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# Advanced Drug Carriers Targeting Bone Marrow

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## 1. Introduction

The progression of nanotechnology has produced engineered fine nanoparticles for use in biomedical applications. Drug delivery systems are in a particularly promising field for the use of the unique properties of nanoparticles in biomedical applications. The pharmacokinetics of nanoparticles differs considerably from that of small drug molecules. Therefore, a drug delivery system based on nanoparticles offers a novel direction of drug discovery as well as an improved delivery system for use with conventional drugs. It has been demonstrated that drug delivery systems using nanoparticles are advantageous for stable solubilization of lipophilic drugs, reduced toxicity, inhibition of enzymatic degradation of the drugs, and so on (Moghimi et al., 2001; Papahadjopoulos et al., 1991; Torchilin, 2005). Nanoparticulate drugs show longer circulation time by avoiding renal excretion compared to drugs with small molecules. Moreover, one important technology termed "PEGylation", surface modification of nanoparticles by polyethylene glycol (PEG) chains, prolonged the circulation time (Klibanov et al., 1990; Owens & Peppas, 2006). These long-circulating nanoparticles are effective to increase the passive delivery of anticancer drugs into tumor tissues having leaky blood capillaries with wide fenestrations (Matsumura & Maeda, 1986; Gabizon et al., 1994).

Practical nanotechnology and methodologies to target a specific organ or cell actively are of current interest in the development of further advanced drug delivery systems. For this purpose, the specific interaction which is typically mediated by the receptors on a cell surface must be ascertained. Furthermore, non-specific interactions must be minimized to achieve high selectivity to the target. These specific and selective mechanisms are working constantly in communication, transport, and metabolism processes in living system. Drug carriers offer a platform to target these biological mechanisms. Several results of studies have shown that the bone marrow is the principal organ for the specific uptake of bioparticles such as senescent cells, lipoproteins, and nuclei from erythroid precursor cells through the mononuclear phagocyte system (Hussain et al., 1989a; Qiu et al., 1995; Rankin, 2010). This importance suggests that the phagocytic activity of bone marrow is a potent target of nanoparticle-based

drug delivery. In fact, it has been demonstrated that nanoparticles modified with a specific molecule on their surface are distributed selectively into bone marrow tissues (Harris et al. 2010; Mann et al., 2011; Moghimi, 1995; Porter et al., 1992; Schettini et al., 2006; Sou et al. 2007, 2010, 2011a). These bone marrow-specific drug carrier systems are expected to improve diagnostic and therapeutic systems to treat hematopoietic disorders. This chapter presents a review of the specific targeting of bone marrow using nanoparticles as carriers. Furthermore, future aspects of their medical applications are discussed.

## 2. Functions of bone marrow for blood cell turnover

Bone marrow, a soft and spongy tissue found in the hollow spaces in the interior of bones, constitutes about 4% of the total body weight of adult humans. Progenitor cell (stem cell) lines in the bone marrow produce new blood cells and stromal cells. It has been estimated that as many as  $4.9 \times 10^{11}$  senescent blood cells are eliminated from blood circulation per day in adult humans (Fliedner et al., 1976, 2002). Figure 1 shows that almost as many blood cells are released from bone marrow to maintain a constant number of circulating blood cells. Bone marrow possesses an efficient system that takes nutrients from blood circulation selectively for blood cell production.

Mononuclear phagocyte systems in liver and spleen have been regarded as the main pathway to eliminate senescent blood cells. However, a recent study has shown that mouse bone marrow is an important organ for eliminating white blood cells, especially neutrophils, where neutrophils are ultimately phagocytosed by bone marrow stromal macrophages (Dalli et al., 2012; Furze & Rankin, 2008; Rankin, 2010). The expression of the anti-inflammatory molecule annexin A1 by resident macrophages is necessary for clearance of senescent neutrophils, which are determined by their higher levels of CXCR4 expression and annexin V binding in the mouse bone marrow (Dalli et al., 2012). Consequently, it can be speculated that annexin A1 on bone marrow resident macrophages specifically interacts with senescent neutrophils. High uptake of white blood cells in bone marrow can also be observed in humans following administration of  $^{111}\text{In}$ -radiolabeled white blood cells that are administered routinely to humans for the detection of occult infection which is first recognized by secondary manifestations such as increased neutrophils in the circulation or fever of unknown origin. Whole body region-of-interest analysis frequently reveals that 60–70% of the administered white blood cells localize to bone marrow, whereas 30–40% localize to liver and spleen (Sou et al., 2011a).

Chylomicrons are large lipoprotein particles that consist of triglycerides, phospholipids, cholesterol, and proteins. Hussain and co-workers reported that rabbit and marmoset bone marrow had significant uptake of chylomicrons labeled with  $^{14}\text{C}$  cholesterol and  $^3\text{H}$  retinol (Hussain et al., 1989b). Perisinusoidal macrophages protruding through the endothelial cells into the marrow sinuses were responsible for accumulation of the chylomicrons in the marmoset bone marrow. In contrast to marmosets, chylomicron clearance from the bone marrow of rats, guinea pigs, and dogs was much less, and the spleen in rats and guinea pigs took up a large fraction of chylomicrons. Consequently, Hussain et al. concluded that the observed differences in chylomicron metabolism result

