A gel-encapsulated bioreactor system for NMR studies of proteins/nucleic acids in living mammalian cells

Overview: In the in-cell NMR applications, the use of gel-encapsulated bioreactors ensures that the NMR experiments are conducted with stable, metabolically active cells [1, 2]. Although there are differences among existing bioreactors' designs, they all are based on the conventional concept of flow-through NMR tube [1-3]: A fresh growth medium continually flows into the cells encapsulated in a biocompatible gel matrix, while metabolic by-products are removed. Compared to the setup of an in-cell NMR experiment based on pelleted cells, a bioreactor's use brings about several advantages. The bioreactor maintains cell viability for up to 24 hours (time-consuming in-cell NMR experiments can be performed) [1-3]. The bioreactor's use also maintains a stable intracellular environment (compared to in-cell NMR spectra recorded using pelleted cells, the in-cell NMR spectra recorded using bioreactor have increased sensitivity and resolution [4,5]). Noteworthy, the bioreactor not only removes toxic by-products of cell metabolism but also removes target molecules that might "leaked" from compromised cells in the course of the in-cell NMR experiment. As Luchinat et al. [6,7] illustrated, the bioreactor might be used to deliver drug-like molecules, in a controllable manner, to the encapsulated cells, which allow real-time (quantitative) in-cell NMR studies of intracellular drug binding/unbinding kinetics.

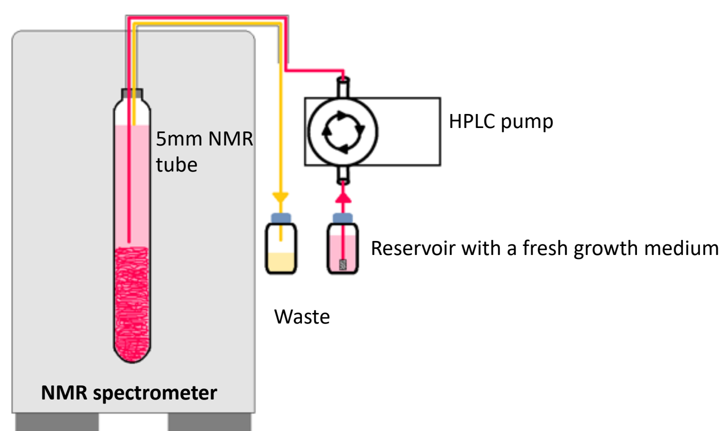

Figure 1. Generalized scheme of a bioreactor system.

