

The weakness of stem cells



Contamination may be the
cause of poor results in
treating Parkinson's disease

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During the last three decades, cell therapy has become a source of enthusiasm and deception for patients suffering from Parkinson's disease, an illness characterized by the progressive death of the neurons responsible for the production of an important chemical substance, the neurotransmitter dopamine. In the 1980s, a controversial approach to treating the disease, i.e., the transplanting of cells extracted from the adrenal gland or immature brain tissue of aborted fetuses, initially seemed promising and was tested on animals and humans in countries such as Sweden, the United States and Mexico. The rationale underlying these surgeries, which were debatable even from an ethical perspective, was to supply the brain structure known as black substance (which is damaged in Parkinson's disease patients due to the progressive loss of dopaminergic neurons) with a new population of cells capable of producing dopamine. In this manner, the main symptoms of Parkinson's disease, including tremors, muscular rigidity, slowness of movements and speaking and writing difficulties, should have been eliminated. The results of this approach were disappointing. In cases that showed an improvement in the well-being of the patients, this change was short-lived. In others, no improvement occurred, and the attempted treatment worsened the disease in certain cases, leading to the death of some individuals.

A group of biologists and neuroscientists from São Paulo may have discovered one of the reasons for the failure of these prior cell treatments for Parkinson's disease and perhaps have determined why the more modern and re-

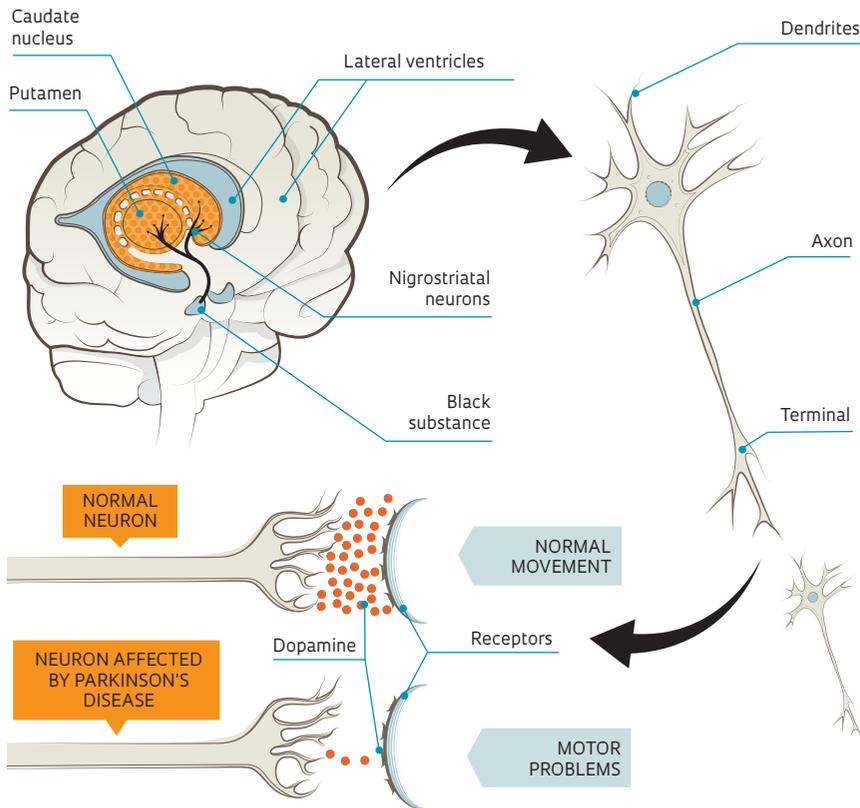


finer versions of this type of experimental treatment, now based on the use of so-called stem cells, continue to yield inconsistent results. The transplants tested in pre-clinical studies in laboratory animals may contain a significant number of fibroblasts, a type of skin cell that is very similar to stem cells but that has completely different properties. On April 19, researchers from the University of São Paulo (USP) and the Federal University of São Paulo (Unifesp) published a study in the on-line issue of *Stem Cell Reviews and Reports* showing that in rats with induced Parkinson's disease, the presence of human fibroblasts cancels out the possible positive effects of implanting mesenchymal stem cells obtained from the umbilical cord tissue of newborns.