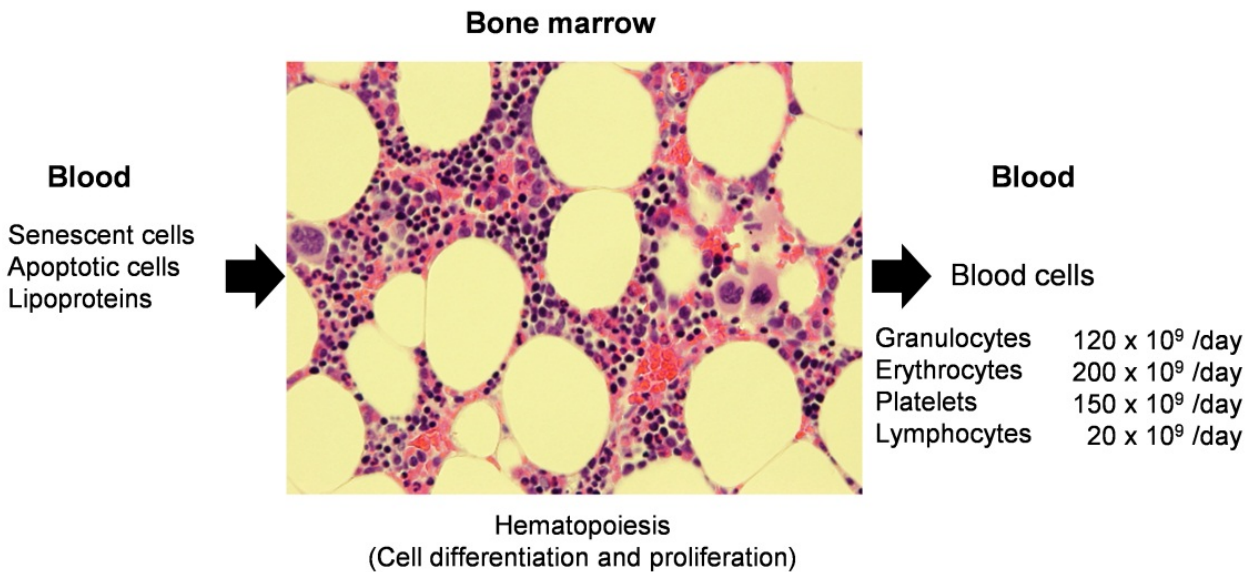
from the presence of perisinusoidal macrophages in bone marrow. It was also believed that the differences between bone marrow and spleen uptake of chylomicrons might provide insights into the role of chylomicron catabolism in these organs, both of which are involved in hematopoiesis. It was speculated that the chylomicrons play a role in the delivery of lipids to the bone marrow and spleen as a source of energy and for membrane biosynthesis or in the delivery of fat soluble vitamins. In addition, bone marrow macrophages, which are associated with erythroblasts in a hematopoietic environment, participate in erythropoiesis control, and engulfment of nuclei from erythroid precursor cells (Chasis & Mohandas, 2008; Qiu et al., 1995; Sadahira & Mori, 1999; Winkler et al., 2010; Yoshida et al., 2005). These features of bone marrow for uptake of senescent blood cells, lipoproteins, and nuclei can be original models of the specific bone marrow targeting system.

The molecular mechanism of the uptake of lipid particles such as lipoproteins and apoptotic cells plays an important role in the transport and metabolism of fats such as cholesterol, triglycerides, and phospholipids. The attractive matter on the molecular mechanism is the ligand-receptor system, which permits the specific interaction of the lipid particles with specific cells in organs. For example, low-density lipoprotein (LDL), a native lipid particles consisting with cholesterol, ApoB-100, phospholipids, and lipophilic vitamins functions to transport cholesterol and vitamins from liver to peripheral tissues. LDL receptors on cells are responsible to the binding and following endocytosis of the LDL where ApoB-100 and ApoE on the LDL act as ligands to the LDL receptors. However, once the LDL is oxidized, scavenger receptors, instead of LDL receptors, act to recognize the oxidized LDL (OxLDL) where ApoB-100 and ApoE are not ligands to the scavenger receptors. Scavenger receptors are typically expressing on the mononuclear phagocyte cells including macrophages, monocytes, and endothelial cells.

It is believed that OxLDL specifically expresses carboxyl groups on its surface and that scavenger receptors bind to the OxLDL via the carboxyl groups as ligands. Phosphatidylserine (PS), a characteristic phospholipid with a carboxyl group on its hydrophilic head group, is a known component of bilayer membrane and is asymmetrically localized in the intracellular membrane of living cells. However, PS is detected specifically on the surface of apoptotic cells that are detectable by the specific binding of annexin V to PS (Koopman et al., 1994; Martinet et al., 1995). Consequently, it can be assumed that the asymmetry of the bilayer membrane disappears on the apoptosis of cells and that PS is exposed on the cell surface by the flip-flop. In addition, previous reports describe that the PS is detected on the activated platelets (Thiagarajan & Tait, 1990), senescent erythrocytes (Schroit & Zwaal, 1991), and nuclei from erythroblasts (Yoshida et al., 2005). A specific receptor to PS has been identified on the phagocytes, enabling phagocytes to recognize the biomembrane exposing PS and to eliminate it (Fadok et al., 1992, 2000).

In selective elimination of lipid particles, oxidation of the unsaturated phospholipids plays a critical role. Natural phospholipids have polyunsaturated fatty acids such as linoleic acid and arachidonic acid. These polyunsaturated fatty acids are susceptible to free radical-initiated oxidation. The general process of lipid peroxidation consists of three stages:

initiation, propagation, and termination (Catala, 2006). The initiation phase of lipid peroxidation includes hydrogen atom abstraction by which radicals such as hydroxyl ( $\bullet\text{OH}$ ) react with polyunsaturated fatty acids to produce a lipid radical ( $\text{L}\bullet$ ), which in turn reacts with molecular oxygen to form a lipid peroxy radical ( $\text{LOO}\bullet$ ). The  $\text{LOO}\bullet$  can abstract hydrogen from an adjacent fatty acid to produce a lipid hydroperoxide ( $\text{LOOH}$ ) and a second lipid radical (Catala, 2006). The  $\text{LOOH}$  thus formed can suffer reductive cleavage by reduced metals such as  $\text{Fe}^{2+}$ , producing a lipid alkoxyl radical ( $\text{LO}\bullet$ ). Both alkoxyl and peroxy radicals stimulate the chain reaction of lipid peroxidation by abstracting additional hydrogen atoms (Buettner, 1993). Finally, the decomposition of the alkoxyl radical produces a terminal carboxyl group. Several kinds of oxidized phospholipids having the terminal carboxyl group have been identified. These oxidized phospholipids are known as ligands or strong agonists to the scavenger receptors, especially CD36 (Gao et al., 2010; Podrez et al., 2002). Further study has indicated that the carboxyl group of oxidized phospholipid is not located in the bilayer membrane but that it extends from the cellular surface like whiskers, in the “Whisker model”, to interact with the receptors (Greenberg et al., 2008; Hazen, 2008). This whisker model is expected to be important to design of surface of the drug delivery carrier, which is expected to interact with the receptors.



