The firefighter Dariusz Fidyka during rehabilitation

Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences

First Steps, Once Again

WOJCIECH FORTUNA
Ludwik Hirszfeld Institute of Immunology
and Experimental Therapy
Polish Academy of Sciences, Wrocław

We talk to Dr. Wojciech Fortuna about a successful operation carried out in 2012 to regenerate a severed spinal column using transplanted cells, a collaborative effort by numerous Polish neurosurgeons and scientists in conjunction with a British team

Academia: The news of your success was reported around the world, provoking enthusiastic reactions.

***Wojciech Fortuna**: Unfortunately not from everyone, although honestly we did not expect that. A letter was recently published in the "Journal of Neurotrauma" in which a certain well-known neurosurgeon from Florida writes very skeptically and critically about our results. My colleagues and I have decided, however, that the best way to respond would be with hard data and research articles. We do not want to get caught up in polemic debates, because no diagnosis can be made over the Internet, without first-hand contact with a patient. In the media portrayal, or even in our published papers, one cannot really see all the work, research, and preparation that went into this successful operation.*

What in your view was the greatest success of the Wrocław-based team of scientists and doctors?

We consider the greatest achievement of the team, led by Dr. Paweł Tabakow and Prof. Włodzimierz Jarmundowicz from the Neurosurgery Clinic at Wrocław Medical University, to be the functional regeneration of continuity in a spinal cord that had been severed by a sharp tool. In functional

terms Dariusz Fidyka's spinal cord was completely severed – although a narrow fragment of tissue remained on the right side, electrophysiological tests showed that it did not convey any nerve signals.
The improvement that has been observed in the patient is not, in our view, a spontaneous regeneration, but the outcome of the neurosurgical intervention and the subsequent very intensive rehabilitation. Unlike other patients who had been operated on by our team in 2008-2010, in Mr. Fidyka's case we transplanted cells from his olfactory bulb. We isolated a suspension of olfactory ensheathing cells (OECs) together with fibroblasts in their natural micro-environment. Also crucial for the success of the operation was the use of technique for "bridging" the severed spine using fragments of a peripheral nerve. In so doing, we were applying in practice the pathway theory developed by the British Prof. Geoffrey Raisman. We therefore achieved a successful application of results achieved in basic research, and on small animals, to a human patient.

Can you tell us something more about Prof. Raisman's work?

In the 1980s and 1990s, Prof. Raisman developed a model of spinal injury in rats. He discovered that it was possible to provoke neuroregeneration of damaged axons within the spinal cord under the beneficial influence of OECs, which enable the axons to pass through the unfriendly, astrocyte-containing environment of the glial scar. In our approach, aside from OECs, we also implanted peripheral nerves – this represents our own original contribution and an enhancement of Prof. Raisman's predictions. This enabled the patient to regain the ability to sense various types of stimuli from areas below the level of injury. Moreover he has shown increased muscle mass in the lower limbs, the ability to willfully produce muscle contractions, and also some of the functions of the automatic nervous system. The most innovative part of the operation, however, was the transplantation of OECs with fibroblasts from the olfactory bulb together with peripheral nerves, which had never been reported in the literature.

Should we understand the essence of the procedure as follows: although the neurons could potentially regenerate, they encounter unfavorable conditions for that at the place where the spinal column has been severed, so that is why glial cells need to be transplanted there?

Precisely so. Olfactory ensheathing cells, together with other cells from their environment, such as fibroblasts, participate in providing the right support for severed, damaged axons. That is the physiological role they play in the case of the olfactory nerve.
In some patients, especially those with partial spinal damage, spontaneous regeneration may occur. But for complete spinal cord damage, such a phenomenon is extremely rare. We considered whether such an improvement might have occurred in the case of our patient, but despite prolonged intensive rehabilitation efforts there had been no sign of it prior to the operation. Indeed, there was also no sign of it immediately thereafter – the improvement only began several months after our intervention.

Why is the location where the cells are taken from so important?

