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Estimation of Electrical Cell–Capillary Admittance During Injection with Frequency Response Method

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Abstract: This paper describes electrical equivalent circuit models of cell–capillary admittance during injection of a living cell and presents a measurement system to estimate corresponding frequency responses during microinjection tests. Since the admittance estimate is calculated from data collected during injection, the amount of data is limited. To overcome this constraint, the approach proposed in this paper takes advantage of properties of periodic pseudo random binary sequence (PRBS) excitation signal and avoids end effect anomalies of correlation calculation. The fast and accurate estimation is used to detect the degree of contact during cell injection and to detect breakage and clogging of capillary during a sequence of multiple operations.

1. INTRODUCTION

Current state-of-the-art cell injection micro-manipulators are typically manual or semiautomatic and joystick controlled, making the manipulations obsolete and inefficient. Furthermore, living cells, especially primary cells, are very sensitive and require experienced and skilful persons to operate. Also, it is not possible manually to treat cells in such quantities that are adequate for molecular biology analyses. Automation of the injection system is important for making a faster, more efficient and reliable research instrument.

A major bottle-neck in the automation of cell injection is the detection of a contact between a microinjection capillary and cell membrane. Presently, there are no commercially available reliable methods for detecting the contact. In manual operation the visual feedback is used to detect the contact. Another obstruction for an efficient microinjection is the lack of means for the detection of the capillary breakage or clogging. A clogged capillary prevents proper microinjection, and since it cannot be detected in currently used injection systems, the operator should – in an extreme case – change or clean the capillary after each injection.

Sensory feedback technologies and data analysis theories have been used successfully in increasing productivity, improving quality, decreasing fluctuations, and replacing laborious and monotonous work phases in many industries. Modern control and automation methods are also applicable to cell injection processes.

This work presents how sensory feedback and a fast Pseudo Random Binary Sequence (PRBS) signal based frequency response method can be used to measure injection manipulations of single cells in real-time and to measure cell properties simultaneously with the manipulations. Since the duration of the manipulation incident is constrained and the properties of manipulation device changes during operation the computational methods conventionally used cannot be applied directly.

Frequency response method is a widely used method in estimation and visualization of dynamic properties of variety of processes (Pintelon and Schoukens, 2001). The maximum length PRBS signal is a well known excitation signal for estimating frequency responses since it has some exemplary properties in respect of periodicity and frequency content (Davies, 1970). With the PRBS excitation signal and appropriate estimation algorithms it is possible to implement fast and accurate frequency response estimation (Miao et al., 2005).

The approach in this work is to estimate a PRBS based frequency response for the electrical admittance of a system comprising a capillary and a cell during microinjections. By including also the capillary into frequency response analysis it is possible to make an estimate of capillary properties during injection process. The hypothesis is that the properties of the capillary and the cell and different injection events can be detected from the frequency response. The events and properties of interest during injection can be listed as follows: a degree of contact between a capillary and a cell; clogging of a capillary; breakage of a capillary; condition of the cell; type of the cell; and injection volume.

The objective in this paper is to demonstrate how the PRBS signal shall be generated and how the frequency response is calculated from measured response to reveal contact, and clogging and breakage of capillary when only limited amount of data is collected.

The estimation results presented in this work are interpreted with equivalent electrical circuits for the cell, capillary, and system that comprise both. Section 2 presents circuits for cell membrane, a capillary and a cell–capillary system before an

injection event and during it. Moreover, transfer functions for admittance before and during injection and for a broken and clogged capillary are derived from these equivalent circuits. Section 3 presents principles of system identification used for fast frequency response estimation and Section 4 presents simulated and measured responses.

2 CELL AND CAPILLARY MODELS

This section describes the models of capillary–cell admittance. Subsection 2.1 discusses the electrical circuit models describing a capillary and a cell and Subsection 2.2 presents a transfer function model made on the basis of the electrical circuit models

2.1 Electrical Circuit Models of Capillary and Cell

When thinking a flow of current to or from a cell, the most important part of the cell is the cell membrane. Inside the cell is cytoplasm, which consists of the intracellular fluid cytosol and cell organelles and since cytosol is mostly water, it does not affect much the flow of current. Therefore, the component having the largest influence on the current is the cell membrane. The cell membrane comprises of a lipid bilayer and ion channels, which go through the bilayer.

