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Design of drug delivery systems for physical energy-induced chemical surgery

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Abstract

Physical energy-induced chemical surgery, a technique that induces antitumor effects by delivering a drug that exerts a therapeutic effect in response to physical energy and irradiating the diseased part with the corresponding physical energy, is a useful method to treat cancers with minimal systemic side effects. Among chemical surgery, photodynamic therapy (PDT) and neutron capture therapy (NCT) require a system that selectively delivers drugs to the diseased site. Although PDT and NCT have a similar concept, drug delivery systems (DDSs) for their purpose need different functions to solve the unique problems derived from the characteristics of respective physical energy. In this review, we will describe recent chemistry-based solutions including ours to overcome these challenges.

Keywords: drug delivery system; photodynamic therapy; photochemical internalization; neutron capture therapy; companion diagnostics

1. Introduction

Killing cancer cells using physical energy including laser, radiation (X-ray, proton beam, and heavy particle beam), and ultrasound (high intensity focused ultrasound) is a useful technique to offer permanent cure of cancer with minimal invasiveness [1-3]. When treating cancer with the physical energy, distinguishing the diseased part clearly from normal tissues is extremely important to selectively damage the target tumor and avoid untoward effects. However, as one cancer patient does not always have one distinguishable tumor, it is sometimes difficult to accurately find and distinguish the tumor from the normal tissue. Particularly in the case of multiple and diffuse cancers such as bladder cancer [4], detection and treatment of all cancer tissues one by one is technically challenging. One of the solutions to treat such cancers is diagnostic agents that can detect cancers with high sensitivity [5, 6]; and another solution is physical energy-induced chemical surgery, a methodology to deliver a drug that exerts a

therapeutic effect in response to physical energy selectively in the cancer and uniformly irradiate the physical energy near the diseased part, thereby preventing failure in treating even tiny cancer that is invisible to naked eyes (Figure 1). Table 1 summarizes the types of physical energy and its application to chemical surgery [7-13]. Development of these technologies is expected to permit permanent cure of cancer without surgical invasion, significantly shorten recovery period of patients after the treatment, and dramatically reduce their physical, mental, and economic burden.

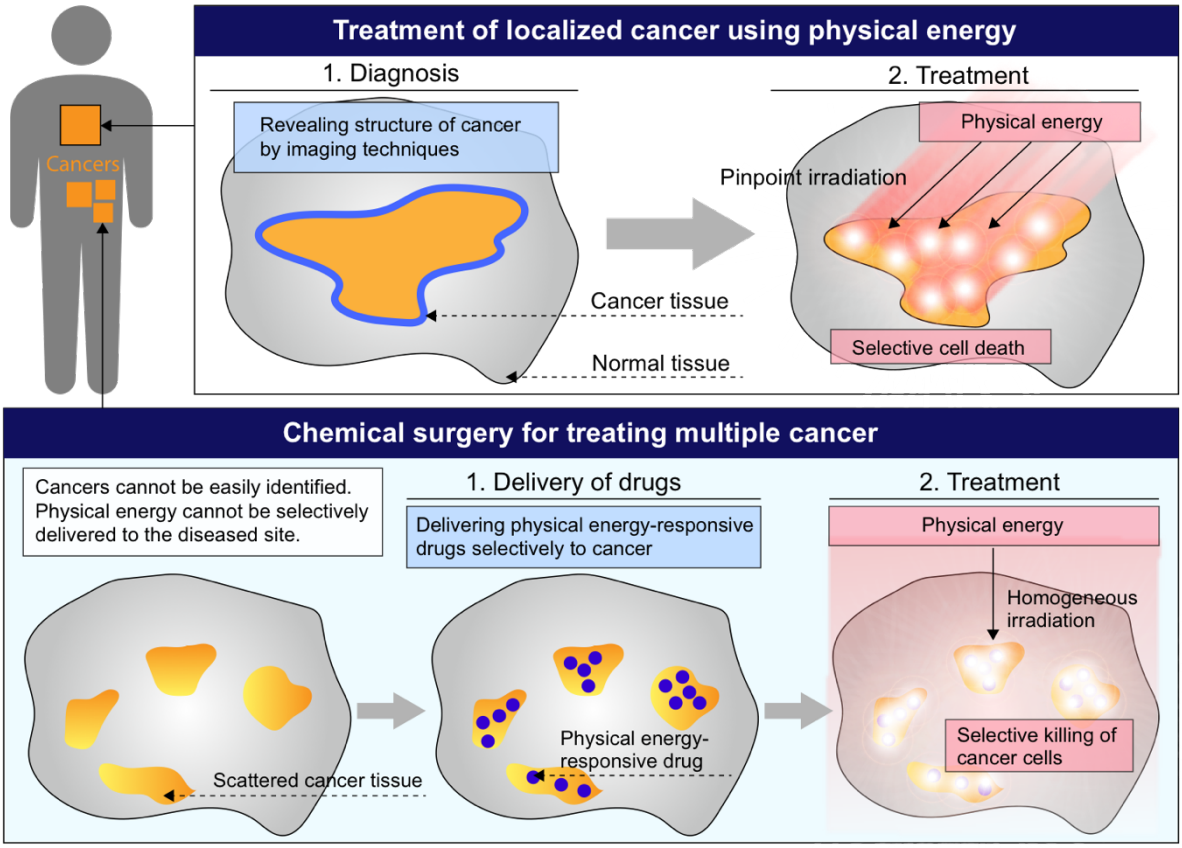

Figure 1. The concept of physical energy-induced chemical surgery.

Table 1. List of chemical surgery.

Physical energy	Drug	Style of chemical surgery
Magnetism	Magnetic particles	Hyperthermia
Ultrasound	Microbubbles, sonosensitizers	Enhanced drug delivery, sonodynamic therapy
Light	Photosensitizers, gold nanoparticles	Photodynamic therapy, photothermal therapy
Neutron	Nuclei that can generate cytotoxic radiation through nuclear reaction	Neutron capture therapy

One of the techniques widely used as the chemical surgery is photodynamic therapy (PDT). Detailed mechanism and clinical outcome of PDT are elegantly summarized by Agostinis *et al.* and MacDonald *et al.* [8, 14]. PDT combines a photosensitizer (PS) and

photoirradiation. In PDT, PS is selectively delivered to the diseased part, and activated by photoirradiation (Figure 2(a)); the excited PS gives the energy to an oxygen molecule and generate cytotoxic singlet oxygen which is one of reactive oxygen species (ROS), thereby killing the target cells. PDT also damages tumor blood vessels as well as the target cells and inhibits blood flow, blocking the supply of nutrients and oxygen to cancer cells and ultimately exerting antitumor effects. Since the travel distance of singlet oxygen within the cell is as short as 10-55 nm, key for successful PDT is selective delivery of PSs to the target site. Upon these therapeutic effects, the dying and dead tumor cells are phagocytosed by dendritic cells, activating immune systems. This PDT-induced immune system is also believed to contribute to antitumor activity [15, 16].

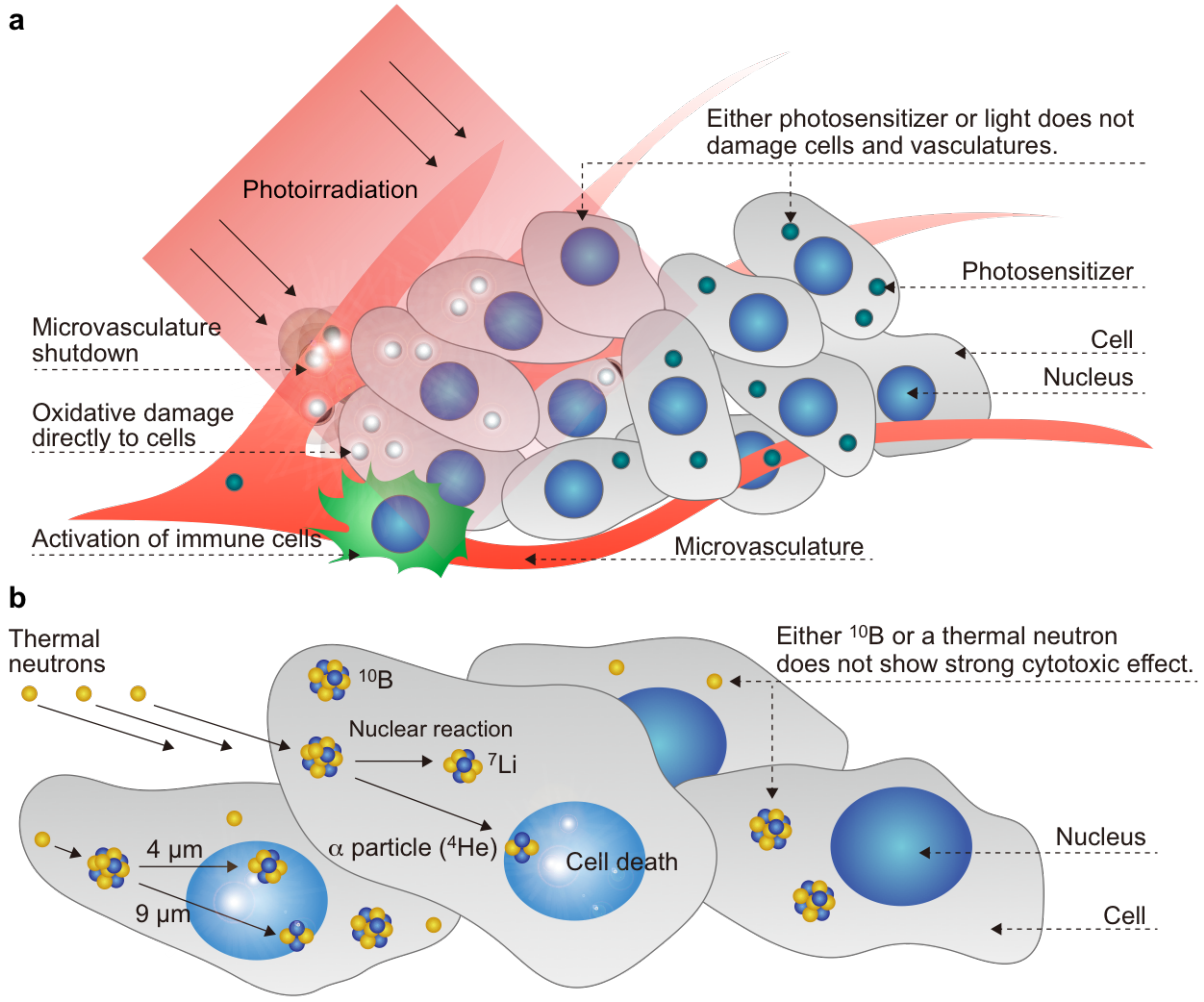

Figure 2. Schematic illustration of therapeutic mechanism of PDT and NCT. (a) Therapeutic mechanism of PDT. PS delivered to the target cells induces ROS upon photoirradiation, thereby giving oxidative damage to the cells. ROS generated by photoactivated PS also damages and shuts down tumor-associated microvasculature, blocking the supply of nutrients and oxygen to the tumor cells. After PDT treatment, some cells produce specific proteins that

activate immune systems. The activated immune systems are believed to contribute to antitumor activity. (b) Therapeutic mechanism of BNCT. BNCT combines delivery of ^{10}B nuclei and irradiation of thermal neutrons. Nuclear reaction of ^{10}B and the thermal neutron generates an α particle (^{4}He) and ^{7}Li. The α particle can travel a distance of 9 μm, the nuclear reaction can induce cytotoxic effect only to the cell that takes up sufficient amount of ^{10}B.