**Figure 1.** Bone marrow in blood cell turnover. Bone marrow can be regarded as a cell culture system in which nutrients for hematopoiesis can be supplied from circulating blood as senescent cells, apoptotic cells, lipoproteins, and so on. The blood cell turnover in an adult human is 20–200×10<sup>9</sup>/day which is estimated from the life time and total number of cells of each type. The image shows a hematoxylin and eosin stained rabbit bone marrow tissue section, where nuclei of hematopoietic cells are stained darkly and the circular blanks are adipocytes.

### 3. Engineered carriers for bone marrow targeting

Intravenous injected nano-sized materials are typically eliminated from blood circulation through a mononuclear phagocyte system (MPS). The main organs for uptake of the nano-



sized materials are the liver and spleen in general. Little attention has been given to the bone marrow though the bone marrow is a part of MPS because the contribution of the bone marrow for uptake of nano-sized materials is believed to be small compared with those of the liver and spleen. However, results of several studies have shown that bone marrow is the most important organ for the uptake of nanoparticles that have been modified with specific molecules on their surface as presented in Table 1.

Carrier type	Targeting	Target cells	Species	Ref
Liposome	Passive (Reduced size)	Macrophages	Dog	Schettini et al., 2006
Liposome	Active (Succinic acid-lipid) and passive (PEG-DSPE)	Macrophages	Monkey, Rabbit, Hamster	Sou et al., 2007; 2010; 2011a
Polystyrene microspheres	Active (Poloxamer 407)	Endothelial cells	Rabbit	Porter et al., 1992; Moghimi, 1995
Polymer complex	Active (Cationic peptide)	Monocytes, T-cell lineage cells	Mouse	Harris et al., 2010
Porous silicon particles	Active (E-selectin thioaptamer ligand)	Endothelium	Mouse	Mann et al., 2011
Nanoparticles of dendritic molecule	Active (Guanidinium group)	Osteoclast precursors	in vitro	Chi et al., 2010
Branched polypeptide	Active (Succinyl group)	Macrophages	in vitro	Szabó et al., 2005

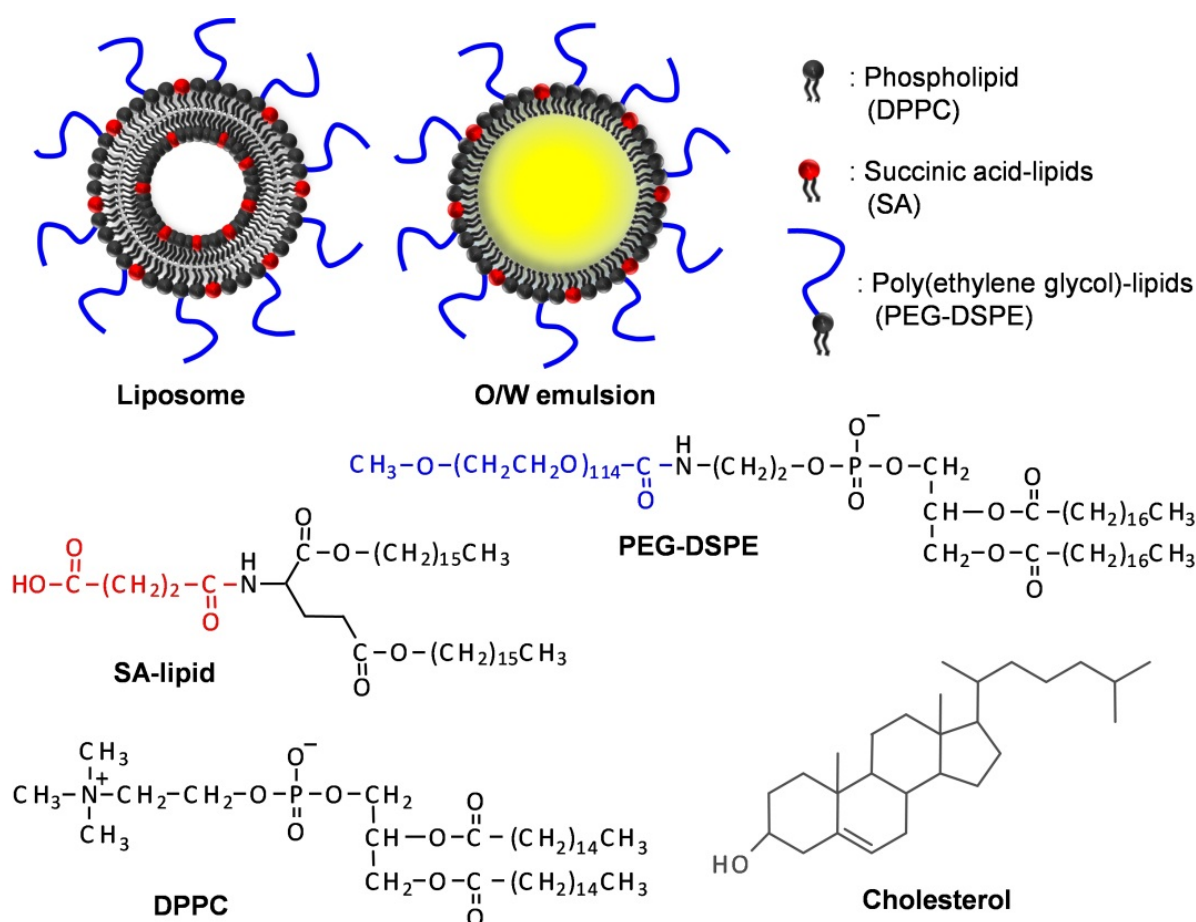
**Table 1.** Proposed drug delivery carriers targeting bone marrow. These carriers based on surface-modified nanoparticles or polymers target bone marrow phagocytes such as macrophages and endothelial cells.