"When we administered just stem cells, the symptoms of the disease in the rats improved," says geneticist Mayana Zatz, one of the authors of the article and coordinator of the Human Genome Studies Center at USP, one of the Centers of Research, Innovation and Dissemination (Cepid) funded by FAPESP, and the National Institute of Stem Cell Science and Technology in Human Genetic Diseases. "But when we also injected fibroblasts, the beneficial effects disappeared, and there was even some deterioration. It's possible that many poor results in scientific work with cell therapy are due to this type of contamination." According to the researchers, their work is the first to demonstrate both the positive effects of using mesenchymal stem cells against Parkinson's disease and the harmful effects of contamination by fibroblasts in the same animal model.

Brain circuit in Parkinson's disease

The signals that control body movements are transmitted by neurons that move from the black substance to the caudate nucleus



two approaches, all other procedures against the disease remain in the trial stage and have not been approved by medical authorities.

Dopamine, a chemical messenger produced by less than 0.3% of the nerve cells, belongs to a class of substances called neurotransmitters. The basic function of neurotransmitters is to pass information from one neuron to another in the form of electrical signals, a communication process known as a synapse. Dopamine acts specifically on brain centers linked to the sensations of pleasure and pain and has proven roles in the mechanisms that generate dependence and addiction as well as the control of movement. In the case of Parkinson's disease victims, motor function is clearly affected due to the lack of the neurotransmitter.

Mixing up fibroblasts and mesenchymal stem cells is very easy, and this confusion may be at the heart of the inconclusive or contradictory results of many attempts to treat Parkinson's disease using cell therapy. Fibroblasts and mesenchymal stem cells have the same origin. They are derived from the mesenchyme, the main connective tissue of the embryo, from which various types of cells are formed. Despite their common origin, fibroblasts and mesenchymal stem cells have different properties. Fibroblasts, which are responsible for the synthesis of collagen, form the basis of the connective tissue in adults. Accordingly, fibroblasts are specialized and differentiated cells. Mesenchymal stem cells, however, are very non-differentiated and can generate many types of tissue, such as bone, cartilage, fat, support cells for the formation of blood and connective fibrous tissue. "It's almost impossible to distinguish these two types of cells if we simply examine them under a microscope," commented biochemist Oswaldo Keith Okamoto from the Human Genome Studies Center and principle investigator of the study published in *Stem Cell Reviews and Reports*. "They grow *in vitro* under the same conditions, and we only distinguish them with the help of markers and specific tests." Mesenchymal stem cells also have an important peculiarity: they have immunosuppressive properties and can minimize the need to take medication to avoid the rejection of transplanted organs and tissues.

In addition to presenting an advance in the basic knowledge on the possible benefits of cell therapies in an organ as complex and delicate as the brain, the result of this study presents a warning to the families of people with Parkinson's disease. In no country in the world is there any officially approved treatment based on stem cells for combating this or any other neurodegenerative disease. "Stem cell research needs to be looked at carefully, and false promises of a cure mustn't be made," says the author of the article, neuroscientist Esper Cavalheiro, from Unifesp, who is a lead researcher the work at the National Institute of Translational Neuroscience, a joint project of FAPESP and the Ministry of Science and Technology (MCT). "Before proposing therapy, we need to understand the entire differentiation mechanism of stem cells in the various tissues in the organism and understand what the brain does to 'talk to' and direct the operation of these cells." Currently, the only diseases that have

a stem cell-based treatment are blood diseases, including cancers (e.g., leukemia). When faced with this type of problem, for decades, doctors have resorted to the transplant of bone marrow rich in hematopoietic stem cells, which are the precursors of blood.

Parkinson's disease continues to lack a cure and is currently controlled with the help of medications, such as levodopa, that the brain can convert into dopamine. In more serious cases, a second alternative may be used: the implantation of electrodes in the brain of patients who are not responding well to the treatment or who experience multiple side effects from the drugs. The electrodes, which are connected to a small generator implanted under the skin, are used to attempt to improve the communication between neurons. The delicate surgery for implanting the electrodes is known as deep brain stimulation, or simply DBS. With the exception of these

There is no hard evidence that mesenchymal stem cells can generate the neurons that are lacking or somewhat dysfunctional in Parkinson's disease patients. Nevertheless, mesenchymal stem cells seem to improve the environment in which the lesions associated with the disease occur, reduce local inflammation and favor the preservation of nerve cells. According to Okamoto, "their effects might be indirect, reducing the inflammation in the brain." This effect was observed by researchers from São Paulo in their experiment with rats. They injected stem cells into the brain of a group of ten rodents with induced Parkinson's disease and, one month later, found that the rats had no symptoms of the disease and were as healthy as the animals in the healthy control group. This result coincides with the conclusions of similar studies conducted both here and abroad.

