



Cell Sense - A reagent to non-invasively track the delivery, migration and persistence of cells administered for therapeutic and diagnostic purposes.

Overview

Cellular therapeutic strategies include using transplanted cells to reconstitute lesioned tissues or to stimulate an immune response. A challenge in the development and translation of these emerging therapies is effective tracking of cells post-transfer in both animal and human subjects. *Cell Sense* is a fluorocarbon-based emulsion engineered to safely and efficiently label cells *ex vivo* without the use of transfection reagents. Labeled cells are transferred into an animal or human subject enabling investigators to non-invasively track the administration and migration of therapeutic cells, quickly and quantitatively, using ^{19}F magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS). The key advantage of ^{19}F detection is that there is no background signal from the host's tissues and only labeled cells are detected. Quantification of the ^{19}F signal yields a fast, accurate marker of the number of transferred cells in regions of interest *in vivo* or in intact, excised tissue samples. A dual-mode version of *Cell Sense*, detectable using either MRI/MRS or conventional fluorescence detection modalities (e.g., flow cytometry and optical microscopy), is available to simplify validation studies. Using the *Voxel Tracker*[™] software package, investigators can visualize and quantify the number of labeled cells in user-defined regions directly from the *in vivo* MRI data. Applications of the *Cell Sense* platform include cell tracking in immunotherapy or regenerative medicine, as well as diagnosis of inflammatory sites by monitoring selected populations of immune cells.



Unmet Need

Cell therapy is the prevention or treatment of human disease by the administration of cells that have been selected, pharmacologically treated, or engineered outside the body (1). Therapeutic cells such as various immune, progenitor, or stem cells, originate from autologous, allogenic, xenogenic, or immortalized cell line sources. Transplanted cells can be used to replace diseased tissues, secrete factors that stimulate tissue regeneration, or regulate (up or down) a desired immune response. Cell therapy research encompasses most of the major human disease states, and a multitude of clinical trials are underway, for example in the treatment of cancer, neurological diseases, autoimmunity, chronic infection and tissue regeneration (1).

Determining the trafficking patterns of transplanted cells is fundamental to the evaluation of efficacy and safety of virtually all emerging cell therapies. A failure to observe a clinical response raises the question of whether a sufficient number of cells were delivered to, and/or persisted at, the desired site(s). Conversely, the manifestation of undesired side effects raises the possibility that large numbers of cells were delivered off-target. In fact, a significant barrier to the successful implementation of cell therapy has often been the inability to target cells to tissues of interest with high efficiency and engraftment (1). In many cases, the biological significance of preclinical model data associated with a particular human disease treatment is questionable due to intrinsic species differences. Hence, the discovery and development process is heavily dependent on data derived from small phase I/II clinical trials. In many cases, these trial results are inconclusive due to the absence of cell trafficking data. Recognizing this deficiency, the FDA's Cellular, Tissue and Gene Therapy Advisory Committee recommended that sponsors develop labeling and non-invasive imaging methods for tracking cells as an integral element of any patient monitoring protocol (2).

Moreover, harvested and transplanted leukocytes can be used for diagnostic purposes. Inflammation associated with infection, disease, and injury often results in localized accumulations of immune cell populations at lesioned sites. Monitoring the trafficking patterns of phenotypically-defined populations of immune cells can be an important diagnostic indicator of disease loci, therapeutic efficacy, and sites of action.

Current methods to track therapeutic cells rely principally on histological examination of excised tissues. In animal studies, histological determination of cell migration often represents a significant bottleneck at the discovery and preclinical stages due to the significant time and labor investment associated with processing and analysis of necropsied tissues. In humans, biopsies are required to assay cell distribution, which are burdensome to the patient and may present significant safety concerns. Moreover, certain tissue types are not readily biopsied, for example tissues of the central nervous system. Alternatives to biopsies often include bioanalysis of circulating markers, which is still semi-invasive and only provide indirect evidence of therapeutic cell location.

