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Diamagnetic chemical exchange saturation transfer (diaCEST) liposomes: physicochemical properties and imaging applications

Kannie W.Y. Chan^{1,2}, Jeff W.M. Bulte^{1,2,3,4,5,6}, and Michael T. McMahon^{1,2,*}

¹The Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

²F. M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, USA

³Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁴Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁵Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

⁶Department of Chemical & Biomolecular Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Chemical exchange saturation transfer (CEST) is a new type of magnetic resonance imaging (MRI) contrast based on labile spins which rapidly exchange with solvent, resulting in an amplification of signal which allows detection of solute protons at millimolar to micromolar concentrations. An additional feature of these agents is that natural organic and biodegradable compounds can provide strong CEST contrast, allowing the development of diamagnetic CEST (diaCEST) MRI contrast agents. The sensitivity of the CEST approach per unit of agent increases further when diaCEST contrast agents are loaded into liposomes to become diaCEST liposomes. In this review, we will discuss the unique and favorable features of diaCEST liposomes which are well suited for *in vivo* imaging. diaCEST liposomes are nanocarriers which feature high concentrations of encapsulated contrast material, controlled release of payload, and an adjustable coating for passive or active tumor targeting. These liposomes have water permeable bilayers and both the interior and exterior can be fine-tuned for many biomedical applications. Furthermore, a number of liposome formulations are used in the clinic including DoxilTM, which is an approved product for treating patients with cancer for decades, rapid translation of these materials can be envisaged. diaCEST liposomes have shown promise in imaging of cancer, and monitoring of chemotherapy and cell transplants. The unique features of diaCEST liposomes are discussed to provide an overview of the applications currently envisioned for this new technology and to provide an overall insight of their potential.

INTRODUCTION

Chemical exchange saturation transfer (CEST) is an emerging magnetic resonance imaging (MRI) contrast mechanism that allows detection of low concentration exchangeable protons indirectly by acquiring the water signal after saturation pulses are applied^{1–3} (Figure 1(a)). Forsen et al. first demonstrated⁵ how to monitor the transfer of saturation from labile protons (Figure 1(b)–(d)). In 2000, Balaban and colleagues showed how saturation transfer can be used to produce MRI contrast, and introduced the term ‘CEST contrast agents’.⁶ The field of CEST imaging has grown rapidly after this pioneering paper. Saturation transfer allows selective detection of molecules with labile protons such as hydroxyls (OH), amines (NH₂), and amides (NH), and ions such as diethylphosphate, Ca(II) and Zn(II).^{2,7,8} The process of imaging these specific pools of exchangeable protons is a useful tool for molecular imaging and has several advantages. First, because proton exchange occurs many times during the saturation pulse, the signal from a small pool of solute protons (μM – mM) is amplified and transferred onto the much larger water signal (110 M for pure water), which improves the detection sensitivity dramatically. Second, the use of frequency selective saturation pulses to irradiate solute protons allows the contrast to be ‘switched on and off at will’, and enables identification of these protons through their chemical shift with respect to water (e.g. Solute A vs Solute B in Figure 1(e)). As a result of these features, different exchangeable protons can be detected simultaneously but also separately identified, e.g. OH versus NH. There are three main types of CEST contrast agents: paramagnetic agents (paraCEST),^{9,10} diamagnetic agents (diaCEST),^{6,11} and hyperpolarized agents (hyperCEST).¹² ParaCEST agents are mainly lanthanide complexes with protons exchanging slow enough for detection, as first shown by Sherry and Aime et al., although complexes which include other metals such as iron are also possible.¹³ This contrast is based on proton exchange of water bound to the metal center and/or exchangeable protons in the vicinity of the metal center with bulk water, with the metal perturbing the offset frequencies of these protons. diaCEST agents are naturally occurring molecules without metal ions, with the contrast dependent on the number and type of labile protons. HyperCEST agents are slightly different, which are cages such as cryptophane designed to trap dissolved hyperpolarized material. Frequency differences are induced in the spins of the hyperpolarized material which naturally passes in and out of the cage structure. During this process, the signal is transferred from the interior of the cage to the exterior. HyperCEST imaging requires the use of a polarizer, and to date, has only been applied using xenon as the agent. CEST contrast depends on the chemical exchange rate, the type of exchangeable spins, their resonance frequency relative to the water concentration, and the relaxation times of the spins, in addition to pH, temperature¹⁴ and magnetic field strength. As a result, CEST contrast is often exquisitely sensitive to changes in the surrounding micro-environment of a CEST probe.

