First electrophysiological studies on mouse embryonic stem cell-derived cardiomyocytes

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There is emerging epidemiological evidence of an increased risk of adverse cardiovascular effects at low or moderate doses of ionizing radiation occurring many years after the exposure [1]. However, essentially no information is available on the potential cardiovascular effects associated with the exposure to heavy ions. To address this issue, we recently established in our laboratory an effective protocol for generating functional (i.e. beating) cardiomyocytes from mouse embryonic stem cells (mESC). In the present study, first electrophysiological measurements were performed employing microelectrode array chips (MEA).

Briefly, commercially available mESC (line D3) were differentiated by means of the hanging drop method [2]. ESC aggregate and form embryoid bodies (EBs). Seven days after initiation of the differentiation process, EBs were plated onto MEAs (1 EB/MEA). The MEAs used have a field with 60 electrodes arranged in an 8x8 grid. Each electrode has a diameter of 30 µm and the interelectrode distance spans 200 µm. The system allows non-invasive electrophysiological measurements. At day 10, EBs start beating, pointing to the formation of cardiomyocyte-networks. In general, the number of beating EBs increased during the subsequent days and declined around day 20. Western Blot analysis and histological staining of cardio-specific markers confirmed the formation of true cardiomyocytes. Accordingly, electrophysiological measurements were conducted between day 10 and 20.

Electrophysiological activity of untreated control samples was measured for 60 sec at a sampling rate of 10 kHz. The obtained data was then analysed with the MATLAB based program *DrCell* developed at the University of Applied Sciences Aschaffenburg [3]. The recorded data were analysed in terms of signal amplitude, shape and beat rate. Additionally, the origin of the signal, its spreading direction and velocity was registered and the number of active electrodes was determined. Representative recordings of the signal spreading, number of active electrodes and beat rate are shown in figures 1-3.

Detailed analysis of the data revealed that the cell system is highly variable; large differences were observed both within a sample and between samples. This is illustrated in figure 2 and 3, where changes in the number of active electrodes and in the beat rate are depicted for 2 EBs. Based on these results we conclude that mESC-derived cardiomyocytes are an unsuitable model system for studying the electrophysiological responses of cardiac cells after radiation exposure which are expected to be small.

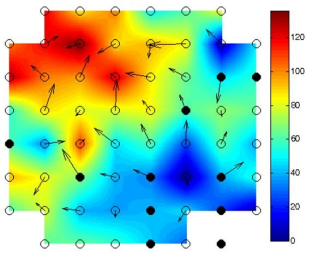

Fig. 1: Pseudocolor plot showing the delay time (ms) of action-potentials. One EB was seeded per MEA chip and 10 days after initiation of the differentiation electrical activity of cardiomyocytes was measured. Dark blue regions indicate the origin of the signal. Circles are electrodes.

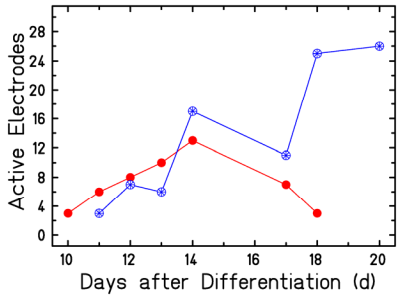

Fig. 2: Number of active electrodes during the observation period (day 10 to day 20). The graphs display recordings from 2 EBs.

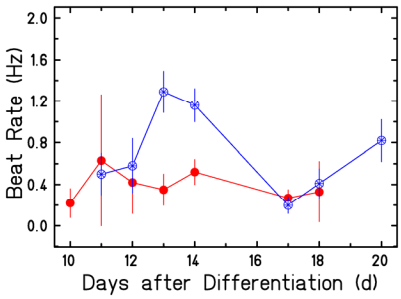

Fig.3. Beat rate of rhythmically contracting clusters in 2 EBs.

References

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*Funded by Euratom 7th Framework Programme (no.295823), Verein für Förderung der Tumortherapie mit schweren Ionen e.V. and HGS-HIRe.