Biosensors and Bioelectronics 26 (2010) 1407–1413

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Studies on neuronal differentiation and signalling processes with a novel impedimetric biosensor

T. Valero[a,*], G. Moschopoulou[a], S. Kintzios[a], P. Hauptmann[b], M. Naumann[c], T. Jacobs[c]

[a] Faculty of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece
[b] Institute of Micro and Sensor Systems, Otto-von-Guericke University Magdeburg, 39106 Magdeburg, Germany
[c] Institute of Experimental Internal Medicine, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 8 July 2010
Accepted 17 July 2010
Available online 23 July 2010

Keywords:
Collagen
Electrical impedance spectroscopy
Impedimetric biosensor
Laminin
Neural differentiation
Three-dimensional cell culture

ABSTRACT

The differentiation of neural cells is an important process during the development of the central nervous system. Studies on the mechanisms of the differentiation process is of special importance, e.g. in the field of regenerative medicine. In this contribution the cellular differentiation of gel matrix embedded neuronal cells was studied. The three-dimensional organization of neuronal cells represents a new cellular model system closer to the physiology than conventional two-dimensional cell cultures. Neuro2a (N2a) neuroblastoma cells were immobilized in different gel matrices and the grade of differentiation was compared. Furthermore, the impact of the cell number and selected differentiation factors were analyzed. Experimental results revealed that gel matrices based on collagen–laminin mixtures in contact with serum free medium enable neural differentiation. Therefore, collagen–laminin gels appear as a suitable three-dimensional model for drug screening in developmental neurobiology. Following optimization of the immobilization process, a novel impedimetric sensor and electrical impedance spectroscopy technique was applied to on-line monitor the differentiation process by means of changes in the dielectric and conductive properties. Experimental results showed an increase in the impedance magnitude that can be mainly attributed to differentiating cells accompanied by an increase in the specific resistivity of the bare gel mixture.

1. Introduction

During the development of the central nervous system (CNS), a coordinated program decides the neural cell fate. Hundreds of neuronal types are generated in an organized manner (Briscoe and Novitch, 2008). Neuronal differentiation processes will determine the development of new neurons, their specialization in a concrete subtype and their interactions.

Classically, neuronal differentiation has been studied based on cellular models, neuroblastoma or pheochromocytoma cell lines. Those models give the advantage of being an endless source of biological material and resemble neuronal differentiation in response to certain stimuli. N2a neuroblastoma cell line is a well-known model of neural differentiation in response to dibutyril cyclic AMP (db-cAMP). However, classical studies on cell lines have a limited prediction power due to the fact that cells are cultured *in vitro* in a two-dimensional (2D) environment, which differs drastically from the physiological reality. Three-dimensional (3D) models, instead, can resemble better the 3D physiological structure, where cells can express their features in a better manner. It is known that cells in 2D cultures express different genes compared to their 3D counterparts and tissue (Birgersdotter et al., 2005; Li et al., 2007; Wang et al., 2009). This can be the reason of the low efficiency index of drugs that can reach the bed side. 3D models, as an intermediate step between classical 2D cell cultures and animal experimentation can bridge this gap (Pampaloni et al., 2007), thus leading not only to a higher probability of successful drug development but also to a reduction of the number of animals sacrificed for experimental purposes and to a reduction of the related costs. Although 3D models appear as physiologically more relevant, their use is limited due to the fact that not many techniques are adapted to the third dimension.

Electrical impedance spectroscopy (EIS) is a well-established technique for the analysis of liquid or solid ionic and dielectric materials by means of a conventional lab-scale or miniaturized impedance analyzer (Barsoukov and Macdonald, 2005; Schroeder et al., 2004). Furthermore, it represents an established analytical method to monitor cell biological processes in 2D cultures, e.g. the spreading of adherent cells to artificial surfaces (Wegener et al., 2000) and viral induced cell death (Campbell et al., 2007). Electrical impedance spectroscopy of two dimension cell cultures is state of the art for monitoring adherent two-dimensional cell cultures

* Corresponding author. Tel.: +30 2105294292; fax: +30 2105294286.
E-mail address: teretavale@yahoo.es (T. Valero).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.07.066

by means of planar electrodes (Lisdat and Schafer, 2008). This technique is, e.g. used to analyze the kinetics of wound healing (Keese et al., 2004). EIS has already proved to be able to detect differentiation of PC12 cells in response to Nerve Growth Factor (NGF), dexamethasone and forskolin in 2D (Slaughter and Hobson, 2009). In this context EIS is better known as electric cell substrate impedance sensing (ECIS). Impedance analysis has also proved to be a powerful tool for 3D applications. EIS has already been applied for the determination of cell toxicity, growth and membrane integrity in 3D (Kyle et al., 1999), response to neurotransmitters in 3D neuronal cultures (Valero et al., 2009), cell growth in 3D matrices (Lin et al., 2009) and the effect of drugs on spheroids (Kloss et al., 2008a). EIS experiments have been commonly conducted at frequencies lower than 1 MHz (Kloss et al., 2008b). To our best knowledge only little is known about changes of alternating electric fields at 1 MHz passing differentiating neuronal cells embedded in a gel matrix. The purpose of this study was to demonstrate that high frequency electrical impedance sensing can be a promising technique to on-line monitor neural differentiation in 3D matrices.

