TAMPERE UNIVERSITY OF TECHNOLOGY

Electric Impedance Assisted Micropipette Aspiration

Katrin Kovanen, Mikko Lukkari, Sami Purmonen, Timo Ylikomi, Jouko Viitanen and Pasi J. Kallio

Abstract—**Micropipette aspiration is a technique to selectively isolate cells from cell cultures using suction pressure. Cells can selectively be isolated one by one from neighboring cells into the micropipette. This paper presents a novel micropipette aspiration system assisted by an impedance measurement system. Furthermore, a method to reduce the adhesion force at a single cell level for a gentler detachment of the cell from a cultivation surface and surrounding cell connections is proposed.**

Index Terms—**Micropipette aspiration, adherent cells, impedance measurement, microinjection, micromanipulation**

I. INTRODUCTION

ASPIRATION, also known as isolation, separation or cell sorting of single cells can be performed on suspension or adherent cells. Some methods for aspiration of single suspension cells are, for example, flow cytometry and magnetic bead supported separation [1]. On the other hand, aspiration of single adherent cells can be performed using laser capture microdissection, mechanical microdissection or micropipette aspiration (MPA) [2, 3].

MPA is closely related to the capillary pressure microinjection (CPM) method, as the same subsystems are used in both: a micromanipulator, a pressure source, a vision and an incubation subsystem. Unlike in the CPM, where a positive injection pressure is applied, in MPA a negative pressure (vacuum) is applied, resulting in a suction force on the cell.

The MPA method is best applied on individual cells in *in-vitro* cell populations as it is a highly selective isolation method with single cell accuracy. MPA has numerous applications on single cell level, shown in Fig. 1: (a) undesired cells can be removed from cultivation, (b) aspirated cells of interest can be transferred to start a new cultivation, (c) the cell can be removed for later studies such as determining the gene expression, or (d) mechanical properties of a cell can be characterized.

K. Kovanen and M. Lukkari are with the Institute of Automation and Control, Tampere University of Technology, 33101 Tampere, Finland.

S. Purmonen and T. Ylikomi are with the Department of Cell Biology, Medical School, University of Tampere, 33014 Tampere, Finland.

J. Viitanen is with the Digital Information Systems, Technical Research Center of Finland, Tampere, FI-33101 Finland

Pasi Kallio is with the Institute of Automation and Control at the Tampere University of Technology, P.O.Box 692, 33101 Tampere, Finland (phone +358-500-525546; fax: +358-3-31152340; e-mail: pasi.kallio@ tut.fi)
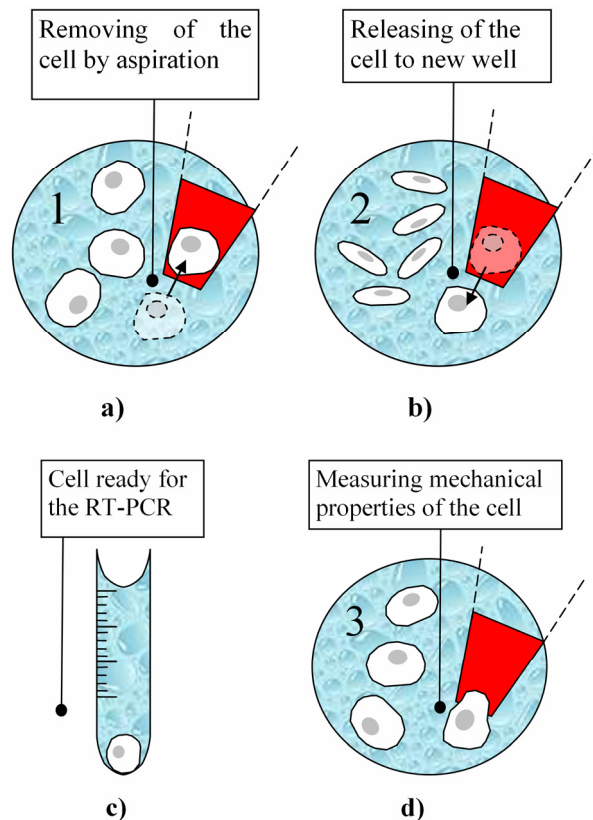
Fig. 1. Micropipette aspiration: the cell is removed from the culture (a), the cell is transferred to start a new cultivation (b), the cell is transferred into a test-tube for next experiments (c), or mechanical properties of the cell is measured (d).

