order to enhance their transgene delivery capacity. DNA/liposome complexes to the brain could be achieved by incorporating antibodies to the transferrin receptor in order to facilitate passage across the blood–brain barrier [15]. As far as the application of siRNAs is concerned, many groups have already reported the usefulness of cationic liposomes or modified liposomes for *in vivo* use, suggesting that liposomal systems are good candidates for siRNA delivery [16].

Monoclonal antibodies are highly specific therapies with low toxicities. In addition to directly inducing cancer cell death, monoclonal antibodies also lead to immune activation, resulting in tumor cell toxicity. Limitations of

Table 1. Overview of different lipid carriers.

Carriers	Size range	Composition	Features and preparation methods
Liposomes	25 nm – few microns	Natural or synthetic phospholipids	Bilayered vesicles containing an aqueous volume entirely enclosed by a membraneous lipid bilayer
			Passive loading (mechanical dispersion, solvent dispersion) and active loading
Solid lipid nanoparticles	50 – 1000 nm	High melting point fats of natural or synthetic origin	Submicron colloidal carriers containing solid hydrophobic core having a monolayer of phospholipid coating
			High pressure homogenization Microemulsion formation
Oily suspensions	Globule size 10 nm – few microns	Natural or synthetic oils	Dispersions of peptides and proteins in oils of high viscosity for sustained-release of proteins
			Dispersion technique
Submicron lipid emulsions	Lipid globules 1 – 100 nm	Lipids, hydrophilic liquid, surfactants	Multicomponent fluid made of water, a hydrophobic liquid, and one or several surfactants resulting in a stable system
			Emulsification technique (o/w, w/o, w/o/w, w/o/o)
Lipid microspheres	0.2 – 100 μm	High melting lipid, phospholipids	Water dispersible solid microparticles composed of a solid hydrophobic fat core stabilized by a monolayer of phospholipid molecules embedded in a microparticle surface
			Melt method, multiple microemulsion, preincorporation into lipophilic carriers

monoclonal antibodies are their size, which may limit tumor penetration, heterogeneous antigen expression and expression of tumor antigens in normal cells [17]. Monoclonal antibodies (mAbs) have come to represent a standard therapy for some tumors, and have proven to be an important extension to the treatment of a broad spectrum of malignancies [18]. Many mAb therapies are already in use for preventing transplant rejection, oncological treatment and antiviral treatments. The success of mAb therapy depends on several factors, including the creation of non-immunogenic mAbs (i.e., mAbs that do not generate a human anti-mAb antibody), the tissue dynamics of the tumor, the integrity of the targeted antigen and the route of administration. For instance, the large molecular weight of antibodies is likely to result in inefficient drug delivery into the brain because the blood–brain barrier prevents their passage into brain parenchyma. For this reason, mAb therapy is often delivered intratumorally rather than systemically. Such intratumoral delivery is generally done via catheter or convection-enhanced delivery methods and might avoid systemic targeting and toxicity. It can be performed into the tumor itself or after resection. Cetuximab binds to the epidermal growth factor receptor (EGFR) and prevents ligand binding, thereby blocking ligand-induced tyrosine kinase activation and stimulating receptor internalization. Cetuximab appears to induce apoptosis and inhibit angiogenesis. In an effort to increase effectiveness, mAbs may be conjugated with drugs, toxins, or radioisotopes. For example, a three-step avidin–biotin pre-targeting approach to target yttrium-90 biotin to tumor cells in patients with high-grade glioma has been reported upon. MAbs against EGFR and EGFRvIII have been used to deliver 125I and have been proposed for use in Phase III studies in patients with GBM [17].

This review will focus on DDS, including monoclonal antibodies, liposomes, emulsions, folic acid, and epidermal and vascular endothelial growth factors (EGF and VEGF).

2. Boron delivery systems for effective BNCT

2.1 Monoclonal antibody

Barth *et al.* first reported the application of a monoclonal antibody for boron delivery systems, using boronated monoclonal antibody 17-1A in potential neutron capture therapy for colorectal cancer [18].

Takahashi and our group reported the application of a monoclonal antibody (mAb) against alpha-fetoprotein (AFP) as a useful tool to deliver boron-10 (^{10}B) to AH-66 hepatoma cells for BNCT [19]. MAb was boronated by mixing

with ^{10}B-compound ($Cs_2{}^{10}B_{12}H_{11}SH$) using *N*-succinimidyl 3(2-pyridyldithio)propionate (SPDP). The maximum number of ^{10}B atoms conjugated to an antibody molecule was approximately 1240. Secondly, using this boronated mAb, ^{10}B was delivered to AH-66 cells, and 11×10^9 ^{10}B atoms were estimated to be on and/or in an AH-66 cell. After irradiation with thermal neutrons, boronated AH-66 cells showed a decreasing uptake of [^{3}H]TdR in proportion to the number of ^{10}B atoms bound to and/or incorporated into tumor cells [19].

Yanagië's group prepared boronated mAb by conjugating 50 mM ^{10}B compound to an anti-AFP mAb (2 mg/ml) using SPDP. Boron concentrations in tumor tissue obtained 12, 24, 72 and 120 h after injection of 3.0 mg ^{10}B-conjugated anti-AFP mAb were 11.10 ± 3.12 (SD, n = 6). 29.30 ± 5.11, 33.02 ± 11.8 and 12.91 ± 5.62 ppm, respectively. The concentrations in blood were less than 0.40 ± 0.10 ppm with anti-AFP mAb. We were able to carry 1.84×10^9 ^{10}B atoms to AH66 rat hepatic tumor cells by using ^{10}B-anti-AFP mAb. ^{10}B-conjugated antitumor mAb could deliver a sufficient amount of ^{10}B atoms to tumor cells to induce cytotoxic effects upon thermal neutron irradiation [20].

Tamat *et al.* reported on the conjugated dicesium-mercapto-undecahydrododecaborate ($Cs_2B_{12}H_{11}SH$) to 225.28S mAb directed against high molecular weight melanoma-associated antigens (HMW–MAA), using poly-L-ornithine as a 'bridge' to increase the carrying capacity of the antibody and to minimize change in the conformational structure of antibody. This method produces a boron content of 1300 – 1700 B atoms per molecule 225.28S, while retaining immunoreactivity [21].

Ranadive *et al.* reported that B72.3 mAb was successfully boronated using mercaptoundecahydro-closo-dodecaborate (boron cage compound), reacting the lysine residues of the antibody with m-maleimidobenzoyl succinimide ester. Michael added the maleimido group to the mercapto boron cage compound to form a physiologically stable thioether linkage. 125I-labeled and boronated B72.3 mAb demonstrated clear tumor localization when administered via tail vein injections to athymic nude mice bearing LS174-T tumor xenografts. Boronated antibody was calculated to deliver 10^6 boron atoms per tumor cell. They calculated that fewer atoms of ^{10}B per tumor cell would be necessary to effect successful BNCT when boron is targeted to the tumor cell membrane [22].

Liu *et al.* described a panel of BsAb reactive with polyhedral borane anions (PBA) and a tumor-associated chondroitin sulfate proteoglycan. Two of BsAb (H6 and B8) also reacted with cells that had been exposed to PBA ($Na_2B_{10}H_{10}$ and ^{10}BSH) and a boronated starburst dendrimer, which contained approximately 250 – 400 B atoms per molecule. The affinity constant (Ka) of BsAb-B8 was 2.57×10^8 M^{-1} on M21 human melanoma cells and 3.49×10^8 M^{-1} on A172 glioblastoma cells; these BsAb recognized the tumor-specific antigen and could potentially be used to target ^{10}B to these tumors for BNCT [23].

Barth *et al.* evaluated the mAb, cetuximab, as a boron delivery agent for BNCT of brain tumors. Cetuximab (IMC-C225) is a mAb directed against both the wild-type (WT) and mutant vIII isoform of the EGFR. Twenty-four hours after intratumoral administration of boronated cetuximab (C225-G5-B(1100)), the mean boron concentrations in rats bearing either F98 (EGFR) or F98 (WT) gliomas were 92.3 ± 23.3 μg/g and 36.5 ± 18.8 μg/g, respectively. The mean survival time (MST) of rats that received C225-G5-B(1100), administered by convection-enhanced delivery (CED), was 45 ± 3 d compared to 25 ± 3 d for untreated control animals. Further enhancement of MST to > 59 d was obtained by administering C225-G5-B(1100) in combination with intravenous BPA. The efficacy of boronated mAb for BNCT of an intracerebral (i.c.) glioma was demonstrated as a paradigm for future studies using a combination of boronated mAbs and low molecular weight delivery agents [24].

2.2 Liposomes

Yanagië's group first applied liposomes as carriers in a boron delivery system for cancer BNCT. Immunoliposomes containing the ^{10}B-compound can act as selective and efficient carriers of ^{10}B atoms to target tumor cells in BNCT. An immunoliposome containing a ^{10}B-compound was examined as a selective drug delivery system in BNCT. A new murine mAb (2C-8) was prepared by immunizing mice i.p. with carcinoembryonic antigen (CEA), producing a human pancreatic cancer cell line. Liposomes, conjugated with mAbs specific for CEA, were shown to bind selectively to cells bearing CEA on their surface. After irradiation with a thermal neutron ($1 \times 10^{11} - 1 \times 10^{13}$ n/cm^2), boronated AsPC-1 cells showed a decreasing uptake of ^{3}H-TdR compared with the control group. The numbers of ^{10}B atoms in liposomes bound to an antibody were in proportion to the dose of ^{10}B compounds added, and the maximum number of ^{10}B atoms was approximately 1.2×10^4/Ab. Immunoliposomes attached to tumor cells suppressed growth *in vitro* upon thermal neutron irradiation and this suppression was dependent upon the concentration of the ^{10}B-compound in the liposomes and on the density of the antibody conjugated to the liposomes. Immunoliposomes could deliver a high amount of ^{10}B atoms to tumor cells and exert a cytotoxic effect by thermal neutrons [25,26]. Yanagië's group found that injection of ^{10}B-immunoliposomes caused the greatest tumor suppression with thermal neutron irradiation *in vivo*. Immunoliposomes could deliver a high amount of ^{10}B atoms to tumor cells and exert a cytotoxic effect by thermal neutrons. BNCT with intratumoral injection of immunoliposomes is able to destroy malignant cells in the marginal portion between normal tissues and cancer tissues (**Figures 2, 3**) [27]. We also noted the growth inhibition of human breast cancer cells in