Our concept involved transplanting the patient's own cells. This avoids the phenomenon of rejection and the need to apply immunosuppressive drugs, as in the case of allogenic transplants (those taken from other individuals). Based on our own pre-clinical tests on animals and on experimental results on using OECs in people, we had grounds to believe that such cells would not provoke an unfavorable immunological response when transplanted into the spinal column.
Although most teams performing similar operations use OECs isolated from the nasal mucosa, we instead removed one of the patient's olfactory bulbs from the base of the brain. This route of access is used in classical procedures, but we used it for the first time for the purpose of obtaining the olfactory bulb to be used in transplantation.
Such an intervention involves enhanced risk of post-operational complications and is more complex than the alternative method – endoscopic collection of segments of the olfactory mucosa from the nasal cavity, from which OECs cells can also be isolated. Despite that, we decided to collect cells from the olfactory bulb rather than the olfactory mucosa because OECs from different locations differ in terms of their regeneration-enhancing properties. Those from the olfactory bulb simply perform much better in this regard.

Regeneration of the spinal cord

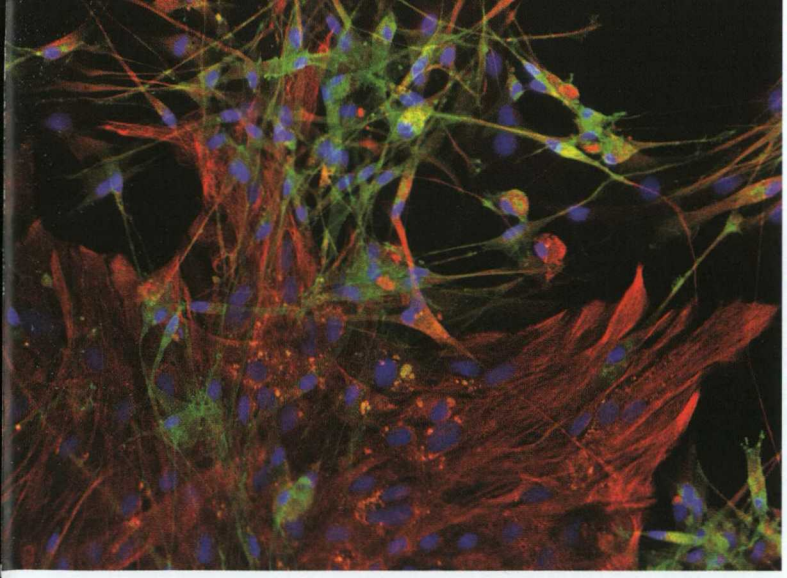
What contribution was made by the team from the PAS Institute of Immunology?
Like in the case of three previous patients, our task was to isolate the cells to be transplanted from the material obtained in the operation. In the previous cases this was from the olfactory mucosa, but for the fourth patient it was from a whole olfactory bulb. We were involved in collecting the material, and then in our laboratory we isolated cells in a several-stage procedure. Next, after 14 days in culture, we prepared a cellular suspension that we transported to the operating room, where by means of an infusion pump connected to a micro-manipulator, under our supervision, those cells were applied to the patient's spinal column at specific locations.

How long do they take to produce in culture?
Two to three weeks. In the case of our most recent patient, it was two weeks, from 16 to 28 April 2012.

Depending on which media source your achievement was reported in, the role played by Poles was correspondingly showcased or downplayed. The Western press, in particular, often merely mentioned the Wrocław team. What was the collaborative effort like in reality?
One might have expected the British press to hail this as a British success. But I will say that we did not end up bickering with one another following the BBC broadcast; rather, we are still working together and are currently talking about preparations for operating on further patients. That attempt to divvy up the credit between Polish and British contributors was completely unnecessary. Prof. Raisman spent a long time looking for collaborators and took an interest in our work. Before we got to know him, we ourselves had learned – based on the available publications – how to cultivate olfactory ensheathing cells and identify them, in the case of both patient-derived cells and those taken from cadaver organ donors. Unlike the professor, we did not devote all our energy and funding into testing spinal damage on an animal model. We decided that in this respect we would rely upon our foreign partner's experience. We took a different tack, and focused on translating that knowledge into the practical, clinical domain.