MPA has been used for various tasks to measure different parameters, by producing controlled deformation of the cell. The parameters include (i) different cell wall studies, such as measuring stiffness and studying nature of the lipid component [4, 5], (ii) studies of deformation behavior and mechanical properties [6, 7, 8, 9, 10, 11, 12] and (iii) studies on adhesion [13, 14, 15, 16, 17].

The size of the micropipette used in the cell removal applications of MPA depends on the application and on the size of the cells. The micropipette can be smaller than the diameter of the cell, for example when measuring the adhesion force. In aspiration, the cell exhibits an initial "jump" in deformation with the applied pressure, followed by a slow asymptotic entry of the cell into the micropipette up to two times its radius [15, 16]. The entry speed of the cell depends on the cell, on the micropipette and on the applied aspiration pressure.

The current major challenges in MPA include the

aspiration of tightly attached adherent cells and the total extent of manual operations in the aspiration process. This paper presents a method to aid the detachment of adherent cells from the bottom of the well and from surrounding cell connections using CPM in connection with the aspiration. Furthermore, a micropipette aspiration system with an additional impedance measurement system is proposed to facilitate automatic aspirations. The paper next discusses the materials and methods used in the experiments including the components of the MPA system in Section II. Then, the results are presented in Section III. Finally, Section IV provides conclusions and proposes future steps.

II. MATERIALS AND METHODS

A. Cell Culture

Experiments were performed on the adherent human breast cell line MCF-7. All cell culture media and supplements were from Gibco Invitrogen Life Sciences (Paisley, UK). Before experimental studies, cells were cultivated 2-3 passages in phenol red free DMD/F12 supplemented with 5% dextran-coated, charcoal-stripped treated, fetal bovine serum (FBS), penicillin-streptomycin, 10ng/ml insulin, and 1nM 17β-estradiol. The day before experiments, cells were plated in 12-well plates at a density of $5 \times 10^4 – 7 \times 10^4$ cells per well. Cells were allowed to attach overnight. For experiments the medium was replaced with L-15 Leibovitz medium (Sigma-Aldrich, Munich, Germany), which required no pH adjustment with carbon dioxide, supplemented with 5% dextran-coated, charcoal-stripped treated, FBS, penicillin-streptomycin, 10ng/ml insulin, 1 nM 17β-estradiol and 2mM L-glutamine.

B. Micropipettes

To perform aspiration experiment, micropipettes CustomTip TypI (Eppendorf, Hamburg, Germany), with an inner diameter of 20μm, were used. For the experiments, the micropipettes where filled with (i) a trypsin/EDTA (ethylenediamine tetraacetate) solution, 0.05% of trypsin and 0.53 mM of EDTA or (ii) a cell medium L-15. Trypsin solutions are commonly used for enzymatic detachment of cells from culture dishes.

C. Micropipette Aspiration System

The system consists of a positioning system, an injection / aspiration system, an impedance measurement system, a vision system, a cell incubation system and control software.

1) Positioning System

The positioning system includes a 3 DOF micromanipulator MAN*i*PEN developed by the research group for the high-precision positioning of the micropipette and a 1 DOF pneumatic positioning device for a course positioning of the micromanipulator [18]. The micromanipulator is fixed to a stand at such an angle that the tip of the micropipette can be located in a well of a 12-well plate, which is hitherto the limit for the smallest well size in

which the manipulation area is reasonable.

2) Injection / Aspiration System

The pressure system used in the aspiration has been developed by the research group. It is fully computer-controlled and provides both positive (0 – 34.6 kPa) and negative (-5.3 – 0 kPa) pressures for injection and aspiration, respectively.

3) Impedance Measurement System

The impedance measurement system, developed by the group, detects a contact between the tip of the micropipette and the cell membrane [19]. The system utilizes the general principle of a current/voltage clamp circuit. The device measures the impedance of the micropipette by supplying a known square-wave voltage signal between a measurement electrode placed inside the micropipette, and a reference electrode placed in a cell culture well, and then measuring the resulting current. The change in the impedance of this "electric circuit" is an excellent marker of many events [20]. For instance, when the micropipette is in contact with the cell, the impedance increases. In this work the impedance measurement is further used for detecting the entrance and the exit of the aspirated cell into and from the micropipette. Only the real, resistive component of the impedance was measured.

4) Vision System and Cell Incubation

The vision system includes an inverted optical microscope with a fluorescence option (TS100F; Nikon Corporation, Tokyo, Japan) and a charge-coupled device (CCD) camera (RT monochrome spot 2.1.1; Diagnostic Instruments, Sterling Heights, MI, USA). The vision system includes a self-made incubator which makes it possible to record the event of aspiration, as well as long-term monitoring of cells, while the cells are in incubation conditions.