A number of non-invasive imaging technologies are now being evaluated for cell tracking studies, including MRI, radioisotope methods such as SPECT and PET, CT, and ultrasound. Adopting these imaging modalities to visualize cells post-transfer is generally a complex problem. Cells must be labeled while in culture, i.e., pre-transfer, to render them visually distinct within in vivo images. Effective cell tracking requires an integrated approach to imaging agent design, tissue culture methods, data acquisition and analysis.

Overall, an effective cell labeling imaging agent should have a number of key characteristics, namely:

- i. non-toxic to cells (no decrease in proliferation, viability, differentiation capacity, or function),
- ii. non-toxic to surrounding tissues,
- iii. indicates the location, migration, and quantity of labeled cells,
- iv. allows for repeated, non-invasive detection,
- v. detectable at physiologically-safe doses,
- vi. long-lasting and durable, but follows a defined elimination pathway,
- vii. effective and safe for both animal and human use, thereby eliminating the need to adopt different imaging biomarkers for preclinical and clinical studies.

The Cell Sense Platform

Cell Sense was designed to satisfy all of the key design criteria listed above. The *Cell Sense* reagent is a fluorocarbon-based emulsion used to label cells ex vivo. Labeled cells are administered to the patient, and cell trafficking is monitored using ^{19}F MRI or MRS in vivo or in fixed tissues (Fig. 1). The key advantage of this platform is that the positive-signal ^{19}F images are extremely selective for the labeled cells with no background. Co-registered conventional proton (^1H) MRI acquired in the same imaging session places the labeled cells into their anatomical context. Furthermore, the absolute number of labeled cells can be measured directly from the in vivo ^{19}F images, thus providing a quantitative biomarker.

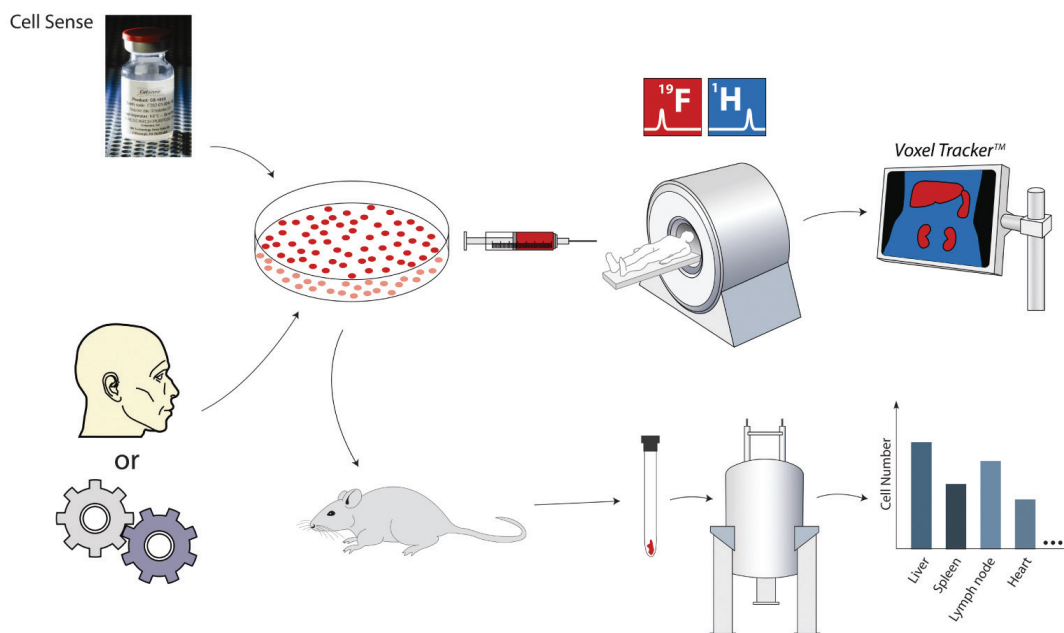


Figure 1. - Schematic of the Cell Sense cell tracking platform. Cells of interest are co-cultured ex vivo with the Cell Sense reagent, resulting in intracellular uptake. The labeled cells are then inoculated into a subject and imaged using MRI. Images of both the ^{19}F (labeled cells) and ^1H (background anatomy) are acquired in the same imaging session. A $^{19}\text{F}/^1\text{H}$ fusion image is constructed showing the regions containing labeled cells (red) in their anatomical context. Image fusion, visualization and cell quantification is performed using the Voxel Tracker™ software program. Alternatively, intact tissues samples excised from the subject are rapidly assayed using ^{19}F MRS to determine cell number or cell density, thereby minimizing histological endpoints.

