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Investigating translatable therapies for the treatment of peripheral nerve injury

Ms Gillian Christina Higgins, MBChB

Submitted in fulfilment of degree of Doctor of Medicine (MD)



UNIVERSITY
of
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Investigating translatable therapies for the treatment of peripheral nerve injury

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Thesis Abstract

Figure 1

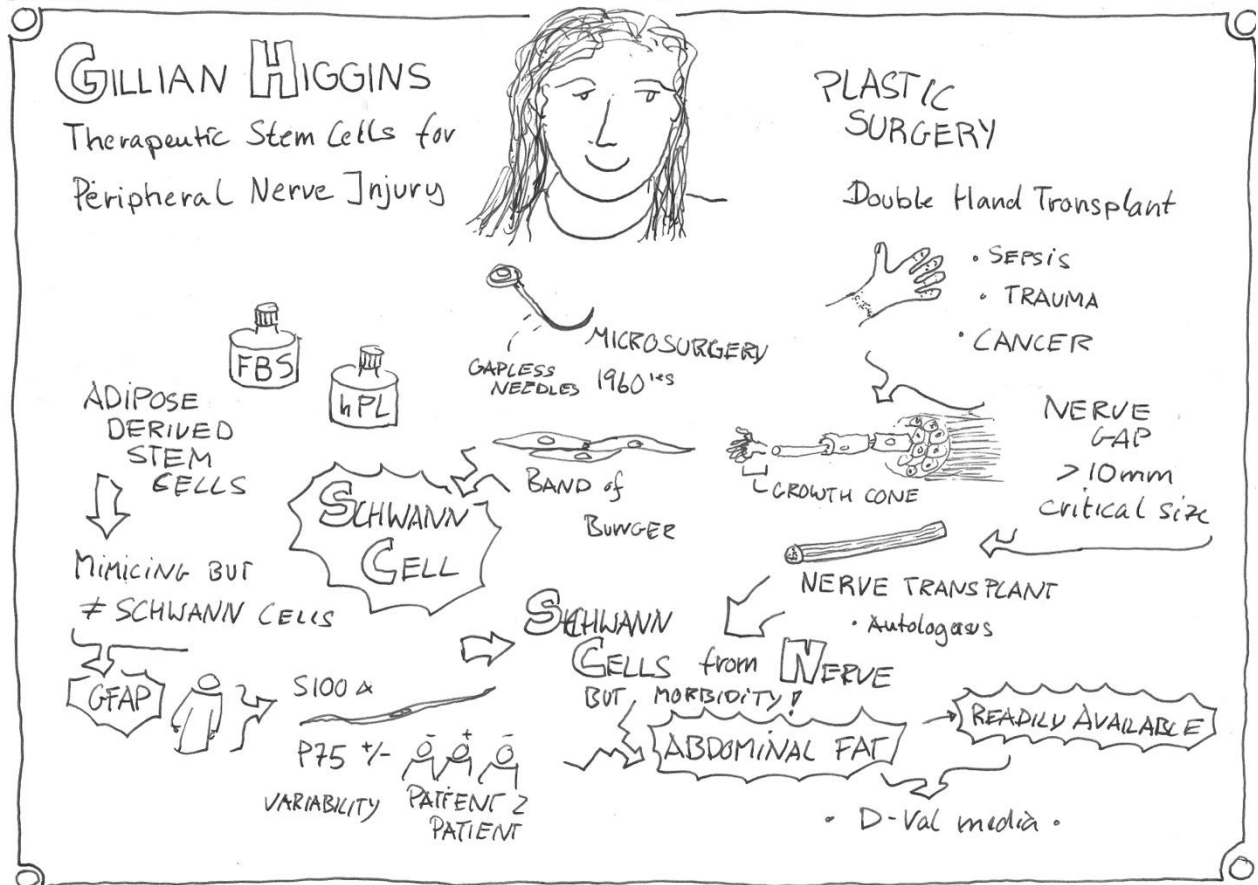


Figure 1 is a visual abstract outlining the scope of this thesis, by Dr Mathis Riehle.

