

# Early Detection of Sepsis by Quantifying SPIO Uptake by LPS-activated Macrophages Using COSMOS

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**Introduction:** Sepsis is the leading cause of death in critically ill patients in the United States.<sup>1</sup> Current diagnosis of sepsis relies heavily on bacteria cultures and the patient's manifestation of septic symptoms. However, as the underlying biological changes of sepsis occur hours to days before the clinical presentation of symptoms, early detection of the biological changes will provide crucial opportunities for early diagnosis and treatment of sepsis.<sup>2</sup> As an alternative to existing techniques to detection, we propose using susceptibility-based MR imaging as a method of quantifiably measuring the activity of the immune system as a measure of sepsis progression. It has been observed that macrophages play a pivotal role in the early systemic response to infection, a condition characteristic of sepsis.<sup>3</sup> Further, it has been observed that phagocytosis by macrophage is a major mechanism by which nanoparticle-based contrast agents, such as Feridex, are cleared from the body.<sup>4</sup> By quantifying the amount of superparamagnetic iron-oxide nanoparticles uptaken by these macrophages and correlating this result to immune system response and the progression of sepsis, we can utilize commonly used contrast agents as marker for diagnosis and monitoring of sepsis.

This study offers an *in vitro* proof of concept; RAW264.7 murine monocytes were treated with lipopolysaccharide to induce a sepsis-like cell condition, were incubated with the FDA-approved contrast agent Feridex IV, and were imaged using the COSMOS technique (Calculation of Susceptibility through Multiple Orientation Sampling) for the quantification of iron.<sup>5</sup>

**Method:** Murine macrophages, RAW264.7 (ATCC, Manassas, VA), cultured for no more than 15 passages, were grown to 80% confluency in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS).  $1 \times 10^6$  cells were seeded, were allowed to incubate overnight at 37°C, 5% CO<sub>2</sub> and were subsequently activated by a treatment of E. coli-derived lipopolysaccharide (LPS) at a concentration of 100ng/mL in fresh media and allowed to incubate for 24 hours; untreated cells were used as negative controls. Feridex IV (Bayer AG, Leverkusen, Germany), a ferumoxide solution, was then added to the media at a concentration of 112ug of Fe/mL and allowed to incubate for 3, 6, 9, 18, and 24 hours. The media was removed by aspiration, and the cell monolayer was washed 3 times with Dulbecco's Phosphate Buffered Saline (DPBS) to remove free Feridex. Cells were trypsinized, collected and counted, and centrifuged into a pellet at the bottom of a 15mL centrifuge tube containing DPBS. The centrifuge tube was positioned in the center, lengthwise, of a 50mL centrifuge filled with DPBS, creating a cell pellet suspended in the middle of a uniform DPBS solution. The cell apparatus was scanned using a 3T Signa GE scanner (General Electric, Milwaukee, WI) with a homemade birdcage coil and using an EFGRE 3D sequence, 4 TEs 3.0/3.8/5.4/10.2ms, TR 30ms, Flip Angle 30°, bandwidth 31.25 kHz, at an isotropic resolution of 500µm<sup>3</sup>. Images were reconstructed and iron content was determined using the COSMOS methodology, as described in Liu 2008.<sup>5</sup>

**Results:** The total detected iron was obtained for each sample after 0, 3, 6, 9, 18 and 24 hours of Feridex incubation over a total of four independent trials; the results were scaled by the number of cells counted in each sample, and averaged over all trials (Figure 1). An estimation of the error of the mean was calculated with a confidence

level of 95%. Representative images of the intensity field (Figure 2), the relative difference in the intensity field, scaled against a reference scan (Figure 3), and the COSMOS reconstruction (Figure 4) are shown for select samples.

**Discussion:** Results from COSMOS reconstruction indicate a statistically significant difference (CI = 95%) in the average amount of Feridex uptaken per cell after 18 and 24 hours of incubation with Feridex; LPS-activated cells uptook, on average, 4.75 times more Feridex than non-LPS-activated control cells after an 18 hour period, and 4.81 times more after the full 24 hour period. Non-LPS-activated cells demonstrated a roughly linear rate of Feridex uptake throughout the 24 hour trial ( $R^2 = 0.977$ ), while LPS-activated cells exhibited a roughly linear rate of uptake in two distinct regimes, separated at a point shortly after 9 hours ( $R^2 = 0.933$  for first regime,  $R^2 = 0.999$  for the second regime). These results agree with previous literature concerning LPS-activated RAW264.7 cells, where it was observed that the presence of LPS, at a concentration of 100ng/mL, maximally activate the macrophages after a comparable delay, accelerating the production of NO products and increasing phagocytic activity of similarly sized SPIO nanoparticles, when compared to that of their inactivated counterparts<sup>4,6,7</sup>.

These observations demonstrate that differences in the phagocytic activity of two macrophage populations under differing conditions can be readily quantified using MR techniques; moreover, it demonstrates that this rate of uptake can be differentiated on a statistically significant level using COSMOS. The study strongly suggests that this technique can be expanded to the *in vivo* measurement of macrophage activity, whereby the administration of Feridex can be used as a marker to directly measure macrophage activity, providing an indicator of overall immune system function as a tool to diagnose systemic immune conditions, such as sepsis.

**References:** [1] Angus DC. Crit Care 2001; 29:1303-10. [2] Barrie PS. Surg Infect 2005; 6:41-54. [3] Gregory SH. J Leukoc Biol 2002; 72:239-248. [4] Fujii H. Magn Reson Imag 2002; 271-276. [5] Liu T. Magn Reson Med, in press. [6] Raynal I. Invest Radiol 2004; 56-63. [7] Schmidt HH. Mol Pharmacol 1992; 615-624.

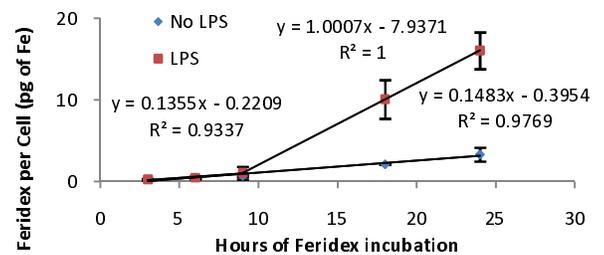


Figure 1. Amount of Feridex (added at a concentration 112ug of Fe/mL) uptaken, averaged by the total RAW264.7 population, with and without the presence of LPS (100ng/mL). Error bars indicate CI 95%. Data fit to linear trends.

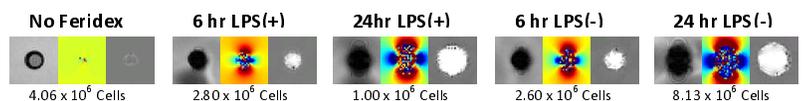


Figure 2. Traditional intensity field images (left), relative difference of the intensity field images scaled by the intensity of a reference phantom (middle), and results of COSMOS reconstruction (right), for cells untreated with Feridex, LPS activated cells with 6 and 24 hrs of Feridex incubation, and inactivated cells with 6 and 24 hrs of Feridex incubation.