

# Capillary Chip Based Characterisation of Small Tissue Samples

**Hagen Thielecke**

Fraunhofer Institute for Biomedical Engineering, St Ingbert, Germany

A novel capillary chip has been developed for in vitro diagnostics and the evaluation of therapies. Its design reportedly enables marker-free, noninvasive and fast characterisation of tissue samples down to a diameter of 100  $\mu\text{m}$ .

Image: Image 100

## The need for tissue-based test systems

Efficient cell- and tissue-based test systems are needed in medicine for the evaluation of new therapies, to determine individualised therapies, for diagnostics and for quality control in cell-therapy processes. Every patient reacts differently to a therapy. For the optimal treatment of diseases, the chosen therapy must be based on the patient's conditions. One approach for determining the optimal therapy for a patient is the identification of small variations in the patient's DNA, that is, single nucleotide polymorphism analysis. However, this merely detects variants of disease based on the tolerances in the genome. The genome is, in principle, a defined and static quantity. However, diseases are caused ultimately by the interaction of proteins. All the proteins that are synthesised in a cell under certain environmental conditions are called the proteome. In contrast to the genome, which is identical in different cells of the body, the proteome differs according to environmental conditions such as temperature, nutrient milieu and the effects of stress or drugs. To determine the best

therapy for a patient, the interaction of all the proteins of a proteome should be taken into account. The only way of doing this is by testing possible therapy options directly on the cells or tissues of the patient.

It has been shown that cell-specific protein pattern stays stable even under the highly artificial conditions of an in vitro culture. Therefore, it is theoretically possible to test therapy options in vitro on cells taken from the patient. For this, the development of an efficient test system based on microelectromechanical systems is required. These test systems must be able to determine, noninvasively and in a short time, the effect of many therapy options on a number of cell parameters. The effect of possible therapies can be tested directly on cell or tissue samples taken from the patient's tissue. In addition, it could be possible to culture and proliferate the patient's cells to generate patient-specific cell and tissue models for therapy evaluation.

## Marker-free cell characterisation

One problem with current techniques for tissue characterisation is that cells are characterised indirectly using chemical marker substances. The use

of marker substances requires cost-intensive and time-consuming pre-treatment, it influences the cell physiology and the functional characterisation of the cell can only be achieved with a great deal of effort. Microtechnologies offer new opportunities to realise marker-free systems. Marker-free techniques characterise a physiological/pathological event in biological systems by measuring a physical quantity that is correlated to the event.

Physiological/pathological events can be determined, for example, by measuring alterations in the pH value related to cell metabolism or in electrical properties caused by changes in cell membranes and cell organelles. This physical quantity is converted by a transducer system into a signal that can be processed further, and ultimately to an electrical signal. Currently, most of the available test systems are based on two-dimensional (2D) cell layers. However, in many cases three-dimensional (3D) tissue models or tissue samples are required to mimic the in vivo situation.

The electrical and dielectric properties of a tissue are determined by its physiological and morphological properties.<sup>1</sup> Because the electrical and

dielectric properties of a sample can be determined by recording the electrical impedance over a frequency range (impedance spectroscopy), the physiological events and morphological properties of biological tissues are determinable by electrical impedance spectroscopy. A capillary impedance measurement system has been developed for marker-free, noninvasive and fast characterisation of small tissue

samples such as biopsies and 3D-tissue models by impedance spectroscopy. Tissue characterisation can take from several seconds to several minutes and depends, for example, on the methods used for impedance measurement. The time typically needed for classical histopathological and histochemical tissue characterisation is in the range of hours and days.