The wavelength of light used for PDT is in the near-infrared region (600-800 nm) because the light in this wavelength range permits relatively efficient penetration in the body compared to that in shorter wavelength. The light at more than 800 nm offers less efficiency in producing ROS. Thus, the light in 600-800 nm is most commonly used in practice. Since the depth at which light can deliver sufficient energy to PS to generate ROS is estimated to be up to approximately 1 cm, PDT allows for selective treatment of superficial cancers including hollow organs.

In the similar approach with PDT, neutron capture therapy (NCT) can treat deeper cancers [10, 17, 18]. NCT kills cancer cells using highly cytotoxic radiation including α and γ rays generated by nuclear reactions induced by irradiating thermal/epithermal neutrons to nuclei with high neutron capture cross sections such as boron (^{10}B) and gadolinium (^{157}Gd) (Figure 2(b)). Since the range of α rays generated by the nuclear reaction of boron and neutron is limited as 5-9 μm, selective boron delivery to the diseased cell is expected to lead to exceedingly confined cancer treatment. Because epithermal neutrons can induce therapeutic nuclear reaction up to a depth of about 8 cm [19], NCT can treat larger and deeper cancers compared to PDT. Excellent clinical outcome of NCT is well summarized in previous works [17, 20, 21]. On the other hand, although hydrogen and nitrogen have small neutron capture cross sections, the body contains huge amount of them; radiation generated by their nuclear reaction with neutrons gives unignorable effect on normal tissues. Hence, minimally invasive cancer treatment by NCT requires selective delivery of an enough number of nuclei capturing neutrons to the target cancer to attain efficient antitumor activity with the acceptable level of radiation exposure of the surrounding normal tissues.

Table 2 summarizes the characteristics of PDT and NCT. In both PDT and NCT, the amount of drug delivered to the tumor determines the balance between a side effect and a therapeutic effect. A particular problem with PDT is that some PSs remain in the skin and cause photosensitivity. It is important to develop PSs that selectively accumulate in tumors with minimal distribution to normal tissues such as skin exposed to light in a daily life [22, 23]. Meanwhile, the most important point in NCT is the selective accumulation of the therapeutic neutron-capturing nucleus in the tumor and its intratumoral concentration considering radiation

exposure of normal tissues. For example, boron NCT (BNCT) requires >25 ppm of intratumoral ^{10}B concentration, and >2.5 of tumor/normal tissue and tumor/blood ^{10}B concentration ratios in clinical application. To achieve these requirements, a large amount of boron should be injected to patients as described later. Thus, drugs need to be safe enough in this huge dose, and should accumulate efficiently to the target tumor. Currently, L-*p*-boronophenylalanine (BPA) and one of the boron clusters, mercaptoundecahydrododecaborate (BSH) are applied to humans in NCT, but development of new drugs is required to expand applicable disease.

Table 2. Comparison of PDT and NCT.

Methods	Drug	Energy / Availability	Depth	Diagnostics	Target	Requirements in designing carriers
PDT	PS	Light (600–800 nm) / Easy	~1 cm	・PS fluorescence detection	Gliomas. Superficial cancers such as head and neck, esophageal, lung, and bladder cancers including recurrent cancers.	Avoidance of untoward photochemical damage to normal tissues. Excitation by NIR light.
NCT	^{10}B ^{157}Gd	Thermal or epithermal neutron / Difficult	~8 cm (up to target)	・Positron emission tomography ・Magnetic resonance imaging	Gliomas, melanomas, head and neck cancers, lung cancers, liver cancers including recurrent cancers.	High tumor/normal tissue drug concentration ratio at a time point for neutron irradiation. Extremely minimal toxicity.

For selective drug delivery to tumors, drug delivery systems (DDSs) such as liposomes and polymeric micelles have attracted great attention [24, 25]. Since enhanced permeability and retention (EPR) effect was reported in 1986 [26], many DDSs have been clinically approved or in clinical trials [27], demonstrating their validity in cancer therapy. However, recent clinical studies have reported that a tumor has regions where DDSs are likely to accumulate and regions where they are not, and such tumor heterogeneity can be also observed between patients [28]. In this context, researchers have recognized the importance of microscopically investigating intratumoral behavior of DDSs, and many studies have been devoted to track DDSs in the tumor over time with high spatial resolution using intravital microscopy and magnetic resonance imaging (MRI); the accumulation of DDSs that was vaguely understood has been discussed in detail [29-31]. Recent studies have also developed techniques for directly visualizing the relationship between the design of DDSs and their *in vivo* behavior, allowing us to clarify critical structures of DDSs to achieve the purpose in complicated *in vivo* condition [32-36]. Furthermore, in addition to these fundamental research, imaging technology can evaluate drug accumulation semi-quantitatively or quantitatively; it has been applied to companion diagnostics to select patients who can be efficiently treated by the corresponding drugs (Figure 3). Particularly in PDT and NCT, in order to make the best use

of the advantage of minimal invasiveness, it is necessary to noninvasively decide the timing at which physical energy is irradiated (the timing at which drugs selectively accumulate at the target diseased site). Thus, companion diagnostics is the key for the success of these treatments.

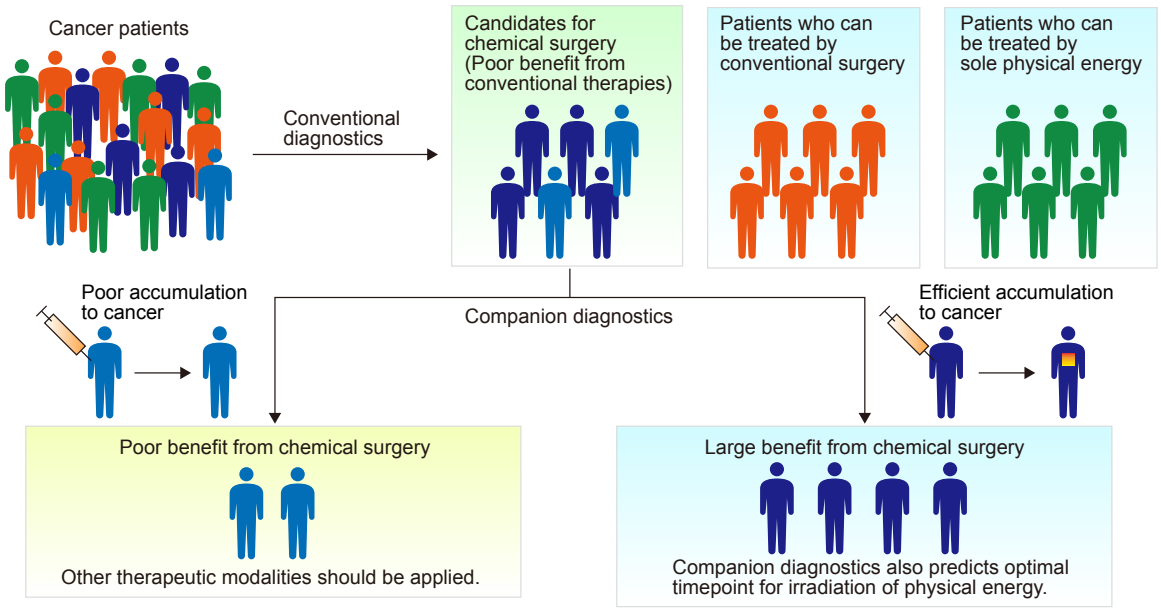

Figure 3. The concept of companion diagnostics. Diagnostic drugs that show the same biodistribution with the corresponding therapeutic drugs can be utilized to select patients who can receive significant benefit from the treatment. In chemical surgery, since selective delivery of drugs to the diseased site is critical for therapeutic effect, companion diagnostics is a useful technique to predict the therapeutic effect. The companion diagnostics also provides information about the timing, at which the drugs efficiently accumulate within the target cancer. This information can be utilized to determine the timing to irradiate physical energy to the target site.

As described above, the DDSs for PDT and NCT need to enable companion diagnostics while delivering the drug selectively at the diseased site. On the other hand, different nanocarrier functions are required to solve respective problems that are derived from the characteristics of physical energy in parallel. Although previous elegant review papers comprehensively summarize DDSs for PDT [37-42] and NCT [10, 17, 43], importance of companion diagnostics in development of DDSs has not been discussed from the viewpoint of chemistry oriented to clinical application, and DDSs for PDT and NCT have not been compared. Because there has been a growing demand for novel therapeutic modality for cancer and enormous efforts have been devoted to develop biomaterials for clinically applicable DDSs, introduction of recent DDSs for PDT and NCT and comparison between these therapeutic

approaches should elucidate the requirements for DDSs aimed at successful clinical application of physical energy-induced chemical surgery (Figure 4). In this review, we thus describe the required functions of DDSs for these types of chemical surgery and chemistry-based method to construct them by introducing related research including our recent studies.

