Specific Device for Cells Sampling

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ABSTRACT

We developed a miniaturized biopsy tool for harvesting molecules of interest in delicate areas such as brain. This tool permits harvesting of intact cells as well permitting genomic analysis on these cells.

The sampling tool is manufactured by microtechnology methods rendering it small enough to not damage surrounding tissues. Microtechnology techniques permit to geometrically structure the surface compensating for miniaturization and to add a functionalization in order to manage a gentle capture of the cells in the location of the sampling. By controlling the chemical surface modification we can manage to sample isolated cells or clusters of cells.

Recovered cells are capable of growth in culture directly on the tool. This particularity avoids recovering or transfer damaging the cells.

Keywords: microtechnology, cells, genomic

1 INTRODUCTION

We first developed a biopsy micro tool for sampling molecules of interest by contact without any damage on tissue for delicate areas such as brain (see Fig. 1). Delicate areas can be reached thanks to a patented innovative method of sampling [1], [2] which keeps tissue intact while only infinitesimal quantity of biological material is gathered on the tool surface.

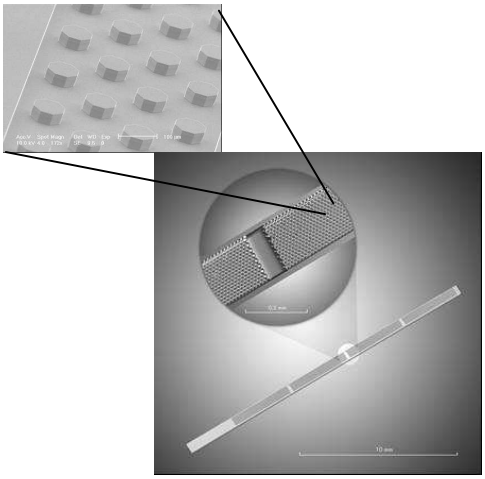

Figure 1: Tool developed for proteins sampling.

Fields of application for this miniaturized technology are oncology and degenerative diseases like Parkinson's disease. In that kind of diseases not only proteomic analysis are important but also genomic information is crucial to understand behaviour of cells and protein expression to implement specific therapy.

The tool developed leads to harvesting of intact cells providing such genomic analysis.

Existing techniques for cells capture are generally based on a kind of scrubber or cutter to tear off cells from tissue [3], [4]. Even if techniques tend to be miniaturized the principle is to disrupt tissue [5], [6]. These techniques lead most of the time to recovering pieces of tissue containing damaged cells or not enough material to make morphological and molecular analysis.

With our tool we can capture delicate cells without any damage. A specific silicon chip is assembled at the end of a surgical stylet which is inserted in a windowed guide (see Fig. 2) to permit active area and tissue contact.

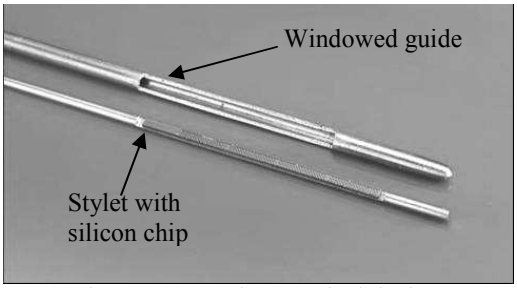

Figure 2: Complete surgical device.

The complete device is inserted and the silicon chip is put in contact with the targeted tissue a few seconds and by specific interactions and affinities cells are "stuck" on the surface of the chip.

Using the windowed system, samples recovered remain intact, there is no friction applied on the surface of the silicon chip during insertion and extraction of the device. Moreover it provides a spatially oriented sample.

2 MANUFACTURING OF THE CHIP

The device is manufactured by microtechnology methods rendering it small enough not to damage surrounding tissues during insertion and extraction.

Moreover, microtechnology techniques permit microstructuration of the surface and addition of a chemical functionalization to manage a gentle capture of cells in the targeted location. Depending on chemical surface modifications isolated cells or clusters of cells can be captured.

2.1 Microtechnological Process

Each tool is designed individually, as a chip in the microtechnologic industry, and manufactured collectively on 200mm silicon wafers.

The process flow consists mainly in standard photolithography and Deep Reactive Ion Etching (DRIE) process running in the LETI MEMS clean rooms.

