Adenoviral Gene-transfer in Cultured Adult Cardiomyocytes as an Alternative Approach to Transgenic Animals

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The enzymatic isolation of cardiac myocytes is known for more than 30 years. Once isolated, the cells dedifferentiate and lose the cardiac specific morphology and gene expression pattern.

We developed a cardiac myocyte culture with minimised dedifferentiation by (i) cultering the cells in serum free medium supplemented with insulin, transferrin and selenite, (ii) plating them on elastic surfaces coated with a mixture of extracellular matrix proteins and (iii) applying a continuos electrical stimulation to keep the isolated cells contracting. Based on this treatment we can conserve rod shaped cells with a distinct cross striation in culture for one week. This provides a time slot to allow an adenoviral mediated gene expression, which typically takes place within 20 to 40 hours. We constructed adenoviruses for (i) a number of fluorescent fusion proteins to visualise subcellular structures in living cells, (ii) genetically encoded biosensors, like the calcium sensors YC3.6 or TN-XL and (iii) signalling molecules such as several isoforms of protein kinase C.

For optical investigations of the living cardiomyocytes we developed 24-well plates and the adjacent hard- and software to allow automated high resolution imaging while cells are electrically stimulated.

In summary we set the scene to go for optical high content screening of genetically modified primary adult cardiomyocytes without the necessity to breed transgenic animals. This allows high impact research and developments with a minimum of sacrificed animals and without animal experiments.