The ion channels transport ions to and from the cell and therefore the channels have certain conductance while they are open. Thus, the ion channels can be described by resistors with a resistance equal to the inverse of the conductance of the channels. Since the lipid bilayer consists of two layers close to each other and charged particles cannot penetrate it, the lipid bilayer can be presented as a capacitor. The hydrophilic heads of the bilayer act like the thin capacitor plates and the hydrophobic tails form the non-conductive part between the plates (Looi and Haque, 2004). Therefore, the cell membrane can be modelled as a parallel connection of resistances and capacitors and further a parallel connection of a single resistor and a single capacitor. This model is presented in Fig. 1.

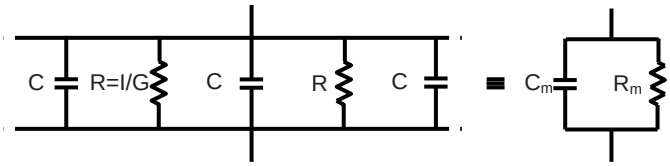

Fig. 1. RC-circuit model of the cell membrane.

Another component to model is the microinjection capillary. It is a sharp glass capillary with a tip diameter smaller than one micrometer. The capillary contains a small portion of injection liquid to be delivered into the cell and an electrode in contact with the liquid. The other end of the electrode is connected to a current measurement circuitry. Stimulus voltage signals are also fed to this electrode. In cell injections, the tip of the capillary is inside a Petri-dish containing the cells to be injected and cell growth medium. The ground electrode of the measurement system is placed in the cell growth medium. Fig. 2 depicts the capillary, the electrodes and the liquids.

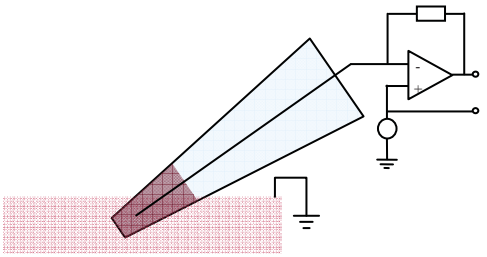

Fig. 2. Capillary, electrodes, measurement circuitry and the liquids.

One route, via which the current can flow from the injection liquid to the grounded medium – that is from the measurement electrode to the ground electrode – is through the capillary tip. Since the injection liquid forms a very narrow column of conductive liquid, it can be modelled as a resistor and its resistance is estimated as follows:

$$R = \rho \frac{4l}{\pi d^2} \tag{1}$$

where ρ is the resistivity of the liquid, l is the length of the narrow tip section and d is the tip diameter of the capillary.

To understand the other route for current between the electrodes, the capillary walls and the liquids have to be considered. The tip of the capillary is an insulating pipe full of and surrounded by conductive media with a potential difference. Therefore, the capillary and the liquids can be thought to form a capacitor whose capacitance can be approximated by the capacitance of a cylindrical capacitor:

$$C = \frac{2\pi\varepsilon_g\varepsilon_0}{\ln\left(\frac{2t+d}{d}\right)} \tag{2}$$

where ε_o is vacuum permittivity, ε_g is the permittivity of the capillary glass and t is the thickness of the glass.

Thus, another route for the current from injection liquid to medium is through the capillary glass as from a capacitor plate to another. This means that also the electrical model of the capillary is a parallel connection of a resistor and a capacitor as presented in Fig. 3.

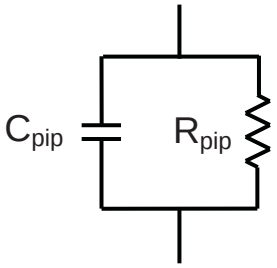

Fig. 3. RC-circuit model for the capillary.

When the capillary is not in contact with the cell, only the circuit in Fig. 3 can be thought to be connected to the electrodes of the measurement device. However, during the

contact, the circuit depicting the cell is also connected to that circuit with two resistors, R_{leak} and R_{patch} (Lukkari *et al*, 2004). This is shown in Fig. 4.

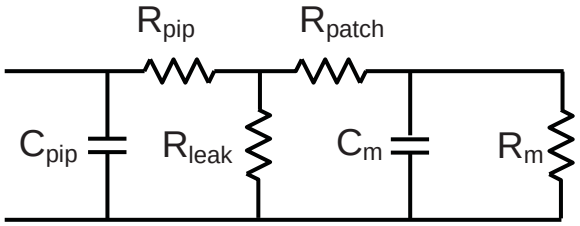

Fig. 4. RC-circuit model of the capillary, cell and liquids.