2. Materials and methods

2.1. Reagents

Neuro2a cell line was originally provided from LGC Promochem (Teddington, UK). Dulbeco's Modified Eagle's Medium (DMEM), L-Alanyl-L-Glutamine, Penicillin/Streptomycin, Pyruvate, Trypsin/EDTA, Hepes were purchased from Biochrom AG (Berlin, Germany) and Foetal Bovine Serum (FBS), UtraPure LMP Agarose from Invitrogen (Carlsbad, CA). Collagen, laminin, basement membrane extract (BME) were provided from Trevigen (Gaithersburg, MD) and Agar bacteriological from Scharlau (Barcelona, Spain). db-cAMP was purchased from Sigma–Aldrich (St. Louis, MO). Finally, dimethylthiazol-2-2,5 diphenyl tetrazolium bromide (MTT) was provided from MP Biomedicals (Solon, OH).

2.2. Immobilization of N2a cells in 3D matrices

Cells were collected with trypsin/EDTA, centrifuged 2 min at 1000 rpm and resuspended in 1% (w/v) agarose or 1% (w/v) agar bacteriological (bactoagar) at 37 °C using a block heater. BME gels were prepared according to the manufacturer's instructions. Immobilization in collagen (CGs), and in collagen–laminin gels (CLGs) was performed according to the manufacturer's instructions with the following modifications. The final composition of the selected CGs and CLGs used was: 10% cells suspended in DMEM, 50% Collagen, 2% NaOH 3N, 1.5% Hepes 1 M, 6.2% DMEM 10×, 2.3% H_2O, 28% DMEM or 28% laminin, respectively. BME, CGs and CLGs were prepared in an ice bath to avoid solidification. 50 μL of each mixture was poured in 96-well plates and after solidification 200 μL of DMEM were added to each well. The final cell concentration for gel comparison experiments was 1×10^6 cells/mL, while the final cell concentration for impedance experiments was 2×10^6 cells/mL. Gels were stored at 37 °C and 5% CO_2. After 24 h of culture, determination of differentiation in 3D gels was performed by inverted optical microscopy.

2.3. MTT viability test

MTT viability test were performed as described previously (Mosmann, 1983) with the following modifications. MTT was added to the supernatant at a final concentration of 0.5 mg/mL. 2 h incubation in the presence of MTT was needed for its diffusion into the gels. After MTT formazan was formed, supernatant was discarded and gels were suspended in 1 mL DMSO and vortexed for 30 min to allow the extraction of the MTT formazan from the gels. Absorbance of 100 μL of this extract was measured at 560 nm in a BioTeck Power Wave 340 plate reader.

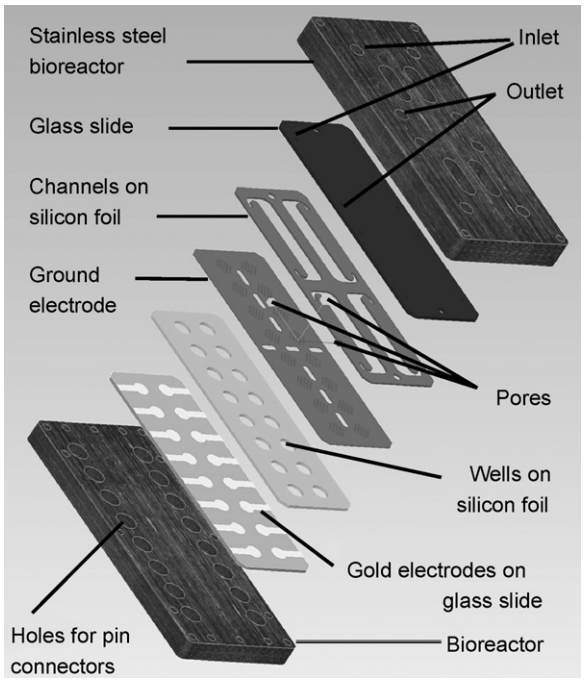

Fig. 1. Impedimetric biosensor electrode set-up. Impedimetric biosensor consisting of 16 gold electrodes on a glass slide and a silicone foil on top with circular wells for the gel matrix and a porous reference electrode on top for impedance measurement with four micro-fluidic channels. It is inserted in a stain less steel bioreactor with four inlets and four outlets on top for medium perfusion and 16 holes for pin connectors on the bottom.