### 3.1. Liposomes

Liposomes are the most studied nanoparticles for drug and gene delivery to date. Great interest has been devoted to interaction between liposomes and cellular components *in vitro* and *in vivo*. Allen et al. studied the uptake of liposomes by cultured mouse bone marrow macrophages as a function of liposome composition (Allen et al., 1991). In this study, surface modification with monosialoganglioside (GM1) and polyethylene glycol-lipid (PEG-PE) greatly decreased DPPC liposome uptake by bone marrow macrophages in a concentration-dependent manner. However, incorporation of PS increased liposome uptake by macrophages substantially in a concentration-dependent manner. These observations were correlated with the *in vivo* behavior of liposomes. Consequently, results of this study showed that liposome uptake by bone marrow macrophages is sensitive to the liposome composition, and that PS, which is a biological marker of apoptotic cells, accelerates the

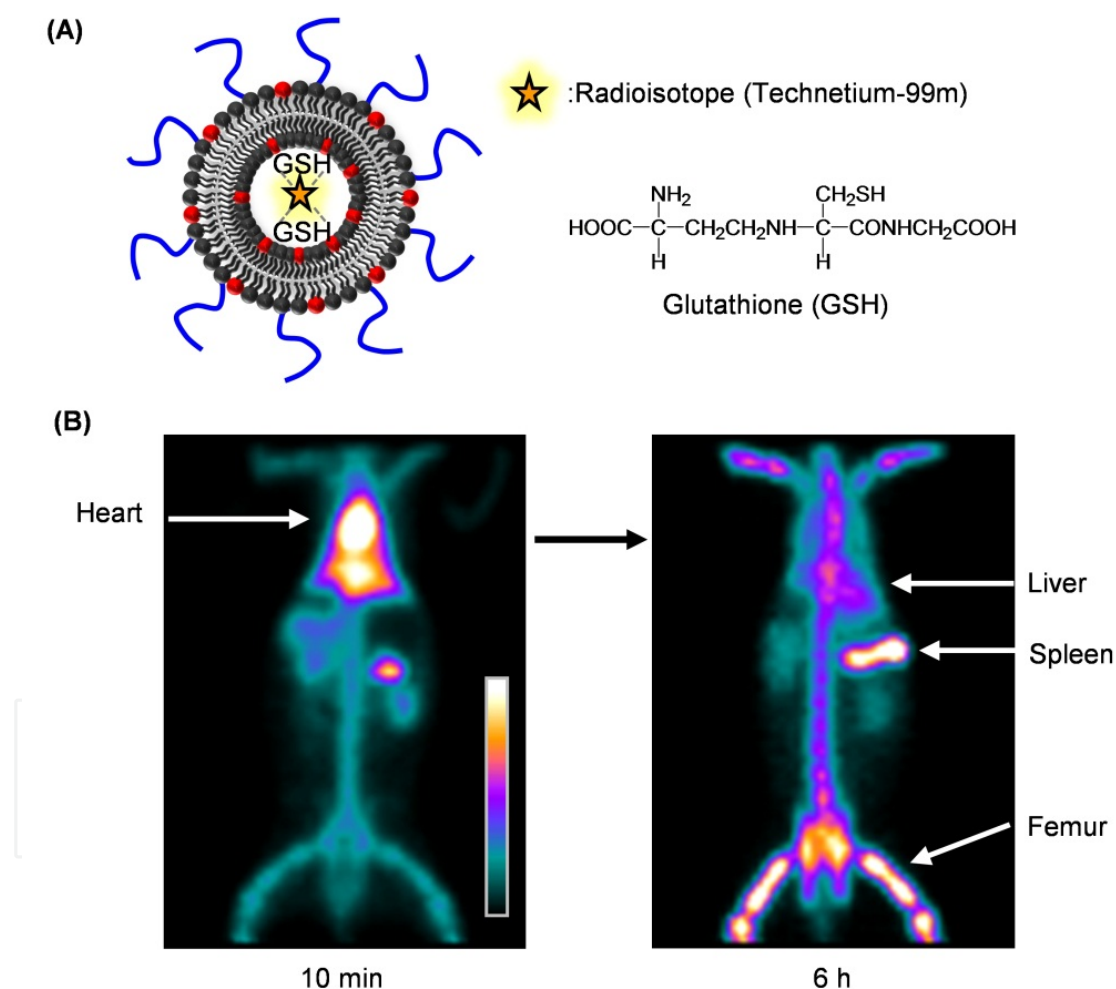
uptake of the liposomes. Several other studies have also shown the accelerated uptake of liposomes containing PS or oxidized PS by macrophages, suggesting a similar molecular mechanism on apoptotic cell clearance (Fadok et al., 1992; Greenberg et al., 2006; Ishimoto et al., 2000).

Schettini and co-workers prepared a novel liposomal formulation of meglumine antimoniate, a drug used for treating leishmaniasis, to deliver the drug to the bone marrow (Schettini et al., 2006). The liposomes were produced from distearoylphosphatidylcholine (DSPC), cholesterol, and dicetylphosphate (molar ratio of 5:4:1). The targeting of antimony to the bone marrow was improved approximately three-fold with the small liposomal formulation compared to the large liposome formulation used in dogs with visceral leishmaniasis. These liposomes had no active targeting factor to bone marrow, but the passive targeting of the liposomes to the bone marrow of dogs was improved by the reduction of vesicle size from 1200 nm to 400 nm.



