

Overview:

The inflammatory process plays a role in the onset and progression of a variety of acute and chronic diseases. The quantification of inflammation using rapid *ex vivo* measurements or non-invasive *in vivo* imaging can facilitate research and aid development of novel therapeutics. V-Sense 1000H (V-Sense) is a perfluorocarbon emulsion (manufactured by Celsense, Inc.) which is phagocytosed by immune cells *in situ*, allowing detection of a general aspect of inflammation: immune cell infiltration to a distal location. A specific and quantitative label, V-Sense has the capacity to simplify and streamline experimental workflow and enable *in vivo* imaging, contributing vivid insight into the inflammatory process.

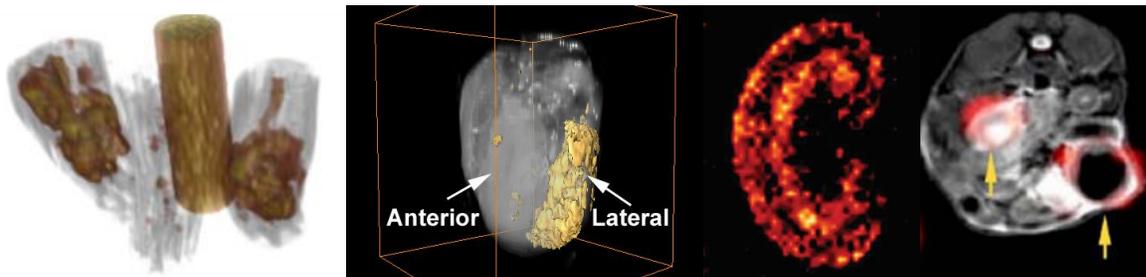


Figure 1. V-Sense applications: From left to right: Inflammation in the ankles of rat in an experimental arthritis model¹. Inflammation surrounding a porcine heart two days after an experimental myocardial infarction. Rejection of a transplanted kidney in a murine model². Inflammation in the ascending and descending colon in a mouse model of inflammatory bowel disease³.

Unmet Need:

Conventional approaches for scoring inflammation in animal tissue, such as histology, often lead to bottlenecks in the therapeutic discovery process. For disease models where external swelling is minimal, often the only method to assess inflammation is direct microscopic evaluation of excised tissue. There are two practical issues which arise using this method. First, the time and labor involved in the extraction, sample preparation, staining, and observation of even a single organ or tissue is substantial, contributing significantly to the cost of even small studies. Second, tissue extraction is typically a terminal procedure in small animal studies, drastically increasing the number of animals required to monitor disease and/or treatment efficacy over time. A non-invasive, quantitative measurement of disease severity would drastically improve the quality of inflammation-related datasets as well as reduce the costs associated with their acquisition.

V-Sense:

V-Sense was developed to alleviate laborious histological processing and allow an *in vivo*, non-invasive imaging option for the assessment of inflammation. V-Sense is a perfluorocarbon emulsion, formulated for direct injection, and optimized for use in conjunction with ^{19}F NMR and MRI methods. Following injection, the emulsion droplets, each laden with many detectable ^{19}F nuclei, are taken up by circulating leukocytes. These tagged cells participate in any inflammatory event *in vivo*, and become detectable only when concentrated at those sites. V-Sense does not actively target macrophage in the typical “lock and key” approach, but, through formulation for specific particle size and surface characteristics, a very selective “passive cell-targeting” mechanism is employed. V-Sense is eventually cleared from the body via the reticuloendothelial system and the lungs. V-Sense has been found to be safe in rodent and porcine animal models, even at doses exceeding 10% of blood volume. V-Sense is not recommended for use in canine models.

V-Sense Labels Host Leukocytes:

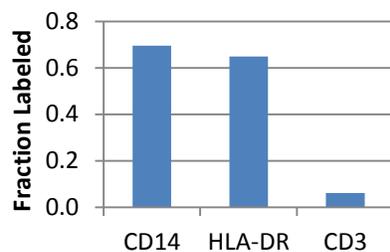


Figure 2. Cells labeled by V-Sense. Flow cytometric analysis of human mononuclear cells after co-incubation with fluorescent V-Sense. The fraction of cells labeled within subpopulations of cells expressing each surface marker is depicted. CD14^+ monocytes and HLA-DR^+ professional antigen presenting cells were labeled extensively, in contrast CD3^+ T cells.