**Sensitive and reproducible impedance measurement**

To measure the impedance of a biological sample, at least two electrodes in an electrolytic environment are required, that is, an electrochemical cell with a biological sample (Figure 1, upper panel). The main components of an equivalent circuit model of an electrochemical cell are the interfacial electrode–electrolyte impedance, the series resistance of the electrolytic solution, the impedance of the biological sample, a shunt resistance and a stray capacitance (Figure 1, lower panel). For reliable and sensitive measurement of the impedance, in practice, the following conditions must be fulfilled:

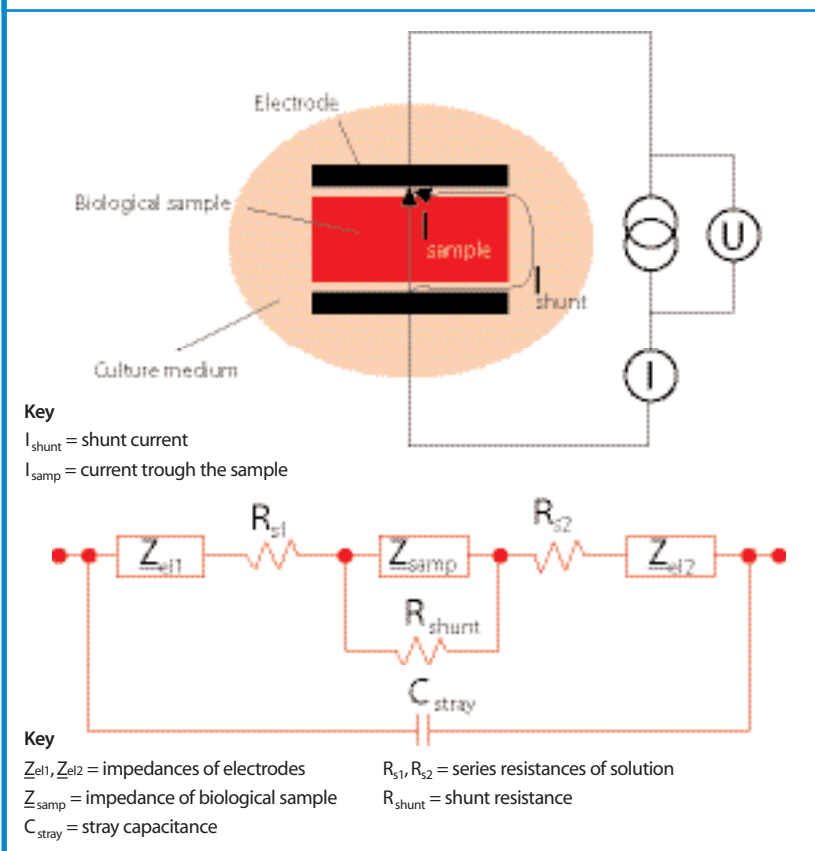
- the resistance of the shunt path must be high and constant
- the impedance of the electrode–electrolyte interface must be low in comparison to the tissue impedance
- the stray capacitance of the measurement cell must be low.

Normally, when the impedance of small samples is to be measured, small electrodes are necessary. The problem with using small electrodes in an electrochemical cell is that their high interfacial impedance can dominate the total impedance of the measurements cell. If this is the case, the impedance of a sample inside the measurement cell is only determinable with a low sensitivity.

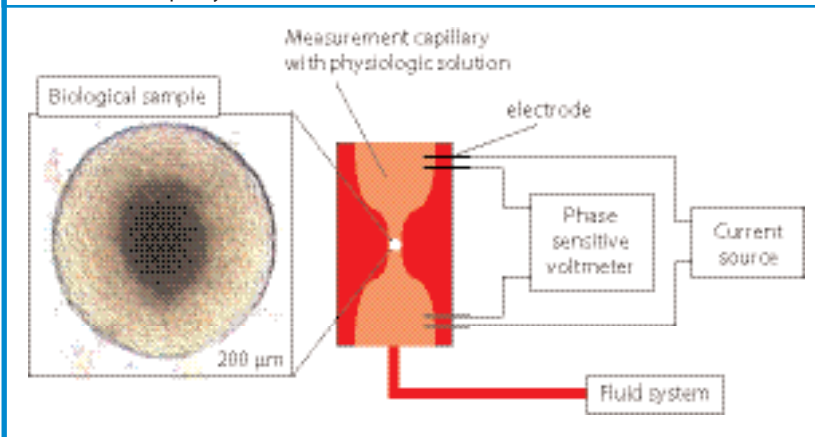
**Design of capillary measurement cell**