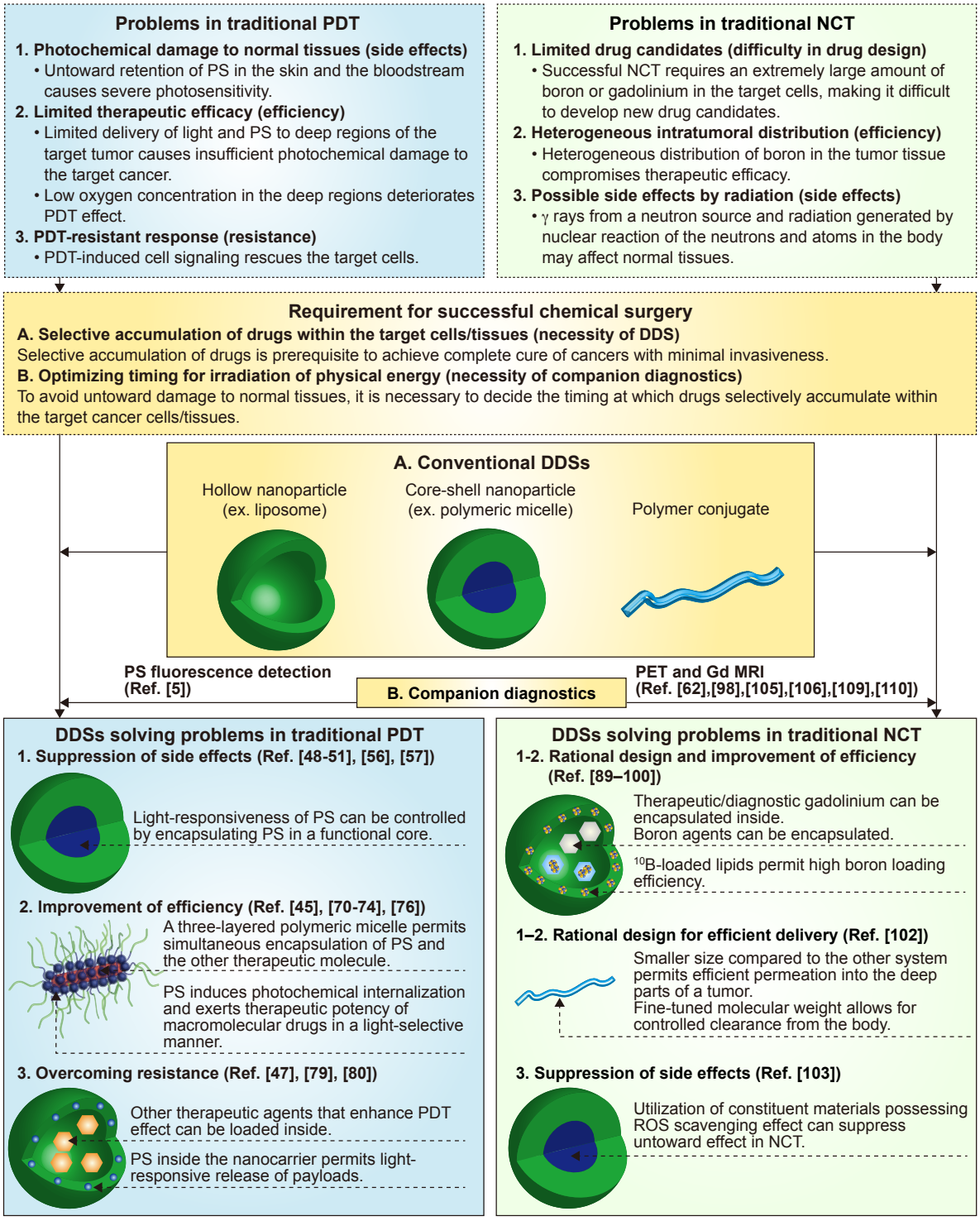

Figure 4. Design of DDSs for chemical surgery.

2. DDSs for PDT

2.1. Design guidelines for DDSs for PDT

As described in Introduction, PS should be excited at near-infrared regions, a chemical structure of PS usually has large π conjugated systems. Clinically approved PSs such as Photofrin, Foscan, Laserphyrin, and Photosense, indeed, have a chemical structure of either porphyrin, chlorin, or phthalocyanine [37]. Although these PSs demonstrated efficient therapeutic effect in clinical application, it takes time for them to be completely cleared from the body, forcing patients to avoid light for several weeks [22]. In addition, when the diseased site is photoirradiated, scattered light would be inevitably delivered to the surrounding normal tissues. Thus, from the viewpoint of preserving normal tissues as much as possible, it is necessary to further improve the selective accumulation of the PSs to the tumor. In this regard, many researchers have developed nanocarriers to deliver PSs selectively to the tumor via EPR effect [37]. However, generally, to attain efficient PS accumulation in the tumor via the EPR effect, the nanocarrier needs to circulate in the bloodstream for a long period, and PSs should longitudinally retain in the blood, which may cause untoward photochemical damage to the normal tissue including skin. As one of the great advantages of PDT is minimal invasiveness, it is important to suppress the side effects associated with this longitudinal retention, which should be a problem unique to PDT using visible light to which our bodies are frequently exposed in a daily life. However, only few studies mentioned photochemical damage of PS-loaded nanocarriers to normal tissues [44]. Thus, we here describe design of recent PS delivery systems to suppress such side effects.

Another advantage of PDT is antitumor effect strong enough to completely cure cancers; however, the permanent cure cannot be achieved in some cases in actual treatment. This compromised therapeutic effect may be caused by insufficient delivery of PSs and light to the target tumor, and intratumoral heterogeneous distribution of PSs [45]. In addition, recent studies reported that cancer cells activate signaling pathways to prevent cell death after PDT, and suppressing these pathways is important for enhancing the PDT effect [46, 47]. In this section, we also describe the design of the nanocarriers devised to enhance the therapeutic effect of PDT.

2.2. Design for reducing untoward photochemical damage

PS-loaded nanocarriers aimed at the EPR effect tend to circulate in bloodstream for longitudinal periods, and the prolonged retention in the normal tissue may cause untoward photosensitivity as described above. Thus, nanocarriers utilizing the EPR effect need to be devised to suppress the photochemical activity of PSs in normal tissues.

In this regard, Choi and Park *et al.* designed a nanocarrier that can suppress photochemical activity of encapsulated PS, utilizing hyaluronic acid conjugated with hydrophilic poly(ethylene glycol) (PEG), hydrophobic 5β-cholanic acid, and black hole quencher (BHQ3) [48]. This polymer can form nanoparticles encapsulating PS, chlorin e6 (Ce6), and the photoactivity of Ce6 could be suppressed by BHQ3, thereby decreasing fluorescence intensity and ROS production efficiency. Meanwhile, hyaluronic acid can target CD44 overexpressed on specific cancer cells, and be degraded by hyaluronidase in cancer cells. Ce6 could be released in target cancer cells overexpressing CD44, and restore fluorescence and ROS production efficiency. This elaborated devise is expected to prevent untoward damage to normal tissues and exhibit photochemical damage selectively the target cancer. In addition, this technique permits facile suppression of the photoactivity of PS by simultaneous inclusion of BHQ3 and PS into the core compartment of core-shell nanoparticles. Because most of PSs have hydrophobic chemical structure, hydrophobic quenchers like BHQ3 would be easily utilized for other PS delivery systems [49].

Such an on/off switch of PS activity could be more simply achieved without quenchers by utilizing quenching that occurs when PS is encapsulated in a nanocarrier. Maeda *et al.* developed poly(*N*-(2-hydroxypropyl)methacrylamide conjugated with zinc protoporphyrin, which can form polymeric micelles in physiological condition. They reported that the micelle formation decreased the fluorescence intensity and ROS production efficiency due to π-π interaction and quenching of PSs [50, 51]. This quenching was expected to be resolved in a tumor; their nanocarrier exhibited strong fluorescence in the tumor after intravenous injection, and demonstrated drastic antitumor activity upon photoirradiation, observing disappearance of the tumor in some cases. Although the detailed mechanism of the elimination of the quench in the tumor was not clarified yet, it was suggested that amphiphilic components such as lecithin of the cell membrane may induce disintegration of the micelle and eventual quenching cancellation. It was also speculated that a part of the polymer might be cleaved within the lysosome and the PS was released. These indications suggest that conjugation of PSs to polymers via internal stimuli-responsive linkers would enhance the tumor-selective dequenching [52-55]. For example, a previous study developed nanoparticles comprising of glycol chitosan conjugated with pheophorbide a (PS) via disulfide linkages [56]. The nanoparticle successfully inactivated the photoactivity of pheophorbide a through self-quenching effect, while, upon cleavage of the disulfide linkages under reductive environment, pheophorbide a exhibited strong fluorescence and efficient generation of singlet oxygen. Since clinical translation of DDSs requires simplicity for manufacture, minimal structure of PS delivery systems proposed in these studies would be key for attaining photoactivity of PS

selectively in the target cancer to avoid unfavorable photochemical damage to normal tissue that was accompanied with conventional PS and PS delivery systems.

Meanwhile, as simply synthesizable nanocarriers that switch on/off the photoactivity of PS in response to an acidic environment without using chemical bonds, we recently developed inorganic-organic hybrid nanocarriers possessing calcium phosphate for their core (Figure 5) [57]. Calcium phosphate is a material capable of incorporating various drugs and releasing the drugs in response to pH by increasing its solubility in an acidic environment [58-64]. Simple mixing of calcium and phosphate ions in aqueous solution forms aggregates of calcium phosphate; however, by simultaneously mixing PEG-poly(aspartic acid), poly(aspartic acid) can be incorporated into the calcium phosphate, forming nanoparticles with PEG shell layer and the calcium phosphate core. Furthermore, addition of PS in the preparation process of these nanoparticles permits encapsulation of PS into the calcium phosphate core. However, we found that the nanoparticles prepared by simple mixing of these materials in aqueous solution were easily disintegrated in physiological condition. We thus utilized hydrothermal synthesis to stabilize the calcium phosphate nanoparticles. Hydrothermal synthesis is a method of increasing the crystallinity of inorganic materials by incubating under high temperature and high pressure[65]. Hydrothermal treatment of these nanoparticles at 120°C for 1 h, indeed, allowed us to construct the hybrid nanocarriers that were stable in physiological condition. Encapsulation of PS in this hybrid nanocarrier blocked access of oxygen molecules to the PS, thereby lowering ROS production efficiency even under light irradiation. Interestingly, unlike the above-mentioned nanocarriers, the decrease in the fluorescence of the PS was slight in this nanocarrier, and the encapsulated PS could be imaged even under physiological condition. This preserved fluorescence intensity can be explained by that the PS could be encapsulated in the hybrid nanocarrier mainly by physical entrapment instead of π- π stacking.

