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Sample patterning on NMR surface microcoils

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Abstract

Aligned microcontact printing for patterning the sample in areas of homogeneous RF-field on the highly sensitive surface of planar NMR microprobes is presented. We experimentally demonstrate that sample patterning allows drastic improvement of the spin excitation uniformity. The NMR microprobes are designed for cell analysis and characterized using lipid vesicles as cell substitutes. Lipid vesicles are advantageous as composition and concentration of the confined solution are precisely controlled and because of their similarity to living cells. Using aligned microcontact printing, a monolayer of lipid vesicles is immobilized on the surface of the planar NMR microprobe in a patterned way. ^{1}H NMR spectra and CPMG spin echoes of sucrose solution confined within the lipid vesicles are successfully recorded. Nutation curves of the sample structured in different patterns demonstrate the impact of patterning on the spin excitation uniformity. The total detection volumes are between 1 and 2 nL and derived with help of a theoretic model based on 3D finite element simulation. This model predicts the signal-to-noise ratio and the progression of the nutation curves.

Keywords: Planar surface microcoil; B_1 homogeneity; Signal-to-noise ratio; Lipid vesicles; Aligned microcontact printing

1. Introduction

There is a growing interest in the manipulation and analysis of cells in small quantities down to single cells. Especially micro- and nano-engineering techniques, which offer new possibilities for cell manipulation and analysis, have stimulated the progress in this field [1]. When analyzing living cells, mostly electrical (impedance) and optical (fluorescent) characterization techniques are used.

Nuclear magnetic resonance is an information rich analysis technique that brings about a lot of potential for new insights and applications in cell analysis [2,3]. Performing NMR analysis of living cells, the cell handling is a very delicate issue. The cells are generally extracted from living tissue or harvested from their cultivation environment in bioreactors or on the surface of Petri dishes or well plates. Due to the principally 2D morphology of living cells, planar coils, where the cells could be directly immobilized or grown on the coil surface, should be better suited for analysis than conventional solenoidal coils, where the sample is confined in the volume of a capillary or tube. Small planar coils can be produced by microfabrication. In addition to the advantages of exact reproducibility and batch-production, microfabrication allows producing extremely small and highly sensitive microcoils for spectroscopy of mass-limited samples and high-resolution imaging.

Planar, microfabricated coils on a GaAs substrate for NMR analysis have been developed first by Peck et al. [4,5]. Trumbull et al. [6] have tried to integrate CE separation and NMR spectroscopy following the concept of micro total analysis systems. Previously, we have optimized the design of planar microcoils for high sensitivity and integrated microfluidic channels in the glass substrate

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[7]. As the focus of these experiments was on chemical analysis, planar microprobes were used confining the sample in a capillary or microchannel instead of using directly the surface of the planar microcoil as sample interface. Besides the aptitude to the analysis of living cells, placing the sample on the surface of the microcoil does not require complicated channel fabrication and allows sample positioning close to the microcoil. This is especially important when using very small microcoils with a limited sensitive region.

Eroglu and Gimi [8] have placed an acrylic container with a flat bottom onto a planar microcoil of 1.5 mm inner diameter. Images of pancreatic rat islets placed in this container have been taken [9]. Due to the inhomogeneous RF-field close to the planar microcoil [10], a compromise had to be made between spin excitation uniformity and sensitivity. The strong RF-field near the coil increases the sensitivity, but the inhomogeneity of the field causes low spin excitation uniformity.

In this contribution, we present the application of a micro-engineering technique to pattern the sample in areas of homogenous RF-field on the microprobe surface close to the NMR microcoil for an improved compromise between spin excitation uniformity and high sensitivity. The microfabricated NMR probe imitates a Petri dish, because integrated cell growing and NMR spectroscopy and imaging of living cells is aimed finally (see Fig. 1).

As the volume of the cells immobilized on the microprobe surface and the concentrations of the various compounds within living cells are not precisely controlled, lipid vesicles that contain a sucrose solution of known concentration are used as cell substitutes to characterize the surface microcoil with respect to sensitivity and spin excitation uniformity. Monolayers of these vesicles are immobilized in different patterns on the microprobe surface. The advantage of using lipid vesicles is, beside the controlled contents, their long-time stability and the similarity to living cells with respect to immobilization techniques and perturbation of the static magnetic field ($\mathbf{B}_0$-field). Knowing the sample concentration, a theoretical model based on finite element simulation of the microcoil's inhomogeneous RF-field is used to calculate the volume of the vesicles and to predict sensitivity and spin excitation uniformity of different sample patterns.