Investigating translatable therapies for the treatment of peripheral nerve injury

Introduction

Peripheral nerve injury (PNI) is common and is increasing globally, resulting in permanent disability and chronic pain in a working-age population. Current surgical solutions do not address the neurobiology of the injury, with nerve regeneration remaining slow, and often incomplete. Current evidence in the literature is predominated by rat models which do not translate into humans. This study investigates patients' perspectives of the treatment of the sequelae of PNI, global surgical community opinion on novel surgical and bioengineered techniques for the treatment of PNI, and experimental laboratory work focussed upon potentially translatable solutions for peripheral nerve repair.

Methods

Retrospective clinical audit case review investigated patient reported outcomes of Qutenza® and Botox® for chronic neuropathic pain, and fascia lata sling for permanent facial nerve palsy secondary to electrical injury. A global survey (Qualtrics) collected surgeon feedback regarding nerve guidance conduits for peripheral nerve repair. Established lab protocols were first optimised to investigate human abdominal adipose as a potential reservoir of therapeutic cells (NHS GGC Biobank 304/314/688). Experiments were conducted to establish impact of Kingham et al. (2007) adipose derived stem cell (ADSC) differentiation protocol to Schwann cell phenotype (dADSC), human platelet lysate (HPL) and superparamagnetic iron oxide nanoparticles (SPIONs) upon ADSC. Flow cytometry to identify cluster differentiation CD markers, immunohistochemistry for Schwann cell markers p75, s100 and GFAP, and morphological quantification was conducted with Cell Profiler. Growth and viability studies were conducted for HPL and SPION labelled ADSC. True cut sampling with s100 and NF200 staining investigated Schwann cell and nerves in human abdominal adipose.

Results

Patients perceived benefits in allodynia, pain, and quality of life with introduction of Qutenza® and Botox® for intractable neuropathic pain. We report the first case of permanent bilateral facial nerve palsy secondary to electrical injury and demonstrate the fascia lata sling as

a reconstructive option to treat the sequelae of chronic PNI, with patient reported improvement in oral competence. Validated patient reported outcome measure tools should be used routinely, to make evidence in the literature meaningfully comparable. Our global study of surgeon perspectives on nerve guidance conduits for peripheral nerve repair highlights that translational gaps and low-level evidence continue, despite technological advancement. There is a need for a collaborative approach, whereby units standardise protocols and outcome assessment, to allow metanalysis to inform clinicians and patients.

Additional biological repeats are necessary to allow for statistical analysis of lab work. The trends in these preliminary studies suggest that dADSC are not translatable, but human platelet lysate (HPL) and 0.01mg/ml superparamagnetic iron oxide nanoparticle (SPION) labelling show potential for clinical translation. Both promote ADSC proliferation and preserve CD expression. Spindle cell morphology was promoted, like that of repair Schwann cells. Further studies to elicit downstream effects on the function of the cells, and whether this may be beneficial (or not) for nerve regeneration are required. Schwann cells were identified from human abdominal adipose. Cell maintenance using D-valine media appeared useful in establishing Schwann cell culture by prohibiting fibroblast overgrowth.

Conclusion

To conclude, this study contributes new low-level evidence in the field of peripheral nerve injury and repair. It highlights the importance of communication and collaboration between patients, scientist and clinicians to ensure that time, resources and energy are focussed upon strategies for peripheral nerve regeneration that may result in clinically feasible solutions for patients with peripheral nerve injury.

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6.3.3 **Figure 51** shows greyscale photographs of different conditions following Schwann cell extraction protocol from abdominal adipose tissue, at day 5 in culture. A=D-Valine Schwann cell medium, B=residual adhered cells in standard Schwann cell medium, following the purification step with Dispase II, and C=purified cell group in standard Schwann cell medium.

Publications and Presentations

Publications

Higgins, G.C., Thomson, S.E., Roditi, G., Riehle, M.O., Murnaghan, C. and Hart, A.M., 2020. Anatomically accurate 3D modelling and printing in a case of obstetric brachial plexus injury. *JPRAS open*, 24, pp.7-11

di Summa, P.G., **Higgins, G.**, Cotrufo, S., Sapino, G., Reekie, T., Leonard, D., Shaw-Dunn, J. and Hart, A., 2022. Distal brachial artery perforator flap: a new chimeric option for complex hand and digits defects. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 75 (2), pp.613-620.

