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Neurons go to great lengths for new therapy

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Stress might actually be good for the nerves. In fact, it has been shown to induce growth in axons by at least 1 cm according to researchers at the University of Pennsylvania Medical Center (UPMC; Philadelphia, PA, USA). Douglas Smith and colleagues have found that applying precise levels of mechanical tension to neurons resulted in enhanced growth across a border between two membranes[1]. This is the first demonstration of stretch-induced growth, which could be exploited to produce materials for neuronal transplantation to treat extensive nerve damage.

The search for a treatment for severe nerve damage such as spinal cord injury (SCI) has attracted intensive research efforts, which have focused primarily on creating a bridge of nerve tissue that can span the injured portion of the spinal cord. However, current transplant methods consist of a 'nerve paste', described by Douglas Smith of UPMC as 'a mushed-up group of dissociated neurons that might include other constituents such as glial cells, which is injected into the area where an axon repair is to be done'. Although these approaches have been shown to have limited efficacy in animal models, it has not been proved that these transplants actually recreate the original three-dimensional (3D) neuron network. Rather, the efficacy has been suggested to be a result of either the release of growth factors from the graft or merely creating a physical support between viable neurons.

Nerve fibre growth

Smith explains the idea behind this latest research: 'We had the idea to recreate the basic structure of the anatomy of the spinal cord. What we've done is to make what we call ''jumper cables'' essentially, where we can grow axons to at least 1 cm while creating the 3D structure of the nerve.'

Smith is surprised that this research has not been done before, 'There are two forms of nerve fibre growth, and yet there is only one form that has been studied until now. Axonal guidance, in which neurons extrude a process to connect with other neurons, has been extensively studied resulting in thousands of publications.' He continues: 'There is another form of growth, however, that begins in the embryo and continues throughout development, in which nerve fibres connect to form large white-matter tracts consisting of many nerve fibres that are differentiated and thus cannot grow from the end of the axon. This means that they must be able to grow from the middle of the axon, and this has never been examined before.'

Douglas believes that the understanding of many diseases might be helped by this approach. An example of this is a rare genetic disease in children called Canavan's disease, in which large areas of white matter degenerate with age. It is not known precisely why the growth of these white-matter tracts cannot be maintained at the same rate as the child's growth.

Neuronal transplantation is often hindered by the body's inhibition of neuron growth. This is part of the body's innate mechanism to control the amount of nerve growth after the nervous system has developed. Therefore, a treatment strategy that relies upon the *in vivo* growth from the end of an axon will have limited success because it will first have to encourage neuron growth by the use of chemoattractants, but will then have to overcome growth inhibition by an array of growth modulating chemicals. Smith points out that by inducing growth in an *ex vivo* environment, there is no inhibition by these factors. He adds: 'We have more control because we can grow cells to specific sizes for the transplant and we don't have to worry about chemoattractants or inhibitory chemicals.'

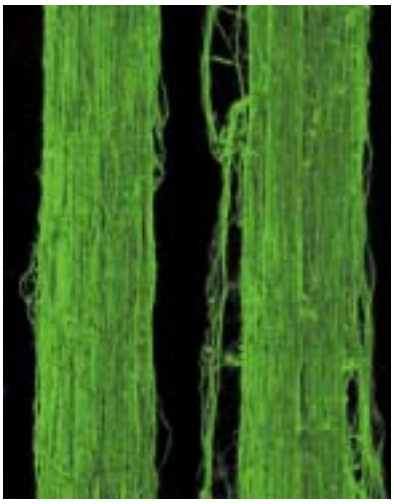

Figure 1. Fluorescence confocal photomicrographs of two fascicular axon tracts (each ~50 μm wide) produced by stretch-induced growth *in vitro*. Each of these tracts contain thousands of axons that were elongated by a remarkable 1 cm via continuous mechanical tension over 10 days. This technique could be exploited to produce transplant materials to bridge extensive nerve damage.