The more novel discovery resulted from the second part of the experiment. The scientists inserted a culture of fibroblasts into another group of ten rats, which also had Parkinson's disease. The result was a disaster. One month after the procedure, the animals started exhibiting additional motor problems, and the number of dopaminergic neurons in the black substance decreased by half. A third group of sick rodents was given a mixture of the two cell types in equal proportions. In this group, no improvement was observed, and it appeared that the fibroblasts cancelled out the anticipated beneficial effects of the stem cells. As stated by Mayana, "the fibroblasts seem to be neurotoxic."

In India, a group of doctors and scientists from the BGS-Global Hospital in Bangalore is testing the use of mesenchymal stem cells in seven human patients aged 22 to 62 who are suffering from Parkinson's disease. Mesenchymal stem cells obtained from the bone marrow of the patients themselves were injected into the damaged brain in accordance with a local protocol designed by the Indian researchers. In an article published in February of last year in *Translational Research*, the researchers reported a reduction in the symptoms of the disease in three of the seven patients and said that the approach appeared safe. The results, however, remain preliminary and must be viewed with reservation. "Mes-

enchymal stem cell transplants may not become a definitive treatment for Parkinson's, but perhaps a complementary one, such as neuroprotection," Okamoto considers. "This type of study can help us understand how to mitigate the degenerative environment in the brain and maybe how to create new drugs against the disease; who knows?"

GENES, ENVIRONMENT AND MYSTERY
Although there are cases of young individuals with Parkinson's disease, such

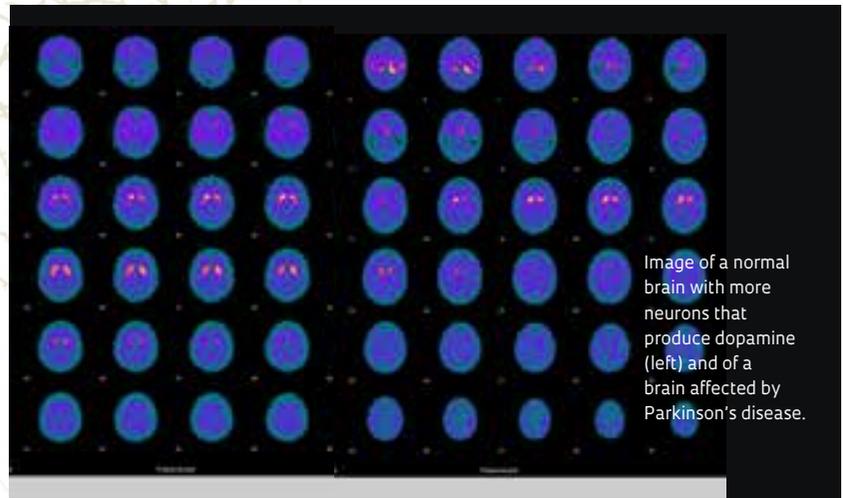


Image of a normal brain with more neurons that produce dopamine (left) and of a brain affected by Parkinson's disease.

Injection of fibroblasts led to a deterioration in the symptoms of the disease in rats with Parkinson's and cancelled out the benefits of the stem cells

as the famous Canadian actor, Michael J. Fox, who was diagnosed at the age of 30, this neurological disorder appears more often in people older than 50 or 60 years of age. "Patients under 50 are seen as precocious and represent some 20% of the total," says the neurologist Luiz Augusto Franco de Andrade from the Teaching and Research Institute at the Albert Einstein Hospital in São Paulo. "But I've already treated a boy of 13 who had Parkinson's."