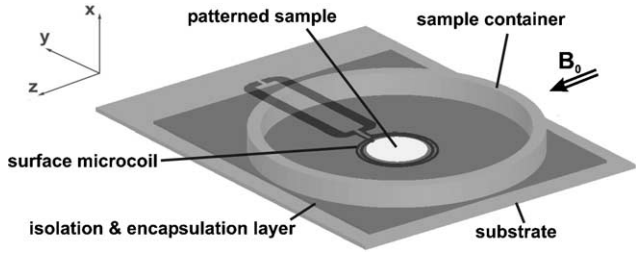

Fig. 1. Design of the NMR surface microcoil with a sample container on top imitating a petri dish. The microcoil has an inner diameter of 1 mm. The sample is patterned in the center of the microprobe surface. The microprobe is introduced into the NMR magnet such that the static magnetic $\mathbf{B}_0$-field lies in the z-direction.

2. Theory

2.1. Theoretic model

We determine sensitivity through the *SNR* produced by an average spin flip angle of 90°. Spin excitation uniformity is quantified by the percentage of the *SNR* produced by a 450° compared to the *SNR* produced by a 90° flip angle. The theoretic model computes the nutation curve of a given coil-sample-configuration according to Eq. (1). We described the derivation of this equation in a previous contribution [7]. The model here is extended to return the final *SNR* monitored by the spectrometer.

$$SNR(\tau_{ex}) = \frac{S(\tau_{ex})}{\sqrt{4kTR_{coil}}} \cdot \sqrt{n_{acq}} \cdot \frac{1}{F'} \cdot \frac{T_2^*}{\sqrt{2T_{acq}}} \tag{1}$$

$S(\tau_{ex})$ denotes the detection signal amplitude dependent on the excitation time τ_{ex}, k is the Boltzman constant, T is the coil's temperature, R_{coil} is the coil's electrical resistance and n_{acq} is the number of acquisitions. F' is the noise factor of the detection electronics, T_2^* is the apparent spin–spin relaxation time and T_{acq} is the acquisition time.

The signal amplitude $S(\tau_{ex})$ is calculated according to the principle of reciprocity [11] by integration of the signal amplitude per unity volume of each point in space $S(\mathbf{r}, \tau_{ex})$ over the whole sample volume V_s:

$$S(\tau_{ex}) = \int_{V_s} S(\mathbf{r}, \tau_{ex}) \mathrm{d}V_s \tag{2}$$

with

$$S(\mathbf{r}, \tau_{ex}) = \omega_0 \cdot M_0 \cdot B_{1u,xy}(\mathbf{r}) \cdot \sin\left(\gamma \cdot \frac{I_{ex}}{2} \cdot \tau_{ex} \cdot B_{1u,xy}(\mathbf{r})\right) \tag{3}$$

ω_0 denotes Larmor frequency and M_0 is the net magnetization of the sample. $B_{1u,xy}(\mathbf{r})$ is the magnitude of the unitary magnetic field ($\mathbf{B}_1$-field) in the xy-plane, γ is the gyromagnetic constant of the excited nuclei and I_{ex} the excitation current.

As the $\mathbf{B}_1$-field is considered in the detection and excitation modeling, the exact progression of the nutation curve can be determined, if the inhomogeneity of the $\mathbf{B}_1$-field is precisely assessed. In a completely homogeneous $\mathbf{B}_1$-field, the nutation curve is an ideal sinusoidal function. In an inhomogeneous $\mathbf{B}_1$-field, the nutation curve is a superposition of sinusoidal functions with different rotation frequencies. This superposition results in a curve, which looses its sinusoidal behavior for high excitation times, as the spins' rotation frequencies disperse. The more inhomogeneous the $\mathbf{B}_1$-field, the faster is the dispersion and the smaller the *SNR* of a 450° excitation with respect to the *SNR* of a 90° excitation.