Perfluoropropane-filled, sorbitan monostearate– and polyoxyethylene 40 stearate–shelled nanobubbles

PFC-S60-PEG40S-NB

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Chemical name:	Perfluoropropane-filled, sorbitan monostearate– and polyoxyethylene 40 stearate–shelled nanobubbles	
Abbreviated name:	PFC-S60-PEG40S-NB	
Synonym:	Nanobubbles	
Agent Category:	Lipids	
Target:	Non-targeted	
Target Category:	Non-targeted	
Method of detection:	Ultrasound	
Source of signal / contrast:	Nanobubbles	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Perfluoropropane (PFC)-filled, sorbitan monostearate (also known as Span 60 (S60))- and polyoxyethylene 40 stearate (PEG40S)-shelled nanobubbles (NB), abbreviated as PFC-S60-PEG40S-NB, is a non-targeted ultrasound contrast agent developed by Xing et al. for

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tumor imaging on the basis of enhanced permeability and retention effect of tumor vasculature (1).

Microbubble agents are the most common type of ultrasound contrast agents and also the only type of these agents to be approved by the United States Food and Drug Administration (2-4). These microbubbles are filled with air or with a water-insoluble gas such as octafluoropropane (C_3F_8), decafluorobutane, or sulfur hexafluoride (5). The microbubble shell, designed to prevent gas diffusion, can be made of lipids, denatured albumin, or polymers (5). Although microbubbles are highly echogenic, the efficiency of *in vivo* contrast enhancement is closely related to the microbubble size, surface charge, shell composition, surface architecture, and gas core, particularly in the context of passive targeting (1, 5, 6). In terms of size, the resonant frequency of a microbubble is directly dependent on its size, and the contrast enhancement is closely associated with the size variance among microbubbles (4, 6). Currently, microbubble-enhanced ultrasound imaging is used to image blood perfusion and to measure blood flow rate in the heart, liver, and other organs (2, 7, 8). The mean size of most microbubble contrast agents is less than or approximately equal to the size of red blood cells, although the shell thickness varies from 10 nm to 200 nm. Microbubbles with a diameter <10 μm exhibit effective flow similar to that of red blood cells in the vascular system.

Tumor vasculature is characterized by the presence of open pores (380–780 nm) (9, 10). In contrast to blood-pool microbubbles, nanobubbles can pass more efficiently through the pores and accumulate within tumors. Therefore, a better imaging contrast may be achieved. Xing et al. developed a biocompatible PFC-filled nanobubble contrast agent by ultrasonication of a mixture of S60 and PEG40S, followed by isolation of the nanobubble subpopulation from the parent suspensions (1). S60 formed a condensed monolayer to prevent PFC escape from the core, while PEG40S was incorporated into the nanobubble shell to serve as a steric stabilizer. PEG40S is known to be a biocompatible, degradable, and nontoxic surfactant that is widely used in food products and pharmaceuticals. This chapter describes the data obtained by Xing et al. with PFC-S60-PEG40S-NB (1).

Related Resource Links:

- FDA alert on microbubble contrast agents
- MICAD chapters on microbubble contrast agents
- Clinical trials of microbubble contrast agents in ClinicalTrials.gov

Synthesis

[PubMed]

Xing et al. first prepared the PFC-filled microbubble suspension with a surfactant shell and a PFC core (1). PFC at 99.99% purity is commercially available. The shell was made of S60 and PEG40S by dissolving them together in phosphate-buffered saline. The PFC core was generated with high-power tip sonication of the vesicle/micelle aqueous solution. Centrifugation at 20*g* (relative centrifuged field) for 1, 2, 3, or 5 min was then applied to

remove the microbubbles and isolate the nanobubble subpopulation. The yield of nanobubbles was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Xing et al. characterized the parental microbubbles and the nanobubbles *in vitro* (1). Preparation of the microbubbles by sonication of the mixed surfactants resulted in a polydispersed suspension. The mean diameter of microbubbles was 1.1–2.0 μm with a bimodal distribution centered at 400–600 nm and 3–5 μm, respectively. Optical analysis confirmed the polydispersity and the bimodal distribution of the parental microbubbles.

Centrifugation at 20*g* for 1 min slightly decreased the mean size of microbubbles to 1.1 μm with a bimodal distribution similar to the parental suspension. Centrifugation for 2 min further reduced the mean size to 820 nm but gave a broad distribution peaked at 400–600 nm. Increasing the time to 3 min markedly decreased the mean size (to 490 nm) and distribution (<1 μm for most bubbles) of the bubbles, and very little precipitate was observed in the samples. However, increasing the time to 5 min did not lower the mean size further, instead resulting in the destruction of the bubbles as well as a decrease in the bubble concentration. The 3-min nanobubbles remained stable at 4°C under a C_3F_8 atmosphere for ~2 weeks without significant variations in the bubble size or morphology. Zeta potentials of the nanobubbles obtained from 3-min and 5-min samples were −35.4 mV and −33.9 mV, respectively. The nanobubbles obtained from 3 min centrifugation were used for *in vivo* imaging.

Animal Studies

Rodents

[PubMed]

Xing et al. evaluated the nanobubbles in New Zealand white rabbits ($n = 4$) by imaging the kidneys in power Doppler mode (1). Upon intravenous injection of the nanobubbles, marked and complete power Doppler enhancement occurred immediately throughout the renal parenchyma. The enhancement lasted >15 min, indicating that the nanobubbles are stable enough for clinical ultrasonic imaging. No adverse effects were observed during the experiments (1).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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