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 organotipic tissues. We have developed a lab-on-chip device allowing monitoring different parameters, as temperature, pH and pO_2 or pCO_2 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 and172 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.

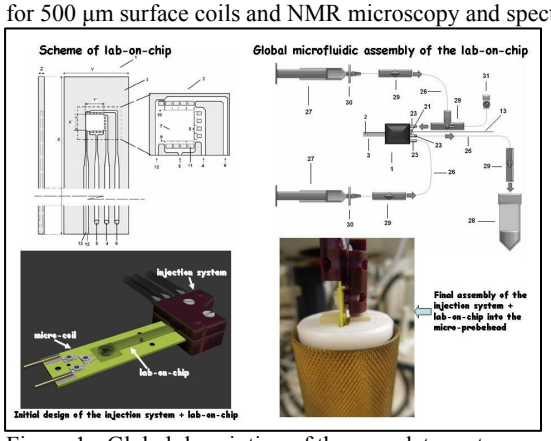

Figure 1.- Global description of the complete system lab-on-chip + injection device + microcoil + probehead

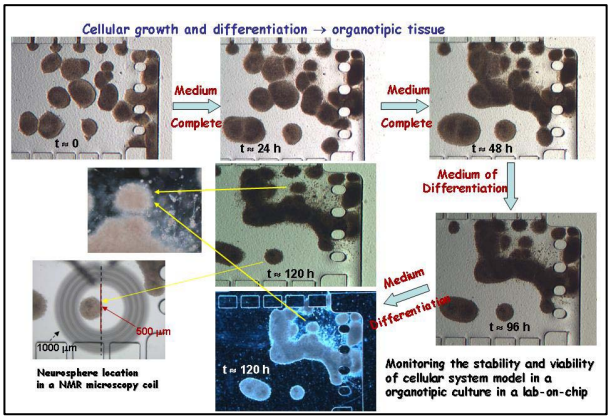

Figure 2.- Example of cellular growth and differentiation inside of the lab-on-chip under temperature control by optical fiber.

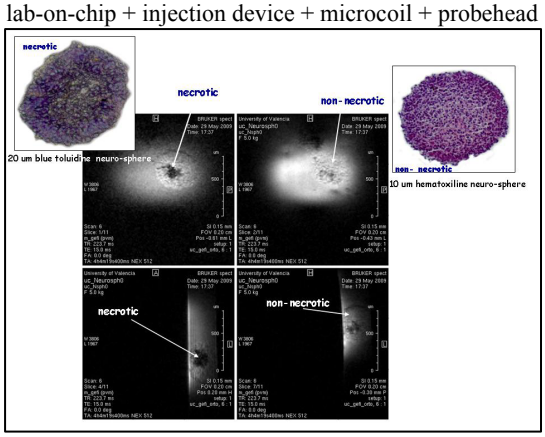

Figure 3.- Example of two slices (150 um) axial (upper part) and sagital (lower part) of a neurosphere of ca.350 um growth inside of the lab-on-chip and obtained by using a 500 um microcoil at 14 T.

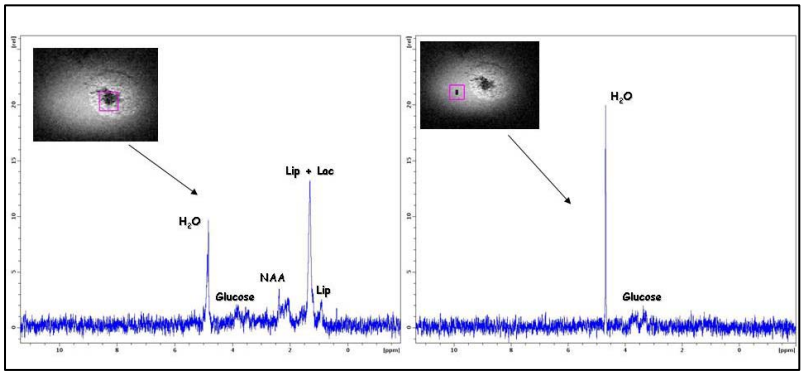

Figure 4.- Examples of spectra of two square SVs of 8 nl dimensions obtained by PRESS at 14T and using a 500 um microcoil. Initial metabolite assignments are shown