Overall, MRI/MRS instruments are widely available. MRI is non-invasive, allows views into deep, opaque tissues at high resolution and does not use ionizing radiation. These characteristics alleviate human safety concerns, thus enabling longitudinal studies. Most MRI scanners can readily be adapted to detect and image ^{19}F with the addition of a suitable $^{19}\text{F}/^1\text{H}$ coil plug-in. In the case of fixed tissue analysis, standard liquid-state MRS (i.e., NMR) spectrometers can be used, and these routinely detect ^{19}F .

^{19}F MRI yields a positive contrast signal from labeled cells with no background from the host, a consequence of the negligible endogenous fluorine content of tissue. This property makes image interpretation straightforward when the ^{19}F image is fused with a conventional ^1H image. Furthermore, the positive ^{19}F signal is of great advantage for cell tracking in regions such as lungs, tissues and organs of the abdominal cavity, and trabecular bone, where ^1H -based MRI can be challenging due to low signal in these regions and/or pronounced intrinsic background contrast.

The first proof-of-principle demonstration of ^{19}F cell tracking using ex vivo labeling was published in 2005 (3); these studies imaged mouse dendritic cells (DCs) in vivo. More recent studies have focused on labeling and tracking phenotypically-defined immune cell populations in the context of early inflammatory events in a rodent model of type 1 diabetes (4), longitudinal studies of an acute inflammation model (5), and the biodistribution of MUC1-specific lymphocytes in irritable bowel disease and colorectal cancer (6). Other studies have employed ^{19}F MRI cell tracking to visualize stem/progenitor cells in vivo in mouse models (7,8).

Most recently (9,10), primary human cell types, relevant to ongoing immunotherapeutic clinical trials, have been labeled, biologically characterized, and imaged. Figure 2 displays image results tracking human DCs in vivo in a xenograft model. Importantly, the same study shows that *Cell Sense* labeling has no significant impact on the viability, phenotype, or functional properties of these human cells (10).

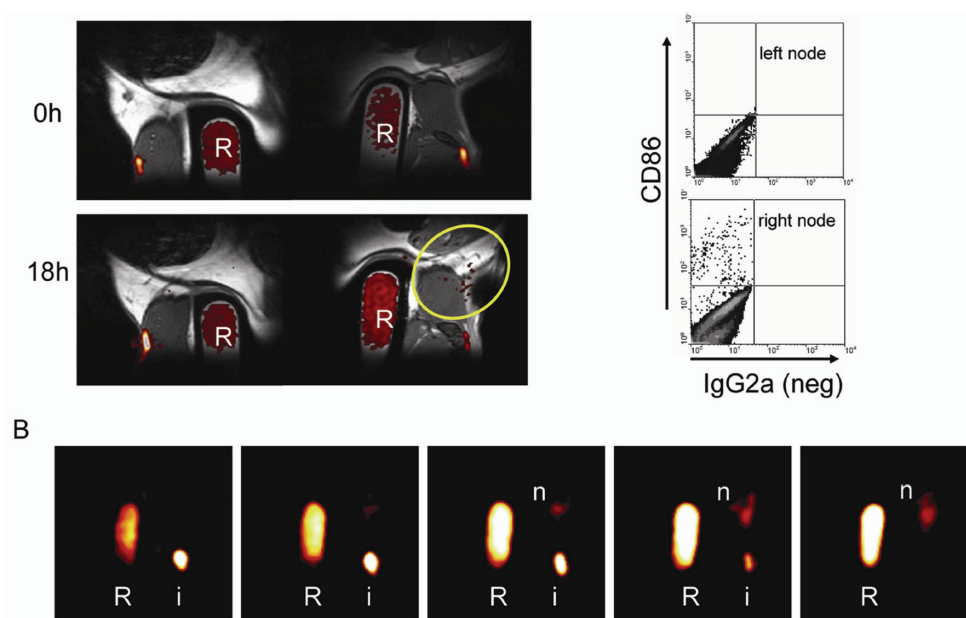


Figure 2. - Detection of human DCs in vivo via ^{19}F MRI in a xenograft mouse model.