Many natural metabolites and macromolecules have exchangeable protons, allowing their use as biocompatible and biodegradable CEST contrast agents. For example, many of the OH protons of sugars resonate at a frequency downfield from water at around 1 ppm, the NH₂ protons of creatine and larginine resonate at around 2 ppm, and the amide protons of peptides resonate at around 3.5 ppm (Figure 1(e)). This unique feature of CEST contrast has fostered the development of a wide range of applications in biomedical imaging, where molecular imaging can play a pivotal role in diagnosis of diseases, monitoring of treatments, and evaluating therapeutic outcomes. In particular, CEST contrast maps of endogenous amide protons which reflect the relative protein concentrations of tissues allow the grading of tumors¹⁵ and monitoring of radiation therapy.¹⁶ clinical MR scanners have now been optimized for CEST imaging, increasing the prospects of translation of new CEST imaging technologies to the clinic.¹⁷

Nanomedicine has attracted attention for its capability of improving current diagnostics and therapeutics with high payload and sensitivity per nanocarrier.^{18,19} Properly designed CEST nanocarriers can result in significant improvements in both therapeutic efficacy and imaging sensitivity, with the large pool of exchangeable protons associated with these carriers allowing detection of the nanocarriers at nanomolar concentrations.^{20,21} Liposomes appear to have unique features to serve these purposes.^{22,23} With all of the developments in CEST imaging and nanotechnology, CEST liposomes have great potential for imaging of molecular and cellular events *in vivo* by making use of the excellent spatial resolution of MRI. In the following, we will discuss the properties of liposomes, how to incorporate CEST contrast agents into these carriers, and how the sensitivity of the resulting probes can be optimized for *in vivo* imaging.

PROPERTIES OF LIPOSOMES

Structure

Liposomes are formed when lipids spontaneously self-assemble in water so that the hydrophobic portions of the lipids cluster together and form a phospholipid bilayer enclosed spherical structure. The first description of liposomes was published in 1965,²⁴ and within a decade these structures were refined for use as drug carriers.²⁵ Figure 2(a) shows the detailed structure of a typical liposome composed of phospholipids stabilized by the presence of cholesterol and an aqueous interior cavity. Both the interior cavity and the surface can carry drugs and/or contrast agents (Figure 2(a)). Early generations of liposomes were multilamellar (~500 nm), until methods were developed to prepare unilamellar liposomes (~100–200 nm), with a greater loading efficiency and a more homogeneous size distribution.^{28,29} The composition of the phospholipid bilayer determines the permeability and hence affects the retention of drugs or contrast agents. Clinically approved liposome formulations employ the following types of lipids with different head groups and chain lengths: DSPC, HSPC, EPC, DOPC, DMPC, DOPE, EPG, DPPG, and DMPG.³⁰ The type of lipids and their ratio with cholesterol determines the fluidity or structural order of the bilayers of the liposomes,³¹ e.g. the melting temperature, T_m , of DSPC (T_m 55 °C) is higher than that of EPC (T_m = 45 °C), and the inclusion of cholesterol is known to reduce the diffusion rate of phospholipids in bilayers.³² Proper formulation of liposomes can minimize unwanted leakage of content, resulting in a controlled release.

In addition to liposomes formulated for slow release, formulations have been developed for a rapid release in response to an external trigger. For example, ThermodoxTM has a phospholipid bilayer that is sensitive to hyperthermia allowing thermally triggered release. DPPC is used in ThermodoxTM because the T_m (41.5 °C) is within an acceptable range for treating patients with local hyperthermia. Moreover, liposome formulations have been developed for other types of triggers, including heat, ultrasound, light, pH, and enzymes.³³ These triggers can be classified as either local (e.g. pH, enzyme), which release based on factors intrinsic to the disease site; or remote (e.g. light, heat, ultrasound), which employ an apparatus to initiate liposomal release. These strategies have been used in drug delivery to minimize toxicity to normal tissue.