**Figure 2.** Lipid components of bone marrow-targeted lipid-based nanoparticles. Liposomes composed by DPPC, cholesterol and SA-lipid have high encapsulation capacity for water-soluble materials (Sou 2011b). Oil-in-water (O/W) emulsions have advantage in embedding lipophilic drugs in their oil core. The uptake of lipid-based nanoparticles by bone marrow phagocytes in rhesus monkeys, rabbits, and hamsters is induced by the incorporation of SA-lipid. PEG-DSPE enhances the uptake of the nanoparticles by bone marrow phagocytes passively by preventing the uptake by hepatic and splenic phagocytes (Sou et al. 2007; 2010; 2011a).

Sou et al. found a liposome formulation that is specifically distributed to bone marrow in rabbits and rhesus monkeys (Sou et al., 2007, 2010, 2011a). Figure 2 shows that the bone marrow-targeted liposomes comprise lipids of four kinds: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), cholesterol, L-glutamic acid, *N*-(3-carboxy-1-oxopropyl)-, 1,5-dihexadecyl ester (SA-lipid), and poly(ethylene glycol) (PEG). The SA-lipid component has been identified as the active factor leading to their phagocytosis by bone marrow phagocytes, presumably macrophages in rabbits (Sou et al., 2007). Furthermore, as little as 0.6 mol% of PEG-DSPE depressed hepatic uptake but did not depress the bone marrow uptake. PEG-DSPE can be incorporated into the outer surface of preformed liposomes using the post incorporation method (Sou et al., 2000; Uster et al., 1996). Otherwise PEG-DSPE is mixed with other lipid components before preparation of liposomes. The active targeting factor of SA-lipid and passive targeting factor of PEG-DSPE appear to increase the distribution of the liposomes to bone marrow cooperatively.



**Figure 3.** Imaging analysis of animal receiving technetium-99m ( $^{99\text{m}}\text{Tc}$ )-labeled bone marrow-targeted liposomes. (A) Preformed liposomes encapsulated glutathione (GSH) can be radiolabeled with  $^{99\text{m}}\text{Tc}$  by a remote loading method (Phillips et al., 1992). (B) Gamma camera images of rabbits acquired at 10 minutes and 6 hours after injection of  $^{99\text{m}}\text{Tc}$ -labeled bone marrow-targeted liposomes (Sou et al., 2007).

The size of the liposomes between 200–270 nm is not a significant factor for uptake by bone marrow. The liposomes were designed to have high entrapment capacity with the interfacial

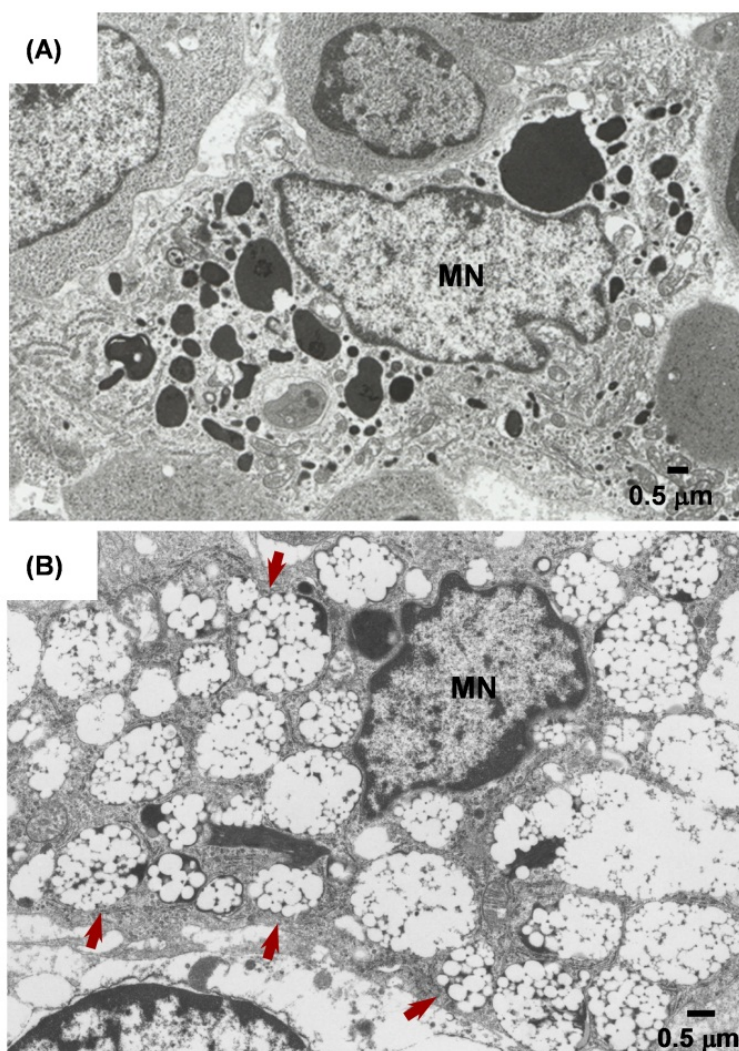


electrostatic interaction to form a unilamellar membrane (Sato et al., 2009; Sou et al., 2003; Sou 2011b). These characteristics are expected to facilitate the application of the bone marrow-targeted liposomes as pharmaceutical carriers to bone marrow.

