Multi-purpose Impedance-based Measurement System to Automate Microinjection of Adherent Cells*

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Abstract **– This paper presents a multi-purpose measurement system developed to automate cellular microinjection. The system is based on measuring impedance between an injection solution in an injection capillary and cell culture medium where cells grow. The system can be used for detecting a contact between a cell and the injection capillary, a broken capillary, a clogged capillary, an aged measurement electrode and faulty injection solution. The measurement system facilitates the development of an expert system for guiding cellular injections, and later a fully automatic microinjection system.**

Index Terms – Automatic microinjection, cell, micromanipulator, contact, impedance.

I. Introduction

Micromanipulation can be defined as manipulation of objects ranging from about one micrometer to a few millimeters in size. The objects can be either natural such as eukaryotic and prokaryotic cells, intracellular components, tissue samples, and paper fibres, or artificial such as miniaturized mechanical parts and electrical and optical components. In microassembly, the handling of miniaturized mechanical, electrical and optical components and their assembly to complete systems is of interest. The application area of the micromanipulation system to be discussed in this paper is biomanipulation. More specifically, it is designed for versatile manipulation of single mammalian cells in adherent cell cultures using contact-based techniques. Various areas of biomicromanipulation and the focus application of the system discussed in this paper are depicted in Fig. 1.

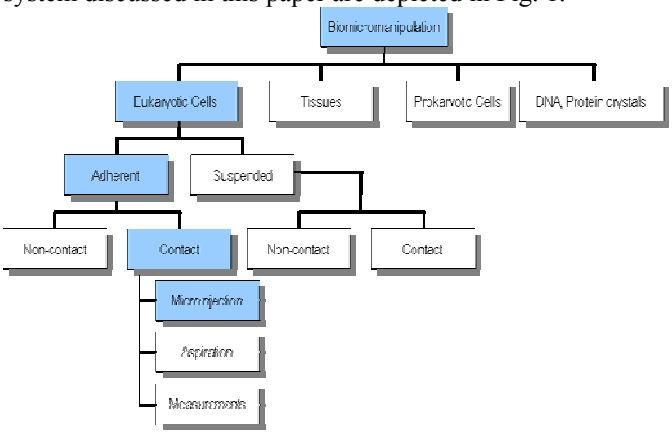

Fig. 1 Classification of biomicromanipulation and the focus areas of the paper indicated with blue boxes.

Depending on the target, different end-effectors and manipulation operations are needed. This paper focuses on the manipulation of eukaryotic cells and more specifically on mammalian cells that grow at the bottom of a cultivation dish and form an adherent cell population. Compared to such suspended cells as egg cells (approximately 100 micrometers in diameter), which are the most typically microinjected suspended cells, the adherent cells are much smaller in size (10 - 30 micrometers in diameter). As illustrated in Fig. 2, manipulation of adherent cells differs from that of suspended cells posing different challenges to the micromanipulation system. Due to the smaller size of the cells, higher positioning and injection accuracies and better visualization system, for example, are needed. The small size of cells also results in a need for very fine injection capillaries. As a diameter of smaller than one micrometer is needed, it is very difficult to visually detect the condition of the capillary, a contact with a cell, an injection depth, etc. On the other hand, a holding capillary is not needed, since the cells adhere to the bottom of the cultivation dish.

One more definition to focus the discussion is needed: microscopic objects can be manipulated either by touching the objects physically or without a physical contact. In non-contact manipulation, objects are manipulated using optic, electric, magnetic or acoustic energy, see for example [1], [2], [3], [4]. Non-contact manipulation is an important way of performing micromanipulation, but the work reported in this paper is based on the contact type manipulation. A microcapillary – or a micropipette – is typically used as an end-effector in contact manipulation of eukaryotic cells. A significant benefit is that the same end-effector, the microcapillary, can be used for injection, aspiration and electrical measurement of the cell. This paper describes an impedance-based measurement system and discusses its potential in automating capillary-based cell injection.

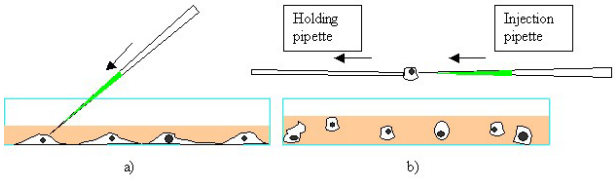

Fig. 2 Microinjection into (a) adherent cells and (b) suspended cells.

