Characterization of an experimental setup to study *in vitro* permeability properties of macromolecular contrast agents by DCE-MRI

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Purpose:
Measurement of microvascular permeability in tumors is of high interest particularly regarding assessment of tumor neoangiogenesis and delivery of therapeutical molecules. Evaluation of permeability is typically performed by dynamic MRI studies after administration of contrast agents (CA). These CA have to be tailored to provide optimum sensitivity for detection of therapy induced permeability changes or to be representative for permeability of specific macromolecular therapeutic agents. In addition, for molecular MRI, new CA targeting tumor cells have to be tested for their permeability. Recently, an experimental setup based on a bundle of hollow porous fibers [1] was developed for *in vitro* evaluation of CA permeability under reproducible conditions. The aim of this study was to characterize the device with respect to flow effects and the ability to distinguish extravasation rates of paramagnetic CA of different molecular weights (MW).

Materials and Methods:
A CellMax® hollow fiber module (Spectrum Labs, USA), mimicking porous capillaries, was connected to a peristaltic pump and a reservoir containing CA diluted in bidistilled water (Fig. 1). Dotarem® (Guerbet, France) [$MW/r_1/r_2$ = 0.5kDa/4.2mM^{-1}s^{-1}/5.1mM^{-1}s^{-1}], Vistarem® (Guerbet, France) [$MW/r_1/r_2$=6kDa/7mM^{-1}s^{-1}/66mM^{-1}s^{-1}] and P846 (Guerbet, France) [$MW/r_1/r_2$=3.5kDa/17mM^{-1}s^{-1}/23mM^{-1}s^{-1}] were studied on an animal MR scanner (Biospec®, Bruker, Germany) at 4.7T. Concentration of each CA was scaled according to r_1 relaxivities. For evaluation of permeability properties, 300mL of the CA solution was pumped through the system at constant flow (5mL/min) yielding a 1h kinetics. One slice at the input and a second one in the fiber containing system, where CA could extravasate, were acquired simultaneously with a dynamic SR-MGE-SNAP (Saturation-Recovery Multi-GradientEcho SNAPshot) sequence [1]. After one global saturation the two slices were acquired at different saturation delays, 200ms and 750ms (voxel=0.6×0.75×2.0mm^3, Δt=1.4s). Aliquots (2mL) were taken at the output of the fiber module every five minutes and their R_1 was measured by an IR-SE sequence. Measurement of R_2*(t) with this sequence allowed R_2* correction of T_{1w} signal intensities, and R_1(t) could be obtained even for CA with high r_2 relaxivity. Extravasation rate k_{R1} was calculated assuming exchange between intra- and extrafiber compartments and obtained by fitting an exponential curve ($f(t) = A(1-e^{-k_{R1}(t-t_{lag})}) + C$) to R_1(t). Furthermore, for Dotarem®, the effect of different flow rates (F=1.0, 1.5, 2.0, 3.0, 5.0, 6.0, 7.5 mL/min) on k_{R1} was assessed.

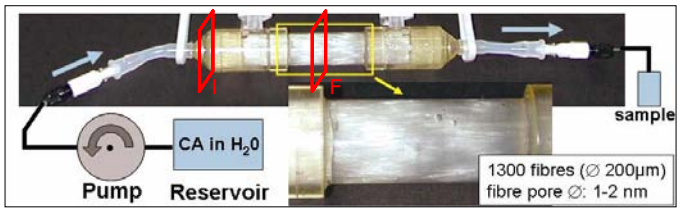

Fig. 1: A hollow fiber module was connected to a peristaltic pump and a reservoir containing CA diluted in bidistilled water. 2 regions were followed during kinetics: the input region (I) and the fiber-containing region where CA were allowed to extravased (F). Samples can be taken at the output.

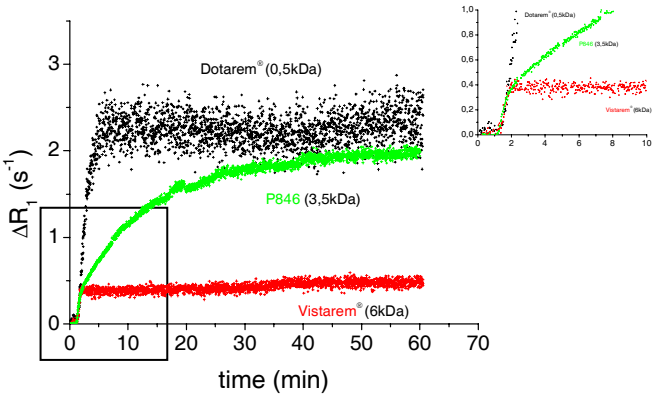

Fig. 2: ΔR_1 time courses: ΔR_1 of Dotarem® reached quickly a plateau at 2.8s^{-1} while ΔR_1 of Vistarem® after an initial fast increase due to fiber filling, rose very slowly which is compatible with a quick extravasation for Dotarem® and rather no extravasation for Vistarem®. P846 showed an intermediate behavior.

Finally, in order to estimate the pore surface fraction of the fiber module, simulations were carried out assuming two compartments separated by a 10µm thick porous membrane, one containing a constant arbitrary concentration of a molecule and the other one containing initially no molecules. Diffusion driven evolution of the system was simulated and k_{R1} values were obtained for different pore fractions. Knowing the diffusion coefficient of the CA in water (assumed to be 2.6x10^{-10}m^{2}s^{-1} for Dotarem®) and the measurement of k_{R1} in the fiber module, simulations led to an estimation of the pore fraction.

Results:
Flow dependency measurements for Dotarem® yielded flow independent k_{R1} values for F ≥ 6.5mL/min (95% of asymptotic value, Fig. 3). Simulations based on k_{R1}(7.5mL/min) provided an estimated pore fraction of 5%.
Fig. 2 shows R_1 evolution for all CA in the fiber region. Extravasation rate for Dotarem® was k_{R1}=(0.52±0.05)min^{-1} and for P846 k_{R1}=(0.098±0.010)min^{-1}. Vistarem® obviously extravasated much slower and k_{R1} could not be determined from the exponential fit.

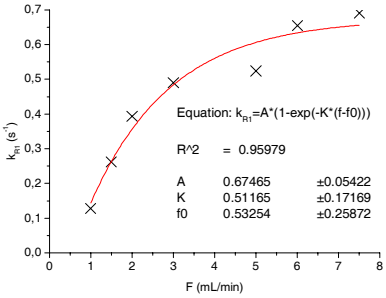

Fig. 3: k_{R1} for different flow rates

Discussion:
A first characterization of the fiber module was presented demonstrating the feasibility of studying permeability properties of macromolecular CA. Extravasation rate constants for Dotarem®, Vistarem® and P846 were compatible with their MW. As flow rate is an adjustable parameter in this system, for each contrast agent, experimental conditions can be set to different flow or permeability limited regimes. In conclusion, the experimental setup allows comparison of permeability characteristics of CA which should help to optimize MW and structure of new CA before performing *in-vivo* measurements.

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References: [1] Heilmann M *et al*. Proc. ESMRMB 2006; 26.