Bio-Impedance Analysis using Minimalistic Hardware

C. Gansauge, D. Echtermeyer, Y. Zaikou, V. Schroeder, M. Saupe, J. Schemberg, U. Pliquett
Institut für Bioprozess- und Analysenmesstechnik e.V., Heilbad Heiligenstadt, Germany
Contact: Chris.Gansauge@iba-heiligenstadt.de

Introduction

The electrical characterization of biological material is a simple way for gaining information about process relevant measures like cell state, cell density or morphology. Although instrumentation for impedance and dielectric measurements is available in great variety, recent developments of applications require cheap, easy to use and miniaturized devices.
Especially single use solutions require often disposable devices, especially in biomedical testing. Moreover, sensory incorporated in packages are usually disposable.
Fast monitoring of electrical properties is an emerging field in biotechnological and medical practice. Here, we monitor the electrical properties of multicellular spheroids passing a nozzle in order to assess the individual behavior.

Materials and Methods

The concept is based on relaxation spectroscopy in time domain where the hardware can be limited to a digital output and an analog-digital converter.

Electrical characterization based on relaxation

The generally used electrical behavior for characterization of biological material is the impedance. For spectroscopic information in frequency domain, a sweep through the interesting frequency range is performed with measurement of magnitude and phase. This takes time and is therefore not suitable for monitoring fast changing objects. Moreover, sophisticated equipment is required which increases both power consumption and cost.
A much faster method is the application of a broad bandwidth signal and monitoring the system response. For LTI-systems (linear, time invariant) Laplace transformation allows the transformation of the result into the frequency domain. This procedure yields a different, maybe more convenient presentation of the data but does not give any additional information.
Common broad bandwidth signals are square wave, multi sine or maximum length sequence. All together, they are steady signals making processing simple by using fast Fourier transformation (FFT). A bottleneck is the requirement of equidistantly sampled signals, yielding huge data volume for broad bandwidth processing and even more pronounced, for continuous monitoring.
Despite of more complicate processing of the response to transient signals, they exhibit great advantages with respect to data reduction and hardware requirements.

Most popular transient signals are Dirac-, step-, and ramp function. The advantage of step function is the robust generation but also the simple data processing.

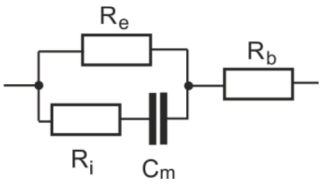

Figure 1: Equivalent circuit for spheroid measurements

In a equivalent circuit for spheroids (Fig. 1), R_e is the resistance of the extracellular medium, R_i mimics the cytosolic electrolytes and C_m depends on the capacitance of membrane structures. R_b is the resistance of the bulk electrolyte in the measuring path. In the ideal case of negligible R_b, the current response to a voltage step of U_0 is simply

$$I = \frac{U_0}{R_e} + \frac{U_0}{R_i} e^{-\frac{t}{R_i C_m}} = I_e + I_i e^{-\frac{t}{\tau}} \qquad (1)$$

where I_i, the current through the interior of the cells, crossing membrane structures at time $t = 0$ is the relaxation strength. The relaxation process, here the charging of the membrane structures, is further characterized by the time constant τ.
In the setup used in our study, however, R_b cannot be neglected.
The current I_e with R_b taken into account is:

$$I_e = \frac{U_0}{R_e + R_b} \qquad (2)$$

For the current I_i through the cells we find:

$$I_i = \frac{U_0 R_e^2}{(R_e + R_b)(R_e R_b + R_i R_b + R_e R_i)} e^{-\frac{t}{\tau}} \qquad (3)$$

The time constant τ is

$$\tau = \frac{C_m (R_e R_b + R_i R_b + R_e R_i)}{R_e + R_b} \qquad (4)$$

The relation between frequency and time domain for LTI-systems is Laplace transformation or in the simpler case of steady state Fourier transformation. A relaxation process in time domain, represented by a time constant and relaxation strength relates in frequency domain to the characteristic frequency and circumference of the frequency dispersion.

The ideal case of single time constant is seldom found in real measurements. Given the nature, for instance of cell based material, each individual cell exhibits electrical behavior which in summary is detected at the electrodes. Due to different cell size but also slight differences in electrolytic composition of the cytoplasm, time constants are distributed. A good approach of this distribution is the Cole-model:

$$Z = R_\infty + \frac{R_0 - R_\infty}{1+(j\omega\tau_0)^\alpha} \quad (5)$$

R_0 is the resistance extrapolated to dc and corresponds to R_e in Fig. 1. R_∞ is the resistance at high frequencies (R_e parallel to R_i) and τ_0 is a frequency normalization factor corresponding to a medium time constant.
Because of the complex form of the analytical solution for transformation into time domain, several well corresponding models are used. The most general approach is the distribution of time constants (DRT):

$$A(t) = A_0 + A_1 e^{-\left(\frac{t}{\tau}\right)^\alpha} \quad (6)$$

where A_0 accounts for a dc-offset and is mostly governed by R_e and R_b in Fig. 1.
In frequency as well as in time domain, α is a dimensionless parameter between 0 and 1. While 1 yields the ideal behavior of simple RC-combination, 0 yields a pure resistor.
For simple data processing, an equivalent circuit consisting of a serial combination of Cole- or RC-elements should be chosen for current excitation while parallel combinations of the corresponding admittances ($\underline{Y} = 1/\underline{Z}$) are preferred together with voltage excitation.