R_{leak} describes the current leaking resistance of the contact. The value of this parameter depends on the tightness of the contact. If the contact is not tight, a great portion of current flows from the capillary to the medium through the gap between them. In this situation, the value of R_{leak} is small and the majority of current is flowing through it and does not flow to the circuit describing the cell. If the contact is tight, the gap is very small and the amount of leaking current is significantly lower. In the model, the value of R_{leak} is high and considerably more current is going to the circuit illustrating the cell. R_{patch} describes the resistance of the section of the cell membrane in contact with the capillary. If the capillary has penetrated the membrane, R_{patch} depicts the access resistance – the resistance between the injection liquid and the interior of the cell. Also, the resistance of the cytoplasm in the flowing route of the current is summed to R_{patch}. The contact can be detected as addition of R_{leak}, R_{patch}, R_m and C_m to the capillary circuit, and R_{leak} describes the degree of the contact. Since the capillary parameters R_{pip} and C_{pip} depend on the geometry of the capillary, the size of the capillary or the changes in the geometry caused by breakage or clogging affect these parameters. Breakage increases the tip diameter, thus it decreases R_{pip}. Also, it decreases C_{pip} since the term $\ln((2t+d)/d)$ in Equation (2) increases while d increases and this term is in the denominator of the capacitance equation. Clogging reduces the tip diameter and thus increases R_{pip}. In addition, in clogging contact remains between the capillary and a small particle and therefore also some change in the capacitance of the circuit takes place.

Tumorous tissue has been found to have lower capacitance than healthy tissue (Looi and Haque, 2004). Thus, changes in the cell membrane capacitance, C_m, could indicate a condition of the cell. Furthermore, cell division and growth result in changes in the membrane area and therefore affect its capacitance (Looi and Haque, 2004). The state of the cell could hence be detected from the value of C_m. Injections increase the volume of a cell and cause also increase in the surface. Thus, injection volume could be detected from the change in C_m. The non-linear relationship of the volume and the area changes and makes this part difficult not to mention the small volume changes caused by injection.

2.2 Transfer Function Model

A transfer function model of the cell–capillary admittance is formed by using the complex admittances, which are functions of the Laplace variable s. For resistors and capacitors, the complex admittances are defined as follows:

$$Y_{resistor}(s) = \frac{1}{R} \tag{3}$$

$$Y_{capacitor}(s) = sC \tag{4}$$

By using Equations (3) and (4) and the basic equations for serial and parallel connections of admittances, the transfer function of admittance of the circuit presented in Fig. 4 can be solved, see Equation (5). This transfer function is non-causal since it has two zeros and one pole. This is a result of the ideal admittance of a capacitor, which is also a non-causal function. To estimate the differences in admittance during different injection phase events, Bode diagrams of Equation (5) with different parameter values can be plot and can be used for analysis of the injection events discussed in Section 2.1.

3. SYSTEM IDENTIFICATION

System identification is a method to model a dynamic system from measurement data. Identification of frequency response consists of three basic steps, which are interrelated: a) designing the excitation signal; b) feeding the excitation signal to the system and measuring the response; c) estimating the frequency response from the measured data.

3.1 Excitation Signal

The stimulus signal used in this work is a pseudo random binary sequence (PRBS). It is a periodical binary signal generated with a shift register (Davies, 1970). The principle of a shift register is shown in Fig. 5.

$$Y_{TOT} = \frac{R_m C_m C_{pip}(R_{leak}R_{pip} + R_{leak}R_{patch} + R_{patch}R_{pip})s^2 + \left[R_m C_m (R_{leak} + R_{patch}) + R_{pip}C_{pip}(R_{leak} + R_{patch} + R_m) + R_{leak}C_{pip}(R_{patch} + R_m)\right]s + R_{leak} + R_{patch} + R_m}{R_m C_m (R_{leak}R_{patch} + R_{leak}R_{pip} + R_{patch}R_{pip})s + R_{leak}(R_{patch} + R_m + R_{pip}) + R_{pip}(R_{patch} + R_m)} \tag{5}$$

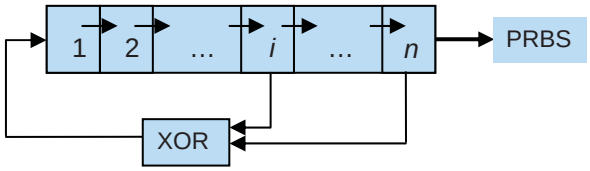

Fig. 5. Structure of a shift register.

