Human neurospheres on microelectrode arrays: a model to investigate ionizing radiation effects on neuronal network communication*

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Motivation

Ionizing radiation is known to induce numerous effects in cells including cell cycle delay, DNA damage, altered gene expression and cell death. Although there are serious impacts on the organisms, the effects on prenatal development are largely unknown. Data regarding potential biological effects on the prenatal development after in utero radiation exposure predominantly stem from atomic bomb survivors and from animal studies. These results indicate that the developing central nervous system is particularly sensitive to ionizing radiation and that exposure can lead to prenatal death, growth retardation, organ malformation or mental retardation [1]. Yet, there is hardly any information on the effects of ionizing radiation on neuronal network communication, the most vital function of neurons.

To fill this gap we developed a protocol that enables electrical characterization of three-dimensional neurospheres (NS) derived from human embryonic stem cells using microelectrode arrays (MEA). This method allows assessing the electrical activity of neuronal tissue in a non-invasive way. Network functionality typically develops in three phases. First, electrical activity can be detected as random single spikes. In a second phase train-like spiking activity evolves, that further develops into burst-like activity. These bursts represent the mature signalling activity of the network [2].

The measurement of electrical activity of neurospheres on MEAs represents a new and promising tool to investigate the effects of ionizing radiation on the neuronal network communication during early brain development.

Material and Methods

Experiments were performed with human embryonic stem cell derived neurospheres [3].

Human embryonic stem cells (hESCs, WA09 line) were obtained from WiCell (Madison, WI, USA) and cultured according to standard protocol [4]. Differentiation was performed in adherent culture to $PAX6^+$ neural progenitor cells. These early central nervous system precursor cells were replated into suspension to develop to round aggregates, socalled neurospheres [3].

The electrical signals of the neurospheres were recorded by microelectrode arrays. Prior to experiments, MEAs were coated with Polyethyleneimine (PEI) and Laminin. After two weeks in culture, neurospheres were harvested and plated on the centre of the coated MEA chips where they attached and neurons migrated out onto the microelectrodes. Thereafter, the electrical signals were recorded for about one week after plating.

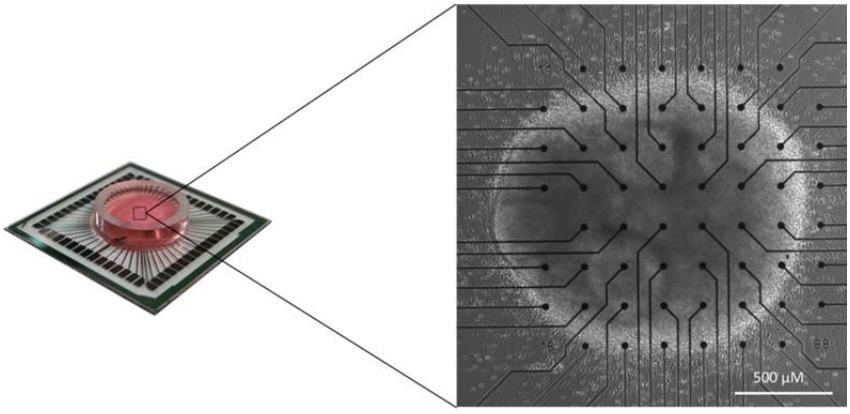

Figure 1: Human embryonic stem cell derived neurospheres on a MEA chip, cultured for two weeks in suspension, prior to plating.