Several studies have been performed that confirm the localization of V-Sense to host leukocytes. Incubation of a fluorescent version of V-Sense with human mononuclear blood cells *in vitro* with flow cytometric analysis identifies monocytes cells as the predominately labeled cell (Figure 2). Histological examination of inflamed tissues after administration of fluorescent V-Sense confirms the co-localization of reagent within host macrophages *in vivo*³. The “passive targeting” employed in V-Sense is highly specific for phagocytic immune cells. This mechanism allows investigators to take advantage of the endogenous cellular immune response to visualize inflammation on the cellular level: the host immune system delivers V-Sense to sites of active inflammation.

Reagent Detection:

V-Sense requires a period of 24 to 48 hours following inoculation to clear the blood stream and accumulate at sites of inflammatory reactions. Detection is performed using fluorine nuclear magnetic resonance (^{19}F NMR) and/or fluorine magnetic resonance imaging (^{19}F MRI). Both methods are non-destructive, allowing further investigation and/or confirmatory experiments on the same samples. Basal fluorine concentrations are negligible in biological tissue, leading to the absence of any appreciable background signal and enabling highly specific detection. As the amount of inflammation increases, the amount of V-Sense delivered to sites of inflammation increases. ^{19}F NMR/MRI

detection of perfluorocarbons is linear (Figure 3), and a linear relationship to the degree of inflammation in tissue has been measured in several models^{1,3}.

¹⁹F NMR used in conjunction with V-Sense is typically performed on intact, excised tissue. ¹⁹F NMR yields a count of the ¹⁹F nuclei in the sample, and, therefore, the extent of inflammation burden in that tissue.

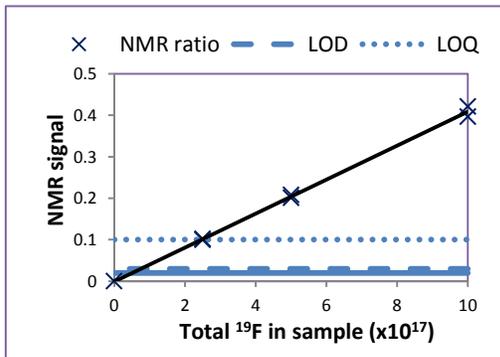


Figure 3. Quantitative measurements. The signal measured by ¹⁹F NMR is directly proportional to the amount of ¹⁹F present, quantified by inclusion of a chemically shifted ¹⁹F reference. ¹⁹F MRI signal is similarly quantitative, and is independent of tissue depth or composition.

Most liquid-state NMR systems are capable of detecting fluorine. It is important to note that quantitative information is obtained only when the repetition time of the NMR sequence is sufficiently greater than the longest magnetic relaxation time in the system to be studied. V-Sense 1000H has been optimized for fast recovery and has a T1/T2 of approximately 500/350 ms at 7T, allowing fast and accurate quantification. An entire rodent organ can be assessed for labeled cells in approximately 5-10 minutes.

V-Sense provides positive contrast for *in vivo* imaging of inflammation by MRI. ¹⁹F MRI is very specific, can be performed at high resolution, does not use ionizing radiation, is not limited by tissue depth, and can be run on most MRI setups with the addition of a fluorine-tunable RF coil. Once tuned to the correct frequency, the MRI instrument detects fluorine atoms specifically, creating a distinct and separate image from that of the anatomy (¹H MRI). Pre-labeling MRI scans are unnecessary, and anatomy can be inspected independently of inflammation. An overlay of ¹⁹F image onto the ¹H two image places inflammation in precise anatomical context. Non-invasive detection allows longitudinal study design with drastically fewer animals, and monitoring the same animal over time allows normalization of subject-to-subject variation inherent in many inflammation models. The images obtained depict the location of inflammation, but also contain quantitative information on the degree of disease/inflammation severity.

