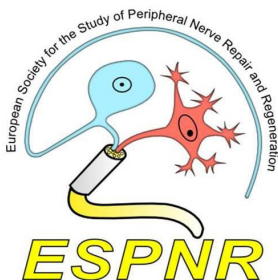


# 5<sup>th</sup> International Symposium on Peripheral Nerve Regeneration

Porto, Portugal  
8<sup>th</sup> and 9<sup>th</sup> of July 2019

## Abstract Book



Hosted by:

**U.** PORTO



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR  
UNIVERSIDADE DO PORTO

# Welcome Note

We are pleased to welcome you to the 5th International Symposium on Peripheral Nerve Regeneration (ISPNR), July 8th - 9th, 2019 in Porto, Portugal. As in previous ISPNRs, this interdisciplinary meeting will bring together experts, researchers and students from diverse disciplines. The symposium is expected to act as a platform to present and discuss new developments in the broad field of nerve repair and regeneration.

The study of peripheral nerve repair and regeneration has gained considerable interest in regenerative medicine.

Despite the scientific advancements, application to the patients is, however, still limited. It appears that in order to optimize the strategy for tissue engineering of the peripheral nerve in the clinical perspective, more basic research is needed. Neuroscientists need to strive for a new level of innovation which brings together (in a multi-translational approach) the main pillars of regenerative medicine, namely 1) Reconstructive microsurgery, 2) Transplantation (of tissues, cells and genes), 3) Material science and bioengineering, 4) Physical therapy and biostimulation, 5) Bioelectronics and bioengineering.

In line with this growing interest, and following the success of the preceding symposia in 2009, 2014, 2015 and 2017 the ISPNR aims at bringing together an international and interdisciplinary panel of scientists who critically address some of the most important issues in this emerging biomedical field.

The organizing committee wishes you an exciting, interesting, and fruitful symposium in Porto!

# Scientific Committee

Professor **Ana Colette Maurício**

Veterinary Clinics Department, ICBAS University of Porto

Professor **Claudia Grothe**

Institute of Neuroanatomy and Cell Biology, Hannover Medical School

Professor **Kirsten Haastert-Talini**

Institute of Neuroanatomy and Cell Biology, Hannover Medical School

Professor **Stefano Geuna**

Department of Clinical and Biological Sciences, University of Torino

Professor **Xavier Navarro**

Institute of Neurosciences, Department of Cell Biology, Physiology and Immunology, UAB



**U** PORTO  
INSTITUTO DE CIÊNCIAS BIOMÉDICAS AMEL SALAZAR  
UNIVERSIDADE DO PORTO



**M-H**  
Hannover Medical School



**M-H**  
Hannover Medical School



 UNIVERSITÀ  
DEGLI STUDI  
DI TORINO



**UAB**  
Universitat Autònoma  
de Barcelona

# Organizing Committee

Professor **Ana Lúcia Luís**

Veterinary Clinics Department, ICBAS University of Porto

**Ana Rita Caseiro**

Vasco da Gama University School

Professor **António Salgado**

Life and Health Sciences Research Institute, School of Medicine, University of Minho

Professor **Artur Varejão**

Veterinary Clinics Department, CECAV, University of Trás-os-Montes e Alto Douro

Professor **Carla Mendonça**

Veterinary Clinics Department, ICBAS University of Porto

Professor **Giovanna Gambarotta**

Department of Clinical and Biological Sciences, University of Torino

Professor **Luís Atayde**

Veterinary Clinics Department, ICBAS University of Porto

**Mariana Branquinho**

Veterinary Clinics Department, ICBAS University of Porto

Professor **Nicoletta Viano**

Department of Clinical and Biological Sciences, University of Torino

**Rui Alvites**

Veterinary Clinics Department, ICBAS University of Porto

**Dr. Sílvia Santos Pedrosa**

Veterinary Clinics Department, ICBAS University of Porto

# Partnerships

**U.** PORTO



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR  
UNIVERSIDADE DO PORTO

**MHH**

Hannover Medical School



UNIVERSITÀ  
DEGLI STUDI  
DI TORINO

**UAB**

Universitat Autònoma  
de Barcelona



ICVS/3B's

Associate  
Laboratory  
University of Minho



**α**  
*alfagene*

  
**Z-MED**

**CON  
CES  
SUS**  
CONCESSUS.SPA

  
**enzifarma**

# Index

8	Programme
10	Keynote speakers
11	Keynote speaker 1
14	Keynote speaker 2
16	Keynote speaker 3
19	Keynote speaker 4
22	Oral Communications
24	Session 1
30	Continuation Session 1
40	Session 2
48	Session 3
52	Session 4
58	Session 5
64	Session 6
74	Poster Sessions
76	Poster Session 1
84	Poster Session 2
92	Poster Session 3
100	Poster Session 4

# Programme

## Sunday, 7th July

19:00 - 20:30 — **Satellite Social Programme: Portologia**  
Commented Port Wine Tasting Experience

## Monday, 8th July

08:30 - 09:00 — Registrations

09:00 - 09:15 — Welcome Note

09:15 - 10:00 — **Keynote Lecture:** Professor Ana Colette  
Maurício *“Pre-clinical trials for the  
development of biomaterials and cell based  
therapies for peripheral nerve regeneration”*

10:00 - 10:45 — **Session 1:** Stem cells and tissue  
engineering for peripheral nerve repair

10:45 - 11:15 — Coffee Break

11:15 - 11:45 — Chaired Poster Session 1

11:45 - 13:15 — **Cont. Session 1:** Stem cells and tissue  
engineering for peripheral nerve repair

13:15 - 14:15 — Lunch

14:15 - 15:45 — **Session 2:** Innovative molecules and  
biomaterials for peripheral nerve repair

15:45 - 16:15 — Chaired Poster Session 2

16:15 - 16:45 — Coffee Break

16:45 - 17:30 — **Session 3:** Artificial nerve prostheses:  
Bioengineering and bioelectronic  
approaches



- 17:30 - 18:15 – **Keynote Lecture:** Professor James Phillips *“Pre-clinical proof of concept testing for off the shelf living engineered neural tissue”*
- 20:00 – **Get-together Dinner:** Capa no Rio Restaurant

## Tuesday, 9th July

- 09:00 - 09:45 – **Keynote Lecture:** Professor Joost Verhaagen *“Repairing injured peripheral nerve by gene therapy”*
- 09:45 - 10:45 – **Session 4:** Genetics and Gene therapy for peripheral nerve regeneration
- 10:45 - 11:15 – Coffee Break
- 11:15 - 11:45 – Chaired Poster Session 3
- 11:45 - 13:00 – **Session 5:** The neurobiology of axonal degeneration and regeneration in the peripheral nerve
- 13:00 - 14:00 – Lunch
- 14:00 - 15:30 – **Session 6:** Cellular molecular basis of axon guidance and regeneration
- 15:30 - 16:00 – Chaired Poster Session 4
- 16:00 - 16:30 – Coffee Break
- 16:30 - 17:15 – **Keynote Lecture:** Professor Stefania Raimondo *“Biomimetic conduits for peripheral nerve repair”*
- 17:15 - 17:30 – Closing Remarks
- 17:30 - 18:30 – General Meeting ESPNR

# Keynote Speakers

# Keynote Lecture 1



Professor **Ana Colette Maurício**

Veterinary Clinics Department, ICBAS University of Porto

# Pre-Clinical Trials for Development of Biomaterials and Cell-Based Therapies for Peripheral Nerve Regeneration

Rui Alvides<sup>1,2\*</sup>; Mariana Branquinho<sup>1,2\*</sup>; Ana Rita Caseiro<sup>1,2,3\*</sup>; Sílvia Pedrosa<sup>1,2</sup>; Tiago Pereira<sup>1,2</sup>; Ana Catarina Sousa<sup>1,2</sup>; Carla Mendonça<sup>1,2</sup>; Luís Miguel Atayde<sup>1,2</sup>; Stefano Geuna<sup>4</sup>; Ana Lúcia Luís<sup>1,2</sup>; Artur Varejão<sup>5,6</sup>; Ana Colette Maurício<sup>1,2</sup>.

1 – Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Rua de Jorge Viterbo Ferreira, n.º 228, 4050-313 Porto, Portugal; 2 – Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Rua D. Manuel II, Apartado 55142, 4051-401, Porto, Portugal; 3 – Escola Universitária Vasco da Gama (EUVG), Avenida José R. Sousa Fernandes, n.º 197 Lordemão, 3020-210 Coimbra, Portugal; 4 – Department of Clinical and Biological Sciences, and Cavalieri Ottolenghi Neuroscience Institute, University of Turin, Ospedale San Luigi, 10043 Orbassano, Turin, Italy; 5 – Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta de Prados, 5001-801 Vila Real, Portugal; 6 – CECAV, Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro (UTAD), Quinta de Prados, 5001-801 Vila Real, Portugal.

\* Considered as first authors.

Recent advances in Tissue Engineering considering neuromuscular system have promoted the development of *scaffolds*, which may convey growth factors from conditioned medium (CM) and cell-based therapies. Mesenchymal stem cells (MSCs) obtained from the umbilical cord matrix, dental pulp and synovial membrane are promising alternatives due to: i) high number of cells obtained, ii) low expression of HLA-ABC antigens and absence of HLA-DR expression, which permits to enlarge the number of available donors, and the use in allogenic and xenografts iii) facility to obtain, cryopreserve, and ethically approved, iv) high number of quality samples cryopreserved in biobanks worldwide. Recently, increasing clinical interest on dental pulp stem cells (DPSCs) has been noticed, due to the large number of orthodontic treatments and number of dental pulp samples cryopreserved. The obtained DPSCs have been demonstrated to have positive effects on bone and neuro-muscular regeneration. The main objective of our research group is to evaluate the therapeutic effect of developed tube-guides with micro- and nano-resorbable structures and MSCs or CM, on neuromuscular regeneration due to nerve injuries. This research approach also allows to adequate MSCs GMP preparation protocols to clinical use. This is therefore, One Health transversal studies, including development of new medical devices and cell-based therapies fully *in vitro* characterized by citocompatibility analysis, multi-lineage differentiation capacity, immunocytochemistry, flow cytometry, secretome, metabolic profile, and RT-PCR, and pre-clinical studies. Our multidisciplinary team has a key role in the pre-clinical trials, overseeing the animal welfare principles. The biodegradable membranes / tube-guides filled MSCs or CM are tested *in vivo*, in rat sciatic nerve axonotmesis and neurotmesis injuries, for initial validation of the

scaffold. Afterwards in the ovine peroneal nerve, allowing the study of critical defects. Outcomes of the regenerated nerves, are assessed by histological analysis and histomorphometry. Functional recovery is assessed serially using video recording of the gait for biomechanical analysis, by measuring extensor postural thrust (EPT), sciatic functional index (SFI) and static sciatic functional index (SSI), and withdrawal reflex latency (WRL). The muscular regeneration after neurogenic atrophy is also evaluated by morphometric and functional assessment. On the other hand, the axonotmesis and neurotmesis injury models are widely used in the evaluation of muscle regeneration via the denervation/reinnervation process, where the rapid restoration of the motor function unit is crucial, permitting to fully access the neuromuscular functional and morphologic recovery. The research team involved in all these ongoing projects is widely experienced in the proposed methodologies, as well as having a relevant experience in science management, providing precious insight on the ethical and regulatory requirements of cell-based therapies for clinical applications in human and veterinary medicine.

# Keynote Lecture 2



Professor **James Phillips**  
UCL Centre for Nerve Engineering

# Pre-Clinical Proof of Concept Testing for Off The Shelf Living Engineered Neural Tissue

James B. Phillips<sup>1</sup>; Melissa Rayner<sup>1</sup>; Adam Day & John Sinden<sup>1</sup>;

1 – UCL Centre for Nerve Engineering, University College London, London, UK.

Engineered Neural Tissue (EngNT) is formed using cellular self-alignment followed by stabilisation in collagen hydrogels. The resulting anisotropic cellular materials mimic the aligned cellular structure of the nerve autograft and have been shown to support regeneration of neurons *in vitro* and *in vivo* [1-5]. To avoid the delay and variability associated with autologous sources of therapeutic cells, a conditionally immortalised human neural stem cell line was used as a source of allogeneic cells for constructing EngNT [6]. The aim of this study was to test 'off-the-shelf' allogeneic EngNT in a critical-length rat sciatic nerve model to provide pre-clinical proof of concept data to support clinical translation. CTX0E03 cells (ReNeuron, UK) were differentiated then used to form EngNT-CTX constructs. These were implanted into athymic nude rats to bridge a 15 mm sciatic nerve gap and compared to autograft controls after 8 weeks and 16 weeks. Histological and electrophysiological analyses showed equivalent motor regeneration between EngNT and autograft groups. In conclusion, EngNT-CTX supported similar nerve regeneration to autografts in this statistically-powered critical-length preclinical model using a range of outcome measures. The use of clinical-grade cells and materials and the development of scalable manufacturing technology means that this study provides robust data to underpin subsequent progression of EngNT-CTX towards clinical testing.

## References:

1. Georgiou, M., et al., *Engineered neural tissue for peripheral nerve repair*. *Biomaterials*, 2013. 34(30): p. 7335-43.
2. Georgiou, M., et al., *Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve*. *Biomaterials*, 2015. 37: p. 242-51.
3. Gonzalez-Perez, F., et al., *Schwann cells and mesenchymal stem cells in laminin- or fibronectin-aligned matrices and regeneration across a critical size defect of 15 mm in the rat sciatic nerve*. *J Neurosurg Spine*, 2018. 28(1): p. 109-118.
4. Sanen, K., et al., *Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair?* *J Tissue Eng Regen Med*, 2017.
5. Schuh, C., et al., *An optimized collagen-fibrin blend engineered neural tissue promotes peripheral nerve repair*. *Tissue Eng Part A*, 2018.
6. O'Rourke, C., et al., *An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells*. *Sci Rep*, 2018. 8(1): p. 2951.

# Keynote Lecture 3



Professor **Joost Verhaagen**

Laboratory for Neuroregeneration, Netherlands Institute for Neurosciences



# Repairing Injured Peripheral Nerves by Gene Therapy

Joost Verhaagen<sup>1</sup>; Ruben Eggers<sup>1</sup>; Fred De Winter<sup>1</sup>;

1 – Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience

Proximal severe peripheral nerve injury leads to permanent functional impairment. In this presentation we will discuss the potential of gene therapy as a strategy to promote nerve regeneration alongside surgical repair. In early work we showed that gene therapy for neurotrophic factors is a powerful strategy to promote axon regeneration (Blits 2004, Eggers 2008). However, uncontrolled expression of a neurotrophic factor in an injured nerve resulted in axon entrapment (candy store effect). Thus, in order to be useful as a therapy, neurotrophic factor expression had to be carefully controlled. The doxycycline-inducible gene switch is one of the most promising systems for regulating therapeutic gene expression. However, an immune response against the transactivator protein (TA) hampered application in peripheral nerve injury models. Immunogenic proteins fused with the Gly-Ala repeat (GAR) of the Epstein-Barr virus Nuclear Antigen-1 have been shown to successfully evade the immune system. We used this strategy to create an immune-evasive - stealth - version of the doxycycline-inducible TA (designated GAR-TA; Hoyng 2014). GAR-TA exhibited an immune-evasive advantage over TA in a bioassay for human antigen presentation. In 2019 we reported the application of a doxycycline inducible stealth gene switch for GDNF (dox-i-GDNF) in a spinal ventral root avulsion lesion (Eggers et al 2019). Time-restricted GDNF expression (1 month) using the immune-evasive gene switch was sufficient to promote long-term motor neuron survival for a post-lesion period of 24 weeks post-lesion, enhanced a limited degree of axon regeneration into the distal nerve and facilitated the recovery of compound motor axon potentials (CMAPs). In contrast, persistent GDNF expression (12 and 24 weeks) impaired axon regeneration by inducing axon entrapment. This study marks an important step in the ongoing development of a neurotrophic factor gene therapy for patients with proximal lesions. Future studies focus on 1. Adaptation of the stealth gene switch for use in an adeno-associated viral vector context, and 2. Exploring the possibility of noninvasive gene transfer to peripheral nerve Schwann cells.

## References:

1. Eggers R et al. *Timed GDNF gene therapy using an immune-evasive gene switch promotes long distance axon regeneration*. Brain 142: 295, 2019
2. Hoyng SA et al. *Developing a potentially immunologically inert tetracycline regulatable viral vector for gene therapy in the peripheral nerve*. Gene Ther 21: 549, 2014
3. Eggers R et al. *Neuroregenerative effects of lentiviral vector-mediated GDNF expression in reimplanted ventral roots*. Mol Cell Neurosci 39: 105, 2008.

4. Blits B et al. *Rescue and sprouting of motoneurons following ventral root avulsion and reimplantation combined with intraspinal adeno-associated viral vector-mediated expression of glial cell line-derived neurotrophic factor or brain-derived neurotrophic factor.* Exp Neur 189: 303, 2004.

**Key Words :** Gene therapy, Stealth gene switch, GDNF, Motor neuron

# Keynote Lecture 4



Professor **Stefania Raimondo**

Department of Clinical and Biological Sciences, University of Torino

# Biomimetic Conduits for Peripheral Nerve Repair

Raimondo Stefania<sup>1,2</sup>; Muratori Luisa<sup>1,2</sup>; Fregnan Federica<sup>1,2</sup>; Ronchi Giulia<sup>1,2</sup>; Biagiotti Marco<sup>3</sup>; Vincoli Valentina<sup>3</sup>; Bassani Giulia Alessandra<sup>3</sup>; Geuna Stefano<sup>1,2</sup>.

1 – Department of Clinical and Biological Sciences, University of Torino, Italy; 2 – Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Italy; 3 – Silk Biomaterials srl, Italy.

The repair and regeneration of peripheral nerve injuries represent a major research field where clinical application of innovative therapies in regenerative medicine should be sought. The more used surgical technique of repair is a primary end-to-end repair, while in nerve gap injuries, when tension precludes a primary repair, a nerve autograft is usually used to provide a scaffold for the regenerating nerve.

The possibility of repairing nerve defects by bridging the gap by means of non-nervous tubes has been widely studied, both experimentally and in clinical practice. This surgical approach is usually called tubulization, which consists in bridging a nerve gap by means of a cylinder-shaped tube. This technique offers also the possibility of manipulate in the laboratory different tissues and organs in order to fashion conduits that mimic some important features of the nerve environment; and to enrich biological or synthetic tubes with various elements (substances, either molecules, cells or tissues) that are considered essential for promoting nerve fiber regeneration. Moreover, in the last years, several studies about the employment of biomaterials for tubulization were published.

In this presentation, results about the use of biomimetic conduits will be presented focusing mainly on the use of a very promising Silk fibroin scaffold.



