ABSTRACT

Despite tremendous progress in medicine, injuries of the adult central neural system remain without satisfactory solution. Regenerative medicine employs tissue engineering, cellular therapies, medical devices, gene therapy, or growth factors with the aim to bridge the lesion, re-establish lost connections and enhance endogenous repair in order to restore neural function.

The aim of my thesis was to evaluate therapeutic potential of two approaches, transplantation of human mesenchymal stromal cells (hMSCs) and biological scaffolds derived from extracellular matrix (ECM) for neural regeneration, particularly in models of spinal cord injury (SCI).

First, hMSCs from various sources - bone marrow (BM), adipose tissue (AT) and Wharton´s jelly (WJ) - were isolated and characterized *in vitro*. All cell types met the minimal criteria for MSC phenotype and displayed similar properties in terms of their surface marker expression, differentiation potential, migratory capacity, and secretion of cytokines and growth factors. On the other hand, the cell yield from WJ and AT was significantly higher, and MSCs isolated from these tissues proliferated better than from BM.

Therapeutic effect of intrathecal application of hWJ-MSCs was then evaluated in SCI compression model in rats. The effect of low (0.5 million) and high (1.5 million) dose of hWJ-MSCs in a single or repeated (3x) application was compared. We demonstrated that transplantation of single as well as repeated application of high cell dose had apparent beneficial effect on neural tissue repair and resulted in a better grey matter sparing and increased number of GAP43+ axons, reduced astrogliosis, and reduced expression of inflammatory and apoptotic markers. Significant recovery of functional outcome was reported in all cell-treated groups except for the single application of the low number of cells. We observed that the effect of hWJ-MSCs was augmented with the dosage and was further potentiated by repeated application.

In the third part of this project, we prepared biologic ECM hydrogels by decellularization of central nervous tissue (CNS: porcine brain and spinal cord) and non-CNS tissues (porcine urinary bladder and human umbilical cord) that were used alone and in combination with hWJ-MSCs to bridge the SCI lesion. Hydrogels from all ECM were characterized in terms of composition, mechanical and biological properties.

In vitro comparison of ECM hydrogels showed that despite the different origin, topography and composition, all ECM hydrogels similarly promoted the migration of hMSCs and differentiation of neural stem cells, as well as axonal outgrowth.

The effect of porcine tissue-derived spinal cord (SC) ECM and urinary bladder (UB) ECM hydrogels was evaluated *in vivo* after their injection into a hemisection cavity in rats. Both ECM hydrogels integrated well into the lesion and stimulated neovascularization and axonal ingrowth. Gene expression analysis revealed significant down-regulation of genes related to immune response and inflammation in both hydrogel types at 2 weeks post-SCI. However, no tissue specific effect was found for CNS-derived SC-ECM when compared to non-CNS UB-ECM. In addition, the combination of hWJ-MSCs with SC-ECM did not further promote ingrowth of axons and blood vessels into the lesion when compared with the SC-ECM hydrogel alone.

In conclusion, results from this project show that both hWJ-MSCs and ECM hydrogels represent promising ways which may contribute to neural tissue regeneration, and therefore, are strong candidates for translation into the clinical setting. We confirmed the beneficial effect of hWJ-MSCs in SCI and proposed repeated intrathecal application as convenient treatment approach. While hWJ-MSCs transplantation has already been transferred into the clinical trials, the production of ECM hydrogels needs to be further optimized for neural tissue applications especially in terms of their fast *in vivo* degradation.

Key words: regeneration, neural tissue, spinal cord injury, mesenchymal stromal cells, bone marrow, adipose tissue, Wharton´s jelly, extracellular matrix, hydrogel, umbilical cord