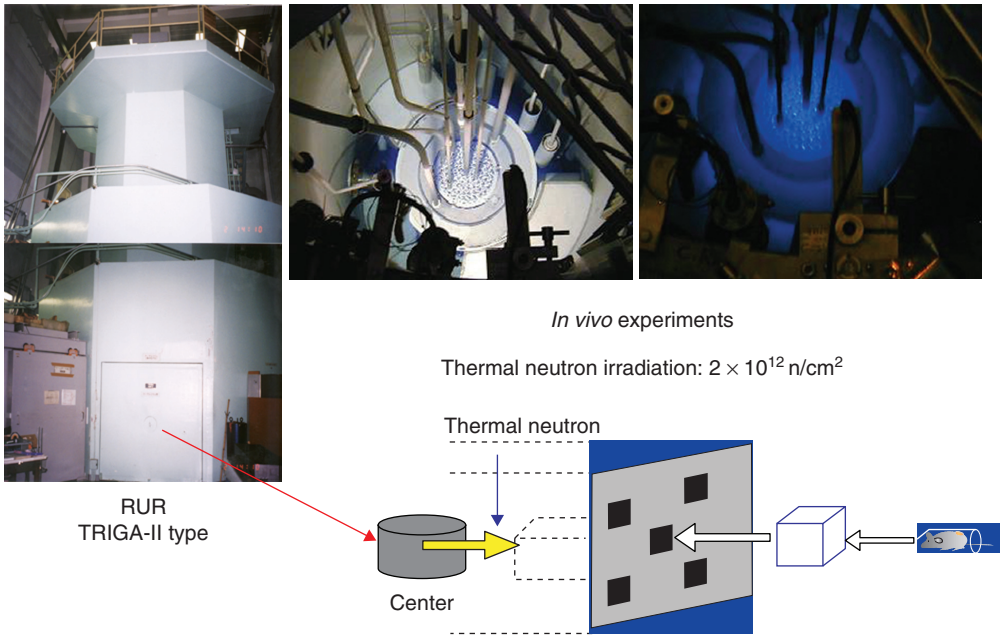


Figure 2. Schema of irradiation on the TRIGA II atomic reactor at Rikkyo University Reactor. AsPC-1 cells reacted, boronated immunoliposomes or boronated stealthliposomes were irradiated with thermal neutrons ($1 \times 10^{11} - 1 \times 10^{13}$ n/cm^2).

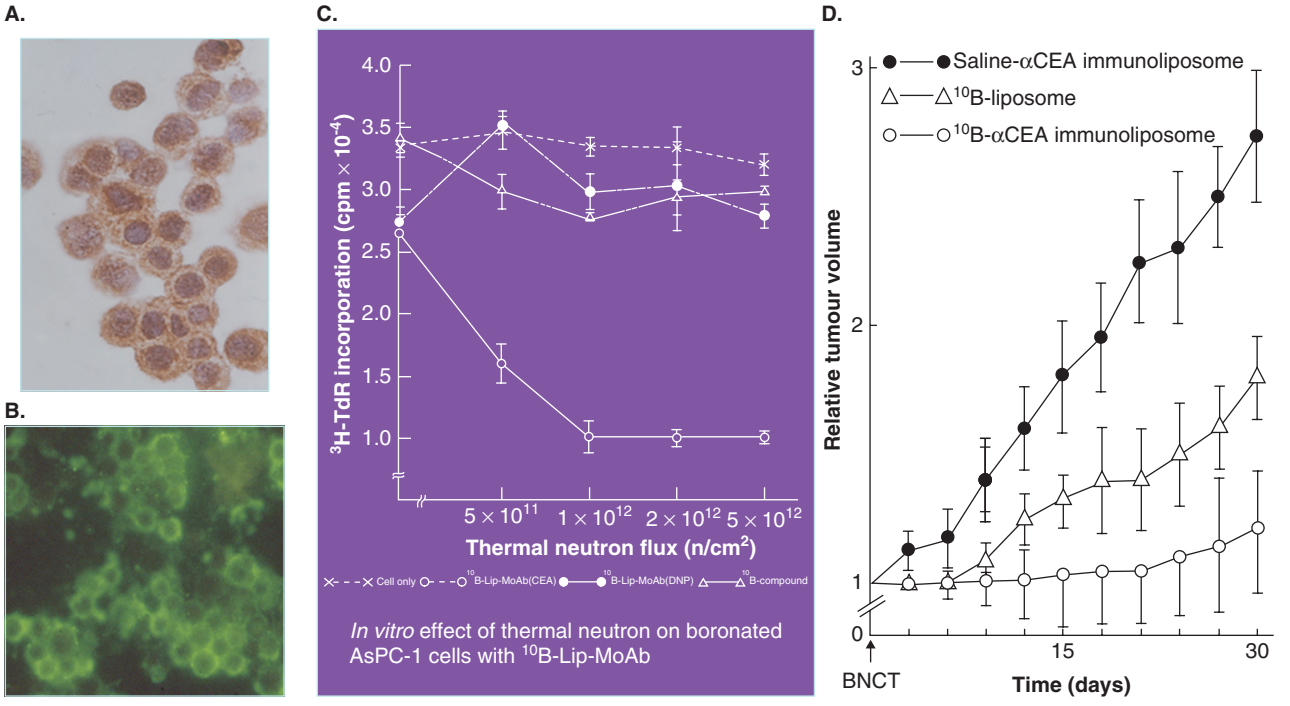


Figure 3. Tumor growth suppression on BNCT with ^{10}B immunoliposomes. Liposomes conjugated with mAbs specific for CEA were shown to bind selectively to human pancreatic carcinoma cells (AsPC-1) bearing CEA on their surface (reaction of boronated anti-CEA mAb-immunoliposomes to AsPC-1 cells by immunostaining) (**A, B**). Immunoliposomes attached to AsPC-1 cells suppressed growth *in vitro* upon thermal neutron irradiation (**C**) (Yanagië *et al.* BJC, 1991). Intratumoral injection of boronated immunoliposomes can increase the retention of ^{10}B atoms in tumor cells, and suppress tumor growth *in vivo* under thermal neutron irradiation (**D**) (Yanagië *et al.* BJC, 1997).

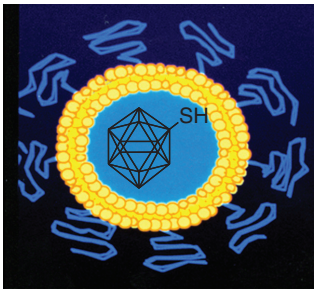
culture by neutron capture reaction using ^{10}B-containing multilamellar liposomes [28].

Shelly *et al.* reported small unilamellar vesicles with mean diameters of 70 nm or less, composed of a pure synthetic phospholipid (distearoyl phosphatidylcholine) and cholesterol encapsulating water soluble ionic boron compounds and a photoisomer of $B_{20}H_{18}^{2-}$. The highest tumor concentrations achieved the therapeutic range (greater than 15 µg of boron per g of tumor) while maintaining high tumor–boron/blood–boron ratios (> 3) [29]. Feakes *et al.* reported that sodium salt of the resulting $[B_{20}H_{17}SH]_{42}$ ion had been encapsulated in small, unilamellar liposomes, which are capable of delivering their contents selectively to tumors *in vivo*, and was investigated as a potential agent for BNCT. The biodistribution of boron was determined after intravenous injection of the liposomal suspension into BALB/c mice bearing EMT6 mammary adenocarcinoma, and the tumor boron concentration increased during the experiment, resulting in a maximum observed boron concentration of 46.7 µg of B per gram of tumor at 48 h and a tumor to blood–boron ratio of 7.7 [30]. They also investigated small unilamellar vesicles, composed of distearoylphosphatidylcholine/cholesterol, 1:1, and incorporating K[nido-7-$CH_3(CH_2)$15-7,8-C_2B_9HI] in the bilayer *in vivo*. They achieved peak tumor boron concentrations of ~ 35 µg of boron per gram of tissue and tumor/blood–boron ratios of ~ 8 by injection with 5 – 10 mg of boron per kg of body weight. Thus, the incorporation of both K[nido-7-$CH_3(CH_2)$15-7, 8-C_2B_9Hll] and the hydrophilic species, Na_3[1-(2′-B_1OH,)-2-$NH_3B_1OH_8$], within the same liposomes demonstrated significantly enhanced biodistribution characteristics, exemplified by maximum tumor–boron concentrations of 50 µg of boron per gram of tissue and tumor/blood–boron ratios of ~ 6 [31,32]. Hawthorne *et al.* described small unilamellar liposomes containing polyhedral borane Na_3[a2-$B_{20}H_1$-$NH_2CH_2CH_2NH_2$]. Liposomes encapsulating this species produced a tumor–boron concentration of 45 µg/g tissue at 30 h post-injection, at which time the tumor/blood–boron ratio was 9.3 [33].

Moraes *et al.* reported that the loading of o-carboranylpropylamine chloride (CPA) into liposomes with an average diameter of 100 nm is estimated at 13,000 molecules per vesicle for the most stable system. CPA toxicity to normal human peripheral blood lymphocytes and to adherent glioblastoma multiform SK-MG-1 cells *in vitro* is observed to decrease as a result of the entrapment of CPA in liposomes [34].

Mehta *et al.* prepared liposomes composed of DPPC/CHOL in a molar ratio 1:1 polyethylene glycol(PEG) (concentration: 5 mol %) with an average diameter in the range of 100 – 110 nm, and 200 ml of liposomes (l.88 mg phospholipid/mouse and 3.5 – 5.8 mg BSH/kg body weight), and injected them into mice via the tail vein. PEGliposomes resulted in a significant improvement in the circulation time of BSH compared to that obtained previously after injecting free BSH. The mean percentage of injected BSH remaining in circulation at the end of 24 h was 19% for PEGliposomes compared to the corresponding value of 7% for conventional liposomes. The mean percentage uptake by the liver and spleen was not significantly different for the two types of liposomes; the blood/RES ratios were higher for PEGliposomes at all time points. The PEGliposomes could be further explored as a means of enhanced boron drug delivery to tumor cells for BNCT [35]. Yanagië's group developed a delivery system consisting of PEG binding liposomes (DPPC/cholesterol/DSPC-PEG2000) with an entrapped ^{10}B-compound. After thermal neutron irradiation of mice injected with ^{10}B-PEGliposomes, the growth of AsPC-1 tumors was suppressed relative to controls (**Figure 4**). Intravenous injection of ^{10}B-PEGliposomes can increase the retention of ^{10}B atoms by tumor cells, causing suppression of tumor growth *in vivo*, after thermal neutron irradiation [36].

