Low cell number perfusion bioreactor system for hyperpolarized MRS in a MRI setting

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Purpose: To develop a MR-compatible bioreactor system for measuring metabolic fluxes in low cell numbers and in various cell types grown in a scaffold with different hyperpolarized ^{13}C bio-probes. The 3D printed scaffold design facilitates a uniform nutrient flow and oxygen supply throughout the cell clusters in the scaffold.

Materials and methods: Bioreactor chambers were designed in SolidWorks™ and 3D printed. The cylindrical growth chamber has a 4.2 mm inner diameter and a 5.0 mm outer diameter and the bottom outer dimension is made with a 6% luer taper conical fitting. The DNP injection fluid path is constituted by a Fluorinated Ethylene Propylene (FEP) tube with a 0.25 mm inner diameter with an IDEX Health & Science LLC PEEK-to-luer connector at both ends and the DNP inlet is connected to an injection site. When the bioreactor chamber is placed in the 5 mm RF solenoid coil, it is positioned into a holster functioning as a fluid outlet. The RF coil is a part of a double tuned NMR Probe and the holster has a 6% luer female conical fitting allowing direct coupling and reproducible placement of the BC in the isocenter of a horizontal 9.4T MRI system. [1-^{13}C] pyruvic acid were polarised in a SpinLab (GE Healthcare, Milwaukee, WI, USA).

The various cell types can be grown in the bioreactor chamber either in suspension or on scaffolds made from polycaprolactone (PCL) and fabricated by fused deposition modeling with a BioScaffolder (Sys+Eng GmbH, Germany). Five mm porous mats were made by a layered deposition of 200 µm thick fibers of PCL melt with an edge-edge distance of 800 µm and an angular displacement between each layer of 106°. To increase surface hydrophilicity and thus improve cell attachment, the scaffold was etched in 5 M NaOH for 3 hours, and then in 70% ethanol for sterilization.

During cell culture the bioreactor champers is placed in an 8-slot holster (designed in SolidWorks™ and 3D printed). Fluid flow is driven by a roller pump generating a flow rate of 0.67 ml/min and set to renew the cell medium 15 minutes every second hour.

Figure 1

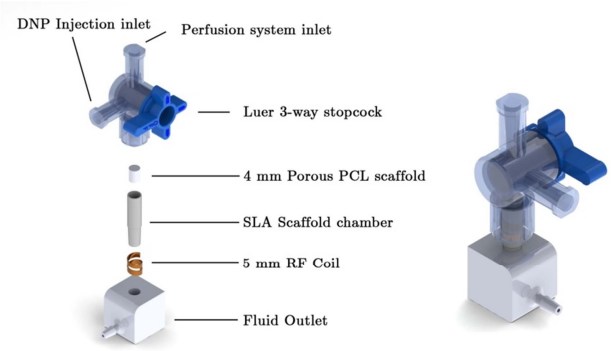
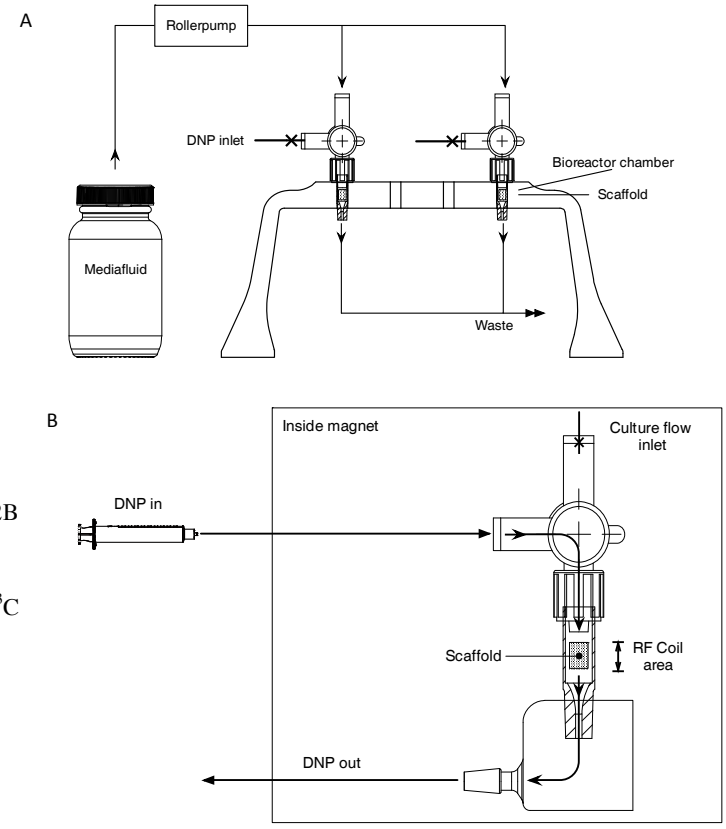


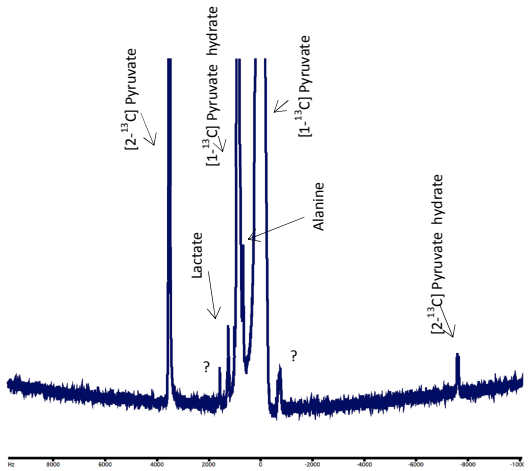
Figure 2



Results: The design of the bioreactor is shown in Figure 1, which highlights every single part of the bioreactor. Figure 2A demonstrate the schematic overview of the bioreactor setup for growing cells and 2B is a representation of the bioreactor system in the RF coil.

Hyperpolarized pyruvate metabolism was tested in the bioreactor on five million pancreas cancer cells in suspension and a representative ^{13}C spectrum is shown in Figure 3. This current setup allows ^{1}H shims below >22 Hz and a sufficient SNR to observe several metabolic derivatives including lactate and alanine.

Figure 3



Conclusion: This study demonstrates the design of a five mm bioreactor system for a horizontal 9.4T MRI system. The bioreactor system is designed to measure cell metabolism of cells in suspension or of cells grown in scaffolds with low cell numbers. The scaffold system facilitates a dynamic cell culture environment with controlled nutrient flow, oxygen supply and a large surface area for growth of adherent cells with minimal disturbance of the cells. Ongoing studies are focusing on evaluation of cell numbers in suspension and scaffold in this bioreactor design.