# **Oral Communications**



# Session 1

## Stem Cells and Tissue Engineering for Peripheral Nerve Repair

### **Chairs:**

#### **Professor Ana Colette Maurício**

Veterinary Clinics Departmente, ICBAS University of Porto

#### **Professor Claudia Grothe**

Institute of Neuroanatomy and Cell Biology, Hannover Medical School



# Exploring the Interactions Between ASCs and HUVECs to Promote Neurite Outgrowth in DRG Explants

Luís A. Rocha<sup>1,2,3</sup>, Eduardo D. Gomes<sup>1,2</sup>, Rui A. Sousa<sup>3</sup>, David A. Learmonth<sup>3</sup>, António J. Salgado<sup>1,2</sup>

1— Life and Health Sciences Research Institute (ICVS), School of Medicine – University of Minho, Braga, Portugal; 2 – ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal; 3 – Stematters – Biotecnologia & Medicina Regenerativa

Disruption of the blood spinal cord barrier is fundamental for the poor prognosis following spinal cord injury. Herein, we developed a hydrogel-based approach consisting on the co-culture of adipose-derived stem cells (ASCs) and human umbilical vein endothelial cells (HUVECs) to promote the vascularization of the injury site. Gellan gum modified with GRGDS (GG-GRGDS) was assessed for its angiogenic capacity in comparison to collagen using the chick chorioallantoic membrane assay (CAM) for 4 days. Afterwards, we developed a co-culture system using ASCs and HUVECs encapsulated in GG-RGDS to assess its capacity in the neurite outgrowth of DRG explants. Cells were encapsulated in a 1:1 ratio 24 hours before DRG isolation and controls included each cell type cultured alone (using the same densities) and the hydrogel without cells. After DRG seeding, the culture system was maintained during 7 days. Immunocytochemistry allowed to evaluate the growth of neurites and vascular organization of HUVECs. The number of vessels converging towards the hydrogels showed no significant differences between each experimental condition, with GG being followed by C and GG-RGDS. Growth of neurites using GG-RGDS hydrogels demonstrated that neurite occupied area was highest for the co-culture condition, being statistically significant from the condition without cells ( $p < 0.01$ ), similarly to the observed for ASCs. Longest neurite followed the same trend, but without statically significant differences ( $p < 0.05$ ). Interestingly, HUVECs clearly tended to organize into vascular-like structures in the presence of ASCs within the GG-RGDS, demonstrating the beneficial effects of the presence of these mesenchymal stem cells for SCI vascularization. Statistically significant differences were found regarding vessel area, vessel percentage area, total vessel length and number of junctions. This co-culture system using GG-RGDS demonstrated potential to be further evaluated in SCI animal models due to its capacity of supporting neurite outgrowth and vascular organization at the injury site.

**Acknowledgements:** Financial support by Prémios Santa Casa Neurociências – Prize Melo e Castro for Spinal Cord Injury Research; Portuguese Foundation for Science and Technology. This work has been supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership. ASCs were kindly provided by LaCell, USA.

**Key Words:** Dorsal root ganglia, 3D Cell culture, Vascularization, Spinal cord injury

# The Effect of Hypoxia on the Ability of Differentiated Human Dental Pulp Stem Cells to Promote Endothelial Cell Proliferation and Neurite Outgrowth *In Vitro*

Kulraj Singh Bhangra<sup>1,2,3</sup>, Magdalena Plotczyk<sup>1,2</sup>, Jonathan Knowles<sup>1</sup>; Rebecca Shipley<sup>3,4</sup>; David Choi<sup>3,5</sup>; James Phillips<sup>1,2,3</sup>

1 – Eastman Dental Institute, University College London, London, UK; 2 – Centre for Nerve Engineering, University College London, London, UK; 3 – Centre for Nerve Engineering, University College London, London, UK; 4 – Mechanical Engineering, University College London, London, UK; 5 - Institute of Neurology, University College London, London, UK

Engineered neural tissue (EngNT) has emerged as a novel tissue engineering strategy for the repair of critical gap peripheral nerve defects [1]. Previous studies have shown that EngNT can be created with differentiated human dental pulp stem cells (d-hDPSCs), which supported neuronal regeneration *in vitro* [2]. However, when tested in a long-gap peripheral nerve repair model the results revealed fewer regenerated fibres compared to autograft but enhanced vascularisation compared to an empty tube [3]. This led to the hypothesis that the hypoxic environment *in vivo* altered the implanted cell phenotype such that angiogenesis, rather than neuronal regeneration, was promoted [3]. The aim of this study therefore was to explore the angiogenic and neurotrophic behaviours of human dental pulp stem cells (hDPSCs) and their differentiated progeny *in vitro* following exposure to different levels of oxygen.

hDPSCs and d-hDPSCs were cultured for 24 h under 1%, 3% and 16 % oxygen conditions after which secretome were collected. The effect of these discrete secretomes were assessed on human umbilical vein endothelial cells (HUVECs) and dissociated sensory neurons obtained from rat dorsal root ganglia (DRG). HUVECs were cultured in each secretome for 24, 48 and 72 h. Proliferation was quantified by counting the number of positive-Ki67 cells in the total population (stained with Hoechst), under a fluorescence microscope. Dissociated DRGs were seeded into a well plate and incubating them for 48 h with each secretome. Following this, neurons were immunostained for anti- $\beta$ III tubulin to quantify neurite length using ImageJ.

The secretome produced from culturing hDPSCs and d-hDPSC in 3% O<sub>2</sub> increased the proliferation rate of endothelial cells compared to 1% and 16% O<sub>2</sub>. At 3% O<sub>2</sub> HUVECs cultured in d-hDPSC secretome revealed significantly higher proliferation rates compared to cells cultured with hDPSCs secretome. Neurons cultured in 1% and 3% O<sub>2</sub> secretome had the shortest neurite length, which increased approximately two-fold in the 16% O<sub>2</sub> condition.

These results support the hypothesis that under low oxygen conditions hDPSCs and d-hDPSCs release increased levels of pro-angiogenic factors but decreased levels of neurotrophic factors compared to their behaviour under standard ambient

cell culture oxygen. This is an important consideration in the design of engineered tissues for implantation into a nerve repair environment, since adequate angiogenesis is required to support implanted cell survival, but neurotrophic factor release must also be maintained in implanted cells.

**References:**

1. Georgiou, M., et al., *Biomaterials*, 2013. 34(30): p. 7335-43.
2. Martens, W., et al., *FASEB J*, 2014. 28(4): p. 1634-43.
3. Sanen, K., et al., *J Tissue Eng Regen Med*, 2017. 11(12): p. 3362-3372.

# In Silico Framework to Optimise the Design of EngNT Nerve Conduits.

Simão Laranjeira<sup>1,2</sup>; Kulraj S. Bhangra<sup>2,3</sup>; James B. Phillips<sup>2,3</sup>; Rebecca Shipley<sup>1,2</sup>.

1 – UCL Mechanical Engineering, London, UK; 2 – UCL Centre for Nerve Engineering, UK.;

3 – Department of Pharmacology, UCL School of Pharmacy, London, UK.

Peripheral nerve injury (PNI) affects 1M people in Europe and the USA p.a.<sup>1</sup> Patients experience debilitating symptoms resulting in loss of end-organ function and morbidity.<sup>1</sup> The current gold standard aims at establishing a connection between proximal and distal nerve stumps through a nerve autograft.<sup>2</sup> However, donor site morbidity, limited availability and poor clinical outcomes are associated with this approach.

To address these concerns Engineered Neural Tissue (EngNT), an engineered anisotropic cellular hydrogel was developed, which has a comparable regenerative potential to the autograft.<sup>3</sup> To improve the efficacy and integration of EngNT, the addition of biomaterial structures is being explored. One strategy consists of embedding the EngNT with rods, which provide durotactic and directional cues to neurites. However, the optimal number and distribution of rods are not known.

Here we seek to inform the design of EngNT by developing computational models of neurite growth in order to identify spatial arrangements of biomaterial within a conduit that maximise the rate of regeneration. We present a new computational model that predicts neurite growth in response to the mechanical environment.

Neurite movement is simulated as a 3D lattice-based random walk. For each time-step of the model the stochastic movement of the neurites is calculated following the Langevin equation, which relates the movement of cells to the forces acting on them.<sup>4</sup> Three forces are considered: 1) the mechanical resistance of cells; 2) a bias to move forward due to cues present in EngNT and 3) a bias towards stiffer material. The parameters, were fitted to *in vivo* neurite counts within EngNT using a Particle swarm algorithm.<sup>5,6</sup> With such a model it was possible to explore the competing factors of providing cues to neurites vs the surface area occupied by the fibres. Preliminary results have shown good agreement with data.

In summary, this is the first parameterised framework in a PNI context that now can be used to explore the optimal arrangement of materials and cells to promote efficient neuronal regeneration following injury, and thus propose new conduit designs.

## References:

1. Chen, S. et al. *Neural Regeneration Research* 10, 1777 (2015).
2. Grinsell, D. & Keating, C. P. *BioMed Research International* (2014).
3. Georgiou, M. et al. *Biomaterials* 34, 7335–7343 (2013).

4. Zubler, F. & Douglas, R. A *Front Comput Neurosci* 3, (2009).
5. O'Rourke, C. et al. *Scientific reports* 8, 2951 (2018).
6. Vaz, A. I. F. & Vicente, L. N. *Journal of Global Optimization* 39, 197–219 (2007).

**Key Words:** In-silico, Biomaterials, Therapy, Peripheral nervous system

# Continuation Session 1

## Stem Cells and Tissue Engineering for Peripheral Nerve Repair

### **Chairs:**

**Professor Stefania Raimondo**

Department of Clinical and Biological Sciences, University of Torino

**Professor Luís Atayde**

Veterinary Clinics Department, ICBAS University of Porto

# Silicon or Silicon Carbide Surface as Novel Cell Culture Device for Neural Stem Cells

Gabriele Bonaventura<sup>1</sup>; Rosario Iemmolo<sup>1</sup>; Valentina La Cognata<sup>1</sup>; Massimo Zimbone<sup>2</sup>; Francesco La Via<sup>2</sup>; Maria Elena Fragalà<sup>4</sup>; Maria Luisa Barcellona<sup>3</sup>; Rosalia Pellitteri<sup>1</sup>; Sebastiano Cavallaro<sup>1</sup>

1 – Institute of Neurological Sciences, Italian National Research Council, Catania, Italy;

2 – Institute for Microelectronics and Microsystems, Italian National Research Council, Catania, Italy; 3

– Department of Pharmaceutical Sciences, Section of Biochemistry, University of Catania, Catania, Italy;

4 – Department of Chemical Sciences, University of Catania, Catania, Italy

**Silicon (Si) wafers have attracted considerable scientific interest as a promising bio-material for the manufacture of implantable medical devices in the neurodegenerative diseases. Since the use of Si involves responsibilities due to its toxicity, the investigation is moving towards a new class of composite semiconductors, such as the Silicon Carbide (3C-SiC). The main concerns in the material research for biomedical applications is to find a suitable material that produces low or no adverse effect when grafted in the body. Si has always been the preferred substrate material for micro-devices due to its low cost and ready availability, but it has several drawbacks that limit its use in biomedical applications, specifically when used *in vivo*. On the contrary, 3C-SiC results a good material for these purposes being biocompatible and hemo-compatible. The aim of our work was to test the biocompatibility levels of Si and 3C-SiC in an *in vitro* model of neural cells derived from both human dental pulp mesenchymal stem cells (DP-NSCs) and mouse Olfactory Ensheathing Cells (OECs), a particular glial cell type showing stem cell characteristics. To assess the biocompatibility of these substrates, we investigated the mitochondrial membrane potential, cytotoxicity and morphological changes through Scanning Electron Microscope analysis. Moreover, we assessed the expression of some markers, such as Nestin, GFAP, S-100, MAP2 and Neurofilament by immunocytochemical procedures. Our results demonstrate that 3C-SiC avoids cell stress both on DP-NSCs and OECs, in addition we found that it was not visible adverse reaction on both mitochondrial membrane potential and morphological modifications. A significant regulation of gene expression was found by qRT-PCR assay in neuronal cells differentiated from DPSC. Therefore, this study shows that 3C-SiC might represented a valid support in long-term implantable biochips having a biocompatibility that overlap with that obtained with other carbon-derived materials. In addition, the synergistic effect of cell transplantation and biomaterial supports implantation could be considered as a promising insight for nerve injury treatment. In conclusion, our findings highlight the possibility to use, as clinical tool for lesioned neural areas, Neural Stem Cells plated on 3C-SiC substrate, indicating these strategies as a future perspective for the development of novel cell therapies that would reach from bench to bedside to serve the neuro-degenerated patients.**

**Key Words:** Silicon surface, Neural stem cells, Biocompatibility, Neuro-regeneration

# Ex Vivo and In Vivo Evaluation of New Detergent-Based Decellularized Nerve Allografts

J Chato-Astrain<sup>1</sup>; F Campos<sup>1</sup>; C Philips<sup>2</sup>; Od García-García<sup>1</sup>; M El Soury<sup>3</sup>; V Domingo-Roa<sup>4</sup>; M Alaminos<sup>1</sup>; A Campos<sup>1</sup>; V Carriel<sup>1</sup>.

1— Department of Histology & Tissue Engineering Group, University of Granada, and Instituto de Investigación Biosanitaria ibs. GRANADA, Granada, Spain; 2 — Tissue Engineering Group, Faculty of Medicine and Health Sciences, Ghent University, Belgium; 3 — Department of Clinical and Biological Sciences, University of Torino, Turin, Italy; 4 — Experimental Unit, University Hospital Virgen de las Nieves, Granada, Spain

Tissue decellularization is a promising alternative in regenerative medicine, including nerve repair [1]. This strategy demonstrate that acellular nerves grafts support and acceptable degree of tissue regeneration and functional recovery. However, more research is needed to determine the potential clinical usefulness of these strategies. In this sense, our aim was to generate novel detergent-based decellularized peripheral nerve allograft for peripheral nerve repair (D-DPNAs). The decellularization rate, structure and biomechanics of the novel D-DPNAs were compared to the Sondell (SD) and Hudson (HD) decellularized allografts *ex vivo*. Furthermore, the D-DPNAs were used to repair 10-mm nerve gap in rats and compared to acellular grafts (SD and HD) and autograft technique at the functional and histological level after 12 weeks.

The *ex vivo* characterization demonstrated an efficient decellularization rate with the new protocol used for the generation of the D-DPNAs, being these results better than SD and HD DPNAs. Histology showed an adequate and comparable preservation of the extracellular matrix in all DPNAs. However, electron microscopy confirmed an inefficient removal of myelin in the D-DPNAs. In addition, tensile test revealed that the biomechanical behavior of the D-DPNAs differed as compared to SD and HD groups, but they were closely comparable to native nerves [2].

*In vivo* evaluation showed comparable clinical and functional profile between DPNAs and autograft technique. However, the mass and volume of the muscles resulted more affected by the use of DPNAs than autograft. Histology confirmed an active tissue regeneration along grafted DPNAs with slightly better results with D-DPNAs, but DPNAs were not fully comparable to the histological pattern observed in autograft.

Finally, this study demonstrated clear signs of nerve regeneration and functional recovery with the use of DPNAs, suggesting that these novel D-DPNAs could be a promising alternative for peripheral nerve repair. However, despite these promising results, we were not able to significantly overcome the effectiveness of the use of autograft. Therefore, future studies focused on the improvement of the biological properties and functionality of DPNAs are needed to create more efficient alternatives for peripheral nerve repair.



**Fundings:** FIS-PI17/393, Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, España.

**References:**

1. *Front Cell Neurosci* (2018). doi: 10.3389/fncel.2018.00427.
2. *Ann Biomed Eng* (2018). doi:10.1007/s10439-018-2082-y

**Key Words:** Nerve repair, Functional recovery, Nerve tissue regeneration, Decellularized allografts, Natural biomaterials

# Interaction Between Exogenous and Endogenous Glial Cells in Engineered Neural Tissue (EngNT)

Titinun Suannun<sup>1</sup>; Jonathan Knowles<sup>1</sup>; James B. Phillips<sup>2</sup>.

1 – Biomaterials and Tissue Engineering Department, UCL Eastman Dental Institute, University College London, London, UK; 2 – Department of Pharmacology, UCL School of Pharmacy, University College London, London, UK

Schwann cells play a critical role in supporting peripheral nerve regeneration by providing support and guidance to regenerating neurons. A promising alternative treatment to the use of autografts for long gap nerve injury is transplanting engineered neural tissue (EngNT), formed from simultaneous self-alignment of Schwann cells and collagen fibrils in a tethered gel resulting in an anisotropic tissue-like structure. Previous studies have focussed on understanding the length and angle of regenerating neurites within EngNT constructs *in vitro* and *in vivo* [1-4]. However, the behaviour of the implanted Schwann cells and their interaction with host Schwann cells remains unclear.

This research aims to explore how the endogenous therapeutic glial cells within EngNT interact with exogenous infiltrating host Schwann cells *in vitro* and after transplantation. Primary glial cells from GFP-labelled rat dorsal root ganglia were seeded onto EngNT containing aligned SCL4.1/F7 Schwann cells. After 3 days in co-culture, the exogenous glial cells had become aligned with the endogenous Schwann cells in the EngNT. In decellularised control EngNT where endogenous cells were killed by freeze-thaw, the alignment of exogenous glial cells was also observed. Further investigations are testing the interactions between Schwann cells within EngNT and host Schwann cells *in vivo*, using a 10 mm-gap rat sciatic nerve model for 3 weeks. Understanding how implanted therapeutic Schwann cells interact with host Schwann cells, particularly at the interface with the proximal and distal nerve stump, will help to inform the development of treatments for nerve injury that involve implantation of cellular materials.

## References:

1. Georgiou, M., et al., *Engineered neural tissue for peripheral nerve repair*. *Biomaterials*, 2013. 34(30): p. 7335-43.
2. Georgiou, M., et al., *Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve*. *Biomaterials*, 2015. 37: p. 242-51.
3. Martens, W., et al., *Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro*. *FASEB J*, 2014. 28(4): p. 1634-43.
4. O'Rourke, C., et al., *An allogeneic & off the shelf & therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells*. *Sci Rep*, 2018. 8(1): p. 2951.

**Key Words:** Engineered neural tissue, Glial cells, Implantation, Hydrogel, Nerve guidance

# Use of PLLA Electrospun Piezoelectric Membranes and Dental Pulp Stem/Stromal Cells in Regeneration of the Sciatic Nerve After Neurotmesis – Functional Results

Rui Alvim<sup>1,2</sup>; Mariana Branquinho<sup>1,2</sup>; Ana Rita Caseiro<sup>1,2,3</sup>; Sílvia Pedrosa<sup>1,2</sup>; Thierry Correia<sup>1,2,3</sup>; Ana Lúcia Luís<sup>1,2</sup>; Stefano Geuna<sup>4</sup>; Artur Varejão<sup>5</sup>; Ana Colette Maurício<sup>1,2</sup>.

1 – Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS); 2 – Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Rua D. Manuel II, Apartado 55142, 4051-401, Porto, Portugal; 3 – Escola Universitária Vasco da Gama (EUVG), Av. José R. Sousa Fernandes 197, Campus Universitário – Bloco B, Lordemão, 3020-210 Coimbra, Portugal; 4 – Department of Clinical and Biological Sciences, and Cavalieri Ottolenghi Neuroscience Institute, University of Turin; 5 – Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD).

**Peripheral nerve lesions are frequent and highly debilitating, affecting the sensory, motor and autonomic components of the nerve, and resulting in different degrees of dysfunction, changes in motion capacity, apraxia and prolonged pain [1]. Over the years there have been several therapeutic approaches developed to reverse the negative effects of this type of injuries and to promote nerve regeneration. Despite all the advances accomplished, the ideal methodology through which the best functional recovery results are achieved has yet to be determined.**

To overcome the drawbacks of conventional techniques, nerve conduits appeared as an alternative capable of guiding nerve regeneration, particularly when applied in combination with other approaches such as cell-based therapies. The latest generation of nerve conduits are electroconducting polymers that stimulate the propagation of endogenous and exogenous electrical signals along their structure, ensuring mechanical support and electrical guidance that stimulates nerve regeneration [2]. More specifically, piezoelectric materials generate electric charges in response to forces of mechanical stress, not suffering structural deformations[3]. The aim of this work is to test the effect of the combined use of Dental Pulp Stem/Stromal Cells (hDPSCs) and PLLA Electrospun Piezoelectric Membranes on promoting functional regeneration of the Rat sciatic nerve after neurotmesis.

Sprague-Dawley rats submitted to a neurotmesis lesion of the sciatic nerve were divided into three experimental therapeutic groups: end-to-end suture of the nerve stumps alone (EtE), end-to-end suture enveloped with PLLA piezoelectric membranes (PLLA) or end-to-end suture enveloped with PLLA piezoelectric membranes impregnated with hDPSCs (DP). Control groups were drawn from uninjured animals (UC). Over 20 weeks, the animals underwent functional tests to determine progression of motor, sensory and behavioral recovery.

After 20 weeks, the results obtained during the study period were analyzed. It was possible to determine that the DP group presented better final values of nociceptive and functional recovery than the other groups. In general, the PLLA group was the one with the worst overall results. These results should, however,

be interpreted in the light of other studies such as kinematic, morphological and histomorphometric analysis.