To evaluate the bone marrow-targeting capability quantitatively, whole body scintigraphic imaging in living animals is a particularly powerful tool (Goins and Phillips 2001, Phillips et al., 2009, 2011). Technetium-99m ( $^{99m}\text{Tc}$ ), which is a good tracer for imaging, is used widely for single photon emission computed tomography with a gamma camera. Figure 3A shows that the  $^{99m}\text{Tc}$  labeling of the liposomes encapsulating glutathione (GSH) can be accomplished using a complex of the  $^{99m}\text{Tc}$  and GSH in inner aqueous phase of the liposomes (Phillips et al., 1992). Stoichiometric analysis has shown a 2:1 molar ratio of GSH and  $^{99m}\text{Tc}$  for stable complex formation (Baba et al., 1999). Images presented in Figure 3B show the distribution of  $^{99m}\text{Tc}$ -labeled bone marrow-targeted liposomes in a rabbit at 10 min and 6 hr after intravenous injection (lipid dose: 15 mg/kg b.w.). The image at 10 min is a typical blood pool image representing large blood pool at heart and liver, where the liposomes exist in blood circulation. At 6 hr, the radioactivity at the bone including marrow is increased, although it is decreased at the heart and liver. At this point,  $69.7 \pm 0.3\%$  ID of bone marrow-targeted liposomes accumulated in bone marrow. At the same time point, the liver and spleen respectively contained much smaller amounts of  $11.5 \pm 2.88$  and  $5.0 \pm 1.19\%$  ID (Sou et al., 2007).

In addition to the macroscopic and quantitative observation of the distribution of the liposomes by scintigraphic imaging, histological observation by microscopic techniques enables further microscopic localization of the liposomes in bone marrow tissues. Transmission electron microscopy (TEM) is the frequent method used to observe the nanoparticle sample to determine their size and size distribution. Observations of liposomes in cells and tissues can be made using TEM (Sakai et al., 2001). Figure 4 shows that microscopic localization studies demonstrate that bone marrow macrophages are the cellular components responsible for clearance of bone marrow-targeted liposomes from circulation and that they are also responsible for their uptake by the bone marrow. The liposomes are located in the phagosomes and lysosomes of bone marrow macrophages, indicating that the bone marrow macrophages capture the liposomes through phagocytosis. Therefore, it can be speculated that bone marrow-targeted liposomes interact with a receptor on the bone marrow macrophages, which stimulates the phagocytic activity of bone marrow macrophages specifically.

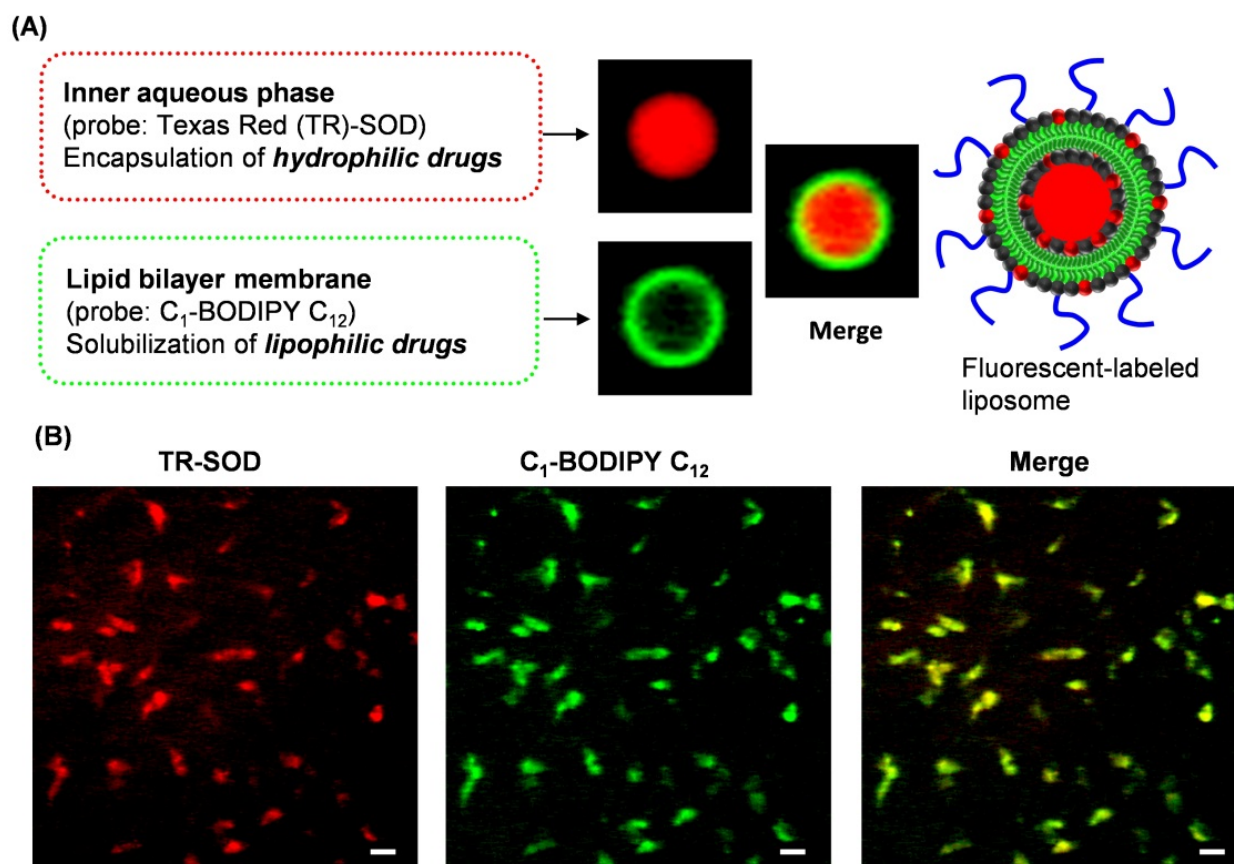
Also flow cytometry analysis is a useful method to determine the targeted cell population quantitatively (Harris et al., 2010). In this method, bone marrow cells will be labeled with markers to identify specific cell subtypes. Flow cytometry analysis might be particularly useful for animal experiment in mice and rats because the markers to identify specific cell subtypes are extensive for these species. Other possible method to identify the cell type is the immunofluorescent staining. The cells type emitting fluorescence from nanoparticle carriers or cargos in tissue section could be determined from the comparative analysis with images of immunofluorescent staining (Longmuir et al., 2009).