2.4. Impedimetric biosensor

For the electrical impedance analysis of gel matrix embedded neurons an impedimetric biosensor with an electrode array was used. The device, as shown in Fig. 1 consists of 16 gold electrodes on a glass slide. Electrode uniformity was guaranteed by a standard sputtering technique with a unique shadow mask. First a chromium layer was sputtered and afterwards a thin layer of inert gold (300 nm ± 20 nm) was deposited. In case of properly fabricated electrodes, no deterioration could be observed over time. An adhesive silicone foil (thickness 1 mm) on top containing circular wells (Ø 5 mm) defines the shape of the gel films for impedance measurement. The bigger diameter of the circular wells compared to the electrode diameter guarantees a constant active measurement area, defined by the uniformity of the gold electrodes. A proper sticking of the silicon foil to the slide was achieved by applying high temperature and pressure during 24 h. As depicted in Fig. 1 a thin sheet of stainless steel placed on the top side serves as grounded reference electrode. Thus, gel films poured in each well are squeezed between both electrodes for a proper electrical contact and a well-defined gel thickness. Multiple small holes in the stainless steel sheet enable medium diffusion into the gels while four micro-fluidic channels made of a silicon foil on a glass slide allow the perfusion of the cells with culture medium and stimuli. Electrodes are divided by the micro-fluidic channels into four groups, with four electrodes each. For the electrical impedance analysis of neural differentiation N2a cells were immobilized in a CLG matrix on top of the gold electrodes of the impedimetric sensor. After preparation of the gels in the wells and solidification of the gel in the incubator, the stack as shown in Fig. 1 was pressed for proper sealing. The array was inserted into a novel custom-made micro-

fluidic bioreactor with four inlets and four outlets which allow the perfusion of the four chambers with different media (Fig. 1). The device, made of stainless steel, was manufactured using precision mechanics. Subsequently the bioreactor was connected to an external flow injection system consisting of a syringe pump and conventional HPLC equipment and inserted into the heating jacket at 37 °C. DMEM without serum was dispensed at a constant flow rate of 0.5 mL/h into the micro-fluidic channel. Electrodes were connected by means of a custom-made multiplexer to a network analyzer in impedance measurement mode (Agilent, 4395A) for impedance measurement of gel matrix embedded cells in the wells. The output power was fixed to −10 dBm. Impedance spectra were acquired in the range of 100 kHz to 50 MHz at a rate of 1 spectrum/min between the signal electrodes and the porous ground electrode. This approach guarantees the penetration of the electrical field through the whole film and the electrical shield of the gel film from the liquid phase. The changes in the dielectric and conductive properties of the bare gel and the cell membrane as well as cellular action potential changes contribute to the sensor response. Multiple electrodes in parallel in a single group enable the detection of enclosed gas bubbles or partly uncovered electrodes due to an improper filling of a well. The overall stability of the measurement system in terms of on-line impedance analysis is mainly limited by the temperature stability. With an appropriate heating jacket and a thermostat we have achieved stability against thermal fluctuations as low as 0.1 K. Experiments were performed in quadruplicates and data were processed and analyzed using MATLAB (The Mathworks, Natick, MA, USA).

2.5. Immobilization of cells in the impedance sensor

CGs and CLGs were prepared as described in the previous chapter. 30 μL CGs or CLGs were poured inside the electrode-cavities (Fig. 1). The gel dispensing procedure was optimized in order to obtain uniform and complete filling of the wells. Gels were dispensed between measurement and ground electrode through the tiny ground electrode pores with the help of a 100 μL pipette. This procedure ensured a homogeneous coverage of all measurement electrodes. The micro-fluidic channel piece was set on the top and all three pieces inserted in a custom-made stain steel bioreactor for medium perfusion. After each experiment rests of biological material were removed with a concentrated solution of trypsin and sterilized by EtOH and 20 minutes UV exposure.

3. Theory

We can use the following model in order to explain the immobilized cell behavior: The matrix–cell sensor system can be considered as a series of n cell/matrix layers acting both as bipolars and as capacitors whereas the potential varies in relation to the total impedance between the measuring electrode and the reference electrode. Each immobilized cell has a conductance $G_{i,j}$ and a capacitance $C_{i,j}$ (where $i = 1 \ldots n$ and $j = 1 \ldots m$) depending on its position within the probe. In addition, and in particular at higher gel densities, there is an additional resistance R of the matrix (gel and pores with solution). Thus the sensor resembles an RC circuit consisting of a group of n capacitors serially connected to each other:

We can calculate an impedance of the system assuming that an electric current in the system travels through the sensor in two ways: (1) through the cell (current entering and exiting the cell), and (2) through the extracellular environment. In this way, we can represent each part of the system as a "unit cell", which consists of an individual cell and its environment (matrix + solution). We assume that a three-dimensional parallelepiped with dimensions L_x, L_y, L_z is packed with such "unit cells". We also assume, that the x dimension contains n such unit cells, and y and z dimensions contain m and k unit cells. Because all impedances through the x-axis are connected in series, we may calculate the total impedance Z_{tot} of such a circuit, taking into account that m and k impedances are connected in parallel:

$$Z_{tot} = \rho \frac{L}{S}$$

and $L = L_x$, $S = L_y \cdot L_z$, all information about the unit cell is contained in ρ, which may be presented as:

$$\rho = z_1 \cdot \frac{\Delta_y \cdot \Delta_z}{\Delta_x}$$

where z_1 is the impedance of the unit cell and Δ_y, Δ_z, Δ_x, are the dimensions of the unit cell, where the actual ('biological') cell is located.