Timeline of work leading up to the operation:

2000 Dr. Paweł Tabakow begins collaborating with Prof. Włodzimierz Jarmundowicz ■ **2002** Neuroregeneration research team established at Neurosurgery Clinic ■ **2003** Collaboration begins with Hirszfeld Institute of Immunology and Experimental Therapy (PAS) in Wrocław, studying the properties of olfactory ensheathing cells (OECs) *in vitro* and *in vivo*, and with the "Akson" Neuro-Rehabilitation Center in Wrocław ■ **2004** First article on the possible use of OECs to treat spinal cord damage in humans is published in the journal *Neurologia i Neurochirurgia Polska* ■ **2004-2008** A KBN grant to study the use of OECs in treating completely damaged spinal cords in humans is received by a Polish team from the Neurosurgery Clinic and the Hirszfeld Institute led by Prof. Jarmundowicz ■ **2004-2008** Recruitment of patents with completely damaged spinal cords to receive transplants of OECs isolated from the nasal mucosa (1st stage clinical research) ■ **2004** Collaboration initiated with Prof. Juliusz Huber from the Motor Organ Pathophysiology Department of the Wiktor Dega Orthopedic-Rehabilitation Hospital (Poznań University of Medical Sciences) on electrophysiologically evaluating patients qualified for participation in the experiment ■ **2005** Dr. Paweł Tabakow and Dr. Ryszard Międzybrodzki meet Prof. Geoffrey Raisman for the first time at the "First International Spinal Cord Injury Treatment and Trials Symposium" in Hong Kong. Patent request filed for original method of isolating OECs from deceased organ donors and their utilization (patent awarded in 2012) ■ **2006** Article published in the journal *Glia* on obtaining OECs from organ donors. Prof. Goeffrey Raisman pays first visit to Wrocław ■ **2007** Ball held at Wrocław Opera House by the Old Fellows fraternal organization in Wrocław, raising 127,000 PLN for further research ■ **2008** First two procedures transplanting a mixed culture of OECs and fibroblasts isolated from the nasal mucosa into patients with completely severed spinal cord continuity are carried out at the Neurosurgery Clinic of the University Clinical Hospital in Wrocław ■ **2010** Prof. Raisman observes another transplant procedure ■ **Autumn 2011 – April 2012** Preoperative rehabilitation of a fourth patient with completely damaged spinal cord, developed by Stefan Okurowski at the "Akson" facility ■ **2012** The University Bioethics Committee approves the removal of the olfactory bulb instead of the nasal mucosa and the use of peripheral nerves to form spinal cord bridges. Dr. Paweł Tabakow and Dr. Marcin Czyż remove an olfactory bulb from the patient ■ **16-28 April 2012** At a laboratory at the Hirszfeld Institute, Dr. Wojciech Fortuna and Dr. Daqing Li from UCL isolate and grow OECs from the olfactory bulb ■ **28 April 2012** Dr. Tabakow, Prof. Jarmundowicz, and Dr. Bogdan Czapiga with the assistance of Dr. Dariusz Szarko perform a transplantation of a mixed culture of OECs and fibroblasts prepared by Dr. Fortuna, Dr. Międzybrodzki and Dr. Li. During the operation