**Figure 4.** Transmission electron microscopic observations of bone marrow-targeted liposomes. (A) Bone marrow macrophages in a femoral bone marrow tissue section, taken from rabbit. Many phagosomes and lysosomes (electron-dense vacuoles) are visible. (B) Bone marrow macrophages in a femoral bone marrow tissue section, taken from a rabbit at 6 hr after intravenous injection of bone marrow-targeted liposomes (lipids: 15 mg/kg b.w.). Arrows indicate phagosome maturation trapping liposomes with the original diameter (average 270 nm) (Sou et al., 2007). MN: macrophage nucleus

Fluorescent techniques enable detection of the distribution of particular components labeled with fluorescent probes in live cells and tissues microscopically, with exquisite sensitivity and selectivity. For liposomes, both the lipid bilayer membrane (green fluorescent C1-BODIPY C12) and inner aqueous phase (red fluorescent Texas Red- superoxide dismutase, TR-SOD) were labeled with fluorescence probes as shown in Figure 5A. At 6 hr after injection of the dual-labeled bone marrow-targeted liposomes in rabbits (lipids: 15 mg/kg b.w.), the femoral bone marrow section was observed using confocal scanning microscopy. Figure 5B shows that the bone marrow sections have fluorescence from both the TR-SOD and C1-BODIPY C12, where the fluorescence from membrane probes and encapsulated probes are co-localized in bone marrow. This observation revealed that the bone marrow-targeted liposomes and encapsulated agents are distributed at the same locations into bone

marrow tissues, clearly indicating that the encapsulated agents were delivered to bone marrow tissues by the liposomes.



**Figure 5.** Histological examination of fluorescence delivered into bone marrow tissues using bone marrow-targeted liposomes as carriers. (A) Fluorescence localization in double fluorescence-labeled large multilamellar bone marrow-targeted liposomes with ca. 10  $\mu\text{m}$  diameter. (B) Confocal scanning images of femoral bone marrow taken from a rabbit at 6 hr after i.v. injection of double fluorescence-labeled bone marrow-targeted liposomes (lipids: 15 mg/kg b.w.) (Sou et al., 2007). Scale bars show 20  $\mu\text{m}$ .

### 3.2. Polymeric nanoparticles

Regarding engineered colloidal particles, Porter and co-workers observed remarkable accumulation of small colloidal particulates (150 nm and smaller diameter) that were coated by the block co-polymer poloxamer-407, a non-ionic surfactant, in the bone marrow after intravenous administration in rabbits (Porter et al., 1992). In this case, the coated colloids were sequestered by the sinusoidal endothelial cells of the bone marrow instead of macrophages. Importantly, no marked uptake was achievable with other block co-polymers having a similar structure to that of poloxamer-407, suggesting the participation of a specific interaction mechanism between the particle and the sinusoidal endothelial cell surface.

Chi and co-workers prepared dendritic amine and guanidinium group-modified nanoparticles for the delivery of model peptide drug into primary osteoclast precursor cells



(bone marrow macrophages) (Chi et al., 2010). It can be speculated that positively charged guanidinium groups have favorable interactions with negatively charged functional groups in the cell membrane of osteoclast precursors (Rothbard et al., 2005). Physicochemical interaction between a positively charged drug carrier and a negatively charged cell surface can enhance the cellular uptake in cells of various kinds with a negatively charged surface. However, the selectivity to specific bone marrow cells might be low in practical applications for drug delivery carriers.

Harris and co-workers studied tissue-specific gene delivery via nanoparticle coating (Harris et al., 2010). They prepared cationic nanoparticles with plasmid DNA and then coated their cationic surface with poly anionic poly(glutamic acid)-based peptides with and without cationic insert. Particles coated with a low 2.5:1 peptide:DNA weight ratio (w/w) form two large micro-sized particles that can facilitate specific gene delivery to the liver in mice. However, the same particles coated at a higher 20:1 peptide with cationic insert:DNA (w/w) form small 200 nm particles that can facilitate specific gene delivery to the spleen and bone marrow. They have confirmed that the terminal sequence insert, cationic amino acid sequence G-dP-dL-G-dV-dR-G, to the poly(glutamic acid)-based peptides is a critical factor enhancing bone marrow and spleen-specificity of gene delivery *in vivo*. Regions of luminescence selected around the femur bones showed nearly 40-fold enhancement, and regions around the spleen showed nearly 30-fold enhancement by the cationic insert. Flow cytometry analysis of bone marrow cells from a mouse tail-vein injected with green fluorescent protein-encoding nanoparticles coated with 20:1 w/w peptide with a cationic insert revealed that green fluorescent protein expression relative to the whole population of bone marrow cells is enriched in monocyte and T-cell lineage cells. This system might be available for bone marrow-specific drug delivery and for gene delivery. The molecular mechanism at work in this system is not obvious.

E-selectin is an attractive molecular target for active targeting of a drug carrier to bone marrow because E-selectin is expressed selectively on endothelial cells of adult and fetal hematopoietic organs (Schweitzer et al., 1996). It has been suggested that the E-selectin plays a role in the homing of hematopoietic progenitor cells and that its constitutive expression on endothelial cells of hematopoietic organs is necessary in the initial step of the homing process. Mann and co-workers identified a thiophosphate-modified aptamer (thioaptamer) against E-selectin (Mann et al., 2010). They confirmed that the thioaptamer ligand selectively binds to E-selectin with nanomolar binding affinity ( $K_D=47$  nM) while exhibiting minimal cross reactivity to P-selectin and L-selectin. Recently, they developed porous silicon particles modified with E-selectin thioaptamer ligands to target bone marrow endothelium (Mann et al., 2011). A mice study demonstrated that the accumulation of the porous silicon particles modified with E-selectin thioaptamer ligands in the bone marrow was eight times higher than control porous silicon particles, which were accumulated primarily in the liver and spleen instead of bone marrow. Histological analysis supported the presence of porous silicon particles modified with E-selectin thioaptamer ligands on the endothelial wall of the bone marrow tissue. The molecular target of this ligand-receptor reaction might be readily apparent in this system and be promising for delivering drugs to bone marrow endothelial cells specifically.

