NMR Microsystem for Label-free Characterization of 3D Nanoliter Microtissues

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Introduction

CMOS microchip technologies were used to implement ultra-compact probes, where multilayer micro-coils are co-integrated on the same chip with the transceiver electronics.[1,2,3] This type of probe allows for an exceptional degree of versatility and state-of-art performance for the analysis of microscopic samples. With a probe having a sensing region of about 200 pL and a spin sensitivity of $1.5 \cdot 10^{13}$ spins/Hz$^{1/2}$ we previously demonstrated direct reading of endogenous compounds in sub-nL eggs of microorganisms, validating its use for bio-oriented applications.[4] More recently, the combination of CMOS with microscale 3D printing allowed for spectroscopy of sub-sections of intact C. elegans worms.[5] We have now evolved this approach into a platform technology for spectroscopy of biological samples having various nature, shape, and a volume ranging from 0.1 to 10 nL. In this work we describe an NMR micro-system consisting of a microchip probe combined with a microscale 3D printed holder for biological samples. With this device we performed spectroscopy of more than 100 single human micro-liver spheroids[6] and we measured markers of lipid metabolism from ^{1}H spectra obtained in 10 minutes (at 7 T). Such an achievement is made possible by a micro-NMR device that is robust, highly sensitive, and easy to use.

CMOS Chip Design and Sensitivity

The microsystem is made by combining a CMOS chip with a 3D micro-structure made with a two-photon polymerization 3D printing technique. This method allows fabricating structures that can easily be adapted to specific sample sizes and shapes. In this work the structure was specifically designed to position and hold MTs that are well approximated by spherical shapes. The micro-system is robust and allows for quick sample loading (< 5 min) with the sole use of a micro-pipette and a stereomicroscope (**Figure 1**).

