Summary

Many progressive neurological disorders are based on the degeneration of motoneurons. These cells exhibit the longest axons of the nervous system and have to navigate very long distances to get from the spinal cord to their muscle target. This fact makes motoneurons much more vulnerable to extracellular and intracellular defects. The disruption of transmitted signals between the motoneuron and the innervated muscle leads to weakness and atrophy of the muscle. Finally the control of voluntary movement is lost. Therefore many neuroscientific questions deal with the degeneration of motoneurons (Jablonka, Wiese et al. 2004; Van Den Bosch, Van Damme et al. 2006; Linker, Lee et al. 2011). Primary cell cultures are able to display specific physiological characteristics of isolated cells. Defined culture conditions allow standardized analysis of specific interactions. Also interactions with other cell populations can be investigated in so called co-cultures. Behind this background the goal of this thesis was the development of an isolation technique for the enrichment of embryonic spinal cord motoneurons and the establishment of a co-culture system. With this co-culture system the influence of extracellular matrix molecules on motoneuron behavior ought to be investigated. Furthermore the investigation of the extra cellular matrix equipment of different cell lines and of the spinal cord and its surrounding tissue during the time of neurogenesis and axon outgrowth should reveal new interaction partners for outgrowing motoneurons.

- With the lectin-based panning procedure a significant enrichment of spinal cord motoneurons was achieved. On average 73 % of the isolated cells were motoneurons. This isolation method guarantees high vitality and a high yield of the cells. The panning technique is easy to reproduce and also suitable for the isolation of motoneurons from individual mouse embryo. In vitro the isolated motoneurons displayed a typical morphology and expressed specific transcription factors and receptors.

- For the characterization of the used cell lines immuncytochemistry and western blot analysis was performed. The different cell lines secreted a dense network of different matrix molecules. The glycoproteins Laminin and Fibronectin were found in all cell lines. TenascinC, the receptor-protein-tyrosine-phosphatase RPTPβ/ζ and the secreted isoform phosphacan, the Chondroitinsulfate epitope DSD-1 and the glycan modification LewisX were found in different amounts in the investigated cell lines.
The expression pattern of the different matrix components in the developing embryo suggests a participation of these molecules in processes of motoneuron differentiation and axonal pathfinding. In vivo, at the time point when mainly neurogenesis occurs the glycoproteins Laminin and Fibronectin were found at the blood vessels in the spinal cord and at the meninges. Also within the whole mesenchyme immunoreactivity was found. With starting gliogenesis both proteins were also detected in the spinal cord. TenascinC was not localized in the spinal cord during neurogenesis, but strong signals were detected in the paraxial mesoderm and in the tissue anterior of the spinal cord. With starting gliogenesis TenascinC expression was also found in the spinal cord, but also in the muscles and bones of the developing limbs. At embryonic day 10,5 RPTPβ/ζ/Pcan and the DSD-1 epitope were mainly associated with the meninges. In the ventral part of the spinal cord both molecules could be detected at the time point E12,5. Both molecules were colocalized with axons in the white matter. A strong RPTPβ/ζ/Pcan signal was also detected at the exit points of the motoraxons outside the spinal cord. At E15,5 RPTPβ/ζ/Pcan was expressed by muscle cells and bones of the developing limb. These signals were associated with neuronal fibers. Both RPTPβ/ζ/Pcan and the DSD-1 epitope were associated with the pathways of neural crest cells. With ongoing development (E15,5) the DSD-1 epitope was detected in the developing limbs and localized next to the neuronal fibers. The LewisX modification was only detected in the spinal cord and not in the embryonic mesenchyme, At embryonic day 12,5 LewisX was localized in the white matter at the ventral part of the spinal cord. The cell generated matrices mediated survival as well as growth promoting properties on cultured embryonic motoneurons. In comparison to the control situation on PDL significant more motoneurons survived an cell generated matrices. The growth promoting properties were mainly mediated by the matrices of the A7 cells and myotubes. On this matrices motoneurons exhibited significant more and longer neurites. A striking observation was made by looking on the growth behavior of the motoneurons on the IMS matrix. Generally significant less and shorter neurites were measured and also the soma size was significant smaller than the soma size of motoneurons grown on MT-matrix.