di Summa PG, Osinga R, Sapino G, Glen K, **Higgins G**, Tay S, Eva Weiler-Mithoff. Fat grafting versus implant-based treatment of breast asymmetry, a single surgeon experience over 13 years: a paradigm shift? *Gland Sure* 2021;10 (6):1920-1930

Douglas JM, MacLean N, Horsley C, **Higgins GC**, Douglas CM, Robertson E. COVID-19: smoke testing of surgical mask and respirators. *Occup Med* 2020;70 (8):556-563

Palombella S, Guiotto M, **Higgins GC**, Applegate LL, Raffoul W, Cherubino M, Hart AM, Riehle MO, di Summa PG. Human Platelet lysate as a potential clinical-translatable supplement to support the neurotrophic properties of human adipose derived stem cells. *Stem Cell Res There* 2020;11 (1):432.

Lindsay KJ, Leonard DA, **Higgins GC**, Robertson E, Perks G. Invited Editorial from the social media editor of JPRAS; Leadership in the time of COVID-19. *J Plas Reconstr Aesthet Surg* 2020;73 (8):1405-1408.

di Summa PG, Sapino G, **Higgins GC**, Guiller D. Freestyle superficial femoral artery perforator (SFAP) free flap combined with a free periosteal medial condyle flap in the Gustillo IIb fracture: overkill of ideal treatment? *J Plas Reconstr Aesthet Surg* 2020;73 (7):1253-1254.

Higgins GC, Thomson SE. The introduction of plastic and reconstructive surgery to the University of Glasgow undergraduate core curriculum. *Postgrad Med J* 2020;96 (1132):64-66. doi: 10.1136/postgradmedj-2019-137046. Epub 2019 Nov1.2019;0:1-3.

Letters

Higgins GC, Thomson SE. Peripheral nerve regeneration: the resolution revolution. *J Plas Reconstr Aesthet Surg* 2021;74 (1):223-243

Higgins GC, Thomson SE, Baker J, Honeyman C, Kearns M, Roberts J, Tay S. COVID-19 lockdown and beyond: Home practice solutions for developing microsurgical skills. *J Plas Reconstr Aesthet Surg* 2021;74 (2):407-447.

Higgins GC, Robertson E, Horsley C, Maclean N, Douglas J. FFP3 reusable respirators for COVID-19; adequate and suitable in the healthcare setting. *J Plas Reconstr Aesthet Surg* 2020;73 (7):1357-1404

Higgins GC, Robertson E, Maclean N, Douglas J. FFP3 reusable respirators; a solution to the problem. *BMJ* 2020;369:m1743

Higgins GC, Thomson SE, AM Hart. Letter to Editor; Botox® in the treatment of Chronic Neuropathic Pain. Published online December 2019 *BMJ Case Reports* 2009;2009:bcr0820080812.

Douglas JDM, **Higgins G**, Robertson E, MacLean N, Horsley C. Smoke Testing of Surgical Masks and FFP3 masks uses photographs to explain risk to Health and Care Workers. BMJ 2021;372:n30.

Case reports

Higgins GC, Thomson SE, Roditi G, Riehle MO, Murnaghan C, Hart AM. Anatomically accurate 3D modelling and printing in a case of obstetric brachial plexus injury. Accepted for publication JPRAS Open Feb 2020, manuscript number JPRASO-D-20-00013.

Published Abstracts

G. Higgins, L. Reid, A. Hart. Outcomes of Onabotulinum toxin A (BOTOX®) therapy for patients with chronic neuropathic pain. British Journal of Surgery, Abstracts from the International Surgical Congress of the Association of Surgeons of Great Britain and Ireland, Liverpool, July 2018;105:113.

G. Higgins, R. Melvin, M. Morrogh. Patent Preparedness for the one Stop Breast Clinic. British Journal of Surgery, Abstracts from the International Surgical Congress of the Association of Surgeons of Great Britain and Ireland, Glasgow, July 2017;104 (6):208.

R. Melvin, **G. Higgins**, M. Morrogh. Effective Weekend Handover: Improving Patient Safety & Clinical Governance. British Journal of Surgery, Abstracts from the International Surgical Congress of the Association of Surgeons of Great Britain and Ireland, Glasgow, July 2017;104 (6):203.