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The rest of the paper is organized as follows. Section II describes a capillary pressure microinjection technique and provides background for the development of the impedance-based measurement system. The micromanipulation system, the impedance-based measurement system and issues needed to be taken into account in their integration are discussed in Section III. Section IV describes tests performed for collecting data in real cell injection experiments. The analysis of the data is presented in Section V and conclusions and visions for future work are provided in Section VI.

II. Capillary Pressure Microinjection

Microinjection is a technique for the delivery of small volumes of compounds into suspended or adherent cells and it has become a prominent experimental approach in biological research. A large variety of molecules like dyes, proteins or nucleic acids can be injected into cells and their activity can be studied [5].Various microinjection methods exist. The most important are capillary pressure microinjection, laser beam injection, electroporation, iontophoresis and various endocytosis methods.

Capillary pressure microinjection (CPM) is a mechanical method, where a thin microcapillary is penetrated through a cell membrane and then liquid is delivered from the capillary to the cell upon a pressure pulse.

Capillary pressure microinjection provides the greatest potential for an exact control of the volume injected and importantly, also large molecules can be injected into the cells. Furthermore, as already mentioned, the same end-effector - a microcapillary - can be used in other operations, such as cell isolation and electrophysiological measurements. Over the last years, the development of the CPM technology has enabled steps towards automation and research groups are developing automatic microinjectors [6], [7], [8], [9]. Some commercial devices such as those provided by Eppendorf, Narishige and Cellbiology Trading are also available. However, even though some systems are already partly automated, a huge amount of manual work is still required by the operator. Due to the involvement of the operator, the number of cells that can be injected in a certain time is limited. This can be a problem if a large number of cells has to be injected for a biochemical assay or when microinjection is used to produce stable transfected cell lines [10]. Furthermore, the reliability, repeatability and accuracy of the method should still be improved: the capillary gets clogged, it breaks without the operator noticing it and the exact volume of the injection is not known.

By increasing the amount of instrumentation and the level of automation, (i) the speed of the method can be increased such that its usage will be feasible even in high throughput applications, (ii) the involvement of the scientist can be reduced such that he/she can concentrate on the analysis of the results and gets them much faster, and (iii) the reliability and accuracy of the system can be increased such that more reliable results will be obtained. Issues in the automation of living cell manipulation in general and capillary pressure microinjection in particular are discussed in more details in [11] and [12], respectively.

The biggest bottle-neck for a versatile and fully automatic microinjection system is the lack information on the contact between a cell and a microcapillary tip. The following section will first describe the micromanipulator system developed by the group and then an impedance-based measurement device which has been developed for detecting a contact between a cell and a microcapillary.

III. Micromanipulation System

The micromanipulation system consists of four subsystems: a manipulator system, an incubation system, a vision system and a control system. A block diagram of the system is shown in Fig. 3. The manipulator subsystem is composed of a 3D micromanipulator named MANiPEN, a course 1D translation stage and an injection/aspiration system. As a result of the work reported in this paper, the impedance-based contact detection device is included in the manipulator subsystem.

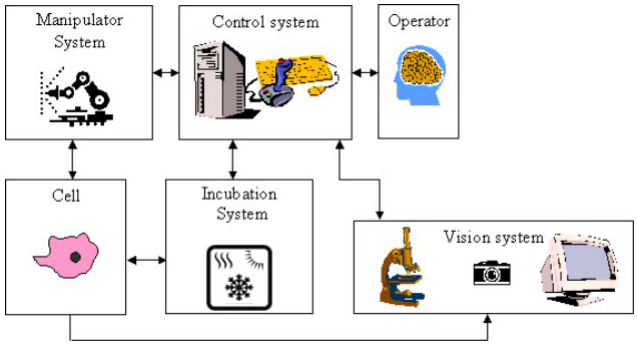

Fig. 3 Block diagram of the entire micromanipulation system.

