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Introducing a nested phase change agent with an acoustic response that depends on electric field: A candidate for myocardial perfusion imaging and drug delivery

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ABSTRACT

We present a voltage-sensitive phase change agent comprising an aqueous emulsion of surfactant-coated liquid perfluorocarbon droplets nested within a negatively charged phospholipid bilayer. The sensitivity to voltage allows, via exposure to an electric field, acoustic activation of the perfluorocarbon droplets at an ultrasound intensity that is otherwise insufficient to cause activation. The result is a phase change agent for which activation depends not on ultrasound intensity but rather on the presence of an electric field. Accordingly, we offer the first enhanced ultrasound contrast agent ("Electrast") that takes advantage of the electrical activity of the heart and leads to selective activation at a fixed mechanical index (MI). Being voltage-sensitive, Electrast activates selectively in the coronary circulation, giving enhanced ultrasound contrast within the myocardium while leaving other regions largely unenhanced. Specifically, in a closed chest swine study, the contrast enhancement between the myocardium and the left ventricle increased by 36.4 dB ± 0.2 upon injection of a charged, nested PCA formulation at a fixed MI of 0.9 (GE Vivid *i*). Similar enhancement was observed in rats, and the contrast-to-tissue ratio increased by nearly 10 dB at an MI of 0.28 upon exposure to an electric field of 1 V/cm in a tissue-mimicking phantom. Additionally, ultrasound-induced leakage of calcein, a water-soluble fluorescent dye, from a nested, charged PCA formulation more than doubled at a peak negative pressure of 0.5 MPa upon exposure to an electric field of 0.25 V/cm. These results suggest that Electrast, a voltage-sensitive phase change agent, is a candidate for myocardial perfusion imaging using ultrasound.

1. Introduction

1.1. Brief history of nesting

We introduced nearly a decade ago a novel microbubble architecture that comprises coated microbubbles nested within the aqueous core of a microcapsule [1]. In subsequent years we described how a nesting shell enables ultrasound imaging that is longer-lasting [2–5] and safer [5,6] than imaging with conventional (that is, un-nested) microbubbles. The monolayer coating of a microbubble slows – by decreasing interfacial tension so as to lower the Laplace overpressure [7,8] and by increasing resistance to diffusion [9] – but does not prevent gas dissolution [9]; as a result, microbubbles do not survive exposure to a mechanical index (MI) > 0.3 [10,11]. When nested inside the aqueous core of a microcapsule, however, a microbubble dissolves only partially. This is because the nesting shell separates the aqueous core from the bulk, and the aqueous interior of the microcapsule becomes sufficiently concentrated with the gas it receives from the microbubble that mass transport ceases; the microbubble, though it shrinks somewhat, persists [3,4]. At MI values sufficiently low that an un-nested microbubble sustains oscillations, the monolayer coating of the microbubble influences the oscillations via dilatational viscosity, which impacts damping, and via area expansion modulus (Gibbs elasticity), which impacts the natural frequency [12]. The presence of a nesting shell further influences microbubble oscillations, allowing greater tuning of microbubble acoustic and cavitation phenomena than is possible with a monolayer coating alone [13,14]. In particular, a nested microbubble requires a higher ultrasound intensity (that is, a greater peak negative pressure) for inertial cavitation than does an un-nested microbubble [13]. In the context of imaging, the nesting shell improves safety both by increasing the inertial cavitation threshold pressure and by absorbing the energy of the shock wave generated by inertial cavitation – thereby protecting nearby cells – if

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inertial cavitation were to occur [6]. Employing a liposomal bilayer as the nesting shell allows theranostic applications of microbubbles beyond imaging, including ultrasound-triggered release as might find use in controlled drug delivery [15,16]. Furthermore, a bilayer nesting shell offers the possibility of fine-tuning the rate of release by tailoring shell composition; for example, one can combine cholesterol and phospholipids in a manner that sets the number and sizes of membrane domains (or lack thereof) [17–19], which in turn prescribes the kinetics and mechanism of release [1,20,21].

Herein we present a new nesting construct with a novel feature, namely voltage activation.

1.2. Voltage activation

In place of microbubbles, the new construct nests an emulsion of phase-change agents (PCAs), which are (typically perfluorocarbon) liquid droplets that have the potential to become gaseous microbubbles (that is, to change phase) once inside the body [22–25]. In the absence of a nesting shell, an advantage of PCAs over microbubbles is smaller size; whereas microbubbles have diameters in the micron range and are therefore limited to the intravascular space, PCAs can be prepared with diameters of nominally hundreds of nanometers such that they have the possibility to access the extravascular space [26]. This advantage of smaller size does not come free; the PCAs require vaporization after delivery into the body if they are to behave acoustically as microbubbles. Indeed, this was a motivation for developing condensation methods to produce PCAs that are easier to vaporize [27]by taking advantage of the fact that the energy required for vaporization depends on the perfluorocarbon species and droplet size [28].

In our view, the necessity to vaporize PCAs offers an opportunity for a new class of contrast agent.