Published Visual Abstracts

VA author GC Higgins. DA Michno, ACS Woollard, NV Kang. Letter to the Editor: Clinical outcomes of delayed targeted muscle reinnervation for neuroma pain reduction in longstanding amputees. JPRAS Open Epub 2019 Oct. PMID: 31133528 DOI: 10.1016/j.bjps.2019.05.005.

VA author GC Higgins. L Qing, P Wu, Y Fang, Z Zhou, J Tang. Sequential chimeric deep circumflex iliac artery perforator flap and flow-through anterolateral thigh perforator flap for one-stage reconstruction of complex tissue defects. JPRAS Open Epub 2019 July. <https://doi.org/10.1016/j.bjps.2019.02.029>.

Poster Presentations

Jun '20 A Multidisciplinary Approach: Utilising a Ward Round Proforma improves Patient Safety in a Tertiary Plastic Surgery Unit. Association of Surgeons of Great Britain and Ireland (online). J Roberts, **GC Higgins**, J Kirkpatrick.

Nov '18 Use of topography to enhance Neurotrophic Potential of Adipose Derived Stem Cells. Glasgow Paediatric Research Day, Queen Elizabeth University Hospital, Glasgow. M Kearns, SE Thomson, **GC Higgins**, P Kingham, AM Hart, MO Riehle. Nanoparticle labelling to facilitate Stem Cell tracking of peripheral nerve regeneration.

Oct '18 International 42nd Annual Conference of the Indian Society for Surgery of the Hand (ISSH), Coimbatore. **GC Higgins**, SE Thomson, M Kearns, S Hosseinzadeh, E Ross, C Berry, JF Riddell, R Wallace, AM Hart, MO Riehle. Onabotulinum Toxin A (Botox) therapy for the treatment of chronic neuropathic pain.

Oct '18 International 42nd Annual Conference of the Indian Society for Surgery of the Hand (ISSH), Coimbatore. GC Higgins, L Reid, AM Hart. Retrospective study of patients with chronic pain and their outcomes following Onabotulinum Toxin A (Botox) therapy.

Sep '17 International Surgical Congress of the Association of Surgeons of Great Britain and Ireland (ASGBI), Liverpool. GC Higgins, L Reid, AM Hart. Nanoparticle labelling to facilitate high resolution non destructive in vivo 3D imaging of peripheral nerve regeneration.

Sep '17 Glasgow Orthopaedic Research Initiative (GLORI), Queen Elizabeth University Hospital, Glasgow. GC Higgins, SE Thomson, M Kearns, C Berry, JF Riddell, R Wallace, A.M. Hart, M.O Riehle. Nanoparticle labelling to facilitate high resolution non-destructive in vivo 3D imaging of peripheral nerve regeneration.

Jul '17 Clinicians in Research Network (CiRN) Annual Meeting, Glasgow.

GC Higgins, SE Thomson, M Kearns, C Berry, JF Riddell, R Wallace, AM Hart, MO Riehle. Use of topography to enhance Neurotrophic Potential of Adipose Derived Stem Cells.

Jul '17 Clinicians in Research Network (CiRN) Annual Meeting, Glasgow.

M Kearns, SE Thomson, **GC Higgins**, P Kingham, AM Hart, MO Riehle. Nanoparticle labelling to facilitate high-resolution non-destructive 3D imaging of peripheral nerve regeneration.

May '17 4th International Symposium on Peripheral Nerve Regeneration, Barcelona.

GC Higgins, SE Thomson, M Kearns, C Berry, JF Riddell, R Wallace, AM Hart, MO Riehle. The Resolution Revolution? Nanoparticle labelling to facilitate 3D imaging of peripheral nerve regeneration.

Oral Presentations

Aug '24 Increasing translatability of Cell Therapy for Peripheral Nerve Injury; reducing animal products by replacing FBS with HPL. GC Higgins, M Kearns, M Guiotto, AM Hart, Mo Riehle. **Culture of Care Day**, University of Glasgow.