D. Proposed Method to the Micropipette Aspiration

Many adherent cells tend attach to the surface of the cultivation well very tightly and therefore, their fast and harmless aspiration is very challenging. This paper proposes the use of trypsin microinjections around the cell prior to the aspiration, as illustrated in Fig. 2. Due to trypsin adhesion forces will decrease, the cell loosens itself from the surface and surrounding cells. This makes it possible to use a reduced aspiration pressure, and hence a smaller mechanical force is applied to the cell, leaving the cell intact for later studies.

Due to the relatively big size of the micropipette, capillary forces are an issue. The balance between the trypsin efflux (from the micropipette) and the cell medium influx (into the micropipette) was controlled by applying backpressure to the pipette. Too low backpressure results in suction force (aspiration) and cells which are loosely in the medium (suspended cells), enter the micropipette, too. By increasing backpressure, the balance between the influx and efflux is sustained.

After the trypsin injection around the cell, a constant aspiration pressure (2kPa) is applied to suck the cell inside

the micropipette. The needed amount and duration of the aspiration pressure depends on the strength of adhesion force of the cell to the surface and the surrounding cells.
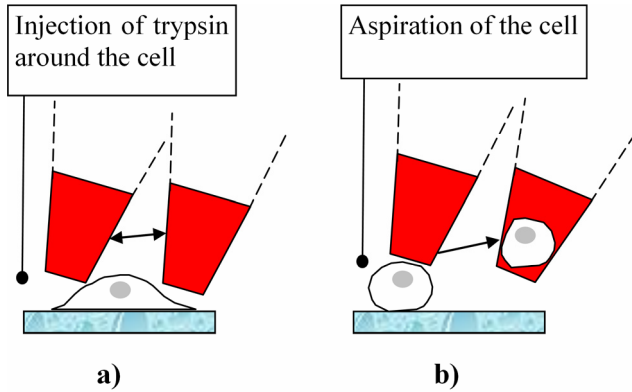

Fig. 2. Step 1: Trypsin is used to decrease the adhesion force of an individual cell (a). Step 2: The cell loosens in a smooth spherical form and is aspirated into the micropipette (b).

III. RESULTS

In the experiments, two injection substances were studied. The hypothesis was that any strong fluid flow has an effect on the cells adhesion force. Trypsin and cell medium L-15 were injected to decrease the adhesion force of the cell prior the aspiration.

Without the injection, the aspiration pressures need to be higher and some mechanical force has to be applied to scrub the cell from the surface. When filling the micropipette with L-15 cell medium, the aspiration result was better than without using any microinjection prior the aspiration. However, using the L-15 medium inside the micropipette did not have similar effect on cells as trypsin. Due to trypsin injection, cells readily detach from the surface in a smooth spherical form, which makes them easy to aspirate. Furthermore, when using L-15 the cells tended to stuck to the micropipette, which was not a problem in case of trypsin.

The aspiration is stopped when the human operator sees visually that the cell enters the micropipette and stops the aspiration pressure. The longer the aspiration pressure is applied, the deeper into the micropipette the cell moves. Also undesired cells may enter the micropipette. In case a certain individual cell of interest is aspirated and further gene expression studies are to be made, the controlled operation of the cell inside the micropipette is needed. Also for automation of micropipette aspiration a feedback from the tip of the pipette could be high of interest.

In next experiments the impedance measurement system was tested to see if any additional information could be found when aspirating the cell.

An example of a micropipette aspiration experiment is illustrated in Fig 3. In Figure 3a, MCF-7 cells to be aspirated are highlighted with white circles. In Figure 3b, the places where those cells have been situated before aspirations are shown with light dots and are numbered. The white bar

