Intervertebral disc (IVD) degeneration is a major cause of low back pain, affecting a large percentage of the population. Given that disc degeneration is a cell-mediated response to progressive structural failure, new treatments which normalize disc cell homeostasis and restore disc function are required. Cell-based therapies have received increasing attention in recent years. Among them, mesenchymal stem cells (MSCs) appear to be ideal candidates, since they can differentiate into various cell types, potentially including IVD cells, and have trophic effects. However, it remains uncertain whether MSCs that are similar to native disc cells are required to offer regenerative potential, and whether cells introduced into the IVD environment would remain alive and fully functional for required periods of time. The aim of this proposal was to evaluate and optimize the fate of injected MSCs in an IVD maintained in an *in vitro* organ culture system under defined nutrient and mechanical loading conditions.

In the first year of the project, we demonstrated that hyaluronan-based thermoreversible hydrogels support human MSC differentiation toward the disc-like phenotype without the need for growth factor supplementation both *in vitro* and *ex-vivo*. In the first period of the second year, we established a suitable model to study IVD regenerative therapies under dynamic load using an endplate approach to perform nucleotomy and optimizing the loading regime for nucleotomized discs. Since the hyaluronan-based thermoreversible hydrogel was not sufficiently mechanically competent, we investigated fibrin gels as alternative carriers for MSCs. Concentrated fibrin gel (60 mg/mL fibrinogen) was able to withstand physiological loading and support survival of MSCs implanted in the IVD.

In the last period, we focused on the contribution of fibrin gel on the restoration of disc height and the role of MSCs implanted in the disc. We found that fibrin gel significantly contributed to disc height restoration under dynamic load. In our experimental setting, application of MSCs to physiologically loaded discs induced an up-regulation of collagen types I and II in the entire disc (nucleus pulposus, inner and outer annulus fibrosus), while only minor changes were observed in catabolic gene expression. In degenerative loaded discs, only the outer annulus fibrosus responded to the MSC treatment. Our data suggest that regenerative effects of MSCs in the IVD are due to the release of trophic factors which positively influence the endogenous disc cells.

To conclude, we found that (1) MSCs are able to survive and to further differentiate when implanted into a whole IVD cultured under simulated-physiological conditions; (2) implanted MSCs induce regenerative effects on degenerative IVDs in organ culture; and (3) dynamic compressive loading shows, depending on the loading frequency, beneficial or detrimental effects.