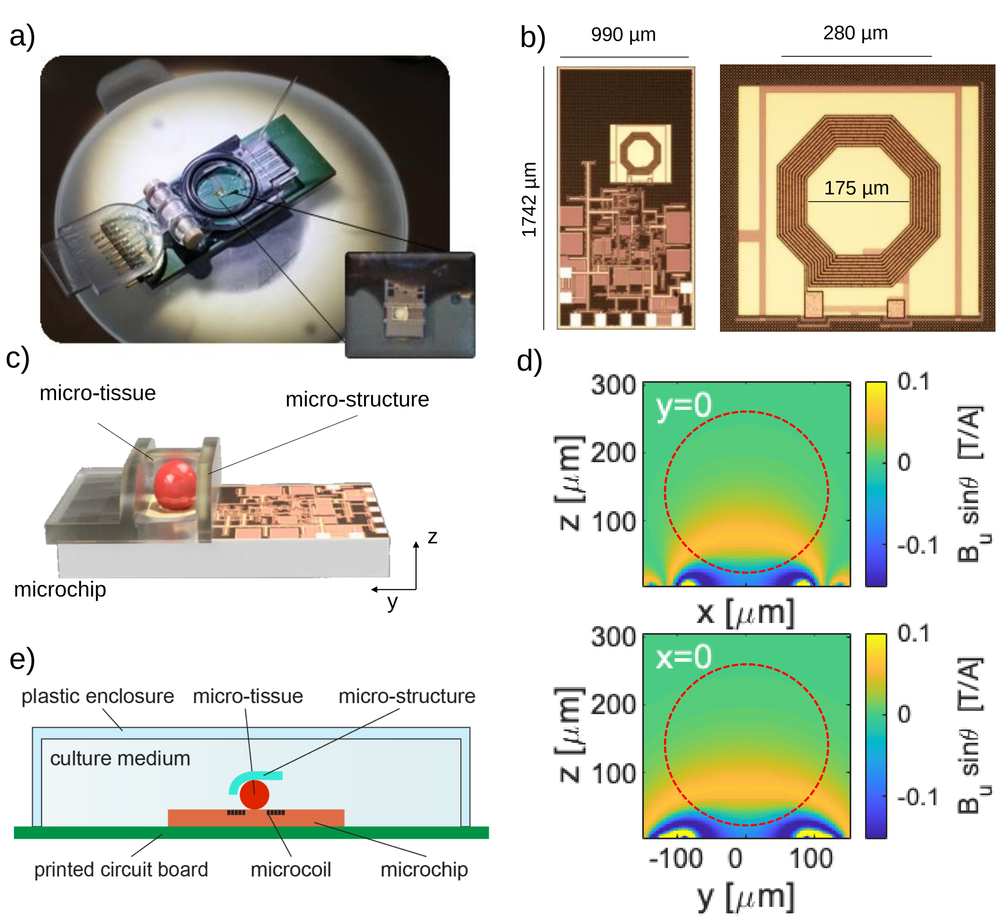

Figure 1: CMOS-based NMR probes for nL 3D MTs. a) Photographs of our CMOS-based NMR sensor designed for *in-vivo/in-vitro* experiments at the nL scale. Expansion highlights the microchip with microstructure housing. b) CMOS microchip and its microcoil. The coil region appears with a different color due to the absence of metal density filling in order to minimize parasitic effects. c) 3D rendering at scale of the micro-system and sample. d) Maps of sensitivity of the integrated microcoil in experimental conditions ($\tau = 20$ µs, $i = 11$ mA) at $y = 0$ and $x = 0$ cross-sections. The excitation current $i = 11$ mA is deduced by matching maximum signal intensity from experiments and sensitivity maps computation. e) Cross-section schematics of the micro-system, defined as crossing transversally the chip at the microcoil center. The curved shape enables placement of MTs of different sizes in the most sensitive area of the microcoil.

Summary and Outlook

In this work we have demonstrated and validated a device concept that enables NMR on single biological entities at the nanoliter scale. Its simplicity of use, sensing performance, robustness and versatility makes this technology suitable for extended high throughput non-invasive investigations of intact microscopic biological samples. The use of this CMOS-based NMR device is demonstrated in an exemplary study, detecting fatty acid metabolism dynamics, label-free, from more than 100 measurements of single nL human liver microtissues. The flexibility of the fabrication methodology makes CMOS-based micro-NMR a promising platform for more widespread use of the technique in different research environments.

3D Human Liver MicroTissue NMR Spectra

1D ^{1}H NMR spectroscopy results of single MTs obtained with our CMOS-based sensor in a field strength of 7 T are shown for acquisition times of 10 minutes. The spectral resolution is about 0.2 ppm, slightly better than what has been observed by previous *in-vivo* liver studies. In order to acquire a sufficiently large set of data to infer statistically significant conclusions, we fixed an experimental time of 10 min per single MT and performed several measurements of single MTs in the time points indicated (**Figure 2**). The MTs are divided in three experimental groups (LEAN, Steatotic On Diet, & Steatotic On Fat), differentiated by the type of culture medium (diet) to which they are exposed for the duration of the experiment.

