7. Summary

The aim of this work was the establishment of the scientific and technical supposition for an efficient electroporation of a large and small number of cells. A part of this work was used for the development of a new electroporation instrument, the Multiporator. For that purpose varying cell types have been transfected with the plasmid pEGFP. The influence of different electrodes materials, of temperature, of electroporation medium composition and of the plasmid concentration on the transfection effeciency was investigated. The results flowed step by step into the development of the Multiporator. In consequence, there is now an electroporation instrument available that enables the transfection of mammalian cells with very high yields, due to an optimized system of technique, components and proceedings.

In contrast to common instruments the Multiporator uses the µ pulse technology (field pulses of high intensity and short duration in microsecond range). The synergy of the instrument and the components (aluminium electrodes, the composition of the hypoosmolal medium of low conductivity) enable the electrotransfection of eukaryotic cells with high efficiency. Under these conditions the Laplace equation is a good predictor of the parameters of electroporation.

With this technique the gene transfer by mammalian artificial chromosomes (MACs) was demonstrated for the first time. The artificial chromosomes were sorted due to a new dye combination, wich allows the sorting of MACs with low cost FACS instruments. The uptake of the chromosomes into cells is based on electrointernalisation. The presence of the MACs in their original morphology in the L929 cells could not been demonstrated, but the expression of the genes from the artificial chromosomes.

Another application for this new electroporation instrument is the transfection of primary cells. In this case only a small number of cells is available. The results of this work have shown that the transfection of cardiofibroblasts and embryonic stem cells is possible with high efficiency. The main point for the high yields is the cell cycle. The results show, that the share of cells in S-phase is proportional to the efficiency of electrotransfection.

The manipulation of the genom and cytosol of only a few cells is more and more important for the biotechnological application of electroporation. Therefor, a concept for a new electroporation instrument was evolved. With the developed apparatus cells can be concentrated in high conductivity medium. Thus, different steps of electrotransfection (centrifugation, pipette) could be saved. The electroporation could be carried out under electrodeformation.

The results of this work can contribute to the development of modern gene therapy. The µ pulse technology and the mechanisme of the gene transfer (gene transfer by artificial chromosomes and the role of cell cycle for the efficiency of electrotransfection) offer the possibility for the genetical manipulation of cells with very high yields. The developed apparatus can be the basis for a new generation of electroporation instruments.