Applications of V-Sense

The inflammatory response underlies many disease states. Nearly any site of inflammation where macrophages are recruited from the circulation can be imaged. Reports of success include models of peripheral nerve inflammation⁴, inflammatory bowel disease³, myocardial infarction, pulmonary inflammation, bacterial infection⁵, rheumatoid arthritis¹, multiple sclerosis⁶, cerebral ischemia, transplant rejection², tumor inflammation, and acute injury⁷. The following examples illustrate the use of V-Sense and the quantitative nature of resulting data.

Rapid evaluation of inflammation in intact tissue

Often, higher-level information, such as the distribution of inflammation in a large tissue or in a whole organism is required. When imaging is not required for fine-scale details, V-sense can be

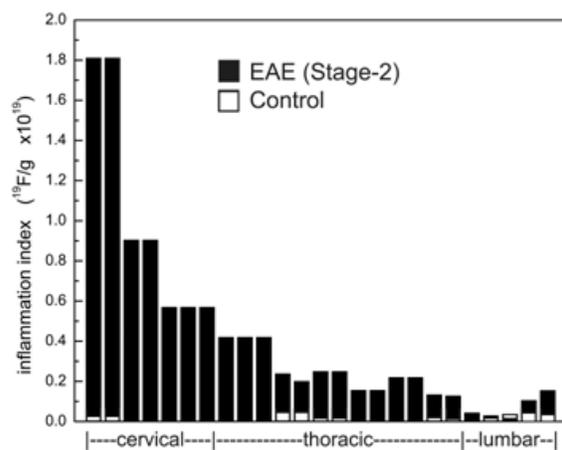


Figure 4. Profile of the inflammation burden along the spine in a rat EAE model. Per vertebra measurements of cell infiltration were made using ¹⁹F NMR in a fraction of the time required by typical histological methods.

used in conjunction with ¹⁹F NMR to assay cell infiltrate on a per-tissue basis. In this example, V-Sense is used to measure the inflammation profile in the spinal cord of a rodent model of multiple sclerosis, experimental allergic encephalomyelitis (EAE). The EAE model was generated in a DA rat using a single subcutaneous inoculation of isogenic spinal cord homogenate mixed with complete Freund's adjuvant. Clinical stage 2 EAE rats were intravenously injected with V-Sense, and intact, fixed segments of the spinal column were assayed for inflammation using conventional ¹⁹F NMR spectroscopy at 470 MHz. The inflammation index of each spinal cord vertebra was calculated as the number ¹⁹F nuclei per tissue weight. Control

animals receiving adjuvant alone had minimal V-Sense uptake. Data are the mean of n=3 animals. In this example, the total preparation and analysis time per animal was approximately 6 hours, representing approximately an order of magnitude in time-savings compared to conventional histological analyses⁶.

V-Sense: enabling non-invasive imaging of inflammation

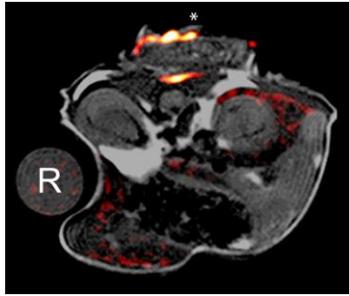


Figure 5. Visualization of the inflammation in an *in vivo* sponge-granuloma model. Shown is an axial slice of a $^1\text{H}/^{19}\text{F}$ fusion image, with the ^{19}F rendered in pseudo-color. 'R' is a ^{19}F reference capillary along the animal's torso.

A polyvinyl alcohol sponge disk was soaked in complete Freund's adjuvant and subcutaneously implanted dorsally in a C57Bl/6 mouse. A single intravenous injection of V-Sense was given on day 4. The anesthetized mouse was imaged on day 5 at 7T. Shown is a $^1\text{H}/^{19}\text{F}$ fusion image, with the ^{19}F rendered in pseudo-color. The data shows an intense concentration of macrophages labeled with V-Sense surrounding the sponge (asterisk). A small amount of V-Sense is also seen in the liver, a major clearance pathway. The short imaging acquisition, intensity, and resolution of the resulting images imply the potential use of V-Sense in a number of systems requiring more sensitive detection of inflammation.

