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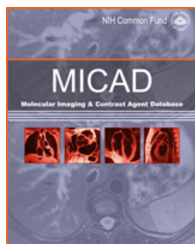

Buried cyclo(Arg-Gly-Tyr(Me)-Lys-Glu) microbubbles

bRGD-MBs

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Chemical name:	Buried cyclo(Arg-Gly-Tyr(Me)-Lys-Glu) microbubbles	
Abbreviated name:	bRGD-MBs	
Synonym:		
Agent category:	Peptide (nanoparticle)	
Target:	Integrin $\alpha_v\beta_3$	
Target category:	Receptor	
Method of detection:	Ultrasound	
Source of signal/contrast:	Microbubble	
Activation:	Yes	
Studies:	• *In vitro*	No structure is currently available in PubChem.

Background

[PubMed]

Microbubbles (MBs) are an aqueous suspension of a hydrophobic gas (perfluorocarbon or sulfur hexafluoride) encapsulated with stabilized shells of lipids, proteins, surfactants, or biocompatible polymers layer (1). The inherent material properties (density and compressibility) generate different speeds of sound in the gas core and the surrounding fluid (2). This leads to a mismatch in acoustic impedance on an order of 10,000, whereas the mismatch between different physiological tissues is around 1% (2). Ultrasound images are reconstructed from tissue-reflected echoes (backscatter) with an amplitude proportional to the impedance mismatch (3). The substantial mismatch between the echogenic MBs and the tissues allows for imaging vasculatures at high sensitivity (4). MBs that are 1–5 µm in diameter exhibit flow characteristics similar to erythrocytes in the blood stream (2). MBs can survive passage through the pulmonary vasculatures and the smallest capillaries (3), and they are removed from the circulation through filtration by the reticuloendothelial system and engulfment by the phagocytic cells (2). Because they are confined to the vascular system, MBs are designed to target the receptors expressed in diseased endothelial cells, including vascular endothelial growth factor receptor 2 (VEGFR2) and $\alpha_v\beta_3$ integrins (angiogenesis), intracellular adhesion molecule 1 (ICAM1) (inflammation), and fibrinogen receptor GPIIb/IIIa (thrombosis) (5). These targeted MBs have various molecular probes attached on the shell surface, such as proteins, peptides, antibodies, and biotin-avidin complexes. Nevertheless, the presence of these probes may trigger immune activation and can lead to accelerated blood clearance (6). Thus, avoidance

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of an immunogenic response becomes important to the targeted MBs for minimizing hypersensitivity and enhancing accumulation at the target (7). A new architecture of the encapsulated shell has been developed for protecting recognition of ligands until they reach the target. This new architecture has utilized the ultrasound radiation force (USRF), also called Bjerknes force, produced from the interactions of the MBs with the ultrasound wave (7, 8).

Under ultrasound, MBs experience a large net radiation force in the direction of ultrasound wave propagation (8). The use of a cycling ultrasound pulse can deflect MBs over a distance of several millimeters as the result of the primary USRF, which concentrates the MBs near a vessel wall (8). At the same time, a secondary USRF that is present between individual MBs causes MBs to attract each other and accumulate along the vessel wall (8). Thus, the use of ultrasound pulse can facilitate the localization of targeted MBs along vessel walls, which has demonstrated a 20-fold improvement in the efficiency of MB adhesion (6). MBs with buried-ligand shell architecture (BLA) respond to the ultrasound differently compared to normal MBs (with the exposed ligand shell architecture (eRGD-MBs)) (9). BLA contain ligands that are tethered to the MB surface by polyethylene glycol (PEG) molecules and buried in a PEG overbrush (PEG with a much longer chain) (9). Without ultrasound, the buried ligands are concealed from blood components such as opsonins and phagocytes. The ultrasound-produced USRF can cause the forward movement of the MB mass center and the expansion of the encapsulated shell, which reveals the ligand to the target epitopes (7). This allows the targeting ligands to be hidden from the milieu until they reach the target site. Once they arrive at the target, the ultrasound pulse can be used to expose of the surface ligands and facilitate the adhesion of MBs. This protocol also reduces the nonspecific interactions between the ligands and the blood pool agents, maintains targeted agent viability, and reduces MB immunogenicity (9).

Buried cyclo(Arg-Gly-Tyr(Me)-Lys-Glu) MBs (bRGD-MBs) comprise a BLA type of ultrasound contrast agent that targets the $\alpha_v\beta_3$ integrin receptors in endothelial cells (7). bRGD-MBs consist of perfluorobutane as the gas core, and distearoyl phosphatidylcholines (DSPC) and PEG conjugated distearoyl phosphatidylethanolamine (DSPE-PEG) as the encapsulated shell. The ligand RGD, which is the cell-recognition motif that is widely used to target the $\alpha_v\beta_3$ integrin (10), is covalently attached to the surface of the shell and buried in a PEG overbrush (PEG5k). The PEG5k is roughly three times thicker than the ligand-bearing inner layer and thus provides sufficient concealment for the ligands (9). This design can be extended to a variety of probes by adapting their surface characteristics to the microenvironment.

