In vivo multimodal tracking of macrophages labelled with Yeast Cell Wall Particles

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Introduction

Tracking cell migration requires a noninvasive imaging modality that has to be both sensitive and capable of yielding high-resolution images. Magnetic resonance imaging (MRI) is an outstanding technique for the *in vivo* visualization of labeled cells. Yeasts are cells whose membrane consists of β-1,3-D-glucan polymer associated with mannose-containing proteins and chitin. Such materials are well tolerated by living systems and can be processed into small fragments by macrophages. Moreover, β-1,3-D-glucan is an excellent targeting vector towards dectin-1 receptor, which is exposed on the membrane of several phenotypes of antigen presenting cells [1]. The peculiar chemical stability of yeast walls can be exploited to undertake a new loading procedure in which the inner cavity of the particle may act as a micro-reactor, thus allowing the formation of large size self-assembling systems (e.g. emulsions) that, once formed, remain entrapped in the particle.

Taking advantage of the high affinity of YCWPs towards immune system cells, Gd and rhodamine labelled macrophages have been tested in an *in vivo* inflammation model.

Methods

Dried YCWPs were prepared according to the published method [2] and then Rhodamine-DPPE-labeled YCWPs were prepared as follows: 20 mg of YCWPs were suspended in 5 mL of chloroform containing 20 µg/mL of Rh-DPPE. The suspension was left under stirring overnight in order to allow the dye to equilibrate between inside and outside the yeast shells. Afterwards, the particles were separated by centrifugation and the pellet, represented by the swollen YCWPs, was added with water. The sudden change in the solvent polarity induces the formation of an oil/water micro-emulsion (behavior observed also in the absence of YCWPs) that entraps the amphiphilic dye inside the particle core. The laser-scanning confocal image reported in Fig. 1 (right), overlaid to the phase contrast image, clearly shows the localization of the fluorescent dye inside the yeast shells that appear as pseudo-spherical particles with a hydrodynamic diameter of ca. 6 µm, as determined by dynamic light scattering measurements.

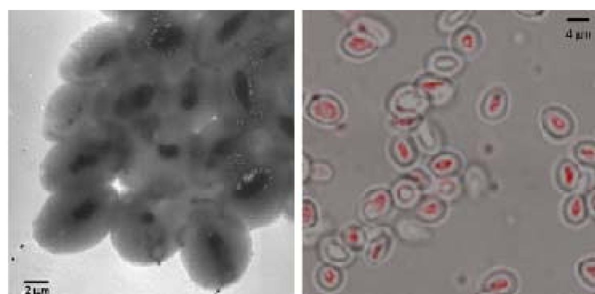

Figure 1 – TEM image (left) and confocal image (middle) of either Gd- or Rhodamine-PE-labeled YCWPs.

The same loading protocol was used replacing the fluorescent dye with the water insoluble paramagnetic complex Gd-DOTAMA$(C18)_2$ selected as a prototype of amphipathic MRI agents. In this case, a ten-fold higher amount of imaging label (2 mg/mL vs. 0.02 mg/mL) was used to balance the difference in the threshold detection between the two imaging techniques. Fig. 1 (left) reports a TEM image of the Gd-loaded YCWPs where the vesicle cores appear filled up with the metal-based emulsion.

Cell uptake experiments were carried out using J774A.1, mouse melanoma (B16-F10) and rat hepatoma (HTC) cell lines. MRI of labeled cells were performed on a Bruker Avance 300 NMR spectrometer and on an Aspect M2™ MRI scanner, operating at 1 T, using a standard multislice multiecho sequences.

Acute liver damage was induced by injecting 8-week-old male C57BL/6 mice with CCl_4, (1 mL/kg) intraperitoneally. MR images were acquired using a T_1-weighted, fat suppressed, multislice multiecho protocol and fat suppression was performed by applying a pre-saturation pulse at the absorption frequency of fat. Representative liver blocks were removed for histological analysis and slices were examined either using a standard light microscope or a ZEISS ApoTome fluorescence microscope.

Results and Discussion

It was estimated that each YCWP is loaded with ca. 1.6×10^7 Gd^{3+} ions, yielding a relaxivity per particle of 3.5×10^8 $s^{-1}mM^{-1}$, thus achieving, to our knowledge, the highest relaxivity ever measured for a Gd-based agent.

Cell based experiments, performed on J774A.1, B16-F10 and HTC cells have demonstrated the high affinity of YCWPs towards macrophages, showing also a fast uptake of the paramagnetic particles with a good temporal persistence of the contrast.

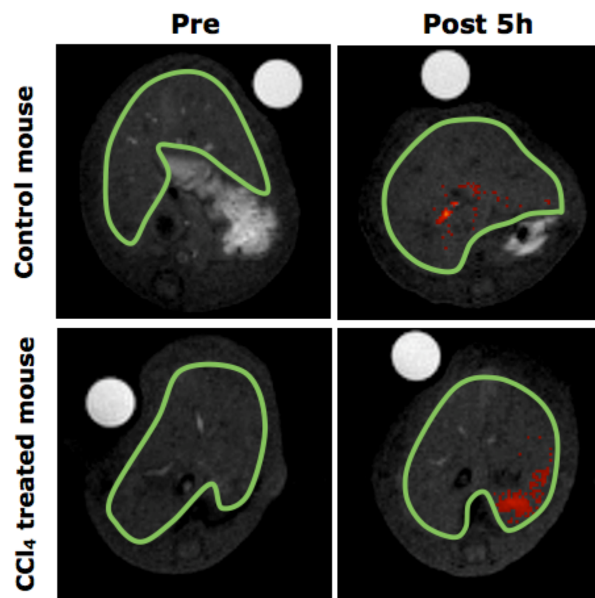

Figure 2 – Fat-suppressed T_1-weighted multislice multiecho MR images of C57BL/6 mice treated with CCl_4 or control group. Images were obtained before and after the injection of J774A.1 cells incubated with Gd-labeled YCWPs.

Using a model of severe hepatocellular liver failure, a marked increase in liver signal was observed after the iv injection of cells previously loaded with labeled YCWPs. The result that the tracked macrophages successfully reached the inflamed site was validated by histological microcopy. In the control groups, no significantly increase in the MRI signal was detected (Figure 2).

Conclusion

YCWPs represent a promising class of carriers for highly sensitive MRI probes. In addition, and analogously to other particulate systems, YCWPs can be loaded with lipophilic probes for other imaging modalities, and, moreover, can be functionalized to endow them with targeting abilities. Our labeling approach is efficient and allows the detection of macrophages in a damaged liver. Moreover, YCWPs can be used as immunomodulator per se or used in combination with several anti-inflammatory drugs, thus potentiating the effective therapeutic outcome.

References

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