Fluorine-19 labelling of stromal vascular fraction cells for clinical imaging applications

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PURPOSE: To develop a clinically suitable method to track autologous stromal vascular fraction (SVF) cell transplants with ^{19}F MRI, and to characterize the final ^{19}F labeled cell product as part of an IND resubmission for clinical trial NCT02035085.

METHODS: Adipose from healthy female patients undergoing liposuction was enzymatically digested in a GID SVF-1 tissue processor. Extracted SVF cells were incubated with the ^{19}F label CS-1000 for 4 hours and washed 3 times. Cells for flow cytometry were stained with antibodies against CD45, CD31, and CD45, along with isotype controls. To determine the ^{19}F content, cells were lysed and samples spiked with trifluoroacetic acid (TFA) in deuterium oxide. The -91.5 ppm CS-1000 peak was integrated against the -76 ppm TFA reference. Labeled SVF cells (0.5-2 million) were injected into a silicone breast phantom 5-10 mm below the surface and were imaged on a Siemens Trio 3 Tesla (T) MRI with a custom-built 4-phase array surface coil. A TrueFISP sequence for ^{19}F MRI included the following scan parameters: echo time/repetition time of 1.6/3.2 ms, 421 x 500 mm field of view, image size of 54 x 64, and 5mm slice thickness.

Table 1: SVF Characterization

	CD45+	CD34+	CD31+	CD45+/ CD34+	CD31+/ CD34+	CD45-/CD31- /CD34-
AVE	25.0	24.6	7.49	3.98	7.20	55.2
SD	15.8	12.5	3.3	3.47	4.41	23.4

Table 2: Characterization of CS-1000 labeled SVF cells

	Total Cells	CD45+	CD34+	CD31+	CD45-/CD31- /CD34-
CS-1000 + (%)	37.1	47.8	87.0	92.7	16.4
CS-1000 - (%)	62.9	52.2	13.0	7.3	83.6

RESULTS: Flow cytometry analysis of SVF cells from four patients (***Table 1***) found that 25.0% of cells were CD45+, 24.6% were CD34+, and 7.5% were CD31+, with 2.13×10^5 nucleated cells obtained per cc of fat. 3.98% of SVF cells were CD45+/CD34+ and 7.20 were CD31+/CD34+, while 55.2% expressed none of the queried markers. SVF cells were 37.1% positive for CS-1000 label (***Table 2***). Nearly all of the CD34+ and CD31+ cells labeled with CS-1000 but only 47.8% of CD45+ and 16.4% of CD45-/CD31-/CD34- cells took up CS-1000. The ^{19}F content was determined to be $2.8 \times 10^{12} \pm 2.0 \times 10^{12}$ atoms per cell by NMR. To confirm detection of cells in a clinically relevant setup, cells were imaged at 3 T, where two million CS-1000 labeled cells could be detected in a breast phantom at a depth of 5 mm (***Figure 1***).

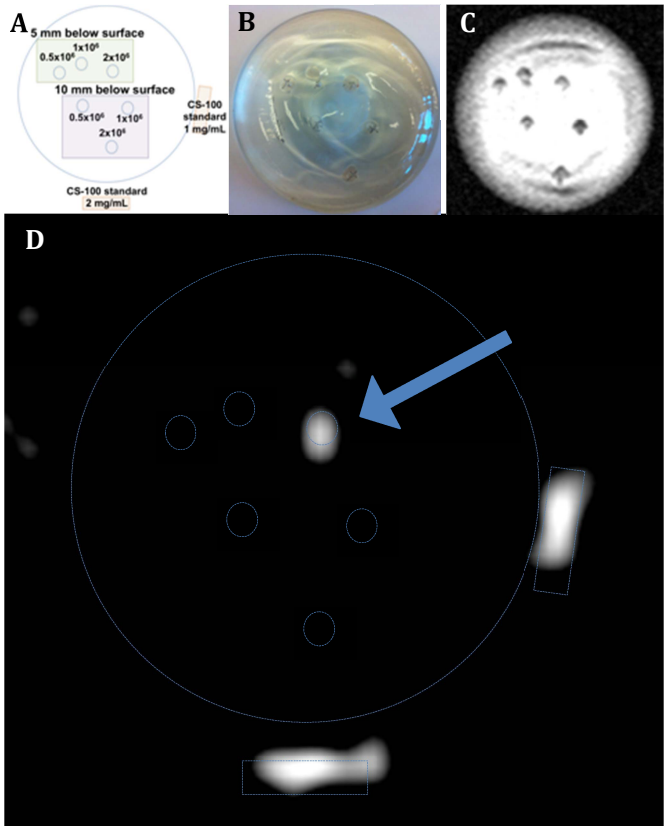

Figure 1: Outline of ^{19}F labeled cells in a silicone breast phantom (**A**, **B**) imaged with ^{1}H (**C**) or ^{19}F (**D**) MRI. Arrow denotes 2 million cells at 5 mm depth.

DISCUSSION: Detecting ^{19}F labeled cells with MRI is emerging as a suitable option for cell tracking, recently demonstrated with a dendritic cell vaccine in patients[1] but previous ^{19}F labeling studies have only investigated relatively homogenous cell populations. Increased efficacy and decreased costs make uncultured SVF cells a preferred option in experimental therapies,[2] necessitating investigation of ^{19}F labeling in highly heterogeneous cells. The near complete labeling of CD34+ indicates that despite SVF heterogeneity, ^{19}F labeling allows tracking of clinically significant stem cells. Previous pre-clinical SVF labeling studies with radiotracers[3] have not investigated subpopulations, despite the current finding that only 37.1% of SVF cells became labeled.

CONCLUSIONS: Clinically significant CD34+ stem cells were labeled with ^{19}F, enabling detection with ^{19}F MRI using a clinical set-up. Based on a successful same-day labeling protocol, ^{19}F-labeled SVF cells will be tracked in patients with radiation-induced fibrosis (RIF) from breast cancer radiotherapy treatment.

REFERENCES

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