

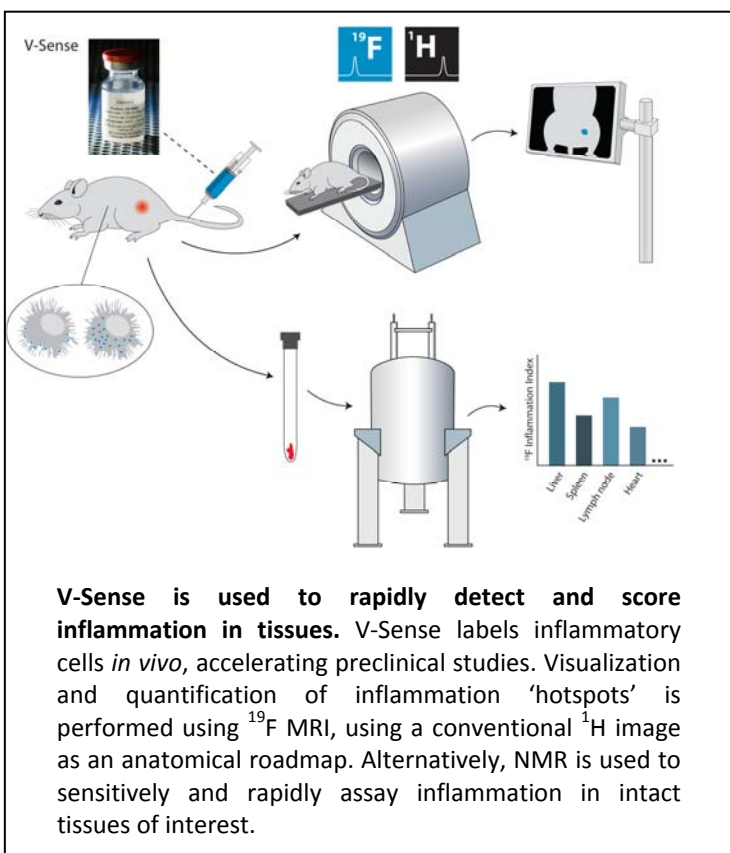
V-Sense

A versatile reagent to accelerate the quantification of inflammation in tissue



Overview

Conventional approaches for scoring inflammation in animal tissue, such as histology, often lead to bottlenecks in the therapeutic discovery process. V-Sense is an enabling reagent used to accelerate the detection and quantification of inflammation. It can minimize the use of laborious histological processing and yield accurate, fast, and quantitative measures of inflammation in intact tissues or *in vivo*. V-Sense is a fluorine-based 'tracer' agent used in conjunction with nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) instrumentation. The heart of V-Sense is a proprietary nanoemulsion containing tiny perfluorocarbon droplets. V-Sense is safe for direct intravenous injection into animals. Following injection, the nanoemulsion droplets are taken up by circulating leukocytes. The fluorine-tagged cells participate in any inflammatory event *in vivo*. When labeled cells accumulate at a site of inflammation, they become detectable using fluorine-19 (^{19}F) NMR in excised tissue (via biopsy or necropsy) or by *in vivo* MRI. The key advantage of using ^{19}F detection is that there is no background signal from the host's tissues, only labeled cells are detected. Quantification of the ^{19}F signal yields a fast, accurate marker of the degree of inflammation present. V-Sense can be used to detect a wide range of lesions and diseases where inflammation is a hallmark.



Biological indications provided by V-Sense:

- *In vitro* measurement of inflammation in intact tissue samples
- *In vivo* visualization of inflammatory sites
- Quantification of inflammation severity

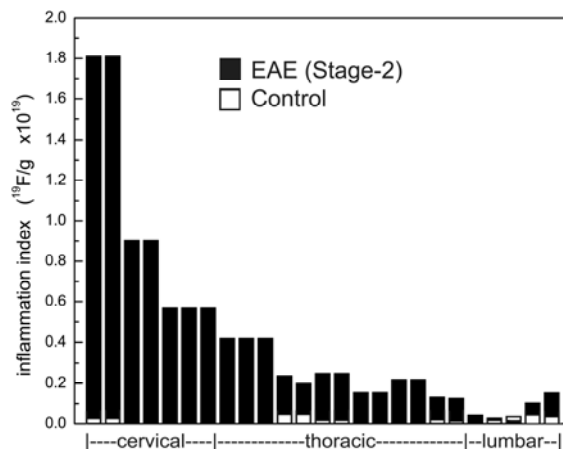
Preclinical applications of V-Sense:

- Autoimmune disease
- Cancer
- Cardiovascular disease
- Chronic obstructive pulmonary disease
- Infectious disease
- Organ, tissue and cell transplant rejection
- Traumatic injury

Rapid quantitative inflammation profiles of excised tissues

V-Sense was developed to accelerate the routine task of inflammation quantification in intact tissue specimens. Following inoculation with V-Sense, tissues of interest are removed from the animal. Inflammation can be assayed in two ways – by using conventional high-resolution NMR or by using MRI. In both methods, one detects the abundance of ^{19}F nuclei in tissue, contained within inflammatory cells. The measured ^{19}F is linearly proportional to the inflammation burden. Most standard NMR instrumentation can routinely detect ^{19}F . NMR provides a sensitive and cost-effective approach for quantifying the degree of leukocyte infiltration in tissue samples. This approach abrogates the need for time-intensive pathological staining and subsequent cellular quantification via microscopy. No special tissue preparation is required, except for an optional fixation step. Additionally, the use of NMR eliminates the potential for histological sampling bias and error, resulting in smaller, higher quality data sets. NMR analysis of excised tissues is non-destructive, and thus the same tissues may undergo conventional histological or biochemical analysis following NMR.