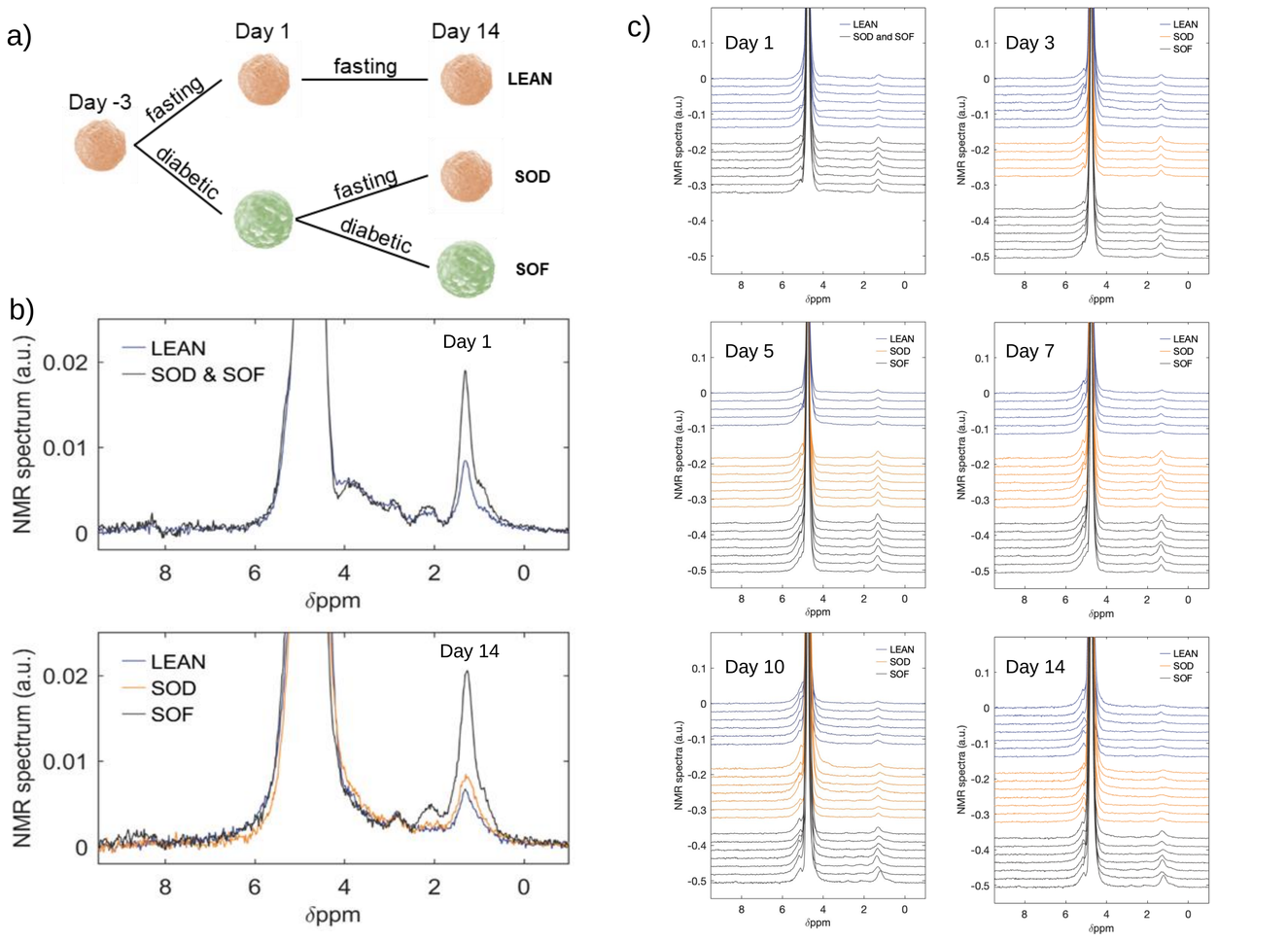

Figure 2: NMR spectroscopy of 3D microtissue cultures. a) Schematic of experimental design timeline for Lean, Steatotic on Diet (SOD), and Steatotic on Fat (SOF) MTs. b) NMR spectra averaged by experimental group at Day 1 (top) and Day 14 (bottom). c) 1D ^{1}H NMR spectra obtained from 117 single MTs in the 6 experimental time points Each spectrum results from a measurement time of 10 minutes, i.e. averaging 300 scans.

Quantitation and Statistical Results of 3D MTs

We identified three spectral regions of interest ranging from 1.18–1.46 ppm (integral L1), 1.80–2.20 ppm (integral L2), and 2.50–3.00 ppm (integral L3), which respectively represent aliphatic chains corresponding to the presence of lipid signatures from fully saturated (L1), mono- (L2) and poly- (L3) unsaturated fatty acids. Three of these markers are defined as ratios of the integrals Li and represent relative concentrations of molecular groups. The fourth, Proportional Lipid Content (PLC), is defined in analogy with conventional MRI-PDFF (Proton Density Fat Fraction, today's gold standard and Endpoint in NASH trials) and compares the content of –(CH2)n groups to those found in LEAN at Day1. The overall dynamics highlight a gradual approach over time of the SOD from a lipid accumulation phase to a lean condition. The SOF shows saturation of lipid content, reaching a maximum value already by Day 5 and remaining constant after that (**Figure 3**).