In this study, we evaluated photochemical damage to the normal skin using intravital microscopy. It is known that photochemical damage to normal blood vessels by intravascular photoactivated PS causes occlusion and decreased velocity of blood flow[66]. Although it is difficult to observe such photochemical damage from macroscopic viewpoint immediately after exciting PS, intravital microscopy permits the real-time monitoring of the effect of the injury at the tissue level. In this work, we intravenously injected the hybrid nanocarriers and excited intravascular PS in the normal skin using laser of intravital microscopy. The photochemical damage was then estimated by tracking fluorescently labeled red blood cells and measuring their velocity in bloodstream over time. As a result, the hybrid nanocarrier maintained the velocity whereas a marked decrease in the velocity was observed with the free PS, indicating that encapsulation of the PS into the hybrid nanocarrier successfully suppressed

the untoward photochemical damage to the normal tissue. On the other hand, this nanocarrier was taken up by cancer cells through endocytosis and localized in the lysosome. The hybrid nanocarrier could restore the photoactivity of the PS in response to the lysosomal acidic environment and showed a strong antitumor effect equivalent to the free PS in a subcutaneous tumor model. As shown in this study, calcium phosphate is the promising inorganic material for the core of nanocarriers permitting pH-responsive on/off switch of photoactivity.

Several studies have been devoted to reduce side effects in PDT as described above. However, only few studies have experimentally demonstrated reduction of side effects including photosensitivity. Although suppression of photoactivity of PS in *in vitro* condition has been revealed in many studies, it does not always indicate that photochemical damage to the normal tissue can be fully avoided by such an approach. Because the actual biological environment is far more complicated compared to *in vitro* condition, functions of developed DDSs have been sometimes not induced only in the target site as indicated by strong PS fluorescence in normal tissues after systemic administration of the nanoparticles encapsulating quenched PS. Thus, *in vivo* evaluation of side effects by scoring photochemical damage to skin like the study by Berg *et al.* [67] would be necessary to clarify the validity of newly developed biomaterials and facilitate clinical translation of PS delivery systems. The aforementioned microscopic evaluation using intravital microscopy developed in our study would be also a powerful tool to elucidate the mechanism of side effects caused by developed DDSs [57].

It should be noted that all the aforementioned nanocarriers possess potential for fluorescence imaging. Many PSs can be applied for fluorescence imaging, and, in particular, porphyrin and chlorin offer excitation in the Soret band and emission in Q band, which leads to clear detection of diseased site with a high signal/noise ratio. Such diagnostic technology utilizing PS has been widely used as PS fluorescence detection (PFD) or photodynamic diagnosis. PFD visualizes tumor accumulation of PS, and prevents cancer cells from being undetected during surgery [5]. The aforementioned nanocarriers utilizing quenching of PS would be utilized to selectively detect the diseased site if dequenching could be induced only in the target site. Meanwhile, our hybrid nanocarriers emit fluorescence even when PS is encapsulated in the nanocarrier; thus, accumulation of PS to normal tissues and target tumors can be tracked over time, and behavior of the nanocarriers in the body could be monitored in detail, which would provide fundamental information leading to improvement of nanocarriers.

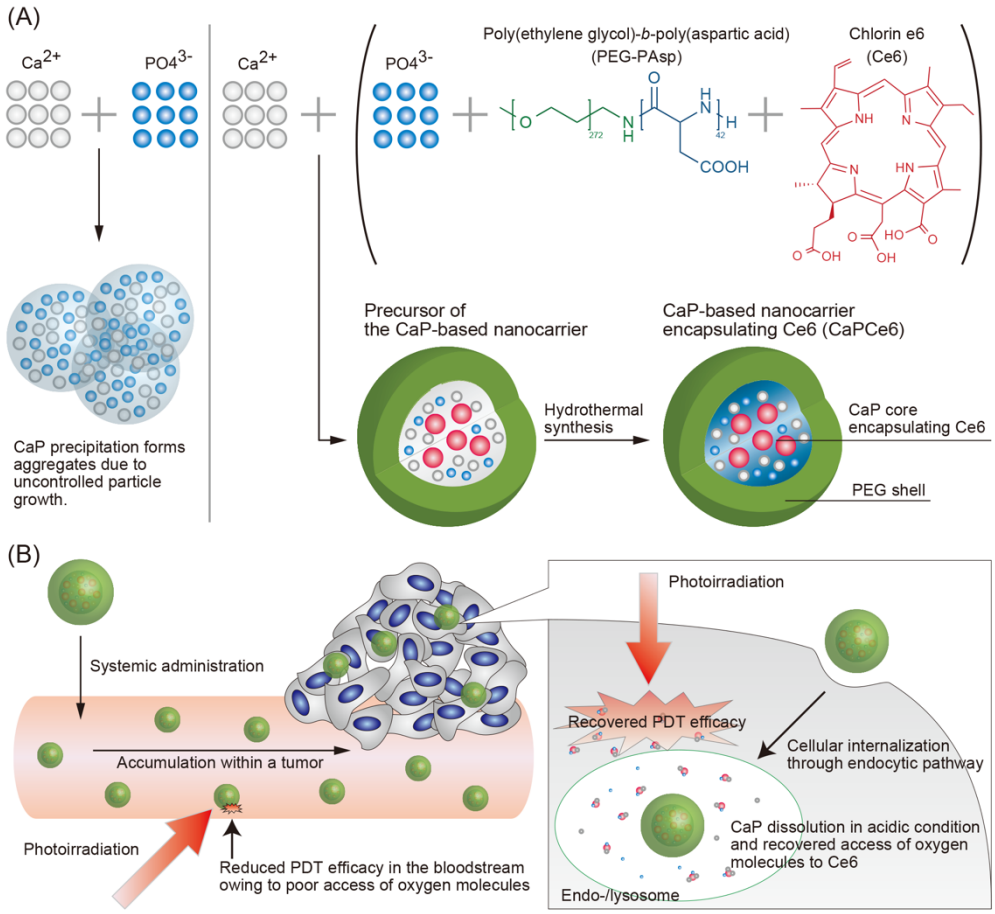

Figure 5. Concept of inorganic–organic hybrid nanocarrier for delivery of PS with pH-responsive on/off switch of photoactivity. (Reprinted from Ref. [57].)

2.3. Design for enhancing PDT effect

Although PDT is a treatment offering permanent cure of cancers, its therapeutic efficacy is compromised by insufficient intratumoral oxygen and limited light penetration, which are necessary for generating cytotoxic singlet oxygen [45]. In addition, recent research reported that some cancers activate signaling pathways to promote angiogenesis and to avoid cell death, indicating the promise of combination of PDT with other drugs [47].

Photochemical internalization (PCI) is a method for efficiently inducing a therapeutic effect in a region where the concentration of oxygen is low and light is difficult to penetrate like the deep part of the tumor [67, 68]. PCI is a technique to damage the endo-/lysosomal membrane by activating PS localized on the endo-/lysosomal membrane, thereby promoting the cytoplasmic delivery of drugs that are entrapped in the endo-/lysosomes and cannot exert their therapeutic potency; PCI enhances therapeutic effect of the drugs in a light-responsive manner. Owing to this unique mechanism, PCI requires less light dose for exerting its effect

compared to PDT, and can be induced even in the deep part of the tumor that cannot receive the sufficient PDT effect. Thus, PCI is expected to compensate for the insufficient therapeutic effect of PDT due to low oxygen concentration and limited light dose. In addition, PCI has potential to overcome drug resistance, as chemotherapeutic resistance for example in MCF-7 human breast tumor cells should be caused by accumulation of drug (doxorubicin) into acidic organelles including lysosomes and subsequent exclusion from the cell, and loss of acidification in such organelles can induce the translocation of drugs to the nuclei[69], which translocation is critical step to attain therapeutic effect. We indeed demonstrated the strong PCI effect in a deep region of a doxorubicin-resistant MCF-7 tumor by the use of doxorubicin and polyion complex (PIC) micelles composed of PEG-poly(L-lysine) and anionic dendrimeric phthalocyanine (DPc) [45]. Because the PIC micelle is internalized into the cell through endocytosis and localized in endo-/lysosomes, it can efficiently induce PCI effect and facilitate the transfer of doxorubicin from the endo-/lysosomes into the nuclei, thereby augmenting the therapeutic efficiency. While sole PDT with the PIC micelle could damage the cells only in a superficial region (roughly 1 mm below the skin) in the subcutaneous tumor, PCI could provide the therapeutic effect even in the deeper region (deeper than 1.6 mm) as shown in Figure 6.

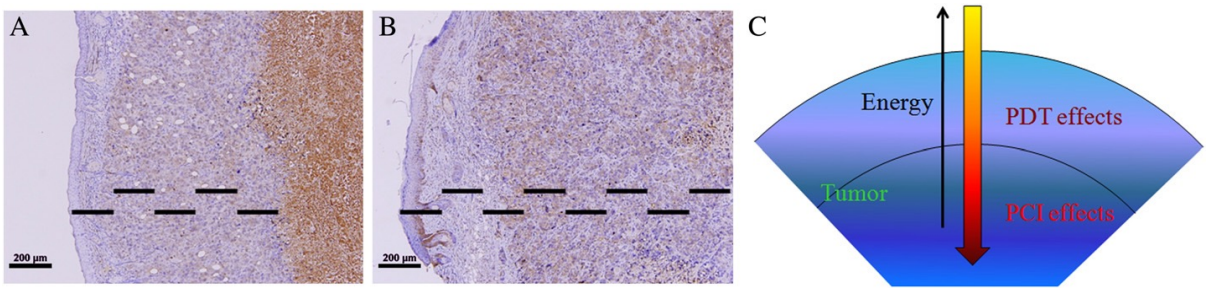

Figure 6. Macroscopic immunohistochemical observation of PCNA in doxorubicin-resistant MCF tumors treated with (A) sole PDT with the PIC micelle or (B) PCI. (C) Schematic illustration of the effect of PDT and PCI. (Reprinted from Ref. [45].)