To satisfy the above-mentioned conditions, a capillary measurement cell has been designed and fabricated. The tissue sample is positioned hydrodynamically into a vertical measurement capillary with funnel-shaped openings (Figure 2). The capillary diameter is constant at the positioning area of the tissue sample. Two electrodes are arranged above and below the funnel-shaped openings. For the impedance measurement, a current is supplied to the outer electrodes and the resulting voltage drop is recorded via the inner electrodes, thus providing measurement in a four-electrode arrangement. This electrode arrangement and →

**Figure 1:** Electrochemical cell for measuring the impedance of biological samples. Upper panel: Schematic drawing. Lower panel: Circuit diagram for the electrochemical cell.

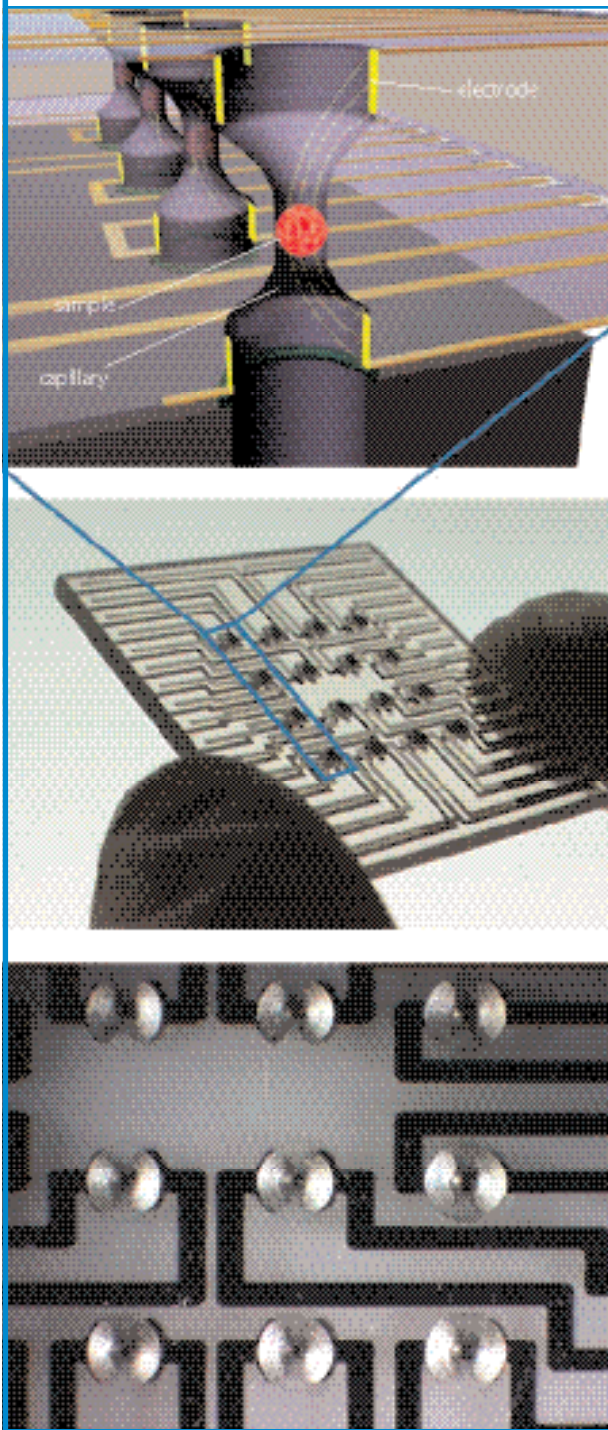


**Figure 2:** Scheme for impedance measurement on small biological samples using a measurement capillary.



→ the geometry of the capillary measurement cell allow impedance measurements with negligible influence of electrode impedances: the electrode areas can be chosen independently from the size of the sample and measurement can be performed in a four-electrode configuration. The

**Figure 3:** Capillary array chip. Upper panel: schematic of a section. Middle panel: the whole chip. Lower panel: cutout photograph of the chip with opening of capillaries, electrodes and interconnecting lines.



shunt resistance is high because the sample is surrounded by the wall of the capillary, and constant because the area of capillary in which the sample is positioned has a constant diameter.

