Aqueous colloidal nanoemulsion of perfluorocarbon polymers

CS-1000

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Chemical name:	Aqueous colloidal nanoemulsion of perfluorocarbon polymers	
Abbreviated name:	CS-1000	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Cells	
Target Category:	Others (cell tracking)	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	^{19}F	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

Cell tracking with magnetic resonance imaging (MRI) is achieved by labeling cells of interest with a contrast agent or by transducing cells to express reporter genes (1, 2). In both preclinical and clinical situations, iron oxide–based agents are typically used and provide a strong change in signal per unit of metal in T2-weighted images without significant effect on labeled cells and on the host. However, MRI quantification of iron oxide concentration is still not reliable because there is no clear correlation between the

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iron oxide signal and the number of labeled cells and measuring a decrease in signal has always been a problem (2, 3).

An alternative development for quantitative cell tracking is the application of ^{19}F in MRI imaging (4, 5). Endogenous fluorine is found primarily in bones and teeth as solid fluorides, and it has a very short T2 relaxation time and results in an undetectable signal with ^{19}F MRI. Cell tracking studies with fluorinated tracers have shown that the labeled cells can be detected on the order of 10^4–10^5 cells per voxel for clinical MRI systems and 10^3–10^4 cells per voxel for high-field animal scanners (3, 6). The MRI images positive for the ^{19}F signal are extremely selective for the labeled cells. The absolute number of the labeled cells could be measured directly from the *in vivo* ^{19}F images, thus providing a quantitative biomarker. The positive ^{19}F signal is also of great advantage for cell tracking in regions such as lungs, tissues and organs of the abdominal cavity, and bones. Although cell division and subsequent dilution of the tracers limit long-term cell tracking and decrease the accuracy of cell quantification, it has been shown that the absolute cell number can be quantified for up to 3 weeks in actively dividing cells in mice and that the underestimation of the cell number is within tolerable limits (usually two- to four-fold lower depending on the cell division rate and length of time) (6). This error range can be reduced if the cell division rate is known. Similar to what has been observed with iron oxide agents, the death of the labeled cells may result in transfer of the tracers to phagocytes, resulting in false positive signals (3, 7). CS-1000 is a commercially available reagent specifically formulated to facilitate its internalization into various cell types *ex vivo* regardless of the cell's inherent phagocytic ability (3, 7). CS-1000 is an aqueous colloidal nanoemulsion of perfluorocarbon polymers. Perfluorocarbon is both hydrophobic and lipophobic, and it does not become associated with cell membranes (3, 8). The perfluorocarbon component maintains its structure at typical lysosomal pH values and is not degraded by cell enzymes found in the body, which allows it to provide long-lasting intracellular labeling. Bonetto et al. investigated the utility of CS-1000 for tracking and quantification of human dendritic cells (DCs) directly from image data (3). The investigators showed that the minimum number of labeled cells detectable at 7 T was ~2,000 per voxel, with 1.7×10^{13} fluorine atoms per cell (3). DCs are antigen-presenting cells of the immune system with key roles in inducing immunity, which forms the rationale of DC immunotherapy (5). Monocyte-derived DCs do not divide (3), which makes DC quantification and tracking studies particularly suitable because there is no signal reduction and data misinterpretation is less likely because the results are not affected by the uncontrolled process of cell division.

Related Resource Links:

- Chapters on ^{19}F MRI in MICAD
- Chapters on MRI cell tracking in MICAD
- Drug information of CS-1000

Synthesis

[PubMed]