In order to obtain high developed surfaces, octagonal micro pillars were designed. They have 80µm diameter, 240µm pitch and are 50µm high. These pillars represent a 50% surface increase compared to a smooth surface.

A controlled thermal oxidation is carried out at the end of the process to cover the silicon with 500nm of silicon dioxide for following chemical functionalization.

The tools are then diced, measuring each 300µm x 600µm x 2mm and are ready for the chemical treatment.

2.2 Surface Modifications

The chemical functionalization process described bellow is used for efficient surface grafting of the tool.

Silanes with different active functions are grafted as coupling agents.

First, the silicon dioxide layer (500nm) of the chip is hydrated for 2 hours with a solution of NaOH 2,5N/Ethanol 40%/60% in volume and rinsed successively in water and ethanol under ultrasonic stirring in order to have hydrophilic silica surface (contact angle < 10°).

The grafting reaction is performed under an inert atmosphere thermo-regulated at 4 °C for 24h with a 10^{-2} M solution of silane dissolved in dry trichloroethylene (see Fig. 3). The chip is then successively rinsed with trichloroethylene, ethanol, and chloroform under ultrasonic stirring to remove the excess of silane and the physisorbed organosilane molecules.

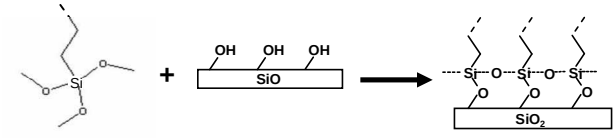

Figure 3: Summary of the chemical grafting.

Four main proprietary chemistries were developed: a hydrophobic surface directly obtained after grafting the silane and a hydrophilic, a cationic and an anionic surface obtained by performing one post-silanisation step. The anionic function is a cation exchanger and the cationic function is an anion exchanger.

Chemical surface modification is composed of a well organized molecular monolayer, which allows much more reproducible results compared to multilayer treatment such as polymer coatings. This reproducibility is a crucial parameter for diagnostic.

3 EXPERIMENTS

Work has been made in two steps: First, we validated the efficiency of surface modifications on glass slides in capturing cells from cells suspension, from animal tissues and from human tissue. Then, we validated cells capture on silicon microstructured surface directly in mice brain.

Cells used in suspension were rat C6 glioma cells at 10^7 cell./mL. Animal tissues used were extracted subcutaneous tumor developed by nude mice during 20 days after injection of C6 tumor cells. Human tissue was an oligodendroglioma obtained extemporaneously during surgery. Mice brains were obtained from healthy nude mice.

Glass slides and silicon chip, after contact with tissue or cells are observed by microscope.

3.1 Capture on Glass Slides

Glass slides have been grafted with the four different surface chemistries by the process reported above.

First, glass slides are put in contact with 10µL of cells suspension that is to say 100 000 cells during 10min at 37°C. Then, slides are washed with culture medium (DMEM). Cells adsorbed are counted before and after washing.

For capture on mice or human tumor, treated glass slides are put in contact with the extracted tissues during a few seconds for mice tissue and a few minutes for human tissue. Then, slides are directly observed by microscope.

3.2 Capture on Silicon Chip

Silicon chips are put in contact with tissue during a few seconds (see Fig. 4).

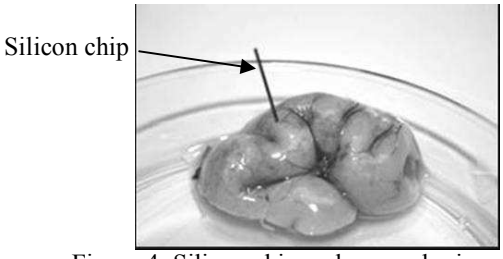

Figure 4: Silicon chip and mouse brain contact.

Chips are directly observed by microscope.

4 RESULTS AND DISCUSSION

4.1 Cells Suspension on Glass Slides

A summary of the results of capture from cells suspension on glass slides are presented in Table 1.