**References:**

1. Yousefi F, Arab FL, Nikkhah K, Amiri H, Mahmoudi M. *Novel approaches using mesenchymal stem cells for curing peripheral nerve injuries*. Life sciences. 2019.
2. Stewart E, Kobayashi NR, Higgins MJ, Quigley AF, Jamali S, Moulton SE, et al. *Electrical stimulation using conductive polymer polypyrrole promotes differentiation of human neural stem cells: a bio-compatible platform for translational neural tissue engineering*. *Tissue Engineering Part C: Methods*. 2014;21(4):385-93.
3. Fine EG, Valentini RF, Bellamkonda R, Aebischer P. *Improved nerve regeneration through piezoelectric vinylidene fluoride-trifluoroethylene copolymer guidance channels*. *Biomaterials*. 1991;12(8):775-80.

**Key Words:** Peripheral nerve injury, Nerve regeneration, PLLA electrospun Piezoelectric membranes, hDPSCs, Functional evaluation

# The Supporting Role of Mesenchymal Stromal/ Stem Cells in Peripheral Nerve Regeneration – The Effects of MSCs Secretome on Endothelial Populations

Ana Rita Caseiro<sup>1,2,3</sup>; Sílvia Santos Pedrosa<sup>1,2</sup>; Mariana Branquinho<sup>1,2</sup>; Rui Alvites<sup>1,2</sup>; Tiago Pereira<sup>1,2</sup>; Ana Colette Maurício<sup>1,2</sup>.

1 – Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Rua de Jorge Viterbo Ferreira, nº 228, 4050-313 Porto, Portugal; 2 – Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Rua D. Manuel II, Apartado 55142, 4051-401, Porto, Portugal; 3 – Escola Universitária Vasco da Gama (EUVG), Av. José R. Sousa Fernandes 197, Campus Universitário – Bloco B, Lordemão, 3020-210 Coimbra, Portugal

**Mesenchymal Stromal/ Stem Cells (MSCs) assume a supporting role to the intrinsic mechanisms of tissue regeneration, a feature mostly assigned to the contents of their secretome. Peripheral nerve regenerative events rely strongly on the re-vascularization of the lesion site, to ensure adequate supply to regenerating tissues and removal of process debris. A comparative study on bioactive molecules/factors content of the secretome of MSCs derived from two expanding sources: the umbilical cord stroma (UC-MSCs) and the dental pulp (DPSCs) is presented and discussed. The secretion profiles of both cellular populations revealed prominent differences in terms of for the bioactive factors production and release into the culturing media, in which FST, GRO, HGF, GRO, IL-8 and MCP-1 dominate in UC-MSCs secretion, while in DPSCs the VEGF-A and FST are more evident. The effects of the produced secretome were assessed *in vitro* on their effects on endothelial populations. The distinct secretory cocktail did not result in significantly different effects on endothelial cell populations dynamics including proliferation, migration and tube formation capacity.**

# Repair Schwann Cells but not Schwann Cell Precursors Have Axon Regeneration Effects After Peripheral Nerve Injury

Takeshi Endo<sup>1</sup>; Ken Kadoya<sup>1</sup>; Tomoaki Suzuki<sup>1</sup>; Yuki Suzuki<sup>1</sup>; Yuki Matsui<sup>1</sup>, Yuan Rufe<sup>1</sup>, Daisuke Kawamura<sup>1</sup>, Norimasa Iwasaki<sup>1</sup>.

1 – Department of Orthopaedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan.

Although peripheral nerve can regenerate, clinical outcomes after peripheral nerve injuries (PNI) are still unsatisfactory, especially in severe and proximal injury cases. Accumulated evidences show that Schwann cell (SC) graft is one of potential approaches for regenerative therapy after PNI. While the graft of glial precursors supports axon regeneration in adult central nervous system (CNS), it remains to be elucidated that SCs at developmental stages promote regeneration of adult axons after PNI. The purpose of the current study is to elucidate the axon promoting effects of developing SCs after PNI. Total of 4 types of SCs were tested, including 1) SC precursors (SCPs), 2) immature SCs (ISCs), and 2 types of mature SCs, which were 3) repair SCs (RSCs) and 4) non-RSCs. All cells were prepared from RFP transgenic Lewis rats. SCPs, ISC, and non-RSCs were harvested from intact sciatic nerves at embryonic day 14 (E14), E18, and postnatal 10-12 weeks. RSCs were isolated from transected adult sciatic nerves at 1 week after injury. One million of prepared cells were grafted into 25 mm long cell-free region with crush injury in a sciatic nerve of a syngeneic Lewis rat. Cell-free area was achieved by repeated freeze and thaw procedures with liquid nitrogen. Injury alone (no cell-free region) and no cell graft groups were used as positive and negative controls respectively. Two weeks after injury and grafts, the RSCs group showed the greatest axon regeneration among all cell graft groups, although its extent was still significantly reduced compared to the positive control. Non-RSCs was the next effective cell type. Surprisingly, SCPs and ISCs failed to support axon regeneration at all, even though they maintained high proliferative ability after grafting. Further, *in vitro* culture of dorsal root ganglion (DRG) neurons at adult and embryonic (E14) stages in combination with RSCs and SCPs demonstrated that RSCs but not SCPs promoted neurite outgrowth of adult DRG neurons and that RSCs and SCPs didn't stimulate neurite outgrowth of E14 DRG neurons. The molecular mechanisms underlying this marked difference of axon promoting effects between RSCs and SCPs were partially verified by transcriptome analysis showing a distinct difference of gene expression profiles and by ELISA of neurotrophic factor productions. These findings indicate that, unlike CNS, SCs at developmental stages don't support regeneration of adult axons after PNI and that mature SCs, especially RSCs, are good candidates as a graft cell type for regeneration therapy after PNI.



# Session 2

## Innovative Molecules and Biomaterials for Peripheral Nerve Repair

### **Chairs:**

#### **Professor Kirsten Haastert-Talini**

Institute of Neuroanatomy and Cell Biology, Hannover Medical School

#### **Professor António Salgado**

Life and Health Sciences Research Institute, School of Medicine, University of Minho

#### **Professor Artur Varejão**

Veterinary Clinics Department, CECAV, University of Trás-os-Montes e Alto Douro



# Suitability of Natural Matrices and Encapsulated BDNF-Producing MSCs for Primary Auditory Neuron Protection and Neurite Regeneration

Jana Schwieger<sup>1</sup>; Noushin Kakuan<sup>1</sup>; Suheda Yilmaz-Bayraktar<sup>1</sup>; Anayancy Osorio-Madrado<sup>2</sup>; Andrea Hoffmann<sup>3</sup>; Ulrike Böer<sup>4</sup>; Theodor Doll<sup>1,5</sup>; Thomas Lenarz<sup>1</sup>; Verena Scheper<sup>1</sup>.

1 – Department of Otolaryngology, Hannover Medical School, Hannover, Germany; 2 – Laboratory for Sensors, Department of Microsystems Engineering, University of Freiburg IMTEK/FMF, Freiburg, Germany; 3 – Department of Orthopaedic Surgery, Hannover Medical School, Hannover, Germany; 4 – Division for Cardiothoracic-, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany; 5 – Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany

**Introduction:** Patients suffering from sensory neural hearing loss are standardly treated with cochlear implants (CI). An electrode is inserted into the fluid-filled scala tympani and electrically stimulates the spiral ganglion neurons (SGN), located in the bony axis of the cochlea, to evoke a hearing sensation. A high number of excitable SGN and a regeneration and guidance of their neurites to the electrode are assumed to improve the hearing outcome. Bridging the remaining gap between electrode and neurons might allow a more focused stimulation. This could be achieved by supply of neurotrophic factors (NF) for neuroprotection and neurite regeneration and a growth matrix in the scala tympani.

**Methods:** Two hydrogels of different viscosities, alginate (soft, 2 mM BaCl<sub>2</sub>-crosslinked; stiff, 2 mM or 20 mM BaCl<sub>2</sub>-crosslinked) and chitosan (0.7, 1, 1.3, 2 wt%), and decellularized equine carotid arteries (dEAC), consisting of tunica adventitia, media and intima, were tested for neurite outgrowth of SG-explants of neonatal rats in culture. A combination of hydrogels and serum or NF (BDNF (brain-derived neurotrophic factor, 50 ng/ml) and CNTF (ciliary neurotrophic factor, 100 ng/ml)) to support neurite outgrowth was analyzed and compared to a control without additives. Additionally a co-culture of alginate-encapsulated, BDNF over-expressing mesenchymal stem cells (MSCs) and SGN was performed and tested for neuroprotection (survival rate, neuronal morphologies) and guided neurite regeneration (neurite number, length, orientation) and compared to pure alginate.

**Results:** The number and length of outgrowing neurites were increased in stiffer compared to softer alginate. On 2.0% chitosan, cells adhered but regenerated no neurites. In contrast, lower concentrations supported the neurites' growth on and into the gel. The combination of hydrogels and NF, especially CNTF, positively influenced the neurite regeneration. On the dEAC layers neurite outgrowth was most prominent on tunica intima, followed by adventitia and media. Compared to negative control, the encapsulated, BDNF-producing MSCs significantly increased the number of mono- and bipolar SGN and their neurite regeneration but did not attract the neurites.

**Conclusions:** Alginate, chitosan and dEAC are promising matrices for SG neurites to bridge the neuron-electrode gap, especially if they are combined with growth factors for neuroprotection and neurite regeneration, which could be delivered by alginate-encapsulated, NF-producing cells.

**Key Words:** Neurite regeneration, Alginate, Chitosan, Decellularized arteries, Cells-based drug delivery

# Interleukin 6 (IL-6) Contributes to Temporal Induction of Pro-Regenerative State in Uninjured Dorsal Root Ganglia Neurons after Sciatic Nerve Lesions

Petr Dubový<sup>1</sup>; Ilona Klusáková<sup>1</sup>; Ivana Hradilová-Svíženská<sup>1</sup>; Václav Brázda<sup>1</sup>; Marcela Kohoutková<sup>1</sup>; Marek Joukal<sup>1</sup>.

1— Department of Anatomy, Cellular and Molecular Research Group, Faculty of Medicine, Masaryk University

Increased IL-6 expression and STAT-3 activation was detected in uninjured dorsal root ganglia (DRG) neurons no-associated with sciatic nerve lesions (1, 2). The results suggest that IL-6 and its signaling could induce regenerative program in DRG neurons without an axonal injury.

In the present experiments we demonstrated that unilateral sciatic nerve lesion of rats by compression or complete transection induced bilaterally increased levels of GAP-43 and SCG-10 not only in lumbar but also cervical DRG. The increased levels of pro-regenerative proteins in cervical DRG following sciatic nerve lesion was associated with enhanced initiation of axonal regeneration distal to the ulnar nerve crush (UNC). In addition, initiation of axonal regeneration distal to the UNC following sciatic nerve lesion was increased by intrathecal administration of IL-6, but decreased by JAK-2 inhibitor AG490. Neurite outgrowth assay of *in vitro* cultivated DRG neurons also confirmed an enhanced initiation of axon regeneration in medium supplemented with IL-6. Protein levels of SCG-10 and activated STAT-3 in cervical DRG, as well as initiation of axon regeneration distal to the UNC were significantly reduced after sciatic nerve lesion in IL-6 knockout mice compared to their wild-type counterparts.

These results suggest that unilateral sciatic nerve lesion is a conditioning stimulus triggering the neuronal regenerative program in uninjured cervical DRG neurons and IL-6 contributes to initiation of this phenomenon.

## References:

Dubový et al. 2013. *J Neuroinflamm* 10:824. 2. Dubový et al. 2018. *Histochem Cell Biol* 150:37–47.

**Fundings:** Supported by grant No. 16-08508S of The Czech Science Foundation.

**Key Words:** Cytokine, Neuroinflammation, Remote, Conditioning

# Systemic Application of Parthenolide Promotes Functional Nerve Regeneration

Dietmar Fischer<sup>1</sup>; Philipp Gobrecht<sup>1</sup>; Jeannette Gebel<sup>1</sup>; Katharina Wiebe-Ben Zakour<sup>1</sup>.

1 – Department of Cell Physiology, Ruhr University of Bochum

We previously demonstrated that intraneurally-applied parthenolide accelerates axon regeneration in the crushed sciatic nerve almost two-fold via the inhibition of microtubule deetyrosination in axonal growth cones. Here we addressed the questions of whether the systemic application of parthenolide or its more water-soluble derivate di-methyl-amino parthenolide (DMAPT) can mimic intraneural injection and whether this treatment route also improves functional motor and sensory recovery.

In cell culture, we found that DMAPT significantly increased the axonal growth rate of adult sensory neurons, the same as parthenolide. *In vivo*, daily intravenous administration of both parthenolide or DMAPT markedly improved regeneration of sensory, motor and sympathetic axons in the mouse and rat sciatic nerve. This application paradigm also led to an accelerated reestablishment of neuromuscular junctions and functional recovery in both rodent models, thus also showing that the regeneration-promoting effects of these compounds are not restricted to just one species. However, while a continuous oral application of DMAPT accelerated functional recovery, parthenolide did not, indicating its superior oral bioavailability. Furthermore, we also showed that parthenolide not only improves regeneration in the crushed sciatic nerve but also increases axon regeneration in a transected, then surgically reattached nerve. These findings demonstrate the general potential applicability of microtubule deetyrosination agents as pharmacological treatments after peripheral nerve injuries.

# Comparing Neural Stem Cells in Biomaterials as a Potential Therapeutic in Nerve Tissue Engineering

[Lavaniya Thanabalasundaram](#)<sup>1,2,3</sup>; Rubén Deogracias Pastor<sup>3</sup>; John Sinden<sup>2</sup>; James B Phillips<sup>2,4</sup>.

1 – Biomaterials & Tissue Engineering, UCL Eastman Dental Institute, University College London, UK; 2 – UCL Centre for Nerve Engineering, University College London, UK; 3 – ReNeuron Ltd, Pencoed, UK; 4 – Department of Pharmacology, UCL School of Pharmacy, University College London, UK.

**Differentiated stem cells embedded in collagen constructs have been shown to mimic some features of Schwann cells and support regeneration of the peripheral nerve [1-3]. A clinical neural stem cell line (CTX, ReNeuron, UK) in stabilized collagen gel models showed differentiation and an upregulation of glial markers *in vitro* [4,5].**

The aim of this work was to test a range of clinically relevant stem cells in biomaterials, using metabolic and neurite outgrowth assays to compare cell behaviour and identify those with greatest potential for use in nerve tissue engineering.

Stem cell lines of neural and neural retinal origin were embedded in tissue engineered constructs and maintained in culture to assess metabolic activity over 72h. The findings suggest that there are differences between alternative stem cell lines in terms of their metabolism in tissue engineered constructs *in vitro*. In the neurite outgrowth assay, the data suggest differences in the neurite lengths of SH-SY5Y human neuroblastoma cells, transduced to express a fluorescent reporter signal, when in coculture with different tissue engineered constructs.

Characterizing cell activity in this way provides an insight into the cellular changes that take place over time *in vitro* during the manufacture of engineered tissue constructs and potentially ensures their quality for therapeutic purposes.

## References:

1. M. Georgiou et al (2013) *Biomaterials* 34: 7335-43.
2. W. Martens et al (2014) *FASEB J* 28: 1634–1643.
3. M. Georgiou et al (2015) *Biomaterials* 37: 242-251.
4. C. Murray-Dunning et al (2015) *European cells & materials* p1.
5. C. O'Rouke et al (2018) *Scientific Reports* 8, 2951

**Key Words:** Clinical grade stem cells, Tissue engineered construct

# Spider Silk Fibers of *Nephila Edulis* Allow Schwann Cell and Fibroblast Attachment, Proliferation and Migration *In Vitro*

Flavia Millesi<sup>1</sup>; Tamara Weiss<sup>1</sup>; Anda Mann<sup>1</sup>; Christine Radtke<sup>1</sup>.

1 – Medical University Vienna, Department of Plastic and Reconstructive Surgery

The spider dragline silk from the spider *Nephila edulis* has unique characteristics advantageous for nerve regeneration. It is easily harvested, non-immunogenic, long- term degradable and demonstrated to encourage axonal outgrowth and locomotor function. Since the current gold standard in nerve reconstruction, nerve autografts, show disadvantages like longer operating time, a second incision and loss of function at the donor site, nerve conduits filled with spider silk fibers could be a readily available alternative that avoid donor site morbidity.

In this study, we cultured primary rat Schwann cells (rSCs) on spider silk and performed live cell imaging to assess their migratory potential by tracking the cells using ImageJ. In addition, combined SC- and proliferation-marker expression was implemented by establishing multicolor immunofluorescence panels.

The results showed that spider silk serves as an adhesive for SC as they were able to attach to the silk within an hour. Furthermore, live cell imaging demonstrated SC movement as well as the velocity and distance covered by the cells. Multicolor images of SCs stained for S100 in combination with vimentin visualized a culture purity over 90%. In addition, EdU staining demonstrated SC proliferation on spider silk.

As of now, nerve conduits are still inferior to nerve autografts - likely because they lack neurotrophic factors and intraluminal filling as well as viable SCs. Our results showed that spider dragline silk serves as a favorable adhesive for SCs allowing attachment, migration and proliferation, which supports the use of spider silk as valuable guiding material in nerve conduits.

**Key Words:** Schwann cells, Nerve regeneration, Spider silk, *Nephila edulis*

# Rapidly Formed Dense Collagen Gel Scaffolds Seeded with Schwann Cells Promote Peripheral Nerve Regeneration

[Papon Muangsant<sup>1</sup>](#); James Phillips<sup>1,2</sup>; Alison Lloyd<sup>3</sup>.

1 – UCL School of Pharmacy, University College London, UK; 2 – UCL Centre for Nerve Engineering, University College London, UK; 3 – MRC Laboratory for Molecular Cell Biology, University College London, UK

Gel aspiration-ejection (GAE) has recently been introduced as a novel approach to rapidly produce aligned dense collagen hydrogels with tissue-like fibrillar densities and microstructure. In this study, a GAE system was applied to produce aligned dense collagen gels containing aligned Schwann cells for potential nerve tissue engineering applications. The mechanical properties of these gels were investigated. Dynamic mechanical analysis (DMA) revealed that the linear viscoelastic range of these gels was similar to that of rat sciatic nerves (1% strain) and they also showed approximate storage modulus ( $E'$ )/loss modulus ( $E''$ )  $\approx 6$  and less frequency dependence which is commonly seen in stable gels. Schwann cells were shown to survive in the gels after the GAE process. Cellular alignment was achieved and maintained throughout a period of 4 days. This construct supported and directed neuronal growth in a co-culture model *in vitro* with either NG108-15 neuronal cells or dissociated rat dorsal root ganglion (DRG) neurons. *In vivo* testing using a rat sciatic nerve repair model (10 mm gap) showed that, at 4 weeks, these aligned dense collagen gels containing aligned Schwann cells had supported greater neuronal regeneration across the gap than control empty conduits. This demonstrated that aligned dense collagen gels with aligned Schwann cells produced using GAE can be potentially used to promote peripheral nerve repair.

**Key Words:** Gel aspiration-ejection, GAE, Schwann cells, Nerve regeneration, collagen gels

# Session 3

## Artificial Nerve Prostheses: Bioengineering and Bioelectronic Approaches

### **Chairs:**

**Professor Fátima Gärtner**

Molecular Pathology and Immunology Department, ICBAS University of Porto

**Professor James Phillips**

UCL Centre for Nerve Engineering



# A Regenerative Cuff Electrode for the Control of Neuroprosthetic Devices

Bruno Rodríguez<sup>1</sup>; Laura Ferrari<sup>2</sup>; Annarita Cutrone<sup>3</sup>; Alberto Bonisoli<sup>3</sup>; Silvestro Micera<sup>5</sup>; Jaume Del Valle<sup>1</sup>; Francesco Greco<sup>4</sup>; Xavier Navarro<sup>1</sup>.