The developed contact detection device utilizes the general principle of a current/voltage clamp circuit (such as presented e.g. in [13]). The device measures the impedance of the capillary by supplying a known square-wave voltage signal ("a stimulus signal") between a measurement electrode placed inside the injection capillary and a reference electrode placed in a cell culture well and by measuring the current. The impedance of the capillary increases, while the capillary is in contact with the cell. Moreover, when the tip of the capillary breaks, the impedance decreases, and when it gets clogged, the impedance increases.

A simplified block diagram of the device is presented in Fig. 4. The *Stimulus Processing and Scaling* block includes adjustable scaling and buffering of the stimulus signal. The *Current Measurement* block consists of a sensitive current-to-voltage converter and a differential amplifier. The *Signal Conditioning and Amplification* block includes a low-pass filter and an adjustable output amplifier. The actual system implementation is separated into two parts: a head stage and a control unit. The head stage placed next to the capillary includes the current-to-voltage converter circuit and the control unit includes all the other functions of the device. A photograph of the head stage and the micromanipulator used in a cell injection experiment is shown in Fig. 5. A more detailed description of the device can be found in [14].

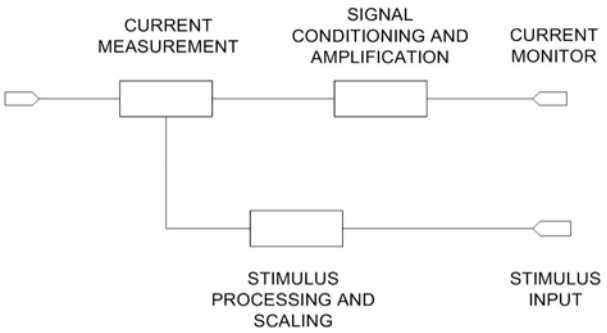

Fig. 4 A simplified block diagram of the contact detection device.

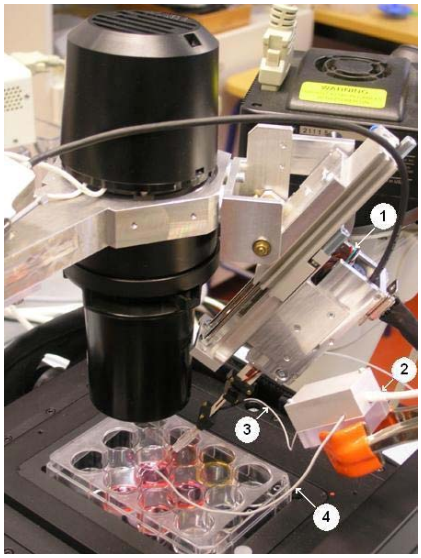

Fig. 5 Photograph of the head stage of the impedance measurement device and the micromanipulator. (1) Micromanipulator, (2) head stage, (3) measurement electrode, and (4) reference electrode.

In the previously reported work, the contact detection device was implemented with a manual hydraulic manipulator and a Faraday's cage in the Institute of Medical Technology at the University of Tampere. This arrangement was favorable in noise reduction and concept testing points of views, but not sufficient to test the applicability of the device in a computer-controlled electric micromanipulator system. As a first step towards fully automatic microinjection, the contact detection device is integrated with the manipulator system.

Due to the very small currents to be measured, grounding and shielding of the wires appeared to play an essential role. By proper grounding and shielding, the noise level was achieved to be reduced to one part of several hundreds. By grounding the microscope, the micromanipulator, the head stage, the head stage holder and the shields around the capillary and the culture well, a noise level of 4 mV (approximately 2 % of the signal) was able to be achieved. The result is excellent, since the signal-to-noise ratio was better than achieved using a commercial patch-clamp device, the hydraulic micromanipulator and the Faraday's cage in the previous work.

IV. Experiments

A. *Effects of Injection Solution and Culture Medium*

Firstly, the effects of an injection solution and a cell culture medium on the measurement signal were studied. Two cell culture media and three different injection solutions used typically in cellular injection experiments were compared.

The two cell culture media compared were DMEM/F12 and Leibowitz 15 used typically in the group's injection tests. DMEM/F12 is a culture medium often used in cell cultivation. It needs CO_2 supply to maintain the pH value of the culture. Leibowitz 15 maintains the pH level without additional CO_2 and is sometimes used in the group's experiments. The results show that the two cell culture media provide very similar measurement signal after a short transition period in the beginning of the experiment. Thus, the contact detection device can be used with both media.