It is necessary to make an estimation of frequencies, where dispersion of impedance may occur. In general, there are three characteristic ranges of frequencies related to the biological objects polarization. In the region of high frequencies (10 GHz) polarization of water is observed, middle frequencies (1 MHz) corresponded to properties of cell membranes and low frequencies range (100 Hz) are related to the cell surface (Hölzel and Lamprecht, 1994).

4. Results and discussion

4.1. Effect of gel composition on the differentiation of N2a cells in 3D gels

The gel composition and matrix structure are important parameters for the cell differentiation similar to the extracellular matrix (ECM) for naturally differentiating cells. Cell survival, organization, growth and differentiation are strongly dependent on cell adhesion to the ECM (Kintzios et al., 2007). For the electrical impedance analysis of cellular differentiation by means of the impedimetric sensor the composition of the gel matrix and the number of cells needed to be optimized. A comparison between five different gel matrices was carried out (Fig. 2) at 24 h after cell immobilization. In a previous study was shown that bactoagar gel matrices can be used for cell immobilization and preservation (Kintzios et al., 2004). CGs are extensively used as 3D matrices (Desai et al., 2006; Lin et al., 2005) while BME possess the natural composition of the extracellular environment. In these series of experiments we compared our CLGs with all those known substrates and observed differentiation. Neither bactoagar, nor agarose nor bare collagen was able to support the differentiation of N2a cells in a period of 48 h. On the contrary, BME and CLGs were able to do it with two main differences: BME promoted the formation of neurites short and robust, while CLGs promoted the appearance of long neurites which tended to touch adjacent neurons.

Preliminary comparative experiments between a range of differentiation stimuli in conventional 2D N2a cultures were performed (data not shown). The most potent differentiation stimulus in 2D tests, dibutyril cyclic AMP (db-cAMP), was selected for the 3D cell cultures. All the conditions were tested in the presence and in the absence of db-cAMP. Qualitative data were obtained by optical microscopy. As shown in Fig. 2, db-cAMP was not able to induce differentiation in those systems that did not have it in its absence as control. On the contrary, it seems to poorly boost the formation of neurites in those systems which support differentiation i. e. BME and CLGs. Comparing the pictures a–e with f–j in Fig. 3, it is interesting to remark that the key factor for differentiation in 3D collagen matrices is laminin and not db-cAMP as expected. A reasonable high differentiation can be obtained without the addition of any differentiating agent when using CLGs. The reason why

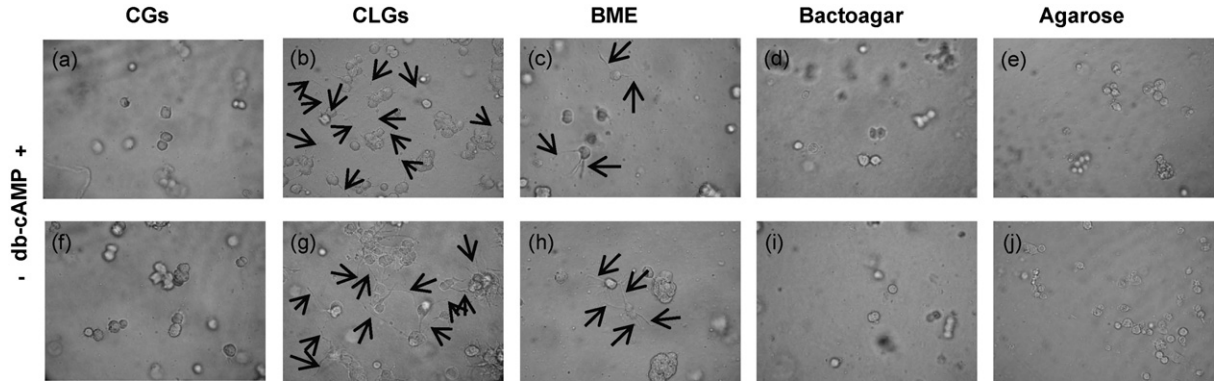

Fig. 2. Differentiation of N2a cells depends on gel composition. Pictures of N2a cells embedded during 24 h in CGs (a and f), CLGs (b and g), BME (c and h), bactoagar (d and i) and agarose (e and j) gels in the presence (a–e) and in the absence (f–j) of 0.3 μM db-cAMP. The arrows point to the neurites.