Jun '23 Increasing translatability of Cell Therapy for Peripheral Nerve Injury; investigating Human Platelet Lysate and SPIONs. **GC Higgins**, SE Thomson, M Kearns, E Robertson, R Wallace, MO Riehle, AM Hart. **Invited talk to represent the school of medicine, veterinary and life sciences (MVLS)**, University of Glasgow

May '23 Increasing translatability of Cell Therapy for Peripheral Nerve Injury; investigating Human Platelet Lysate and SPIONs. **GC Higgins**, SE Thomson, M Kearns, E Robertson, R Wallace, MO Riehle, AM Hart. **ERC (European Association of Plastic Surgeons Research Council Meeting)**, Stockholm.

Jun '22 Increasing translatability of Cell Therapy for Peripheral Nerve Injury; investigating Human Platelet Lysate and SPIONs. **GC Higgins**, SE Thomson, M Kearns, E Robertson, R Wallace, MO Riehle, AM Hart. **Tissue and Cell Engineering Society**. Birmingham University, Birmingham.

Jun '21 Protective Solutions for airborne COVID-19, **Department of Health and Social Care and Infection Prevention and Control Cell UK government policy makers**, online. **GC Higgins**, R Gallagher (RCN), C Peters (NHS Freshair), K Gadhok (RCSLT), B Jones (BAPEN).

Jun '20 Benefits of P3 respirators for addressing PPE inequalities, **UK NHS Innovation Hub**. GC Higgins.

Jun '20 How MedSupplyDrive UK Scotland formed and functioned, **Glasgow Science Festival**, GC Higgins

May '19 A Multidisciplinary Patient Safety Checklist improves Medicines Reconciliation in a Tertiary Plastic Surgery Unit. **Association of Surgeons of Great Britain and Ireland (online)**. J Roberts, **GC Higgins**, J Kirkpatrick.

Jun '20 Adherence to Venous Thromboembolism Prophylaxis in Plastic Surgery Patients; A team approach Improves Patient Safety, **Association of Surgeons of Great Britain and Ireland (online)**. D Hughes, **GC Higgins**, J Kirkpatrick.

May '19 Nanoparticle labelling of therapeutic stem cells. **EURAPS (30th Annual Meeting of the European Association of Plastic Surgeons), Helsinki**. **GC Higgins**, SE Thomson, M Kearns, S Hosseinzadeh, E Ross, C Berry, JF Riddell, R Wallace, AM Hart, MO Riehle.

Nov '18 Human Platelet Lysate (HPL) animal free supplemented medium improves survival, stemness and neurotrophic potential of Human Adipose Derived Stem Cells. **BAPRAS Celtic Plastic Surgeon's Meeting, Dunkeld**. **GC Higgins**, W Raffoul, AM Hart, MO Riehle, P di Summa.

Jun '18 Outcomes for patients with neuropathic pain following Onabotulinum toxin A (Botox) Therapy. **BAPRAS Celtic Plastic Surgeon's Meeting, Dunkeld**. **GC Higgins**, L Reid, AM Hart.

May '18 Super paramagnetic iron oxide nanoparticles (SPION) labelling of differentiated adipose derived stem cells (dADSCs) to facilitate development of non-invasive radiography for peripheral nerve injury. **Multidisciplinary Biomaterials Seminar, University of Glasgow, Glasgow**. **GC Higgins**, M Kearns, SE Thompson, S Hosseinzadeh, S Barnett, AM Hart, MO Riehle.

May '18 The Utilisation of Onabotulinum Toxin A (Botox) therapy in patients with neuropathic pain. **ERC (29th European Association of Plastic Surgeons Research Council Meeting), Madrid**. **GC Higgins**, L Reid, AM Hart.

Oct '17 Patients with chronic intractable pain and their outcomes following Qutenza therapy.
ERC (29th European Association of Plastic Surgeons Research Council Meeting), Madrid.
GC Higgins, E Meland, AM Hart.

