OLFACTORY STEM CELLS: A UNIQUE AND PROMISING SOURCE
FOR TREATING SCI AND PARKINSON’S DISEASE

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Abstract

RhinoCyte™ Inc. has developed a breakthrough adult autologous stem cell technology that
repairs damage resulting from spinal cord injury and also shows great promise for treating
Parkinson’s and other neurodegenerative diseases. The technology is straightforward: Cells are
cultured from the olfactory regions of the nasal passageways via outpatient surgery to allow the
isolation of progenitors, which after growth in the laboratory can be transplanted where
necessary such as, for example, into the injury site.

The RhinoCyte™ technology is differentiated from the competition in several critical ways. As an
autologous cell source — that is, the patient is both the donor and the recipient — olfactory
stem cells avoid the time patients must wait while suitable donors are found. They also
eliminate the need for immunosuppressive drugs, which have numerous side effects. The
olfactory stem cells remain viable, regardless of the patient’s age or sex, and can be stored
indefinitely for future use. Additionally, they are less likely to develop into tumors than other
stem cell sources when used for neurodegenerative conditions.

Just as importantly, stem cells taken from the nose of an adult do away with any ethical
concerns that have hampered the development of human embryonic stem cells.

A PROMISING, NON-CONTROVERSIAL STEM CELL SOURCE

Stem cells represent one of the most exciting discoveries of our lifetime. The fact that these cells
have the potential to become any type of tissue under the right conditions presents an
unlimited number of scientific and medical applications.

RhinoCyte™, based in Louisville, Kentucky (USA), has developed a breakthrough adult
autologous stem cell technology that repairs damage resulting from spinal cord injury (SCI) and
also shows great promise for treating Parkinson’s and other neurodegenerative diseases. The
technology is straightforward: Cells are cultured from the olfactory regions of the nasal
passageways via outpatient surgery to allow the isolation of progenitors, which after growth in
the laboratory can be transplanted into the injury site.

The results of animal testing have demonstrated anatomical regeneration and functional
recovery.
Why Olfactory Stem Cells Are Different

The RhinoCyte™ technology is different from the competition in several important ways. As an autologous cell source — that is, the patient is both the donor and the recipient — olfactory stem cells avoid the time a patient must wait while a suitable donor is found, which can be critical to the outcome of the patient’s treatment. RhinoCyte’s™ therapeutic approach begins when the patient has no spontaneous improvement for several months.

Being an autologous source also eliminates the need for immunosuppressive drugs, which have numerous negative side effects. The olfactory stem cells also remain viable, regardless of the patient’s age or sex, and they are less likely to develop into tumors when used for neurodegenerative conditions than other proposed sources.

Just as importantly, stem cells taken from the nose of an adult do away with the ethical concerns that have hampered the development of human embryonic stem cells.

Following successful entry into the SCI market, RhinoCyte™ will expand commercialization efforts to Parkinson’s disease. Additional “proof of concept” studies have been completed that demonstrate the feasibility of this technology to potentially treat amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases, as well.

Proof of Concept
Two recent peer-reviewed studies reveal the promise and potential of the RhinoCyte™ therapy.

The first, entitled “Human Olfactory-Derived Neural Progenitors Diminish Locomotory Deficits Following Spinal Cord Contusion Injury,” was published in the past issue of the Journal of Neurodegeneration and Regeneration. It detailed promising results on the effectiveness of olfactory stem cells in repairing spinal cord damage resulting from the most common cause of these injuries — contusions (bruising) due to major trauma such as that seen in auto accidents, falls or combat.

This could have major implication for the estimated 5 million people worldwide affected by spinal cord injuries — 40,000 of them in the UK. In fact, according to the Spinal Injuries Association, every day in Great Britain three people are permanently paralyzed by damage to their spinal cord, most of them young people between 21 and 30 years old. The cost for their treatment is estimated at more than £500 million (US $807 million) each year, according to the SCI support group Aspire. In the United States, 1.275 million have spinal cord injuries and the cost of treatment exceeds £25 billion (US $40.5 billion) each year [1,2].

Current treatment options are limited to retaining and retraining mobility; no drug therapies are available, but studies pertaining to stem cell treatments are showing great promise for these as well as other neurodegenerative conditions.