Ishida *et al.* suggested that transferrin (TF) pendant-type PEGliposomes (TF-PEGliposomes) was useful for *in vivo* cytoplasmic targeting of chemotherapeutic agents or plasmid DNAs to target cells. TF-PEGliposomes, bearing approximately 25 TF molecules per liposome, readily bound to mouse Colon 26 cells *in vitro* and were internalized by receptor-mediated endocytosis. Electron microscopic studies in Colon 26 tumor-bearing mice revealed that extravasated TF-PEGliposomes were internalized into tumor cells by receptor-mediated endocytosis [37]. Maruyama and our group had reported unilamellar BSH-encapsulating, TF-PEGliposomes as selective boron-targeting liposomes. When TF-PEGliposomes were injected at a dose of 35 mg ^{10}B/kg, we observed a prolonged residence time in the circulation and low uptake by the reticuloendothelial system (RES) in Colon 26 tumor-bearing mice, resulting in enhanced accumulation of ^{10}B into solid tumor tissue. TF-PEG liposomes maintained a high ^{10}B level in the tumor, with concentrations over 30 µg/g for at least 72 h after injection. This high retention of ^{10}B in tumor tissue indicates that binding and concomitant cellular uptake of extravasated TF-PEG liposomes occurs by TF receptor and receptor-mediated endocytosis, the plasma level of ^{10}B decreased, resulting in a tumor/plasma ratio of 6.0 at 72 h after injection. Intravenous injection of TF-PEG liposomes can increase the tumor retention of ^{10}B atoms, which were introduced by receptor-mediated endocytosis of liposomes after binding, causing tumor growth suppression *in vivo* upon thermal neutron irradiation (**Figure 5**) [38]. Ogura and our group reported that neutron capture autoradiographic images obtained by cold neutron irradiation were highly superior in quality to those of thermal neutron beams [39]. Yanagië's group reported a technique of neutron capture autoradiography (NCAR) of sliced whole-body samples of tumor bearing mice using CR-39 plastic track detectors in order to achieve an accurate measurement of ^{10}B concentrations in biological samples. We can increase



Schema of
^{10}B PEG liposome

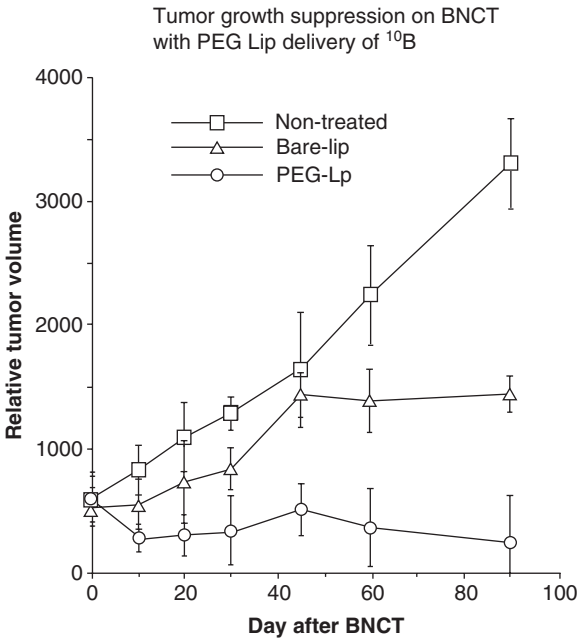


Figure 4. Tumor growth suppression with BNCT after intravenous injection of ^{10}B-PEG liposomes. We have reported that the ^{10}B-PEG-liposome and ^{10}B-TF-PEG-liposome have the possibility of retention to tumor cells and providing sufficient ^{10}B atoms for the BNCT by systemic injections. Intravenous injection of ^{10}B-PEG-liposome and ^{10}B-TF-PEG liposome inhibited tumor cell growth with thermal neutron irradiation *in vivo*.
Yanagië *et al.* Biomed Pharmacother 2006; Maruyama *et al.* J Control Rel 2004.

the accumulation of ^{10}B atoms in tumor tissues by binding PEG chains to the surface of liposomes, which increases retention in the blood flow and escapes phagocytosis by RES, therefore, we will be able to apply the NCAR technique to select an effective ^{10}B carrier in BNCT for cancer (**Table 2**, **Figure 5**) [40]. Doi *et al.* reported on BSH-encapsulating TF-PEGliposome, which decreased ^{10}B concentration in blood and normal tissue, while maintaining a high ^{10}B concentration in tumor tissue for a couple of days [41].

Pan *et al.* reported folate receptor (FR)-targeted liposomes as potential carriers for a series of boron-containing agents. Two highly ionized boron compounds, $Na_2B_{12}H_{11}SH$ and Na_3 ($B_{20}H_{17}NH_3$), were incorporated into liposomes by passive loading with encapsulation efficiencies of 6 and 15%, respectively. They also investigated boronated polyamines, spermidine derivatives and spermine derivatives. These were incorporated into liposomes by a pH-gradient-driven remote-loading method with varying loading efficiencies. The *in vitro* uptake of folate-derivatized, boronated liposomes was investigated using human KB squamous epithelial cancer cells, which have amplified FR expression. Higher cellular boron uptake (up to 1584 µg per 10^9 cells) was observed with FR-targeted liposomes than with non-targeted control liposomes (up to 154 µg per 10^9 cells) in human KB squamous epithelial cancer cells [42]. FR-targeted liposomes were saturable and could be blocked by 1 mM free folic acid [43]. Pan *et al.* also reported folate binding liposomes as boron delivery agents. Mice that received FR-targeted and non-targeted control liposomes showed indistinguishable levels of tumor boron uptake (up to 85 µg/g tumor), which reached a maximum at 24 h, while the tumor-to-blood (T/B) ratio continued to rise until 72 h [44]. Sudimack *et al.* reported that large unilamellar vesicles (-200 nm in diameter) were prepared with the composition of egg PC/chol/K[nido-7-$CH_3(CH_2)$15-7,8-$C_2B_9H_{11}$] (2:2:1, mol/mol), with an additional 0.5 mol % of folate-PEG-DSPE or PEG-DSPE added for FR-targeted or non-targeted liposomal formulations, respectively. Boron-containing FR-targeted liposomes readily bound to KB cells, an FR-overexpressing cell line, and were internalized via FR-mediated endocytosis. Boron uptake in cells treated with these liposomes was approximately 10 times greater than those treated with control liposomes. Most of the boron compound was found in either the cytosol/endosomal or cell membrane fractions, indicating efficient internalization of liposomal boron [45]. Stephenson *et al.* reported on the evaluation of $Na_3(B_{20}H_{17}NH_3)$ entrapped FR-targeted liposomes as a boron delivery carrier in mice bearing xenograft implants of FR (+) KB subcutaneous tumor. The folate receptor is amplified in a variety of human tumors, including over 90% of ovarian carcinoma. Mice that received FR-targeted liposomes containing boron showed the highest tumor boron levels at 24 h (6.1 µg/g) and tumor/blood boron ratios continued to rise for up to 120 h [46]. Thirumamagal *et al.* prepared three novel carbonyl cholesterol derivatives as lipid bilayer components for the

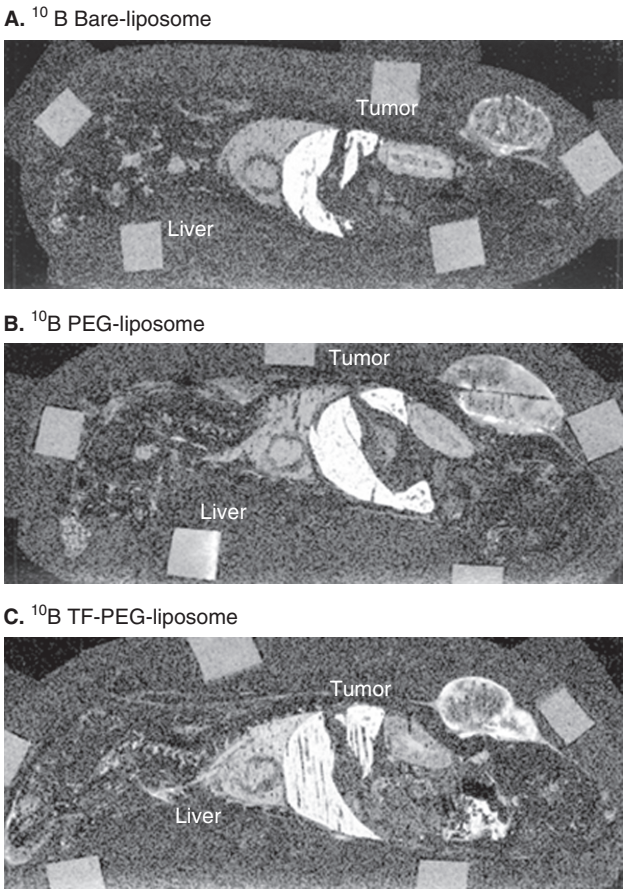


Figure 5. Neutron capture autoradiography (NCAR) of mouse whole body sections after intravenous injection of ^{10}B stealth liposomes. About 700 µg of ^{10}B-liposome solutions were injected. When mouse colon cancer, Colon 26, was transplanted to the back of Balb/c mice, the tumor had hypervascularity instead of AsPC-1 has hypovascularity. Imaging of the Col 26 bearing mice showed that ^{10}B accumulation was increased with PEGylation of liposomes (**B, C**) compared to bare liposomes (**A**), and superior selectivity of accumulation of ^{10}B in the tumor tissue was acquired by binding of transferrin to the PEG chains (**C**).

construction of non-targeted and receptor-targeted boronated liposomes for BNCT. One of the synthesized boronated cholesterol mimics was stably incorporated into non-, FR- and vascular endothelial growth factor receptor-2 (VEGFR-2)-targeted liposomes. FR-overexpressing KB cells and VEGFR-2-overexpressing 293/KDR cells were incubated with boronated FR- and VEGFR-2-targeted liposomes, respectively, so that the former accumulated extensively in KB cells and the latter effectively interacted with VEGFR-2 [47].