Such anticancer drug delivery is the most advanced in PCI research. PCI of anticancer drugs with Amphinex (PS) is in phase I/II of clinical trial. To enhance the effect of PCI with more reduced systemic side effects, nanocarriers are promising techniques. Considering that nanocarriers are internalized into cells through endocytosis, their application to PCI appeared to be a rational approach. In this context, a recent study reported the polymeric micelle composed of PEG-poly(L-lysine) conjugated with camptothecin (CPT) via disulfide linkers [70]. This micelle was designed to release CPT in response to reducing environment of cytoplasm, and, to exert this function, the micelle needs to escape from endo-/lysosomes. The researchers thus utilized Photofrin to induce PCI of the polymeric micelles. In a subcutaneous tumor model, combination of free CPT and PDT with Photofrin markedly decreased the body weight, indicating the severe side effect; however, PCI with the polymeric micelle did not induce such loss of the body weight, and exhibited strong antitumor efficacy equivalent to free CPT and PDT with Photofrin. Note that clinically approved Photofrin was used to induce PCI in this study; however, it is well known that this PS retains in the skin and forces patients to avoid light for a long period. To improve safety of PCI, anticancer drug and PS should be delivered selectively to the target site. One of the approaches for this purpose may be simultaneous incorporation of anticancer drug and PS into a delivery system.

In designing such DDSs for PCI, in addition to utilizing internal stimuli for drug release as described above, functionalization of nanocarriers with light-responsive drug release is expected to enhance the selectivity of this therapeutic approach. As a polymer degrading in response to light as well as an acidic environment, Pasparakis *et al.* constructed PEG-polyacetal in which PEG is introduced into a polymer synthesized by condensation polymerization of 2-nitroresorcinol and divinyl ether derivatives, and, by the use of this PEG-polyacetal, developed nanoparticles containing hydrophobic CPT and hematoporphyrin (HP) [71]. They proposed that the nanoparticles encapsulating CPT and HP could be decomposed in the low pH environment in endosomes, and the carboxyl groups of HP could be protonated to increase hydrophobicity facilitating its localization to the endosomal membrane. Photoactivation would induce PCI by HP on the endosomal membrane, and promote decomposition of polyacetal, thereby further releasing CPT.

PCI is useful not only for anticancer drugs but also for nucleic acids whose translocation from the endo-/lysosome to the cytoplasm is regarded as a bottleneck for efficient transfection [72-74]. Although many studies reported PCI of nucleic acids in *in vitro*, only few studies succeeded in the PCI in *in vivo* because systemic nucleic acid delivery requires nanocarriers having multiple functions to overcome numerous biological barriers [75]. In addition, PSs and nucleic acid-loaded nanocarriers should be delivered to the same target cells

and organelles to attain efficient PCI. To accomplish such simultaneous delivery of PSs and nucleic acids, these molecules should be integrated into one nanocarrier. Simple encapsulation of these molecules however may cause unfavorable photochemical damage to the incorporated nucleic acids by the near PSs under photoirradiation. We thus synthesized ABC-type triblock copolymer, PEG-PAsp(DET)-PLys, and constructed a three-layered polyplex micelle composed of PEG outer shell, cationic PAsp(DET) intermediate layer accommodating anionic DPc, and pDNA/PLys PIC core (Figure 7) [76]. The outer PEG shell prevents unfavorable interaction with biological components, and the core compartment protects pDNA from enzymatic degradation, while DPc in the intermediate layer induces strong PCI effect. In this design, DPc and pDNA were compartmentalized to prevent oxidative damage to pDNA by ROS generated from the photoactivated DPc. Although the generated ROS still can reach the incorporated pDNA, its oxidative damage could be significantly reduced. In addition, while DPc shows anionic characteristics in physiological condition owing to 32 carboxyl groups in its periphery and could be accommodated into the cationic PAsp(DET) intermediate layer, in an acidic environment in the endo-/lysosomes, the carboxyl groups of DPc are protonated, facilitating its release from the micelle and interaction with the endo-/lysosomal membrane [72]. Super-resolution microscopic observation of subcellular distribution of this three-layered polyplex micelle in the cultured cells indeed revealed that DPc was localized on the endo-/lysosomal membrane while pDNA was entrapped in the endo-/lysosomes. Upon photoirradiation, DPc induced PCI, and transfection efficiency was enhanced by more than 100 times. More importantly, in a subcutaneous tumor model, the intravenously injected three-layered polyplex micelle exhibited enhanced gene transfection after photoirradiation to the tumor, thereby demonstrating first success of light-selective transfection after systemic administration.

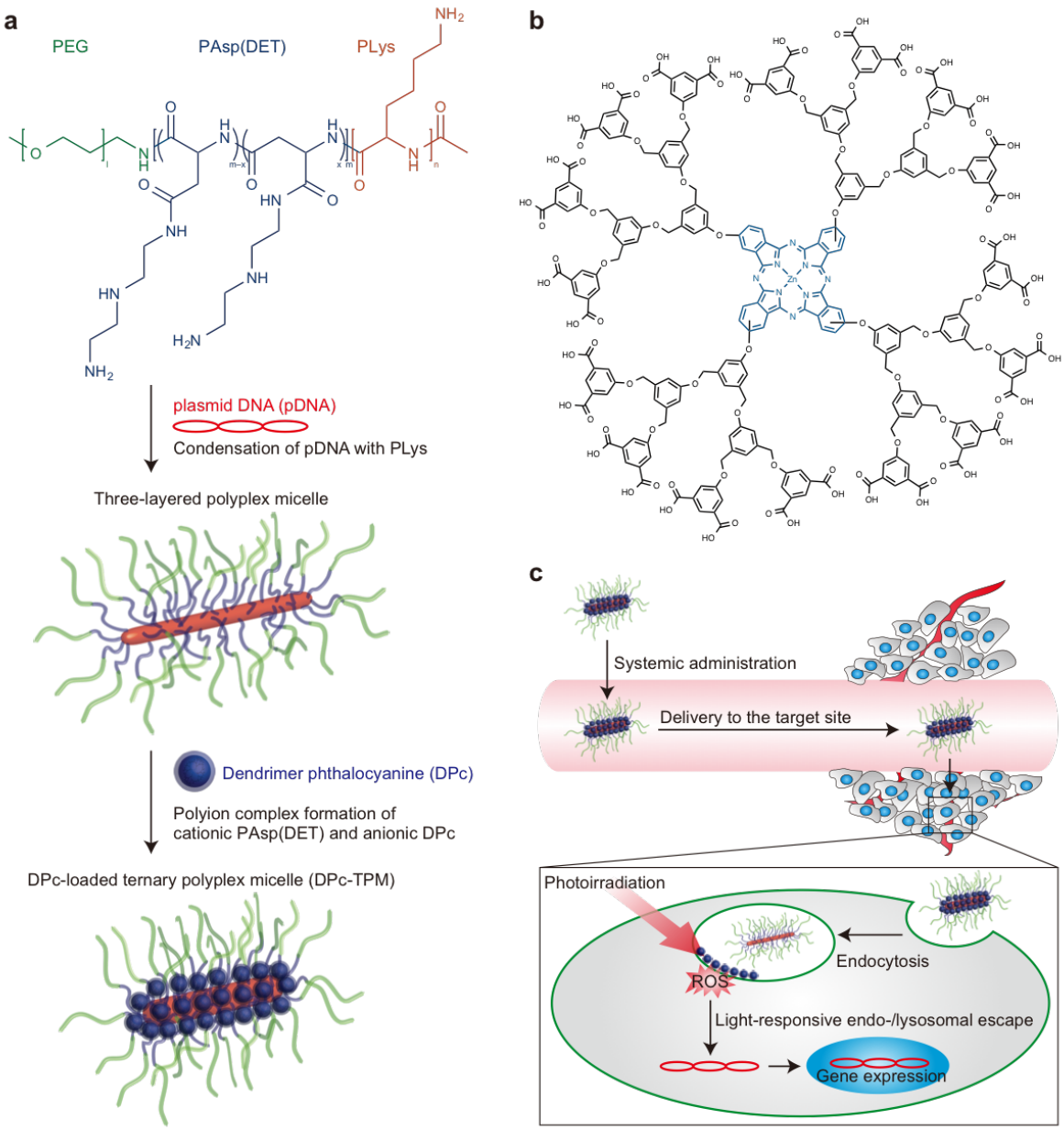

Figure 7. A three-layered polyplex micelle encapsulating dendrimeric PSs in the intermediate layer that can exert strong PCI effect. (Reprinted from Ref. [76].)

Another approach to enhance PDT effect is suppression of tumor signaling pathways promoting angiogenesis and cell death avoidance. For example, it is well known that shutting down microvasculature and oxygen consumption by PDT induce hypoxia and activates hypoxia inducible factor-1α, leading to up-regulation of VEGF [77, 78]. Administration of the inhibitors for such signaling after PDT appears to be a facile method to achieve this purpose; however, PDT-induced microvasculature shut-down should compromise delivery of the inhibitors administered after PDT. Hence, such inhibitors should be delivered to the target tumor in parallel with PDT treatment. In this approach, tumor-associated microvasculature

would be occluded when the inhibitors reached the target tumor, leading to prolonged tumor retention of the inhibitors. Many studies reported nanocarriers that can release drugs in response to light, but most nanocarriers respond to the light in the low wavelength region such as ultraviolet, which cannot offer sufficient penetration in the body.

In this context, Hasan *et al.* developed a near-infrared light-responsive liposome that can induce PDT and release anti-VEGF monoclonal antibodies [79]. The lipid bilayer of the liposome incorporates hydrophobic benzoporphyrin derivatives (BPD) in a monomeric form and the antibodies inside. Activation of BPD by near-infrared light irradiation (690 nm) causes a photochemical reaction in the lipid bilayer membrane and destroys liposome integrity, thereby releasing the antibodies. The liposome successfully co-delivered BPD and the antibodies to a subcutaneous pancreatic tumor in a mouse, and exhibited significantly higher antitumor activity compared to Visudyne with the antibody. This research group has recently reported a more advanced delivery system using sophisticated material design. By the use of aforementioned technique, Spring, Sears, and Hasan *et al.* developed a near-infrared light-responsive liposome that can damage tumor cells and tumor-associated vasculature by PDT and release drugs inhibiting PDT-induced signaling of cell death avoidance (Figure 8) [47]. The liposome whose membrane incorporates BPD encapsulates the PLGA nanoparticle loaded with a hydrophobic multi-kinase inhibitor, cabozanitinib (XL184). By loading XL184 into the PLGA nanoparticles, its encapsulation efficiency into the liposomes could be increased, and the lipid bilayer membrane could inhibit hydrolysis of the PLGA nanoparticles. Activation of BPD by near-infrared light irradiation (690 nm) causes a photochemical reaction in the lipid bilayer membrane and destroys liposome integrity, thereby promoting release of XL184 from the nanoparticles. XL184 can suppress the MET signaling that is activated to prevent cell death after PDT, and the VEGFR signaling that contributes to tumor angiogenesis. Integration of these multiple functions into one nanocarrier permitted a higher antitumor effect than simple administration of each drug. Their nanocarrier indeed demonstrated a remarkable antitumor effect in a subcutaneous tumor model, and, more importantly, succeeded in dramatic suppression of tumor growth and metastasis into the liver and lymph node even in an orthotopic pancreatic tumor model.

