Feasibility study of localized oxygen consumption measurements in a microcavity array based 3D cell culture system by ^{19}F MRI

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Target audience: nX-Nuclei in Practice

Purpose: The application of new drugs in the clinical stage can result in toxicities like mitochondrial dysfunction[1]. A drug-induced mitochondrial dysfunction decreases the oxygen consumption of cells. Monitoring the oxygen consumption of cells in a well-controlled environment (bioreactor system[2]) represents an optimal method to detect drug-induced mitochondrial dysfunction already in the preclinical stage. The combination of a bioreactor system with ^{19}F spectroscopic T_1 measurements of perflubron was previously used[3] to measure the oxygen consumption during a perfusion stop. For the detection of a drug-induced mitochondrial dysfunction, a control experiment is always needed. By measuring locally T_1, one compartment of the bioreactor system can be used as a control experiment and in the second compartment a drug can be applied. Therefore, this study compares T_1 mapping with a spectroscopic T_1 measurement and verifies the feasibility of T_1 mapping in the bioreactor system during a perfusion stop in the presence of a bacterial contamination which was induced by an insufficient heat sterilized emulsion.

Methods: A 9.4T preclinical small animal scanner (Bruker Biospec 94/20, Ettlingen, Germany) combined with a custom built rectangular loop ^{19}F surface coil were used for the experiments. The bioreactor[2] was equipped with an empty polymer chip (DYNARRAYS, 300MICRONS GmbH, Karlsruhe, Germany) and was actively perfused (400 µl/min) under normoxic conditions (Fig.1). A heat sterilized emulsion consisting of 40% v/v perflubron, 2% w/v perfluorodecylbromide (both Apollo Scientific, Bredbury, UK), 2% w/v soybean lecithin (dm-drogerie markt, Karlsruhe, Germany) and distilled water was prepared and 15ml was added to 65ml cell culture medium. An insufficient heat sterilized emulsion resulted in a bacterial contamination. Therefore, an increase of T_1 from (1655.9±13.5)ms (~14% oxygen concentration)[3] to (2857.6±29.9)ms (~0% oxygen concentration)[3] at the polymer chip inside the bioreactor was measured. The influence of the contamination on this feasibility study could be disregarded as no oxygen was present at both bioreactor compartments leading only to elevated T_1 values.

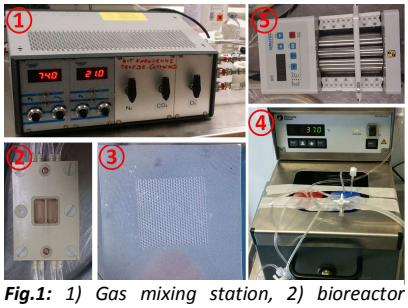

Fig.1: *1) Gas mixing station, 2) bioreactor housing, 3) polymer chip (DYNARRAYS(R)), 4) water bath and 5) perfusion pump are shown*[3].

For T_1 mapping a FAIR-RARE sequence (T_R=16s, T_E=47.7ms, 2 avg., 20 T_I values, 16 RARE factor, 32x18 matrix and FoV 5.8x5.8cm^2) and for non-localized T_1 measurements an inversion recovery sequence (T_R=16s, 25 T_I values and 2 avg.) was used, followed by a 3-parameter least squares fit. The T_1 values of both sequences were compared during active perfusion and only T_1 mapping was done during a perfusion stop to verify the possibility of localized oxygen consumption measurements. The perfusion influence was determined by subtracting the T_1 values of the last T_1 map and the first T_1 map of the perfusion stop measurement. The slice for the T_1 mapping was placed on pre-measured localizing images (FLASH sequence: T_R=100ms, T_E=7.5ms, 60 avg., 256x256 matrix and FoV 5x5cm^2). Both sequences measured the T_1 value of the CF_3 resonance. The shown T1 maps include only the area of the two compartments (red box Fig.1).