Kullberg *et al.* reported PEG-stabilized EGF liposomes containing water soluble boronated phenanthridine, WSP1, or water-soluble boronated acridine, WSA1, for EGFR targeting as a pegylated ligand liposome delivery vehicle. WSA1 was obtained with ligand-dependent uptake of inglioma cells. Approximately 10^5 boron atoms were contained in each liposome. After internalization in tumor cells, WSA was distributed mainly in the cytoplasm and was shown to have long cellular retention, with 80% of the boron remaining after 48 h. Since it is known that, for a therapeutic effect, approximately $10^8 - 10^9$ boron atoms are needed in a single tumor cell, $10^2 - 10^3$ receptor interactions are needed to meet the demand. Tests applying cultured glioma cells indicate, without optimization of the delivery conditions, that boron uptake is in the ppm range, which is necessary for successful BNCT [48,49]. Kullberg *et al.* also applied EGF-tagged liposomes loaded with water soluble boronated acridine developed for BNCT. The cellular uptake of boron reached 90 ppm and it was determined by subcellular fractionation that most of the cell-associated boron was located outside of the nucleus. At fluence of 3×10^{12} neutrons/cm^2, the cell-killing effect of boron-containing EGFliposomes was about 10 times higher than for neutrons only [50]. Carlsson *et al.* reported ligand liposomes targeting folate or EGF receptors, or PDGFR and EGFRvIII [51]. Pan *et al.* reported the construction of EGFR targeting cetuximab–immunoliposomes for targeted delivery of boron compounds to EGFR(+) glioma cells for neutron capture therapy.

Immunoliposomes were synthesized using a novel cholesterol-based membrane anchor, maleimido-PEG-cholesterol (Mal-PEG-Chol), to incorporate cetuximab into liposomes. Much greater (approximately eightfold) cellular uptake of boron was obtained using cetuximab-immunoliposomes in EGFR(+) F98EGFR compared with non-targeted human IgG-immunoliposomes [52]. Wei *et al.* investigated HER-2-targeted boron-containing liposomes as a potential drug delivery vehicle for BNCT. Trastuzumab was conjugated to the distal end of PEG-DSPE-NHS in micelles and Trastuzumab-PEG-DSPE were then transferred to preformed liposomes, either empty or loaded with water soluble boronated acridine (WSA). The conjugates showed specific binding to HER-2 receptors of SK-BR-3 cells, reaching 132 ppm of boron in targeted cells after 24 h. WSA was distributed mainly in the cytoplasm and was shown to have long cellular retention, with 90 and 67% of the boron remaining in the cells after 24 h and 48 h, respectively. The conjugate – Trastuzumab-liposome-WSA – was considered to be a potent drug delivery system for BNCT [53].

Peacock GF *et al.* reported a new cholesterol–carborane conjugate (BCH) synthesized as a potential targeting agent for BNCT of cancers. The compound is extremely water insoluble, and studies have indicated that the cellular uptake of BCH in conventional and PEG liposomal formulations was 49.1 and 45.9 µg boron/g cells, respectively. Therefore, this compound, formulated in both liposomal formulations, delivered sufficient levels of boron to cancer cells *in vitro*, indicating that BCH is a promising approach for use in BNCT [54].

Table 2. Track density of organs after injection of ^{10}B-Liposomes.

	Brain	Heart	Kidney	Liver	Lung	Tumor
Cont	780 ± 121	736 ± 261	864 ± 54	509 ± 52	741 ± 12	1045 ± 7
BSH	779 ± 91	873 ± 193	1370 ± 85	2460 ± 99	1245 ± 120	1370 ± 85
Bare-lip. 48	737	1290 ± 14	7865 ± 2750	39300 ± 424	3715 ± 1845	4450 ± 113
Bare-lip. 60	830	861 ± 309	5290	41100 ± 8909	1335 ± 276	3030 ± 240
PEG-lip. 48	1002 ± 139	3455 ± 502	6515 ± 232	21850 ± 919	4255 ± 148	5705 ± 289
PEG-lip. 60	0	2470 ± 1032	5700	25100 ± 1555	3170 ± 339	5000 ± 890
TF-PEG-lip. 48	1190 ± 85	6455 ± 275	9790 ± 268	18100 ± 424	5540 ± 268	6995 ± 403
TF-PEG-lip. 60	752	2030 ± 113	3925 ± 1096	14300 ± 141	1875 ± 148	6670 ± 848

Track densities under each organ after injection of ^{10}B-PEGliposome/^{10}B TF-PEGliposome/^{10}B Bare-liposome on AsPC-1 tumor bearing mice. Track densities of the AsPC-1 bearing mice showed that ^{10}B accumulation was increased with PEGlation of liposomes compared to bare liposomes or BSH solution.

Rossi *et al.* reported the evaluation of perturbations induced by beta-5-o-carboranyl-2′-deoxyuridine (CDU) on the structure and dynamics of egg phosphatidylcholine (EPC) and EPC/cholesterol liposomes with electron spin resonance (ESR) spectroscopy of long-chain nitroxides (5-, 7- and 16-doxyl stearic acid) [55]. Nakamura *et al.* had developed a double-tailed nido-carborane lipid, which was found to form a stable vesicle [56]. Miyajima *et al.* reported that synthesis of transferring conjugated PEG liposome by introducing nido-carborane lipid (Tf(+)-PEG-CL liposomes). Nido-carborane lipid 2 as a double-tailed boron lipid was synthesized from heptadecanol. The lipid 2 formed stable liposomes at 25% molar ratio toward DSPC with cholesterol. A boron concentration of 22 ppm in tumor tissues was achieved by the injection of Tf(+)-PEG-CL liposomes at 7.2 μg/kg body weight boron in tumor-bearing mice. Longer survival rates were observed in mice treated with Tf(+)-PEG-CL liposomes [57,58].

Justus *et al.* reported on two lipids – *S*-(*N,N*-(2-dimyristoyl oxyethyl) acetamido) thioundecahydro-closo-dodecaborate (2-) (B-6-14) and *S*-(*N,N*-(2-dipalmitoyloxyethyl) acetamido) thioundecahydro-closo-dodecaborate (2-) (B-6-16) – both of which have a double-tailed lipophilic part and a head group carrying two negative charges. When incorporated into liposomal formulations with equimolar amounts of distearoyl phosphatidylcholine (DSPC) and cholesterol, stable liposomes are obtained. Zeta-potential measurements indicate that both B-6-14- and B-6-16-containing vesicles are negatively charged. The liposomes are of high potential value as transporters of boron to tumor cells in treatments based on BNCT. Liposomes prepared from B-6-16 were not toxic even at 30 mM [59]. Martini *et al.* reported that (1)H and (13)C NMR was used to study the insertion of BPA in mixed liposomes, made up of positively charged 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and zwitterionic 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE). The boronated drug was distributed between the water phase and the liposomes [60].

Morandi *et al.* reported on the possibility for cationic (di-oleoyltrimethyl ammonium propane, DOTAP)/(L-alpha-dioleoyl-phosphatidyl-ethanolamine, DOPE) liposomes to act as carriers of boronated compounds such as 1,2-dicarba-closo-dodecaboran (12)-1-ylmethyl (beta-D-galactopyranosyl)-(1- > 4)-beta-D-glucopyranoside and 1,2-di-(beta-D-gluco-pyranosyl-ox)methyl-1,2-dicarba-closo-dodeca-borane(12). Computer analysis of ESR spectra from carborane-loaded liposomes enabled identification of the increase of the order degree in the liposome bilayer with increasing carborane concentration, together with decreased mobility [61]. Ristori *et al.* reported that carboranes are efficient boron delivery agents in BNCT. Cationic liposomes were prepared using the positively charged DOTAP and the zwitterionic DOPE as helper lipids, and can be considered appropriate vectors for BNCT [62]. Our group reported that BSH-entrapped cationic liposome solution (COATSOME-EL-C01) showed the retention of boron compound in AsPC-1 tumors 3 ~ 6 h after intratumoral injection [63,64].

Nakai *et al.* reported that HVJ-liposome, which is liposome fused with HVJ-E, had high boron trapping efficiency. The HVJ envelope (HVJ-E) vector system is a novel fusion-mediated gene delivery system based on inactivated hemagglutinating virus of Japan (HVJ; Sendai virus). Preliminary experiments showed that the cellular ^{10}B concentration after 60-min incubation with HVJ-E containing BSH was 24.9 μg/g cell pellet for BHK-21 cells and 19.4 μg/g cell pellet for SCC VII cells. HVJ-E fused with the tumor cell membrane and rapidly delivered boron agents, so it was hoped to apply this HVJ-E-mediated delivery system, including liposomes, for BNCT [65].

2.3 Polymer

Shukla *et al.* reported targeting the folate receptor on cancer cells using folic acid conjugates of boronated PEG containing polyamidoamine dendrimers to obtain ^{10}B concentrations

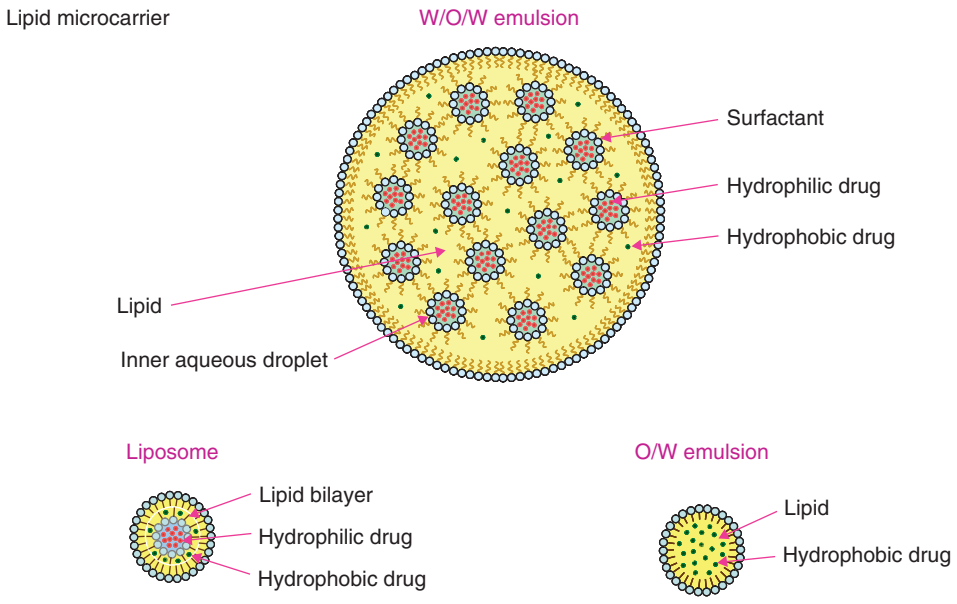


Figure 6. Schema of water-in-oil-in-water (W/O/W) emulsion, liposome, oil-in-water (O/W) emulsion.

necessary for BNCT by reducing uptake of these conjugates by the RES. They prepared polyamidoamine dendrimers containing approximately 13 decaborate clusters, approximately 1 PEG(2000) unit, and approximately 1 PEG(800) unit with folic acid attached to the distal end. *In vitro* studies using folate receptor (+) KB cells demonstrated receptor-dependent uptake of the conjugate [66].