Moghimi reviewed the clearance mechanism of particulate materials from the circulation by bone marrow (Moghimi, 1995). The endothelium of bone marrow sinusoids can remove particles from circulation by both transcellular and intercellular routes. The intercellular route occurs through the fenestrate in the endothelial wall. Therefore this mechanism is strongly dependent on the particle size. The intercellular distance is less than 2 nm for the tight junctions in capillaries and less than 6 nm for post-capillary venules under normal conditions (Simionescu et al., 1978; Bundgaard, 1980). In contrast, the size of the fenestrate in the endothelial wall is reportedly 85–150 nm (Huang, 1971). These fenestrated capillaries exist in bone marrow as well as in the liver and spleen. Therefore, the intercellular route is a possible pathway to target bone marrow through circulation. Liposomes consisting of DSPC, cholesterol, PEG(5000)-DSPE, and  $\alpha$ -tocopherol prepared in various sizes (136–318 nm diameter) have been tested for organ distribution in rabbits. None of these liposomes show a significant accumulation in bone marrow (Awasthi et al., 2003). Therefore, the passive diffusion of nanoparticles from blood circulation to extravascular space through an intercellular route is not an important factor in bone marrow targeting when no active targeting factor exists. Indeed, based on the proposed drug delivery carrier described above, the surface modification of nanoparticles with specific molecules is a critical factor to achieve bone marrow targeting with nanoparticles, although further study is necessary to ascertain details of its molecular mechanism. Consequently, discovery of receptors specifically expressing in bone marrow tissues facilitates the development of drug carriers targeting bone marrow.

#### **4. Future prospects for bone-marrow-targeted drug carrier systems**

The balance between blood cell production and removal in blood cells of each type is important to maintain the number of circulating blood cells. When blood cell production is suppressed, erythrocytopenia, leukopenia, and thrombocytopenia are induced. The blood cell production suppression results from the direct bone marrow dysfunction or indirect factor such as deficiency of the hematopoietic growth factors such as erythropoietin (EPO), granulocyte colony-stimulation factor (G-CSF), and thrombopoietin (TPO). These hematopoietic growth factors, EPO and G-CSF, have been established as recombinant products. Recombinant EPO products are used clinically for the renal anemia patients or autologous blood. These growth factors function with hematopoietic cells in bone marrow, so that a drug delivery system to target bone marrow can be expected to offer an improved therapeutic system.

Recently, Winkler and co-workers reported that the bone marrow macrophages are pivotal to maintain an endosteal hematopoietic stem cell niche and that the loss of such macrophages engenders the egress of hematopoietic stem cells into the blood (Winkler et al., 2010). They administered clodronate-loaded liposomes intravenously to deplete the bone marrow macrophages. After the macrophage depletion, hematopoietic stem cells were found in the blood. These findings provide evidence supporting the critical role that macrophages play in the support of hematopoietic cells in bone marrow. Such specific biology of bone marrow macrophages can present a therapeutic target for the treatment of hematopoietic disorders.



Leishmaniasis is an infectious disease caused by a protozoan parasite that is parasitic on resident macrophages. Promastigotes, which are injected into the skin by a sandfly, are phagocytized by macrophages. Thereafter, promastigotes transform into amastigotes inside macrophages. The amastigotes multiply in cells, including in macrophages, at various tissues. The typical symptoms in leishmaniasis are damage to the spleen and liver, and anemia by damage to bone marrow. Therefore, macrophages in bone marrow as well as in the liver and spleen are target cells in leishmaniasis treatment. Thus several nanoparticles loaded with therapeutic agents such as nanoparticles loaded with amphotericin B (Gupta & Vyas 2007; Nahar et al., 2010), PLGA nanoparticles loaded with saponin (Van de Ven et al., 2012), lipid nanoparticles loaded with oryzalin (Lopes et al., 2012), PLGA nanoparticles loaded with kinetoplastid membrane protein-11 (Santos et al., 2012) have been developed to deliver the drugs in leishmania-infected macrophages. Drug carriers targeting bone marrow, especially bone marrow macrophages, would have a great potential to deliver these therapeutic agents in leishmania-infected macrophages.

The abnormal increase of cancerous cells such as leukemia cells, which are immature white blood cells in bone marrow, suppress normal hematopoiesis. Different from a solid cancer, surgical resection is ineffective as a treatment for leukemia. Most cases of leukemia are treated using chemotherapy. Therapies are typically combined into multi-drug chemotherapy. Drug delivery systems targeting bone marrow can increase the efficacy of such methods. Moreover, bone marrow is sensitive to chemotherapy and radiation therapy. Several pharmaceuticals are available to protect soft tissues from chemotherapy and irradiation. Drug carriers targeting bone marrow offer a promising platform to deliver such pharmaceuticals to bone marrow effectively.

## 5. Conclusion

Conventional drug delivery to bone marrow is uncontrolled passive diffusion of drugs into bone marrow through blood circulation. The fraction of drugs reaching the target site through such passive diffusion is negligibly small. Bone marrow-targeted drug delivery carriers provide a platform to develop more efficient and safer diagnostic and therapeutic systems. These therapeutic systems can target bone and bone marrow diseases such as rheumatoid arthritis, bone regeneration and repair, bone metastases, osteoporosis, infectious diseases, multiple myeloma, and hematopoietic dysfunction.

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