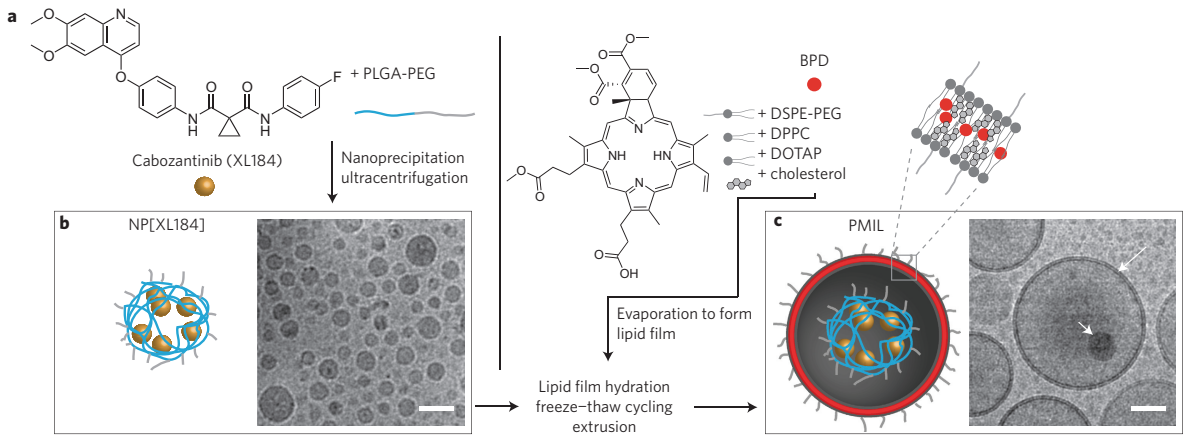

Figure 8. A liposome that can release inhibitors suppressing tumor regrowth and treatment escape signaling pathways in a light-induced manner. (Reprinted from Ref. [47] with permission from Springer Nature.)

Like this elegant study by Hasan *et al.*, it may be a promising approach for accomplishing efficient chemical surgery to induce PDT effect and in parallel release of encapsulated drugs from a DDS in response to near-infrared light. In 2014, we also succeeded in development of such a near-infrared light-responsive nanocarrier [80]. Our nanocarrier termed PICsome is a hollow nanoparticle that possesses crosslinked PIC membrane comprising PEG-poly(aspartic acid) and poly([5-amino-pentyl]-α,β-aspartamide) and encapsulates Al(III) phthalocyanine disulfonate (AlPcS2a) as a PS. Although photochemical activity of the encapsulated AlPcS2a is lowered by concentration quenching, photoactivation of AlPcS2a gradually gives photochemical damage to the PIC membrane and releases AlPcS2a, thereby recovering photochemical activity of AlPcS2a. Furthermore, inside a cell, AlPcS2a could be located on the endo-/lysosomal membrane owing to its amphiphilic property, which is favorable to induce PCI; and continuous photoactivation could permit translocation of AlPcS2a into the cytosol. This cascade of photochemical activity ultimately induced light-selective cell death in cultured cells. While this study demonstrated light-induced release of PS and subsequent PCI of the AlPcS2a-loaded PICsome, the PICsome also enables tumor-target delivery of other functional molecules including proteins [81]. Our PICsome may have great potential as a systemically injectable nanocarrier that permits cytosolic delivery of emerging biopharmaceuticals such as nucleic acids, proteins, and peptides in a light-selective manner using PCI.

The aforementioned studies using the liposomes and PICsomes [47, 79, 80] indicate important approach in designing PS delivery systems. These studies not merely delivered PS but also utilized it as the trigger of light-induced release of other therapeutic molecules, while

traditional design of PS delivery systems has been mainly aimed at simple delivery of PS selectively to target sites. Since numerous PSs have been developed in these decades[82] and they respectively have unique physicochemical characteristics including hydrophobicity, excitation wavelength, and photostability, DDSs for PDT should be designed to make the best use of these characteristics. Conventionally, hydrophobic property of PS was considered to be unfavorable property because poor water-solubility requires solubilizing agents and hydrophobic interaction of these molecules compromises ROS production efficiency. However, Hasan *et al.* utilized this hydrophobicity to accommodate PS in the lipid bilayer, thereby accomplished near-infrared light-induced release of the therapeutic molecules [47, 79]. We also utilized amphiphilic property of AlPcS2a, and demonstrated sequential disruption of the PIC membrane and the cell membrane to induce PCI [80].

Note that PS also has potential to control drug distribution in photoirradiated regions as the co-delivery by the aforementioned liposomes encapsulating BPD and XL184-loaded nanoparticles demonstrated higher antitumor activity compared to separate administration of BPD-loaded liposomes and XL184-loaded nanoparticles. This enhancement might be explained by the entrapment of XL184-loaded nanoparticles induced by PDT-mediated microvasculature shut-down, and subsequent sustained release of XL184. As well as such light-induced entrapment, previous studies reported the potential of PS to enhance permeability of macromolecules in a light-selective manner [83-86]. Although precisely controlled induction of either of microvasculature shut-down or enhanced permeability still requires further fundamental analysis, exploiting such functions to control drug distribution would offer new light-selective therapeutic modalities.

In summary, simple enhancement of intratumoral PS concentration does not always satisfy the requirements in designing DDSs for PDT. Since the clinically approved PSs can already accomplish strong therapeutic effects, the function required for the nanocarriers is rather to reduce side effects [23] and improve therapeutic efficiency in the tumor that resists sole PDT or in the site that light cannot sufficiently reach. Thus, DDSs for PDT should possess function to respond to internal/external stimuli including pH, redox, enzyme, and light to reduce possible photochemical damage to the normal tissue, and other drugs should be strategically combined to completely kill all the tumor cells. In addition, function of developed DDSs should not be examined only in monolayer cultured cells, because strong phototoxicity of PS is prerequisite and the problems in PDT have been raised in complicated 3D structure of tumors as described above. In this regard, 3D cell culture models may be useful tools to examine potential of newly developed DDSs [87].

3. DDSs for NCT

3.1. Design guidelines for DDSs for NCT

In the field of NCT, BSH and BPA have been clinically used as the drugs. BSH has high hydrophilicity and can accumulate selectively within brain tumors through disrupted blood-brain barrier, but it has no targetability to the other tumor. On the other hand, BPA can be internalized into cells through amino acid transporters that are overexpressed on many types of tumor cells, thereby showing high selectivity to the tumor. However, extension of the range of NCT application requires development of novel drugs. Thus, also in the development of drugs for NCT, nanocarriers have attracted attention in recent years.

Before designing nanocarriers for NCT, it should be noted that the dose of drug for NCT is much higher than the other therapeutic modalities. For example, to attain antitumor effect using ^{10}B, intratumoral ^{10}B concentration should be more than 20 ppm (20 μg/g tumor). Assuming that a nanocarrier achieves delivery of 4% dose/g tumor in a mouse model, ^{10}B should be administered at a dosage of 500 μg; and if the weight fraction of ^{10}B in the whole nanocarrier is 5 wt%, 10 mg of the nanocarriers will be administered to a mouse weighing 20 g. This dosage corresponds to 500 mg ^{10}B/kg, where 30 g of the nanocarriers would be administered to a human with a body weight of 60 kg. Since this dosage is extremely large, the material composing nanocarriers should be safe even when such large doses are administered, and the nanocarrier should have a high boron weight fraction. These are the most important functions in developing nanocarriers for NCT. Thus, it is sometimes difficult to simply divert a nanocarrier platform for the other therapeutic modalities to that for NCT. In this context, many studies have been devoted to reduce the total dose of a nanocarrier by increasing the weight fraction of ^{10}B in the nanocarrier, which is the unique characteristics of NCT research. Note that ^{157}Gd can also capture neutrons and generate cytotoxic γ rays. The dose of ^{157}Gd is however limited due to heavy metal toxicity. Hence, the nanocarriers for ^{10}B delivery are more enthusiastically studied, and boron clusters including BSH have been used as boron sources because of their high boron weight fraction. Because the α ray generated by the nuclear reaction between ^{10}B and the neutron has short range and plays the main role in killing tumor cells in BNCT, ^{10}B should be distributed as uniformly as possible within the tumor to accomplish permanent cure [88].

Up to now, as boron delivery systems, researchers have reported active targeting low molecular weight drugs, antibody-drug conjugates, polymer conjugates, polymeric micelles, liposomes, nanoparticles, and carriers utilizing biological components [10]. Here, we focus on studies demonstrating systemic application of boron delivery systems by the use of liposomes,

polymer conjugates, polymeric micelles, or carriers utilizing biological components, in order to elucidate biomaterial-related chemistry used in recent NCT research.