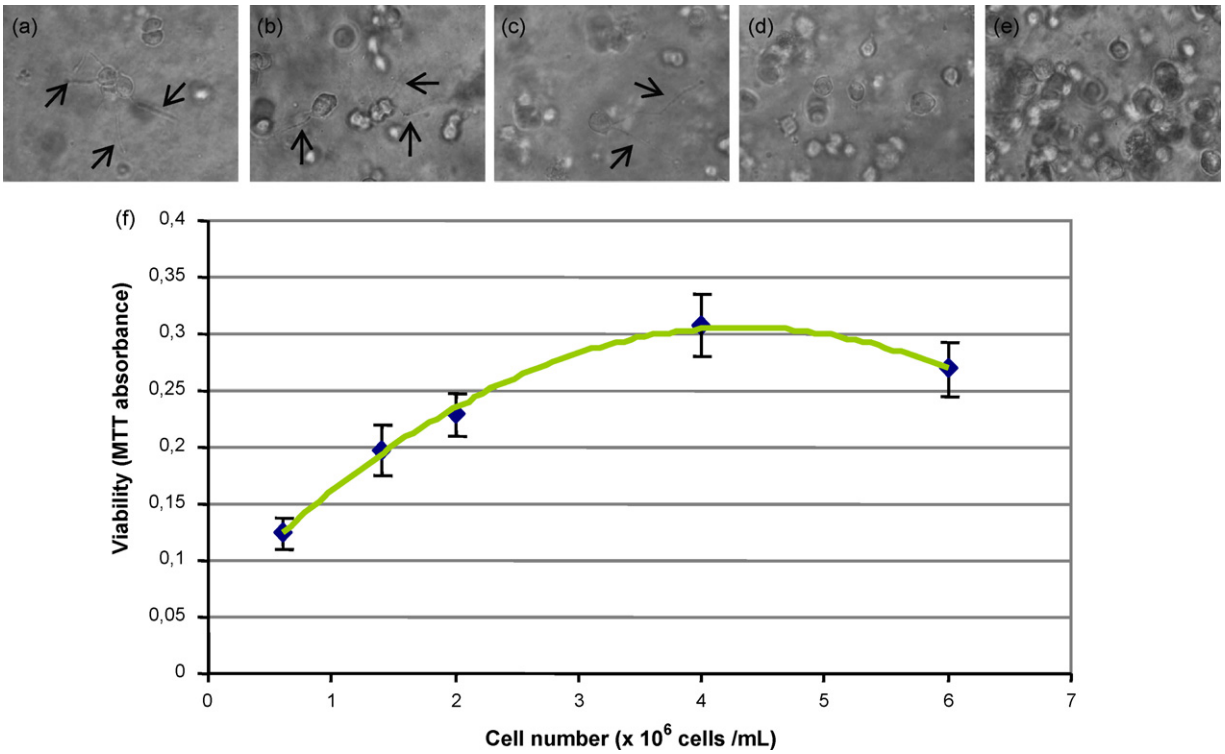

Fig. 3. Differentiation and viability of N2a cells is dependent on cell density in 3D CLGs. Pictures (a)–(e) are representative examples of cells immobilized at different densities. (a) 6×10^5 cells/mL, (b) 1.4×10^6 cells/mL, (c) 2×10^6 cells/mL, (d) 4×10^6 cells/mL, (e) 6×10^6 cells/mL. The arrows point to the neurites. (f) Depicts viability as the absorbance of MTT formazan depending on the cell density.

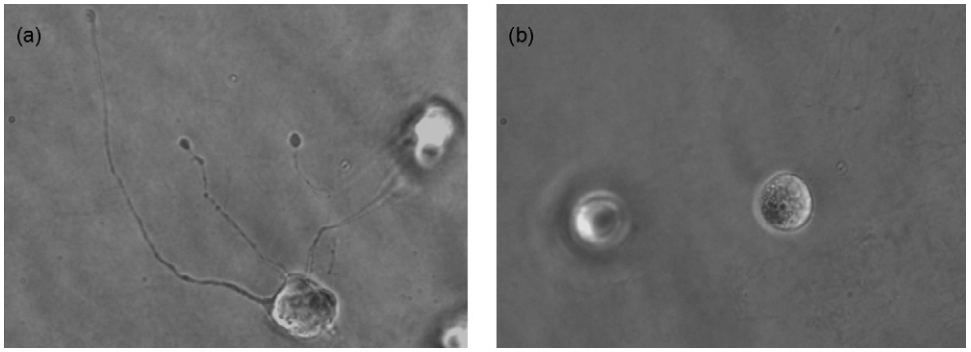

Fig. 4. N2a cells differentiate inside the impedimetric biosensor in CLGs. Differentiated (a) and non-differentiated (b) N2a cells embedded in a CLG and a CG after the end of the experiment in a single well of the impedimetric sensor.

bactoagar and agarose do not support differentiation could be the absence of anchorage proteins in these matrices, which are necessary for a proper dendrite elongation (Cullen et al., 2007). Other authors have used the strategy of mixing different matrices and components of the extracellular matrix in order to favor neurite extension (Cullen et al., 2007; Lin et al., 2005) with similar results on other cell models.

4.2. Effect of cell density on viability and differentiation

In order to optimize the 3D cell culture conditions in the novel CLGs to reach the maximum differentiation degree, different cell densities were studied: 0.6×10^6, 1.4×10^6, 2×10^6, 4×10^6, and 6×10^6 cells/mL. As shown in Fig. 3(a–e), a high differentiation degree was found in cells cultivated at densities up to 2×10^6 cells/mL while this differentiation was partially lost when cell density was increased above that level. These results suggest that differentiation degree is strongly dependent on the concentration of cells within the gel matrix. This effect can be attributed to a decrease in diffusion processes, to cell-to-cell contact inhibition, or simply to the lack of nutrients and excess of waste products. In order to understand the effect occurring at higher cell densities, MTT assays were carried out to discover if the lack of differentiation was correlated to a loss in cell viability. As shown in Fig. 3(f), the increase in cell concentration leads to an increase in MTT absorbance which has a maximum at 4×10^6 cells/mL, while above this concentration, viability is reduced although more cells are immobilized in the gel. Thus, two effects are simultaneously taking place in N2a cells embedded in CLGs. On the one hand, differentiation is lost when cell density rises above 2×10^6 cells/mL. On the other hand, relative viability (viability on per cell basis) is continuously decreasing with increasing cell densities and this loss in viability is especially critical above 4×10^6 cells/mL. The loss of differentiation at high cell concentrations is attributed to the increase in cell death, probably due to nutrient starvation.