The shift register that consist of n bits generates a periodical signal with the period length of $N = 2n-1$ steps. The minimum frequency $f_{\min}$ and the frequency resolution Δf depend on the length of the sequence N and the operation frequency of the signal generator. If the sampling frequency F_s equals to the operation frequency of the shift register the minimum frequency and the frequency resolution of the PRBS signal are

$$f_{\min} = \Delta f = \frac{F_s}{2^n - 1} = \frac{F_s}{N} \tag{6}$$

The maximum frequency $f_{\max}$ of the signal below Nyquist frequency is:

$$f_{\max} = floor\left(\frac{2^n - 1}{2}\right)\Delta f = floor\left(\frac{N}{2}\right)\Delta f \tag{7}$$

The PRBS can be tailored to the identification task of a system by using (6) and (7) when the dynamics of the system are known or can be estimated.

3.2 Measurement System

The software used in the measurements was Matlab xPC Target and the PRBS was generated with a Simulink model. xPC Target is a platform for rapid prototyping and its maximum sampling frequency is 50 kHz. The interface board communicating between the computer and the actual measurement device was National Instruments PCI-6052E. The maximum sampling frequency and the maximum digital to analog converting frequency of the card are both 333 kHz. The actual measurement device of the system was the contact detection device (CDD) developed in the group (Lukkari *et al*, 2004). The operation of the device in a nutshell is the following: first, it buffers the stimulus signal with a voltage follower; secondly, it scales it down with resistors; thirdly, it feeds the scaled signal in the system; fourthly it measures the current yielded and converts it to voltage; and finally, it conditions and amplifies this voltage signal. The CDD is connected to the system measured with two Ag/AgCl electrodes. One is a measuring electrode and another is a ground electrode. Fig. 6 illustrates the structure of the contact detection device. The capillary is positioned and injection is performed using a MANiPEN micromanipulator (Kuncova and Kallio, 2004).

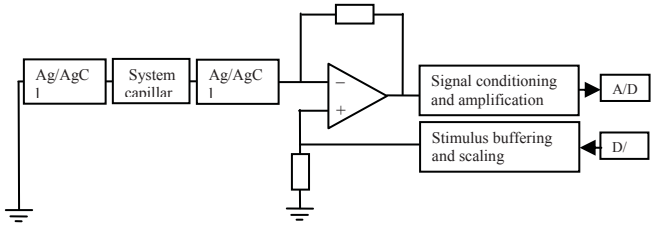

Fig. 6. Operation principle of the contact detection device.

3.3 Calculation Algorithm

A typical approach to define a frequency response is following. The dynamic system may be expressed as (Davies, 1970)

$$y(t) = \int_{-\infty}^{\infty} g(s)x(t-s)ds \tag{8}$$

where $y(t)$ is the output signal, $x(t)$ the input (excitation) signal and $g(s)$ system's impulse response function. The cross-correlation function of two time-varying signals $x(t)$ and $y(t)$ is denoted by $\phi_{xy}(\tau)$ and is defined as

$$\phi_{xy}(\tau) = \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{T} x(t)y(t+\tau)dt\,. \tag{9}$$

Cross-correlation with the signal itself is called the autocorrelation function. With these definitions the dependence of cross correlation and auto correlation can be written as (Davies, 1970)

$$\phi_{xy}(\tau) = \int_{-\infty}^{\infty} g(s)\phi_{xx}(\tau - s)ds\,. \tag{10}$$