Consider the following three facts: (1) a PCA of a given composition, droplet size, and interfacial coating has a fixed energy for vaporization; (2) vaporization generates a gaseous bubble that is larger than the initial liquid droplet; and (3) water is largely incompressible.

Taken together, the first two facts above mean that vaporization of a PCA liquid droplet requires both an energy input and room for expansion. Now imagine a PCA liquid droplet nested inside the aqueous core of a vesicle or microcapsule. The third fact then means that the PCA droplet cannot vaporize unless the nesting shell responds to provide the necessary room for expansion. In other words, the nesting shell poses an added resistance to vaporization. On one hand, this added resistance could be overcome with ultrasound alone simply by using a higher MI (than is needed to vaporize an un-nested PCA). On the other hand, one could design a nested PCA that works with both ultrasound and a second modality to activate PCAs in a selective manner; ultrasound of a given intensity would be sufficient to activate only those liquid droplets in nests acted on by the second modality, which interacts with the nesting shell to allow for acoustic activity.

This article describes the use of an electric field as the second modality to interact with charged nesting shells. Fig. 1 illustrates the concept. We demonstrate that a given formulation can be relatively dark (strictly speaking, silent) or relatively bright (noisy) at a given MI, the difference being due to the absence or presence of an electric field, respectively. We call this phenomenon, which occurs with electric field strengths that are orders of magnitude weaker and at least an order of magnitude slower (in the case of alternating current) than those associated with electroporation, “voltage activation.”

Applications involving voltage activation can proceed anywhere there is an electric field (e.g., endogenous or applied), need not induce a phase change to be visible with ultrasound, and need not be limited to ultrasound theranostics. In fact, one can think of voltage activation applications that do not use ultrasound; in the case of nested PCAs, a modality other than ultrasound could supply the energy for the phase change, and PCAs are not the only entities that could be nested inside a voltage-activated nesting shell. Still, the potential of using a voltage-activated ultrasound contrast agent for myocardial perfusion imaging - where the heart supplies the electric field - did not escape our notice.

1.3. Myocardial perfusion imaging

The idea of using ultrasound contrast agents for myocardial perfusion imaging is not new. Indeed, some echocardiographers with specialized skills are able to employ commercial microbubbles in an off-label manner, taking advantage of the aforementioned fact that microbubbles do not survive MI > 0.3 [11,29,30], some investigators have used PCAs directly [31], and others have used tethered PCAs and microbubbles, a construct they refer to as acoustic cluster therapy [32]. Additionally, there have been at least two commercial attempts to develop ultrasound agents for myocardial perfusion imaging [33].

We offer the first agent that utilizes the electrical activity of the heart.

As a result, ours is the first agent that activates selectively in the myocardium for a given set of ultrasound parameters, leaving the ventricle relatively dark while brightening the myocardium. We are optimistic that our approach has the potential to rival single photon emission computed tomography (SPECT) and positron emission tomography (PET) at lower cost and lower risk without a loss in sensitivity or specificity. We recognize that “the history of contrast echocardiography has been characterized by cycles of enormous expectations and subsequent disappointment,” [33,34] yet do not believe that success necessarily “… starts with the microbubble itself, which must be predictably destroyed by high mechanical index (MI) ultrasound but provide stable, high signal-to-noise ratio across a wide range of lower intensity imaging.” [33] We contend that a single MI suffices and that what is needed is an agent that takes advantage of the heart’s electrical activity to allow selective activation.

The voltage-activated phase-change agent described herein satisfies that need.

2. Theory

There are two key points to bear in mind at the outset. First, the electrical fields employed in this work are orders of magnitude lower – even after accounting for particle size – than those associated with electroporation [35–41], and the applied electric field (alternating current, ac) frequency employed in this work is at least an order of magnitude slower than frequencies utilized for ac electroporation [36,42,43]. Second, the (zeta) potentials reported herein are not transmembrane potentials.

Voltage activation begins with making the nesting shell charged to interact with the electrical activity of the heart. The surface potential of the nesting shell depends on the surface charge density, and the weakening of this potential with distance away from the nesting shell depends on the ionic strength of the solution and the radius of the nest. In the case of a bilayer, it is possible for both the inner and outer leaflets to be charged and for the ionic strength to be different in the different aqueous compartments that exist inside and outside the bilayer. The situation is similar to that of spontaneous vesicles comprising mixtures of cationic and anionic surfactants [44].

When a charged entity (e.g., an ion, a charged nest, or a charged molecule or charged domain within a nesting shell) experiences an electric field, E, that entity can move (e.g., translation or rotation) or deform, or both. In the case of translation, the entity accelerates until the viscous drag force matches the force that caused the acceleration. What is relevant for the current work is that the force due to electric field depends on charge, whereas the drag force depends on size. Accordingly, the various charged entities in the system can therefore experience different accelerations, depending on the charge-to-size ratio, and this can lead to structural shifts.

The entity eventually reaches a terminal velocity, v, that is proportional to the electric field as described by Eq. (1).