3.2. Development trend of boron delivery systems

Among boron delivery systems, liposomes have been most enthusiastically studied [89-100]. As described above, in BNCT, an extremely large amount of boron should be delivered to the diseased part, and increase of the weight fraction of boron in liposomes is important to lower the dosage of lipids. Simple encapsulation of a large amount of boron cluster into the liposomes, however, causes osmotic pressure and destabilizes liposomal membrane, limiting stable boron delivery to the target site. Nakamura *et al.*, have attempted to solve this problem by conjugating boron clusters on the liposome membrane [92, 93, 95-97]. They have synthesized BSH-conjugated lipids and succeeded in preparation of the boron-loaded liposomes that could stably maintain the liposomal structure even when they are administered in *in vivo*. This liposome achieved the intratumoral boron concentration necessary to obtain an antitumor effect of 22.7 ppm in a subcutaneous tumor model at a dose of 20 mg B/kg, and by performing neutron irradiation, the tumor growth could be remarkably suppressed. Meanwhile, they found that the tumors treated by BNCT with their liposomes exhibited regrowth after the temporal shrinkage. By analyzing intratumoral distribution of the fluorescently labeled liposomes, they elegantly illustrated that the tumor regrowth might be caused by proliferation of the tumor cells that were not exposed to sufficient α rays due to heterogeneous intratumoral distribution of the liposomes [97]. To overcome the compromised therapeutic efficacy, they further encapsulated BSH into the inside of the liposome [98]. The inside BSH is expected to efficiently penetrate to the deep region in the tumor upon release, and in parallel contributes to increase of the boron weight fraction of the liposome. The liposome encapsulating BSH indeed demonstrated strong antitumor activity with neutron irradiation, and more importantly revealed the disappearance of the tumor, indicating that successful BNCT requires homogeneous intratumoral distribution of ^{10}B. Consistent with this study, Ono *et al.* previously reported that BPA is not efficiently taken up by quiescent cells, compromising therapeutic effect of BNCT with BPA [88]; and combination of BPA and BSH may overcome this challenge because BSH can homogeneously distribute [101]. BSH however does not have tumor targetability. Thus, delivery of free BSH using the aforementioned liposomes appears to be a rational approach.

While the liposomes have been highly functionalized as described above, we focused on polymer conjugates as boron delivery systems with efficient tumor penetration because the size of polymer conjugates is smaller compared to that of liposomes and polymeric micelles, which is expected to permit the efficient penetration into the deep region of the tumor. In

addition, in a polymer conjugate, multiple boron clusters could be conjugated to one macromolecule, enabling the increased boron weight fraction. We constructed the polymer conjugate for boron delivery by utilizing a biocompatible polymer, PEG-poly(L-glutamic acid), as a platform, and introducing BSH to the side chains of poly(L-glutamic acid) via disulfide linkers (Figure 9) [102]. Cellular uptake study in cultured cells revealed that the polymer conjugate exhibited higher boron internalization than conventional BSH, and *in vivo* study showed the enhanced tumor accumulation. In addition, this polymer conjugate has a molecular weight of 23,000, which is subject to glomerular filtration; it can be cleared out from the bloodstream earlier than a liposome and a polymeric micelle, improving a tumor/blood boron concentration ratio. Furthermore, probably owing to this small size, the polymer conjugate could be distributed throughout the tumor even in a stroma-rich tumor, in which Doxil could not efficiently move away from the vasculature. Hence, the polymer conjugate may have potential to overcome the aforementioned heterogeneous intratumoral distribution and compromised antitumor activity. In vivo study using a subcutaneous C26 tumor model demonstrated a high tumor/blood boron concentration ratio and efficient antitumor activity of the polymer conjugate, indicating that the polymer conjugate is a powerful platform to develop a boron delivery system that permits high tumor accumulation and early disappearance from normal tissues, and ultimately reduces untoward radiation exposure to the normal tissues in BNCT.

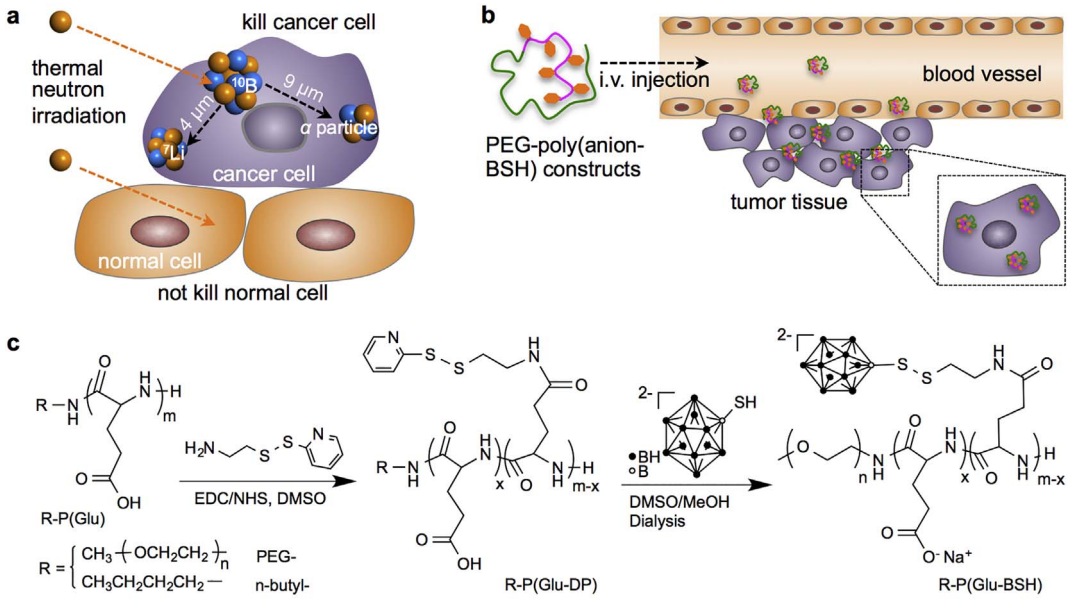

Figure 9. A polymer conjugate having BSH via disulfide bonds (Reprinted from Ref. [102].)

Focusing on side effects caused by radiation exposure to normal tissues, Nagasaki *et al.*, constructed a unique polymeric micelle (Figure 10) [103]. In NCT, neutron irradiation is generally performed using nuclear reactors, and γ rays included in such neutron sources and radiation generated by nuclear reaction of the neutrons and atoms in the body are known to produce ROS, which may induce inflammation and damage normal tissues. In this regard, Nagasaki *et al.* used a functional polymer scavenging ROS as a material composing a polymeric micelle for delivering boron clusters. Specifically, this boron delivery system is the PIC micelle composed of PEG-*b*-poly((closo-dodecaboranyl)thiomethylstyrene) (PEG-b-PMBSH) and PEG-*b*-poly(4-(2,2,6,6-tetramethylpiperidine-*N*-oxyl)aminomethylstyrene) (PEG-b-PMNT), where the styrene structure enhances the stability of the PIC micelle and the 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) in the PMNT side chain scavenges ROS. The intravenously injected micelle delivered boron to a subcutaneous C26 tumor in a mouse through the EPR effect, and, 48-72 hours after the injection, the micelle accomplished the efficient tumor accumulation of 5.5% dose/g tumor. BNCT with this micelle revealed the antitumor effect equivalent to that with conventional BPA, and the significant enhancement could be obtained compared with BSH. It is noteworthy that the number of leukocytes, which is one of the indicators of inflammation, in the mouse treated with neutron irradiation with the micelle was comparable to that of a healthy mouse, whereas neutron irradiation with either of PBS or BPA significantly increased the number of leukocytes. This result suggests that the micelle could successfully suppress the untoward damage to the normal tissues. Introduction of such a function for suppressing side effects to the material composing DDSs may be a useful approach for realizing minimally-invasive chemical surgery.

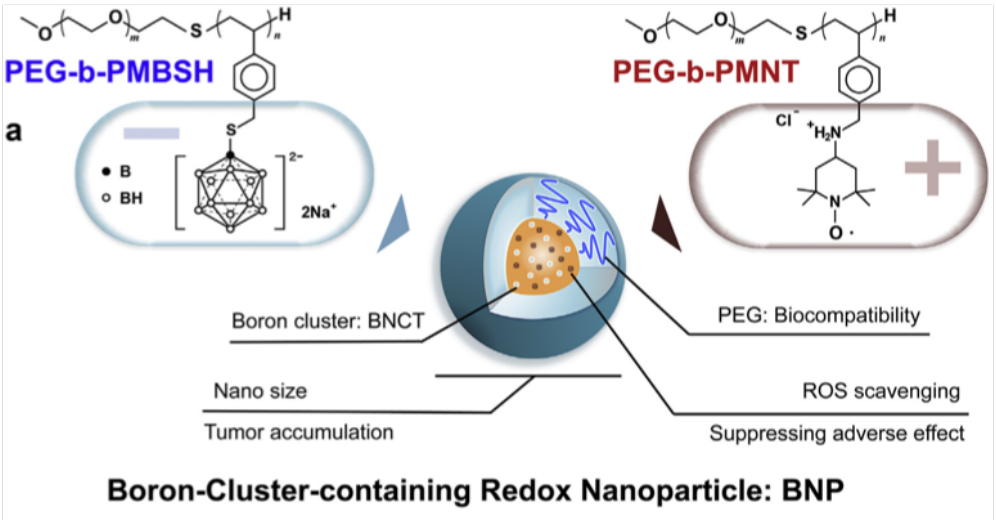

Figure 10. A polymeric micelle scavenging ROS induced by untoward radiation exposure. (Reprinted from Ref. [103] with permission from Elsevier.)

From the viewpoint of the safety of materials constituting boron delivery systems, biological components in the body may offer great promise. Also, it is well known that the association of drugs to biological components including blood proteins like Abraxane (the albumin-bound paclitaxel) improves the tumor accumulation of the drugs. In this regard, Nakamura *et al.* developed maleimide-functionalized closo-dodecaborate (MID) possessing a simple chemical structure for efficient introduction of the boron cluster to albumin [104]. MID can be conjugated to thiol group and amino group of albumin via the maleimide group. In assessing tumor accumulation in a subcutaneous tumor model, MID-conjugated bovine serum albumin (BSA) achieved strikingly high intratumoral boron concentration of 62 ppm at a dose of 30 mg B/kg. The MID-conjugated BSA also exhibited strong antitumor effect with neutron irradiation. Because of its simple chemical structure, MID has high boron weight fraction and is expected to be a promising boron delivery system with high safety. In addition, they demonstrated that intravenously injected MID could conjugate to serum albumin in the bloodstream, indicating that MID-albumin conjugates can be prepared from serum albumin collected from patients at time of use. This facile preparation procedure is a great advantage for efficient production because the manufacturer needs to synthesize only MID. The final product, MID-albumin conjugates can be constructed in a hospital simply by mixing MID and the albumin of the patient, which avoids possible contamination accompanied with blood products.

