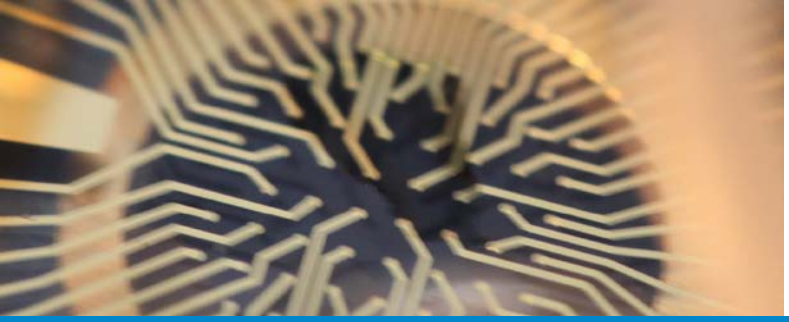

NMI TT Pharmaservices

Case Study: Influence of Cell Density on Field Action Potential and Pharmacological Properties using MEA Technology

Abstract

The health of cardiomyocytes cultivated on MEA is not only determined by the correct plating and culture medium, but also on the cell density. We investigated the influence of cell density of different commercially available cardiomyocytes on the electrophysiological properties and sensitivity to cardioactive drugs. We demonstrated that the field action potential (fAP) duration was highly dependent on the plating density. The cardiomyocytes plated at 9k and 18k cells/↔l displayed fAP durations of 473 ✣ 12 and 387 ✣ 9 ms, respectively. Resulting in a difference of ~ 22%. Furthermore, other commercially available hiPS-CM showed similar values, indicating that high cell densities result in a decreased cardiac action potential duration. Moreover, we observed that drug sensitivity is also in function of cell density. For instance, the application of the specific hERG inhibitor E4031 at 30 nM resulted in a fAP prolongation of 124 ✣ 15% at 6k and 44 ✣ 9% at 12k cells/↔l, which is also in line with findings using other hiPS-CMs. Additionally, the occurrence of proarrhythmic markers was increased at lower cell densities. Altogether, these findings demonstrate the importance of highly standardized plating and cultivation of cardiomyocytes on MEA chips, especially when data needs to be compared between different labs (like in the running CiPA study).

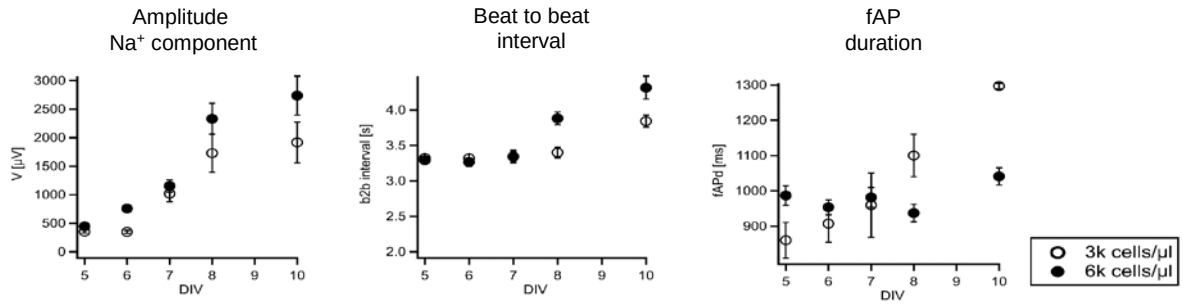

Figure 1. Field action potential (fAP) properties depend on cell plating density. We compared the fAP properties from cultures that initially contained 3 and 6 thousand cells per microliter. Interestingly, we observed that the amplitude of the Na^+ component was higher in the wells that contained a higher cell density. Furthermore, the beat to beat interval and the fAP duration indicate that the beat was faster in cultures that contained a higher cell density.

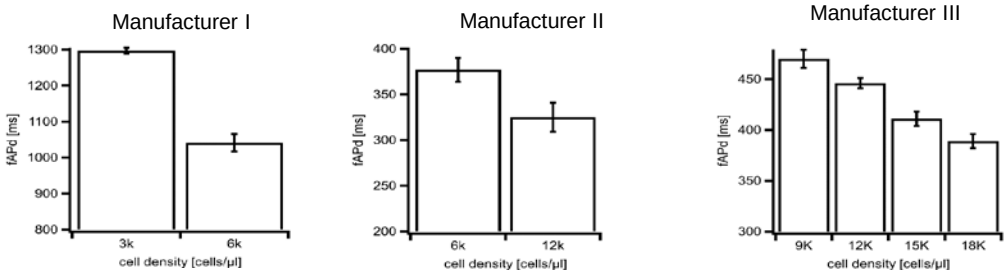

Figure 2. Cell density influences the fAP properties of hIPS cardiomyocytes from different commercial manufactures. We were interested to compare the cell-density dependency from different commercially available hIPS cardiomyocytes. Thus, we used cultures from three different manufacturers. Without exception, we observed that the denser-cell cultures presented a shorter fAP duration, which leads to a faster beat.