Applying Fourier transform yields (Ljung, 1999)

$$\Phi_{xy}(j\omega) = G(j\omega)\Phi_{xx}(j\omega)\,. \tag{11}$$

Hence the frequency response of the system is obtained as

$$G(j\omega) = \frac{\Phi_{xy}(j\omega)}{\Phi_{xx}(j\omega)}\,. \tag{12}$$

In computation of correlation in Equation (9) it is assumed that the signals are long. However, time is constrained during injection and only a limited number of samples can be taken. To avoid the end effects caused by zero padded short signals in correlation calculation, a better approach is to first produce the fast Fourier transformation $U(k) = \mathrm{DFT}\left(u(t_k)\right)$ and $Y(k) = \mathrm{DFT}\left(y(t_k)\right)$ from excitation and output signals and then frequency response is given by (Pintelon and Schoukens, 2001)

$$G(j\omega_k) = \frac{Y(k)}{U(k)} \tag{13}$$

4. SIMULATIONS AND MEASUREMENTS

4.1 Materials

Injection experiments were performed on an insect cell line SF-9. Before experiments, cells were plated in 6-well plates. Microcapillaries FemtoTip II (Eppendorf, Hamburg, Germany), with an inner diameter of 0.5±0.2 µm, were used in the experiments.

The model parameters were determined using the FemtoTip II capillaries, L-15 Leibovitz medium (Sigma-Aldrich, Munich, Germany) and a fluorescent dye Fluorescein Isothiocyanate (FITC).

4.2 Results

The parameters for equivalent electrical circuit component are needed. The capacitance of capillaries filled with Leibovitz medium and FITC was measured with Mastech MY-64 multimeter and gave a result of 8 – 17 pF. The rest of unknown values for the parameters are found in literature. Capillary resistance, R_{pip}, of the FemtoTip II capillaries used has been reported to be 10 MΩ by the manufacturer.

Membrane resistance, R_m, has been reported to be over 1 GΩ Contact detection test(Barnett, 1997). In the work of (Thompson *et al.*, 2001), the conductance of the cell membrane of a RBL-2H3 cell was measured to be from 300 to 500 pS. This corresponds to resistances 2 – 3.33 GΩ. Membrane capacitance, C_m, has commonly a value of 5 – 10 pF (Barnett, 1997). In the injection tests made with the MANiPEN micromanipulator and the contact detection device this far, R_{leak} has usually been more than 5 MΩ when a capillary has been in contact with a cell. Sometimes even a gigaseal have been formed. The value of R_{patch} is hard to approximate since the injection conditions affect it very much. It is approximated to be in the order of mega ohms while the capillary is inside the cell.

Bode diagrams in Fig. 7 calculated using (5) for cell–capillary dynamics show how admittance changes when R_{leak} in increased from a very low value to the value of 100 MΩ. In this simulation low resistance describes non-contact situation and a high value tight contact between cell and capillary.

The Bode diagrams also show that in contact the gain of the impedance decreases at low frequencies and phase advances at frequencies between 10 and 1000Hz.

In the measurememtns the excitation signal is 1023 step PRBS signal. During injection contact three consecutive 1023 period sequences are fed with frequency of 2000 Hz, thus, the duration of excitation and data collection is 1,5 seconds. The response from each sequence is averaged to reduce measurement noise. Also, the frequency resolution and the lowest frequency calculated by (6) and (7) are 1.955 Hz and highest below Nyquist frequency is 999.0225 Hz.

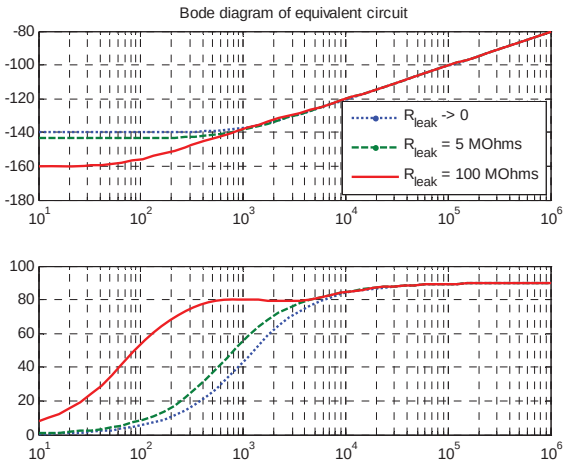

Fig. 7. Simulated effect of parameter R_{leak} on the frequency response of admittance.

