

CLAIMS

1. An ultrasound contrast agent comprising:
 - (a) microbubbles of perfluorocarbon, which microbubbles are stabilised by a membrane of phospholipid; and
 - (b) a buffering agent;wherein the ultrasound contrast agent has a bulk pH of from about 7.5 or above, preferably about 8.5 or above.
2. The ultrasound contrast agent according to claim 1, which is for long-term storage.
3. The ultrasound contrast agent according to any preceding claim, which is ready to use in a clinical setting, such as for *in vivo* imaging, diagnosing and/or treatment of a subject.
4. The ultrasound contrast agent according to any preceding claim, wherein the buffering agent is selected from a group consisting of tris(hydroxymethyl)aminomethane (Tris), sodium phosphate, ammonium chloride, diethanolamine, glycine, triethanolamine, and sodium carbonate.
5. The ultrasound contrast agent according to any preceding claim, wherein the buffering agent has a concentration of from about 1 mM to about 10 mM.
6. The ultrasound contrast agent according to any preceding claim, wherein the membrane of phospholipid has a net negative charge.
7. The ultrasound contrast agent according to any preceding claim, wherein the ultrasound contrast agent further comprises a tonicity agent.
8. The ultrasound contrast agent according to any preceding claim, wherein the ultrasound contrast agent further comprises a viscosity agent, and/or a flotation-reducing agent.
9. The ultrasound contrast agent according to any preceding claim, wherein the perfluorocarbon is selected from perfluorobutane, perfluoropropane, and perfluoropentane.
10. The ultrasound contrast agent according to any preceding claim, wherein the perfluorocarbon is perfluorobutane and the phospholipid is hydrogenated egg phosphatidyl serine.

11. A method for preparing an ultrasound contrast agent, comprising the following steps:
- (i) Homogenising perfluorocarbon continuously in a sterile aqueous dispersion of phospholipid to generate phospholipid-stabilised microbubbles of perfluorocarbon dispersed in an aqueous dispersion;
 - (ii) Adjusting the size distribution of microbubbles in the aqueous dispersion to a median size in the range of from 1 to 6 μm , preferably from 2 to 5 μm ;
 - (iii) Optionally adding a tonicity agent to the aqueous dispersion;
 - (iv) Adding a buffering agent to the aqueous dispersion to adjust the bulk pH of the aqueous dispersion to a pH of from about 7.5 or above, preferably about 8.5 or above;
 - (v) Adjusting the concentration of microbubbles in the aqueous dispersion to achieve a target concentration of microbubbles of about 6-10 $\mu\text{l/ml}$;
 - (vi) Dispensing the aqueous dispersion into a vial and flushing the headspace of the vial with perfluorocarbon.
12. The method according to claim 11, wherein steps (iii) and (iv) are performed in any order.
13. The method according to any one of claims 11-12, wherein step (v) is performed before or after any one of steps (ii), (iii) and (iv), with the proviso that step (v) is performed after step (i) and before step (vi).
14. The method according to any one of claims 11-13, wherein the ultrasound contrast agent is for long-term storage and/or is ready to use in a clinical setting, such as for *in vivo* imaging, diagnosing and/or treatment of a subject.
15. The method according to any one of claims 11-14, which does not include a step of freeze-drying.
16. An ultrasound contrast agent prepared according to the method as defined in any one of claims 11-15.
17. A method for improving the contrast of an ultrasonic image of tissue in a subject, comprising injecting the ultrasound contrast agent according to any one of claims 1-10 or 16 into said subject and carrying out an ultrasound scan of said tissue.

18. A method for *in vivo* imaging of tissue in a subject, comprising injecting the ultrasound contrast agent according to any one of claims 1-10 or 16 into said subject, carrying out an ultrasound scan of said tissue and generating an image of said tissue.
- 5 19. A method for diagnosing of a subject, comprising injecting the ultrasound contrast agent according to any one of claims 1-10 or 16 into said subject, carrying out an ultrasound scan of a region of interest in said subject, generating an image of said region of interest and assessing said image in order to make a diagnosis.
- 10 20. An ultrasound contrast agent for use in a method according to any one of claims 17-19.
21. Use of an ultrasound contrast agent according to any one of claims 1-10 or 16 for the manufacture of a medicament for use in a method according to any one of claims 17-19.