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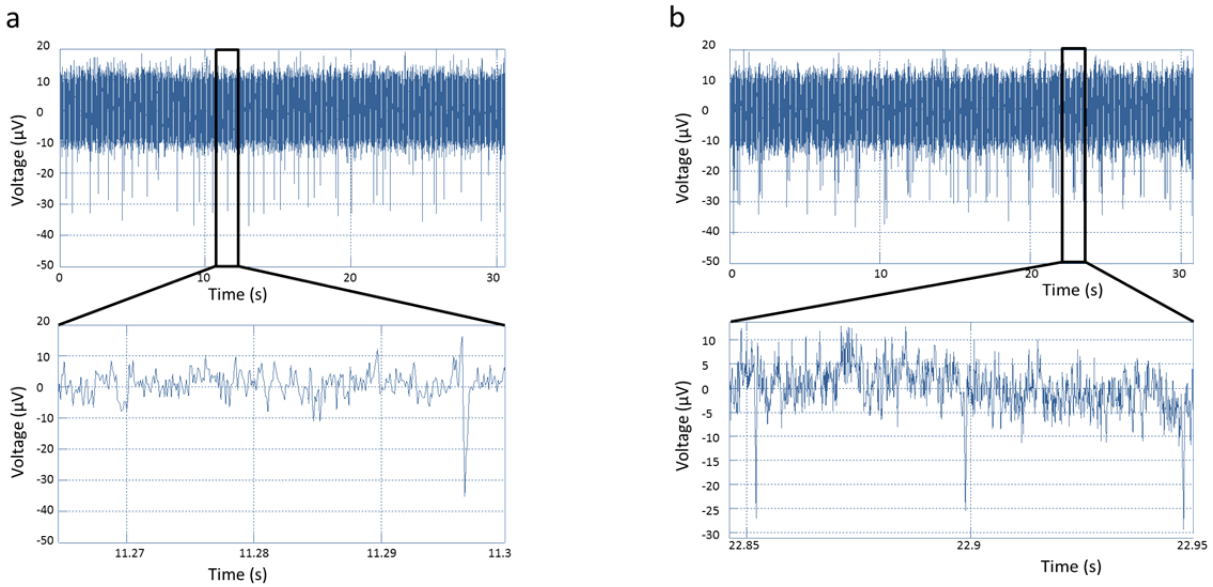

Figure 2: Development of neuronal activity of neurospheres on MEA Chips. (a) One day after the neurospheres are plated on the centre of the MEA chip the first signals are detected. They are random single spikes. (b) After two days of cultivation the cells started to express train-like spiking activity.

Results

PAX6 positive neural progenitor cells re-aggregated to neuronal networks in a 3D neurosphere system. The neurospheres reached sizes up to 600 µm just one day after precursor cells were replated into suspension. After two weeks in culture their size ranges from 600 µm to about 1500 µm. At this stage, neurospheres were plated onto the centre of the MEA chips where they attached to the coated surface and covered the whole electrode array as can be seen in Figure 1.

The first electrical signals could be recorded just one day after the neurospheres were placed on the MEA Chip. At this time electrical activity could be detected as random single spikes (Fig. 2a). After two days the cells started to express train-like spiking activity as can be seen in Figure 2b. These spike-trains consisted usually of three spikes and shows interspike intervals of about 50 ms. The signal amplitude remained constant and ranged between -30 and -40 µV.

However, in this first experiment spikes-trains have not developed into burst-like activity which is defined as at least four spikes with a maximum idle time between those spikes of 100 ms.

Conclusion

Our preliminary results show, that human embryonic stem cell-derived neurospheres represent a promising tool to investigate neuronal network communication in a three dimensional cell culture system.

The model used in this experiment provides many advantages. The differentiation from human embryonic stem cells towards neuronal precursor cells in neurospheres mimics early human brain development [3]. We have shown here that it is possible to detect electrical activity on MEAs and it may be feasible to treat the cells in different stages of differentiation, for example with ionizing radiation, and to analyse electrical activity on MEA chips to determine whether the treatment influenced the neuronal network communication. Another important advantage of this model is that neurospheres can be cultured over a period of several weeks enabling chronic exposure studies.

The fact that no bursts were detected could be related to the short culture and maturation period on the MEA chip of just one week. It is planned to extend this period. In addition, neurospheres in various stages could be plated on the MEA chips to evaluate optimal maturation for electrophysiological studies.

References

[1] McCollough, C.H. et al., “Radiation Exposure and Pregnancy: When Should We Be Concerned?”, RadioGraphics 2007, 27: 909-918

[2] Heikkilä, T.J. et al., “Human embryonic stem cell-derived neuronal cells form spontaneously active neuronal networks in vitro”, Exp Neurol. 2009, 218: 109-116

[3] Hoelting, L. et al., “A 3-dimensional human embryonic stem cell (hESC)-derived model to detect developmental neurotoxicity of nanoparticles”, Arch Toxicol. 2013, 87 (4): 721 - 733

[4] Thomson, J.A. et al., “Embryonic stem cell lines derived from human blastocysts”, Science 1998, 282 (5391): 1145-1147

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