Monitoring disease severity and therapeutic response with V-Sense:

Following two immunizations with Type II collagen, at the onset of clinical symptoms, V-Sense was administered to naïve and arthritic rats, followed by $^{19}\text{F}/^1\text{H}$ MRI of the hind limbs. Naïve rats lacked detectable ^{19}F signal in the joints, while diseased animals had strong accumulation of

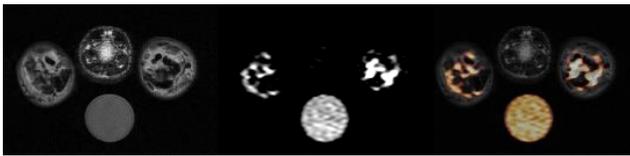
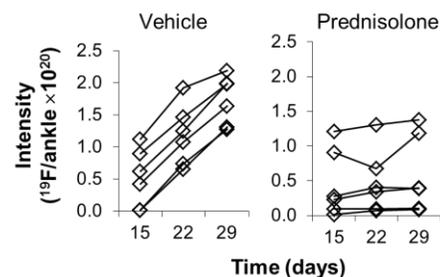
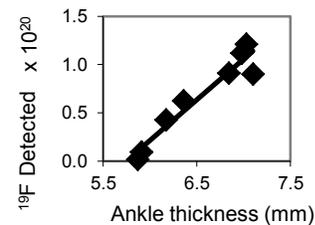


Figure 6. V-Sense in a rat model of rheumatoid arthritis. **Top:** From left to right, ^1H MRI of rat ankle anatomy, ^{19}F MRI of V-Sense accumulation in the joint, and the overlay of the co-registered images. **Upper right:** Strong, quantitative correlation between the ^{19}F signal intensity versus ankle swelling in diseased animals. **Lower right:** serial imaging of arthritic rats undergoing progressive disease (vehicle control) or treated with prednisolone resulting in resolution of the inflammatory process (both clinically and histologically) reflects the ability of V-Sense and ^{19}F MRI to quantitatively monitor changes in the disease process



the contrast agent. The high resolution images of inflammatory infiltrate (^{19}F signal) concentrates specifically in the bone interstitial space in the diseased limbs. Assessments correlating ^{19}F signal versus thickness of the joint, the standard clinical measurement of disease severity, provide strong evidence that this non-invasive biomarker measurement is an accurate indicator of the severity of disease. Serial imaging with sequential administrations of V-Sense depict this approach as an effective tool for monitoring the efficacy of therapeutic intervention and as a quantitative diagnostic tool for drug discovery¹.

Unique Features of V-Sense:

- Quantitative measure of inflammation
- Optimized ^{19}F MR detection moiety
- “Passive Targeting” of host immune cells
- High specificity, depth independent measurement
- Non-destructive measurements

V-Sense Enables ^{19}F NMR/MRI for:

- *In vivo*, non-invasive visualization of inflammatory sites
- Longitudinal evaluation of disease state
- Serial monitoring of response to therapy
- Rapid and accurate quantification of inflammation in intact excised tissue
- Use as surrogate measure of disease severity

Preclinical Applications:

- Autoimmune disease
- Cancer
- Cardiovascular disease
- Infectious disease
- Organ, tissue, and cell transplant rejection
- Biomaterial rejection
- Traumatic injury
- Other inflammatory conditions
- Drug discovery/drug development

Related Products:

V-Sense Dual-Mode: V-sense formulated with a green, red, or near-infrared fluorescent moiety, allowing optical detection (flow cytometry, fluorescence histology, fluorescence microscopy, etc.) for confirmatory experiments and/or *in vivo* measurement.

Voxel Tracker: A web-based software package which provides tools for superposition of the ^{19}F and ^1H MRI data and cell quantification. Enables study management and secure data sharing through cloud-computing.

Product Support: Celsense, Inc. provides detailed protocols for our products and live support through a team of PhD scientists. Our expertise enables researchers to focus on answering their hypotheses rather than technicalities. Celsense also invites proposals for contracted research.

References

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