Yanagië's group constructed boronated PEG-binding bovine serum albumin (BSA), and examined the delivering capacity of PEG-BSA to carry ^{10}B atoms to a human pancreatic cancer cell line (AsPC-1). Boronated PEG-BSA was synthesized using γ-maleimidobutyryloxy-succinimide (GMBS). The fluorescence intensity of AsPC-1 cells using FITC-PEG-BSA increased with the incubation time. Phagocytosis of the monocytes was prevented with PEG-conjugated BSA. Boronated PEG-BSA will reach the effective ^{10}B concentration with repeating contact with ^{10}B-PEG-BSA and conjugation of more ^{10}B atoms to PEG-BSA compound [67].

Hepatocellular carcinoma (HCC) is difficult to cure by operation, chemotherapy, or radiation therapies. Iodized poppy-seed oil (IPSO) has a property of depositing itself selectively in HCC cells. The usefulness of iodized poppy-seed oil (IPSO) for detecting or treating liver cancer has been reported. Kanematsu *et al.* reported a method that mixed a water soluble antitumor agent with IPSO [68]. In their reports, an aqueous solution of an anticancer drug, doxorubicin, was mixed with 60% urografin, a water soluble contrast medium, before the solution was mixed with the oil (IPSO) [69,70]. Suzuki *et al.* reported the efficacy of administering an emulsion of a BSH and lipiodol via a hepatic artery using a rat liver tumor model. Boron concentrations in the liver tumors and the tumor/liver (T/L) boron concentration ratio at 1, 6, and 12 h after administration of BSH/lipiodol emulsion (concentration: T/L ratio) were 479.2 ppm: 4.0, 197.3 ppm: 14.9, and 96.5 ppm: 6.6, respectively. The intra-arterial administration of BSH/lipiodol emulsion is an effective method for delivering a high concentration of ^{10}B selectively to liver tumors [71]. Suzuki *et al.* also reported intra-arterial administration of BSH with another embolizing agent, degradable starch microspheres (DSM) [72]. Yanagië's group reported the preparation of ^{10}BSH-entrapped water-in-oil-in-water (WOW) emulsion for selective intra-arterial infusion for HCC. WOW emulsion has been used clinically as a carrier of anti-cancer agents in intra-arterial injections. Higashi *et al.* prepared a long-term, inseparable WOW emulsion for use in arterial injection therapy to treat patients with HCC (**Figures 6, 7**) [73,74]. WOW was prepared by a membrane emulsification technique using a controlled pore glass membrane [75,76]. Emulsification using a fine-pore glass membrane of equal pore size is a new technique for preparing lipid microdroplets of equal size (monodispersed) containing aqueous fine microdroplets to form WOW. WOW emulsion was administrated by intra-arterial injection via proper hepatic artery and compared with ^{10}BSH-lipiodol mix emulsion or ^{10}BSH solutions in VX-2 rabbit hepatic tumor models. ^{10}B concentrations in VX-2 tumors with WOW emulsion were superior to those by conventional lipiodol mix emulsion. Electro-microscopic figures of WOW emulsion show the accumulation of fat droplets of WOW emulsion at the tumor site, but no accumulation of fat droplets in lipiodol emulsion. These results show that ^{10}B-entrapped WOW emulsion is most useful for intra-arterial boron delivery carrier in BNCT for cancer [77]. Chou *et al.* constructed phenylboric acid derivative-entrapped lipiodol nanoparticles (PBAD-L

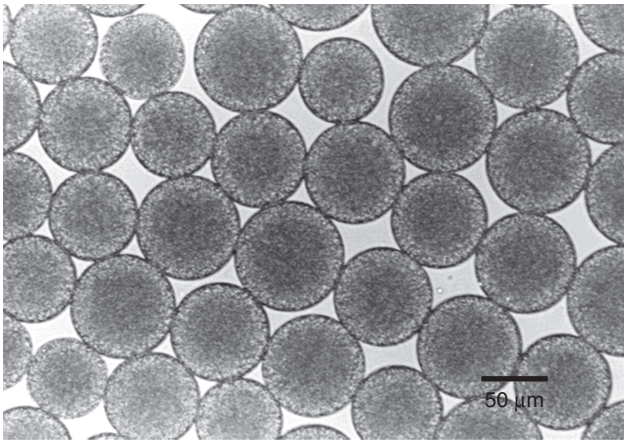


Figure 7. WOW was prepared by a membrane emulsification technique using a controlled pore glass membrane. Emulsification using a fine pore glass membrane of equal pore size is a new technique for preparing lipid microdroplets of equal size (monodispersed) containing aqueous fine microdroplets to form WOW. Yanagië's group reported on the preparation of ^{10}BSH-entrapped water-in-oil-in-water (WOW) emulsion for selective intra-arterial infusion for hepatocellular carcinoma.

nanoparticles) with boric acid. The boron concentration in HepG2 cells treated with PBAD-L nanoparticles for 18 h and then combined with boric acid for 6 h was 158 ppm. After neutron irradiation, the surviving fraction of hepG2 cells treated with PBAD-L nanoparticles was 12.6%, while with the combined treatment it was 1.3%. It was reported that this combination therapy had the potential for higher therapeutic efficacy of BNCT on hepatoma [78].

Kahl *et al.* reported that tumor bearing rats receiving boronoporphyrins (BOPP) by convection-enhanced delivery (CED) showed no evidence of toxic effects. CED of 1.5 mg BOPP produced an average tumor boron level of 519 µg/g and a tumor/blood ratio of ~ 1850:1, and tumor/brain ratios of ~ 9:1 (ipsilateral) and ~ 41:1 (contralateral) were found. The method of BOPP delivery from i.v. to CED significantly enhanced the boron concentration in tumors and produced very favorable tumor/blood and tumor/brain ratios with no concomitant systemic toxicity [79]. Matsumura *et al.* reported the synthesis of boronated porphyrin (STA-BX909) and uptake of boron atoms *in vitro* and *in vivo* [80]. Vincente *et al.* reported the synthesis and characterization of closo-carboranylporphyrins bearing either amino or phosphoric acid substituents for adequate solubility in aqueous solution, and achieved porphyrins containing 30 – 38% boron by weight [81]. Kawabata *et al.* reported the biodistribution of three carboranyl–porphyrins, H2DCP, H2TCP and H2TBP, following intracerebral (i.c.) administration by means of CED to F98 glioma-bearing rats. Tumor boron concentrations immediately after CED were 36 and 88 µg/g for H2DCP and H2TCP, respectively, and 103 and 62 µg/g for H2TCP and H2TBP, respectively, 24 h after termination of CED. Rats that received H2TCP by CED, followed by BNCT at MITRR, had a median survival time (MST) of 35 ± 4 days and animals that received H2TBP had a MST of 44 ± 10 days, compared to the untreated control rats that all died within 23 days [82].

Azab *et al.* reported a series of boronated cationic copolymers, composed of different ratios of acrylamide, *N*-acryloyl-3-amino-phenylboronic acid (APB) and *N*-acryloyl-diaminoethane (the cationic moiety) (polymeric APB). Direct analysis of tissue boron levels showed that polymeric APB uptake was higher in colonic polyps than in the surrounding normal tissues. When tested in the normal jejunum and colon of the rat, polymeric APB uptake was directly proportional to the molar content of the cationic monomer in copolymers [83]. Wu *et al.* reported that a heavily boronated, fifth generation polyamidoamine (PAMAM or "starburst") dendrimer, G5-B1100, was linked to oligo-saccharide moieties, which were distant from antigen binding sites of cetuximab, by means of the heterobifunctional reagents *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and *N*-(k-maleimidoundecanoic acid) hydrazide (KMUH). The localization of C225-G5-B1100 in rats bearing intracerebral implants of either F98EGFR or F98WT gliomas was determined 24 h after direct intratumoral (i.t.) injection, at which time 92.3 ± 23.3 µg B/g tumor was localized in F98EGFR gliomas versus 36.5 ± 18.8 µg B/g tumor in F98WT gliomas and 13.4 ± 6.1 µg in the normal brain [84]. Wu *et al.* also had evaluated the anti-epidermal growth factor mAb cetuximab (IMC-C225) as a delivery agent for BNCT of a human EGFR gene-transfected rat glioma (F98(EGFR)). A heavily boronated polyamidoamine dendrimer was chemically linked to cetuximab. The amount of boron retained by F98(EGFR) gliomas 24 h after CED or i.t. injection was 77.2 and 50.8 µg/g, respectively, with normal brain and blood boron values < 0.05 µg/g. The corresponding MST were 54.5 and 70.9 days ($p = 0.017$), respectively [85,86]. Yang *et al.* evaluated a boronated EGFRvIII-specific monoclonal antibody, L8A4, for BNCT of the receptor-positive rat glioma, F98(npEGFRvIII). A heavily boronated PAMAM dendrimer (BD) was chemically linked to L8A4 by two heterobifunctional reagents, SPDP and KMVH. Rats that received BD-L8A4 by CED in combination with i.v. BPA (500 mg/kg) and BNCT at Massachusetts Institute of Technology Research Reactor-II had a mean ± SE survival time of 85.5 ± 15.5 days with 20% long-term survivors (> 6 months) and those that received BD-L8A4 alone had a mean ± SE survival time of 70.4 ± 11.1 days with 10% long-term survivors compared with 40.1 ± 2.2 days for i.v. BPA and 30.3 ± 1.6 and 26.3 ± 1.1 days for irradiated and untreated controls, respectively [87].