Another boron delivery systems utilizing biological components is based on low density lipoproteins (LDL) [105, 106]. LDL is known to target LDL receptors overexpressed on tumor cells, which is expected to improve boron delivery to the tumor. Aime and Crich *et al.* indeed succeeded in obtaining a superior therapeutic effect using the nanocarrier composed of LDL and the chemical compound having a boron cluster and the complex of Gd^{3+} and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (Gd-DOTA). In their study, they also performed unique evaluation of boron accumulation utilizing this Gd as described in the next section.

3.3. Companion diagnostics in NCT

In NCT, in order to minimize radiation exposure to normal tissues and effectively kill target cells, neutrons should be irradiated at a timing when boron accumulates in the tumor at the sufficient level for obtaining a therapeutic effect with the acceptable tumor/normal tissue boron concentration ratio. In this regard, noninvasive technique to track boron in the body is essential in NCT. This technique is also important in distinguishing patients who are likely to benefit

from NCT and those who are not. Boron MRI has been proposed as such an attempt to track boron in the body [107], but improvement is still necessary to obtain a practical signal/noise ratio.

Currently, positron emission tomography (PET) using ^{18}F-BPA is the most practical tool to track the boron and is now under clinical trials [108]. Because ^{18}F-BPA is considered to show similar biodistribution with BPA, accumulation of BPA to the diseased site can be quantitatively imaged by PET with ^{18}F-BPA, illustrating the contrast of boron accumulation between the target site and the surrounding normal tissue. The PET with ^{18}F-BPA thereby permits estimating radiation exposure in the normal tissue and making a rational treatment plan.

Gadolinium-loaded nanocarriers have recently attracted increased attention because of their potential for companion diagnostics and NCT with Gd (GdNCT). For example, Yanagie *et al.* developed a gadolinium-entrapped liposome using Gadoteridol (paramagnetic agent) and Coatsome EL-01-N liposomes (slightly anionic empty liposomes with the average particle size of 100–300 nm) [109]. MRI with their liposomes qualitatively illustrated the accumulation of Gd within the target tumor, which accumulation was further quantitatively supported by inductively coupled plasma-mass spectroscopy (ICP-MS) measurement of Gd concentration in the tumor. The liposome achieved Gd concentrations of 40.3 μg/g in the tumor 2 h after intravenous injection, and exhibited efficient tumor suppression by neutron irradiation without apparent side effects despite high Gd concentration in the blood at this time point (55.7 μg/mL). Although more detailed analysis about damage to normal tissues still needs to be required to clinical translation of GdNCT, therapeutic outcome and diagnostic application of Gd were sufficient to encourage researchers to develop Gd deliver systems.

In this context, we also have constructed a hybrid nanocarrier stably encapsulating diethylenetriaminepentaacetic acid gadolinium (III) (Gd-DTPA) in the inner core (Figure 11) using the same technique as the inorganic-organic hybrid nanocarrier [57] described in the PDT section (Figure 5) [62, 110]. While Gd-DTPA in a free form could not efficiently reach the target tumor and was quickly cleared from the body after systemic administration, this hybrid nanocarrier stably encapsulated Gd-DTPA in the core and delivered Gd to the tumor (3.9% injected dose/g tumor tissue). The stable encapsulation of Gd-DTPA into the core also contributed to the increase of r_1 relaxivity, probably because the encapsulation may disturb water exchange between bulk water and inner-sphere water, thereby slowing down tumbling motion of Gd-DTPA [111, 112]. Owing to these functions, the nanocarrier exhibited clear contrast enhancement in the tumor, and ultimately showed strong antitumor activity with neutron irradiation, demonstrating the promise of theranostic application of gadolinium-loaded nanocarriers.

Because γ rays used in GdNCT can efficiently penetrate tissue, it may be not necessary to deliver Gd only to the target cancer cell. In this regard, GdNCT may have advantage compared to BNCT, in which boron should be delivered to all the target cells. However, it should be noted that mechanism of antitumor effect of GdNCT should be more investigated in detail. Although the optimal ^{157}Gd concentration in tumors in GdNCT was reported to be 50–200 μg/g tumor [113], effective suppression of tumor growth could be still observed even at much lower Gd concentration in another study [114]. In this study, correlation between Gd concentration in a tumor and therapeutic effect could not be observed; thus, rationale design of DDSs for GdNCT requires more detailed analysis from the viewpoints of physics and nuclear medicine.

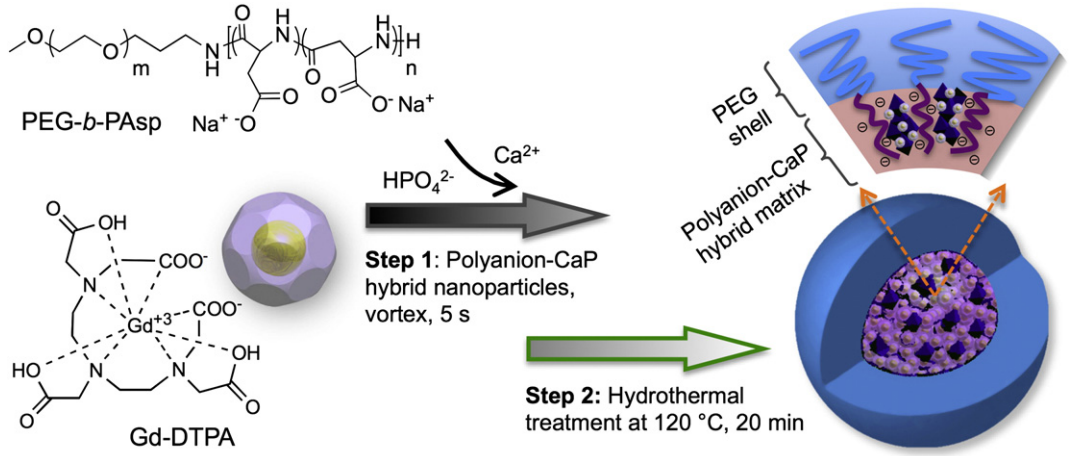

Figure 11. A calcium phosphate-based hybrid nanocarrier encapsulating Gd-DTPA (Reprinted from Ref. [62].)

Gd can be utilized not only for GdNCT but also for companion diagnostics of boron delivery systems. As aforementioned, ^{18}F-PET has been used as the companion diagnostics in BNCT; however, the half-life of ^{18}F is short (roughly 1.83 h). Thus, it is usually difficult to integrate ^{18}F into the DDSs that require multiple synthetic steps, and Gd is an attractive material to construct the function of companion diagnostics in boron delivery systems.

By the use of the nanocarrier composed of LDL and the chemical compound having a boron cluster and Gd-DOTA, Crich *et al.* attempted to quantify the concentration of boron in the target tumor by quantitative analysis of MRI [106]. In this study, boron concentration in the tumor and the surrounding tissue were quantified by MRI in a lung metastatic cancer model. Such a technique to find the optimal timing for neutron irradiation should be greatly useful in clinical application. In addition, the developed nanocarrier possesses boron and gadolinium, which may permit dual NCT generating α and γ rays. Nakamura *et al.* also developed the Gd-

loaded liposome comprising BSH-introduced lipids, and succeeded in *in vivo* tracking of the liposome [98]. Their system is also expected to be able to perform the companion diagnostics and NCT using both Gd and B.

MRI of Gd is a powerful tool in NCT, but toxicity of Gd should be carefully considered. While relatively low toxicity of boron-based drugs has been widely reported, Gd has toxicity peculiar to heavy metals although it has a higher neutron capture cross section than boron; it is difficult to administer Gd in the same amount of boron. Thus, the inclusion fraction of boron and Gd in a nanocarrier should be carefully determined in the dual NCT.

4. Perspectives

In this review, we mainly highlighted systemically applicable DDSs. Because target cancers of physical energy-induced chemical surgery are multiple and diffuse cancers that cannot be precisely located, intratumoral injection is generally not available. However, local injection of drugs and DDSs is a powerful technique to gain efficient tumor accumulation with reduced systemic side effects. For example, Suzuki and Yanagie *et al.* demonstrated that intra-arterial administration permits efficient boron accumulation in hepatocellular carcinoma for BNCT [115-118]. This approach allows for selective delivery of the high amount of drug to the target site, which cannot be achieved by systemic administration. Thus, strategic local administration should have great potential for accomplishing the concept of physical energy-induced chemical surgery, and may require new design of DDSs for this purpose. This topic may be discussed elsewhere in future.

In the development of such systemically/locally applicable nanocarriers directed to physical energy-induced chemical surgery, unlike nanocarriers for chemotherapy such as anticancer drug delivery, the timing of irradiation of physical energy critically affects the therapeutic effect. At the level of animal experiments, diseased areas can be collected and quantified. However, it is not realistic to quantify drug distribution by collecting tissues at each site over time when applied to humans, which requires development of noninvasive methods to quantify drug accumulation with spatiotemporal information. For this purpose, imaging technique appears to be most useful.

In PDT, PFD is most convenient. Although it is difficult to track PS fluorescence from the body surface, remarkable development of endoscopy in recent years has enabled minimally invasive imaging. The fluorescence imaging can provide qualitative or semi-quantitative information about PS accumulation, and clarify the contrast between the target site and the surrounding normal tissue even during PDT treatment. Because PDT is a treatment that can be performed multiple times, integration of the function monitoring the therapeutic effect in real time into the nanocarriers may permit further advanced chemical surgery.

In the case of NCT, radiosensitivity varies depending on organs and γ rays contaminated in the neutron source may cause untoward effects in normal tissues, limiting neutron irradiation dose that can be used for the treatment. Thus, it is required to develop a thorough treatment plan before neutron irradiation, and a quantitative method evaluating drug accumulation is necessary for this purpose. PET using ^{18}F-BPA provides quantitative information, which should be the diagnostic method compatible with NCT. On the other hand, it is important to distribute drugs to the whole tumor in order to achieve permanent cure of cancers with BNCT, and MRI with higher spatial resolution than PET may be useful for

evaluation of such intratumoral distribution. By quantitatively evaluating drug distribution with advanced spatiotemporal resolution using these imaging techniques, it would be possible to perform chemical surgery that minimizes burden on patients.

The advance of these imaging technologies depends on the development of nanocarriers oriented toward this purpose. That is, nanocarriers for chemical surgery in the future should have the function as highly sensitive diagnostic agents. These technological developments contribute not only to chemical surgery but also to cancer therapy by the sole physical energy mentioned at the beginning of Introduction. To make progress in this research, it is important to understand the characteristics of each physical energy and to select suitable chemistry.

5. References

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6. Acknowledgements

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