4.3. Electrical impedance analysis of 3D gel matrices with embedded N2a cells

Based on the experimental results using conventional cell culture plates presented in the previous sections, the electrical impedance analysis of cellular differentiation in CLG was focussed. An array of 16 electrodes inserted in 16 wells was designed for the analysis of neural differentiation in 3D matrices. In order to validate the novel electrode array for this application, CLGs and CGs were prepared as described in Section 2.4. Results of CLGs with differentiating N2a cells were compared with cells immobilized in bare CG as a control experiment for non-differentiating cells. Impedance spectra of the electrodes were acquired for a period of 12 h, while DMEM without serum was continuously dispensed into the microfluidic channels. At the end of each experiment the viability, the grade of differentiation and the structure of the deposited gel film were characterized by means of MTT tests and light microscopical pictures. Fig. 4(a) shows CLGs embedded N2a cells in a single well of the impedimetric sensor after the end of the impedance spectrum acquisition. Microscopical pictures revealed that cells further differentiate in the impedimetric biosensor accompanied by the formation of neurites in CLGs but not in CGs as shown in Fig. 4(b). Parallel experiments in an identical bioreactor were performed in an incubator in order to determine the effect of current flow on the cells. When comparing immobilized cells with and without AC-current flow, no differences could be observed based on the viability tests and cell morphology. These results are in accordance to the results observed in conventional cell culture plates as shown in Fig. 2(g) and (f), respectively. In all the cases cells were found to be differentiated when cultured in CLGs while cells in CGs did not differentiate. Hence, the effect of the AC-current flow and the impact of medium flow compared to stop flow regime on the cell behavior is regarded as negligible.

Based on preliminary experiments, in the presence and in the absence of gel, a significant increase in the magnitude of the impedance due to the gel was found. The presence of the gel on the electrodes reduces ion mobility and increases the ohmic resistance. Fig. 5(a and b) shows the relative impedance shift referred to the initial impedance of two representative electrodes (E1,E2) at 1 MHz of 2×10^6 N2a cells/mL in bare collagen (CG). During the first two hours an increase of around 7 Ω in the magnitude of the impedance was observed that is attributed to thermal equilibration. After two hours the temperature was stable at 37 °C and a stable baseline is present that remains constant in the time frame of impedance measurement. Thus, within 12 h no significant changes in the dielectric or conductive properties of bare collagen and the immobilized N2a cells could be observed. Fig. 5(c and d) shows the corresponding measurement curves of four representative electrodes (E1–E4), when N2a cells were immobilized in CLG. During thermal equilibration a local minimum occurs and subsequently the impedance magnitude starts to increase, while the phase remains almost constant. Kinetics of the sensor signal shift as shown in Fig. 5(c and d) correspond to the time scale of cellular differentiation as observed in 96-well plates. The reduction up to 2 Ω in the initial impedance (Fig. 5c) might be attributed to physiological changes in preparation for differentiation such as ion channel opening. Similar results were obtained by Slaughter and Hobson in 2009, who detected similar curves during NGF-induced PC12 differentiation to sympathetic neurons. In their study a decrease in normalized impedance followed by a gradual increase and stabilization was observed. However, the subsequent increase in the impedance magnitude can be attributed to the formation of neurites during differentiation. Neurites are thin projections of the neuronal body and thus extensions of the cellular membrane surface. If we consider the cell as a resistor/capacitor (Kyle et al., 1999), an increase in the number of neurites would result in a permanent increase in the ohmic resistance and thus in an increase in the magnitude of the impedance. Fig. 5(e) shows the impedance spectrum of N2a cells 2×10^6 cells/mL embedded in a laminin–collagen matrix without compensation of parasitic electric elements. At frequencies higher than 100 kHz and less than 10 MHz the magnitude of the impedance is almost frequency-independent. The phase switches from capacitive to ohmic and inductive behavior. Hence, the sensor response as shown in Fig. 5(c and d) is mainly mediated by a shift in the specific resistivity of the gel matrix with embedded cells.