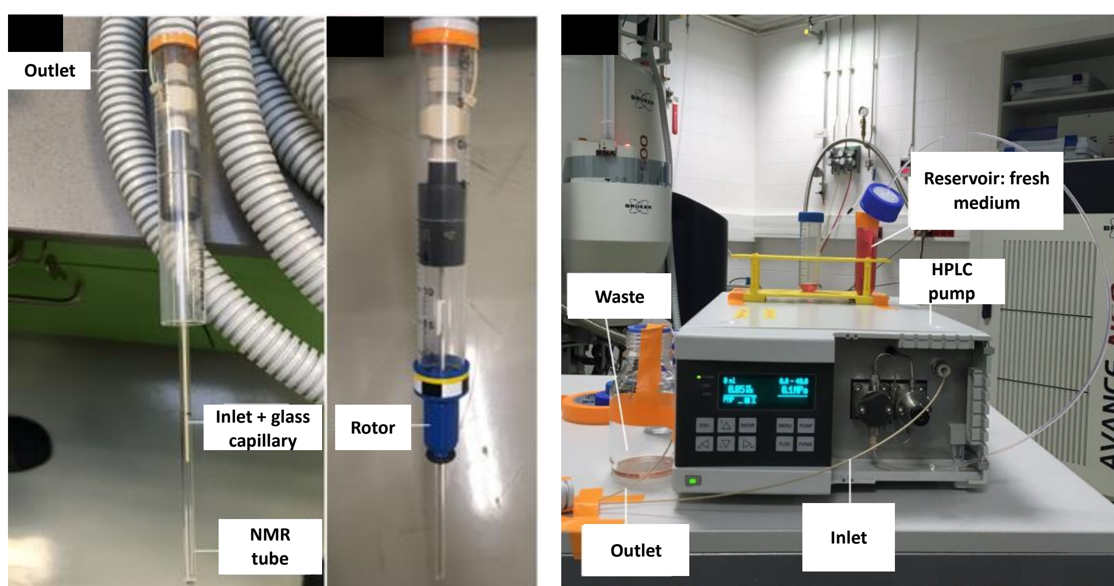

Figure 2. Installed (in-house built) bioreactor at Josef Dadok NMR Centre (Central European Institute of Technology, Masaryk University, Czech Republic).

The viability of cells in the course of the in-cell NMR experiment is assessed from the changes in intracellular ATP levels (Figure **3**).

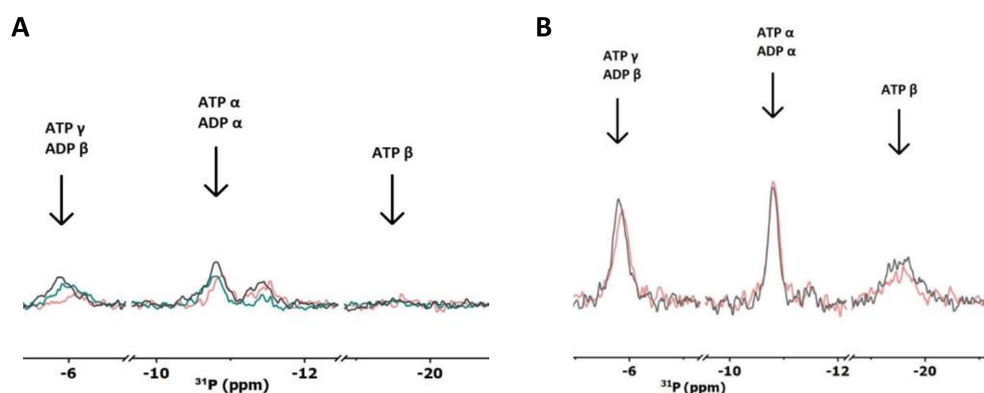

Figure 3. ATP-specific region of the 1D 31P NMR spectra of HeLa cells encapsulated in low-melting agarose acquired without (A) and with (B) the bioreactor's use, respectively. In A), black, green, and red lines correspond to the spectra acquired at 30 min, 5 hours, and 6 hours, respectively. In B), black and red lines correspond to the spectra obtained at 30 min and 10 hours, respectively.

Bioreactor type I (B-HEAD I) (Figure **4**) – a bioreactor based on the original Kubo's design [2]. It allows a continuous supply of a fresh medium to the cells encapsulated in a biocompatible gel matrix. In contrast, metabolic by-products and the biomolecular target leaked from compromised cells are removed. The bioreactor is suited for the vast majority of in-cell NMR applications ranging from time-consuming in-cell NMR experiments to quantitative real-time in-cell NMR interaction studies. The bioreactor assembly follows a procedure described in ref. [8].