Growing human olfactory ensheathing cells from the olfactory bulb

Prof. Raisman devoted his entire professional life to neurobiology and experimentally demonstrated the neuroplasticity of the brain. He has vast experience in basic research and animal testing. But our part in this success – the contribution made by various researchers from Poland, from different centers, not just from the Polish Academy of Sciences – was to create the conditions necessary to perform a cell transplant in a patient with a severed spinal cord. We had to think about what kind of damage we would study, find the right patient, plan out his therapy and rehabilitation, work out how we would evaluate his condition and possible improvement, and how we would carry out the operational procedure itself. We did all of this ourselves, drawing upon the reports from a team in Australia one year previously. This was compounded by many technical issues: how to implant the cells, at what rate, using what kind of needles, etc. We were helped greatly here ("we" meaning myself and my colleague from the Institute, Dr. Ryszard Międzybrodzki) by experience working with animal models. Our knowledge, combined with the experience of the neurosurgeons, each of whom contributed something of their own, came together into an excellent collaborative effort. Considering the innovative solutions utilized during the operation itself, and during the neuro-rehabilitation, I would estimate that our contribution accounted for about 60%, with the remaining 40% being due to Prof. Raisman's team. But that does not change the fact that it was Prof. Raisman's work that inspired this effort in the first place. He had the idea, he argued that neuroregeneration was possible in an adult human, and we proved capable of making that idea into a reality.

Basing ourselves critically on Prof. Raisman's theory, we considered which cells would be the best to transplant. First we worked using OECs

they create a peripheral nerve bridge across the site of spinal cord damage. Observing the procedure are Prof. Raisman and Dr. Li ■ **May 2012** Postoperative neurorehabilitation begun for the fourth patient at the "Akson" facility ■ **October 2012** Patient's neurological condition improves. On the ASIA scale, which measures sensation, muscle strength, and control over sphincter muscles, the patient's condition turns from the preoperative category A to category B. Category A means complete damage of the spinal cord, i.e. with no motor or sensory function below the level of injury, including in segments S4-S5, whereas category B stands for incomplete damage (i.e. no motor function below the level of injury but with sensory function preserved (this also applying to segments S4-S5) ■ **2013** Prof. Raisman organizes financial support to cover the costs of postoperative rehabilitation for the fourth patient from the Nicholls Spinal Injury Foundation ■ **2013-2014** Dr. Fortuna and Dr. Daqing Li work at a UCL lab on standardizing the conditions for OEC culture ■ **March 2013** Further improvement in patient's neurological condition, from category B to C on the ASIA scale (incomplete damage, motor function preserved below the level of injury and more than half of key muscle functions below the level of injury having a Lovette muscle grade of less than 3) ■ **April 2013** Collaborative ties established with Prof. Kazimierz Gąsiorowski from the Department of Basic Medical Sciences at the Department of Basic Medical Sciences at Wrocław Medical University. Funding of 940,000 PLN secured from the National Science Centre under a Harmony grant for international research on applying OECs in treating complete spinal cord injuries in humans (project led by Dr. Fortuna) ■ **April 2013** Paper published in the journal "Cell Transplantation" presenting the completed first-stage research results for patients transplanted with OECs from the nasal mucosa ■ **July 2013** The *British Journal of Neurosugery* publishes an article on the technical aspects of operationally accessing the olfactory bulb ■ **December 2013** The journal *Cell Transplantation* carries a key paper, explaining the advantage of using OECs from the olfactory bulb over those from the nasal mucosa in achieving functional neuroregeneration in a severed rat nerve root model. ■ **September 2014** Dr. Bogdan Czapiga earns a DSc degree (*habilitation*) for research evaluating the safety of applying OECs to the spinal cord in an animal model ■ **21 October 2014** BBC One broadcasts the documentary "To Walk Again" ■ **21 October 2014** The journal *Cell Transplantation* publishes a paper presenting the results of the experimental therapy for the fourth patient, who received a transplant of a mixed culture of OECs and fibroblasts from the olfactory bulb and a peripheral nerve bridge of the spinal cord, in conjunction with intensive rehabilitation ■ **22 October 2014** Announcement made of planned start in **2015** of selecting two more patients with completely severed spinal cords. Their procedures will aim to repeat the observed clinical effect in the fourth patient now in rehabilitation.

from the nasal mucosa, but later we decided to switch to material from the olfactory bulb. That, as I said, represents our own original achievement. On top of that, we came up with the idea of bridging the spinal cord using peripheral nerve fragments. Here we followed a previously known method, used in peripheral nerve surgery.