The injection solutions were (i) pure KCl, and KCl mixed with (ii) FITC (Fluorescein Isothiocyanate) and (iii) GFP (Green Fluorescence Protein) dyes. FITC is a fluorescent dye used by the group in studies of the injection success. If the injection is successful the cell is fluorescent immediately after the injection. The GFP dye is composed of plasmid DNA (pBabe-Gem2) containing a GFP gene. Approximately 24 hours after injection, the cell starts to express Green Fluorescence Protein (GFP) if the injection has been successful and if the cell has survived the injection. Therefore, the GFP dye is used in the analysis of the cell survival rate. In addition to a typically used KCl solution, GFP was also diluted in water to test if errors in the injection solution can be recognized with the device.

Pure KCl provides slightly larger signal amplitude and offset levels than KCl mixed with FITC or GFP dyes but in overall the results are similar. Water based solution results in a clearly different measurement signal: it has large peaks in the beginning and end of the pulse. The difference between measurement signals obtained with water and KCL based GFP solutions and Leibowitz 15 cell culture medium is illustrated in Fig. 6 and Fig. 7. As a conclusion, the contact detection device can be used with injection solutions (FITC diluted in KCl and GFP diluted in KCl) typically used in the group's experiments. Furthermore, seems that faults in the solution can be detected with the device, too.

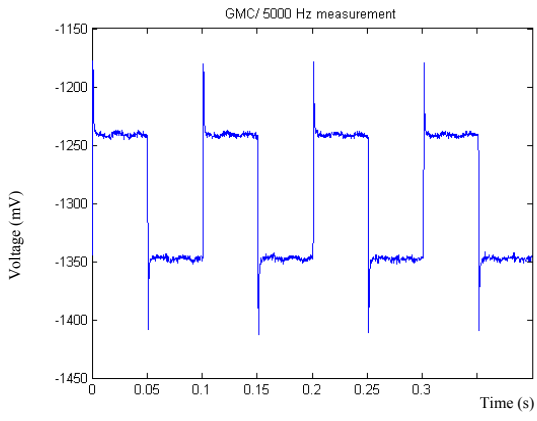

Fig. 6 Measurement signal obtained with water-based GFP solution and L15 cell culture medium.

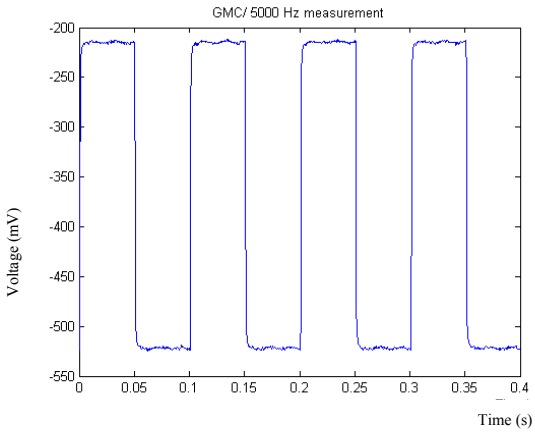

Fig. 7 Measurement signal obtained with KCl-based GFP solution and L15 cell culture medium.

B. Cell Contact Experiments

The cell contact experiments were performed to collect data in real cell injections. Numerous injection experiments were performed and data was collected on (i) various kinds of contacts between the cell membrane and the capillary tip, and on possible (ii) capillary breakages and (iii) cloggings. The cells used in the experiments were cancerous human breast cancer cells MCF-7. The injection solution was FITC diluted in KCl and the cell culture medium was Leibowitz 15. The amplitude of the square-wave stimulus signal used was 5 mV and the frequency 10 Hertzs. The data was recorded with a sampling frequency of 5 kHz.

Fig. 8 shows an example of the measurement signal recorded in a cell-capillary contact and Fig. 9 provides a close-up in the beginning of the contact. Fig. 10 provides an example of the capillary breakage.