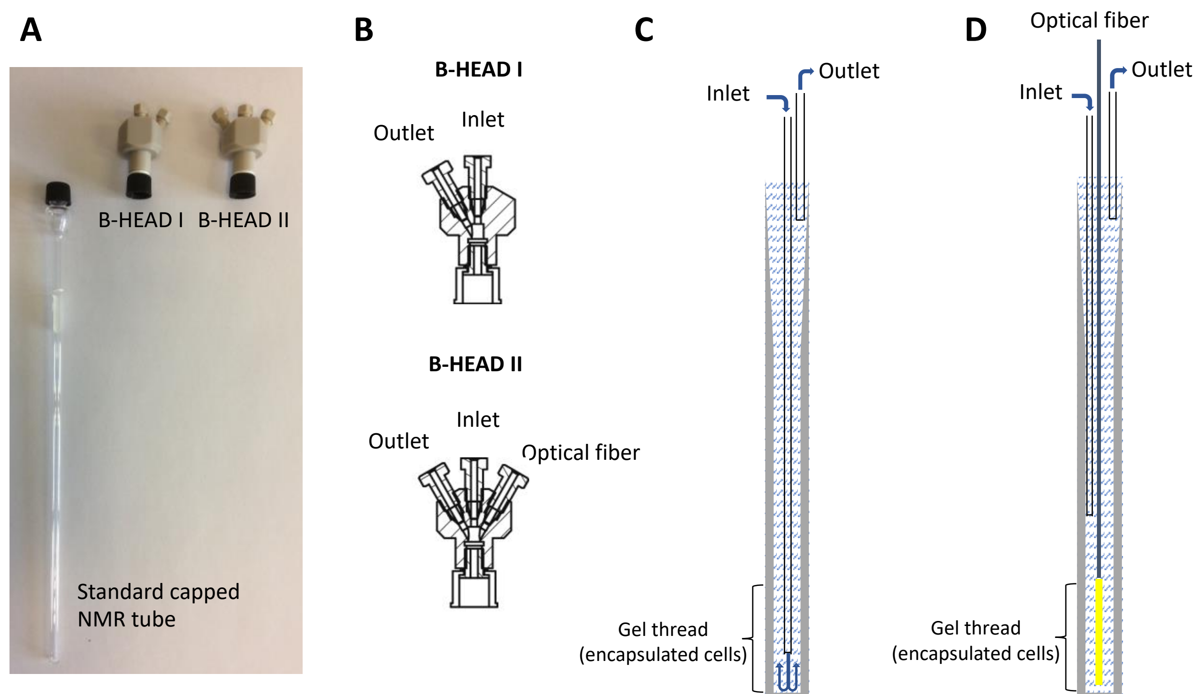

Figure 4. A) Standard capped NMR tube is used with both B-HEAD I and II. B) Schematic representation of B-HEAD I (top) and B-HEAD II (bottom). C) and D) Detail of the placement of inlet/outlet capillaries in B-HEAD I and II, respectively.

Bioreactor type II (B-HEAD) (Figure **4**) – an extension of HEAD I type bioreactor for in-cell NMR applications requiring light-control in the course of in-cell NMR data acquisition (Figure 2). Next to the inlet and outlet for media input/output, the HEAD II features a port allowing optical fiber insertion into a space occupied by encapsulated cells (active NMR coil volume). Note that B-HEAD II's capillary inlet placement is distinct from that in B-Head I (Figure 4C, D). In B-HEAD II, the optical fiber is placed into active coil volume to achieve efficient light delivery to cells. In contrast to B-HEAD I, the inlet capillary in B-HEAD II is positioned above cells encapsulated in the gel matrix. In this arrangement, the medium exchange is inefficient, and cell viability and metabolic stability get compromised much faster than in the B-HEAD I setup. As a result, the in-cell NMR measurements' active time window shortens to about 1.5-2 hrs.

Accessibility: B-HEAD I and II bioreactors are accessible via the Open Access entry points of iNEXT-Discovery (https://inext-discovery.eu/submit-proposal/) @ **Josef Dadok NMR Centre (CEITEC, Masaryk University, Brno, Czech Republic) and @ BMRZ, Goethe University, Frankfurt am Main, Germany).**

Contact access information:

Assoc. Prof. L. Trantirek, Central European Institute of Technology – Masaryk university, Brno, Czech Republic (lukas.trantirek@ceitec.muni.cz)
Prof. H. Schwalbe, BMRZ, Goethe University, Frankfurt am Main, Germany (schwalbe@nmr.uni-frankfurt.de).

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