Phase contrast based MR Microscopy of glial tumor cells using microcoils

N. Baxan[1], U. Kahlert[2], H. Weber[1], M. Mohammadzadeh[1], J. Hennig[1], and D. von Elverfeldt[1]
[1]Diagnostic Radiology, Medical Physics, University Hospital, Freiburg, Germany, [2]Stereotactic Neurosurgery, University Hospital, Freiburg, Germany

INTRODUCTION

The contrast mechanism employed for differentiating structures in micron-scale samples is of great interest especially when is combined with high-resolution MRI and an adequate SNR. In this study, phase contrast together with the SWI [1] technique were performed for imaging living glial tumor cells using MR surface microcoils. Biochemical spectroscopy investigations were performed as well within a timeframe not detrimental for preserving cells viability.

MATERIALS AND METHODS

Patient derived primary cell line established from an intra-operative resection of malignant (WHOIV) glial tumor was used for assessing the microcoils applicability in analyzing living cells. The cell line was established as a neurosphere assay with EGF and bFGF growth factors (20 ng/ml). Immediately after dispensing the cells (10^5 cells/µl in ~ 2 µl medium), the sample container was closed with an adhesive film. Experiments were performed on a 9.4T Bruker Biospec system using a 500µm diameter surface microcoil developed by Bruker Switzerland. 2D GE (T_2^* weighted, TR/TE=400/10 ms, resolution 10x10x200 μm^3, scan time 13 min 39 s) and 2D SE images (T_1 weighted, TR/TE=700/16 ms, resolution 15x20x200 μm^3, 17 min 57 s scan time) were acquired. GE data acquisition was followed by reconstruction and processing of the phase images to remove unwanted background phase variations [2]. Spatial high-pass filtering was used for preserving the small-scale phase variations caused by local cell structure (Fig 1c). Moreover, the contrast was enhanced by combining phase and magnitude images as described in the susceptibility weighted imaging (SWI) technique [1]. Maximum intensity projection (MIP) was computed on 400µm (2 slices) on both pure phase images (Fig 1d) and SWI images (Fig 1e). Cells metabolic profile (Fig 1g) was established by non-localized spectroscopy methods since the microcoil active volume has already one voxel size (*one pulse*: TR =1800 ms, 512 averages, scan time 15 min 21 s). The water signal was suppressed by variable power RF pulses with optimized relaxation decays (VAPOR) [3]. The removal of residual water components was performed in a pre-processing step using the Hankel-Lanczos Singular Value Decomposition algorithm-HLSVD (*http://www. mrui.uab.es/mrui*).

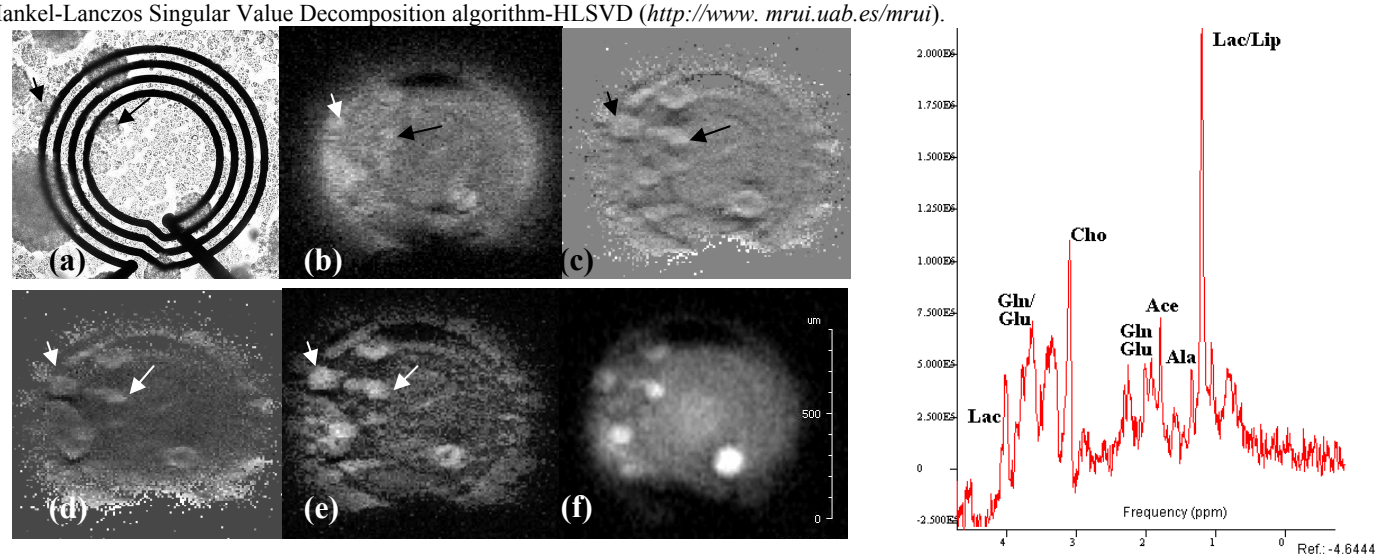

Figure 1: (a) Light microscopy image of glioma cells as they are distributed on the microcoil surface. Single-cell diameter is below 10 µm and clusters have a diameter between 100-120 µm. T_2^* weighted GE (b) magnitude (c) phase image (d) MIP on phase image and (e) on SWI image of cells. (f) T_1 weighted SE coronal view of cell clusters with the imaging slice positioned approximately 30 µm above the surface of the coil. As expected, both pure phase images and SWI images yield higher contrast than magnitude GE images (see arrows). (g) Metabolic profile of primary cultured glioma cells acquired in 15 minutes. Several metabolites are represented: lactate, choline, glutamate, glutamine and alanine.

RESULTS/DISCUSSION

Cell clusters are clearly depicted in the phase based MRI images (Fig 1c,d,e) proving that phase imaging provides high contrast between cells and medium after a relatively short scanning time. Positive frequency shifts of 4.49±0.9Hz were measured within the neurosphere structure. Since phase information is not directly dependent on T_1 and T_2 parameters [4], it may also provide quantitative measurements of cell structure properties. T_1 values of each cell cluster can be determined as well based on the high-resolution T_1 weighted images (Fig. 1f). Taking into account that the measured microcoil sensitive volume is 0.12 µl [5], the metabolic profile of a small amount of cells (~$1.2x10^4$ cells) within this volume was possible after 15 minutes of scan time. Minimum linewidth at half height was of 13 Hz (Cho). Increased Choline (3.2 ppm) signal was observed, which could be related to an elevated tumor cell growth [6] and increased Lactate (1.3 ppm) signal.

CONCLUSION

The presented results demonstrate our ability to obtain high-resolution images of glial tumor cells in a reasonable acquisition time for preserving cell viability. Our method combines the benefits of exploiting the phase MR signal for contrast enhancement and the sensitivity optimization by using MR microcoils. While NMR extraction protocols often require 10^8 cells [7], MR microcoils proved their efficiency to record spectra from much smaller sample volume. Further studies will be performed to correlate possible metabolite variations with the degree of tumor malignancy.

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