Sep '17 Use of topography to enhance Neurotrophic Potential of Adipose Derived Stem Cells.
BAPRAS Scottish Plastic Surgeon's Meeting, Dunkeld. M Kearns, SE Thomson, **GC Higgins, P Kingham, AM Hart, MO Riehle.**

Jun '17 Patients with chronic intractable pain and their outcomes following Qutenza therapy.
Canniesburn 50th Anniversary Meeting, Royal college of Physicians and Surgeons of Glasgow. **GC Higgins, E Meland, AM Hart.**

May '17 Use of topography to enhance Neurotrophic Potential of Adipose Derived Stem Cells.
4th International Symposium on Peripheral Nerve Regeneration, Barcelona.
 M Kearns, SE Thomson, **GC Higgins, P Kingham, AM Hart, MO Riehle.**

Awards

Feb '24 Hack the ARC commission £2,500 to curate a concert, Q+A and exhibition series to celebrate creativity and community

Sep '22 Oct 21 Giving Voice Award; Royal College of Speech and Language Therapy for work in campaigning for PPE for health and social care workers

Jan '21 Tenovus Scotland Pump Priming Grant, £20,000

Nov '19 Stephen Plumpton Foundation Grant, £1,000, for research materials and consumables.

Aug '19 Greater Glasgow and Clyde Travel Grant, £500, to travel to National Reconstructive Plastic Surgery and Burns Centre, Korle-Bu Teaching Hospital, Accra, Ghana as member of faculty for Microsurgery course for Surgeons and Faculty for Postgraduate Diploma Certificate of Burns and Plastic Surgery Nursing.

Oct '18 Best Poster Presentation Prize 42nd Annual Conference of the Indian Society for Surgery of the Hand (ISSH), Ganga Hospital, Coimbatore, India. Nanoparticle labelling to facilitate Stem Cell tracking of peripheral nerve regeneration. **GC Higgins**, SE Thomson, C Berry, JF Riddell, R Wallace, AM Hart, MO Riehle.

Oct '18 BSSH Travel Bursary Award, £2,236

Jan '18 Best Poster Presentation Prize Clinicians in Research Network (CiRN) Annual Meeting, Glasgow. Nanoparticle labelling to facilitate high resolution non-destructive in vivo 3D imaging of peripheral nerve regeneration. **GC Higgins**, SE Thomson, R Wallace, M Riehle, C Berry, AM Hart.

Sep '17 Pump Priming Project grant, £10,000, Royal College of Surgeons of Edinburgh. Investigation of strategies to enable objective quantification of neural regeneration through tissue engineered conduits in vivo, preparing for clinical trials. SE Thomson, **GC Higgins**, R Wallace, M Riehle, C Berry, AM Hart.

Acknowledgements

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Thank you to my long-suffering supervisors Prof Andy Hart and Dr Mathis Riehle, for standing by me, and for your wise guidance. We did not foresee that there would be a pandemic, and that I would return to full-time clinical life, and volunteer in ICU due to a staffing crisis. To Kay, Christine, and Eleanor, I don't know what I would have done without you. It was of great surprise that we had to campaign at the upper echelons of Government for safe PPE for health and care workers, and with a grassroots approach, that we'd collect and distribute over half a million pieces of donated PPE, for free, all over the UK. This included 3D printed and laser cut visors made by the University of Glasgow Engineering Department, that travelled as far as colleagues in need, in Ghana. If it had not been for my research training, encouraging creative "if you can't find one, make one" problem solving, these things may not have come to pass. To all the kind souls at the University of Glasgow and beyond, who worked tirelessly to help us during those desperate times, *thank you*.

To maintain our friendship and our sanity my friend and I made music over lockdown. CLR theory. To Hannah, and our bandmates Ali, Ruth and Nico, *thank you* for being my chosen family and being there for me always. Your friendship and this music gave me the confidence to pivot from my career in academic plastic reconstructive surgery, to that of a professional musician, and kept me going whilst writing up this body of work. Free education and music tuition and what a colourful life it has given me, what choices, what a gift. Truly lifechanging.

Just as I was hanging up my surgical hat, I suffered my second bout of COVID and subsequently spent over a year of being mostly bed bound. Having been a keen hill walker and a runner in-marathon-training, I am now registered disabled and use a walking stick. Add to this a late diagnosis of ADHD at the ripe old age of 35, it is no small miracle that I managed to get this work written up. *Thank you* to Jonathan, John, Gosia, Sally, Jaime, the Disability Service and the Students Awards Agency Scotland for advocating for me and supporting me.