#### Evaluation of anticancer therapies

The first applications of the capillary measurement cell are the evaluation of new anticancer therapies using 3D in vitro tissues models. Impedance spectra are recorded of tumour models, which are threaded with an anticancer therapeutic gene construct<sup>2</sup> and with different radiation doses.<sup>3</sup> The parameters of an equivalent circuit model for the tumour models are determined from the impedance data. In the case of anticancer therapy, the decrease of the extracellular resistance has already indicated that the therapy has the desired effect. This parameter is related to the extracellular volume ratio of tissue (ratio of the volume of extracellular space to the volume of the intracellular space). The aim of an anticancer therapy is to stop cell proliferation and to induce apoptosis. Both effects lead to an increase of the extracellular volume ratio and hence to a decrease in the extracellular resistance.

The electrochemical measurement cell is not only suitable for tissue characterisation by impedance spectroscopy, but also for recording field potentials of electrophysiological active samples such as samples from heart-muscle tissue or neuronal tissue models. In these applications, the electrodes of the measurement cell are connected to an electrical potential recording system instead of an impedance analyser.<sup>4</sup>

In the first evaluation of the design of the single capillary measurement cell, a prototype made of glass was used. However, a capillary chip has also been developed with 16 vertically arranged measurement capillaries and integrated electrodes in plastic using microinjection-moulding technology (thinXXS GmbH, Zweibrücken, Germany), see Figure 3. For a noninvasive and sensitive characterisation of a biological sample, the diameter of a capillary

should be adapted to the biological sample. If the diameter of the capillary is too small, the sample is deformed; if the diameter is too large, the shunt resistance is too small. The capillary chip can contain capillaries down to a diameter of 100  $\mu\text{m}$ .

#### Summary

There is a clear need for cell- and tissue-based test systems for in vitro diagnostics and therapy evaluation. The advantages of tissue-based test systems are that the complex cell response is taken into account and that proteins are in their natural environment. The capillary measurement cell enables impedance measurement of small tissue samples with negligible electrode impedances and a high constant shunt resistance so that biologically relevant tissue parameters are determinable.

#### References

1. H.P. Schwan, "Mechanisms Responsible for Electrical Properties of Tissues and Cell Suspensions," *Med. Prog. Technol.*, **19**, pp.163–165 (1993).
2. H. Thielecke, A. Mack and A. Robitzki, "A Multicellular Spheroid-Based Sensor for Anti-Cancer Therapeutics," *Biosens. Bioelectron.*, **16**, pp.261–269 (2001).
3. H. Thielecke et al, "In Vitro Tissue Based Test System for Evaluation of AntiCancer Radiotherapies," Paper Number: 3770.00, World Congress on Medical Physics and Biomedical Engineering, 24–29 August 2003 Sydney, Australia.
4. A. Reininger-Mack, H. Thielecke and A. Robitzki, "3D-Biohybrid System: Application in Drug Screening," *Trends Biotechnol.*, **20**, pp.56–61 (2001). [mdt](#)

#### Hagen Thielecke

is Group Manager Cell-based Sensors and Biomonitoring at Fraunhofer Institute for Biomedical Engineering, Ensheimer Strasse 48, 66386 St Ingbert, Germany, tel. +49 6894 980162, fax +49 6894 980400 e-mail: hagen.thielecke@ibmt.fhg.de www.ibmt.fraunhofer.de

Published in the November 2003 issue of *Medical Device Technology*.