MICRONEEDLE BASED SAMPLING FOR BREAST CANCER TISSUES

A. Hokkanen[1*], I. Stuns[1], P. Schmid[2], A. Kokkonen[1], A. Steinecker[2], J. Budczies[3], P. Heimala[1] and L. Hakalahti[1]

[1]VTT Technical Research Centre of Finland, FINLAND, [2]CSEM Swiss Center for Electronics and Microtechnology, SWITZERLAND, and [3]Charité - Universitätsmedizin Berlin, GERMANY

ABSTRACT

We have developed a microneedle based system that is capable of extracting cell membrane lipids from real breast cancer tissues. We show that it is possible to extract phosphatidylcholine (PC) and phosphoethanolamine (PE) lipids from the cancer tissue samples with the microneedle system and analyse them with mass spectrometry (MS). PC and PE lipids act as biomarkers for breast cancer. In future such system can help surgeons in their decision making during operations or they can be used as device for tissue research at high spatial resolution.

KEYWORDS: Microneedle, sampling, tissue, cancer

INTRODUCTION

Currently surgeons rely on pre-operative imaging scans and results received from frozen sections during surgery to remove the cancerous tissue. This approach is relatively slow and often requires further surgery after the later examination. Real-time imaging tools are needed to help oncologists characterize tumour tissue during surgery. For this purpose Takats et al. have developed MS based technique to distinguish between cancerous and noncancerous tissue during surgery [1]. In another approach the Delamarche group has developed a method for staining tissue sections at the micrometre scale to extract more high-quality information from tissue sections [2].

It has been shown in the literature that small specific fatty acids incorporated into different cell membrane phospholipids can be used as a biomarker for the onset of cancerous growth [3, 4]. Here we propose to use a disposable silicon microneedle for sampling cell membrane lipids from tissue. Combining the sampling with MS analysis could provide a screening method for cancer after taking a biopsy or during surgery as the cancerous tissue can be rapidly identified. We have reported earlier that the microneedle together with a MS is capable for sampling and analysing standard lipids [5]. Here we demonstrate sampling and analysing lipids from real tissue samples using microneedles and the mass spectrometer.

EXPERIMENTAL

In situ extraction of lipids from tissue samples is performed with a two-port microneedle (Figure 1). The microneedle is fabricated using silicon micromachining technologies. Its outer diameter is 250 µm and its height is about 200 µm. The needle has two separate fluidic channels (ports) with 60 µm diameter and they are 80 µm apart from each other. Both fluidic channels open into a shallow 30 µm well on top of the needle head.

The two-port microneedle is integrated on a polymethyl methacrylate (PMMA) polymer microfluidic chip (Figure 2) so that both needle ports are connected to their own fluidic channels in the polymer chip. The inner liquid volume within the microneedle is only ~5 nL but with the polymer channel the total fluidic volume that can be handled with the needle is increased to >10 µL. The polymer chip has been structured using hot embossing. The channels are 100 µm deep and 400 µm wide.

Tissue sampling is done so that the extracting solvent MTBE (Methyl tert-butyl ether) is first aspired to one of the microneedle ports. Then the two-port microneedle is pressed against the tissue section fixed on a microscope slide. The shallow well on top of the needle forms a flow cell structure on top of the tissue. Next the MTBE solvent is forced to flow over the tissue sample to extract the cell membrane lipids while the other needle port is used to simultaneously collect the extracted lipids sample into the needle. After the

extraction process the collected sample is moved from the needle to the mass spectrometer for the lipid analysis. For the MS analysis Waters TQ (Triple Quadrupole) mass spectrometer is used. The polymer microfluidic chip with silicon microneedle is a disposable device and can easily be changed between each single analysis to avoid cross-contamination between the samples.

A micromanipulator based handling system is developed for the microneedle actuation [5]. The positioning of the microneedle can be controlled in three dimensions with a ~10 µm accuracy. Camera vision gives feedback from the microneedle position with respect to the tissue sample both in plane and in distance from the surface. A special pressure/vacuum control system is built for precise pneumatic control of the liquid flows in the microneedle chip. The polymer microfluidic chip acts as an interface between the microneedle and pressure controlled sampling system. It is a closed system on the micromanipulator side and there is no risk on contamination between subsequent sampling events.