Nabeta *et al.* reported that nanoparticles of hydrophobic gadopentetic derivatives (Gd-nanoGR) were prepared with a wet ball-milling process for gadolinium neutron capture therapy. The particle size of Gd-nanoGR was 63 nm,

and i.v. injection of Gd-nanoGR gave higher gadolinium accumulation in the tumor (109 μg Gd/g wet tumor at 6 h after administration) [88]. Fukumori *et al.* reported that Gd-nanoCPs was prepared based on the w/o emulsion-droplet coalescence technique. The most highly Gd-containing (22%) and finest (155 nm) Gd-nanoCPs prepared at 0.5% of 10 kDa chitosan exhibited the strongest tumor cell growth suppression of B16F10 malignant melanoma cells [89].

2.4 Others

Ono *et al.* investigated heterogeneous microdistribution of boron compounds in tumors and its significance for tumor cure by a radiobiological procedure, and the role of quiescent (Q) cells in tumors. Fifty percent tumor control was obtained with 10.2×10^{12} n/cm^2 in BPA-BNCT. In Q cells, BSH-BNCT yielded higher micronucleus frequency than BPA-BNCT and NCT alone. BPA was distributed in tumors heterogeneously. Q cells especially might not accumulate BPA. The accumulation mechanism of BPA and BSH are different (BPA: transporter uptake; BSH: passive diffusion), so simultaneous injection of BPA and BSH can distribute the boron atoms in different subpopulation in tumor tissue [90]. To decrease the possible disadvantage of BPA-BNCT, the combination of BPA with BSH or another neutron capture element that emits particles with longer ranges, for example, gadolinium, should be used [91].

Ferro *et al.* reported on the application of boronated glutamate–lysine polymer in conjunction with biotin and streptavidin [92]. Bench *et al.* reported that antibacterial protein avidin self-associates with the boric acid gel polymer, and avidin-coated gel particles in the micrometer and sub-micrometer size ranges. Gel particles carry large amounts of boron-10, and avidin-coated gel particles eventually cross-link, forming a solid matrix. With shorter exposure times, rapid agglutination-like reactions were observed with biotinylated bovine albumin, suggesting that two-stage pre-targeting of specific tissues should be possible with biotinylated antitumor antibodies [93].

Barth *et al.* reported that the major challenge in the development of boron delivery agents has been the requirement for selective tumor targeting to achieve boron concentrations (approximately 20 μg/g tumor) sufficient to deliver therapeutic doses of radiation to the tumor with minimal normal tissue toxicity [94]. Yang *et al.* evaluated a boronated dendrimer (BD)-epidermal growth factor bio-conjugate (BD-EGF). Twenty-four hours after CED of (125) I-labeled BD-EGF, 47.4% of the ID was retained in F98 (EGFR) gliomas, compared to 12.3% in F98 (WT) (wild-type) receptor-negative tumors. Boron concentrations in F98 (EGFR) gliomas were 22.3 and 11.7 μg/g following CED and i.t. injection, respectively. The MST of rats that received BD-EGF either alone or in combination with boronophenylalanine (BPA), injected i.v., were 53 ± 13 d and > 61 ± 14 d, respectively, compared to 40 ± 5 d for BPA alone and 31 ± 4 d for irradiated controls. CED improved the radiobiological effectiveness of BD-EGF [95].

Voge *et al.* reported the first example of DNA-binding molecule containing a dodecaborate cluster. BNCT is most effective when boron is directly located in the cellular nucleus, so that interaction with thermal neutrons can directly damage the DNA. Voge *et al.* connected ammonioundecahydrododecaborate(1-) to DNA-binding structures such as 2,5-*bis*(4-formylphenyl)furan via the Schiff-Base reaction [96].

Yanagië's group reported a novel boron compound, ($H_{15}[V_{12}{}^{10}B_{32}O_{84}Na_4]\cdot 13H_2O$; $^{10}B_{32}$), as the structure of polyoxometalates. Polyoxometalates are negatively charged inorganic substances which contain early transitional metal ions such as tungsten, molybdenum, etc., and which make a cluster with the surrounding oxygen atoms. Cytotoxic effects of $^{10}B_{32}$ on the proliferation of AsPC-1 cells were shown with thermal neutron irradiation in colony formation assay. Tumor growth suppression of $^{10}B_{32}$ was seen in AsPC-1-bearing mice of a BNCT model. We indicated that $^{10}B_{32}$ functioned as a novel neutron capture agent [97].

3. Conclusion

Many types of boron carriers have been investigated for application in BNCT. The most important factor is the increase of boron atoms in the cells. The feasible boron delivery target of the cell is the nucleus, so boron delivery systems have shifted to the construction of internalized carriers, and the retention of boron atoms in cells is also important. To expand boron delivery systems to BNCT clinical trials, it is necessary to construct a good laboratory product (GLP) or good commercial product (GCP) for boron delivery carriers and boron compounds for preclinical and clinical trials. Further collaborations with researchers, clinicians and pharmaceutical companies are necessary to apply DDS in BNCT trials.

4. Expert opinion on DDS for BNCT

Expectations for BNCT have increased because it may become the major modality for the next generation of radiation therapy, cell-selective charged-particle therapy. No other radiation therapy can overcome cell selectivity in high LET charged particle therapy other than BNCT. With the establishment of a hospital-based accelerator for BNCT and the development of new DDS technologies, BNCT may become an effective tool for cancer therapy.

Many DDS materials are being developed, so it is necessary to select DDS carrier materials to advance clinical trials. The most urgent and important points for clinical trials of BNCT are the productions of a boron compound, including BSH and BPA, and boron delivery carriers of GCP grade.

Monoclonal antibodies can be applied to BNCT for production with gene-engineering techniques. Cetsuximab is

a well-known molecular-targeting anti-cancer agent, and its safety and toxicity have been confirmed, so if GCP grade boronated cetuximab can be developed, preclinical and clinical trials can be performed with the combination of drip infusion of BSH or BPA in BNCT. Several receptors are expressed on cancer cell surfaces, for example folate receptor, HER-2/neu, transferrin receptor and EGFR. For targeting methods, it is preferable to select ligands that can be internalized into cells with endocytosis. In addition, it is better to select a human-type monoclonal antibody for targeting, because it may be able to produce idiotype antibodies and therefore a decrease in antibody activity and allergy responses in the body, if zeno-monoclonal antibodies are used. Liposomes are now applied clinically in many anti-cancer drug entrapment chemotherapies for cancer, so it is easy to advance to preclinical and clinical trials with boron-entrapped liposome of GCP grade. To elevate boron concentrations in targeted tumors, it is necessary to evaluate the toxicity of liposome administrations. For the safe administration of boron-entrapped liposomes or boronated monoclonal antibodies, it is also necessary to evaluate the boron uptake of cancer cells as to whether the concentration of boron can reach the level of effective BNCT.

Emulsions constructed with lipiodol are now commonly applied in clinical use. Emulsions are also easily applied in clinical trials of BNCT using intra-arterial infusions to tumoral arteries. Micro emulsions are able to entrap highly concentrated boron compounds, and can accumulate many boron atoms in tumors by arterial infusion techniques, so we plan to apply them for BNCT clinical trials for HCC.

Recently, sequential BSH and BPA drip infusion was proposed for patients with malignant brain tumors to maintain effective boron concentrations in tumor tissues with different uptake mechanisms into cancer cells (BSH: passive diffusion; BPA: transporter uptake). DDS will enable boron atoms to be transported into cells by intracellular delivery mechanisms, for example, endocytosis. Boron delivery with DDS will also be combined with continuous drip infusion of BSH and BPA. It has become to clear that the transportation of boron atoms into the nucleus is important for effective BNCT using intracellular boron delivery systems (intra-nuclear delivery techniques), so the development of boron compounds will shift to the development of nuclear transportable boron compounds, for example, the application of NLS-binding boron compounds. The uptake mechanisms of boron compounds with DDS into cancer cells should also be analyzed in detail, for example, endocytosis and membrane fusion.

We are convinced that liposomes, monoclonal antibodies and emulsions will be able to be utilized as boron delivery systems for BNCT clinically, in accordance with the preparation of GCP grade materials. We are planning BNCT using intra-arterial injections of boronated WOW emulsions.

To apply boron delivery using DDS, it is necessary to cooperate with researchers, clinicians and pharmaceutical companies, because the development of new compounds and new boron delivery systems for clinical trials is expensive. We hope to establish boron delivery systems with high throughput selection of carriers for BNCT trials in the near future.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hatanaka H, Nakagawa Y. Clinical results of long-surviving brain tumor patients who underwent boron neutron capture therapy. Int J Radiat Oncol Biol Phys 1994;28:1061-66
2. Nakagawa Y, Hatanaka H. Boron neutron capture therapy – clinical brain tumor study. J Neurooncol 1997;33:105-15
3. Mishima Y. Treatment of malignant melanoma by single thermal neutron capture therapy with melanoma-seeking 10B compound. Lancet 1989;39:325-33
4. Nakagawa Y, Pooh K, Kobayashi T, et al. Clinical review of the Japanese experience with boron neutron capture therapy and a proposed strategy using epithermal neutron beams. J Neuro Oncol 2003;62:87-7
5. Kato I, Ono K, Sakurai Y, et al. Effectiveness of BNCT for recurrent head and neck malignancies. Appl Radiat Isot 2004;61:829-33
6. Miyatake S, Kawabata S, Kajimoto Y, et al. Modified boron neutron capture therapy for malignant gliomas performed using epithermal neutron and two boron compounds with different accumulation mechanisms: an efficacy study based on findings neuroimages. J Neurosurg 2005;103:1000-9
7. Nakagawa Y. Boron neutron capture therapy in Japan – from the past to the future. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. President's Address of ICNCT-12, Published by International Society for Neutron Capture Therapy; 2006
8. Kageji T, Mizobuchi Y, Nagahiro S, et al. Comparison of BNCT radiation dose between BSH-based intra-operative BNCT and BSH, BPA-based non-operative BNCT. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 62-3
9. Ono K, Masunaga S, Kinashi Y, et al. Neutron irradiation under continuous BPA injection for solving the problem of heterogeneous distribution of BPA. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in Neutron Capture Therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 27-30
10. Imahori Y, Ueda S, Ohmori Y, et al. Positron emission tomography-based boron neutron capture therapy using boronophenylalanine for high-grade gliomas: part I. Clin Cancer Res 1998;4:1825-32
11. Birgit Romberg, Wim E, Hennink, Gert Storm. Sheddable coatings for long-circulating nanoparticles. Pharm Res 2008;25(1): 55-71