1 — Group of Neuroplasticity and Regeneration, Department of Cell Biology, Physiology and Immunology, Institute of Neurosciences, Universitat Autònoma de Barcelona, Bellaterra, Spain; 2 — Center for Micro-BioRobotics @SSSA, Istituto Italiano di Tecnologia; 3 — The Biorobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy; 4 — Institute of Solid State Physics Graz University of Technology Petersgasse 168010 Graz Austria; 5 — Bertarelli Foundation Chair in Translational Neuroengineering, Center for Neuroprosthetics and Institute of Bioengineering, School of Engineering, École Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland.

**Loss of function after an amputation or a nerve injury prevents patients from carrying out part of the daily activities. Neuroprostheses are one of the most advanced therapeutic strategies used to improve patients' quality life, that can restore motor function and can even provide sensory feedback. Within a neuroprosthesis, nerve electrodes are a key element as they link the nervous system with the machine. According to their invasiveness a broad number of neural electrodes (extraneural, intraneural and regenerative) have been developed. Among them, regenerative nerve electrodes are limited by its low lumen transparency which can produce nerve compression and chronic pain when the nerve regenerates. In this study, we tested a new regenerative cuff electrode (RnCE) with high transparency based on a flexible polyolefin tube with a conductive polymer (PEDOT:PSS) patterned on the tube lumen. High conductivity exerted by this polymer combined with soft mechanical properties make RnCE an ideal interface for biological systems. First, we tested if the device components were suitable to be used as a regenerative nerve guide. To evaluate functional recovery after nerve section and repair with the RnCE, the sciatic nerve of rats was transected and the bridge was repaired with the tubes of the RnCE. Results of neurophysiological tests (nerve conduction, pinprick, and pain sensibility) for 90 days showed that the RnCE can function as a nerve guide and allow regeneration of the sciatic nerve similar to silicone standard conduits. On the other hand, the possibility to stimulate the sciatic nerve to activate different muscles was investigated with the RnCE. Both motor axons just after nerve section and regenerated motor axons 90 days after section and repair were able to be stimulated with the electrode inducing contraction of the different muscles tested in the paw. These results present a new class of regenerative electrodes that can be used for several applications in terms of nerve stimulation and repair.**

**Key Words:** Neuroprosthesis, Stimulation, Cuff electrode, Regenerative electrode, Sciatic nerve, Peripheral nerve interface, PEDOT:PSS

# Long-Term Functionality of Transversal Intra-neural (TIME) Electrodes is Improved by Dexamethasone Treatment

Jaume Del Valle<sup>1,2</sup>; Natàlia De La Oliva<sup>1</sup>; Bruno Rodríguez-Meana<sup>1</sup>; Matthias Mueller<sup>3</sup>; Thomas Stieglitz<sup>3,4,5</sup>; Xavier Navarro<sup>1</sup>.

1 – Institute of Neurosciences, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, and Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Spain; 2 – Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, Spain; 3 – Laboratory for Biomedical Microtechnology, Department of Microsystems Engineering IMTEK, Albert-Ludwig-University Freiburg, Freiburg, Germany; 4 – BrainLinks-BrainTools Cluster of Excellence, Albert-Ludwig-University Freiburg; 5 – Bernstein Center Freiburg, Albert-Ludwig-University Freiburg, Freiburg, Germany.

Neuroprostheses aimed to restore lost functions after a limb amputation are based on the interaction with the nervous system by means of neural interfaces. Among the different designs, intraneural electrodes implanted in peripheral nerves represent a good strategy to stimulate nerve fibers to send sensory feedback and to record nerve signals to control the prosthetic limb. However, intraneural electrodes, as any device implanted in the body, induce a foreign body reaction (FBR) that results in the tissue encapsulation of the device. The FBR causes a progressive decline of the electrode functionality over time due to the physical separation between the electrode active sites and the axons to interface. Modulation of the inflammatory response has arisen as a good strategy to reduce the FBR and maintain electrode functionality. In this study transversal intraneural multi-channel electrodes (TIMEs) were implanted in the rat sciatic nerve and tested for 3 months to evaluate stimulation and recording capabilities under chronic administration of dexamethasone. Dexamethasone treatment significantly reduced the threshold for evoking muscle responses during the follow-up compared to saline-treated animals, without affecting the selectivity of stimulation. However, dexamethasone treatment did not improve the signal-to-noise ratio of the recorded neural signals. Dexamethasone treatment allowed to maintain more working active sites along time than saline treatment. Thus, systemic administration of dexamethasone appears as a useful treatment in chronically implanted animals with neural electrodes as it increases the number of functioning contacts of the implanted TIME and reduces the intensity needed to stimulate the nerve.

**Key Words:** Dexamethasone, Foreign body reaction, Nerve stimulation, Neuroprosthesis, Intraneural electrode

# Design and Development of an Implanted Biohybrid Device for Muscle Stimulation Following Lower Motor Neuron Injury

Mariann Angola Rojas<sup>1,2</sup>; Nick Donaldson<sup>2</sup>; Henry T. Lancashire<sup>2</sup>; James B. Phillips<sup>1</sup>.

1 – Department of Pharmacology, UCL School of Pharmacy, University College London, London, UK; 2 – Implanted devices group, Department of Medical Physics and Biomedical Engineering, University College London, London, UK

In the absence of innervation caused by lower motor neuron injuries, skeletal muscle undergoes an inexorable course of degeneration and atrophy<sup>1</sup>. The most apparent and debilitating clinical outcome of denervation is the immediate loss of voluntary use of muscle. Electrical stimulation has been implemented as a clinical rehabilitation technique in patients with denervated degenerated muscles offering some remarkable improvements in muscle function<sup>2</sup>. Nevertheless, this approach has multiple limitations and side effects triggered by the delivery of high intensity electrical pulses<sup>3</sup>.

Combining innovative approaches in the fields of cell therapy and implanted electronics offer the opportunity to develop a novel biohybrid device to stimulate muscles in patients with lower motor neuron injuries. We hypothesise that, by incorporating stem cell-derived motor neurons with implanted electrodes, it will be possible to stimulate muscles in a physiological manner and circumvent limitations and side effects associated with direct electrical stimulation of muscle. By having motor neurons as an intermediate between stimulator and muscle, the electrical signal can be converted into a biological action potential and muscle re-innervated via neuromuscular interaction.

We identified a suitable stem cell candidate and developed a differentiation protocol to generate motor neurons. In addition, thick-film printing technology and laser ablation techniques were implemented to manufacture implanted electrode arrays with features in the micrometer scale. Bespoke electrodes were electrochemically characterised and stem cell-derived motor neurons incorporated to create biohybrid devices. *In vitro* results indicated cell survival and neurite outgrowth on implanted electrodes and initial *in vivo* testing has begun to explore their implantation in a rat nerve injury model. This work represents a novel approach for fabricating biohybrid devices that may enhance stimulation of denervated muscles.

## References:

1. Carlson, B. M. (2014). "The Biology of Long-Term Denervated Skeletal Muscle." *European Journal of Translational Myology* 24(1): 5-11.
2. Kern, H., et al. (2002). "Functional Electrical Stimulation (FES) of Long-Term Denervated Muscles in Humans: Clinical Observations and Laboratory Findings." *Basic and Applied Myology* 12(6): 291-299.
3. Pieber, K., et al. (2015). "Optimizing stimulation parameters in functional electrical stimulation of denervated muscles: a cross-sectional study." *Journal of NeuroEngineering and Rehabilitation* 12: 51.

# Session 4

## Genetics and Gene Therapy for Peripheral Nerve Regeneration

### **Chairs:**

#### **Professor Ana Lúcia Luís**

Veterinary Clinics Department, ICBAS University of Porto

#### **Professor Joost Verhaagen**

Laboratory for Neuroregeneration, Netherlands Institute for Neuroscience

# Therapeutic Genome Editing for Charcot-Marie-Tooth 1A

Jae Young Lee<sup>1</sup>

1 – ToolGen Inc.

Charcot-Marie-Tooth 1A (CMT1A) is the most common inherited neuropathy without a known therapy, which is caused by a 1.4 Mb duplication on human chromosome 17, which includes the gene encoding the peripheral myelin protein of 22 kDa (*PMP22*). Overexpressed *PMP22* protein from its gene duplication is thought to cause demyelination and subsequently axonal degeneration in the peripheral nervous system (PNS). Here, we targeted TATA-box of human *PMP22* promoter to normalize overexpressed *PMP22* level in C22 mice, a mouse model of CMT1A harboring multiple copies of human *PMP22*. Direct local intraneural delivery of CRISPR/Cas9 designed to target TATA-box of *PMP22* before the onset of disease, downregulates gene expression of *PMP22* and preserves both myelin and axons. Notably, the same approach was effective in partial rescue of demyelination even after the onset of disease. Collectively, our data present a proof-of-concept that CRISPR/Cas9-mediated targeting of TATA-box can be utilized to treat CMT1A.

**Key Words:** CRISPR/Cas9, Charcot-Marie-Tooth 1A, Gene editing

# DA-VRA1-Congenetic Rats Display Increased Schwann Cell Apoptosis, but Unaffected Nerve Regeneration Compared to Parental DA Rats

Lena Stenberg<sup>1</sup>; Yaobin Yin<sup>1</sup>; Alfredo Dueñas Rey<sup>2</sup>; Maria Swanberg<sup>2</sup>; Lars B Dahlin<sup>1,3</sup>.

1 – Department of Translational Medicine – Hand Surgery, Lund University, Malmö;

2 – Department of Experimental Medicine, Lund University, Lund, Sweden; 3 – Department of Hand Surgery, Skåne university Hospital, Malmö.

**Background:** New cellular and mechanistic insights in nerve injury and repair are needed to improve outcome. The rat *Vra1* locus on chromosome 8 is the first genetic region from where naturally occurring variants can be linked to neurodegeneration. The *Vra1*-congenic strain (DA rats having PVG alleles in *Vra1*) displays a 50% increased motoneuron survival after ventral root avulsion (VRA) compared to parental DA rats. We hypothesize that PVG alleles in the *Vra1* locus are neuroprotective with innate neuronal effects conferring protection to a peripheral nerve injury after repair.

**Methods:** The sciatic nerve was unilaterally transected and repaired with sutures in *Vra1*-congenic and DA rats. After 6 days the nerves were harvested together with dorsal root ganglia (DRG). Axonal outgrowth (neurofilament staining) was measured from the site of repair. Activated (activating transcription factor 3; ATF3) and apoptotic (cleaved caspase 3 staining) Schwann cells, total number of DAPI stained cells and expression of Heat Shock Protein 27 (HSP27) were quantified just distal to the site of repair (where regenerating axons are present) and in distal nerve segment (where axons are not present). Expression of ATF3 and HSP27 were also quantified in DRGs.

**Results:** ATF3 and HSP27 were significantly upregulated in the ipsilateral DRG in both strains. A higher percentage of Schwann cells expressed cleaved caspase 3, i.e. were apoptotic, in the distal sciatic nerve of *Vra1*-congenic compared to parental DA rats. There were no differences between the strains concerning axonal outgrowth, numbers of activated Schwann cells, DAPI stained cells or expression of HSP27 in the distal nerve segment. No differences between the strains were observed in the DRGs.

**Conclusion:** A higher number of apoptotic Schwann cells are present in the distal nerve segment in *Vra1*-congenic strain rats, which harbours neuroprotective alleles, but the global nerve regeneration process is not different from parental DA rats.

**Key Words:** *Vra1*-congenic strain rats, Nerve regeneration, Activated Schwann cells, Axonal outgrowth, Apoptosis

# KIF4 - Studying a Potential Spinal Cord Repair Gene Using Regenerative Peripheral Nerve Injury Models

Patrícia D. Correia<sup>1</sup>; Bárbara M. De Sousa<sup>1</sup>; Jesus Chato-Astrain<sup>2</sup>; Raquel Silva<sup>1</sup>; Veronica Estrada<sup>3</sup>; Hans Werner Müller<sup>3</sup>; Vitor Carriel<sup>2</sup>; Frank Bosse<sup>3</sup>; Sandra I. Vieira<sup>1,4</sup>.

1 – Institute of Biomedicine/iBIMED, Department of Medical Sciences, Universidade de Aveiro, 3810-193 Aveiro, Portugal; 2 – Department of Histology & Tissue Engineering Group, University of Granada, and Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain; 3 – Molecular Neurobiology Laboratory, Department of Neurology, University-Hospital Düsseldorf, Heinrich-Heine-University, Düsseldorf, Germany; 4 – The Discovery CTR, University of Aveiro Campus, 3810-193 Aveiro, Portugal.

**Spinal cord injury (SCI) often leads to loss of motor and sensorial functions and so far has no known effective treatment. However, non-regenerative central nervous neurons can improve their regeneration ability when grown in a more permissive environment, as the peripheral nervous system (PNS) one and/or by modulation of animal SCI models. A meta-analysis of the transcriptomic data of three such experimental studies using manipulated rodent SCI models, previously described in the literature, retrieved us a series of up- and down-regulated genes common to the three studies, that may lead to the observed functional recovery. Amongst them was KIF4A, an anterograde kinesin that is essential during development, but downregulated postnatally. KIF4A is found unaltered in the spinal cord tissue of animals that suffered a spinal cord injury with no further treatment, while it is up-regulated in the above mentioned studies, where rodents regain some lost locomotor functions. The levels and distribution of KIF4A were first analysed in two rat lesion paradigms of the peripheral nervous injury (PNI) - crush and transection of the sciatic nerve. Sciatic nerve crush is naturally followed by spontaneous regeneration, while sciatic transection is less regenerative due to stump suture. Fluorescence immunohistochemical (IHC) stainings demonstrated KIF4A localization in tissue sections of spinal cord, dorsal root ganglia (DRG) and sciatic nerve. KIF4A localized in the axons and in nuclei of neurons, and also in Schwann cells. qRT-PCR experiments revealed that KIF4A is expressed in sciatic nerve and DRG, and that following PNI its expression is induced in both, already at 2dpi. At 7dpi, KIF4 levels increase more than 15 times in the distal stump of the lesioned sciatic nerve. A subsequent IHC analysis targeting the staining and distribution of KIF4A in another regenerative rodent sciatic nerve model, where a collagen conduit is used to bridge a nerve transection, is being performed. Sections of unlesioned spinal cord were also analysed, with KIF4 staining being again observed in neurons' nuclei, potentially correlating with an anti-apoptotic role. Data from all models will be here integrated and discussed, with the aim to characterize this protein and its potential neuroregenerative role.**

**Funding and Acknowledgments:** This work was supported by a) FCT (Portugal), Centro 2020, the COMPETE program, QREN, and the European Union (FEDER program) via Institute for Biomedicine iBIMED UID/BIM/04501/2013, fellowship SFRH/BD/139974/2018 and project GoBack PTDC/CVT-CVT/32261/2017; b) Project: FIS-PI17/393, Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, España.

**Key Words:** Spinal cord injury, Peripheral nerve injury, KIF4, qRT-PCR and IHC

# Satellite Glial Cells in Neuropathic Pain: a RNAseq Study

Sara Jager<sup>1,2</sup>; Franziska Denk<sup>2</sup>; Mette Richner<sup>1</sup>; Nadia Concalves<sup>1</sup>; Lone Pallesen<sup>1</sup>; Stephen McMahon<sup>2</sup>; Christian Vaegter<sup>1</sup>.

1 – Aarhus Univeristy; 2 – King's College London

Neuropathic pain is a chronic condition seen in patients suffering a direct injury to the peripheral or central nervous system or an indirect injury due to, e.g., diabetes. Current treatment options fall short of preventing or completely relieving patients of their pain. To enable development of new and better treatments we need to increase our understanding of the underlying mechanisms responsible for the pathogenesis. For years, research has focused on understanding the role of neurons in neuropathic pain pathogenesis while overlooking the role of supportive cells in general and satellite glial cells (SGCs) in the dorsal root ganglion in particular. These cells not only buffer the neuronal microenvironment they are also believed to be involved in controlling the electrical activity flowing through the neurons and in neuropathic pain pathogenesis. The aim of this project is to understand the role of SGCs in neuropathic pain and thereby aid the identification of new drug targets. The SGCs turned out to be challenging to study with traditional neuroscience methods so we decided to develop a protocol for fluorescently activated cell sorting (FACS). This proved to be difficult, but we finally succeeded in purifying SGCs from adult mice. We then ran RNA sequencing on SGCs after peripheral nerve injury to compare their transcriptome to that of uninjured cells at different time points. The results have confirmed that the SGCs react to peripheral nerve injury and have for the first time identified the regulated genes, which turned out to be involved in cholesterol biosynthesis and immune system regulation. Next, we investigated the immune cells in the dorsal root ganglion with flow cytometry and immunohistochemistry. We found that macrophages take over the physical location of some SGCs after nerve injury. The cellular reorganization of the dorsal root ganglion may have implications for the development of neuropathic pain. Furthermore, in immunohistochemical analysis the reorganization may lead to macrophages being wrongly identified as SGCs after nerve injury in turn resulting in wrong conclusions regarding SGC function.

In summary our results hints that after a nerve injury SGCs may contribute to the development of neuropathic pain by recruiting macrophages. Further research into the SGC functions identified by our research will deepen our understanding of how SGCs contribute to neuropathic pain and can lead to identification of new treatment options.

**Key Words:** Pain, Satellite glial cells, Nerve injury





# Session 5

## The Neurobiology of Axonal Degeneration and Regeneration in the Peripheral Nerve

### **Chairs:**

**Professor Carla Mendonça**

Veterinary Clinics Department, ICBAS University of Porto

**Professor Stefano Geuna**

Department of Clinical and Biological Sciences, University of Torino

# Massive Loss of Proprioceptive IA Synapses in Rat Spinal Motoneurons and Mild Inflammatory Response after a Peripheral Nerve Injury in the Postnatal Period

Sara Bolívar<sup>1,2</sup>; Ariadna Arbat-Plana<sup>1,2</sup>; Xavier Navarro<sup>1</sup>; Francisco J Alvarez<sup>2</sup>; Esther Udina<sup>1</sup>.

1 – Institute of Neurosciences, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Spain; 2 – Department of Physiology, Emory University, Atlanta, Georgia 30322.

Peripheral nerve injuries (PNI) during the postnatal period lead to a massive loss of motoneurons, severely limiting functional recovery. However, not many studies have evaluated the effects of axotomy in surviving motoneurons. In the adult, PNI induces a massive synaptic stripping, mainly due to the loss of proprioceptive Ia afferents that directly synapses with the motoneuron constituting the stretch reflex. This loss has been related to the severity of the injury and to the associated microglia reactivity and neuroinflammation.

The purpose of this study was to characterize central changes after postnatal sciatic nerve injury in 10 days old Sprague Dawley rats. We applied a crush injury, which in the adult leads to a moderate neuroinflammatory response and a mild loss of proprioceptive afferents. We evaluated reinnervation of gastrocnemius (GM) muscle by electrophysiological tests and histological counting of muscular endplates, Ia synapses in back-labeled GM motoneurons and the inflammatory response in the lumbar spinal cord at different time points after injury. Reinnervation of GM muscles was  $68.16 \pm 3.2\%$  at 60 days, with almost full recovery of reinnervated end plates ( $96.67 \pm 3.33\%$ ). At 7 and 14 dpi, there was a massive loss of synapses, mainly VGLUT1, loss that was maintained at 60 days. Microgliosis in the ventral horn was observed at 7 dpi, and decreased over the following weeks. We did not see an upregulation of microglial ED-1 and CCL2 nor infiltration of CD45+ cells, suggesting a mild neuroinflammatory response. Thus, the long-lasting disorganization of central synaptic circuitry was not associated with a strong microglial reaction. These results suggest a different time course or mechanism for deletion of Ia afferent synapses after PNI in neonates compared to adults.

**Key Words:** Peripheral nerve injury, Postnatal, Synaptic stripping, Motoneuron, proprioceptive afferents

# Novel Insight Into the Phagocytic Potential of Human Repair Schwann Cells

Tamara Weiss<sup>1</sup>; Victoria Brandel<sup>1</sup>; Sabine Taschner-Mandl<sup>2</sup>; Rudolf Oehler<sup>3</sup>.

