Fluorinated Cyclodextrin as a Novel ^{19}F Contrast Agent for Labeling Cells

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Introduction: Imaging modalities which are able to track the proliferation of cancer cells are especially important for metastasizing tumors like Ewing's sarcoma. ^{19}F-MRI is highly specific for the tracking of cells labeled with substances containing fluorine, as in biological systems no fluorine signal is present that can be detected with conventional MRI. Cyclodextrins (CDs) are a group of chemical compounds with several properties that make them interesting as novel potential MR contrast agents. CDs can be fluorinated with a high content of ^{19}F and can be made soluble in water. It was shown previously for other unfluorinated CDs that they penetrate cells and can be used as transfection agents [1].

Methods: Fluorinated β-cyclodextrin (βCD) was synthesized on-site. The molecular structure is shown in Fig. 1. Each molecule contains 21 ^{19}F atoms that contribute to a single spectral line at a total molecular weight of 2.7 kDa. This is advantageous as in fluorinated compounds which contain many ^{19}F atoms the signal is often spread over a wide frequency range due to strong J coupling. For labeling $2x10^6$ Ewing's sarcoma cells (cell line VH-64) were incubated in 11 ml of cell culture medium which contained 0.5 or 1 mmol/l cyclodextrin for 24 hours at 37°C in a 5% CO_2 humidified atmosphere. In different incubations the influence of adding 20 µl of a lipophilic transfection reagent (TA) (Roti-Fect, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and/or 9µl mercaptoethanol (ME) on the cell viability and βCD uptake was investigated. After incubation cells were washed repeatedly with βCD-free medium, trypsinized and centrifuged. The resulting cell pellets were placed in 2 ml Eppendorf caps and a small amount of βCD-free medium was added.
^{1}H and ^{19}F MR images as well as ^{19}F NMR spectra were acquired. The MR measurements were performed on a 3T clinical whole-body MRI scanner (Intera Quasar Dual, Philips Medical Systems B.V., Best, The Netherlands) equipped with a double resonant ^{19}F/^{1}H rat coil with an inner diameter of 7 cm.

Results: From spectroscopic measurements on a solution of βCD in water T_1 of the ^{19}F NMR signal was determined to be approx. 140 ms. A ^{19}F NMR spectrum is shown in Fig. 2. ^{19}F MR images could be acquired with a 2D gradient echo sequence with TE/TR=2/24 ms and flip angle of 35° with a 2x2 mm resolution and slice thickness of 30 mm in a total measurement time of 12 hours (Fig. 2). Most ^{19}F uptake was observed in cells that were incubated only with 1mmolar βCD, reduced uptake was observed in the presence of the lipophilic transfection agent. ME was not tolerated well, the cells that were incubated with ME were dead after incubation. The results are shown in table form in Fig. 4.

Discussion: Our results show that βCD is a promising candidate for new contrast agents for cell labeling. Ewing sarcoma cells could be successfully labeled by incubating them with a solution of βCD. The short T_1 of the ^{19}F-signal of βCD as well as its high content of ^{19}F contributing to a single spectral line allow for fast imaging and SNR-efficient measurements with short repetition times. At a clinical MRI scanner with relatively low field strength of 3T ^{19}F MR images of the labeled cells could be acquired.
Furthermore, CDs can be functionalized and they can be used as a host molecule that can form an inclusion complex with a guest molecule. In future, CDs could be used to transport additional fluorinated compounds or markers for other imaging modalities.

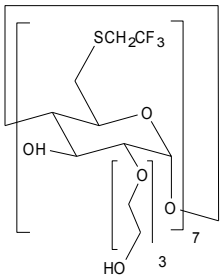

Fig. 1: Chemical structure of βCD

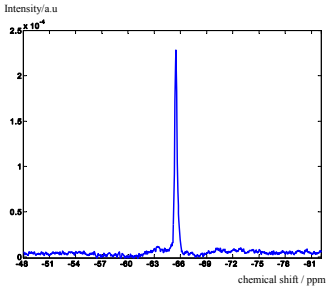

Fig. 2: ^{19}F NMR spectrum of βCD

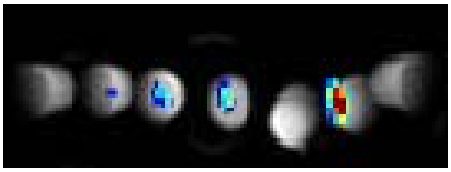

Fig. 3: ^{1}H MR image of tubes with labeled cells (grayscale) and ^{19}F MR image (colored scale). Tubes contained (from left to right): 0.5mmolar βCD + ME + TA, βCD + TA, βCD + ME + TA, βCD + ME, control w/o βCD, βCD, 0.5 mmolar βCD + TA (see text for abbreviations)

βCD	ME	TA	viability	βCD uptake
1 mmolar	+	+	-	+
1 mmolar	-	+	+	0
1 mmolar	+	-	-	+
1 mmolar	-	-	+	++
0.5 mmolar	+	+	-	-
0.5 mmolar	-	+	++	-

Fig. 4: Uptake of βCD and cell viability for different incubation media.

References: [1] SA Cryan, J. DRUG DEL. SCI. TECH., 14 (1) 57-62 2004