A cell perfusion system for MR spectroscopic investigations with horizontal magnets

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Magnetic resonance spectroscopy (MRS) of perfused cell systems is a useful method to investigate a variety of physiological problems. With the growing importance of in vivo MRS in biomedical studies, complementary examination of perfused cell systems can contribute to a better understanding of in vivo data. Up to now, perfused cells were almost exclusively studied using vertical high-field magnets for two reasons. On the one hand, no tomographs suitable for spectroscopy were available when initial studies of this kind were conducted. On the other hand, vertical magnets are generally better suited for this purpose due to spectral dispersion, field homogeneity and adjustability of probe temperature.

We present a cell perfusion system for MRS using MR tomographs (horizontal magnets). The compact construction of the system secures easy handling. A constant perfusion temperature (± 0.1 °C) was achieved by integrating all necessary elements into a temperature-adjusted unit, while a solenoid coil was used to improve the S/N ratio, compared with vertical magnet systems.

Keywords: MRS, cells, perfused cells, perfusion apparatus, 31P MRS (phosphorus MRS), 19F MRS (fluorine MRS), in vitro MRS.

INTRODUCTION

MR spectroscopic studies of living organisms have been performed since the early 1970s. Besides a variety of physiological problems, the main subjects were tumor biology and therapy. Designs comprise in vitro measurements of perfused tumor cells [1-12], a multitude of in vivo animal experiments [13-17] as well as clinical trials in patients [18-24].

In particular, MRS of perfused tumor cells has proven efficient in investigating different biochemical problems. Thus, Glickson et al. [13] were able to demonstrate response patterns of tumours to different therapies. Up to now, standardizable studies of this kind could only be performed with vertical high-field systems and therefore were restricted to adequately equipped centres specializing in MR.

The introduction of MRI into clinical practice has increased the number of tomographs suitable for spectroscopy, and thus, for experimental purposes.

The usual field strength is between 1.5 and 4 T. However, shortcomings compared to high field systems are limited spectral dispersion, probes not adjustable to temperature and poorer S/N ratio. Perfusion systems as used with vertical magnets by D.L. Foxall [8] and others are inadequate for horizontal systems without adjustment. Stray fields are considerably larger than in vertical systems, due to the horizontal main B0 axis. Thus, depending on the size of the stray field, all ferromagnetic objects (e.g. peristaltic pumps, thermostats) must be placed at a certain distance from the magnet. Constant defined study conditions are rendered very difficult by the resulting longer tube connections with increased heat and gas exchange.

When constructing our perfusion system, we had to consider and compensate for these shortcomings as far as possible. Requirements were as follows:

(1) easy application of substances and collection of samples;
(2) compact construction, good handling;
(3) suitability for use of solenoid coils;
(4) constant and controlled temperature inside the perfusion chamber;
(5) sterilizable.

The result was a compact system for MRS of perfused
cells with horizontal magnets under defined conditions. The construction will be described and some experiments presented to demonstrate the efficiency of the system.

MATERIAL AND METHODS

The perfusion system was developed for a Bruker Biospec 24/40 with 2.4 T field strength, but can be used with any horizontal magnet system with a minimum bore of 25 cm. The only materials used were PVC and glass. The construction is shown in Fig. 1. The perfusion unit is mounted on a PVC plate fitted with all components, including the measuring coil, but not the thermostat, peristaltic pump and medium reservoir.

Fig. 1. The entire system including the coil and the adapter switch.

Perfusion unit — construction

Components of the perfusion unit (Fig. 2):
- heat exchanger no. 1 (A)
- oxygenator (B)
- heat exchanger no. 2 (C)
- bubble trap (D)
- perfusion chamber (E)
- fiberoptic thermometer (K)

These are contained in a temperature-adjusted PVC chamber (F) supplied with warm water via silicon tubes by a thermostat pump outside the HF cabin.

Description of components

Heat exchanger. The heat exchangers consist of a glass cooling coil with 10 turns persistently circumcirculated by warm water from the heating cycle. The first exchanger is used to heat up the perfusion medium prior to oxygenation to nearly the same temperature as in the perfusion chamber. This avoids the gradual formation of gaseous carbon dioxide (CO₂) or oxygen inside the temperature perfusion chamber.

Oxygenator. The oxygenator is composed of a 6 m gas-permeable Silastic catheter (Dow Corning 602-235) wound around a 10 cm wide temperate PVC tube within a persistently Carbogen gassed chamber (95% O₂ 5% CO₂).

Bubble trap. Consists of two parts, the lower connected with the perfusion chamber and the second heat exchanger. The upper threaded tube is closed by a screw cap with a piercable membrane, permitting application of substances and collection of samples directly before the perfusion chamber.

Perfusion chamber. Two glass tubes were used for construction. A ground-in joint in the inner tube (20 mm) permits easy removal of the chamber from the unit. The embedded cells are positioned on fritted glass melted into the lower third. The perfusion chamber is closed by a glass stopper for exit of the medium. Fritted glass in the stopper prevents exist of the gel cell beads.

To minimize heat loss within the perfusion chamber by room-temperature air, the inner tube is insulated with a second glass tube (32 mm). This outer chamber is also supplied with warm water (39°C) via a thermostat.

Fiberoptic thermometer. Used to measure perfusion temperature. A photoconductor was introduced in the perfusion chamber via a hole in the silicon membrane of the bubble trap (Fig. 2K).

The thermometer was developed at the University of Moscow in connection with the agreement on mutual scientific and technological exchange with the former Soviet Union, and makes continuous measure-