1 – Department of Surgery, Research Laboratory of the Division of Plastic and Reconstructive Surgery, Medical University of Vienna, Vienna, Austria; 2 – Department of Tumor Biology, Children's Cancer Research Institute, Vienna, Austria; 3 – Department of Surgery, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria.

**Introduction:** The remarkable plasticity of Schwann cells (SCs) allows them to transform into dedicated repair cells after peripheral nerve damage. This transient *repair SC phenotype* is specialized to the needs during wound healing such as the autophagic clearance of myelin, attraction of phagocytes and the expression of cell surface proteins and trophic factors to promote axon re-growth and pathfinding. However, there is still a lot to learn about their neuroprotective and regenerative strategies and how those can be exploited to improve therapeutic approaches for nerve regeneration. Especially the accumulating evidence for an immunomodulatory role of SCs holds yet to be determined implications in regenerative medicine. Repair SCs can act as antigen presenting cells via MHC-II, hence, understanding the underlying phagocytic mechanisms and resulting response is of valuable interest to assess how repair SCs affect immune cells after injury.

**Aim:** We aim to investigate the phagocytic competences and resulting inflammatory response of human repair SCs to shed light on their immunoregulatory effect during peripheral nerve regeneration.

**Methods & Results:** We developed a comprehensive phagocytosis assay, comparing receptor mediated and receptor independent phagocytic processes, and established a flow cytometry based read-out that quantified the phagocytic potential of human primary repair SC cultures *in vitro*. The phagocytized material was validated by 3D confocal laser scanning microscopy and an imaging flow cytometer. Furthermore, we visualized the internalization process by live-cell imaging and scanning electron microscopy. Expression data of human repair SCs suggested a receptor mediated phagocytosis pathway similar to microglia, which was confirmed by pathway inhibition experiments.

**Conclusion & Outlook:** We quantified and validated the phagocytic potential of human repair SCs and could demonstrate that it goes beyond the well-known clearance of myelin with yet unknown consequences. Acting as non-professional antigen presenting cells with a profound phagocytic capacity, we expect repair SCs to emerge as important players in regulating inflammatory processes during nerve regeneration. We are currently analyzing the expression of immunomodulatory molecules of human repair SCs in reaction to different stimuli.

**Key Words:** Schwann cell, Phagocytosis, Immunomodulatory, Nerve regeneration

# Regulators of Peripheral Nerve Repair in Schwann Cells

Xinpeng Dun<sup>1</sup>; Carr Lauren<sup>1</sup>; Patricia Woodley<sup>1</sup>; Mindos Thomas<sup>2</sup>; [David Parkinson](#)<sup>1</sup>.

1 – Plymouth University; 2 – Leibniz Institute for Ageing, Fritz Lipmann Institute, Jena, Germany.

Our recent work has focused upon two regulators of peripheral nerve repair in Schwann cells, the tumour suppressor Merlin and the transcription factor Sox2.

Loss or mutation of the Merlin tumour suppressor increases the likelihood that individuals will develop tumours of the nervous system including schwannomas and meningiomas. Using an *in vivo* transgenic mouse model, we have recently shown that loss of Merlin (NF2<sup>-/-</sup>) leads to a complete breakdown in the regenerative capacity of peripheral nerves to repair and that this failure is due to abnormal activity of Hippo signalling through YAP/TAZ effectors in Schwann cells. Loss of the Hippo effectors YAP or TAZ in Merlin null Schwann cells restores PNS repair following injury. We have also studied interactions between Merlin and YAP/TAZ function in peripheral nerve development and myelination.

This model system now allows us to identify signalling pathways on Schwann cells that direct PNS repair following injury in order to attempt to both boost PNS repair and understand processes of schwannoma tumour development in the PNS. We have performed RNA sequencing (RNAseq) from mouse nerves before and after injury. By both identification of differentially regulated genes and signalling pathways that correlate with PNS repair following injury, our objective is to characterise new therapeutic targets to improve nerve repair in patients as well as understanding events in schwannoma tumorigenesis.

Our recent work with Sox2 has shown that it is a major regulator in Schwann cells to mediate axonal pathfinding and nerve repair following nerve transection. Our work has identified signalling by Robo/Slit axon guidance molecules, and regulation of Robo1 by Sox2 in Schwann cells, as key to correct axon regeneration. Following nerve transection a nerve bridge, consisting of Schwann cells, macrophages, nerve fibroblasts and endothelial cells, forms between the proximal and distal nerve stumps.

We find that macrophages form the outermost layer of the nerve bridge and secrete high levels of Slit3, while migratory Schwann cells inside the nerve bridge express the Robo1 receptor in a Sox2-dependent manner. In line with this pattern of Slit3 and Robo1 expression, we observed multiple axon regeneration and cell migration defects in the nerve bridge of Sox2<sup>-</sup>, Slit3<sup>-</sup>, and Robo1-mutant mice. Our findings have revealed important functions for macrophages in the peripheral nervous system, utilizing Slit3-Robo1 signaling to control correct peripheral nerve bridge formation and precise axon targeting to the distal nerve stump following injury.

**Key Words:** Schwann, Merlin, Sox2, Hippo, Robo, Slit

# Direct Peripheral Nerve Repair Animal Models: a Systematic Review of New Millenium Literature

Francisco Javier Vela<sup>1</sup>; Guadalupe Martínez-Chacón<sup>1</sup>; Alberto Ballestín<sup>1</sup>; José Luis Campos<sup>1</sup>; Francisco Miguel Sánchez-Margallo<sup>1</sup>; Elena Abellán<sup>1</sup>.

1 – Jesús Usón Minimally Invasive Surgery Centre

**Background:** Peripheral nerve repair is required after traumatic injuries to solve this common condition that represents a major public health problem. Despite of precise microsurgical repair, nerve lesion has not always a proper functional recovery, therefore, the regeneration process is a subject of research. Pre-clinical animal models are essential for testing these techniques for peripheral nerve repair prior to implementation in clinical practice.

**Objective:** The focus of this systematic review according to PRISMA statement was to analyze the different animal models used from 2000 to these days in pre-clinical peripheral nerve repair studies.

**Data Sources:** A systematic review of English original articles using Web of Science, Scopus and PubMed among 2000 to 2018 was performed.

**Study Selection:** Only preclinical trials about direct nerve repair were included in this review.

**Data Extraction:** The articles were extracted by the two first authors with a pre-defined data fields.

**Data synthesis:** Once the screening was finished 49 records were analyzed in order to study the techniques and the animal models used.

**Conclusion:** Suture techniques are the first option and the gold standard nowadays, but more research in this field is needed because the bioengineering is growing more and more and maybe glues or laser could replace the suture in a nearby future. The animal model to study peripheral nerve repair most used is the rat. Other animal models as rabbit, mice, etc. are also used but the cost benefit of rat is higher. Suture techniques are the first option and the gold standard nowadays, but glues or laser could replace the suture in a nearby future. Hence more research in this field is needed because the bioengineering is growing more and more.

**Key Words:** Peripheral nerve, Animal model, Repair, Anastomosis, Reconstruction, Microsurgery

# The Role of Wnt Signaling Pathway in PNS Regeneration

Benayahu Elbaz<sup>1</sup>; Nikita Mehta<sup>1</sup>; Anna Kolarzyk<sup>1</sup>.

1 – The Department of Neurology, The Center for Peripheral Neuropathy, University of Chicago, Chicago, Illinois 60637, USA.

Peripheral nervous system (PNS) injury initiates a sequence of events, termed “Wallerian degeneration”, in the nerve distal to the site of injury. In these processes, the axons distal to the site of injury degenerate, Schwann cells break down the myelin and with the help of infiltrating macrophages remove the debris by phagocytosis. This is followed by Schwann cell proliferation and guidance of the regenerating axons to their original targets. The re-innervation is accompanied by Schwann cell re-differentiation and remyelination, which leads to functional recovery. With age, Schwann cells lose their ability to activate the transcriptional repair program after injury, fail to undergo dedifferentiation and to mediate myelin clearance, resulting in perturbed PNS regeneration. Therefore, it is of utmost importance that we find ways to enhance the ability of Schwann cells to activate the transcriptional repair program after injury. The Wnt signaling pathway is involved in many developmental processes. In the absence of Wnt ligand, the  $\beta$ -catenin destruction complex mediates the degradation of  $\beta$ -catenin resulting in Wnt signaling pathway inactivation. The  $\beta$ -catenin destruction complex is composed of the proteins Casein Kinase 1 (CK1), Glycogen Synthase Kinase 3 (GSK3), Axin2 and Adenomatous Polyposis Coli (APC). In the presence of Wnt ligand, the  $\beta$ -catenin destruction complex disassociates, resulting in Wnt signaling activation. We have shown that the tumor suppressor gene APC is crucial for Schwann cells for their timely differentiation, lamellipodia formation, processes extension and hence, for developmental myelination of the PNS. Based on the critical role that APC and Wnt signaling play in developmental myelination of the PNS, we are now exploring the role of Wnt signaling in the response of the adult PNS to nerve injury. We find that Wnt signaling pathway is activated in Schwann cells three to seven days post crush injury. Nevertheless, the role of this activation in the repair process is unknown. We are using genetic and pharmacological approaches to explore the role of the  $\beta$ -catenin destruction complex in PNS regeneration, and we are investigating its therapeutic potential as a target for pharmacological intervention in order to enhance PNS regeneration. In humans, PNS regeneration following injury is slow and incomplete. Therefore, in the long term, our research may be beneficial to patients suffering from PNS injury.

**Key Words:** Wnt signaling, PNS regeneration, Schwann cells

# Session 6

## Cellular and Molecular Basis of Axon Guidance and Regeneration

### **Chairs:**

#### **Professor Giovanna Gambarotta**

Department of Clinical and Biological Sciences, University of Torino

#### **Professor Xavier Navarro**

Institute of Neurosciences, Department of Cell Biology, Physiology and Immunology, UAB

#### **Professor Tiago Pereira**

Veterinary Clinics Department, ICBAS University of Porto



# Nerve Fibroblasts Colonizing Tubular Conduits Express High Levels of Soluble Neuregulin1, a Growth Factor Strongly Involved in Peripheral Nerve Regeneration

Benedetta Elena Fornasari<sup>1,2</sup>; Marwa El Soury<sup>1</sup>; Alessia Fucini<sup>1</sup>; Ilaria Lombardo<sup>1</sup>; Giulia Ronchi<sup>1,2</sup>; Alessandro Crosio<sup>3</sup>; Stefania Raimondo<sup>1,2</sup>; Stefano Geuna<sup>1,2</sup>; Giovanna Gambarotta<sup>1</sup>

1 – Department of Clinical and Biological Sciences, University of Torino, Torino, Italy; 2 – Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Torino, Italy; 3 – UO Microchirurgia e Chirurgia della Mano, Ospedale Gaetano Pini, Milano, Italy.

Tubular conduits for peripheral nerve repair have been proven to be an excellent alternative to autografts because they act as physical guidance for the regenerating axons and provide a protective environment for axonal growth. Autograft, being a transplanted nerve, yet contains most of the players involved in Wallerian degeneration and nerve regeneration, while an hollow tube requires to be colonized by Schwann cells, fibroblasts, endothelial cells and so on. It has been shown that nerve fibroblasts *in vivo* colonize hollow conduits early and release extracellular matrix, and that grafted nerve fibroblasts can positively influence Schwann cell behaviour during nerve repair, while *in vitro* primary cultures of nerve fibroblasts release soluble Schwann cell pro-migratory factors, including Neuregulin1 (NRG1). NRG1 is a glial growth factor playing pivotal roles in the peripheral nervous system during development and after injury. In the peripheral nerves, axons express transmembrane NRG1 isoforms which direct Schwann cell myelinating fate, while soluble isoforms are released after injury and promote Schwann cell survival and trans-differentiation. In this study, we investigated the expression of different genes involved in nerve regeneration within an hollow tube. To this purpose, chitosan tubes were used to repair a 10mm nerve gap in rat median nerves and were analysed 7, 14 and 28 days after repair. In the early time points after repair, the expression of nerve fibroblast markers was observed in the hollow tube, while Schwann cell marker expression was barely detectable. Soluble NRG1, which can be expressed by Schwann cells and nerve fibroblasts, was strongly expressed, while the NRG1 co-receptors ErbB2-ErbB3, usually expressed by Schwann cells, were not expressed. To better investigate the expression of NRG1 isoforms and ErbB in nerve fibroblasts, a primary culture of sciatic nerve fibroblasts was obtained from rat and analyzed at mRNA and protein level. Our data show that nerve fibroblasts express high levels of different NRG1 isoforms: soluble,  $\alpha$ ,  $\beta$ , type a and b, while NRG1 receptors are not expressed, thus indicating that nerve fibroblasts signal in a paracrine (and not in autocrine) manner. In conclusions, the presence of different soluble NRG1 isoforms inside the tube in the early steps after injury, suggests that NRG1 released by nerve fibroblasts might play a key role in the following Schwann cell migration inside the tube.

**Key Words:** Neuregulin1, Schwann cell, Nerve fibroblast, Nerve regeneration, Chitosan conduit

# Characterising Cellular and Molecular Features that Underpin the Regenerative Capacity of the Human Peripheral Nervous System

[Matthew Wilcox](#)<sup>1</sup>; [Kristjan Jessen](#)<sup>1</sup>; [Rhona Mirsky](#)<sup>1</sup>; [Tom Quick](#)<sup>1</sup>; [James Phillips](#)<sup>1</sup>

<sup>1</sup> – University College London

Nerve regeneration is a key biological process in those recovering from neural trauma. From animal models it is known that the regenerative capacity of the peripheral nervous system (PNS) relies heavily on the remarkable ability of Schwann cells to undergo phenotypic shift from a myelinating phenotype to one that is supportive of neural regeneration. A great deal is known about the cellular and molecular mechanisms that underpin this process in rodents; a number of transcription factors (c-Jun, Krox-20 and Sox-10) and neurotrophin receptors (such as p75 NTR) are involved. However, ethical and practical challenges associated with studying human nerve injury have meant that little is known about the molecular signals that control human nerve regeneration.

The present study addresses this issue, analysing 27 denervated and healthy human nerve samples liberated during reconstructive nerve procedures. Using immunohistochemistry and Real-Time quantitative Polymerase Chain Reaction (RT-qPCR), the expression of c-Jun, Krox-20, Sox-10 and p75NTR was assessed in denervated samples and compared to healthy nerve.

We found, first, that two major genes associated with repair Schwann cells in rodents, c-Jun and p75NTR, are also up-regulated in acutely injured human nerves, an observation that encourages the view that rodent models are relevant for learning about human nerve injury. Second, as in rodents, the expression of both of these genes declines during long-term denervation. In rodents, diminishing c-Jun and P75NTR levels mark the general deterioration of repair cells during chronic denervation, a process thought to be a major obstacle to effective nerve repair. The down-regulation of c-Jun and P75NTR reported here provides the first molecular evidence that also in humans, repair cells deteriorate during chronic denervation, and provides markers with which this critical process can be monitored.

**Key Words:** Human, Nerve regeneration, Immunohistochemistry, RT-qPCR, Surgery, Nerve transfer, Cellular and molecular mechanisms

# Quantitative Proteomics on Human Peripheral Nerve Biopsies from Elderly Males with Type 2 Diabetes and Healthy Controls

Erik Ising<sup>1</sup>; Emma Åhrman<sup>2</sup>; Niels O.B. Thomsen<sup>1</sup>; Johan Malmström<sup>2</sup>; Lars B Dahlin<sup>1</sup>.

1 – Department of Translational Medicine – Hand Surgery, Lund University, Malmö, Sweden;

2 – Division of Infection Medicine, Department of Clinical Sciences, Lund University, Lund, Sweden.

Type 2 diabetes (T2D) is connected to multiple microangiopathic complications, such as diabetic peripheral neuropathy (DPN). DPN affects several peripheral nerves, where the sural nerve is mostly studied, including biopsies. However, other peripheral nerves, like the posterior interosseous nerve (PIN) at wrist level, are more suitable for a nerve biopsy. Searching for biomarkers connected to DPN, in which degeneration of nerve fibres are prominent and signs of regeneration, such as regenerative clusters, are present, can be time consuming using immunohistochemistry, but with methods like mass spectrometry screening for biomarkers can be performed resulting in vast amounts of data. This in turn leads to new challenges in managing and analysing of data. Our aim was to quantitatively describe the protein profile of PIN from human elderly male T2D subjects and healthy controls, where morphometric data previously have been described, using shotgun liquid chromatography-tandem mass spectrometry (LC-MS/MS). Uncompressed PINs from elderly male T2D subjects (n=9) and healthy controls (n=6) undergoing surgery for carpal tunnel syndrome were harvested and subsequently prepared for morphometry (data previously published) and LC-MS/MS analysis. Characteristics, expressed below as median [quartiles] of subjects and controls, were analysed. Age for controls and T2D subjects, all males, were 72 [67.5 – 74.75] and 74 [74 – 77] years, respectively. No differences in height, weight or BMI ( $p=0.478$ ,  $=0.289$  and  $p=0.140$ , respectively) were seen between controls and T2D subjects. HbA1c for controls and T2D subjects were 28.5 [25.8 – 32] and 40 [29 – 61.5] mmol/mol, respectively ( $p=0.028$ ). A large number of proteins were identified. In total, 2617 different proteins were identified in the PINs. The median [quartiles] number of proteins identified were 2521 [2507 – 2553] for controls and 2555 [2538 – 2567] for T2D ( $p=0.099$ ). LC-MS/MS can be used to determine proteins occurring in human nerves of T2D subjects and healthy controls, where detailed morphometric data of the same nerve biopsies have been published. Comparing the protein profiles of T2D subjects and healthy controls may reveal potential biomarkers associated with DPN, where both degeneration of nerve fibres and regenerative clusters are present.

**Key Words:** Nerve biopsy, Mass spectrometry, Diabetes, Nerve degeneration, Nerve regeneration

# Optimization of RNA Extraction for Comparative Neuronal-Specific Transcription Responses after Central and Peripheral Injuries

Patrícia D. Correia<sup>2</sup>; Sara Ramos<sup>1</sup>; Gabriela Moura<sup>1</sup>; Hans Werner Müller<sup>3</sup>; [Frank Bosse](#)<sup>3</sup>; Sandra I. Vieira<sup>2,4</sup>.

1 – iBiGen – Genomics Facility, iBiMED, Department of Medical Sciences, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal; 2 – Cell Differentiation and Regeneration group, Institute of Biomedicine (iBiMED), Department of Medical Sciences, Universidade de Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal; 3 – Molecular Neurobiology Laboratory, Department of Neurology, University-Hospital Düsseldorf, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany; 4 – The Discovery CTR, University of Aveiro Campus, 3810-193 Aveiro, Portugal

**As injured adult neurons from central nervous system (CNS) fail to regenerate, traumatic spinal cord injury (SCI) has devastating functional consequences. Previous observations that a) regenerative-associated genes (RAGs), expressed upon peripheral nerve injury (PNI), can lead to neuroregeneration, and that b) RAG modulation in CNS neurons can improve their regrow capacity even under an inhibitory environment, paved the way for the identification of targets for CNS repair. The first hours after injury are of extremely importance for this differential response and so it is of main interest to investigate the early transcriptomic changes in the affected neuronal cell populations. Despite various studies that already profiled spinal cord tissue after lesion, motor neurons in the grey matter comprise only <10% of the total cell population, becoming difficult to depict their transcriptional profile from studies of the entire spinal cord.**

The GoBack project aims to gain insight into the main regulatory mechanisms driving the RAG program, by collecting injury-affected neurons by laser capture microdissection and comparing the transcriptional responses of different neuronal populations to different lesion paradigms. The high-throughput RNA sequencing (RNA-Seq) method will be further used to provide insight into the transcriptome of a cell population and discover transcripts highly relevant for the RAG program. The small number of motor neurons in the grey matter raised a challenge on obtaining RNA of quantity and quality enough to deliver reliable results upon RNA sequencing.