- Cell Sense* labeled human immature and mature DCs were injected subcutaneously into the left and right quadriceps, respectively, in an NOD-SCID mouse. ^{19}F and ^1H images of each quadriceps were captured at time 0 h (top panels) and 18 h (bottom panels) post-administration. In these composite images the ^{19}F is rendered in 'hot-iron' pseudo-color and the ^1H is in grayscale. The circled region shows the draining lymph node region.
- Contiguous ^{19}F images slices through the mature DC injection site and proximal inguinal node region at 18 h post-injection. The panels display the ^{19}F reference capillary (R), injection site (i), and the inguinal node (n). The slice thickness is 0.5 mm. Migration of DCs from the injection site to the lymph node is observed, and quantitative image analysis using Voxel Tracker™ software reveals that ~5% of the cells reach the node.
- Flow cytometry analysis using an anti-human CD86 marker validating human DC presence in the draining lymph node from the same mouse. Images were adapted from (10).

The ^{19}F MRI-active ingredient of *Cell Sense* is a proprietary perfluorocarbon polymer. This molecule is optimized for MRI applications and can be used with conventional, fast-imaging methodologies. The perfluorocarbon is formulated into emulsion droplets, having a mean diameter ~170 nm, suspended in an aqueous buffer. *Cell Sense*'s composition has high chemical stability and is not degraded by any known enzyme found in the body. Its fluorocarbon component maintains its structure at typical lysosomal pH values (11), thereby providing long-lasting intracellular labeling. The perfluorocarbon is both hydrophobic and lipophobic and does not become associated with cell membranes. The low toxicity of perfluorocarbon materials is well understood and documented in the literature (11).

In typical applications, *Cell Sense* is used in minuscule quantities per body weight, i.e., contained within the transferred cells. The reagent has been rigorously tested and is biologically safe, presenting no observed adverse effects to viability or function in cells (9,10) and is non-toxic in vivo.

Cell Sense is formulated to be 'gently' taken up by virtually any cell in culture, even non-phagocytic cells, without the use of transfection agents. Cell labeling by co-incubation can conveniently be incorporated into existing tissue culture protocols used for preparing animal or human cells. The exact *Cell Sense* labeling protocol may vary by cell, medium, and vessel type, and must be empirically determined. Initially, the cell labeling protocol is derived by examining a dose response of reagent in culture for a fixed time period, typically >3 hours. Following incubation and wash, *Cell Sense* uptake is readily assayed via quantitative ^{19}F MRS measurements on labeled cell pellets (4,5,12); cell uptake is normally expressed as the average number of fluorine atoms (^{19}F) per cell. Celsense's biologics laboratory is focused on delivering optimized cell labeling protocols, and we welcome customer's inquiries about their own protocol development.

Generally, the ^{19}F signal detected from *Cell Sense* is independent of the cell or tissue type that it resides in. Because there is negligible ^{19}F background, any ^{19}F signal detected is from labeled cells. The ^{19}F image detects localized pools of cells at arbitrarily low signal levels, and the ^1H underlay provides the detailed anatomical context. Studies have shown that the minimum cell detection sensitivity for ^{19}F cell tracking is of order 104 to 105 cells per voxel for clinical MRI systems and 103-104 cells per voxel for high-field animal scanners (3,4,7,8). Experimental details, such as the image acquisition methods, magnetic field strength, and detector coil configuration determine the actual sensitivity for a particular experiment. Single-voxel, in vivo MRS of the labeled cells, or fixed sample MRS detection, yields even higher sensitivity to sparse cell numbers.