Synthesis

[PubMed]

Borden et al. reported the detailed synthesis of bRGD-MBs (7). First, the construction of different shell architectures was initiated with mixing a variety of lipids in chloroform. bRGD-MBs contained 90 mol% DSPC, 2 mol% lipopolypeptide-cyclo(Arg-Gly-Tyr(Me)-Lys-Glu) (LPP-RGD), and 8 mol% DSPE-N-[methoxy(PEG)-5000] (DSPE-mPEG5000). The eRGD-MBs contained 90 mol% DSPC, 2 mol% LPP-RGD, and 8 mol% DSPE-N-[methoxy(PEG)-2000] (DSPE-mPEG2000). The control MBs contained 90 mol% DSPC and 10 mol% PEGylated species. These lipid solutions were dried and suspended in phosphate-buffered saline containing 1.0 mg lipid/ml as the precursor solution. Then the solution was exchanged with perfluorobutane and shaken in a mechanical device for 45 s to produce a milky, white suspension of microbubbles. The obtained microbubbles were diluted and washed three times with centrifugation flotation for 3 min at 300*g*. The size of these MBs was measured with an optical particle counter. The distribution of the size was centered at 1.8 μm diameter with secondary peaks at ~4.5 and ~7.5 μm diameter.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Borden et al. studied the binding efficiency of bRGD-MBs in the presence of USFR *in vitro* (7). The cell adhesion integrin $\alpha_v\beta_3$ expressed in human umbilical vein endothelial cells (HUVEC) was used to provide sites for binding RGD at high affinity. The lipophilic dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI-C_{18}(3)) was incorporated into the MB lipid shells for fluorescence detection. The MBs were raised by buoyancy to reach the HUVEC cells that adhered on the top of an acoustic chamber in 5 min. At this time point, the static experiment was conducted as the control; the acoustic experiment was performed by applying USRF pulse via a single piston transducer. The USRF pulse was composed of two sequential 5-s bursts with an ultrasound frequency of 2.5 MHz and a peak negative pressure (PNP) of 40 kPa. After application of the USRF pulse, the adhesion of MBs on the cells was imaged with epifluorescence and bright-field microscopy. The eRGD-MBs adhered to the cells to approximately the same extent through either buoyancy or USRF pulse. In contrast, the bRGD-MBs adhered to the cells at a density ~5.5-fold higher with the USRF pulse than with buoyancy. The adhered amount of bRGD-MBs under USRF was similar to that of the eRGD-MBs. In the static experiments, very few bRGD-MBs were observed to adhere to the cells.

Animal Studies

Rodents

[PubMed]

No publication is currently available.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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References

1. Schneider M. Molecular imaging and ultrasound-assisted drug delivery. J Endourol. 2008; **22** (4):795–802. PubMed PMID: 18366315.
2. Dayton P.A., Rychak J.J. Molecular ultrasound imaging using microbubble contrast agents. Front Biosci. 2007; **12** :5124–42. PubMed PMID: 17569635.
3. Hernot, S. and A.L. Klibanov, *Microbubbles in ultrasound-triggered drug and gene delivery.* Adv Drug Deliv Rev, 2008
4. Klibanov A.L. Ultrasound molecular imaging with targeted microbubble contrast agents. J Nucl Cardiol. 2007; **14** (6):876–84. PubMed PMID: 18022115.

5. Sboros, V., *Response of contrast agents to ultrasound.* Adv Drug Deliv Rev, 2008
6. Lum A.F., Borden M.A., Dayton P.A., Kruse D.E., Simon S.I., Ferrara K.W. Ultrasound radiation force enables targeted deposition of model drug carriers loaded on microbubbles. J Control Release. 2006; **111** (1-2):128–34. PubMed PMID: 16380187.
7. Borden M.A., Zhang H., Gillies R.J., Dayton P.A., Ferrara K.W. A stimulus-responsive contrast agent for ultrasound molecular imaging. Biomaterials. 2008; **29** (5):597–606. PubMed PMID: 17977595.
8. Zhao S., Borden M., Bloch S.H., Kruse D., Ferrara K.W., Dayton P.A. Radiation-force assisted targeting facilitates ultrasonic molecular imaging. Mol Imaging. 2004; **3** (3):135–48. PubMed PMID: 15530249.
9. Borden M.A., Sarantos M.R., Stieger S.M., Simon S.I., Ferrara K.W., Dayton P.A. Ultrasound radiation force modulates ligand availability on targeted contrast agents. Mol Imaging. 2006; **5** (3):139–47. PubMed PMID: 16954028.
10. Ruoslahti E., Pierschbacher M.D. Arg-Gly-Asp: a versatile cell recognition signal. Cell. 1986; **44** (4):517–8. PubMed PMID: 2418980.