We have developed a strategy where spinal cord motor neurons, cortex pyramidal cells from layer V, and sensory peripheral neurons from Dorsal Root Ganglia (DRG) are dissected by laser capture, following spinal cord or sciatic nerve injuries, to compare PNS and CNS neuronal transcriptional responses at early time points. A protocol was developed for the dissection of the cells and total RNA extraction to obtain satisfactory RNA yields and RIN. Upon optimization steps, RNA yields of 6-20 ng with RIN  $\geq 7$  could be obtained from  $\sim 350$  laser-dissected cells. The subsequential RNA sequencing will provide an insight into the RAG regulatory

network and help to identify critical and relevant key regulators, capable to robustly stimulate and improve regeneration in injured neurons.

**Funding and acknowledgments:** This work was supported by a) Fundação para a Ciência e Tecnologia (Portugal), Centro 2020, the COMPETE program, QREN, and the European Union (FEDER program) via Institute for Biomedicine iBiMED UID/BIM/04501/2013, fellowship SFRH/BD/139974/2018 and project GoBack PTDC/CVT-CVT/32261/2017.

**KeyWords:** PNI and SCI, Lasermicrodissection, RNA sequencing, Neuroregeneration

# Proteomic Characterization of Curcumin Beneficial Effect on Rat Sciatic Nerve Regeneration after Crush Injury

Stéphanie Durand<sup>1,4</sup>; Martial Caillaud<sup>2,3</sup>; Emilie Pinault<sup>1,5</sup>; Laetitia Vignaud<sup>2</sup>; Alexis Desmoulière<sup>6</sup>; Fabrice Billet<sup>2</sup>.

1 – EA7500 PEIRENE, University of Limoges, France; 2 – EA6309 Myelin Maintenance & Peripheral Neuropathies, University of Limoges, France.; 3 – Department of Pharmacology and Toxicology, Virginia Commonwealth University, USA; 4 – BISCEM Bioinformatics Platform, University of Limoges, France; 5 – BISCEM Mass Spectrometry Platform, University of Limoges, France; 6 – EA6309 Myelin Maintenance & Peripheral Neuropathies, University of Limoges, France.

We have previously shown that a continuous and local low-dose curcumin treatment accelerates functional recovery and nerve regeneration after sciatic nerve crush (SNC), and that this beneficial effect mainly relies on curcumin ability to protect Schwann cells (SC) from reactive oxygen species (ROS) through direct scavenging. The present study was carried out to confirm this mechanism and to potentially reveal new molecular targets for curcumin. Experiments were performed from sciatic nerve homogenates of rats submitted 35 days earlier to SNC and treated with curcumin (0.2 mg/day for 28 days). By SWATH analysis, a quantitative label-free liquid chromatography mass-spectrometry (LC-MS/MS) approach, 459 proteins mapped to Swissprot or trEMBL Uniprot databases were identified and quantified. Differential expression analyses were conducted using non-parametric approaches (Kruskall-Wallis and subsequent post-hoc test for all pairwise comparisons). Functional enrichment analyses were performed using Gene Ontology terms. The results showed that 89 proteins are significantly altered in at least one of the following groups: sham, SNC/saline and SNC/curcumin. More specifically, the expression of 62 proteins was significantly modified in SNC/saline rats in comparison with sham animals. Functional enrichment revealed that these proteins are mainly related to neurogenesis and axon regeneration. Interestingly, among these 62 proteins, 10 were related to response to oxidative stress, confirming our previous study underlining the presence of ROS production up to 35 days after SNC. In SNC/curcumin animals, the expression of 24 proteins was significantly modified in comparison with SNC/saline group. Among these 24 proteins, 7 showed an expression level similar to that of sham animals (return to normal values), while their expression was altered in SNC/saline animals in comparison with sham group. These 7 proteins appear therefore as closely associated with the curcumin beneficial effect. Functional enrichment indicated that 4 of these proteins are related to response to oxidative stress, thus validating our previous results. Among the 3 remaining proteins, 2 can be associated with structure, adhesion and function of myelinating SC. The function of the last protein is less clear and could be related to myofibroblast activation and/or integrity of

intraneural vascularization. Taken together, these results confirm that the mechanism of curcumin beneficial effect mainly results from its antioxidant properties, which are not limited to a direct ROS scavenging activity. This proteomic analysis also revealed unexpected molecular targets that we will explore in the next future.

**Key Words:** Sciatic nerve crush, Curcumin, Proteomic analysis

# Exploring the Importance of Splenic Nerve Sympathetic Fibers in the Inflammatory Response to Spinal Cord Injury

Susana Monteiro<sup>1</sup>; Rui Lima<sup>1</sup>; orge Cibrão<sup>1</sup>; Andreia Pinho<sup>1</sup>; José Graça<sup>1</sup>; Cláudia Miranda<sup>1</sup>; Margarida Correia-Neves<sup>1</sup>; António Salgado<sup>1</sup>; Nuno Silva<sup>1</sup>.

1 – Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; 2 – ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Spinal Cord Injury (SCI) has a devastating impact on motor, sensory and autonomic functions. One less explored consequence of SCI is the disruption of the immune function. Indeed, SCI patients are more susceptible to infections or autoimmune disorders. Sympathetic nerve fibers innervating lymphoid organs are central for the neural control of immunity regulating important steps of inflammation such as the magnitude and resolution of this response. Curiously, those are the most dysfunctional aspects of the inflammatory response mounted after SCI. The main hypothesis of this study is that SCI affects the central-to-peripheral neural control of immunity consequently hampering the development of a balanced immune response. We focused our study on a particular lymphoid organ – the spleen – since this is the origin of the first infiltrating innate cells at the spinal cord after injury.

We investigated the plastic changes occurring in sympathetic axons terminals of the splenic nerve. We observed a dynamic remodelling of the spleen innervation at 24h, 7d, 15d and 30 days post-injury. Moreover, we also observed an altered profile of expressed genes associated with axonal growth, guidance and repulsion in the spleen, being these alterations also dynamic in time following SCI. Since sympathetic fibers of the splenic nerve are noradrenergic in nature, we measured and found increased levels of norepinephrine in the spleen after SCI. We then dissected the effect of norepinephrine *in vitro*, by stimulating splenocytes with different concentration of this neurotransmitter. We found that neutrophils rapidly respond to norepinephrine by increasing their numbers. To assess the importance of these spleen changes to SCI recovery, we compared the motor, sensory and inflammatory profile of mice submitted to a splenectomy or sham surgery before being exposed to a traumatic SCI. We observed that splenectomised mice present less neutrophil infiltration at the spinal cord together with a better spontaneous motor recovery. This study points for the importance of a peripheral lymphoid organ, the spleen for the modulation of SCI local inflammation shifting the target of immunomodulation from the classic central to the peripheral nervous system. In the future, new therapeutic opportunities may be developed based on this strategy.





# Poster Sessions



# Poster Session 1

## **Chairs:**

### **Professor Giulia Ronchi**

Department of Clinical and Biological Sciences, University of Torino

### **Professor Srinivas Madduri**

Center for Bioengineering and Regenerative Medicine, USB

# 1. A Simple, Reproducible and Cost-Effective Method for *In Vitro* Studying of Cell Migration and Cell-Cell Interactions

Pascal Achenbach<sup>1</sup>; José Luis Gerardo-Nava<sup>1,2</sup>; Haktan Altinova<sup>1</sup>; Antonio Sechi<sup>3</sup>; Joachim Weis<sup>1</sup>; Gary Anthony Brook<sup>1</sup>.

1 – Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany; 2 – DWI-Leibniz-Institute for Interactive Materials, Aachen, Germany; 3 – Institute for Biomedical Engineering – Cell Biology, RWTH Aachen University Hospital, Aachen, Germany.

The extracellular matrix (ECM) plays an important role in influencing cell motility and cell-cell-interactions. Therefore reliable *in vitro* approaches for investigating aspects of cell migration or how particular substrates influence the interactions of between different populations of cells are of substantial interest in many research fields. Since established techniques for such investigations, such as the widely used scratch assay or commercially available silicon inserts sold by different companies did not meet our experimental requirements, we developed a novel, cost-effective and reproducible method for studying cell migration and cell-cell interactions in confrontation assays. This approach uses simple, commercially available elastic bands clamped into a customised petri dish to create either a clean and well defined gap of preserved ECM-coating between identical or different populations of cells or also to set up a straight and well defined cell front which acts as a starting point for migration into the cell-free, substrate-coated territory. In our approach, the elastic band applies pressure to a substrate-coated glass coverslip placed within the petri dish, allowing the separation of cell populations that can be seeded onto each side of the coverslip and separated by the elastic band. After giving the cells sufficient time to adhere to the substrate, elastic bands can be removed, and migration or confrontation behaviour of the cells monitored. Since the ECM substrate is preserved in this approach the assessment of cell migration (in the absence of confounding and often over-looked damage to the substrate) can be monitored. The number and density of cells seeded onto each side of the separator can be readily controlled, allowing studies to proceed under standardized and repeatable conditions. Cell migration from the defined start points can easily be quantified by marking the position of the cell fronts on the lower (non cell-seeded) side of the coverslips. With a little practice the preparation of the constructs and positioning of the elastic bands only takes few minutes per sample, allowing the generation of large numbers of samples. Therefore, our technique may serve as a low-cost alternative to already published techniques and provides the added value of allowing investigations of cell migration and cell-cell interactions in the absence of defects to the substrate.

**Key Words:** Migration assay, Confrontation assay, Gap assay, Two-field assay

## 2. The Phenotype of Schwann Cells Differentiated from Human IPCSs

Rebecca Powell<sup>1,2</sup>; Rickie Patani<sup>1,3,4</sup>; Parmjit Jat<sup>1,5</sup>; Kristjan Jessen<sup>1,6</sup>; James Phillips<sup>1,2</sup>.

1 – UCL Centre for Nerve Engineering, University College London, Gower Street London WC1E 6BT; 2 – Department of Pharmacology, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX; 3 – The Francis Crick Institute, 1 Midland Road, London NW1 1AT; 4 – Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, London, UK; 5 – MRC Prion Unit at UCL, Institute of Prion Diseases, Courtauld Building, 33 Cleveland Street London W1W 7FF; 6 – Department of Cell & Developmental Biology, UCL, Bloomsbury, London WC1E 6AP.

**Background:** Engineered neural tissue (EngNT), made with aligned differentiated human neural stem cells, supports axon regeneration after critical nerve gap injury in rats 1. While therapeutic, those cells were CNS-derived and differed from peripheral nerve Schwann cells. After injury, Schwann cells naturally adopt a repair phenotype that guides and supports axon regeneration 2. By using cell and tissue engineering to generate therapeutic cells that mimic this phenotype we aim to improve the efficacy of EngNT.

**Methods:** Human induced pluripotent stem cells (hiPSCs, UK Cell and Gene Therapy Catapult) were expanded in Essential 8 Flex media before differentiating to Schwann cell precursors and Schwann cells 3. Cells were characterised by RT-qPCR using key markers of pluripotency and Schwann cell differentiation 4. 18S was used as the reference gene.

**Results:** There was a significant 8-fold decrease in mRNA expression of the pluripotency marker Oct4 in Schwann cell precursors and Schwann cells. This occurred alongside a significant, 7-fold increase in mRNA expression of Sox10.

**Conclusions:** The increased mRNA levels of Sox10 indicates that the differentiation protocol induces hiPSCs down a neural-crest lineage. Further characterisation of these cells will determine the phenotype of the Schwann-like cells obtained.

### References:

1. O'Rourke, C. et al. *An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells*. *Sci. Rep.* 8, 2951 (2018).
2. Jessen, K. R. & Mirsky, R. *The repair Schwann cell and its function in regenerating nerves*. *J. Physiol.* 594, 3521–3531 (2016).
3. Kim, H. S. et al. *Schwann Cell Precursors from Human Pluripotent Stem Cells as a Potential Therapeutic Target for Myelin Repair*. *Stem Cell Reports* (2017). doi:10.1016/j.stemcr.2017.04.011
4. Jessen, K. R., Mirsky, R. & Lloyd, A. C. *Schwann Cells: Development and Role in Nerve Repair*. *Cold Spring Harbor perspectives in biology* (2015). doi:10.1101/cshperspect.a020487

### **3. *In Vitro* Analysis of Schwann Cell Expression Profiles within Guiding Regenerative Gel (GRG) for Cell Transplantation into Peripheral Nerve Guides**

Zhong Huang<sup>1,2</sup>; Nina Dietzmeyer<sup>1,2</sup>; Tobias Schüning<sup>1,2</sup>; Svenja Kankowski<sup>1,2</sup>; Shimon Rochkind<sup>3</sup>; Mara Almog<sup>3</sup>; Kirsten Haastert-Talini<sup>1,2</sup>.

1 – Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany; 2 – Center for Systems Neuroscience, Hannover, Germany; 3 – Division of Peripheral Nerve Reconstruction, Department of Neurosurgery, Tel-Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel.

We have been conducting a study evaluating a composite nerve graft containing a novel Guiding Regenerative Gel (GRG) introduced into chitosan nerve guides *in vivo* (see Poster Dietzmeyer et al., this conference). Based on hyaluronic acid (HA), the GRG should remodel the extracellular matrix and was meant to also provide a supportive milieu for the transplantation of naive or genetically engineered Schwann cells (SCs), overexpressing fibroblast growth factor-2 (FGF2-SCs). Our *in vivo* data, however, do not demonstrate any effect of co-transplanted SCs or FGF2-SCs on the regeneration outcome. Therefore, we investigate here, the expression profile of SC or FGF2-SCs cultured in different hydrogels with regard to their repair Schwann cell phenotype. We designed primers to detect the expression of RNA levels for key proteins or transcription factors important for the regeneration process: C-Jun, interleukin (IL)-1beta, IL-6, brain derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), FGF-2 and nerve growth factor (NGF).

Neonatal Schwann cells were obtained from rat pups at three days after birth and purified by removing fibroblasts from the cultures using anti-Thy1-coated magnetic beads. Purified SC cultures at passage 10-15, will be cultured for 3 days in HA, GRG, Matrigel (a hydrogel we previously used to transplant SCs), or gel-free control conditions. Total RNA will be extracted according to the manufacturer's instructions (RNeasy Plus Mini Kit, Qiagen, Germany). The eluate of 12 µl will be used completely for cDNA synthesis using the iScript Kit (BioRad, Germany). Quantitative RT-PCR will be performed with Power SYBR-Green PCR Master Mix (Applied Biosystems) on a StepOnePlus instrument (Applied Biosystems, Darmstadt, Germany).

The same procedures will be applied to FGF2-SCs, which have been obtained from purified SCs from passage 8-11, genetically modified using the nucleofection technique (Basic glial cell nucleofection kit and program T-20 of AMAXA II device, LONZA, Cologne, Germany) to introduce the non-viral plasmid encoding for FGF-2 and cultured for 24 hrs specific culture medium to recover from transfection.

The evaluation is still ongoing and results will be presented on the meeting together with a critical discussion of the value of unstructured GRG for cell transplantation purposes.

**Acknowledgement:** For their excellent technical assistance, we thank Jennifer Metzen and Maïke Wesemann from the Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany.

**Key Words:** Peripheral nerve regenerative, Guiding regenerative Gel, Schwann cell, Expression profile



# 4. *In Vivo* Evaluation of Guiding Regenerative Gel as a Three-Dimensional Matrix Filler for Advanced Peripheral Nerve Guides

Nina Dietzmeyer<sup>1,4</sup>, Shimon Rochkind<sup>2</sup>, Mara Almog<sup>2</sup>, Zvi Nevo<sup>3</sup>, Shimon Rochkind<sup>3</sup>, Kirsten Haastert-Talini<sup>1,4</sup>.

1 – Institute of Neuroanatomy and Cell Biology, Hannover Medical School, Hannover, Germany; 2 – Division of Peripheral Nerve Reconstruction, Department of Neurosurgery, Tel-Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel; 3 – Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 4 – Center for Systems Neuroscience, Hannover, Germany.

This study evaluates a composite nerve graft containing a novel Guiding Regenerative Gel (GRG) introduced into otherwise hollow chitosan nerve guides (CNGs). The GRG in adapted concentration should serve as an internal growth-permissive scaffold and support axonal guidance either mechanically or by remodeling the extracellular matrix, thus providing a neurotrophic milieu. Two-chambered chitosan-film enhanced CNGs (CNG[F]s), which have previously proven to enhance regeneration, displayed the second investigated device. Hyaluronic acid (HA) served as control for GRG, which was eventually loaded with naive or genetically engineered Schwann cells, overexpressing fibroblast growth factor-2 (FGF2-SC). Hereby, we want to explore if the graft containing GRG alone already provides the needed properties and/or if transplantation of naive or FGF2-SC further supports functional regeneration.

15 mm rat sciatic nerve gaps were reconstructed with either the gold standard autologous nerve graft, ANG, (n=6), CNGs or CNG[F]s equipped with 0.2% HA (n=5) or 0.2% GRG alone or combined with naive or FGF2-SC (n=8). Electrodiagnostic recordings of evocable compound muscle action potentials (CMAPs) from the anterior tibial and plantar muscles and Von Frey algometry were performed repeatedly over 120 days. After this period, gastrocnemius and anterior tibial muscles were explanted and muscle weight rations (MWRs) were calculated.

ANG treatment was superior to all artificial nerve guides with evocable CMAPs in 100% of the animals. Groups reconstructed with combinations of CNG[F]s revealed higher recovery rates (37.5-87.5%) when compared to groups treated with combinations of CNGs (0-25%). However, 75% of the animals, which received CNGs combined with FGF2-SC, revealed no signs of regeneration, creating the impression that cell transplantation rather impaired regeneration. This finding was histologically confirmed, as smallest numbers of myelinated fibers were calculated for distal nerve segments of animals with CNGs combined with FGF2-SC. In contrast to the CNG[F] groups, all CNG groups performed significantly worse when compared to the ANG groups.

The present study shows that CNG[F]s filled with GRG alone allow successful target reinnervation. However, adding naive or genetically engineered Schwann cells did not provide any further benefit. Therefore, in an additional attempt, we g<sub>1</sub>

evaluated if GRG has an impact on the expression profile of suspended Schwann cells and eventually silences their repair phenotype *in vitro* (see poster contribution Huang et al., this conference).

**Acknowledgement:** For their excellent technical assistance, we thank Jennifer Metzen, Maïke Wesemann, Silke Fischer, and Natascha Heidrich from the Institute of Neuroanatomy and Cell Biology. Financial support was provided by the German Israeli Foundation for Scientific Research and Development (G-1350-409.10\_2016, to KHT) and the Konrad Adenauer Stiftung (to ND).

**Key Words:** Chitosan nerve guide, Rat sciatic nerve, Functional recovery, Hydrogel, Fibroblast growth factor-2, Cell transplantation

## 5. A Novel Experimental Model to Determine the Axon-Promoting Effects of Grafted Cells After Peripheral Nerve Injury

Takeshi Endo<sup>1</sup>; Ken Kadoya<sup>1</sup>; Tomoaki Suzuki<sup>1</sup>; Yuki Suzuki<sup>1</sup>; Yuki Matsui<sup>1</sup>; Yuan Rufe<sup>1</sup>; Daisuke Kawamura<sup>1</sup>; Norimasa Iwasaki<sup>1</sup>.

<sup>1</sup> – Department of Orthopaedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan.