Results/Discussion: Fig.2 shows a ^{19}F localized coronal image of both bioreactor compartments, a T_1 map and a T_1 fit uncertainty map during active perfusion. The mean T_1 value was (2804.2 ± 36.6)ms, excluding the first and last row, whereas for the non-localized T_1 measurements a value of (2840.7 ± 32.1)ms was measured. The T_1 uncertainty map (Fig.2) indicates elevated uncertainties at the inlet and outlet (first and last row) of the two bioreactor compartments. Also, these T_1 values were prone to large fluctuations between succeeding measurements. Therefore, the first and last row was disregarded for the T_1 map of the perfusion stop experiment. A good correlation of the T_1 values between both sequences could be obtained but increased T_1 uncertainties were measured at the upper and lower edges. These resulted from large signal variations at the first T_I increments leading to increased fit uncertainties. The reason for the increased uncertainties could be a larger perfusion influence combined with partial volume effects of the inlet and outlet tubes. Nevertheless, a homogenous T_1 map (Fig.2) was obtained where residual fluctuations arose from different perfusion and temperature influences excluding the middle column which suffered from partial volume effects of both compartments.

Switching off the perfusion allowed for determining the influence of the perfusion on each voxel (Fig.3). The upper voxels experienced a larger T_1 change and for the left compartment the influence of the perfusion was almost identical for all voxels. The time until T_1 was constant was 29.82min. After the influence of the perfusion was gone the T_1 uncertainty of the disregarded rows decreased (Fig.3) and the T_1 values in each compartment were almost identical but elevated values at the lowest row were measured (Fig.3). For the conversion of T_1 values to oxygen concentrations during active perfusion, the influence of the perfusion is of importance whereas for the oxygen consumption measurement only the time until T_1 is constant is important. The influence of the bacterial contamination on the perfusion stop experiment was negligible as the mean T_1 value (2906.9±43.8)ms of Fig.3 corresponded to (-0.13±0.96)% oxygen concentration (assuming T=38°C)[3]. Therefore, no oxygen could be consumed by the bacteria (no additional T_1 change). The decrease in the T_1 uncertainty of the disregarded rows confirmed that the signal variations came from the influence of the perfusion. Therefore, these T_1 values might also be included in the determination of the local oxygen consumption. The residual differences in the T_1 values should come from slight deviations in the local temperature especially at the lowest row.

Conclusion: These results demonstrate the possibility for a separate T_1 measurement in each bioreactor compartment despite the small size of each compartment. As a result one compartment can be used as a control experiment and in the other compartment a drug can be applied. The next step is to induce a mitochondrial dysfunction in one compartment and measure locally the oxygen consumption of each compartment.

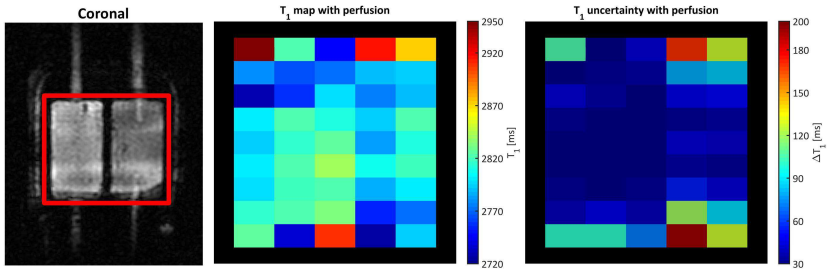

Fig.2: *The left localizer image shows the separation of both compartments (distance 1mm). The red box indicates the area of the shown T_1 maps. The T_1 map includes the disregarded rows for the perfusion stop experiment which were prone to large variations between succeeding scans and large fit uncertainties whereas the middle column contained both compartments.*

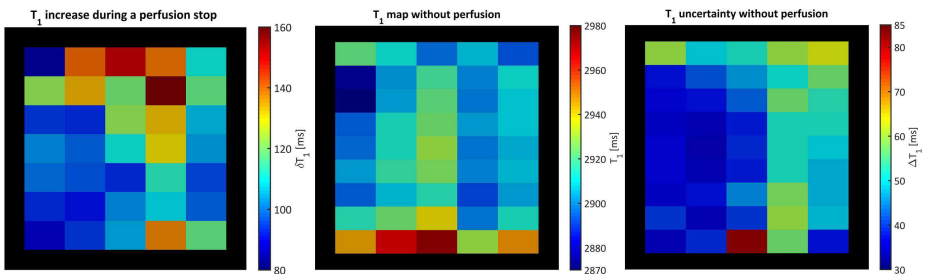

Fig.3: *Larger T_1 changes were observed at the inflow area of the compartments (left image). The T_1 map without perfusion shows only small variations in the T_1 values in each compartment and decreased uncertainties in the disregarded rows (right image).*

References: 1) Hussaini et al, Expert Opin Drug Saf 2007,6:673-684; 2) Gottwald et al, Z Med Phys,2013,23:102-110 3) Kleimaier et al, ISMRM,2018,1518