Yeast Cell Wall Particles: a promising class of nature-inspired microcarriers for multimodal imaging

Sara Figueiredo, João Nuno Moreira, Carlos F.G.C. Geraldes, Silvio Aime, Enzo Terreno*

Supplementary material

Preparation of yeast cell wall particles

β-1,3-D-glucan shells were prepared as described elsewhere.[10] Briefly, Saccharomyces cerevisiae were suspended in a 1 M solution of NaOH and heated at 80ºC for 1 h under continuous magnetic stirring. The insoluble material was then collected by centrifugation and suspended in acidic water (pH 5) and stirred for 1 h at 55ºC. The insoluble material was collected again by centrifugation and washed in water, isopropanol and acetone. The product obtained was dried at room temperature to a fine powder.

Labeling of yeast cell wall particles

Dry glucan particles were incubated overnight at room temperature with a chloroform solution containing water insoluble Gd-DOTAMA$(C18)_2$ (Chart S1) or rhodamine-DPPE dye (Avanti Polar Inc, Alabaster, AL, USA), respectively. The resulting labeled particles were washed three times in PBS buffer, re-suspended in the same buffer and counted with a hematocytomer.

Characterization of yeast cell wall particles mean size

The mean size of yeast particles was determined by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS instrument (Malvern, UK) and corroborated by the analysis of TEM images, obtained on a Philips CM10 operating at 100 keV.

Confocal Microscopy

Confocal microscopy was performed with a confocal laser scanning microscopy system (CLSM) equipped with an argon-ion laser (Leica TCS SP5 (Leica Microsystems s.r.l.). All images were acquired using the same exposure time and

brightness/contrast settings.

Relaxometric characterizations of yeast cell wall particles

The water proton longitudinal relaxation times of YCWPs were measured using the inversion-recovery technique, on a Stelar Spinmaster Relaxometer operating at variable frequencies between 20 and 80 MHz and on a Stelar Field Cycling Relaxometer in the frequency range 0.01–20 MHz, at 25 ºC. The water proton longitudinal (T_1) and transverse (T_2) relaxation times of β-1,3-D- glucan shells were measured using, respectively, an inversion-recovery or a Carr-Purcell-Meiboom-Gill sequence. NMRD profiles were obtained over a range of magnetic fields from 0.24 mT to 1.6 T (0.01-70 MHz).

The measured T_i value (T_{iobs} and $R_{iobs}=1/T_{iobs}$) is the result of both paramagnetic and diamagnetic contributions, as shown by the equation 1.

$$R_{iobs}=r_i\times[Gd]+R_{idia} \text{ (eq. 1)}$$

where the relaxivity r_i is the paramagnetic relaxation rate enhancement per mM concentration of the Gd(III) species. The concentration of gadolinium in YCWPs was determined by ICP-MS after mineralization of the sample.

***In vitro* MRI experiments**

Glass capillaries containing YCWPs were placed in an agar phantom and MRI-T_1 and T_2-weighted images were performed on a Bruker Avance 300 spectrometer equipped with a microimaging probe operating at 7 T and on a portable Aspect (Netanya, Israel) operating at 1 T. The images were obtained using a standard T_1-weighted multislice multiecho sequence. T_1-weighted 1H-MR image acquired at 7.1 T (TR/TE/NEX (250/7.9/10), FOV 1 cm, 1 slice 3 mm), T_2-weighted 1H-MR image acquired at 7.1 T (TR/TE/NEX (5000/26.4/10) FOV 1 cm, 1 slice 3 mm), T_1-weighted 1H-MR image acquired at 1T (TR/TE/NEX (250/7.2/10), FOV 2 cm, 1 slice, 3 mm) and T2-weighted 1H-MR image acquired at 1T (TR/TE/NEX (5000/26.4/10), FOV 2 cm, 1 slice, 3 mm).

The T_1 measurement of particles was performed using a saturation recovery sequence and T_2 values were measured using a multiecho sequence (TR/TE/NEX 5000/3.3/1, number of echoes 500).

In vivo MRI experiments

C57Bl6 mice (Charles River Laboratories, Calco, Italy) were inoculated subcutaneously in the left flank with 0.2 ml of a suspension containing 1×10^6 B16 murine melanoma cells. B16 cells were obtained from ATCC (Manassas, VA, USA) and were grown in a DMEM medium supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Solid tumors formed in about 7–10 days were used for imaging evaluation. Prior to MRI examination, animals were anesthetized by injecting a mixture off tiletamine/zolazepam (Zoletil 100, Virbac, Carros, France), 20 mg/kg and xylazine (Rompun, Bayer S.p.a. Milan).

All MR images were acquired on a Bruker Avance300 spectrometer (7 T) equipped with a Micro 2.5 microimaging probe (Bruker BioSpin). The system is equipped with two birdcage resonators with 30- and 10-mm inner diameter, respectively.

T_2-weighted images were acquired using a RARE sequence (TR/TE/NEX 5000/3.3/4 rare factor 64). T_1-weighted, fat suppressed, images were obtained using a multi slice multi echo protocol (TR/TE/NEX 250/3.3/6). Fat suppression was performed by applying a pre-saturation pulse (90° BW = 1400 Hz) at the absorption frequency of fat (-1100 Hz from water). MRI was recorded before administration and 5, 24 and 48h after administration of contrast to evaluate the biodistribution.

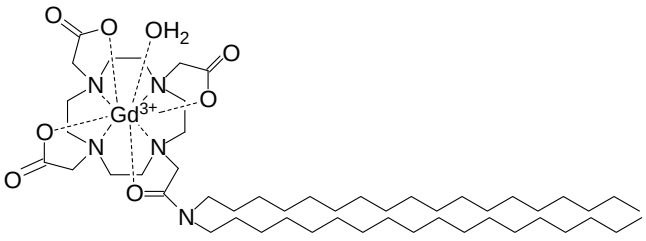

Chart S1. Gd-DOTAMA(C18)$_2$ complex.