Acta Biomaterialia xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Echogenicity enhancement by end-fluorinated polylactide perfluorohexane nanocapsules: Towards ultrasound-activable nanosystems

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ARTICLE INFO

Article history:
Received 18 June 2017
Received in revised form 29 September 2017
Accepted 2 October 2017
Available online xxxx

Keywords:
Nanocapsules
Perfluorohexane
Fluorinated polymers
Ultrasound imaging
Focused ultrasound
Fluorous interactions
Cell viability

ABSTRACT

Polylactide (PLA) polymers containing five distinct lengths of fluorinated (from C_3F_7 to $C_{13}F_{27}$) and non-fluorinated (C_6H_{13}) end-groups were successfully synthesized by ring-opening polymerization of D,L-lactide. Fluorination was expected to increase the encapsulation efficiency of perfluorohexane (PFH). 150 nm nanocapsules were obtained and ^{19}F nuclear magnetic resonance revealed that nanocapsules formulated with fluorinated polymers increased by 2-fold the encapsulation efficiency of PFH compared with non-fluorinated derivatives, without any effect of fluorine chain length. Fluorination of the polymers did not induce any specific *in vitro* cytotoxicity of nanocapsules towards HUVEC and J774.A1 cell lines. The echogenicity of fluorinated-shelled nanocapsules was increased by 3-fold to 40-fold compared to non-fluorinated nanocapsules or nanoparticles devoid of a perfluorohexane core for both conventional and contrast-specific ultrasound imaging modalities. In particular, an enhanced echogenicity and contrast-specific response was observed as the fluorinated chain-length increased, probably due to an increase of density and promotion of bubble nucleation. When submitted to focused ultrasound, both intact and exploded nanocapsules could be observed, also with end-group dependency, indicating that PFH was partly vaporized. These results pave the way to the design of theranostic perfluorohexane nanocapsules co-encapsulating a drug for precision delivery using focused ultrasound.

Statement of Significance

We believe that Acta Biomaterialia is an appropriate journal for our article since we present new and original experimental research in the field of nanomaterials for biomedical applications. In particular, we have synthesized novel fluorinated polyesters and formulated them into nanocapsules of perfluorohexane as ultrasound contrast agents. This nanosystem has been thoroughly characterized by several techniques and we show that fluorination of the biodegradable polymer favors the encapsulation of perfluorohexane without producing further reduction of cell viability. Contrary to nanocapsules of perfluoroctyl bromide formulated with the fluorinated polymers [32], the presence of the fluorinated moieties leads to an increase of echogenicity that is dependent of the length of the fluorinated moiety. Morevover, the ability of nanocapsules to explode when submitted to focused ultrasound also depends on the length of the fluorinated chain. These results pave the way to theranostic perfluorohexane nanocapsules co-encapsulating a drug for precision delivery using focused ultrasound.

1. Introduction

Ultrasound contrast agents (UCAs) are efficient intravascular echo-enhancers currently indispensable to safe and accurate

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https://doi.org/10.1016/j.actbio.2017.10.002
1742-7061/

diagnosis of many diseases, including kidney cists [1], acute myocardial ischemia [2] and solid tumors [3]. All the commercially available UCAs, such as Definity® and Sonovue®, are microbubbles constituted by a perfluorinated gaseous-core stabilized by a monolayer of phospholipids [4]. Unfortunately, the imaging and therapeutic applications of such materials are severely limited by (1) the fast-diffusing gas component, responsible for a relatively short distribution half-life in the bloodstream (~60 s after bolus injection) and an elimination half-life of about 6 min [5,6], and (2) their inherent micrometer size range that prevents extravasation into solid tumors.

To overcome these limitations, research has focused on nanosystems that can be more stable and are able to diffuse beyond the vascular compartment. Since gas nanobubbles are difficult to stabilize [7], the strategy has consisted in encapsulating liquid perfluorocarbons (PFCs) into nanoemulsions using surfactants or in polymer nanocapsules [8]. Although interesting, this approach leads to a significant reduction of the echogenicity. Indeed, as the scattering cross section of a particle is defined according to Eq. (1), a reduction of size and the replacement of the gas core by a liquid one, induce a decrease of the scattering cross section and subsequently of echogenicity.

$$SCS = \frac{4\pi}{9} k^4 R^6 \left[\left(\frac{\kappa_d - \kappa}{\kappa} \right)^2 + \frac{3}{4} \frac{(\rho_d - \rho)^2}{(\rho_d + \rho)^2} \right] \quad (1)$$

where k is the wavenumber, R is the radius of the particle, κ_d and κ the compressibilities of respectively the particle and the medium, ρ_d and ρ the densities of respectively the particle and the medium [9].

To provide strong and long-lasting ultrasonic echoes, liquid PFCs of low boiling point, such as perfluorohexane (PFH) or perfluoropentane, have been selected for their ability to experience a liquid-to-gas transition once exposed to high acoustic pressures [10]. During this phenomenon – known as acoustic drop vaporization (ADV) – the PFC vapor phase undergoes oscillations around an equilibrium radius, which enhances the ultrasound scattering intensity of nanometric systems by several orders of magnitude [11–16]. An advantage of nanocapsules over microbubbles is their ability to co-encapsulate a drug for further therapeutic use [17]. As previously reported, PFC-containing nanocapsules can preserve their integrity and initial diameter after intravenous administration and passively accumulate in tumor tissues through the enhanced permeability and retention effect (EPR) [17–20] provided this effect occurs in patients [21]. After reaching tumor tissue, the release of a co-encapsulated drug might be triggered by focused ultrasound (FUS), causing local PFC cavitation/vaporization and may be followed by capsule shell rupture with subsequent drug release [22]. This strategy is currently investigated to deliver higher drug concentrations in the tumor vicinity and increase the chemotherapy efficacy while avoiding unwanted toxicity to healthy cells [23]. As FUS is clinically employed to ablate and eradicate tumor cells, particularly in prostate [24] and hepatocellular carcinomas [25], it represents an ideal trigger for drug delivery because it is a non-invasive technique, safe to adjacent tissues and provides a precise spatiotemporal control over the thermal and mechanical energy dissipation [26].