There is growing evidence that environmental and genetic factors may be implicated in the appearance of the disease, at least in certain cases. A study by Harvard Medical School researchers, published in October of last year in *Science Translational Medicine*, showed that hundreds of genes linked to the function of the mitochondria, organelles that are the organism's power plant, are less active in Parkinson's disease patients. Even people who are in the initial or pre-Parkinson's disease stage appear to have these changes. If this connection between the mitochondria and the disease is confirmed, drugs that act on these genes may become useful in treating the problem.

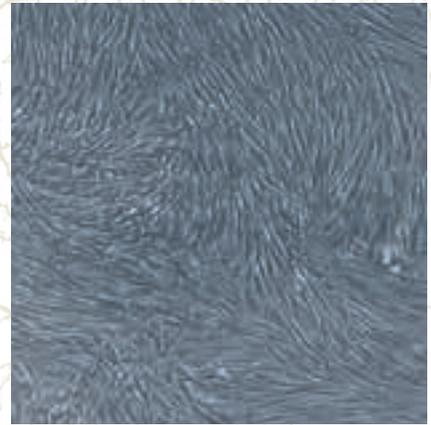
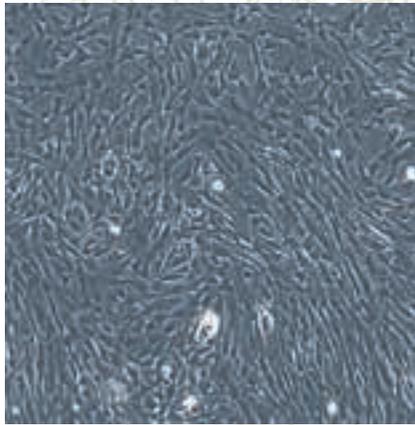
TWICE AS MANY PATIENTS BY 2030
Along a similar line of investigation, a study published in September 2010 by the National Institutes of Health (NIH) of the United States suggested that individuals with a certain version of the GRIN2A gene might benefit from the consumption of coffee and tea. In a person with this genetic profile, the intake of beverages containing caffeine might act as a protective factor against Parkin-

son's disease. The search for substances that help to maintain neurons is a strategy adopted by many research groups. The administration of the GDNF protein, which acts in this manner, has been the target of long-term tests to examine its possible action against the disease.

Despite localized advances in our understanding of the possible mechanisms implicated in its genesis, Parkinson's disease continues to maintain its general status as a neurodegenerative disease with a cause that is both mysterious and unexplained. No one knows for certain why the neurons that produce dopamine begin to die or stop functioning correctly at a particular point in the life of certain people. The only concrete knowledge is a palpable fact of the current reality: an ageing population is a great risk factor for Parkinson's disease. This issue is of particular concern for developing nations, which have an age structure that is rapidly changing before they become more wealthy.

Currently viewed as a nation of young people, Brazil's demographic profile will change dramatically over the next few decades. A report from the World Bank published last month indicates that its population of persons 65 or older will increase from the current 11% to 49% by 2050. Thus, in a period of 40 years, the number of old people will triple, surging from fewer than 20 million to approximately 65 million persons. "The speed of population ageing in Brazil will be significantly greater than that in more developed societies in the last century," say those responsible for the report, *Envelhecendo num Brasil bem mais velho* [Growing old in an older Brazil]. In France, it took more than a century for the population of persons 65 years or older to increase from 7% to 14% of the total population. "Over the last few years, modern gerontology has emphasized the increase of the ageing process more than its physical and mental losses," says anthropologist Guita Grin Debert from the State University of Campinas (Unicamp), who studies issues linked to women and old age. "We have experts in diseases but not many in the ageing process."

A review paper published in January 2007 in the scientific journal *Neurology* analyzed data from 62 different studies and concluded that the number of



Mesenchymal stem cells (left) and fibroblasts: it is difficult to distinguish between these types of cells.

The number of cases of the disease is growing faster in developing countries than in richer nations

cases of Parkinson's disease in people older than 50 should double over the next 20 years in 15 countries throughout the world. This review analyzed statistics from the world's most highly populated nations, including Brazil and the five largest countries in Europe. In 2005, this group of countries had between 4.1 and 4.6 million patients with Parkinson's disease. In 2030, there will be between 8.7 and 9.3 million cases of the disease. During this same period, the number of individuals with Parkinson's disease in Brazil will increase from 160,000 to 340,000. According to this review article, the estimated growth rates of the incidence of Parkinson's disease in developing countries such as China, India and Brazil, whose populations are only now undergoing an ageing process, will be greater than 100%. In developed economies that already have a large number of old people, such as Japan, Germany, Italy and the United Kingdom, the number of people with Parkinson's disease is expected to increase by less than 50%.