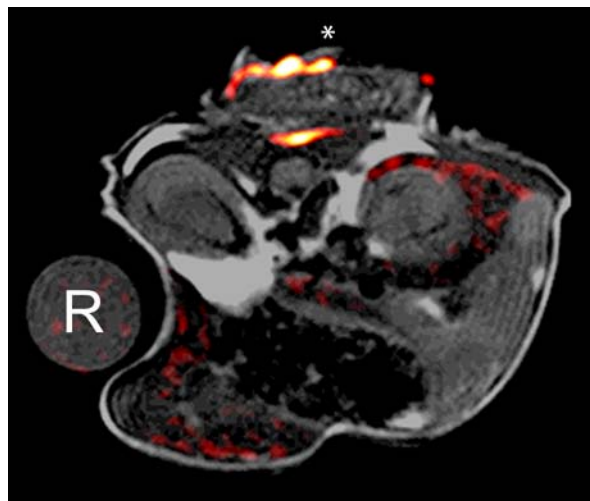
Example 1. V-Sense is used to measure the inflammation profile in the spinal cord (SC) of a rodent model of multiple sclerosis, experimental allergic encephalomyelitis (EAE). The EAE model was generated in a DA rat using a single subcutaneous inoculation of isogenic spinal cord homogenate mixed with Complete Freund's Adjuvant (CFA). Clinical Stage-2 EAE rats were intravenously injection with V-Sense, and 48 hours later intact, fixed segments of the SC were assayed for inflammation using conventional ^{19}F NMR spectroscopy at 470 MHz. The *Inflammation Index* represents the inflammation density of each spinal cord vertebra, calculated as the number ^{19}F nuclei per tissue weight. Also shown are control animals receiving CFA, but no SC homogenate, showing minimal V-Sense uptake. Data are the mean of n=3 animals. In this example, the total preparation and analysis time per SC was approximately 6 hours, representing approximately an order of magnitude in time-savings compared to conventional histological analyses.



V-Sense: an *in vivo* inflammation biomarker

V-Sense also enables non-invasive *in vivo* detection of inflammation 'hot-spots'. Using *in vivo* MRI, scientists can assay fewer animals, longitudinally, with no depth penetration limitations. This approach can be used to quickly score inflammation response to a therapeutic test article. V-Sense offers a vivid and dynamic approach to detecting inflammation within the anatomical context of a living subject — by combing inflammation-specific ^{19}F images with conventional, proton (^1H) MRI.

Example 2. V-Sense is used to detect inflammation in a sponge granuloma model using *in vivo* MRI. A PVS sponge disk was soaked in Complete Freund's Adjuvant (CFA) 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. 'R' is a dilute ^{19}F reference capillary along the animal's torso. Control animals with the sponge soaked in saline displayed negative ^{19}F signal after V-Sense administration. These data show V-Sense's high specificity for inflammation. Data courtesy of MIR, Inc.



About V-Sense

The principal ingredient of V-Sense (VS-1000) is a proprietary perfluorocarbon polymer that is designed for high ^{19}F NMR/MRI sensitivity and low toxicity. A novel formulation yields a stable nanoemulsion with a mean droplet diameter of approximately 145 nm and a 20% volume fraction in buffer. The perfluorocarbon has high chemical stability and is not degraded *in vivo*. The perfluorocarbon is both lipophobic and hydrophobic and does not incorporate into cell membranes. V-Sense is formulated for direct intravenous injection and a long circulation time in blood. This reagent is eventually cleared from the body via the reticuloendothelial system and the lungs. V-Sense is currently packaged in sterile vials with a 4 mL fill size.

Celsense also provides alternative formulations of the V-Sense nanoemulsion, manufactured with different perfluorocarbon molecules, including:

VS-580 (perfluoro-15-crown-5 ether)

VS-462 (perfluorodecalin)

Voxel Tracker™ software

The *Voxel Tracker™* software program, offered by Celsense, maximizes the potential of V-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 ^{19}F images. Powerful, built-in, computational tools enable inflammation quantification in regions of interest, thus enriching the utility of *in vivo* data. The software also serves as a platform for managing workflow and imaging studies. *Voxel Tracker™* is currently offered via convenient web-based access. For more information and demo see www.voxeltracker.com.

Celsense, Inc.

info@celsense.com

603 Stanwix Street Suite 348

Pittsburgh, PA 15222 USA

(412) 263-2870

www.celsense.com