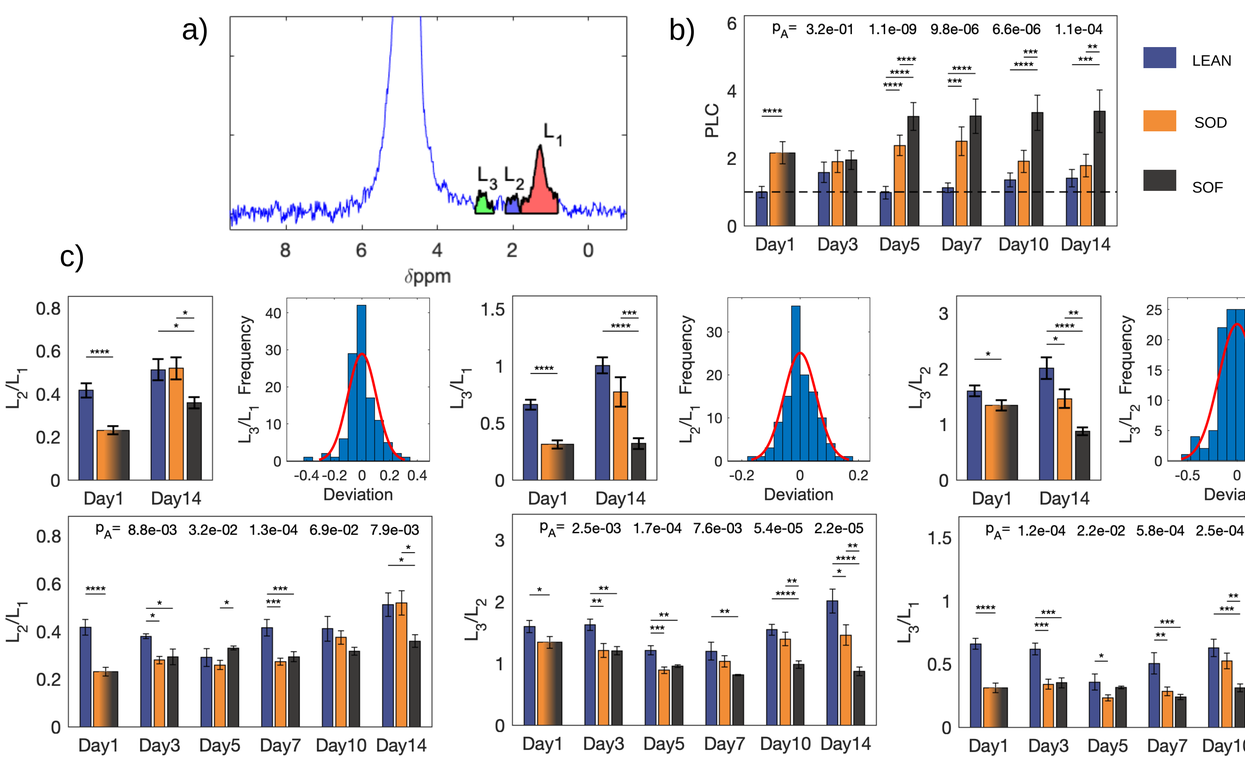

Figure 3: Label-free detection and evolution of lipids. a) Typical spectrum with highlighted biomarker regions. L1: integral area from 1.18 to 1.46 ppm. L2: integral area from 1.8 to 2.2 ppm. L3: integral area from 2.5 to 3 ppm. b) Complete time evolution of PLC (L1(t)/L1_lean(t=0)). c) Time evolution and normal distribution of L2/L1, L3/L2, L3/L1 biomarkers.

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