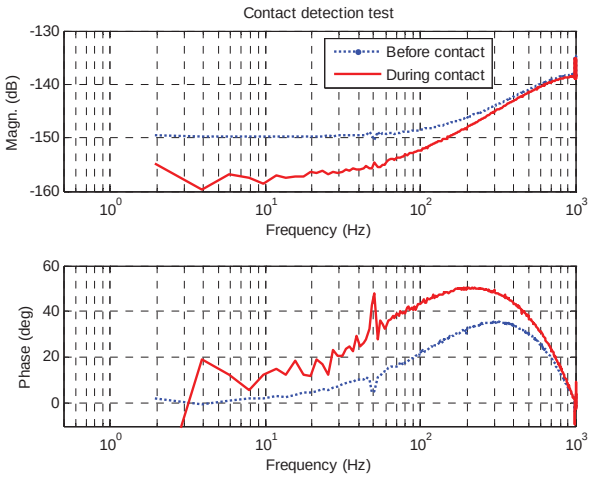

Fig. 8. Bode diagrams of admittance before and during injection in a typical injection experiment.

Frequency responses estimates calculated from non–contact and contact measurements show similar gain and phase behaviour in Fig. 8 than in simulations (Fig. 7). Since the sampling frequency is 2000Hz the Nyquist frequency is 1000Hz. The antialias filtering in CDD attenuates admittance at high frequencies.

Similarly, Bode diagrams can be calculated for broken and clogged capillary using (5). Differences in Bode diagrams for normal and broken capillary indicate increase for the gain at low frequencies and decrease for the phase at higher frequencies after a breakage of the capillary. Frequency response calculated from experiment measurements show similar behaviour as illustrated in Fig. 9.

In the experiment the clogging of the capillary is arranged by making a contact to a cell with the capillary tip and by applying negative pressure to the capillary. The difference between simulated Bode diagrams for normal and clogged capillary is similar to difference during the contact with a cell. Also, the experiment shows that when the capillary is

clogged the change in the measured frequency response is similar to change during contact as can be seen in Fig. 10.

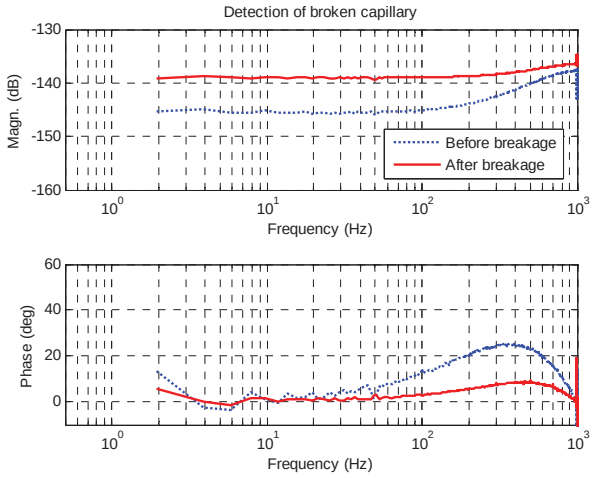

Fig. 9. Bode diagrams of admittance before and after capillary breakage while capillary is not in contact.

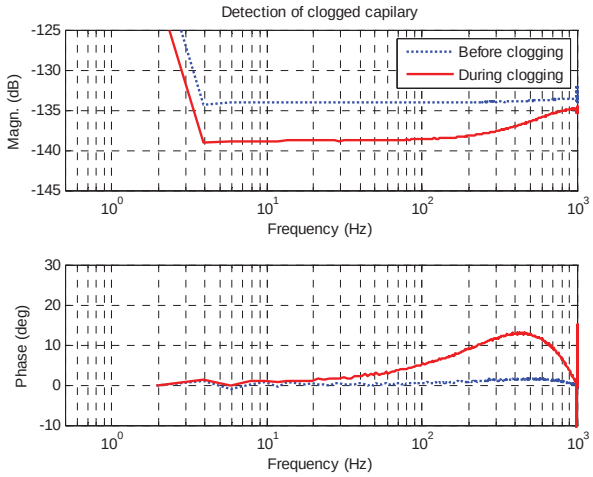

Fig. 10. Bode diagrams of admittance before and after clogging.

CONCLUSION

This work presents how a fast frequency response method can be applied to estimation of cell–capillary admittance during cell injection. Moreover, the paper presents the equivalent electric circuit models of a cell-capillary system and analyses how the admittance should change during various injection events.

The experiments prove that measured changes in admittance can be used for detecting contact between a cell and a capillary as well as for detecting if the capillary is broken or clogged.

Initial results show that detection of a cell cycle phase or an abnormal state of the cell might be possible using the proposed method. Thus, the future work will include the development of the method for detecting the type and condition of the cell.

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