It takes a village to write a thesis. To Aunty Janet, *thank you* for your unwavering support. *Thank you* to my family. To Malcolm for the lifts to feed my cells at all hours. To Suzanne for taking me under your wing, and for all the opportunities. To Marie and Martino for being brilliant team-mates. To Monica, Oana, Al, Euan and all my lab-mates for sharing your time, your wisdom (and your cake). To Frazer Bell, Dr Angelika Frances and the histopathology team. To my study buds Eleanor and Carla and to my wonderful flatmate Peggy-May, *thank you* for the heart to hearts and for putting the world to rights with me. And to my love Andrew, for seeing me and loving me just as I am. I couldn't have done it without you. I am incredibly grateful to every individual whose help has allowed me to contribute this small piece of the puzzle, working towards better outcomes for patients with peripheral nerve injuries. *Thank you*.

Author's Declaration

"I declare that, except where explicit reference is made to the contribution of others, this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution"

Ms Gillian Christina Higgins

5/5/26

Abbreviations

ADSC	Adipose derived stem cell
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
cAMP	Cyclic adenosine monophosphate
CD	Cluster of Differentiation marker
CNP	Chronic neuropathic pain
CT	Computed tomography
dADSC	Differentiated adipose derived stem cell
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
ECM	Extracellular matrix
EGF	Epidermal growth factor
F-Actin	Filamentous actin ¹⁷
FBS	Foetal bovine serum
FDA	Food and Drug Administration
G-Actin	Globular Actin
GDNF	Glial cell-derived neurotrophic factor
GFAP	Glial fibrillary acid protein
GGF-2	Glial growth factor 2
HPL	Human platelet lysate
ISCT	International Society for Cellular Therapy
KDE	Kernal Distribution Estimation
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stem cell
NGF	Nerve growth factor
NHS	United Kingdom National Health Service
Nrg1	Neuregulin 1

p75NTR p75 neurotrophin receptor

PCL Polycaprolactone¹⁹

PDGF Platelet derived growth factor

PLL Poly-L-lysine

PLGA Poly lactic-co-glycolic acid

PNI Peripheral nerve injury

SNAP Synaptosome-associated protein of 25 kDa

SNARE Soluble N-ethylmale-imide-sensitive factor-attachment protein receptors

SPION Superparamagnetic iron oxide nanoparticles

TRPV1 Transient Receptor Potential Vanilloid 1

VEGF Vascular endothelial growth factor

UK United Kingdom

US United states of America

Chapter 1 Introduction: investigating translatable therapies for the treatment of peripheral nerve injury

1.1 Peripheral nerve injury: the clinical conundrum

Peripheral nerves are the biological cables of the electrical grid of the body that relay information to and from the brain. This includes the signal for movement from the brain to muscles (motor nerves) and the sense of touch from the skin back to the brain (sensory nerves). If we use the analogy of a pylon cable running electricity from the power station (the brain) to the house (the fingertips), the nerves are the pylon cables, and inside them are bundles of insulated cables (fascicle groups). These contain bundles of smaller insulated cables (fascicles) which contain the copper wires that carry the electrical signal (axons) (figure 1). If the pylon cable is damaged in a storm, we would not expect the lights of the house to come back on by simply coapting the ends of the pylon cable back together. What are the chances of the copper wires finding their corresponding end on the other side of the damage, and completing the circuit effectively with this crude approach? In the human anatomy equivalent of this analogy, despite the gold standard best treatment available, the literature suggests the rate of axons finding their target across the injury site with this approach is just 50% (Kim et al., 2020), and the chance of function returning to the person's normal preinjury state, is just 10% (Brushart, 2011).

Peripheral nerve injury (PNI) is common, considerably more common than spinal cord injury (Battiston et al., 2005). In a study of patients in England's National Health Service (NHS), there was a mean national incidence of 11.2 PNIs per 100,000 population, per year (2005-2020) (Murphy et al., 2023). The rates of PNI are on the increase, in both incidence and severity. This reflects global population increase, alongside concomitant increases in traffic, industrial accidents, violence, and battlefield injuries (Kaplan et al., 2015). PNI may also occur secondary to disease such as cancer or diabetes, toxins, ischaemia or trauma such as iatrogenic causes. Males are reported to be twice as likely to sustain a PNI, and upper limb nerves at or distal to the wrist are most commonly involved (Murphy et al., 2023). PNIs present with varying degrees of involvement, which dictate treatment and prognosis (expected patient outcome). In less severe injuries, natural healing is often successful in regenerating the injured portion of a nerve, and full