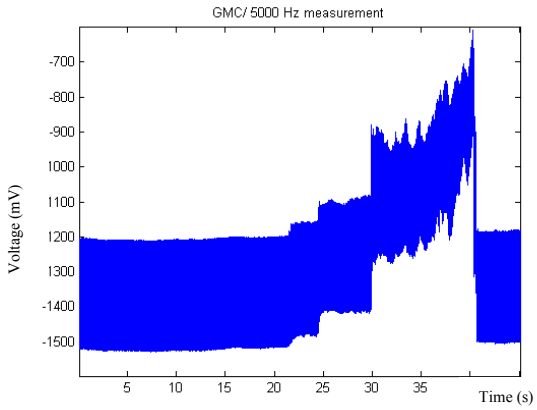

Fig. 8 Measurement signal in a cell-capillary contact.

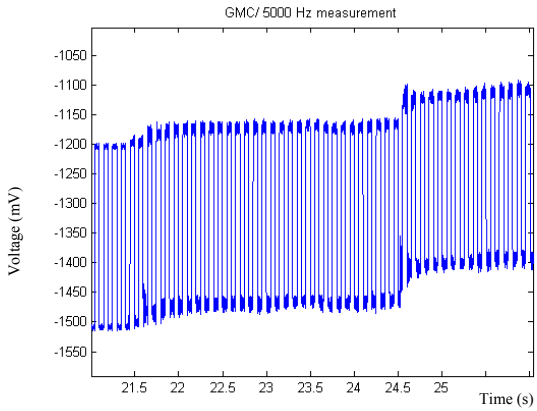

Fig. 9 A close-up in the beginning of the contact.

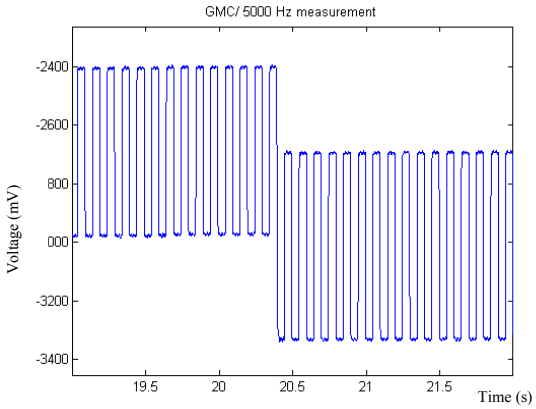

Fig. 10 Capillary breakage detected with the contact detection device.

V. Data Analysis

Section IV discussed the collection of raw data in cell experiments and illustrated the raw measurement signals. This section will discuss the injection events which can be detected using the device and the features in the measurement signal which can be used to detect a certain event. Firstly, the detection of a cell-capillary contact will be discussed in Section A, and then the detection of a capillary breakage, a capillary clogging, an aged electrode, a faulty injection solution and a volume injected will be discussed in Section B, Section C, Section D and Section E, respectively.

A. Cell Contact Experiments

In order to develop an algorithm for an automatic detection of the contact between a cell and an injection capillary, the data was analyzed with Matlab. Four contact indicators were studied: (i) a change in the pulse amplitude, (ii) a change in the pulse offset, (iii) a change in the noise of the high signal value and (iv) a change in the noise of the low signal value. A standard deviation was used as a measure for the noise. Fig. 11 shows an example how the values of the four indicators change in a cell-capillary contact. The values are scaled such that maximum is one. Zero value indicates no contact and the larger the value the stronger the contact.

In Fig. 11, the pulse amplitude and the pulse offset show comparable results. However, the offset can vary quite a lot during the measurement and it seems to depend on many different variables. Firstly, when the electrodes are in

use for a long period of time they loose the chloride, get "dirty" and increase the offset. Furthermore, after the change of electrodes or the injection solution, the offset level drifts for some minutes until the electrodes stabilize. The amount of solution in the capillary and different solutions used also change the offset. Because the offset is not very controllable and all the reasons for changes in the offset level cannot be determined, the offset is not as reliable indicator for the contact as the pulse amplitude.

The contact detection using an increase in the noise appeared to be even more unreliable than the offset change. Therefore, the contact detection was decided to be implemented using the change in the pulse amplitude.