Variations that appear in between electrodes can be mainly attributed to electrode fabrication tolerances, variations in the number of cells and deviations in the manual gel film preparation. In case one bubble could eventually appear within the gel, it was detected by the impedance measurement as a big increase of the output signal due to the increased resistivity of the whole gel (an entrapped bubble behaves like an insulator) and those signals were discarded from the analysis. The presence of four parallel electrodes for the same treatment allows the exclusion of non-properly filled electrodes. In addition after each experiment gels were visually inspected by with a Leica macroscope (MZ16 APO) and with a conventional inverted microscope. Further experiments with bare CLGs revealed that also the mixture of laminin and collagen without cells changes mainly its conductive properties and thus contributes to the sensor response. Insofar, the sensor signal is a summation of two effects that happen simultaneously: the differentiation and formation of axons as well as alterations of the collagen–laminin matrix. According to our results both contributions cannot be fully distinguished with the actual sensor signal processing. This takes into account that a bare gel and gel matrix embedded N2a cells

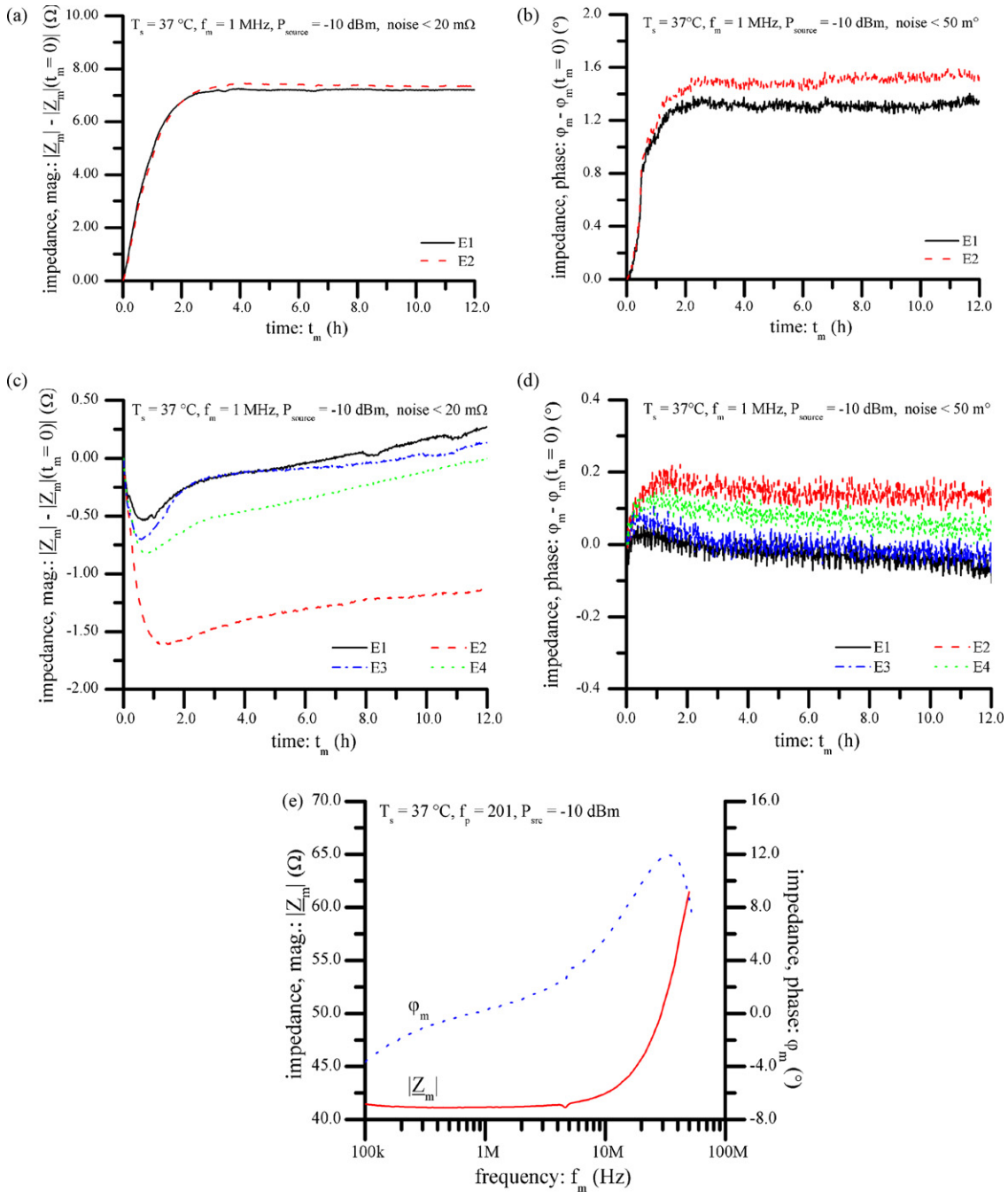

Fig. 5. Impedance recordings of differentiating vs. non-differentiated N2a cells in 3D matrices. Relative impedance shift of two representative electrodes (E1–E2) at 1 MHz during 12 h of non-differentiated cells (2×10^6 N2a cells/mL in CGs) referred to the initial impedance at $t_m = 0$: magnitude (a) and phase (b). Relative impedance shift of four representative electrodes (E1–E4) at 1 MHz during cellular differentiation (2×10^6 N2a cells/mL in CLGs) referred to the initial impedance at $t_m = 0$: magnitude (c) and phase (d). Impedance spectrum of N2a cells at a concentration of 2×10^6 cells/mL embedded in a laminin–collagen matrix in a frequency range between 100 kHz and 50 MHz (e).

can not be treated as a linear superposition of individual electrical properties.