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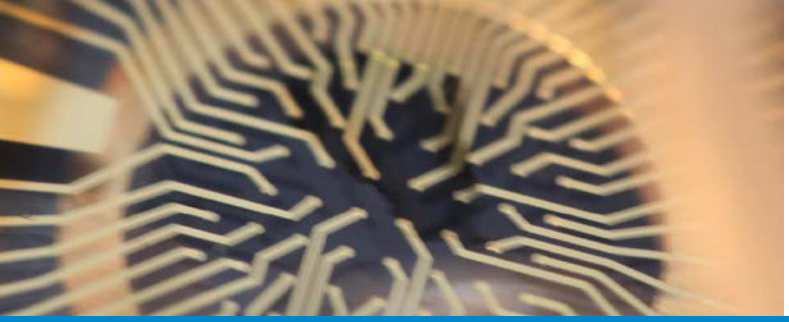

Case Study: Influence of Cell Density on Field Action Potential and Pharmacological Properties using MEA Technology

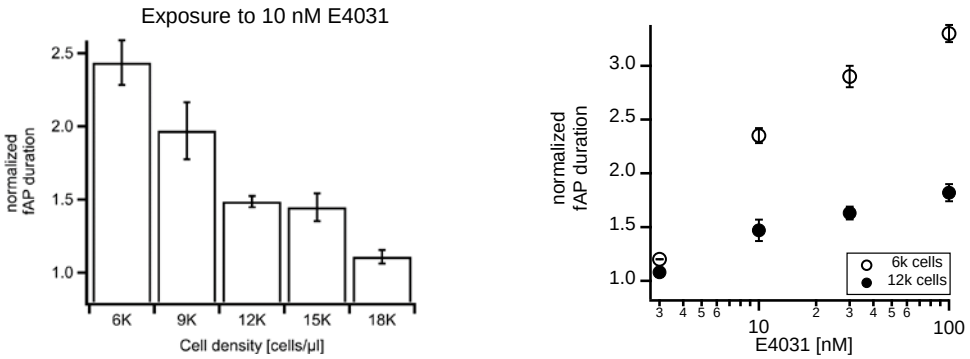

Figure 3. The hERG blocker effect on the fAP duration is influenced by the cell density. For these experiments we used E4031, a hERG blocker that induces a fAP prolongation. We observed that the hERG blocker effect is attenuated in high cell density cultures.

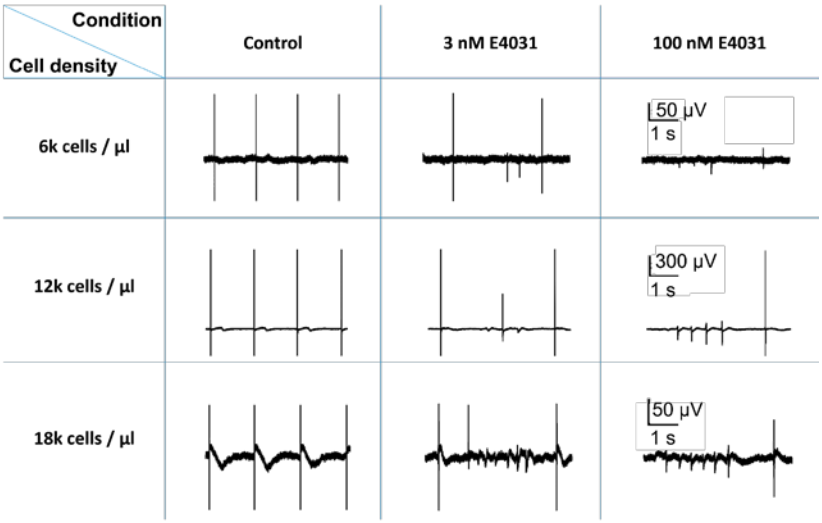

Figure 4. The occurrence of proarrhythmic events induced by a hERG blocker is cell-density dependent. Representative traces using three different cell densities in the absence and presence of the hERG blocker. We observed that the cell density also affects the identification of proarrhythmic effects.

Conclusion

Cell density influences the electrophysiological and pharmacological properties of MEA-cultivated cardiomyocytes. **Remember, a well defined and optimized cultivation protocol is the key for reliable experiments!**

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