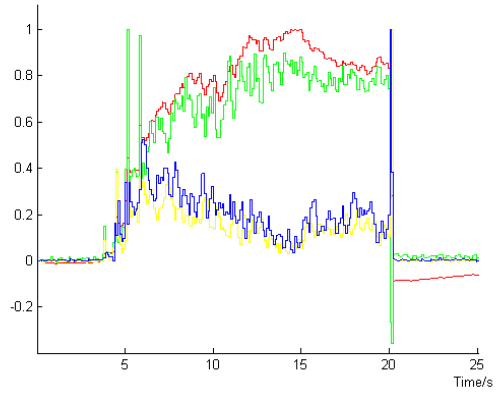

Fig. 11 Cell-capillary contact: the pulse amplitude (green), the pulse offset (red), noise in high pulses (blue) and noise in low pulses (yellow).

The experiments performed indicate that a change in the pulse amplitude corresponds to the force used to make the contact. If the contact is very gentle or the capillary barely touches the cell, the changes in the pulse are quite small. If the capillary is pushed more to the cell the changes in the pulse become much larger (even 50% or more). Furthermore, the cell type, the capillary tip and the angle of the capillary among other variables affect the amount the amplitude reduces in contact. However, the variation of the amplitude when the capillary is not in contact with a cell is very small and therefore, the parameters can be adjusted in such a way that the contact is detected from a very small change in the pulse. The offline contact detection was made even with 5 % reduction in the amplitude and it worked well without false detections of contact. Thus, the reduction of the pulse amplitude seems to be the most reliable way to detect the contact. Since the detection of the contact from the pulses is calculated by subtracting the following offset level from the pulse level and the change in the offset is to the positive direction, the changes in the offset when releasing the contact is included in the current algorithm making the contact detection even more sensitive.

B. Capillary Breakage

The breakage of the capillary can be detected using an increase in the pulse amplitude. Even if the breakage is very small (even barely visible with a microscope) the increase in the amplitude is relatively large and the detection of the breakage can be done easily. Since the normal amplitude variation is small, the breakage detection limits can be set to very small values, such as 5 %.

The breakage of the capillary should be analyzed using three pulses to avoid a faulty detection of the contact when the capillary is leaving the cell. The faulty detection could otherwise take place, since the offset usually increases when the capillary leaves the cell increasing the amplitude change.

C. Capillary Clogging

The clogging of the capillary can also be detected using the change in the pulse amplitude. If the contact detection device still indicates a contact, i.e. an increased impedance, and it is known that the capillary is already removed from the cell, it can be concluded that the capillary is clogged by a particle and the capillary should be cleaned or changed.

D. Changing Electrodes

When the electrodes are used for some time, they usually become dirty, loose the chloride coating and the half-cell potentials change, all causing an increased offset. The detection algorithm can be used to give a notification to change the electrodes when the offset becomes too large.

E. Injection Solution

As was discussed in Section IV, a water-based solution results in a clearly different signal compared to KCl-based solutions which are advised to be used in cell injections. Therefore, the contact detection device can also be used for detecting whether a correct injection solution is used in the injections.

E. Injection Volume Measurement

The size of the commercial capillary tips varies significantly (0.5 µm ±0.2 µm). Therefore, with the same injection parameters, the result is different with different tips. The developed system can be used for determining proper injection parameters for each tip, since the amplitude of the pulse corresponds to the diameter of the tip. Furthermore, initial observations have indicated that when injections are performed using a broken capillary, the offset of the signal changes. This could be used to measure the injection volume. A successful measurement of the volume injected would be a significant leap in the way toward full automation of cellular injections. However, further experiments are needed to determine the accuracy and resolution of the measurement.

VI. Conclusions and Future Work

This paper has described an impedance-based contact detection device developed for detecting a contact between a cell and an injection capillary. The paper showed that the contact be can reliably and repeatably detected using the developed device together with an algorithm determining the amplitude change in the measurement signal. Furthermore, the paper discussed how to extend the device to a multi-purpose measurement system which can detect a broken capillary, a clogged capillary, an aged electrode, a faulty injection solution and probably the volume of the injected solution.

The future work will focus on developing an expert system which will be based on the work reported here. Such

an expert system will instruct the user in the injection process to achieve higher injection success rates. In a longer term, the device and algorithms described here will be further developed to facilitate fully automatic injection of single adherent cells.

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