Although peripheral nerves can regenerate, clinical outcomes after peripheral nerve injuries are not always satisfactory, especially in cases of severe or proximal injuries. Further, autologous nerve grafting remains the gold standard for the reconstruction of peripheral nerves, although this method is still accompanied by issues of donor-site morbidity and limited supply. Cell therapy is a potential approach to overcome these issues. However, the optimal cell type for promoting axon regeneration remains unknown. Here, we report a novel experimental model dedicated to elucidation of the axon-promoting effects of candidate cell types using simple and standardized techniques. This model uses rat sciatic nerves and consists of a 25 mm-long acellular region and a crush site at each end. The acellular region was made by repeated freeze/thaw procedures with liquid nitrogen. Importantly, the new model does not require microsurgical procedures, which are technically demanding and greatly affect axon regeneration. As a result, this model showed great range to detect axon regeneration with minimum variability compared to conventional experimental model using autograft. To test the actual utility of this model, red fluorescent protein-expressing syngeneic Schwann cells (SCs), marrow stromal cells, or fibroblasts were grafted into the acellular area, followed by perfusion of the rat 2 weeks later. All types of grafted cells survived well. Quantification of regenerating axons demonstrated that SCs, but not the other cell types, promoted axon regeneration. Thus, this model is useful for differentiating the effects of various grafted cell types in axon regeneration. Interestingly, regardless of the grafted cell type, host SCs migrated into the acellular area, and the extent of axon regeneration was strongly correlated with the number of SCs. Moreover, all regenerating axons were closely associated with SCs. These findings suggest a critical role for SCs in peripheral nerve axon regeneration. Collectively, this novel experimental model is useful for elucidating the axon-promoting effects of grafted cells and for analyzing the biology of peripheral nerve axon regeneration.

# Poster Session 2

## **Chairs:**

### **Professor Carla Mendonça**

Veterinary Clinics Department, ICBAS University of Porto

### **Professor Petr Dubový**

Department of Anatomy, Masaryk University

## 6. Multiple Sessions of Photobiomodulation Therapy in the Repair of Peripheral Nerve Lesions Using Fibrin Biopolymer

Daniela Vieira Buchaim<sup>1</sup>; Rogerio Leone Buchaim<sup>2</sup>; Karina Torres Pomini<sup>3</sup>; Marcelie Priscila De Oliveira Rosso<sup>3</sup>; Bruna Botteon Della Coletta<sup>3</sup>; Geraldo Marco Rosa Junior<sup>4</sup>; Rui Seabra Ferreira Junior<sup>5</sup>; Benedito Barraviera<sup>5</sup>; Jesus Carlos Andreo<sup>3</sup>.

1 – University of Marilia (UNIMAR) and University Center of Adamantina (UniFAI); 2 – University of Marilia (UNIMAR) and University of Sao Paulo (USP); 3 – University of Sao Paulo (FOB/USP); 4 – University Ninth of July (UNINOVE) and University of Sacred Heart (USC); 5 – Center for the Study of Venoms and Venomous Animals (CEVAP/UNESP Botucatu).

**Injuries or accidents that cause facial paralysis and consequently loss of facial expression cause aesthetic and functional changes that can alter the emotional state and impair the social interaction of the affected individuals. The objective of this study was to evaluate morphologically and functionally the process of end-to-end repair of lesions of branches of the facial nerve repaired by two methods: 10-0 nylon epineural suture or coaptation with the use of fibrin biopolymer derived from snake venom, and to analyze if the use of the photobiomodulation therapy (PBMT) with multiple low-level laser sessions influences this process. 42 male rats were randomly divided into a untreated group (UG; n=10), in which the facial nerve without lesion was collected, and four treated groups: Suture Group (SG) and Biopolymer Group (BG; n=32): Composed of 16 animals, in which the buccal branch of the facial nerve was sectioned on both sides of the face, and on the right side the epineural end-to-end suture was performed and on the left side the fibrin biopolymer was used for coaptation of the extremities; Sutura Photobiomodulation Group (SPG) and Biopolymer Photobiomodulation Group (BPG): Constituted by 16 animals, in which the animals were submitted to the same surgical procedures as SG and BG, associating with the application of multiple laser therapy sessions (wavelength of 830nm, energy density 6.2J/cm<sup>2</sup>, power output 30mW, power density 0.26W/cm<sup>2</sup>, total energy 2.16J; Laserpulse, Ibramed, Brazil). PBMT was applied for 24s/site at 3 points on the skin's surface, performed immediately post-surgery and 3 times a week. The animals were submitted to functional analysis of the whisker movements and euthanized five and ten weeks after surgery for histomorphological analysis of the distal stump of the repaired nerves. The histomorphological analysis showed the growth of axons, predominantly myelinic, into the distal stump of the facial nerve in all treated groups, with a similar appearance to the fibers of the untreated group. The SPG and BPG groups presented better scores on the functional analysis of whisker movements at 5 and 10 weeks post-surgery compared to SG and BG groups. It was concluded that the two techniques allowed the growth of axons**

and that photobiomodulation therapy increased morphological and functional recovery after end-to-end repair of peripheral nerve lesions.

**Key Words:** Photobiomodulation therapy, Low-level laser therapy, Fibrin biopolymer, Nerve regeneration, Facial nerve

## 7. Establishment of an Ovine Model for Induction of Lesions in the Common Peroneal Nerve

Mariana Branquinho<sup>1,2\*</sup>; Rui Alvites<sup>1,2\*</sup>; Catarina Sousa<sup>1,2</sup>; Carla Mendonça<sup>1,2</sup>; Luís Atayde<sup>1,2</sup>; Ana Rita Caseiro<sup>1,2,3</sup>; Sílvia Pedrosa<sup>1,2</sup>; Stefano Geuna<sup>4</sup>; Artur Varejão<sup>5</sup>; Ana Colette Maurício<sup>1,2</sup>.

1 – Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS); 2 – Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Rua D. Manuel II, Apartado 55142, 4051-401, Porto, Portugal; 3 – Escola Universitária Vasco da Gama (EUVG), Av. José R. Sousa Fernandes 197, Campus Universitário – Bloco B, Lordemão, 3020-210 Coimbra, Portugal; 4 – Department of Clinical and Biological Sciences, and Cavalleri Ottolenghi Neuroscience Institute, University of Turin; 5 – Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD); \* Considered as first authors.

**Peripheral Nerve Lesions occur frequently in the modern world, affecting both humans and animals. Despite the efforts and advances made in recent years, it has not yet been possible to establish an effective therapy that promotes the complete and effective regeneration of the injured peripheral nerve and consequent functional recovery [1]. End-to-End (EtE) sutures remain the gold standard method for treating lesions in which complete nerve transection occurred [2], but in recent years several therapeutic alternatives have been developed and explored in order to achieve an ideal method of peripheral nerve regeneration after injury. These new approaches include the use of cell therapies with mesenchymal stem cells and their metabolic products and also the use of biomaterials in the form of nerve conduits with diameters and characteristics adapted to the local, nerve and type of lesion observed [3].**

Most of the studies in the field of peripheral nerve regeneration are performed in rodents, but the anatomical, physiological and behavioral interspecies differences make it difficult to translate the results achieved into clinical practice [4]. The sheep model has been considered as a good option for the transition between rodents and human due to the intrinsic characteristics of these species. Besides being a species with great availability, easy to maintain and to manipulate due to easy temperament, the sheep also presents peripheral nerves with anatomical and physiological characteristics identical to those of humans [5]. In addition, the common peroneal nerve has been insufficiently explored as an injured nerve for further regeneration studies in this species, and access and injury induction protocols have not yet been established [6].

In this work, a surgical protocol for access to the common peroneal nerve in the sheep model was established. Furthermore, axonotmesis and neurotmesis lesion models were established for the same nerve, allowing the application of different therapeutic approaches such as EtE sutures and nerve conduits. The establishment and standardization of this lesion protocol will allow the use of the ovine model for later studies of regeneration of the common peroneal nerve after injury.

**Key Words:** Peripheral nerve injury, Ovine model, Common peroneal nerve

## 8. Analysis of Muscle Recovery after Tubulization Technique in Repair of Peripheral Nerve Lesions

Rogério Leone Buchaim<sup>1</sup>; Cleuber Rodrigo De Souza Bueno<sup>2</sup>; Daniela Vieira Buchaim<sup>3</sup>; Hugo Nary Filho<sup>4</sup>; Leticia Rossi Dare<sup>5</sup>; Daniel Ventura Dias<sup>6</sup>; Mizael Pereira<sup>7</sup>; Geraldo Marco Rosa Junior<sup>8</sup>.

1 – University of Marília (UNIMAR) and University of Sao Paulo (FOB/USP); 2 – University of Sacred Heart (USC/Bauru) and University of Sao Paulo (FOB/USP); 3 – University of Marília (UNIMAR) and University Center of Adamantina (UniFAI); 4 – University of Sacred Heart (USC/Bauru); 5 – Federal University of Pampa (UNIPAMPA); 6 – Federal University of the Minas Gerais Triangle (UFTM); 7 – University of Sao Paulo (FOB/USP); 8 – University Ninth of July (UNINOVE) and University of Sacred Heart (USC/Bauru).

Lesions of peripheral nerves with loss of nervous tissue are common and always cause impairment of parts of the body. The treatment considered gold standard is the autologous nerve graft, although this technique presents a favorable result, it exhibits in some cases limiting factors. The tubulization technique has been shown to be an appropriate and alternative method in clinical practice, providing guidance and an environment conducive to regeneration. Several authors defend the use of venous grafts in the repair of the peripheral nerve gaps, however its functional potentiality is discussed. On the other hand, laminin and collagen considered as trophic factors for the regenerative process are present in the external region of the veins, and in this way, it was thought by inverting the vein and exposing these trophic elements to the graft lumen could generate a more conducive to nerve regeneration. Therefore, the comparison is necessary to obtain answers about the real benefits of inverting the vein at the morphological and functional levels for the target organ. The objective of this study was to compare the results in nerve regeneration using inside-out vein graft and standard venous graft in an attempt to establish which provides better microenvironment for this process of muscle recovery, both morphologically and functionally. 48 rats were randomly divided into 4 groups (n=12 each): denervation control group (DCG), experimental group that received inside-out jugular vein (EGIOV), experimental group that received standard jugular vein (EGSV) and normal control group (NCG). The evaluations were performed in two periods, at 6 and 12 weeks postoperatively, subdividing the groups (n=6) for each studied period. The results were evaluated by morphometric analysis using the smaller diameter and muscle fiber area and the connective tissue area. Functional analysis was performed using the Functional Sciatic Index. The results of morphometry, connective tissue density and functional analysis show that there was no difference between the EGIOV and EGSV groups in the studied muscles and that the soleus muscle suffers a greater atrophy when compared to the extensor digitorum longus muscle (EDL). It was concluded that both the standard and inside-out veins are alternatives for nerve regeneration in tubulization technique and there seems to be no difference in whether to reverse the vein or not in the response of muscle and functional regeneration.

**Key Words:** Nerve regeneration, Sciatic nerve, Muscle, Tubulization technique



# 9. Bioactive Fibrin Conduits for Repair and Reconstruction of Nerve Gap-Injuries

K Prautsch<sup>1,3</sup>, D Schaefer<sup>1,2</sup>, R Guzman<sup>2</sup>, D Kalbermatten<sup>1</sup>, S Madduri<sup>1,2,3</sup>

1 – Department of Plastic, Reconstructive Surgery, University Hospital Basel, Spitalstrasse 21, 4021, Basel, Switzerland; 2 – Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4021, Basel, Switzerland; 3 – Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

**INTRODUCTION:** Repair and reconstruction of the injured nerves is critical and challenging due to lack of efficient therapeutic options (1). Here, we present bioactive fibrin nerve conduits for supporting both trophic and cellular requirements of adult axonal regeneration.

**METHODS:** Adipose derived stem cells (AdSCs) were pre-treated with NGF (AdSC<sup>NGF</sup>) and the resulting secretome was used for treating the dorsal root ganglion (DRG) neuronal cultures. Axonal outgrowth response was analyzed by measuring the length and area. For *in vivo* study, 15mm rat sciatic nerve gap was artificially created<sup>1,2</sup> and reconstructed using the 5 variants of fibrin conduits (FC) and autograft (n=4 per group). Animal response was analysed by measuring anatomical and behaviours recovery at 4 weeks after surgery. The wet-weight of the muscles was measured and nerve tissue was processed for immunostaining and microscopic analysis (1).

**RESULTS:** The secretome derived from AdSC<sup>NGF</sup> promoted significant axonal outgrowth *in vitro*. For animal studies, 5 different conduits were fabricated and coded as FC, FC-AdSC in lumen, FC-AdSC in wall, FC-AdSCNGF in lumen and FC- AdSCNGF in wall, according to the growth factors treatment of AdSC and delivery site of the stem cells. Immunohistochemical analysis of the regenerated nerves revealed the significant regeneration in the proximal nerve segments of animals treated with FCs. These results are comparable to the level of autografts, although the distal nerve regeneration appeared to be incomplete.

**DISCUSSION & CONCLUSIONS:** Secretome of AdSC<sup>NGF</sup> showed synergistic effect on axonal regeneration. Although regenerating axons within the 4 weeks could not cross the 15 mm long gap, enhanced axonal regrowth in proximal part of the nerve shows the potential of bioactive fibrin conduits for bridging the long-gaps injuries. Thus, the fibrin conduits for treating long nerve gaps will be further evaluated in a long-term study.

## References:

(1) Madduri. et al., Biomaterials. 2010, 31:2323-34

# 10. Distinct Effects of Neurotrophic Factors on Axonal Growth and Elongation

K Prautsch<sup>1,3</sup>, L Degrugillier<sup>1,3</sup>, D Schaefer<sup>1,2</sup>, R Guzman<sup>2</sup>, D Kalbermatten<sup>1</sup>, S Madduri<sup>1,2,3</sup>

1 – Department of Plastic, Reconstructive Surgery, University Hospital Basel, Spitalstrasse 21, 4021, Basel, Switzerland; 2 – Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, 4021, Basel, Switzerland; 3 – Department of Biomedical Engineering, University of Basel, Gewerbestrasse 14, 4123 Allschwil, Switzerland

**INTRODUCTION:** The promotion of neuronal survival and axonal regrowth is crucial in order to ensure peripheral nerve regeneration and has therefore been subject of intensive research during the past 20 years. One of the factors that drive neurite elongation are neurotrophic factors (NTF) which are retrogradely transported from the injured axonal tip to the neuronal cell soma. In this *in vitro* study, these survival and growth stimulatory factors were to be investigated for their neurotrophic potential on chicken embryonic dorsal root ganglia (DRG).

**METHODS:** DRG explants from 10 days old chicken embryos were isolated and cultured in culture medium enriched with either nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3) or neurotrophin-4 (NT-4) at a concentration of 10ng/ml. As control, DRG explants were grown in NTF-free culture medium. After 48h of incubation time, axonal outgrowth was visualized by tubulin beta-III immune staining and the growth response was quantitatively analyzed by measuring the length and area of neurite outgrowth<sup>1,2,3</sup>.

**RESULTS:** Striking differences can be observed in the axonal outgrowth of DRG explants for distinct experimental conditions in terms of axonal count, elongation, density and branching. Interestingly, NT-3 and BDNF exerted the most potent neurotrophic effect as evidenced by axonal length and growth-area when compared to all the other conditions.

**DISCUSSION & CONCLUSIONS:** This *in vitro* study revealed the regenerative potential held by NT-3 and BDNF that even exceeded the stimulatory effect of potent and widely acknowledged growth factors such as NGF and GDNF. Further experiments in order to study the growth kinetics and the cell survival rate of NT-3 and BDNF treated neuronal cells are in progress.

## References:

- [1] Madduri. et al., Biomaterials. 2010, 31:2323-34
- [2] Madduri. et al., Biomaterials. 2010, 31:8402-09
- [3] Madduri. et al., Journal of controlled release. 2010, 143:168-174



# Poster Session 3

## **Chairs:**

### **Professor Giulia Ronchi**

Department of Clinical and Biological Sciences, University of Torino

### **Professor Srinivas Madduri**

Center for Bioengineering and Regenerative Medicine, USB

# 11. Characterization of the Cellular and Molecular Mechanisms that Mediate Perineurial Glial Bridging Following Peripheral Nerve Injury

Kimberly Arena<sup>1</sup>; Sarah Kucenas<sup>1</sup>.

1 – University of Virginia.

Motor nerves are necessary for organismal locomotion and survival. In zebrafish and most vertebrates, these peripheral nervous system (PNS) axons naturally regenerate following injury. However, the cellular and molecular mechanisms that mediate this process are poorly understood. Our lab has demonstrated that one crucial cell type—perineurial glia (PG)—is necessary for the earliest regenerative steps by establishing glial bridges across the site of injury that are later followed by axon regrowth. Intriguingly, without PG bridging, regeneration is impaired. In addition to PG, Schwann cells (SC), the cells that ensheath and myelinate the nerve, have their own injury response that is essential for debris clearance and axon guidance. Previous studies from our lab demonstrate that in the absence of SC, PG exhibit perturbed bridging, suggesting that there is communication occurring between these two cell types during the injury response. While the presence and importance of these glial bridging events is known, the mechanisms that underlie them remain a mystery. Understanding the cellular and molecular interactions that drive PG bridging is crucial to unlocking the mechanisms underlying successful motor nerve regeneration. The purpose of this proposal is to elucidate the mechanisms that mediate PG bridging following motor nerve injury by investigating: (1) the role of transforming growth factor beta-1 (TGFB-1) in PG bridging, (2) additional factors involved in the PG injury response, and (3) the physical interactions between PG and SC following injury. I will utilize zebrafish (*Danio rerio*) as a vertebrate model system to investigate the mechanisms that underlie PG bridging. Zebrafish are an optimal model for studying nerve regeneration due to their ability to regenerate nerves naturally, easy genetic and pharmacological manipulation, and unrivaled *in vivo* imaging. Using pharmacological and genetic perturbation, antibody staining, laser axotomy, and *in vivo* imaging I will analyze the effect TGFB-1 has on PG bridging and elucidate the physical interactions between PG and SC during motor nerve regeneration. Together, my studies will provide further insight into the cellular and molecular mechanisms driving regeneration of motor axons in the PNS and the crucial role that glial cells play in this process. Dissecting these mechanisms could provide novel targets for developing therapies for patients with spinal cord injuries, neurodegenerative diseases, or nerve damage.

**Key Words:** Glia, Peripheral nerve regeneration, Zebrafish

# 12. Effect of Low Oxygen Exposure on the Survival of Therapeutic Cells

Despoina Eleftheriadou<sup>1</sup>; Dr Rebecca J Shipley<sup>2</sup>; Dr James B Phillips<sup>3</sup>.

1 – 1UCL Centre for Nerve Engineering, London, UK., 2 Department of Pharmacology, UCL School of Pharmacy, London, UK. 3 UCL Department of Mechanical Engineering, London, UK; 2 – 1UCL Centre for Nerve Engineering, London, UK., 3 UCL Department of Mechanical Engineering, London, UK; 3 – 1 UCL Centre for Nerve Engineering, London, UK., 2 Department of Pharmacology, UCL School of Pharmacy, London, UK.

**Introduction:** Despite recent developments in microsurgical techniques and increase in knowledge regarding the physiopathology of peripheral nerves, repair of critical gap injuries remains a clinical challenge. Recent studies have focused on the potential of tissue mimetic *scaffolds*, that combine therapeutic cells with biomaterials, in encouraging nerve regeneration<sup>1 2</sup>. One of the major determinants of the regenerative success of cellular nerve repair constructs is the local microenvironment, particularly the native low oxygen conditions which can affect implanted cell survival<sup>3</sup>. Nevertheless, most *in vitro* studies assessing the behaviour and pro-regenerative capacity of engineered nerve constructs, have been performed at conventional 21% oxygen concentrations. Here, we aim to evaluate differences in cellular behaviour under controlled oxygen conditions, including key biological features such as oxygen metabolism, proliferation and death.

**Methods:** Clinically relevant neural stem cells (CTX0E03, ReNeuron Ltd, UK) were embedded in thin, stabilized collagen constructs to represent the implanted tissue structure, at a range of cell densities (0.5- 2 10<sup>6</sup>cells/ml) and subsequently cultured at a physiologically-relevant range of oxygen concentrations (1-7%) as well as standard atmospheric oxygen conditions. The impact of these culture conditions on cell survival and proliferation was determined via live/dead staining, flow cytometry and CellTiter-Glo metabolic assay. Oxygen consumption was measured using an optical fluorescence sensor.