In other words, to sum things up, we could say that this proved to be an ideal combination of theory and practice, with practice accounting for a larger share...

I think that is precisely it. Prof. Raisman is a scientist, one with huge experience, but his achievements are limited to what can be seen under an electron microscope and what can be done using animal models. We took things a step further and began to move towards clinical applications before Prof. Raisman came to visit our operating theater at the University Clinical Hospital in Wrocław. But we should also stress that there was a whole team working on the Polish side – apart from the neurosurgeons, there were more than a dozen people involved from various institutions, honing their skills over years. The success therefore brought together the work of teams from the Institute of Immunology, the Neurosurgery Clinic, the "Akson" Rehabilitation Center operating under a foundation devoted to individuals with spinal illnesses, and also many specialists from the various clinics of the University Clinical Hospital, including radiologists and psychologists. When Prof. Raisman saw our determination and the collaboration among all these centres, he admitted that he had never before witnessed such a thing anywhere in the world and would be honored to collaborate with us.

So what are the prospects for the future, in the wake of such a great success?

One important issue is funding for research and operations. The cost of therapy for the previous patients was covered by the Neurosurgery Clinic of Wrocław Medical University, using its own funds and from an R&D grant, awarded by what was then known as the Polish Committee for Scientific Research (KBN). We also gathered funding thanks to assistance from various foundations. Mr. Dariusz Fidyka was operated on after the grant was finished, and so we tapped into the Neurosurgery Clinic's statutory research funding. We also had the support of Polish foundations, and over the past two years Mr. Fidyka's rehabilitation has been sponsored by a British foundation.

We are currently entering the next stage – we have published the results of the operation and we are observing the results of rehabilitation, the gradual improvement in the patient's condition. We are also intensively preparing to take on two more patients. Fortunately, so far we have been able to afford the same therapy in their case as Mr. Fidyka received. Our team has also obtained an international Harmony grant from the National Science Centre, to fund our continued cooperation with the London-based researchers.

We would also very much like to get the government interested in our results, so that we can carry out further research and treat more patients. We need guarantees against something potentially going wrong – there is always a risk of complications, cancer, etc.

You have all definitely chalked up a great scientific success, but this was also a great achievement for the patient himself. The improvement the operation has made in his life, it seems, is like a huge reward for you?

I think so. The patient would definitely tell you that himself, if you asked him. ■

Inteview by **Agnieszka Kloch**

For more information, see the online version of the article at: www.naukaonline.pl.

The team of Polish researchers taking part in the operation:

Dr. Paweł Tabakow, Department and Clinic of Neurosurgery, Wrocław Medical Univeristy (WMU) ■ **Dr. Wojciech Fortuna,** Department and Clinic of Neurosurgery, WUM, and Bacteriophage Laboratory, Hirszfeld Institute ■ **Dr. Marcin Czyż,** Department and Clinic of Neurosurgery, WUM ■ **Dr. Juliusz Huber,** Department of Pathophysiology of Locomotor Organs, Poznań University of Medical Sciences (PUMS) ■ **Dr. Paweł Szewczyk,** Department of General Radiology, Interventional Radiology, and Neuroradiology, WUM ■ **Stefan Okurowski,** MS, "Akson" Rehabilitation Center ■ **Dr. Ryszard Międzybrodzki,** Bacteriophage Laboratory, Hirszfeld Institute, and Department of Clinical Immunology, WUM ■ **Bogdan Czapiga,** Department and Clinic of Neurosurgery, WUM ■ **Beata Salomon,** University Clinical Hospital in Wrocław ■ **Agnieszka Halon,** Department of Pathomorphology and Oncological Cytology, WUM ■ **Joanna Lipiec,** Department of Pathophysiology of Locomotor Organs, PUMS ■ **Aleksandra Kulczyk,** Department of Pathophysiology of Locomotor Organs, PUMS ■ **Prof. Włodzimierz Jarmundowicz,** Department and Clinic of Neurosurgery, WUM