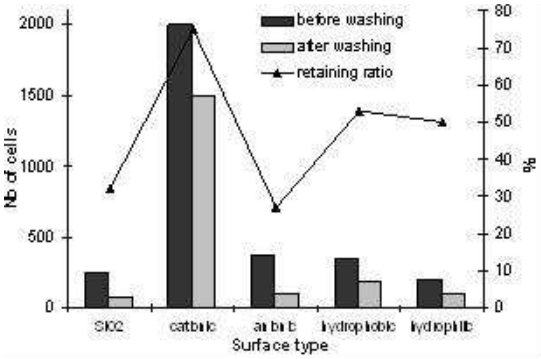

Table 1: Summary of the results on capture from cells suspension on glass slides.

The table shows that cationic surface retains more efficiently cells than other surfaces. On one hand, it retains more cells before washing and on the other hand, its retaining ratio after washing is the highest.

Indeed, cells membranes because of the presence of membrane proteins are mostly negatively charged. So they generate stronger electrostatic interactions with positively charged surfaces than with others. This analysis is confirmed by the low retaining ratio of the anionic surface. Cells membrane and anionic surface tend to repel each other.

This means that the electrostatic interactions are important in cells capture. That's why, for further experiments, we have focused our attention on charged surfaces that is to say, cationic and anionic surfaces.

4.2 Mouse Tumor on Glass Slides

Experiments on glass slides with mouse tumor show that cationic surfaces retain specifically isolated cells (see Fig. 5) whereas anionic surfaces retain mostly organized clusters of cells (see Fig. 6) which are similar to micro-explants.

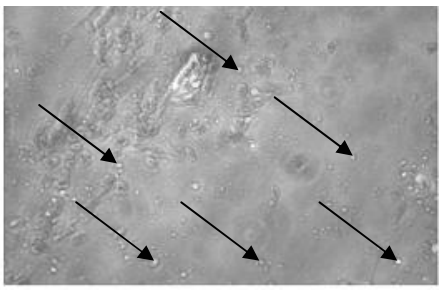

Figure 5: Isolated cells captured on cationic surface.

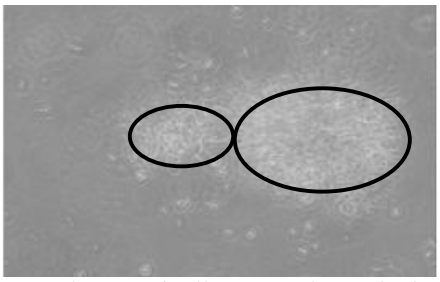

Figure 6: Clusters of cells captured on anionic surface.

As we said, cells membranes have electrostatic affinities with cationic surfaces.

Extra cellular matrix is mostly composed of collagen which is a positively charged protein. That observation explains why clusters of cells and not isolated cells are captured preferentially by anionic surfaces. Extra cellular matrix has thus a key role in capture process. Its interaction with the anionic surface lead to capture an organized cluster of cells and note damaged or scratched cells.

This confirms results obtained on cells suspension on the electrostatic nature of interactions at stake.

4.3 Human Tumor on Glass Slides

Captures on glass slides with human tumor confirm the correlation between the surface type and the recovered sample type.

Once again isolated cells are mostly captured by cationic surfaces (see Fig. 7) whereas clusters are on anionic surfaces (see Fig. 8).

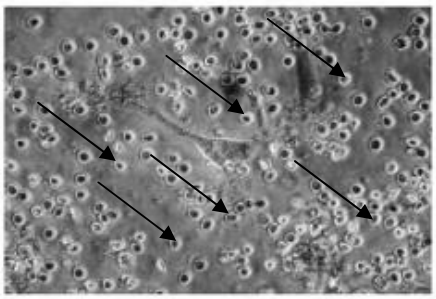

Figure 7: Isolated cells captured on cationic surface.

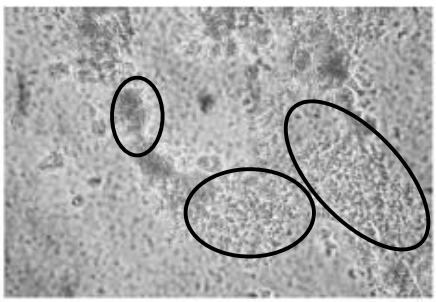

Figure 8: Clusters of cells captured on anionic surface.

Cells and extra cellular matrix in clusters are well organized and can be considered as micro-explants.

4.4 Mouse Brain on Silicon Chip

Experiments on silicon microstructured chip were carried out both on chips without any chemical treatment and on chips with cationic surface to compare.