In labeled cells having a mitotic phenotype, cell division and subsequent dilution of *Cell Sense* can potentially limit long-term cell tracking studies of itinerant cells and/or decrease the accuracy of quantification of absolute cell numbers. Cell death of labeled cells can potentially result in transfer of the emulsion droplets to resident phagocytes (e.g., macrophage) engulfing the dying cell body. If a large number of these labeled phagocytes remain in a region of interest, false positive signals could result. We note that studies suggest that the *Cell Sense* reagent does not maintain its ability to label non-phagocytic cells once released from a dying cell. Furthermore, no evidence for active exocytosis of *Cell Sense* has been observed.

Cell Sense in the Clinic

Cell Sense is currently manufactured in the United States under cGMP conditions and is suitable for use in clinical trials. *Cell Sense* is the subject of an open Drug Master File (DMF) with FDA, and an Active Substance Master File (ASMF) is currently being reviewed by the European Medicines Agency (EMA). These Master Files contain detailed data on the chemistry and manufacturing of the reagent, the results of Celsense sponsored cGLP acute toxicity animal studies, and in vitro toxicity data on multiple cell types. *Cell Sense* has been designated a drug by the FDA, and future IND applications incorporating *Cell Sense* will likely be reviewed and regulated by the Center for Biologics Evaluation and Research (CBER). Investigators are encouraged to contact Celsense about cross referencing the *Cell Sense* Master Files in their pending regulatory filings.

Dual-mode Cell Sense: MRI & Fluorescence Detection

Investigators using *Cell Sense* often require a tool to validate that cells detected using MRI or MRS are the original cells that were labeled and transferred. With this validation concern in mind, Celsense offers a dual-mode version of *Cell Sense* that can be detected by both MRI/MRS and fluorescence. In the dual-mode product, the fluorocarbon polymer is covalently bound to a bright fluorescent dye, ensuring coincident MRI/MRS and fluorescence signals. Currently, dual-mode *Cell Sense* is offered as a conjugate with the FITC fluorophore (fluorescein isothiocyanate), with excitation and emission wavelengths at approximately 495 and 521 nm, respectively. Importantly, the dual-mode agent's uptake efficiency in vitro is the same as the MRI-only *Cell Sense* reagent.

This true dual-mode agent can aid in the adoption of the *Cell Sense* platform in both preclinical and clinical studies. In preclinical studies, it enables investigators to positively identify the fate and phenotype of labeled cells following 19F MRI or MRS, days and weeks after cell transfer. Fluorescence detection (e.g., using optical microscopy) can validate the in vivo cell tracking results in biopsied or necropsied tissues. Moreover, flow cytometry of single-cell suspensions can elucidate potential phenotypic changes of the labeled cells or identify possible in vivo transfer of the label to other cell types.

In a clinical setting, the dual-mode agent can be used as a powerful tool for quick validation of a patient's labeled cells. In a small subset of the therapeutic cells, labeling validation is accomplished using a fluorescence microplate reader, flow cytometer, or microscope. This step also allows for true individualization of the cell labeling protocols by identifying potential patient-to-patient variations in the cell labeling efficiency. The fluorescent cells are not delivered into patients directly, but used in the clinic as an in vitro diagnostic. Subsequently, the bulk of the therapeutic cells destined for the patient are labeled with the MRI-only version *Cell Sense*. The dual-mode technology accelerates cell labeling protocol development and enables routine consistency checks, quickly, without the use of MRS instrumentation.

Voxel Tracker™ Software

The *Voxel Tracker*™ software program, offered by Celsense, maximizes the potential of *Cell Sense* MRI. It enables rapid visualization and quantification of labeled cells in their anatomical context. The image processing toolbox fuses and visualizes three-dimensional 1H and 19F images. Powerful, built-in, computational tools enable cell quantification in regions of interest, thus enriching the utility of the in vivo data. Since the 19F image is exquisitely selective for labeled cells, the absolute amount of 19F, and hence number of labeled cells, can readily be calculated using *Voxel Tracker*™; this calculation uses the average 19F/cell parameter measured in vitro following cell labeling. The software also serves as a platform for managing imaging study workflow. *Voxel Tracker*™ is currently offered via a convenient web-based access. For more information, see www.voxeltracker.com.