Surface Modification and Targeted Delivery

The surface of liposomes plays a critical role in determining their circulation time, with long circulation times necessary for both drug and contrast agent applications. The classical strategy to prolong circulation is to decorate the surface with polyethylene glycol (PEG), creating so-called 'stealth' liposomes^{34,35} (Figure 2(a)). Insertion of this hydrophilic component allows liposomes to circulate for days, while nonpegylated liposomes are cleared within hours. Several clinical formulations employ this strategy,³⁶ including DoxilTM,³⁷

which was the first clinically approved stealth liposomal drug for treatment of cancer. Doxil™ is a liposome composed of HSPC, cholesterol and PEG 2000-DSPE (56:39:5 molar ratio), and is loaded with the chemotherapeutic doxorubicin. It can remain in the circulation for up to 55 h. Stealth liposomes have also been employed as MRI contrast agents.^{38,39} Enhanced permeability and retention (EPR) is the main mechanism enabling the accumulation of nanocarriers in tumors, with this mechanism based on the abnormal and leaky tumor blood vessels^{40–42} and impaired lymphatic drainage. Size is also an important parameter for passive targeting of tumors.^{19,43} Liposomes of ~100 nm are favorable for tumor targeting via EPR, although larger nanocarriers can also accumulate in tumors.

An alternative targeting strategy developed for liposomes involves the attachments of antibodies or peptides to either the surface of liposomes or the terminus of PEG molecules.^{44,45} These actively targeted liposomes can accumulate in tumors via binding of targeting groups to receptors on either cancer cells or endothelial cells.¹⁸ Liposomes have also been designed for targeting atherosclerotic plaques, with results dependent on the size of these active targeting liposomes.⁴³ Studies have shown that active targeting is more efficient than passive targeting only if internalization by macrophages or other cells is avoided and the binding-site barrier is overcome.⁴⁶ In many cases active targeting may not necessarily result in better selectivity than passive targeting. In fact, only a few targeted nanoparticles are in clinical trials.⁴⁷ From an MRI perspective, image contrast can be reduced upon internalization depending on the type of contrast agent and on which cellular compartment is occupied by the agent.⁴⁸ CEST and T1 contrast agents will produce less contrast if they reside in endosomes,⁴⁹ while for T2 contrast agents this compartment does not pose a challenge.⁴⁸

Preparing Drug or Contrast Agent-Loaded Liposomes

As mentioned above, the composition of the phospholipid bilayers affects their permeability and retention of liposomal content. The amount of a given molecule encapsulated and retained also depends on its hydrophilicity (Figure 2(a)). Phospholipid bilayers are more permeable to hydrophobic molecules,⁵⁰ which results in a challenge for retaining hydrophobic molecules. There are two major methods for loading liposomes: active loading, which refers to the encapsulation of molecules during the formation of vesicles, and remote loading, which refers to the encapsulation of molecules after formation of vesicles. Actively loaded liposomes can be prepared through the hydration of lipid films at temperatures above their melting temperature, and using either sonication or extrusion to form small unilamellar vesicles.⁵¹ The advantage of remote loading is that the drugs or contrast agents can be loaded at a different location and time from the liposome formation. One effective remote loading method involves the creation of transmembrane pH gradients. Doxorubicin is efficiently loaded into liposomes using this method,³⁷ because it readily precipitates after reaching a high concentration inside the liposomes. Remote loading is also useful for integrating radioactive metals into liposomes through the presence of strong chelates.⁵²

The requirements for liposomes formulated as therapeutic nanocarriers or diagnostic agents are not exactly the same. It is desirable for both types of liposomes to have a high payload and release a minimal amount of the content when the nanocarriers are in circulation. For therapeutic liposomes, encapsulated drugs are regarded as inactive and should only be released at the target sites to be effective. This results in a reduction in toxicity towards normal tissues after systemic administration, however, this release is not desirable for diagnostic agents. For diagnostic liposomes, the liposomal MRI contrast agents, maximal retention of contrast material is desired after reaching the target site. In the following section, we describe the features of liposomal MRI contrast agents.