Chips without chemical treatment permit to capture from mouse brain some filamentous structures coiled around the micro pillars. It's certainly some smashed cells residues (see Fig. 9) which probably come from the local micro mechanical action of the tool during capture.

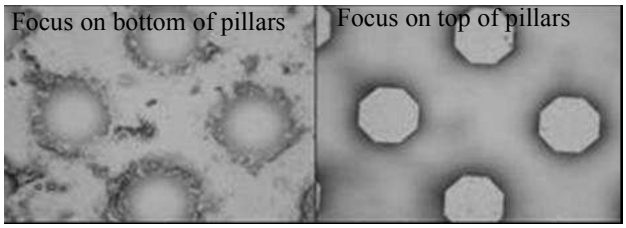

Figure 9: Cells residues captured on microstructured silicon chip without any chemical treatment.

However silicon chips with cationic surface allow sampling some intact cells gathered around the micro pillars (see Fig. 10).

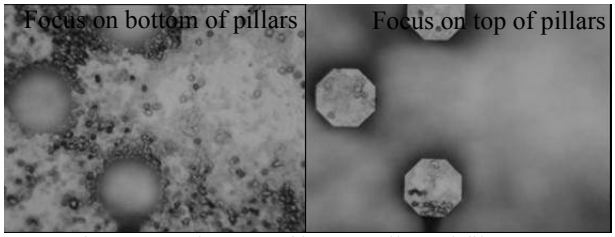

Figure 10: Pictures of intact cells and filamentous structures on cationic microstructured silicon chip.

The electrostatic interactions between cells and cationic surface coupling to the microstructuration of the silicon lead to capture intact cells. Once cells adsorbed, electrostatic interactions may prevent of the micro mechanical smashed of the cells during contact between chip and tissue.

4.5 Cells Culture

Cells cultures have been realized on treated glass slides.

Cells suspension and mouse tumor have been put in contact with cationic and anionic surfaces. After washing, retained cells are then growth in culture directly on the surfaces in DMEM with serum during 8 and 15 days.

In both case, cells are adherent and able to proliferate. Cellular motility and extra cellular matrix production have been observed.

This is a first result permitting to be confident on the non-toxicity of our surface modifications.

These experiments have to be confirmed by growing cells on silicon chip with surface modifications. We are also confident on this point because silicon chip is covered by silicon dioxide which is similar to glass.

5 CONCLUSION

We have demonstrated that we could capture isolated cells or kind of organized micro-explants in controlling surface modifications.

Capture process allow recovering intact cells in order to make genomic analysis.

Major advantage of this new tool is the lack of damage to surrounding tissues during the sampling. Delicate or functional areas could be reached to be investigated at a genomic level.

An interesting and important point is the viability of captured cells on the modified surfaces. It gives a first proof of the inocuity of our tool which is an important point regarding the final application. The cell culture is the first step on investigation of the biocompatibility of a new medical device.

Finally we validate the concept of cell microbiopsy using a specific silicon chip which could be combined with conventional genomic analysis and approaches and easily assembled with existing biopsy tools.

AKNOWLEDGEMENTS

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REFERENCES

[1] A. Bouamrani, D. Ratel, A-L Benabid, J.-P. Issartel, F. Berger, "Apparatus and methods for obtaining a molecular fingerprint", W.O. Patent 2006090220.

[2] P. Caillat, M.L. Cosnier and F. Martin, "Device for contact molecular sampling", W.O. Patent 2006082344.

[3] R.L. Goodale, T.D. Dressel and J.W. Borner, "Methods and apparatus for exfoliative cytology", US Patent 4243049.

[4] N.A. Godshall and R.R. Anderson, "Method and apparatus for disruption of the epidermis", US Patent 5879326.

[5] S.J. Moon, Y.H. Choi and S.S. Lee, "A micro machined biopsy tool for a capsule type endoscope", 13th International Conference on Solid State Sensors, Actuators and Microsystems, (Transducers' 05), Seoul, Korea, 368-371, 2005.

[6] S. Park and al, "A disposable MEMS-based micro-biopsy catheter for the minimally invasive tissue sampling", IEEE International Conference on Intelligent Robots and Systems, pp3045-3050, 2005.