A previous study by the RhinoCyte™ team made national headlines when lab rats whose spinal cords had been partially cut in the region of the animal’s neck in a way that disabled their front right paws were able to regain significant use of their paws after being injected with olfactory stem cells.
The investigative team took the cells from the olfactory neurosensory epithelium — the part of the nose that initiates the sense of smell — in adult volunteer donors who were already undergoing elective sinus surgery. The removal of the stem cells had no effect on the patients’ ability to smell. Also, the minimally invasive surgeries were frequently performed on an outpatient basis so the cells were readily available and, as such, were a potentially promising source of therapeutic stem cells.

The researchers isolated the stem cells and increased their numbers in the laboratory by growing them in an enriched solution. The cells were then injected into a group of lab rats. Twelve weeks later, these animals had regained control of their affected paws while a control group that received no cells had not.

This latest study continued the original work by concentrating on contusions caused by blunt force trauma such as that resulting from an automobile accident or a fall. Spinal cord and head trauma are common among soldiers suffering serious combat injuries, too.

Two independent sets of experiments were conducted, beginning two weeks after the rats had received contusions administered in a computer-controlled surgery. In the first group, 27 out of 41 rats were injected with olfactory stem cells, while the remainder received none. In the second group, 16 rats were treated with olfactory stem cells, 11 received no treatment and 10 received stem cells grown from human skin to see how the olfactory cells compared with another stem cell source.

The results once again showed great promise, with 40 percent of the rats treated with the olfactory-derived stem cells showing significant improvement, which began after just six weeks, compared to 10 percent of those treated with human skin-derived cells and only 9 percent of those receiving no treatment at the conclusion of the study. In addition, the olfactory stem cell-treated rats showing the highest rate of improvement recovered much faster than the other groups and had greater levels of improvement.

The RhinoCyte™ team followed that work with a study, “Adult Human Olfactory Epithelial-Derived Progenitors: A Potential Autologous Source for Cell-Based Treatment for Parkinson’s Disease,” that shows how olfactory-derived stem cells can be used to treat Parkinson’s disease. It will be published in the June issue of the journal STEM CELLS Translational Medicine (SCTM).

Parkinson's disease is a progressive neurological disorder that results in tremors and difficulty with movement and coordination. It occurs when the nerve cells in the brain that produce dopamine, a chemical that helps control muscle movement, are slowly destroyed. Without dopamine, the cells cannot properly send messages, which leads to the loss of muscle function.

Mostly afflicting people over the age of 50, Parkinson’s is one of the most common nervous system disorders of the elderly. One person in every 500 has the disease, according to Parkinson’s UK which, in the UK, comes to about 127,000 people. The Danish Parkinson Foundation estimates it costs the UK an estimated £509 million to £3.7 billion (US $823 million to US $6 billion) each year. In the United States, these numbers increase to 1 million people affected and an economic impact also reaching £3.7 billion (US $6 billion) annually for drug therapy alone. Associated costs such as rehabilitation and home care can exceed £93,000 (US $150,000) per patient per year in the most severe cases[4].
Therapeutic options treat the symptoms associated with Parkinson’s disease, but do not stop the progression or cure the disease. Current medications are directed at increasing the bioavailability of dopamine.

Previous studies using human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSC) on rat models with Parkinson’s provided proof of concept that cell replacement is a viable strategy for treating the disease. Stem cells have potential for cell-replacement therapy for Parkinson’s due to their capacity for self-renewal and ability to differentiate into other cell types.[5]

However, finding a safe source of human cells is essential before beginning clinical trials. While both hESCs and hiPSCs showed promise in treating Parkinson’s, they also had significant drawbacks. The hESCs relieved the symptoms in the rat model, but they frequently resulted in teratomas (a type of tumor containing tissues from three germ layers). Transplantation of hiPSCs also diminished the Parkinsonian behavioral deficits in rodent models; however, like hESCs, in many cases — 50 percent or more — they, too, generated teratomas within 6 to 9 weeks [6,7,8].

What is needed, then, is a cell source to replace lost or damaged dopaminergic neurons that is stable, will not form tumors and eliminates the ethical concerns associated with embryonic tissues. The RhinoCyte™ therapy using human olfactory nasal progenitors (hONPs) is a viable candidate.