LIPOSOMAL MRI CONTRAST AGENTS

Classification of MRI Contrast Agents

Several classes of liposomal MRI contrast agents have been developed, including T1, T2, and CEST agents. The contrast produced by liposomal MRI agents depends on the type of contrast agents, the environment of these liposomes, the bilayer permeability,²⁷ and the size of liposomes.²⁶ T1 contrast is generated by paramagnetic centers that shorten the longitudinal relaxation times of water protons.^{39,50,53,54} A number of clinically approved Gd-complexes have been developed for contrast-enhancement of various pathologies. They have also been used to prepare paramagnetic liposomes that can generate T1 contrast.⁵⁵ The preparation and utility of liposomal T1 agents has been reviewed recently.⁵⁶ Briefly there are two main designs for liposomal T1 agents: insertion of hydrophilic Gd chelates inside the lipid bilayer and introducing lipids with Gd complexes attached to their headgroups. T2 contrast is generated through a reduction in transverse relaxation times of water protons,^{57,58} with iron oxide particles of various sizes commonly used as T2 imaging agents. Liposomal T2 agents have been prepared as early as 1988,^{59,60} using two designs: small liposomes containing iron oxide(s) only or larger vesicles containing a mixture of water and iron oxides. These agents have been reviewed recently.⁶¹ CEST contrast depends on proton exchange between sites with different chemical shifts. For diaCEST liposomes, saturation is transferred first through chemical exchange between labile protons on the CEST agents and intra-liposomal water, with exchange between intra-liposomal and extra-liposomal water also affecting the contrast (Figure 2(b) upper panel). For paraCEST liposomes, intra-liposomal water is shifted allowing selective saturation of this water, so that saturation transfer is accomplished exclusively through the exchange between intra-liposomal and the extra-liposomal water⁶² (Figure 2(b) lower panel). Alternatively, paraCEST agents (instead of paramagnetic shift agents), with protons exchanging in the slow to intermediate regime on the NMR time scale, can be loaded into liposomes to create CEST contrast through saturation transfer of protons bound to the agent which first transfer to the intra-liposomal water and then to the extra-liposomal water. Small liposomes possessing large surface-to-volume ratios and water permeability can produce more contrast.²⁶ As shown in Figure 2(b), the saturation transfer process can be quite different between diaCEST liposomes and the paraCEST liposomes developed by Aime, Terreno, Castelli, and others. Many groups have developed lanthanide complexes as paraCEST agents for sensing changes in pH and other physiological parameters.^{63–67} Comprehensive reviews have been written describing paraCEST agents, with one recent review covering paraCEST nanocarriers recently.²¹ In the following sections we will focus on diaCEST liposomes.

Sensitivity of CEST

diaCEST liposomes possess a large pool of intra-liposomal exchangeable protons, which results in a higher MRI sensitivity per liposome than that of free agents.²² The concentration of intra-liposomal contrast agents, the size of liposomes, and the permeability of the bilayers^{27,26} all influence the contrast. Other factors that determine the sensitivity of small molecule CEST agents also apply for diaCEST liposomes, such as the relationship between the exchange rate and frequency difference between the exchangeable protons and water protons ($\Delta\omega$), the relaxation times, temperature, and pH.^{1,2,68} CEST contrast is typically determined indirectly through the magnetization transfer ratio (MTR; $MTR = 1 - (S_{sat} - S_0)$), and calculated using asymmetry analysis, i.e. $MTR_{asym}(\Delta\omega) = [S_{sat}(-\Delta\omega) - S_{sat}(+\Delta\omega)]/S_0$, where $S_{sat}(-\Delta\omega)$ is the signal in the presence of saturation at a frequency of $-\Delta\omega$ and S_0 is the signal in the absence of saturation (Figure 1(d)). Analytical expressions can be derived based on the Bloch Equations, which relate the proton transfer ratio (PTR or CEST contrast) to the parameters influencing MTR_{asym} .⁶⁹ According to the two-pool model, and in the absence of back exchange and spillover effects (or direct saturation), $PTR = X_S \cdot$

$\alpha \cdot k_{SW} \cdot T_{1W} (1 - e^{-t_{sat}/T_{1W}})$, where X_S is the fractional concentration of solute protons, i.e. the ratio of the concentration of exchangeable protons to that of the water protons, α is the saturation efficiency, k_{SW} is the chemical exchange rate from the agent to water, and T_{1W} is the longitudinal relaxation rate of water.⁶⁹ Other methods are also available for the analysis of CEST spectra.^{68,70,71} However, MTR_{asym} can be produced by multiple processes, including semisolid tissue magnetization transfer. This complicates the evaluation of CEST contrast *in vivo*, especially when $\Delta\omega$ is small. Unfortunately this is the case for most currently developed diaCEST contrast agents. New imaging schemes have also been developed to better highlight CEST contrast.^{70,72–75}

