Investigation of Regenerative Effects of CRISPR/Cas9 Functionalized Piezoelectric Nerve Conduits on *in vitro* and *in vivo* Spinal Cord Injury Models

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Spinal cord injury (SCI) is a debilitating condition with long-term personal, social and economic costs. Patients with SCI experience serious clinical problems, such as partial or complete paralysis, spasticity, pain, loss of bowel/bladder/sexual functions, and difficulty in breathing. Ongoing research for SCI comprises pharmacological therapies and cell transplantation. Combinatorial treatment strategies makes partial recovery possible, but there is no extensive treatment for regeneration. PIECRISCI proposes a novel, functionalized biomaterial-based treatment to reduce glial scar formation and induce neuronal regeneration by incorporating CRISPR-Cas9 complexes into piezoelectric fibers.

Piezoelectric materials generate local electric fields in response to mechanical stresses, and this effect is used to stimulate neuronal regeneration. After SCI, the inflammation observed in the injury zone creates an inhibitory environment for neurons and myelin formation. PIECRISCI hypothesizes that this inflammatory microenvironment can be controlled using CRISPR-Cas9 gene editing technology.

Within the scope of PIECRISCI, we have identified genes that are associated with reduction of inflammation and gliosis by analyzing the transcriptome changes after SCI. We have created CRISPR-Cas9 complexes to edit these identified genes, and the effectiveness of these complexes are tested on *in vitro* cell models. Selected complexes were encapsulated into engineered lipid nanoparticles (LNPs), and further loaded into fibrous piezoelectric conduits. The piezoelectric fibers are tested *in vitro* for increased neurite formation and length in neuron cultures. Results of *in vitro* evaluations will be presented.

LNP-mediated delivery of CRISPR-Cas9 offers the advantage of protection of the genetic editing material and increased delivery efficiency. Incorporation of LNP/CRISPR-Cas9 complexes into fiber conduits allow precise control of local delivery of necessary cargo in the most needed area. Next step is testing of functionalized fibers *in vivo*.

At the end of the project, PIECRISCI aims to propose a new bioengineered treatment approach that promotes neuronal and axonal regeneration through piezoelectric stimulation, while reducing glial scar formation and inflammation through local gene editing.