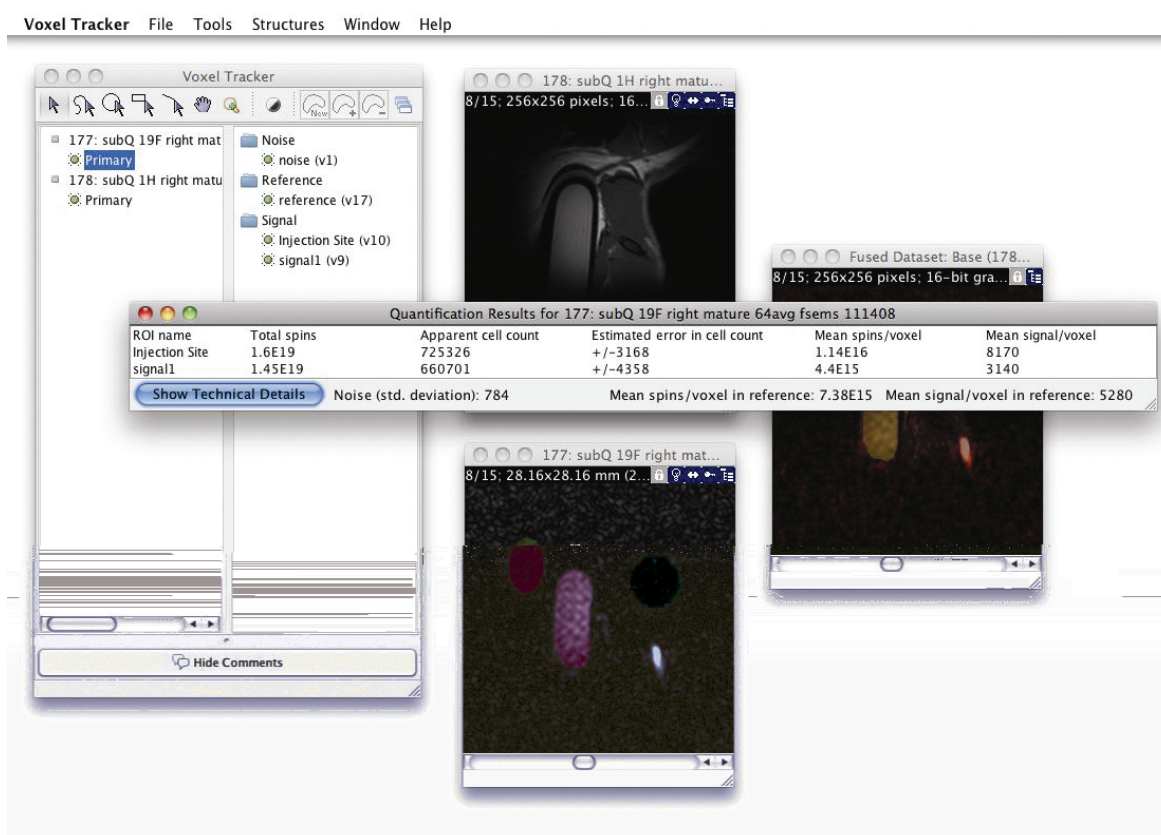


Figure 3. - *Voxel Tracker*™ workstation fuses and visualizes 19F/1H images, quantitatively analyzes apparent cell numbers in regions of interest, and manages imaging studies.

Summary

The accurate delivery of transplanted cells is fundamental to the efficacy and safety of cellular therapy. Tracking the delivery, migration, and persistence of cellular therapy with MRI is now possible using the *Cell Sense* reagent. The *Cell Sense* reagent is an ideal MRI probe for cell labeling due to its low toxicity, its high selectivity versus unlabeled tissues, its ease of use, and the ability to quantify the number of apparent cells in regions of interest.

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