Overall, an estimated 1% of the planet's inhabitants older than 65 suffers from Parkinson's disease. However, this figure may vary according to the characteristics of the population analyzed. A study conducted in 2006 in Bambuí, Minas Gerais, found a high incidence of Parkinson's disease, i.e., a rate of greater than 7.2% in a group of 1,186 individuals older than 64. This figure is three to four times higher than that reported in similar studies performed in Europe, Asia and the United States. Approximately half of the cases of the disease in Bambuí have been caused by the uncontrolled use of drugs against psychoses and dizziness. "We currently believe that the

number of cases of the disease arising from the uncontrolled use of drugs has decreased,” says Francisco Cardoso from the Federal University of Minas Gerais (UFMG), coordinator of the study. “Control of the sale of drugs in the country has improved.”

Scientists are not betting on stem cells alone to improve treatments for Parkinson’s disease. There are no prospects of a cure in the short-term. However, researchers hope that preventing the evolution of this neurological disturbance, or at least slowing its progress, will be possible by developing new drugs and more effective and less invasive surgery. “At present, we try to offset the effects of Parkinson’s by administering oral medication,” says Cardoso. “But the way in which we replace dopamine is not good.” For example, when the patient takes the drug levodopa, a precursor of dopamine, his brain comes into contact with high concentrations of the neurotransmitter. As time passes, the amount of the substance decreases. Consequently, the treated patient experiences cycles of excesses and shortages of the neurotransmitter, i.e., a type of chemical see-saw, with dopamine highs and lows.

Several drugs have been developed in an attempt to regulate the moment at which dopamine, produced artificially via the ingestion of levodopa, becomes available for use by the brain of the patient. However, the control of this process requires further refinement, and the imitation of the physiological mechanism is imperfect. The situation becomes even more complicated when the drugs no longer control the Parkinson’s disease symptoms or produce side effects. The prolonged use of dopamine precursors can occasionally causes involuntary and repetitive movements, called dyskinesia, which may cause patients to bite their lips, poke out their tongue or blink rapidly. In these cases, deep brain stimulation (DBS) surgery may be indicated.

Two years ago, the team of the Brazilian neuroscientist Miguel Nicolelis from Duke University (USA) and founder of the Edmond and Lily Safra International Institute of Neurosciences of Natal (IINN-ELS), suggested that electrical stimulation might pro-



Less invasive surgery to improve communication between neurons is another bet against parkinson’s disease

duce good results against Parkinson’s disease without the need for open-brain surgery. In an article that was featured on the cover of the scientific journal *Science* on March 20, 2009, Nicolelis reported a successful experiment on rats and mice with induced Parkinson’s disease. The installation of small electrodes on the surface of the spinal medulla of the animals helped them recover their normal locomotive capacity. According to the report, the procedure for placing the electrodes takes 20 minutes, is not excessively invasive (only the skin of the animal is cut) and is safe. This new approach, which is now being tested on monkeys, was the first attempt at treating Parkinson’s disease without directly acting on the brain.

The prediction of whether new Parkinson’s disease treatments will arise from studies such as those of the teams of Mayana Zatz at USP and Esper Cavalheiro at Unifesp. For the time being, this work and that of other scientists still continues to comprise lines of research that should be explored rather than considered as immediate treatment possibilities. Nonetheless, doctors who care for people with Parkinson’s disease see no cause for pessimism. Patients are living with the disease for an increasingly long time, even up to decades, although the side effects of the medication continue to be an important issue. Nevertheless, the electrodes and batteries used in DBS surgery are becoming smaller and more efficient. “We still don’t know how the neurons ‘talk’ to each other, but today we can record the activity of a larger number of brain cells,” says neurosurgeon Manoel Jacobsen Teixeira, a professor at USP and member of the Institute of Teaching and Research at the Sirio-Libanês Hospital in São Paulo. ■

Scientific articles

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2. VENKATARAMANA, N. K. *et al.* Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson’s disease. **Translational Research**. v. 155 (2), p. 62-70. Feb. 2010.