CS-1000 is commercially available. The total fluorine content is 100 mg/ml, and the droplet size of the nanoemulsion is ~180 nm (3).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Bonetto et al. analyzed the cell labeling efficiency with DCs and CS-1000 (3). DCs were generated from mononuclear peripheral blood cells from donor blood. CS-1000 (50–200 µl/million cells) was added to immature DCs on day 3 of culture, and the cells were incubated for 3 days before the addition of maturation factors. DCs took up CS-1000 effectively, without a need for transfection aids. Cellular uptake appeared to be saturated at 140 µl (fluorine content, 100 mg/ml) CS-1000 per million cells. Significant toxicity was observed only at high concentrations (a threshold value was not provided) of CS-1000. Labeling with 75 µl CS-1000 per million cells was used by investigators for their experiments. This yielded $1.7 \pm 0.1 \times 10^{13}$ ^{19}F atoms/cell with an acceptable cell viability of 80 ± 6%. Cell labeling was homogeneous on the basis of the ^{19}F content/cell among independent samples. CS-1000 had no evident side effects on DC viability, antigen expression, or maturation at the concentration of 75 µl CS-1000 per million cells.

Detection sensitivity was evaluated with a 7 T MRI system, and a comparison was performed among CS-1000, ProHance (a gadolinium (Gd)-based agent, 1 mM/5 x 10^5 cells) and Ferumoxide (a superparamagnetic iron oxide (SPIO)-based agent, 200 µg/5 x 10^5 cells) (3). Labeled cells were suspended in gelatin. The minimum detectable density of ^{19}F-labeled cells was comparable to that of Gd-labeled cells at <2,000 cells/voxel when assessed visually. The detection limit with SPIO was lower at ~125 cells/voxel. However, the detection sensitivity is related to the amounts of F-19, Gd, and Fe used for cell labeling.

The cell number was determined after cells were labeled with Gd, SPIO, or ^{19}F and suspended at various densities in gelatin (3). A linear relationship between the ^{19}F intensity and the number of cells per voxel was observed. A similar linear relationship existed between the signal/noise ratio and the cell density. Thus, the cell number can be calculated directly from the image data if the label content per cell is known. For the Gd- and SPIO-labeled cells, complex relationships were observed between T1 and T2* relaxation times and the relative contrast in the images. The change in contrast began to saturate at ~8,000 cells/voxel with Gd and as early as 800 cells/voxel with SPIO. These issues complicated the cell number quantification with Gd- and SPIO-based contrast agents but not with ^{19}F labels.

The detection sensitivity over tissue background was evaluated after injection of the labeled DCs (1.5 million) as a bolus into isolated bovine muscle. The unlabeled cells

showed a typical nonhomogeneous background from the biological tissue. The contrast due to SPIO was apparent, and the SPIO-labeled cells displayed a sharp decrease in the signal intensity. The Gd-labeled cells were not readily detected in this image over the background. The ^{19}F-labeled cells were clearly visible and were readily identified and differentiated against the ^{1}H tissue underlay. The detection sensitivity was on the order of 2,000 cells/voxel, confirming that the sensitivity and quality of ^{19}F images are not affected by the presence of tissue background (3).

Animal Studies

Rodents

[PubMed]

The feasibility of *in vivo* ^{19}F cell tracking was tested after ~3 × 10^{6} mature, labeled, human DCs were injected subcutaneously into the quadriceps muscle of a female NOD-SCID mouse. Labeled cells were clearly visible in the ^{19}F image, and at 12 h after injection cells were observed to be localized in a region anatomically consistent with the proximal draining inguinal lymph node, indicating cell migration away from the injection site.

In summary, the detection limit with CS-1000 was 2,000 cells/voxel at 7 T with 1.7 ± 0.1 × 10^{13} ^{19}F atoms/cell, which translates to a detection limit of ~9,000 cells/voxel at 3 T. A typical DC vaccination study utilizes an intranodal or intradermal injection of ~10 million cells, with 30,000–200,000 cells migrating to secondary lymph nodes as detected with scintigraphy on ^{111}In-labeled DCs (9). Detection of 30,000 cells *in vivo* at 3 T with a reasonable signal/noise ratio for ^{19}F images would require <30 min with a conventional fast spin-echo sequence, as calculated by Bonetto at al (3). This is well within acceptable limits for clinical studies. Bonetto et al. concluded that ^{19}F MRI for quantitative cell tracking in a clinical setting has great potential (3).

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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