Lab-on-chip for NMR microscopy: description and application for neurospheres imaging and spectroscopy studies

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Introduction

Extensive and continuous monitoring is one of the main challenges in the study of cell cultures and organotypic tissues. We have developed a lab-on-chip device allowing monitoring different parameters, as temperature, pH and pO2 or pCO2 by using optical fibres (Patent: PCT_ES2011_070173_ISRWO). Additionally, optical, fluorescence and confocal microscopy can be used together with NMR microscopy and spectroscopy. The aim of this communication is to describe the lab-on-chip and demonstrate the possibility of obtaining high resolution images and spectra at very low volumes by using NMR microscopy in a single neurosphere.

Subjects and Methods

A set of neurospheres, from sub-ventricular zone (SVZ) of postnatal rats (P0-P5) and at passage 3, were introduced in the lab-on-chip system (Figure 1). The neurospheres were allowed to growth and differentiated by a continuous flow of DMEM growth medium (180 nl/minute) (Figure 2) the temperature was monitored by a fibre optic (LUXTRON 16521812). A surface coil of 500 μm of diameter (Figure 1, lower right part), allowing the insertion of the lab-on-chip device, and a set of gradients of 60 Amp were used for obtaining images and spectra in a Bruker 600 MHz instrument.

Gradient Echo sequences were used for obtaining NMR microscopy images: i) two with TE and TR 15 and 172 ms, respectively, and 64x64 (43s) and 128x128 (7m 16s) matrix with a resolution of 16 x 16 x 150 um; and ii) one with a matrix of 256 x 256 (2h 43 s) with 8 x 8 x 50 um resolution and TE = 7 ms and TR = 100 ms. PRESS with VAPOR water suppression was used for SV spectra acquisition, TE and TR were 12 and 1500 ms, respectively, and 0,2 x 0,2 x 0,2 mm dimensions with a nominal volume of 8 nl were used. (Figure 6)

Results and Discussion

Highly resolved images were obtained for the single neurosphere developed in the lab-on-chip system by using a 500 μm surface coil (Figure 3).

Theses images allowed to clearly differentiate heterogeneous parts, the surface and necrotic centre, of a ca 350 um neurosphere located in the field of view of the coil. Although with limited S/N, different resonances were initially assigned in the growth medium and in the neurosphere (Figure 4). In particular, high levels of lipids were observed in the necrotic centre of the neurosphere.

Conclusions

Different regions and metabolic information from a single neurosphere can be obtained by the combined used of a particular lab-on-chip developed for 500 μm surface coils and NMR microscopy and spectroscopy.