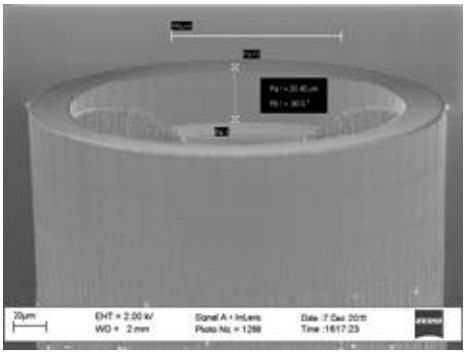

Figure 1: An SEM image of the multiport silicon microneedle.

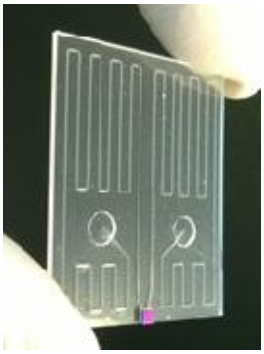

Figure 2: A two-port microneedle (in the bottom edge) integrated with a polymer microfluidic chip.

RESULTS AND DISCUSSION

Tissue sampling experiments were made from bulk tissue samples, both normal and cancerous, on microscope slides. Successful lipid extraction from the tissue was achieved with 1 min extraction time. We observed that the tissue morphology remains unmodified after the lipid extraction (Figure 3) and the MS results showed that cell membrane lipids were extracted.

The MS results of microneedle extracted samples are shown in Table 1. There is a normal sample (26N) and a tumour sample (26T) from the same bulk tissue sample. Blue coloured rows indicate the PC and PE lipids that are candidates for breast cancer specific biomarkers [4]. The relative changes in the amount of these lipids in the tissue sample are indicative for the cancerous growth. As seen in the results, increased values of those lipids were found in the tumour samples anticipating cancerous growth. There can be seen changes also in the levels of other lipids but to our knowledge there is no indication on their relevance for breast cancer.

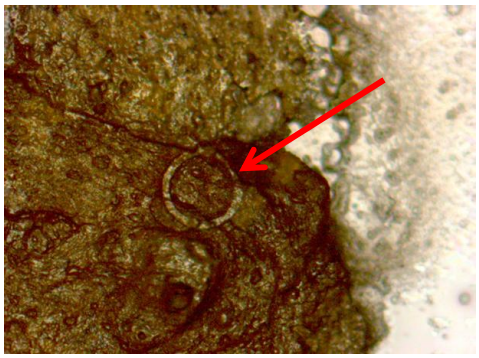

Figure 3: A footmark of the 250 um blunt microneedle on the site of extraction in the cancerous tissue.

Table 1: MS result from the samples extracted with the microneedle sampling system.

Average m/z	Name	26N, Normal	26T, Tumour
782.57	PC(16:0/20:4)	5	505
808.59	PC(18:1/20:4)	2	184
734.56	PC(32:0)	45	209
762.60	PC(34:0)	3	32
760.59	PC(34:1)	6	1 324
758.57	PC(34:2)	5	245
788.62	PC(36:1)	8	236
786.61	PC(36:2)	7	263
782.58	PC(36:4)	24	171
812.62	PC(38:3)	44	135
810.60	PC(38:4)	5	270
796.62	PC(38:4e)	270	241
792.61	PC(38:6e)	169	170
720.55	PE(17:0/17:0)	4	24
768.57	PE(38:4)+PC(35:4)	2	194
752.56	PE(p18:0/20:4)	4	132

CONCLUSION

It was shown that it is possible to extract PC and PE lipids from the breast cancer tissue samples with the microneedle based sampling system. Our next step is to develop an automated robotic microneedle sampling system with cancer tissue imaging. In this way it can be used to help surgeons in making decisions about the extent of the surgery to fully remove the cancerous tissue without sacrificing too much of the healthy tissue.

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CONTACT

* A. Hokkanen; phone: +358-40-7655-795; ari.hokkanen@vtt.fi