Sampling regime and Hardware

A key feature of the step response of biological material in general – for current and voltage excitation – is the monotonically developing response with fast changes immediately after the step occurred and slowing down with time. This means that fast sampling is necessary at the beginning of the response while the distance between sampling points can continuously increase with time (Fig. 2).

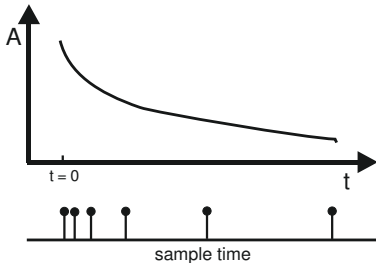

Figure 2: Signal with sample times indicated

Although it works well for signals without noise, serious problems arise in measurement practice. The reason is the violation of the Kotelnikov's sampling theorem which becomes increasingly pronounced with larger distances between sampling points.
Simply an anti-aliasing filter will comprise the high frequency behavior. The way out is an adaptive anti-aliasing filter starting with high cutoff frequency at early times while it decreases with time proceeding.
A simple hardware solution is the partial integration of the signal between sampling points.
In practice, between 3 - 10 sample points per decade in time are required for good signal reconstruction. This means, for a broad-bandwidth measurement over 6 decades (e.g. 10 Hz - 10 MHz) only 18 AD-conversions could be sufficient. In our case, we use 10 sampling points for a range from 20 kHz to 4 MHz.

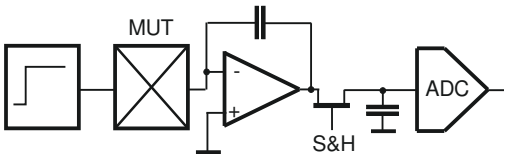

Figure 3: Signal chain consisting of excitation source (step generator), material under test (MUT), integrator, sample and hold (S&H) device and analog/digital converter (ADC)

The practical solution involves a microcontroller with S&H and ADC incorporated. For excitation, a digital output is used and the current is directly fed into the integrator which is wired to the input of the ADC. For circumventing problems with electrochemical reactions but also field effects on biological material, a frontend ensuring low voltage (<100 mV) across the MUT and charge compensation was used.

Characterization of multicellular spheroids

Multicellular spheroids are 3D-cell conglomerates commonly used as model for tissue. Embryonic kidney cells (HEK 293) were grown in culture flasks according to established protocol until a confluent monolayer was reached. DMEM (Dulbecco's Modified Eagle's medium) was used as culture medium throughout all experiments. After trypsination, cells were washed and transferred to a spinning disc culture for three days. Within this time spheroids grow up to an average diameter of 100 µm and more (Fig. 4).
For electrical characterization, spheroids were harvested and pumped through a glass nozzle with a diameter of about 100 µm (Fig.5) which was mounted inside a capillary of 900 µm diameter. At both sides of the nozzle, Ag/AgCl-electrodes were mounted for electrical connection.

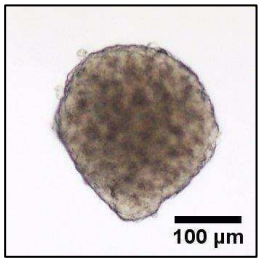

Figure 4: Bright-field microscopy image of a HEK293 spheroid.

In order to validate the electrical measurements, a camera monitored the movement of spheroids through the nozzle. Impedance measurements using a voltage controlled square wave were conducted continuously with a repetition frequency of 1 kHz. Only the positive steps were used for further processing. An additional sample was taken in the negative half wave for offset compensation.

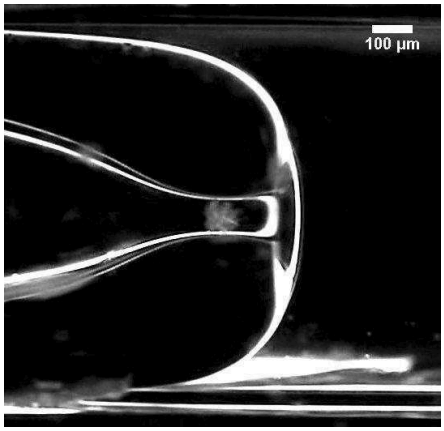

Figure 5: Optical image of a spheroid in the glass nozzle. The spheroid is visible in the narrowest part of the nozzle.