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Stretching neurons

Smith and colleagues calculated how fast axons could grow and used these parameters to find the limits of neuron growth and the optimum speed of growth for viability. Neurons were plated onto adjacent membranes and allowed to integrate across a 50 μm border between the membranes. The neurons were then progressively stretched by using a microstepper motor system to separate the two membranes at a rate of 3.5 μm per 5 min. The neurons not only stretched to at least 1 cm in length after 10 days, but also formed bundles comprised of thousands of axons, thus generating a nerve fibre structure similar to that found *in vivo* (Fig. 1). This represents an insight into the mechanism of growth of white-matter tracts during development.

Smith says this was an unexpected finding: 'The first thing I had in my mind was that we would have a long single cell, but instead, they kept grouping more and more together, coalescing into larger and larger bundles that you can actually see with the naked eye, about the size of a human hair.' Smith thinks that this is part of what occurs in development: first, you have a group of neurons signaling to each other and as they grow, they cluster together to form bundles. This was an important goal for Smith and colleagues: to accomplish not only length, but number as well because, as with many cell types, it can be speculated that strength in numbers can afford growth advantages to the cells.

Future studies

Smith and colleagues now intend to study the mechanisms of stretch-induced growth and will be testing their stretched-axon technology in animal models to show that these neuron grafts are connecting with the host tissue with a complete connection; this could be demonstrated by flowing a dye across the graft region and showing that an electrical current is able to traverse the graft. The ultimate aim is to develop successful transplant therapies for neurodegenerative diseases such as Parkinson's disease, CNS injury and optic nerve damage, and also to expand their studies to include peripheral nerve repair.

Reference

1 Smith, D.H. *et al.* (2001) A new strategy to produce sustained growth of central nervous system axons: continuous mechanical tension. *Tissue Eng.* 7, 131–139

The end for diabetic kidney disease?

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Preclinical studies suggest that an inhibitor of amadorase, the enzyme at the centre of a newly discovered metabolic pathway that contributes significantly to the development of diabetic nephropathy, could offer treatment hopes to diabetic patients. The compound, DYN12, could help to delay, or even prevent, the onset of serious kidney problems. This approach is one of several currently being developed to tackle the range of life-shortening complications that arise in diabetics. 'Antioxidants and inhibitors of the β isoform of protein kinase C are being considered for cardiovascular and renal complications, and specific anti-angiogenic drugs, such as integrin antagonists, are being developed for proliferative diabetic retinopathy,' says Michael Brownlee (Albert Einstein College of Medicine, Bronx, NY, USA). However, Brownlee is impressed by the potential of DYN12 and considers it to be 'one of the more exciting new strategies'.

What is amadorase?

Amadorase, a previously unknown fructosamine kinase, was discovered, purified and characterized by scientists who later founded Dynamis Therapeutics (Wyndmoor, PA, USA). 'We have shown that amadorase is responsible for the production of 3-deoxyglucosone (3DG), a highly reactive dicarbonyl sugar that is a precursor to the advanced glycation end-products (AGEs),' explains Annette Tobia, President and CEO at Dynamis. AGEs were first shown by Brownlee to form on the surface of proteins, and to enable them to cross-link[1]. These cross-linked proteins cause much of the damage to the glomerular basement membrane that leads to problems in the kidneys of patients with diabetes. Previously, it was thought that 3DG resulted exclusively from non-enzymatic rearrangement, dehydration and fragmentation of a fructoselysine-containing protein (Fig. 1). Tobia and colleagues have now shown that 3DG is also produced as a by-product of a pathway that recovers lysine from fructoselysine (Fig. 1). 'This enzymatically-controlled process is the major source of 3DG in the body,' confirms Tobia.

There is considerable clinical and experimental evidence that 3DG is a major factor in the development of diabetic nephropathy. For example, elevated levels of 3DG and 3-deoxyfructose (its

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