Before pushing forward these theranostic applications, the efficient encapsulation of low boiling point PFCs is required. Contrary to high boiling point PFCs such as perfluorooctylbromide, the entrapment of low boiling point PFCs such as PFH into polymeric nanosystems is a challenging process due to their fluorophilic character and high vapor pressure [27,28]. Perfluorinated compounds are usually immiscible with hydrophilic or hydrophobic solvents due to favored interactions between fluorinated domains [29]. Accordingly, perfluorinated liquids tend to phase-separate, resulting in low encapsulation efficiencies – from 3 to 9% in nano-sized formulations [30,31] – which represents a limitation for ultrasound imaging and ultrasound-triggered drug delivery. To promote better PFH encapsulation into nanocapsules, we have synthesized polylactide (PLA) polymers terminated by linear fluorinated endgroups of distinct lengths. The presence of the fluorinated moiety is expected to increase PFH encapsulation efficiency by playing on fluorous-fluorous interactions as it was observed for another PFC: perfluorooctyl bromide [32]. Polymer synthesis, nanocapsule formulation and characterization are reported. Finally, the nanocapsule echogenicity and ability to be destroyed by focused ultrasound is evaluated.

2. Materials and methods

2.1. Materials

D,L-lactide was purchased from Polysciences (Germany) and perfluorohexane (purity >98%) was acquired from Alfa Aesar (Germany). 1-hexanol from Acros Organics (Belgium), 2,2,3,3,4,4-hepta fluoro-1-butanol, 1H,1H perfluoro-1-heptanol, 1H,1H perfluoro-1-nonanol, 1H,1H perfluoro-1-dodecanol and 1H,1H perfluoro-1-tetradecanol were acquired from Fluorochem (United Kingdom). Acetone, tetrahydrofuran (THF) and dichloromethane were purchased from Carlo Erba Reactifs (France), chloroform and diethyl ether from VWR (France). Stannous octoate, dry toluene, D_2O, sodium cholate, trifluoroacetic acid (TFA) and polyvinyl alcohol (PVA, 30–70 kDa, 87–90% hydrolyzed) were provided by Sigma-Aldrich (France). Deuterated solvents (CDCl3 and acetone-d) were purchased from Eurisotop (France), Cell culture reagents such as DMEM (Dulbecco's modified Eagle's medium), RPMI 1640 (Roswell Park Memorial Institute medium), FBS (Fetal Bovine Serum), trypsin-EDTA solution and PBS (Ca^{2+} and Mg^{2+} free phosphate buffer) were purchased from Sigma Aldrich (France). The ultrapure water was produced by a Millipore Synergy 185 apparatus coupled with a RiOs5™ (Millipore, France) with a resistivity of 18.2 MΩ. cm. The NMR sample tubes and coaxial inserts were obtained from CortecNet (France).

2.2. Polymer synthesis

All fluorinated (PLA-C_3F_7, PLA-C_6F_{13}, PLA-C_8F_{17}, PLA-$C_{11}F_{23}$ and PLA-$C_{13}F_{27}$) and non-fluorinated (PLA-C_6H_{13}) derivatives of polylactide polymers were synthesized by ring opening polymerization (ROP) with the presence of stannous octoate as catalyst [33,34]. All glassware and stir bars were flame-dried and cooled under argon flow. Briefly, in a 10 mL schlenck tube equipped with a magnetic stir-bar, the D,L-lactide (10.4 mmol, 1.5 g) and corresponding initiator (0.075 mmol) – 1-hexanol for PLA-C_6H_{13}, 2,2,3,3,4,4-hepta fluoro-1-butanol for PLA-C_3F_7, 1H,1H perfluoro-1-heptanol for PLA-C_6F_{13}, 1H,1H perfluoro-1-nonanol for PLA-C_8F_{17}, 1H,1H perfluoro-1-dodecanol for PLA-$C_{11}F_{23}$ or 1H,1Hp-1-tetradecanol for PLA-$C_{13}F_{27}$ – were added to the flask under argon flow. The tube was sealed with a rubber cap and a stannous octoate solution (0.05 mmol, 20 mg) dissolved in 2 mL of dried toluene was added through the septum. The tube was purged with argon for 0.5 h and the polymerization reaction was conducted with continuous stirring at 130 °C for 55 min in an oil bath under argon flow. The reaction was quenched by immersing the flask in a cold water bath. Afterwards, the solvent was evaporated under reduced pressure for 1 h and the material was dissolved in 5 mL of chloroform. The product was purified by precipitation as previously described [20]: all polymers were precipitated into cold diethyl ether (80 mL), next, PLA-C_6H_{13} was dissolved into THF (5mL), whereas the fluorinated polymers were dissolved in acetone (20 mL) and precipitated again

Please cite this article in press as: G. Picheth et al., Echogenicity enhancement by end-fluorinated polylactide perfluorohexane nanocapsules: Towards ultrasound-activable nanosystems, Acta Biomater. (2017), https://doi.org/10.1016/j.actbio.2017.10.002