12. Laura J. Waters and Evangelos V. Pavlakis: In vitro controlled drug release from loaded microspheres – dose regulation through formulation. J Pharm Pharmaceut Sci 2007;10(4):464-72

13. Mian Li, Michael J Hanford, Jin-Woo Kim, Tonya L Peeples. Amyloglucosidase enzymatic reactivity inside lipid vesicles. J Biol Eng 2007;1:4, 1-9

14. Ishida T, Kiwada H. Accelerated blood clearance (ABC) phenomenon induced by administration of PEGylated liposome. Yakugaku Zasshi 2008;128(2):233-43

15. Adam M, Sonabend, Ilya V, Ulasov, Maciej S. Lesniak: gene therapy trials for the treatment of high-grade gliomas. Gene Ther Mol Biol 2007;11(A):79-92

16. Kawakami S, Hashida M. Targeted delivery systems of small interfering RNA by systemic administration. Drug Metab Pharmacokinet 2007;22(3):142-51

17. Butowski N, Chang SM. Small molecule and monoclonal antibody therapies in neurooncology. Cancer Control 2005;12(2):116-24

18. Barth RF, Alam F, Soloway AH, et al. Boronated monoclonal antibody 17-1A for potential neutron capture therapy of colorectal cancer. Hybridoma 1986;5(Suppl 1):S43-50

• **First application of boronated monoclonal antibody to BNCT for cancer.**

19. Takahashi T, Fujii Y, Fujii G, Nariuchi H. Preliminary study for application of anti-alpha-fetoprotein monoclonal antibody to boron-neutron capture therapy. Jpn J Exp Med 1987;57(2):83-91

20. Yanagië H, Fujii Y, Sekiguchi M, et al. A targeting model of boron neutron-capture therapy to hepatoma cells in vivo with a boronated anti-(alpha-fetoprotein) monoclonal antibody. J Cancer Res Clin Oncol 1994;120(11):636-40

21. Tamat SR, Moore DE, Patwardhan A, Hersey P. Boronated monoclonal antibody 225.28S for potential use in neutron capture therapy of malignant melanoma. Pigment Cell Res 1989;2(4):278-80

22. Ranadive GN, Rosenzweig HS, Epperly MW, Bloomer WD. A technique to prepare boronated B72.3 monoclonal antibody for boron neutron capture therapy. Nucl Med Biol 1993;20(1):1-6

23. Liu L, Barth RF, Adams DM, et al. Bispecific antibodies as targeting agents for boron neutron capture therapy of brain tumors. J Hematother 1995;4(5):477-83

24. Barth RF, Wu G, Yang W, et al. Neutron capture therapy of epidermal growth factor (+) gliomas using boronated cetuximab (IMC-C225) as a delivery agent. Appl Radiat Isot 2004;61(5):899-903

25. Yanagië H, Fujii Y, Takahashi T, et al. Boron neutron capture therapy using 10B entrapped anti-CEA immunoliposome (Article in Japanese). Hum Cell 1989;2(3):290-6

26. Yanagië H, Tomita T, Kobayashi H, et al. Application of boronated anti-CEA immunoliposome to tumour cell growth inhibition in in vitro boron neutron capture therapy model. Br J Cancer 1991;63(4):522-6

•• **First application of immunoliposome as targeting boron delivery system to BNCT for cancer.**

27. Yanagië H, Tomita T, Kobayashi H, et al. Inhibition of human pancreatic cancer growth in nude mice by boron neutron capture therapy. Br J Cancer 1997;75(5):660-5

28. Yanagië H, Kobayashi H, Takeda Y, et al. Inhibition of growth of human breast cancer cells in culture by neutron capture using 10B-containing liposomes. Biomed Pharmacother 2002;56:93-9

29. Shelly K, Feakes DA, Hawthorne MF, et al. Model studies directed toward the boron neutron-capture therapy of cancer: boron delivery to murine tumors with liposomes. Proc Natl Acad Sci USA 1992;89(19):9039-43

30. Feakes DA, Shelly K, Knobler CB, Hawthorne MF. Na3[B20H17NH3]: synthesis and liposomal delivery to murine tumors. Proc Natl Acad Sci USA 1994;91(8):3029-33

•• **New boron compound Na3[B20H17 NH3] has a possibility to remain in cancer cells.**

31. Feakes DA, Shelly K, Hawthorne MF. Selective boron delivery to murine tumors by lipophilic species incorporated in the membranes of unilamellar liposomes (boron neutron capture therapy/borane/vesicle). Proc Natl Acad Sci USA 1995;92:1367-70

•• **Application of small unilamellar liposomes incorporating boron compounds in the bilayer of liposomal membrane.**

32. Feakes DA, Waller RC, Hathaway DK, Morton VS. Synthesis and in vivo murine evaluation of Na4[1-(1*-B10H9)-6-SHB10H8] as a potential agent for boron neutron capture therapy. Proc Natl Acad Sci USA 1999;96:6406-10

33. Hawthorne MF, Shelly K. Liposomes as drug delivery vehicles for boron agents. J Neurooncol 1997;33(1-2):53-8

34. Moraes AM, Santana MH, Carbonell RG. Preparation and characterization of liposomal systems entrapping the boronated compound o-carboranylpropylamine. J Microencapsul 1999;16(5):647-64

35. Mehta SC, Lai JC, Lu DR. Liposomal formulations containing sodium mercaptoundecahydrododecaborate (BSH) for boron neutron capture therapy. J Microencapsul 1996;13(3):269-79

36. Yanagië H, Maruyama K, Takizawa T, et al. Application of boron-entrapped stealth liposomes to inhibition of growth of tumour cells in the in vivo boron neutron-capture therapy model. Biomed Pharmacother 2006;60(1):43-50 [Epub 2005 Sep 21]

37. Ishida O, Maruyama K, Tanahashi H, et al. Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. Pharm Res 2001;18(7):1042-8

• **Explanation of endocytosis and EPR effect using transferring PEG liposome.**

38. Maruyama K, Ishida O, Kasaoka S, et al. Intracellular targeting of sodium mercaptoundecahydrododecaborate (BSH) to solid tumors by transferrin-PEG liposomes, for boron neutron-capture therapy (BNCT). J Control Rel 2004;98(2):195-207

•• **Application of transferring PEG liposome as receptor targeting boron delivery system to BNCT *in vivo*.**

39. Ogura K, Yanagië H, Eriguchi M, et al. Neutron capture autoradiographic study of the biodistribution of 10B in tumor-bearing mice. Appl Radiat Isot 2004;61(4):585-90

40. Yanagië H, Ogura K, Takagi K, et al. Accumulation of boron compounds to tumor with polyethylene-glycol binding liposome by using neutron capture autoradiography. Appl Radiat Isot 2004;61(4):639-46

• **Neutron capture alpha-autoradiography is able to apply to seek the cancer targeting DDS materials *in vivo*.**

41. Doi A, Miyatake S, Iida K, et al. Intracellular targeting of mercaptoundecahydro-dodecaborate (BSH) to malignant glioma by transferring-PEG liposomes for boron neutron capture therapy (BNCT). In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 203-6

42. Pan XQ, Wang H, Shukla S, et al. Boron-containing folate receptor-targeted liposomes as potential delivery agents for neutron capture therapy. Bioconjug Chem 2002;13(3):435-42

• **Application of folate receptor-targeting liposome to BNCT.**

43. Pan XQ, Wang H, Shukla S, et al. Boron-containing folate receptor-targeted liposomes as potential delivery agents for neutron capture therapy. Bioconjug Chem 2002;13(3):435-42.

44. Pan XQ, Wang H, Lee RJ. Boron delivery to a murine lung carcinoma using folate receptor-targeted liposomes. Anticancer Res 2002;22(3):1629-33

45. Sudimack JJ, Adams D, Rotaru J, et al. Folate receptor-mediated liposomal delivery of a lipophilic boron agent to tumor cells in vitro for neutron capture therapy. Pharm Res 2002;19(10):1502-8

46. Stephenson SM, Yang W, Stevens PJ, et al. Folate receptor-targeted liposomes as possible delivery vehicles for boron neutron capture therapy. Anticancer Res 2003;23(4):3341-5

47. Thirumamagal BT, Zhao XB, Bandyopadhyaya AK, et al. Receptor-targeted liposomal delivery of boron-containing cholesterol mimics for boron neutron capture therapy (BNCT). Bioconjug Chem 2006;17(5):1141-50

48. Kullberg EB, Nestor M, Gedda L. Tumor-cell targeted epidermal growth factor liposomes loaded with boronated acridine: uptake and processing. Pharm Res 2003;20(2):229-36

49. Kullberg EB, Carlsson J, Edwards K, et al. Introductory experiments on ligand liposomes as delivery agents for boron neutron capture therapy. Int J Oncol 2003;23(2):461-7

50. Kullberg EB, Wei Q, Capala J, et al. EGF-receptor targeted liposomes with boronated acridine: growth inhibition of cultured glioma cells after neutron irradiation. Int J Radiat Biol 2005;81(8):621-9

51. Carlsson J, Kullberg EB, Capala J, et al. Ligand liposomes and boron neutron capture therapy. J Neurooncol 2003;62(1-2):47-59

52. Pan X, Wu G, Yang W, et al. Synthesis of cetuximab-immunoliposomes via a cholesterol-based membrane anchor for targeting of EGFR. Bioconjug Chem 2007;18(1):101-8

53. Wei Q, Kullberg EB, Gedda L. Trastuzumab-conjugated boron-containing liposomes for tumor-cell targeting; development and cellular studies. Int J Oncol 2003;23(4):1159-65