Results

The electrical behavior of multicellular spheroids passing through a nozzle was assessed by electrical means. For ensuring high repetition of the measurement, time domain spectroscopy was used. This allows for multiple measurements during the passage of a single spheroid and therefore the development of electrically assessed parameters during the passage. For instance, shunting pathways around the cells decline when the pressure increases due to narrowing of the area inside the nozzle. Using the electrical parameters, biophysical behavior like cell density or viability can be deducted from the measured data.

The passage of a spheroid through the nozzle was detected by an overall decrease of the current between the electrodes. The step response during the passage was then fitted with the DRT model (Eq. 6) taking into account the serial bulk resistance R_b. The parameter α exhibited considerable variability when fitted together with the other parameters A_0, A_1 and τ. Since this uncertainty reflects throughout all the parameters, a reasonable region for α around 0.7 was identified. In order to ensure a stable fit throughout the experimental data, the parameter α was set to a value of 0.7. A typical plot of the fit parameters during the passage is shown in Figure 6.

When there was no spheroid present, the resistance of the bulk medium R_b was determined by the direct current resistance. With a spheroid present in the nozzle, all parameters were determined by a DRT fit. Only R_e is shown for the whole measurement, while R_i and C_m are shown only, when a spheroid is present. R_b was set to the value of the dc-resistance of the medium before the spheroid entered the nozzle.

The resistance of the extracellular medium R_e was increasing rapidly at the entrance of the spheroid to the nozzle. Spheroids with a diameter larger than the nozzle diameter were deformed und slowed down. During the passage, the intracellular resistance R_i and the membrane capacity C_m showed no clear trend.

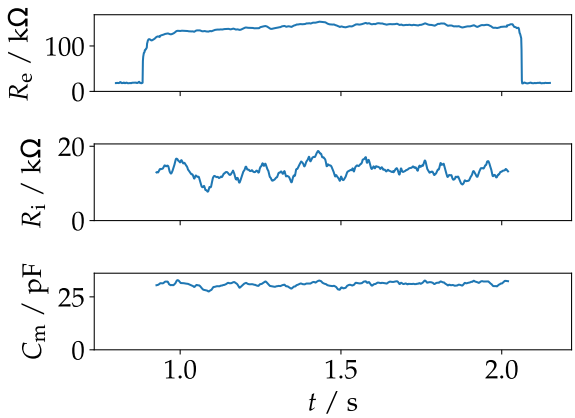

Figure 6: Passage of a single spheroid through the nozzle. R_e – Extracellular resistance, R_i – Intracellular resistance, C_m – Membrane capacity.

Discussion

The fast acquisition of the electrical relaxation due to a voltage step makes it possible to analyze the properties of spheroids during the passage through the nozzle in detail.

The extracellular resistance R_e includes the resistance of the medium around the spheroid and the resistance of the medium between the cells. The tremendous increase of R_e during the passage can be attributed to changes in the resistance of the medium around the spheroid. This part was increasing because the spheroid covered the nozzle and blocked the current between the electrodes through the medium. After the spheroid covered the nozzle completely, R_e was further increasing. This might be explained by the continuing adaptation of the spheroid's shape to the opening.

The intracellular resistance R_i exhibits a large percentual variability due to the low absolute value of the parameter. The missing trend of the parameters R_i and C_m suggest that the internal structure of the cells in the spheroid is unchanged during the passage through the nozzle. Fluctuations in these values might arise form mechanical stressing during the nozzle. This makes the system ideal for measurements of spheroids with varying properties like diameter, incubation time, and the application of drugs.

By adjusting the flow rate, a fast and continuous flow of spheroids can be obtained. The spheroids in flow can then be characterized at a very high velocity.

Conclusions

In this paper, we have shown that we can use a minimalistic hardware setup to measure the bio-impedance of spheroids. The setup is suitable for high-throughput measurements.

Further experiments could include measurements of spheroids with varying parameters like diameter, incubation time, and the application of drugs.

References

[1] S. Grimnes and Ø.G. Martinsen. *Bioimpedance and Bioelectricity Basics*, (2014)

[2] H. Thielecke, A. Mack, and A. Robitzki. *Biosensors and Bioelectronics* 16, Nr. 4 (2001): 261–69.

[3] R. Garrappa, F. Mainardi, and M. Guido. *Fractional Calculus and Applied Analysis*19, Nr. 5 (2016): 105– 1160.

Acknowledgements

We thank Robert Römer for the microscopy images of the spheroids. This research is funded by the Thüringer Aufbaubank (FKz: 2016 FGR 0040).