indicates 40 μm.
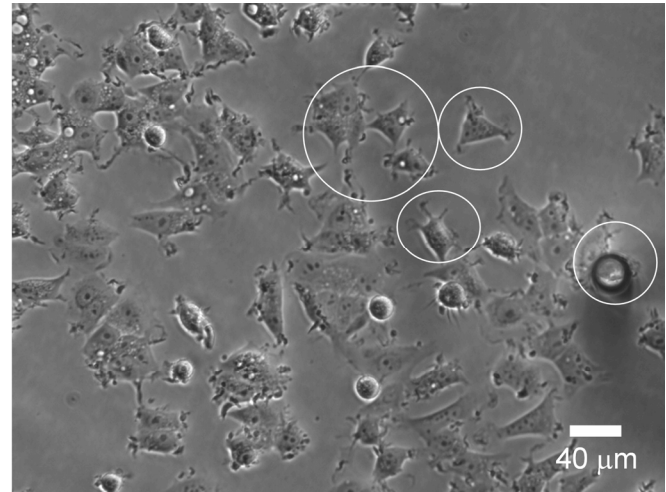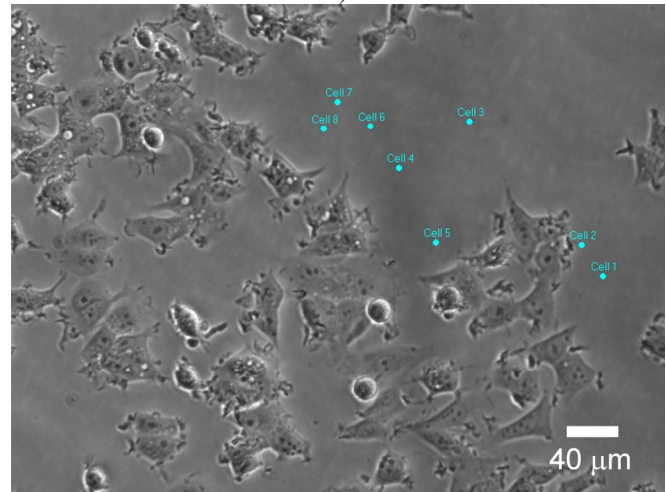
Fig. 3. Illustration of the aspiration test – MCF-7 cells before (a) the micropipette aspiration experiment, highlighted with white circles. The places where cells have been aspirated are shown with light dots (b). The white bar indicates 40 μm.

The use of the electric impedance measurement gives feedback from the events at the tip of the micropipette during the micropipette aspiration. Fig. 4 presents the cell aspiration and then injection out of the micropipette. The contact between the cell and micropipette lasts from 118 second till 128 second (10 seconds), before the cell enters the micropipette. The moments when the cell enters and exits (is injected out) the micropipette are clearly seen on the signal in 139 second timeline.

A high peak can be seen, when the cell is detached and goes inside the micropipette. This corresponds to the increased resistance between the electrode inside the tip and the reference electrode in the free liquid of the well when the insulated pipette rim seals against electrically insulating cell membrane. The event is seen as correspondingly higher voltage over the sealing resistance, over which the pipette current is flowing.
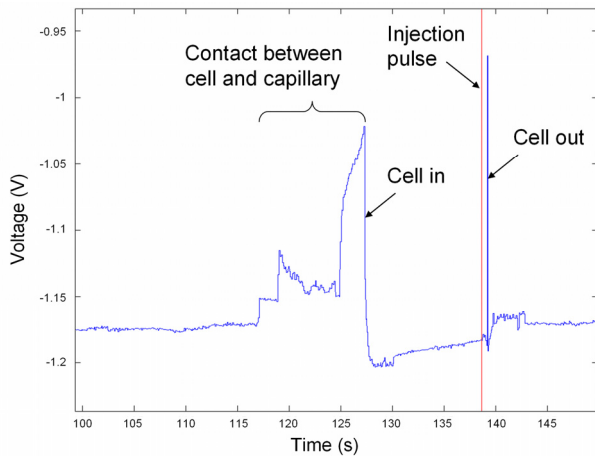
Fig. 4. The voltage over the electric impedance between the tip electrode and the reference electrode in the free liquid of the incubation well (blue line) measured in the aspiration experiment. The vertical (red or light gray in black and white print) line indicates the time when injection pulse is applied to release the aspirated cell.

IV. DISCUSSION AND CONCLUSION

By using the electrical impedance measurement system, automation of the micropipette aspiration can be implemented. The measurement signal provided by the system can be used to quicker stop/reduce the application of aspiration pressure as soon as the connection between the cell and the micropipette reaches a certain level. This will make it easier to inject an individual cell out of the micropipette in a controlled way, without the need to empty the micropipette. On the other hand, in gene expression studies, for example, several tens of cells can be aspirated into the micropipette, and the whole interior of the micropipette is of interest.

The proposed aspiration system combined with the trypsin microinjection method has several application possibilities. For example, in functional genomics studies the cells can be injected with cDNA or siRNA molecules and their activity can be followed. After the microinjection and necessary follow-up, the cells of interest can be aspirated for gene expression studies using single cell RT-PCR, for example.

Stem cell research provides another interesting application domain. During the differentiation of the stem cells, either the differentiated cells, undifferentiated cells or cells differentiated undesirably may need to be isolated. For these purposes, the developed MPA method and the corresponding system provides a technology platform for automatic selective cell isolation in single cell level.

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