^{23}Na Magnetic Resonance Spectroscopic Imaging (MRSI) on a High-Density 3d-cell culture on chip (3^{D}-KITChip)

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Introduction

Cellular functions such as signal transduction and the regulation of cellular proliferation strongly depend on the sodium (^{23}Na) concentration gradient across the cell membrane [1]. The Na/K ATPase pump is playing a major role in maintaining this gradient [2]. To understand the underlying mechanisms of the Na/K ATPase it is necessary to observe the sodium concentration in a controlled living environment. Sodium magnetic resonance spectroscopic imaging (^{23}Na – MRSI) has the potential to monitor ionic concentrations *in vivo* without disturbance of the biological environment. Experiments like in [3] mostly suffer from uncontrollable variations in physiological parameters and therefore a tissue resembling phantom with manually adjustable physiological parameters is desired. Up to now, no adequate *in vitro* setup has been available to keep a cell culture alive and to allow influence on substantial parameters like pH, temperature and composition of the culture medium during MRSI experiments. In this study we use a setup which is a further development of the setup used in [4] and has therefore two separated compartments which can be perfused independently (c.f. Fig. 4).

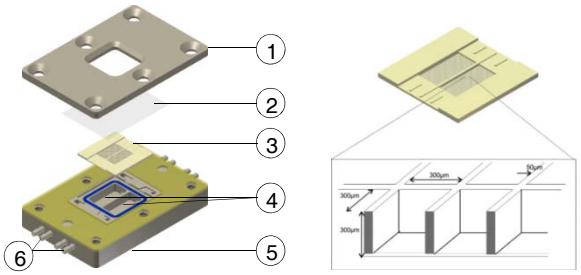

Figure 1: Left: Exploded view of a 3^{D}-KITChip-containing MRI-compatible bioreactor. 1=upper lid, 2=microscope cover slip, 3=3^{D}-KITChip, 4=independently perfused compartments, 5=bioreactor housing, 6=connection for perfusion. Right: 3^{D}-KitChip with flat bottom (f-3^{D}-KitChip) in detail.

Materials and Methods

Bioreactor: The Bioreactor (Fig. 1 left) is equipped with the f-3^{D}-KITChip (Fig. 1 right) and supplied with cell culture medium via pump outside the MRI scanner room.

Cell culture: Hep G2 (ATCC, HB-8065, Manassas, VA, USA) cells are passaged according to standard procedures. Briefly, 3x10^{6} cells in a drop of 75µl cell culture medium are placed on one half of the micro structured grid (c.f. Figure 3) and incubated as described in [4]. Minimal Essential Medium (MEM) with the following additives is used for the experiments: 10% FCS (fetal bovine serum), 1% Pen/Strep, 1% Na-Pyruvate, 1% Glutamax, 1% non-essential amino acids, 0,1% Phenol red

MRI Experiments: All MRI experiments where performed on a 9.4 T small animal scanner (Biospec®, Bruker, Germany). ^{1}H imaging was performed by use of a T_1 weighted RARE sequence the following parameters: T_R = 3000 ms, T_E = 67.7 ms, FoV = 2.8x2.8 cm, Number of Averages = 32, RARE factor = 144, acquisition time = 1 min 36 s. ^{23}Na – MRSI experiments were performed by use of a CSI sequence with FID readout with the following parameters: T_R = 120 ms, acquisition delay T_{AC} = 0.38 ms, number of scans = 1200, sampling time for each FID = 400 ms, FoV = 32x32x40 mm³, matrix size = 25x25x15, number of averaged images = 8, time for each scan = 10 min, spatial resolution = 2x2x4 mm³, acquisition time = 25 min

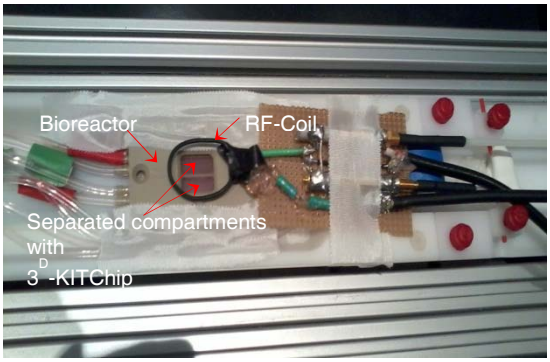

Figure 2: Bioreactor with custom made ^{23}Na surface

Results and Discussion

Figure 2 shows the complete setup for the ^{23}Na measurements. The bioreactor can be placed on the heated animal bed of the scanner which ensures, with the heated medium supply, a constant temperature for the cell culture. The custom made surface coil is placed directly on top of the cover slip. Therefore, the distance between coil and reactor is minimal. A proton image of the 3^{D}-KITChip within the reactor is shown in the left side Figure 3. As can be seen, the micro cavities can be resolved by ^{1}H – MRI. Our obtained ^{23}Na – CSI signal is shown on the right side of Figure 3. The image has been zero filled to reach a resolution of 0.2x0.2x4 mm². It can be seen that the ^{23}Na – CSI sequence is capable of resolving the two compartments. However, the ^{23}Na image shows no difference between the upper, cell loaded, and the lower reference compartment. The time domain analysis of the single voxels showed that our experiment suffers from partial volume effects. These effects arise from the relatively big voxel size compared to the size of the chip cavities, cause oscillations in the time domain signal, affecting the estimation of T_2* as well as the analysis in the frequency domain and cancel out the intensity differences between loaded and unloaded proportion of the chip.

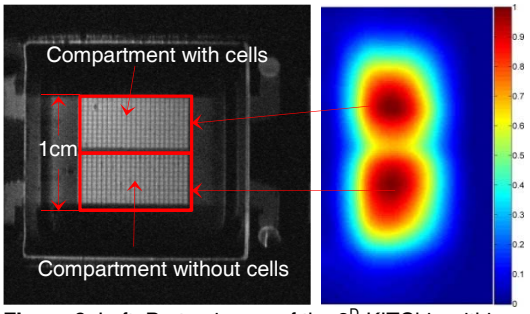

Figure 3: Left: Proton image of the 3^{D}-KITChip within the bioreactor. Right: Corresponding ^{23}Na – CSI signal of the two compartments.

Conclusion

In this experiment we show that we are capable of performing *in vitro* ^{23}Na – MRSI with a tissue like cell culture. Parameters like temperature, pH, and the composition of culture medium can be well controlled. The design of the reactor with its two compartments allows us to vary these parameters in just one compartment while the non-varied compartment serves as internal reference. The availability of an internal reference is of crucial importance for the quantitative, time dependent analysis of cellular processes. To sum it up, we present a unique setup with a tissue like sample in a completely controllable environment within the MR scanner.

References

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