5. Conclusions

In this contribution different gel matrices were studied as candidates for the development of a new 3D model of neural differentiation. Cell density as well as gel composition was optimized mainly in terms of viability and grade of differentiation. Differentiation was on-line monitored by means of a novel impedimetric biosensor especially designed for 3D cell cultures. Viability tests and cell morphology showed no effect of current flow on cell behavior. The electrical impedance analysis of N2a cells in CLG revealed that the differentiation process is mediated by an increase of the impedance magnitude, which is not observed in non-differentiated cells on CGs. In addition to the formation of neurites, changes in the specific resistivity of the bare CLG contribute to sensor response. This model appears as a promising tool for drug screening in developmental neurobiology.

Acknowledgments

This work was funded by the EU Marie Curie Research Training Network “On-chip cell handling and analysis”, ref-

erence: MRTN-CT-2006-035854. The authors acknowledge the contribution of Professor Alex Simonian (Auburn University, AL, USA/Bioengineering and Engineering Healthcare Cluster, National Science Foundation) and Vareli Arakelian (Yerevan Institute of Physics, Armenia) in the theoretical modeling of the cell behavior within the impedimetric sensor, as well as the contribution of Jimmy Lopez (Agricultural University of Athens, Greece) in the analysis of the results.

References

Barsoukov, E., Macdonald, J.R., 2005. Impedance Spectroscopy: Theory, Experiment, and Applications, 2nd ed. Wiley-Interscience.
Birgersdotter, A., Sandberg, R., Ernberg, I., 2005. Semin. Cancer Biol. 15 (5), 405–412.
Briscoe, J., Novitch, B.G., 2008. Philos. Trans. R. Soc. Lond. Ser. B 363 (1489), 57–70.
Campbell, C.E., Laane, M.M., Haugarvoll, E., Giaever, I., 2007. Biosens. Bioelectron. 23 (4), 536–542.
Cullen, D.K., Lessing, M.C., LaPlaca, M.C., 2007. Ann. Biomed. Eng. 35 (5), 835–846.
Desai, A., Kisaalita, W.S., Keith, C., Wu, Z.Z., 2006. Biosens. Bioelectron. 21 (8), 1483–1492.
Hölzel, R., Lamprecht, I., 1994. Neural Netw. World 3, 327–337.
Keese, C.R., Wegener, J., Walker, S.R., Giaever, L., 2004. Proc. Natl. Acad. Sci. U.S.A. 101 (6), 1554–1559.
Kintzios, S., Bem, F., Mangana, O., Nomikou, K., Markoulatos, P., Alexandropoulos, N., Fasseas, C., Arakelyan, V., Petrou, A.L., Soukouli, K., Moschopoulou, G., Yialouris, C., Simonian, A., 2004. Biosens. Bioelectron. 20 (4), 907–916.
Kintzios, S., Yiakoumetis, I., Moschopoulou, G., Mangana, O., Nomikou, K., Simonian, A., 2007. Biosens. Bioelectron. 23, 543–548.
Kloss, D., Fischer, M., Rothermel, A., Simon, J.C., Robitzki, A.A., 2008a. Lab Chip 8 (6), 879–884.
Kloss, D., Kurz, R., Jahnke, H.G., Fischer, M., Rothermel, A., Anderegg, U., Simon, J.C., Robitzki, A.A., 2008b. Biosens. Bioelectron. 23 (10), 1473–1480.
Kyle, A.H., Chan, C.T.O., Minchinton, A.I., 1999. Biophys. J. 76 (5), 2640–2648.
Li, G.N., Livi, L.L., Gourd, C.M., Deweerd, E.S., Hoffman-Kim, D., 2007. Tissue Eng. 13 (5), 1035–1047.
Lin, P.W., Wu, C.C., Chen, C.H., Ho, H.O., Chen, Y.C., Sheu, M.T., 2005. J. Biomed. Mater. Res. Part B 75B (1), 146–157.
Lin, S.P., Kyriakides, T.R., Chen, J.J.J., 2009. Biomaterials 30 (17), 3110–3117.
Lisdat, F., Schafer, D., 2008. Anal. Bioanal. Chem. 391 (5), 1555–1567.
Mosmann, T., 1983. J. Immunol. Methods 65 (1–2), 55–63.
Pampaloni, F., Reynaud, E.G., Stelzer, E.H.K., 2007. Nat. Rev. Mol. Cell Biol. 8, 839–845.
Slaughter, G.E., Hobson, R., 2009. Biosens. Bioelectron. 24 (5), 1153–1158.
Schroeder, J., Doerner, S., Schneider, T., Hauptmann, P., 2004. Meas. Sci. Technol. 15 (7), 1271–1278.
Valero, T., Jacobs, T., Moschopoulou, G., Naumann, M., Hauptmann, P., Kintzios, S., 2009. Procedia Chem. 1, 734–737.
Wang, L., Wu, Z.Z., Xu, B.Q., Zhao, Y.P., Kisaalita, W.S., 2009. Sens. Actuators B 140 (2), 349–355.
Wegener, J., Keese, C.R., Giaever, I., 2000. Exp. Cell Res. 259 (1), 158–166.