In vitro and *In vivo* Evaluation of Neural Stem Cells Seeded on Neural Probes

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Statement of Purpose:

Silicon based neural electrode arrays (neural probes) that permit recording and stimulation of specific sites in the brain are known to experience failure in chronic long-term recording partly due to biocompatibility issues. Surface modification of these devices is needed in order to improve their integration within the host brain tissue. We propose a method in which adult neural stem cells (NSCs) will be seeded on the surface of the probe to improve brain tissue integration, repair electrode induced injury, and minimize scar formation, by presenting a surface with tools 'to heal'. Assessment of NSC growth, adhesion, differentiation *in vitro,* and integration *in vivo,* will be performed to understand the role they play in integration and biocompatibility.

Methods:

In vitro experiments: The surface of the neural probes consists mostly of silicon dioxide, which is not prone to NSC attachment. In order to improve the adhesion of NSCs, laminin was covalently immobilized on silicon dioxide samples using silane chemistry and GMBS crosslinker. After laminin immobilization, the samples were immersed in polyethylene glycol (PEG)-NH_2, to inactivate GMBS and inhibit non-specific protein adsorption.

The NSCs were isolated from the subventricular zone of rat brains and labeled with GFP. For all samples, the stem cells were seeded at 50,000 cells/ml in growth media (GM) or in differentiation media (DM). The cell-seeded samples were incubated at 37^{o}C, 5% CO_2 in humidified air for a period of 3, 7, or 14 days. To evaluate the growth and adhesion of cells, a shear force was applied to half of the GM laminin immobilized samples and half of the unmodified samples using an orbital shaker. The number of cells on the surface before and after shaking was quantified. To asses NSC differentiation, the DM samples were stained for neurons, astrocytes, oligodendrocytes, and neural stem/progenitor cell marker (nestin) after different time points. Fluorescence images were taken and quantifications were done using the image analysis software Metamorph®.

In vivo experiments: Laminin immobilized neural probes (NeuroNexus Technologies) were seeded with NSCs and incubated in growth or differentiation media for 14 days. Implantation of stem cell-covered probes in Fisher 334 rats was performed following IACUC regulations. The probes were implanted in the motor cortex of the rats, one day or one week, followed by perfusion and extraction of their brains for immunohistochemistry and quantification purposes. For one sample, two-photon microscopy was performed to visualize the GFP-NSC seeded probe within the brain tissue slice. For all other samples, neural probes were removed, stained, and further analyzed using fluorescent microscopy. The extracted brains were cut into 20 μm thick horizontal sections using a cryostat. The sections were stained for GFP, astrocytes, neurons, microglia, and macrophages. The stained slices were then imaged using confocal microscopy. Integrated intensity and distance of the cells around the probes was quantified using Metamorph®.

Results / Discussion:

In vitro results: A large number of cells were found to be adhered on the laminin immobilized surface compared to the unmodified control. The adhesion was further assessed by comparing the number of cells on the surface in the applied force (AF) group of the laminin immobilized samples vs. control (without applied force). It was observed that the number of cells increases as incubation time increases. There was a significantly greater percent cell coverage on the control samples than the AF samples at day 7 ($p<0.05$). However, at day 14, there was no significant difference between the two groups ($p>0.05$) suggesting that 14 day is the incubation time needed for the best surface coverage and adhesion of NSCs before implantation. The DM samples showed that astrocyte differentiation increased with increase of incubation time. Nestin positive cells decrease with incubation, suggesting increased differentiation of the stem cells over time. The percentage of neurons was observed to be higher at day 3 and decreased later. Oligodendrocyte differentiation seemed to follow the same patterns as neuron differentiation.

In vivo results: The 2-photon microscopy and fluorescent images of the probes showed that NSCs incubated in GM adhere well to the laminin modified surfaces of the neural probes. Some of the cells remained attached after insertion and they seemed to stay attached even after extraction. The immunostained tissue sections show less astrocyte activation around the stem cell seeded probes than control after 1 week. This suggests that the stem cells may help to reduce astrocytic tissue reaction around the probe implanted in the brain.

Conclusions:

The greater percent cell coverage of laminin immobilized samples vs. clean silicon samples is due to the high affinity of laminin for stem cells. This also contributes to the increased adherence of the stem cells on the laminin immobilized samples. Based on *in vivo* results, some NSCs seeded on the probe survived the shear forces applied on the probe during implantation insertion and remained alive after being in the host tissue. These results are encouraging and further analysis will be taken to track the fate of these cells (where they go and what type of cells they become) after longer period of times. In addition, the host tissue reaction to the NSC seeded probes will be further compared to the unseeded ones at longer time points.