Investigator Profile

The hope: healing damaged neurons with genetically-modified bone marrow-derived stem cells

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– Professor Donald Sakaguchi

Professor Donald Sakaguchi's lab in the Department of Genetics, Development, and Cell Biology at Iowa State University recently adopted high-content imaging to expedite development of stem cell-based approaches to rescue and repair the damaged nervous system.

Professor Sakaguchi, can you describe the neuroregenerative approaches in development at your lab?

We are genetically modifying bone marrow-derived stem cells to be delivery vehicles for therapeutic proteins following transplantation into the damaged or diseased nervous system. From a clinical standpoint, the use of bone marrow-derived stem cells would offer an important advantage for autologous transplantation. Much of our work has focused on eye diseases, such as glaucoma, and transplanting modified stem cells into the glaucomatous eyes of rodent animal models. The transplanted cells are used as delivery vehicles for neurotropic growth factors.

In addition, we are studying peripheral nerve regeneration. We're developing a biodegradable conduit, a multifunctional construct that can be used to stimulate and help facilitate peripheral nerve regeneration. We take this device that has been seeded with different cell types and we implant them in a peripheral nerve model of traumatic nerve injury in a rat.

How is that biodegradable conduit for facilitating neural regeneration constructed?

We use polymers, such as polylactic acid, to create a thin film that is micro-patterned with grooves. We roll that into a conduit and seed it with bone marrow-derived mesenchymal stem cells (MSCs) that have been transdifferentiated into Schwann-like cells. In future studies we anticipate also using genetically modified stem cells over-expressing either brain-derived neurotrophic factor (BDNF) or glial cell-derived neurotrophic factor (GDNF).

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It's important that we take the time upfront and characterize these cells before we use them for our transplant or implant studies. We've been doing some pilot studies at the moment trying to optimize the conditions. For example, optimizing the conduit itself, finding materials that will facilitate cell survival, as well as being tolerated well by the animal. As we want to culture our cells in a relatively native type of environment, we compare different types of extracellular matrix molecules' effect on the growth and migration properties of the cells.

Is that screening requirement the impetus behind the high-content assay that you developed and published in the Journal of Visualized Experiments?

Yes, high-content screening (HCS) is creating great opportunities in the field of stem cell biology and drug discovery. We incorporated the HCS approach into our Department of Defense grant application, which we received together with collaborators here at Iowa State University and the University of Nebraska Medical Center.

We published in the Journal of Visualized Experiments a proof of principle of using HCS as a platform for screening populations of mesenchymal stem cells. In that study, we compared five different populations of adult stem cells, four being therapeutic populations expressing BDNF and/or GDNF as well as GFP as a fluorescent marker, on six different substrates. Those populations of cells were plated across a 96-well plate coated with various substrates.

The plated stem cells were maintained in the ImageXpress Micro System's environmental chamber for 48 hours, during which transmitted light and fluorescence images were captured every 5 minutes at two sites per well. After 48 hours, the sample wells were then fixed and assessed for cell differentiation, proliferation and survival using multi-wavelength imaging on the ImageXpress Micro System.

To quantify the neurotrophic factors that the cells secreted, the cell-conditioned culture media was collected at interim and terminal time points and analyzed by ELISA.

What advantage does HCS afford?

Having the HCS system in our lab has allowed us to expand the types of experiments we can conduct.

In the past, our experiments would be on a single cell type or a single cell line. We would set a culture chamber on a conventional upright fluorescence microscope with a heated stage, identify a region of interest, and hope that the cells in that region did something interesting by the end of two to three days of time-lapse imaging. As you can imagine, the site often turned out not to be particularly interesting and the experiment would be a bust. Typically data collection for this type of experiment would last several months and result in about 1,200 images for our analyses.

With HCS, we're able to conduct that type of comparison in a period of a week or so. In a routine experiment, we might use a 96-well plate and perhaps culture in about 60 wells and image two sites per well. At the end of four days of culture, time-lapse imaging would generate about 275,000 images.

For those considering HCS systems, what advice do you have for selecting the right instrument?

Since this is the only HCS system here at Iowa State, our group was sort of the pioneer in establishing this technology here.

We were able to assess multiple models of these HCS systems. The system we purchased has an environmental chamber within the microscope itself, allowing us to conduct time-lapse imaging on multi-well plates over the course of days if we would like. That opened up new avenues for conducting cell behavior types of experiments.

Another important feature is phase contrast optics. We often conduct multiple-day experiments using phase contrast imaging combined with fluorescence imaging.

What challenges should one prepare for when developing high-throughput assays?

One of the more obvious challenges for us is the amount of data that we generate. Now we have a server with a number of external hard drives to help archive and maintain the data. However, it is reassuring to know that we have all of this information available for future analyses.

One challenge that is not necessarily immediately apparent is assay development. A big part of conducting the experiments on the HCS system is designing the experiments right from the beginning. In the end, this process made our experimental approach much more robust, contributing to a reduction in the variability across experiments.

What are your next steps using the HCS assay platform you developed?

We've expanded the types of experiments we conduct to other cell types including neurons, glial cells, neural stem cells and retinal stem cells. Furthermore, this HCS platform has opened the door to new collaborations. We collaborate with colleagues from the Departments of Chemical and Biological Engineering, Biochemistry, Biophysics and Molecular Biology, as well as the Department of Biomedical Sciences at the ISU College of Veterinary Medicine. As we move forward, we anticipate training more members of the lab in HCS and also staff from collaborating labs. Research projects with collaborators will further lead to an expansion of the cell types that we work with, and perhaps eventually leading into whole animal imaging using zebrafish as a model experimental system.

Over the next several years, we will continue our studies focused on developing strategies for repair of the damaged nervous system. In addition to stem cell-based approaches, we plan to screen compounds and nano-based therapeutics that may have neuroprotective and neuroregenerative benefits for nervous system repair.

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