Previous studies demonstrated that hONPs can differentiate into neurons in response to their local environment [9,10]. They also act as biological mini-pumps, releasing neurotrophins — the proteins responsible for the growth and survival of developing neurons and the maintenance of mature ones — that create a permissive regenerative environment [11] and potentially arrest the progressive nature of the disease and facilitate improvement without the drawbacks of hESCs and hiPSCs.

In the study outlined in SCTM, four groups of animals (with a total of more than 140 animals) were evaluated including a control group, a fibroblast-engrafted group, a group that was injected with genetically modified hONPs and one that was injected with unmodified hONPs. The rats were injected in areas of their forebrains at three different sites to ensure broad and uniform distribution of the cells or medium. These sites were located between the four injection sites of the neurotoxin that destroyed the dopaminergic neurons.

Based on an initial pilot series of studies, the minimal dose that produced a maximal response was determined to be 15,000 cells in a total volume of 6 μl. This concentration was substantially below the widely used levels of other cell types, as hONPs tend to maintain a high level of viability following their engraftment while many other cell types undergo dramatic reductions in number in the host environment.

Twenty-four weeks later, approximately 35 to 40 percent of the rats with Parkinson-like symptoms engrafted with hONPs demonstrated improved behavioral recovery as well as better mobility and coordination. In contrast, the animals receiving no hONPs or a cellular control group showed no significant improvement.
After six months in cryostorage, the hONPs retained their ability to produce and release dopamine, giving them the distinct potential to serve as a stable population for cell therapy for Parkinson’s [12]. To their surprise, the researchers learned that the genetically modified and unmodified hONPs worked equally well.

They also found that hONPs are likely to have a dual role: cellular protection, in addition to replacing the damaged or dead dopamine-producing cells. They also enhanced the existing progenitor cell populations, which speeds up the regeneration process.

As a result of the study, the RhinoCyte™ team determined that hONPs represent a potential ideal population for Parkinson’s cell therapy because of their capacity to survive, produce dopamine and provide a permissive regenerative environment with neurotrophins. Furthermore, hONPs have a significant advantage over other stem cell sources since they can be harvested from the patient’s olfactory epithelium without highly invasive surgery and, thus, represent an autologous cell source that is free of the side effects that result from donor cells.

Future studies will be required to optimize engraftment parameters and to determine if hONPs have a positive action in patients with Parkinson’s disease.

**What’s Next?**
The RhinoCyte™ breakthroughs described here are the culmination of more than 40 years of research experience in neurobiology by Dr. Roisen and his experienced team. The company has completed a comprehensive IND enabling plan for the treatment of spinal cord injuries (SCI) and is now conducting the final pivotal toxicology/biodistribution studies to submit the IND to the FDA for the lead indication. An IND submission for SCI is targeted for the final quarter of this year and for Parkinson’s disease in the final part of 2013. The initiation of the Phase I SCI clinical trial is set to begin early next year, with a Phase I PD clinical trial start date targeted for 2014.

RhinoCyte™ received a USA Orphan Drug Designation (ODD) for the treatment of spinal cord injury in patients with ASIA Impairment grades of A, B, or C in February 2008 and for ALS in December 2009. These ODD designations confer strategic and competitive advantages: seven years of market exclusivity, access to FDA sponsored clinical trial grants and tax credits that can be utilized to offset costs associated with development.

In addition to the clinical development plan for spinal cord injury and Parkinson’s disease, concept studies are under way for amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases.

**About RhinoCyte™**

RhinoCyte™ Inc. (www.rhinocyte.com), located in Louisville, Kentucky (USA), is a biopharmaceutical company focused on developing an innovative stem cell platform. Founded in 2005, the company is engaged in the discovery and development of products to extend and enhance the quality of human life. Dr. Fred Roisen is the chief science officer and co-founder of RhinoCyte™ Inc., and a professor and chair at the University of Louisville School of Medicine’s Department of Anatomical Sciences and Neurobiology. He co-founded RhinoCyte™ with UofL colleagues Dr. Chengliang Lu and Dr. Kathleen Klueber. The original work forming the basis for
the contusion and Parkinson's studies was conducted by Dr. Roisen's group at UofL and has been licensed to RhinoCyte™. RhinoCyte™ currently has three patents for olfactory stem cell treatments approved in the United States, Australia and Israel, with other patents pending.

References:

1. Sahni V, Kessler JA: Stem cell therapies for spinal cord injury. NAT REV NEUROL 2010; 6(7); 363-372.