Natural molecules, such as D-glucose,^{76–78} glycosaminoglycan,⁹⁰ glutamate,^{79,80} creatine,⁸¹ and L-arginine^{22,82} are attractive diaCEST agents because of their low toxicity and the possibility of imaging their metabolism *in vivo*. These natural compounds have exchangeable protons at $\Delta\omega \sim 1–2$ ppm. The overall contrast-to-noise (CNR) of MTR_{asym} maps tend to be lower for these agents because signal loss occurs by spillover of the saturation pulse to water at these lower frequencies. This direct saturation can be reduced through choosing a CEST agent with a larger $\Delta\omega$. Endogenous macromolecules such as glycosaminoglycan ($\Delta\omega=1$ ppm)⁹⁰ and peptides or proteins with faster exchanging protons ($\Delta\omega=3.5$ ppm) can produce CEST contrast.^{16,83} The exchange rate of amide protons is slower than that of NH_2 or OH , and the direct saturation is smaller for these protons. Clinical X-ray contrast agents such as Iopamidol possess suitable labile protons resonating at $\sim 4–5$ ppm from water,⁸⁴ similarly their contrast has less direct water saturation. Macromolecules such as poly-L-arginine (PLR), glycogen, Poly(rU),⁸⁵ poly-L-lysine (PLL),⁸⁶ and glycosaminoglycan⁹⁰ have a large number of exchangeable protons per molecule, making these very sensitive agents. Poly(rU) displays the largest $\Delta\omega$ (~ 6 ppm) for a diaCEST polymer to date,⁸⁵ while the hydroxyl (OH) and amine (NH) protons of PLL and PLR resonate at $1–3$ ppm (Figure 1(f)). In general, a large exchangeable proton pool, optimized k_{SW} , and a large $\Delta\omega$ are preferred to maximize the sensitivity and specificity of the CEST contrast. k_{SW} can be measured for a given agent using either the quantification of exchange using saturation power (QUESP), the quantification of exchange using saturation time (QUEST) experiments,⁸⁶ or a derivative method.^{87,88} Although CEST is a contrast mechanism that amplifies the signal from low concentrations of solute protons through rapid exchange with the water signal, the low concentrations and small $\Delta\omega$ of diaCEST agents are still challenging for *in vivo* studies.⁸⁹ Hence, stealth diaCEST liposomes with a high payload are important, as these have a longer circulation time to provide a wide imaging window and are able to localize in targeted regions through selective biodistribution.

'Multicolor' Imaging Strategies

diaCEST liposomes can be engineered to carry CEST agents with different types of exchangeable protons, enabling the use of 'multicolor' CEST imaging to identify multiple diaCEST liposomes simultaneously⁴ (Figure 1(e)). This attractive strategy could lead to numerous biomedical applications. diaCEST agents with different $\Delta\omega$ can be selectively saturated and assigned an artificial color in CEST contrast maps (Figure 1(e)). By co-registering the individual saturation frequency image and assigning a color to a pixel based on the frequency dependence of MTR_{asym} , the location of each agent can be identified with high spatial resolution *in vivo* as shown by Liu et al.²² In this study (Figure 3), glycogen liposomes ($\Delta\omega=1$ ppm), L-arginine liposomes ($\Delta\omega=2$ ppm), and PLL liposomes were prepared ($\Delta\omega=3.6$ ppm). Their frequency-dependent MTR_{asym} *in vitro* is shown in Figure 3(a). To test whether this frequency dependence could be observed *in vivo*, L-arginine and PLL liposomes were injected into the right and left footpad, respectively. The liposomes were found to accumulate in the popliteal lymph nodes through lymphatic drainage. In this case, 'two-color' MR images containing the left and right lymph nodes were produced

through examining the ratio of CEST contrast at the two different $\Delta\omega$ for the CEST agents. If voxels displayed a ratio of $\text{MTR}_{\text{asym}}(3.6 \text{ ppm})/\text{MTR}_{\text{asym}}(1.8 \text{ ppm}) > 1$, they were assigned a green color; for voxels displaying ratios < 1 , they were assigned a yellow color. A representative 'two-color' image is shown in Figure 3(b), and Figure 3(c) shows the MTR_{asym} as a function of frequency for the left and right popliteal nodes. Essential technical developments for obtaining high quality *in vivo* CEST contrast maps in this work include B_0 correction,^{91–93} CNR filtering⁹⁴ and NOMAR filtering,⁹⁵ while additional post-processing methods have also been developed recently.^{68,71,96} By using color encoding, the distribution of the diaCEST liposomes can be determined. This strategy has also been reported using paraCEST agents.^{49,97}