**Results & Conclusions:** Cells responded to local oxygen environments with oxygen- and density-dependent changes in their survival. Hypoxia-induced death was more pronounced at lower oxygen concentration (1%) and higher density (2 10<sup>6</sup>cells/ml) and correlated with a metabolism-related increase in oxygen consumption. Together, these results suggest that oxygen plays a critical role in the regulation of physiological processes and highlight the importance of studying cellular function under conditions that closely mimic those of the native microenvironment.

**References:**

1. Sarker, M., Saman, N., McInnes, A. D., Schreyer, D. J., & Xiongbiao, C. (2018). *Regeneration of peripheral nerves by nerve guidance conduits: Influence of design, biopolymers, cells, growth factors, and physical stimuli*. *Progress in Neurobiology*, (February), 1–26.
2. Rourke, C. O., Day, A. G. E., Thanabalasundaram, L., & Cowan, J. (2018). *An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells*, (January), 1–11. <https://doi.org/10.1038/s41598-018-20927-8>
3. Lim, T. K. Y., Shi, X. Q., Johnson, J. M., Rone, M. B., Antel, J. P., David, S., & Zhang, J. (2015). *Peripheral Nerve Injury Induces Persistent Vascular Dysfunction and Endoneurial Hypoxia, Contributing to the Genesis of Neuropathic Pain*. *The Journal of Neuroscience*, 35(8), 3346 LP-3359.

**Key Words:** Controlled oxygen, Cell survival, Metabolism, Therapeutic cells, Peripheral nerves

# 13. Serotonin and Glutamate Requirements during Nerve Regeneration in Planarian Flatworms

Mohamed Ali<sup>1</sup>; Ellie Mcguire<sup>1</sup>; Anthony J Walker<sup>1</sup>; [Francesca E Mackenzie](#)<sup>1</sup>.

1 – Kingston University London.

Planaria (a type of flatworm) share many neuronal proteins with humans, but remarkably can very successfully regenerate their nervous system after injury, making them a useful model with which to study neuronal regeneration. Recent studies e.g.<sup>1</sup> have suggested that classical neurotransmitter proteins have other roles during neuronal regeneration outside of their functions in neural synaptic transmission. We examined the requirement for classical neurotransmitters in planarian neural regeneration. Pharmacological disruption of the serotonin and glutamate pathways in planaria (*Dugesia japonica* and *Schmidtea mediterranea*) caused abnormal locomotion and movement, as expected, and additionally showed varied effects on head/CNS or eye regeneration in transected or eye-ablated planaria. Planaria are a useful invertebrate model that may provide insights into how human neuroregeneration may be encouraged.

## References:

<sup>1</sup>Romaus-Sanjurjo, D et al (2018) *Cell Death & Disease* 9: 663

**Key Words:** Serotonin, Glutamate, Planaria, Model, Invertebrate, Genetics



# 14. Attempted Repair of 15mm Experimental Adult Rat Sciatic Nerve Lesions with Orientated Nanofiber-Fibrin Hydrogel-Containing Conduits

Brook G.A<sup>1</sup>; Hodde D.<sup>1</sup>; Gerardo Nava<sup>2</sup>; Altinova H.<sup>1</sup>; Steinbusch H. W.<sup>3</sup>; Moller M<sup>2</sup>; Mey J.<sup>4</sup>; Achenbach P.<sup>1</sup>; Weis J.<sup>1</sup>.

1 – Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany; 2 – DWI-Leibnitz-Institute for Interactive Materials, Aachen, Germany; 3 – European Graduate School of Neuroscience, University of Maastricht; 4 – Hospital Nacional de Paraplejicos, Toledo, Spain.

Peripheral nerve injuries (PNI) that cause gaps larger than a few millimeters require the interposition of a bridging material in order to support axon regeneration and tissue repair. The current gold standard treatment for such larger gaps, the autologous nerve graft, is associated with significant limitations and does not guarantee a useful degree of functional recovery. Recent advances in tissue engineering have opened up novel opportunities towards the development of alternative strategies to replace the autograft. The generation of complex three-dimensional (3D) *scaffolds* capable of mimicking the molecular and topographical cues of the extracellular matrix (ECM) found in native tissue is expected to improve the efficacy of clinically-approved materials. The aim of the present study was to develop a biomimetic, nanofiber-based implantable nerve guide intended to enhance functional recovery in an *in vivo* model of PNI. Electrospun arrays of orientated polycaprolactone (PCL) nanofibers were stacked in multiple layers and embedded in a fibrin hydrogel. The nanofiber-hydrogel composite (with or without autologous Schwann cells seeding) was then loaded into a hollow collagen conduit, implanted into a 15mm resection injury of the adult rat sciatic nerve. The integration and performance of the implant was assessed using morphological and functional measures over a period of 3 months. The experimental design compared implants of nanofiber-hydrogel containing conduits with simple hollow entubulation, autografted sciatic nerve and no repair. There was remarkable spatial reorganisation of seeded Schwann cells and regenerating axons within the scaffold during the study. The presence of orientated nanofibres within the fibrin hydrogel and autologous Schwann cells promoted a degree of repair that was similar to the autograft in terms of the numbers of animals capable of showing evoked compound muscle action potentials of the affected hind-paw, and that these numbers were greater than those of the non-cell seeded implant and the simple hollow conduit but was inferior to that of the autograft. However, for almost all other morphological and behavioural parameters, the autograft proved to be the best. Although these nanofiber-based conduits were incapable of reaching the performance of that supported by the autograft, future modifications such as the choice of hydrogel may offer the possibility of optimizing scaffold characteristics and promoting greater nerve regeneration.

# 15. *Ex Vivo* Characterization of Genipin-Crosslinked Decellularized Peripheral Nerve Allografts

Od García-García<sup>1</sup>; M El Soury<sup>2</sup>; J Chato-Astrain<sup>1</sup>; A Irastorza-Lorenzo<sup>1</sup>; Od Durand-Herrera<sup>1</sup>; D Sánchez-Porras<sup>1</sup>; M Alaminos<sup>1</sup>; S Geuna<sup>2</sup>; F Campos<sup>1</sup>; V Carriel<sup>1</sup>

1 – Department of Histology & Tissue Engineering Group, University of Granada, Granada, Spain; 2 – Department of Clinical and Biological Sciences, San Luigi Gonzaga Hospital, University of Turin, Turin, Italy.

Nowadays, an efficient alternative to the use of nerve autograft in peripheral nerve repair is still needed and decellularized nerve allografts (DPNA) emerged as a potential alternative for these patients. Recent studies demonstrated that the decellularization process affect the 3D structure and extracellular matrix with a negative impact on the biomechanical properties of DPNAs [1,2]. In this sense, the aim of this study was to investigate the impact of the crosslinking with Genipin (GP) on the histological pattern and biomechanical properties of DPNAs.

For this study, Wistar rats' sciatic nerves were decellularized with Sondell (SD, 3% Triton X-100 and 4% of sodium deoxycholate) and our new combined technique (CT, 1% Triton X-100 and a mix of DNase, RNase and Trypsin) [2]. DPNAs were then crosslinked with GP (0.1 and 0.25%) [3] and compared to non-crosslinked SD and CT DPNAs used as controls. All DPNAs and native nerves were subjected to tensile test (Instron) and processed for histology.

Tensile test revealed that the use of both concentration of GP significantly increased the stress at fracture as compared to controls ( $p < 0.05$ ). The Young modulus was significantly increase with CT, but not in SD group. In addition, biomechanical differences between the use of different concentrations of GP were not statistically significant ( $p > 0.05$ ). Histology revealed a successful decellularization with both decellularization techniques (SD and CT) which once the DPNAs were subjected to GP crosslinking process histology demonstrated a proper preservation of the 3D histological organization of the peripheral nerves. Furthermore, histochemistry confirmed the high content of collagens and partial removal of some proteoglycans.

Finally, this *ex vivo* biomechanical and histological study demonstrate that the use of GP could be a promising alternative to improve the biomechanical properties of SD and CT DPNAs. The use of GP did not altered the 3D structure and ECM composition, however, further *in vivo* preclinical studies are still required to demonstrate the potential usefulness of these crosslinked DPNAs in peripheral nerve repair.

**Fundings:** FIS-PI17/393, Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, España.

**References:**

1. *Front Cell Neurosci* (2018). doi: 10.3389/fncel.2018.00427.
2. *Ann Biomed Eng* (2018). doi:10.1007/s10439-018-2082-y
3. *Biomed Mater.* 2018 Feb 8;13(2):025021.

**Key Words:** Nerve allograft, Chemical decellularization, Histology, Biomechanical properties, Natural biomaterials

# Poster Session 4

## **Chairs:**

### **Professor Carla Mendonça**

Veterinary Clinics Department, ICBAS University of Porto

### **Professor Petr Dubový**

Department of Anatomy, Masaryk University

# 16. Local Immunosuppression for Peripheral Nerve Allografts with Zero-Order Drug Eluting Materials

Victoria Robertson<sup>1,2</sup>; Holly Gregory<sup>1,2</sup>; Ukrit Angkawinitwong<sup>1,2</sup>; Gareth Williams<sup>1,2</sup>; James Phillips<sup>1,2</sup>

1 – UCL School of Pharmacy, , 29-39 Brunswick Square WC1N 1AX, London, UK; 2 – UCL Centre for Nerve Engineering, University College London, WC1E 6BT, London, UK.

Repair of nerve gap injuries is currently achieved through nerve autografting which, although successful, is affected by limited tissue supply and donor site morbidity. An attractive alternative is to use allografted nerve from organ donors for gap repair, providing an abundant tissue source. However, delivering a nerve allograft requires immunosuppression of the patient to prevent rejection of therapeutic donor derived cells within the transplanted nerve. Currently systemic immunosuppression is avoided in non-life-threatening conditions such as peripheral nerve injury due to associated side effects. Using novel material formulations to allow local delivery of drugs at the site of transplantation can potentially avoid systemic effects of immunosuppression. We have successfully encapsulated the immunosuppressant tacrolimus (FK506) as a core within poly- $\epsilon$ -caprolactone (PCL) shell fibers using an optimised method of coaxial electrospinning to achieve zero-order release kinetics<sup>1</sup>. Tacrolimus-loaded fiber sheets were characterised using scanning electron microscopy (SEM), thermal methods and X-ray diffraction. Drug release *in vitro* was assessed over a 12 day period using UV-Vis spectroscopy showing zero-order release characteristics and no evidence of burst release. Fiber sheets (1cm<sup>2</sup>) were subsequently assessed *in vivo* by implanting into a rat sciatic nerve allograft model to demonstrate proof of concept for this approach. Male Lewis rats (haplotype RT1<sup>l</sup>) received sciatic nerve autografts, or allografts from male Dark Agouti (haplotype RT1<sup>av1</sup>) donors. Nerve grafts were wrapped with either tacrolimus-loaded or control fiber sheets prior to wound closure. Nerves were harvested at 3 and 8 weeks post-transplantation (n=4 per group per time-point) and the host immune response and extent of regeneration were assessed by electrophysiological assessment of nerve conduction and histological analysis. Implanted fiber sheets were well tolerated by the animals with no adverse effects in those receiving drug-loaded or control fibers. At 3 weeks post-transplantation increased T cell infiltration was found in nerve allografts compared to autografts, which was reduced in allografts wrapped with tacrolimus-loaded fibre mats. Electrophysiology at 8 weeks post-transplantation showed return of nerve conduction continuity in all animals.

**References:**

1. Angkawitwong, U., Awwad, S., Khaw, P. T., Brocchini, S. & Williams, G. R. Electrospun formulations of bevacizumab for sustained release in the eye. *Acta Biomater.* 64, 126–136 (2017).

**Key Words:** Allograft, Biomaterials, Immunosuppression, Transplantation, Controlled drug release

# 17. Generation and Characterization of a New Decellularized Peripheral Nerve Allograft: a Possible Future Alternative for Peripheral Nerve Repair

Marwa El Soury<sup>1,2</sup>; Òscar Dario Garcia Garcia<sup>2</sup>; Stefano Geuna<sup>1,3</sup>; Arianna B Lovati<sup>4</sup>; Marta Bottagisio<sup>4</sup>; Alessandro Crosio<sup>5</sup>; Antonio Campus<sup>2</sup>; Simonetta Odella<sup>5</sup>; Pierluigi Tos<sup>5</sup>; Ferdinando Campos<sup>2</sup>; Victor Carriel<sup>2</sup>; Stefania Raimondo<sup>1,3</sup>.

1 – Department of Clinical and Biological Sciences, San Luigi Gonzaga Hospital, University of Turin, Turin, Italy; 2 – Department of Histology & Tissue Engineering Group, University of Granada, and Instituto de Investigacion Biosanitaria, Ibs.GRANADA, Granada, Spain; 3 – Neuroscience Institute Cavalieri Ottolenghi (NICO); 4 – Cell and Tissue Engineering Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; 5 – Reconstructive Microsurgery and Hand surgery Unit, ASST Pini-CTO Milano, Italy.

Many efforts are being done to improve the process of peripheral nerve regeneration following injury. Nerve autografts remains the gold standard for nerve repair. An alternative to autografts is needed to overcome the accompanied limitations of this technique such as second operation and donor site morbidity. In this context, attention has been given to bridge the nerve gap using allografts from donor cadaver. Immunosuppressants were supplied to overcome the adverse immune response and rejection of the allograft but it could be dangerous to a patient's life. Decellularization is a promising solution, where nerve allografts are processed to get rid of the existing antigenic components of the cells in a nerve grafts ,yet retaining the extracellular matrix as a natural scaffold for peripheral nerve regeneration [1-3].

In this study, we generated and characterized *in vitro* decellularized peripheral nerves from rats and human. A new decellularization technique that was originally optimized for tendons was applied on these nerves and was compared to another protocol that was published before in literature. Light and transmission electron microscopy (TEM) analysis, resistance to mechanical tension, DNA quantification and histology analysis were performed on rat samples, while a preliminary light and TEM analyses were performed in human samples.

The new protocol showed a better conservation of the extracellular matrix structure with the presence of integral endoneuronal tubes, the myelin was destroyed in both protocols, but axons were absent only in the new one. It also showed a higher resistance for mechanical tension and a lower quantity of DNA.

It is planned to perform the other analysis on human samples too, and in the future *in vivo* analysis could be performed to test the efficacy of these nerves as a possible allograft for bridging large nerve gaps.

**Fundings:** FIS-PI17/393, Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, España, and by the Fondazione Cassa di Risparmio di Torino (Turin, Italy), protocol 702 number 2017.AI190.U219, RF: 2016.2388.

**References:**

1. *Front Cell Neurosci* (2018). doi: 10.3389/fncel.2018.00427.
2. *Ann Biomed Eng* (2018). doi:10.1007/s10439-018-2082-y  
J Neural Eng. 2018 Apr;15(2):021003.

**Key Words:** Decellularization, Allografts ,Peripheral nerve regeneration, Nerve repair



# 18. Maturation of Perineuronal Nets Around Spinal Motoneurons in Postnatal Rats and After Peripheral Nerve Injury

Delphine Morisio<sup>1</sup>; Ariadna Arbat-Plana<sup>1</sup>; Judith Sánchez-Ventura<sup>1</sup>; Xavier Navarro<sup>1</sup>; Esther Udina<sup>1</sup>.

1 — Institute of Neurosciences, Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Spain.

It is well known that peripheral injuries during the postnatal period, in contrast to adulthood, induce a massive loss of spinal motoneurons. However, less is known about the effects of these injuries on survival motoneurons. Perineuronal nets (PNN) are an extracellular matrix that encapsulates the soma of most motoneurons. Since PNN have been deeply studied in the brain, it is known that their formation is related to the end of the critical period during development, favouring the stabilization of the circuitry in the mature brain. As physical exercise increases PNN around spinal motoneurons, the progressive increase of activity in motoneurons during the postnatal period would facilitate the maturation of these nets.

The aim of our study is to evaluate the development of spinal PNN in postnatal rats and how nerve injury is affecting their normal maturation when applied during this period. Therefore, we applied a crush injury at different postnatal days, and we evaluated motoneuron death and characterised PNN by means of different immunomarkers in the lumbar spinal cord.

Considering motoneuron death, the most significant loss occurred in animals lesioned at postnatal day 2 (P2) compared to animals injured at P21 ( $54\pm 17\%$  vs  $16\pm 9\%$ ) and P14 ( $54\pm 17\%$  vs  $22\pm 10\%$ ).

Regarding the normal maturation of PNN, immunoreactivity to WFA, a general marker for PNNs, started to be visible at P7-P9, increased at P14, P21, P28 and became highly defined at P35. Aggrecan, the most PNN-specific lectican marker, began to be apparent at low levels at P14, increased at P21 and was abundant at P28 and P35. Link protein, another component of PNN, could be detected at P21 and increased in intensity at P28 and P35. After injury, an important reduction of PNN around axotomized motoneurons could be observed. Motoneurons that survived to injury at 10 postnatal days, did not have clear WFA immunostaining 14 days afterwards (P24), thus indicating that axotomy was affecting the normal development of spinal PNN. These motoneurons also suffered an important synaptic stripping, mainly from glutamatergic synapses, with a compensatory increase of inhibitory GABAergic ones. Whereas normal PNN could be observed 60 days after injury in axotomized motoneurons, the altered synaptic content was maintained.

**Key Words:** Perineuronal nets, Peripheral nerve injury, Postnatal, Development

# 19. *In Vivo* Peripheral Nerve Regeneration with Decellularized Nervous Matrix

Sara Bolívar<sup>1</sup>; [Estefania Contreras](#)<sup>1</sup>; Núria Nieto-Nicolau<sup>2</sup>; Patricia López-Chicón<sup>2</sup>; Oscar Fariñas<sup>2</sup>; Anna Vilarrodona<sup>2</sup>; Ricardo Casaroli-Marano<sup>2</sup>; Xavier Navarro<sup>1</sup>; Esther Udina<sup>1</sup>

1 – Department of Cell Biology, Physiology and Immunology, Institute of Neurosciences, Universitat Autònoma de Barcelona, and Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Barcelona, Spain; 2 – Barcelona Tissue Bank (Banc de Sang i Teixits) – IIB Sant Pau, Barcelona, Spain.

**Autografts are the current gold standard for repairing peripheral nerve injuries, despite presenting several drawbacks such as limited length, loss of function of the donor nerve and morbidity in the extraction area. Although allografts are more accessible, they require prolonged use of immunosuppressants to avoid immunological rejection towards Schwann cells and myelin sheaths. Immunogenicity of allografts has been reduced in experimental models through decellularization processes, rendering non-immunogenic grafts while maintaining the connective structure preserved, acting as a scaffold for axonal regeneration. However, there is a need to standardize these approaches in humans to generate suitable grafts for clinical utilization at a reasonable cost.**

In collaboration with the Barcelona Tissue Bank (*Banc de Sang i Teixits*) we aim to optimize a decellularization protocol for human nerve allografts from donor corpses to be used in clinic. These matrices could be an alternative to currently commercialized similar products that are quite expensive. We developed chemical decellularization protocols for human and rat nerves. These acellular allografts preserved their extracellular structure and presented laminin, an essential tropic factor for axonal regeneration. DAPI staining revealed a significant cell reduction and double-stranded DNA was below 50 ng per mg of dry weight, indicating an appropriate decellularization.

To evaluate the capacity of the grafts to support regeneration *in vivo*, we grafted 15 mm rat allogenic acellular nerves in a rat model of sciatic transection. Nerves processed with the optimized protocol were compared to cellular autografts. Extent of regeneration was assessed by monthly electrophysiological tests and histological analysis at 120 days. Preliminary results of the on going *in vivo* study show that distal reinnervation of plantar muscle is present in 3/3 animals repaired by autograft and in only 1/5 of the acellular group at 60 days. At 90 days, all animals in both groups had distal reinnervation, although amplitudes were higher in autograft group. Besides the superior capacity of autografts for supporting axonal regeneration, our results indicate that acellular nerve allografts can become a reasonable alternative to autografts in clinics.

**Key Words:** Peripheral nerve, Decellularization, Nerve graft, Allograft