•• **Application of boron entrapped immunoliposome to BNCT using molecular targeting agents.**

54. Peacock GF, Ji B, Wang CK, Lu DR. Cell culture studies of a carborane cholesteryl ester with conventional and PEG liposomes. Drug Deliv 2003;10(1):29-34

55. Rossi S, Schinazi RF, Martini G. ESR as a valuable tool for the investigation of the dynamics of EPC and EPC/cholesterol liposomes containing a carboranyl-nucleoside intended for BNCT. Biochim Biophys Acta 2005;1712(1):81-91

56. Nakamura H, MiyajimaY, Takei T, et al. Synthesis and vesicle formation of a nido-carborane cluster lipid for boron neutron capture therapy. Chem Commun (Camb) 2004;17:1910-11

• **Synthesis of liposomes constructed with double tailed nido-carborane lipid.**

57. Miyajima Y, Nakamura H, Kuwata Y, et al. Transferrin-loaded nido-carborane liposomes: tumor-targeting boron delivery system for neutron capture therapy. Bioconjug Chem 2006;17(5):1314-20

58. Nakamura H, Miyajima Y, Kuwata Y, et al. Transferrin-loaded nido-Carborane liposomes: synthesis and intracellular targeting to solid tumors for boron neutron capture therapy. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 195-8

59. Justus E, Awad D, Hohnholt M, et al. Synthesis, liposomal preparation, and in vitro toxicity of two novel dodecaborate cluster lipids for boron neutron capture therapy. Bioconjug Chem 2007;18(4):1287-93 [Epub 2007 Jun 15]

60. Martini S, Ristori S, Pucci A, et al. Boronphenylalanine insertion in cationic liposomes for boron neutron capture therapy. Biophys Chem 2004;111(1):27-34

61. Morandi S, Ristori S, Berti D, et al. Association of sugar-based carboranes with cationic liposomes: an electron spin resonance and light scattering study. Biochim Biophys Acta 2004;1664(1):53-63

62. Ristori S, Oberdisse J, Grillo YI, et al. Structural characterization of cationic liposomes loaded with sugar-based carboranes. Biophys J 2005;88:535-47

63. Yanagië H, Ogura K, Matsumoto T, et al. Neutron capture autoradiographic determination of 10B distributions and concentrations in biological samples for boron neutron capture therapy. Nucl Instr Method A 1999;424(1):122-8

64. Yanagië H, Ogura K, Nonaka Y, et al. Alpha-autoradiographic determination of ^{10}B concentrations in cancer bearing mice for boron neutron-capture therapy. In: Hawthorne MF, et al. editors, Frontiers in neutron capture therapy. Kluwer Academic/Plenum Publishers, New York; 2001. p. 945-51

65. Nakai K, Yamamoto T, Matsumura A, et al. Application of HVJ envelope system to boron neutron capture therapy (BNCT). In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 207-10

66. Shukla S, Wu G, Chatterjee M, et al. Synthesis and biological evaluation of folate receptor-targeted boronated PAMAM dendrimers as potential agents for neutron capture therapy. Bioconjug Chem 2003;14(1):158-67

67. Yanagië H, Sato T, Nishi H, et al. Boron delivery to tumors mediated by polyethylene glycol-binding BSA. Proceeding of 7th international symposium on neutron capture therapy for cancer. In: Larsson B, et al., editors, Advances in neutron capture therapy. Elsevier Science; 1997. p. 403-7

68. Kanematsu T, Inokuchi K, Sugimachi K, et al. Selective sffects of lipiodolized antitumor agents. J Surg Oncol 1984;25:218-26

69. Kanematsu T, Furuta T, Takenaka K, et al. A 5-year experience of lipiolization: selective regional chemotherapy for 200 patients with hepatocellular carcinoma. Hepatology 1989;10:98-102

70. Katagiri Y, Mabuchi K, Itakura T, et al. Adriamycin-lipiodol suspension for i.a. chemotherapy of hepatocellular carcinoma. Cancer Chemother Pharmacol 1989;23:238-42

71. Suzuki M, Masunaga S, Kinashi Y, et al. Intra-arterial administration of sodium borocaptate (BSH)/lipiodol emulsion delivers B-10 to liver tumors highly selectively for boron neutron capture therapy: experimental studies in the rat liver model. Int J Radiat Oncol Biol Phys 2004;59(1):260-6

•• **Application of boron BSH/lipiodol emulsion by intra-arterial injection to hepatic tumor.**

72. Suzuki M, Nagata K, Masunaga S, et al. Biodistribution of 10B in a rat liver tumor model following intra-arterial administration of sodium borocaptate (BSH)/degradable starch microspheres (DSM) emulsion. Appl Radiat Isot 2004;61(5):933-7

73. Higashi S, Shimizu M, Setoguchi T. Preparation of new lipiodol-emulsion containing water soluble anticancer agent by membrane emulsification technique (In Japanese with English abstract). Drug Deliv Syst 1993;8:59-61

74. Nakashima T, Shimizu M, Kukizaki M. Membrane emulsification by microporous glass. Inorgan Membr 1991;ICIM2-92:511-6

75. Higashi S, Shimizu M, Nakashima T, et al. Arterial-injection chemotherapy for hepatocellular carcinoma using monodispersed poppy-seed oil microdroplets containing fine aqueous vesicles of epirubicin. Cancer 1995;75:1245-54

76. Nakashima T, Shimizu M, Kukizaki M. Development of membrane emulsification and its applications to drug delivery systems (In Japanese with English abstract). Maku (Membrane) 1999;24:278-89

77. Yanagië H, Higashi S, Ikushima I, et al. Selective enhancement of boron accumulation with boron-entrapped WOW emulsion in VX-2 rabbit hepatic cancer model for BNCT. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 211-4

•• **Application of WOW emulsion for high concentration boron compound delivery to hepatic tumor by intra-arterial infusion.**

78. Chou FI, Chung HP, Wen HW, et al. Synthesis of PBAD-lipiodol nanoparticles for combination treatment with boric acid in boron neutron capture therapy for hepatoma in vitro. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 153-6

79. Kahl SB, Ozawa T, Afzal J, et al. BOPP revisited: a study on the toxicity, biodistribution and convection enhanced delivery of BOPP in the 9L intracerebral rat glioma model. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 199-202

80. Matsumura A, Shibata Y, Yamamoto T. A new boronated porphyrin (STA-BX909) for neutron capture therapy: an in vitro survival assay and in vivo tissue uptake study. Cancer Lett 1999;141:203-9

81. Vincente MGH, Easson M. Syntheses of phosphonate-and amine-substituted carboranylporphyrins for boron neutron capture therapy of tumors. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 231-3

82. Kawabata S, Barth RF, Yang W, et al. Evaluation of carboranylporphyrins as boron delivery agents for neutron capture therapy. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 123-6

83. Azab AK, Srebnik M, Doviner V, Rubinstein A. Targeting normal and neoplastic tissues in the rat jejunum and colon with boronated, cationic acrylamide copolymers. J Control Rel 2005;106(1-2):14-25

84. Wu G, Barth RF, Yang W, et al. Boron containing macromolecules and nanovehicles as delivery agents for neutron capture therapy. Anticancer Agents Med Chem 2006;6(2):167-84

85. Wu G, Barth RF, Yang W, et al. Site-specific conjugation of boron-containing dendrimers to anti-EGF receptor monoclonal antibody cetuximab (IMC-C225) and its evaluation as a potential delivery agent for neutron capture therapy. Bioconjug Chem 2004;15(1):185-94

• **Application molecular targeting monoclonal antibody as boron carrier.**

86. Wu G, Yang W, Barth RF, et al. Molecular targeting and treatment of an epidermal growth factor receptor-positive glioma using boronated cetuximab. Clin Cancer Res 2007;13(4):1260-8

87. Yang W, Barth RF, Wu G, et al. Molecular targeting and treatment of EGFRvIII-positive gliomas using boronated monoclonal antibody L8A4. Clin Cancer Res 2006;12(12):3792-802

88. Nabeta C, Ichikawa H, Fukumori Y. Biodistribution of nanoparticles of hydrophobic gadopentetic-acid derivative prepared with a planetary ball mill for neutron-capture therapy of cancer. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 221-3

89. Fukumori Y, Ichikawa H, Nakatani Y, et al. Gadolinium-loaded chitosan nanoparticles for cancer neutron-capture therapy: pharmaceutical characteristics and in vitro antitumor effect. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 224-7

• **Pioneer of gadolinium neutron capture therapy (GdNCT).**

90. Yokoyama K, Miyatake S, Kajimoto Y, et al. Analysis of boron distribution in vivo for boron neutron capture therapy using two different boron compounds by secondary ion mass spectroscopy. Radiat Res 2007;167(1):102-9

91. Ono K, Masunaga SI, Kinashi Y, et al. Radiobiological evidence suggesting heterogeneous microdistribution of boron compounds in tumors: its relation to quiescent cell population and tumor cure in neutron capture therapy. Int J Radiat Oncol Biol Phys 1996;34(5):1081-6

•• **Discussion about heterogeneous distribution of boron compounds in tumor tissue.**

92. Ferro VA, Morris JH, Stimson WH. A novel method for boronating antibodies without loss of immunoreactivity, for use in

neutron capture therapy. Drug Des Discov 1995;13(1):13-25

93. Bench BJ, Johnson R, Hamilton C, et al. Avidin self-associates with boric acid gel suspensions: an affinity boron carrier that might be developed for boron neutron-capture therapy. J Colloid Interface Sci 2004;270(2):315-20

94. Barth RF, Coderre JA, Vicente MG, Blue TE. Boron neutron capture therapy of cancer: current status and future prospects. Clin Cancer Res 2005;11(11):3987-4002

95. Yang W, Barth RF, Wu G, et al. Neutron capture therapy of epidermal growth factor receptor (EGFR)vIII positive gliomas using boronated monoclonal antibody L8A4. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p 251-2

96. Voge A, Hoffmann S, Gabel D. Synthesis of furan-based DNA binders and their interaction with DNA. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 228-30

97. Yanagië H, Mitsui S, Ogata A, et al. Production of polyoxisoboronate as novel boron compound. In: Nakagawa Y, Kobayashi T, Fukuda H, editors, Advances in neutron capture therapy 2006. Published by International Society for Neutron Capture Therapy; 2006. p. 115-8

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