APPLICATIONS OF diaCEST LIPOSOMES

diaCEST Imaging and Cancers

CEST imaging has shown great promise in cancer imaging. In the absence of administering contrast agents, CEST contrast maps at $\Delta\omega = 3.5 \text{ ppm}$ can be used to detect tumors,⁸³ for tumor grading, and to discriminate between radiation necrosis and tumor recurrence¹⁶ (Figure 4(a)). Intravenous injection of natural, small molecule CEST probes such as D-glucose⁷⁷ and glutamate⁸⁰ (Figure 4(b)) can highlight tumors, and potentially image tumor metabolism. Tumors are very heterogeneous, and an insufficient delivery of drugs to solid tumors can be a source of treatment failure. Therefore, understanding the tumor microenvironment using molecular imaging approaches is important for evaluating therapeutic outcomes. As mentioned in previous section, liposomes with a size of $\sim 100 \text{ nm}$ (e.g. DoxilTM used for treating cancer patients) can accumulate in the extravascular and extracellular space of tumors via leaky vasculature and EPR.⁹⁸ In principle, diaCEST liposomes can be injected to evaluate tumor permeability and retention prior to administration of the nanotherapeutic using either passively targeted liposomes or actively targeted liposomes with the RGD peptide conjugated to the surface⁹⁹ (Figure 4(c)) or other targeting ligands.¹⁰⁰ A proper evaluation of tumor retention through contrast agents requires administering a large enough amount of the contrast agent, to detect signal changes and also that the amount of contrast agent administered is similar to the amount of therapeutic. This is feasible for therapeutic nanocarriers such as DoxilTM using diaCEST liposomes due to the amount of contrast generated by these nanocarriers, but more challenging to evaluate the tumor retention of free drug such as doxorubicin through administration of free small diaCEST agents.

Theranostic nanoparticles are particles containing both imaging agents and therapeutics, which are another potential application of diaCEST liposomes in cancer imaging.¹⁸ The advantage of this strategy is the use of noninvasive imaging to evaluate repeated administration of therapeutics, which may allow the refinement of treatment protocols. Theranostic diaCEST liposomes can be designed with CEST probes either loaded into the bilayers or the intraliposomal cavity, while the therapeutic agents are covalently attached to the surface of liposomes or loaded inside (Figure 2). If more than one type of liposome is needed, the 'multicolor' strategy can be employed (Figure 1). It is also possible to combine CEST imaging agents with other contrast agents to allow monitoring of both liposome location and triggered release of its contents. This dual probe strategy can address some of the sensitivity issues *in vivo* and can be used for internal referencing. This concept was demonstrated using liposomes containing both CEST and fluorine contrast agents.¹⁰¹ With the development of coils that are capable of collecting both fluorine and proton images,¹⁰² the combination of fluorine and CEST agents seems promising.

Monitoring Cell Therapy

Cellular therapies have shown promises as a therapeutic strategy for a number of otherwise untreatable disorders, including cancer, neurological disease, heart disease, liver failure, chronic pancreatitis, and diabetes.^{103,104} An effective means of monitoring both the location and fate of the cell transplants *in vivo*, either through direct or indirect cell labeling is essential^{57,105–107} for clinical translation. Bioluminescence imaging is a robust method in pre-clinical studies. The combination of this and other imaging modalities enables evaluation of the fate of transplanted cells and reveals how these cells interact with their host environment.^{106,108} One important question that needs an answer for all such therapies is: 'How long have the cells survived after transplantation?' Using the unique properties of diaCEST liposomes and pH sensitivity of L-arginine, we designed a nanosensor-impregnated alginate hydrogel for viability sensing during cell therapy (Figure 5(a)).⁸² The nano-sensor, i.e. L-arginine diaCEST liposomes, is in the proximity of the transplanted cells, so that no direct labeling of the cells is required for MRI visualization. The contrast produced by these diaCEST capsules is sensitive to changes in pH over the physiological range from 6.0 to 7.5. Changes in cell viability should result in pH changes, i.e. the pH will drop below 7.4 when cell death occurs. Hence, pH sensitive contrast produced by diaCEST liposome containing hydrogels can indicate transplanted cell death. We have shown that the CEST contrast produced by these capsules correlated with the viability for encapsulated hepatocytes *in vivo*, with the cell viability monitored and validated using conventional bioluminescence imaging (Figure 5(b)).⁸²

