POLYMERSOMES: A NEW TOOL IN THE ARMOURY OF CEST AGENTS

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Purpose

To test the potential of polymeric nanovesicles endowed with higher *in vivo* stability (polymersomes) as highly-sensitive paramagnetic MRI-CEST agents (PolymerCEST).

Introduction

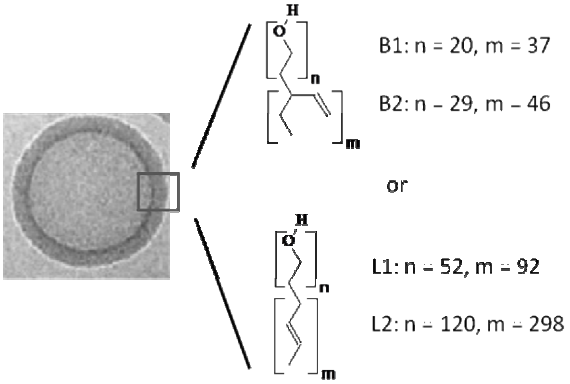
CEST (from Chemical Exchange Saturation Transfer) agents possess the unique property of yielding a "frequency-encoded" MR contrast that may allow the visualisation of different agents in the same image voxel.[1-3] It has been reported that the use of paramagnetically loaded liposomes can substantially improve the sensitivity (up to sub-nanomolar scale) of the system owing to the extremely high number of mobile intraliposomal water protons, properly shifted by the presence of an encapsulated paramagnetic Ln(III)-based shift reagent (SR), that can be selectively saturated.[4] *In vivo*, liposomes are quite avidly taken up by macrophages (RES, circulating or tumor-associated), and this event is highly detrimental for the detection of CEST contrast. Polymersomes (nanovesicles made of amphiphilic diblock copolymers)[5] display much higher *in vivo* stability because, differently from liposomes, their bilayer can be fully pegylated.[6] To our knowledge, the use of polymersomes as nanoplatform for MRI applications has not yet been explored.

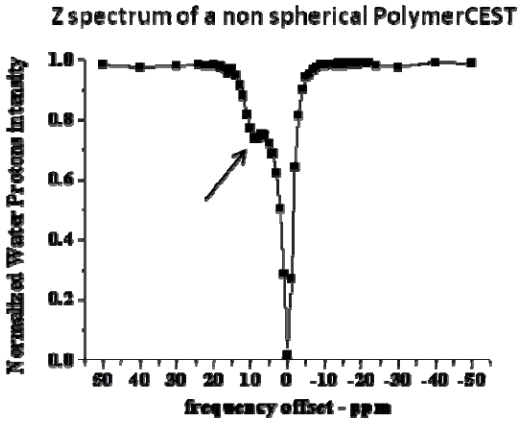
Methods

Polymersomes were prepared by using the thin film rehydration method,[5]followed by extrusion on polycarbonate filters (pore diameters 200 nm). The non encapsulated material was purified by exhaustive dialysis. The hydrodynamic size of the vesicles was determined by dynamic light scattering measurements. The mean diameter of the polymersomes lies in the 200-250 nm range. Relaxometric measurements were performed at 0.47 T on a Stelar Fast-Field Cycling instruments. CEST experiments were carried out at 7 T on a Bruker Avance 300 spectrometer equipped with a microimaging probe.

Results and Discussion

First of all, the water permeability, P_w, of polymersomes made of different diblock copolymers (based on PEG-poly-butadiene copolymers) have been determined by relaxometric measurements upon encapsulation of Gd-HPDO3A. The used copolymers display noticeable differences either in their length or in their structure (linear, L, or branched, B for the hydrophobic component, see the scheme below). Water permeability values were inversely proportional to the bilayer thickness. They are in the range of the values measured for liposomes made of DPPC and POPC/Chol (the two typical membrane formulations for LIPOCESTs), thus providing support to the view that polymersomes may be used as CEST agents. In analogy to the different classes of LIPOCEST agents proposed so far, three different PolymerCEST agents were prepared: i) a spherical one encapsulating, as shift reagent, a high concentration of Tm-DOTMA, ii) an osmotically shrunken one encapsulating Tm-HPDO3A, and iii) an osmotically shrunken one encapsulating Tm-HPDO3A and incorporating an amphiphilic paramagnetic shift reagent (see the Z-spectrum reported below). The chemical shift of the intravesicular water protons were in all cases similar to those observed for the analogous LIPOCESTs. Furthermore, also the sensitivity displayed by these systems was in the nanomolar range. Finally, the stability of this new class of nano-sized CEST agents against macrophages activity has been tested *in cellulo* and *in vivo* on a tumor (melanoma) mouse model.





References

[1] J. Zhou and P.C.M. van Zijl, *Prog. NMR Spectr.* **2006**, *48*, 109-136.
[2] M. Woods, D.E. Woessner, A.D. Sherry, *Chem. Soc. Rev.* **2006**, *35*, 500-511.
[3] S. Aime, C. Carrera, D. Delli Castelli, S. Geninatti Crich, E. Terreno, *Angew. Chemie Int. Ed.* **2005**, *44*, 1813-1815.
[4] S. Aime, D. Delli Castelli, E. Terreno, *Angew. Chemie Int. Ed.* **2005**, *44*, 5513-5515.
[5] B.M. Discher, Y-Y Won, D.S. Ege, J.C-M Lee, F.S. Bates, D.E. Discher, D.A. Hammer, *Science*, **1999**, *284*, 1143-1146.
[6] P.J. Photos, L. Bacakova, B Discher, F.S. Bates, D.E. Discher, , *J. Controlled Release*, **2003**, *90*, 323-334.