CONCLUSION

Challenges and Opportunities

Numerous strategies have been applied to optimize the sensitivity of CEST imaging, including more specific detection of chemical exchange⁶⁸ and the development of responsive agents and nanoparticle-based agents.²¹ Endogenous molecules can generate CEST contrast, moreover, changes in physiological or pathological parameters attenuate this contrast, which poses challenges to *in vivo* CEST imaging. The use of liposomes as nanocarriers of CEST agents can address some of these issues and provide an ideal platform to optimize properties of CEST contrast for *in vivo* studies.

CEST imaging is a budding research field, with much progress in both imaging sequences/strategies and formulation of diaCEST liposomes. There are many opportunities for improving both aspects of this technology and then translating these improvements into patients. Current investigations have focused on the use of diaCEST liposomes to monitor the biodistribution of nanocarriers and sense whether changes in pH have occurred. With the unique 'multicolor' CEST imaging features of these probes and the capability to design 'smart' diaCEST liposomes that are sensitive not only to pH, but also to ion, metabolite and enzyme concentrations, and temperature, we envision a wide range of biomedical applications for this technology. diaCEST liposomes are an emerging platform in the field of nanomedicine, which we expect to open up new avenues through imaging.

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FIGURE 1.

(a–d) Principle and measurement of chemical exchange saturation transfer (CEST). (e) Each CEST contrast agent has a unique frequency offset from water, allowing the use of the frequency to identify the type of exchangeable protons, such as amine at 2 ppm and amide at 3.5 ppm (f) The color-encoded CEST contrast of polypeptides, including PLT, PLK and PLR. (Reprinted with permission from Refs 1 and 4). Copyright 2011 and 2008 Wiley Inc.

FIGURE 2.

Structure of liposomes shows (a) its components as nanocarriers for contrast agents and/or drugs. (b) Contrast mechanisms for diaCEST liposomes where the RF pulses saturate exchangeable protons on the encapsulated contrast agent (top); in case of paraCEST liposomes, the RF pulses saturate the interior water whose frequency has been shifted by the encapsulated contrast agent (bottom). The amount of contrast generated depends on the size²⁶ and permeability²⁷ of liposomes, in addition to the concentration of protons and their relaxation rates.

FIGURE 3.

In vivo 'multicolor' imaging of diaCEST liposomes. (a) The CEST contrast of liposomes at distinctive frequency offsets. (b) CEST MR image of these liposomes at the lymph nodes with color encoding. (c) Obtained CEST contrast within the lymph nodes. (Reprinted with permission from Ref 22). Copyright 2012 Wiley Inc.

FIGURE 4.

Cancer imaging using diaCEST liposomes. (a) The endogenous contrast of amide protons of proteins for therapeutic evaluation (Reprinted with permission from Ref 16). Copyright 2011 Nature Publishing Group. (b) Tumor imaging using the exogenous diaCEST agent D-glucose (Reprinted with permission from Ref 77). Copyright 2012 Wiley Inc. (c) The angiogenesis targeting liposomes in brain tumors. (Reprinted with permission from Ref 99). Copyright 2013 Wiley Inc.

FIGURE 5.

CEST imaging in cell therapy. (a) The design of diaCEST liposomes containing microcapsules (left), which immunoprotect transplanted cells. (b) CEST images showing a decrease in contrast upon cell death as validated by bioluminescence imaging (c). Indicated are the CEST contrast of empty capsules (–Cells) and capsules with hepatocytes (+Cells); transplanted mice received immunosuppression (+IS) or no immunosuppression (–IS). (Reprinted with permission from Ref 82). Copyright 2013 Nature Publishing Group.