functional recovery may be expected without intervention.<https://pmc.ncbi.nlm.nih.gov/articles/PMC8560160/> However, with more severe injury, prolonged deficiency of neuronal input from the distal nerve stump can significantly reduce the regenerative success of nerves and increases the symptom burden for the patient (MacKay et al., 2021). These injuries frequently affect young and working-age adults and may lead to lifelong motor and/or sensory functional impairment with associated risks of chronic pain, disability, reduction in quality of life and substantial socioeconomic consequences[3]. In the United States of America (US), the indirect costs of a brachial plexus injury reportedly reach a mean of \$1,113,962 per patient, and approximately 40% of patients with a significant upper limb nerve injury will not return to work by 1 year postinjury (McMorrow et al., 2024).

The natural process of nerve regeneration is complex and dynamic, involving the coordination of multiple biological systems. Schwann cells coordinate this process, physiologically and physically guiding the regenerating axons (Nadi M, 2026). Despite this innate capacity for peripheral nerve regeneration (which is lacking in the central nervous system), recovery is very slow (around 1mm per day) and often incomplete (Kim et al., 2020). Clinical intervention aims to expedite and improve this process and reduce the risk of neuroma formation (a neuroma is a mass of tangled nerve endings trying to regenerate, which is often painful (Sisti et al., 2024).

Of note, nerve injuries are highly time sensitive, and early recognition, accurate localisation, and prompt intervention remain critical determinants of patient recovery (MacKay et al., 2021). The target sensory and motor end-organs deteriorate irreversibly over time. Suboptimal recovery from PNI may also be due to upstream degeneration secondary to neuronal cell death in the spinal motor neurons (in proximal nerve injuries such as the brachial plexus) and/or the dorsal root ganglia (distal sensory nerve injuries). Changes in the cerebral cortex of the brain are known to develop in cases of prolonged neuronal deficiency. In addition, in order to inhibit or prevent painful neuroma formation, careful nerve repair inhibits the formation of inflammatory reactions, peripheral fibrosis, and scar tissue. Upon time of injury, the immune system is activated, and proinflammatory cells including neutrophils, mast cells, monocytes, macrophages and lymphocytes migrate to the wound site within the first few hours (Zhou et al., 2023). Therefore, nerve injuries should be treated as soon as possible (within 24 hours), especially for

mixed or motor nerve injuries; the literature suggests sensory nerve injuries should be considered acutely (within 14 days of injury) when possible to prevent painful neuroma formation, and repair within 15 days of digital nerve injury has been associated with significantly improved sensory outcomes (MacKay et al., 2021).

1.1.1 Complex architecture and neurobiology of peripheral nerve injury

An understanding of the complex microanatomy and neurobiology of healthy and injured peripheral nerves is essential for determining patient prognosis, planning surgical strategy and for innovating novel bioengineered, clinically translatable solutions for peripheral nerve repair.

Peripheral nerves are composed of layers of connective tissue (figure 1). The paraneurium (also known as mesoneurium) is the outermost loose areolar layer, which allows gliding of the nerve within its tissue bed. The next layer is called the epineurium, which has a dense outer connective tissue layer which protects the nerve from mechanical stress (the epifascicular epineurium) and an inner layer which houses blood vessels and runs between and encloses the fascicle groups (interfascicular epineurium). The perineurium encloses each fascicle and forms the principal component of the blood–nerve barrier via the extrinsic vessels. The inner endoneurium, is a delicate matrix which surrounds individual axons and provides mechanical and metabolic support, houses unmyelinated nerve fibres, myelinated nerve fibres, and intrinsic blood vessels. The myelinated axons are wrapped in Schwann cells which insulate them, increasing conduction velocity, and providing nutrients (Nadi M, 2026). In turn, neuregulin-3 expression on axons is essential for survival and maturation of Schwann cell precursors, and the degree of myelination depends on the amount of neuregulin on the surface of the axon (Nadi M, 2026). The non-myelinated fibres (such as C fibres which conduct pain, temperature, itch, light touch) are surrounded by Remak Schwann cells, which separate them from the surrounding tissue (Jessen and Mirsky, 2016).

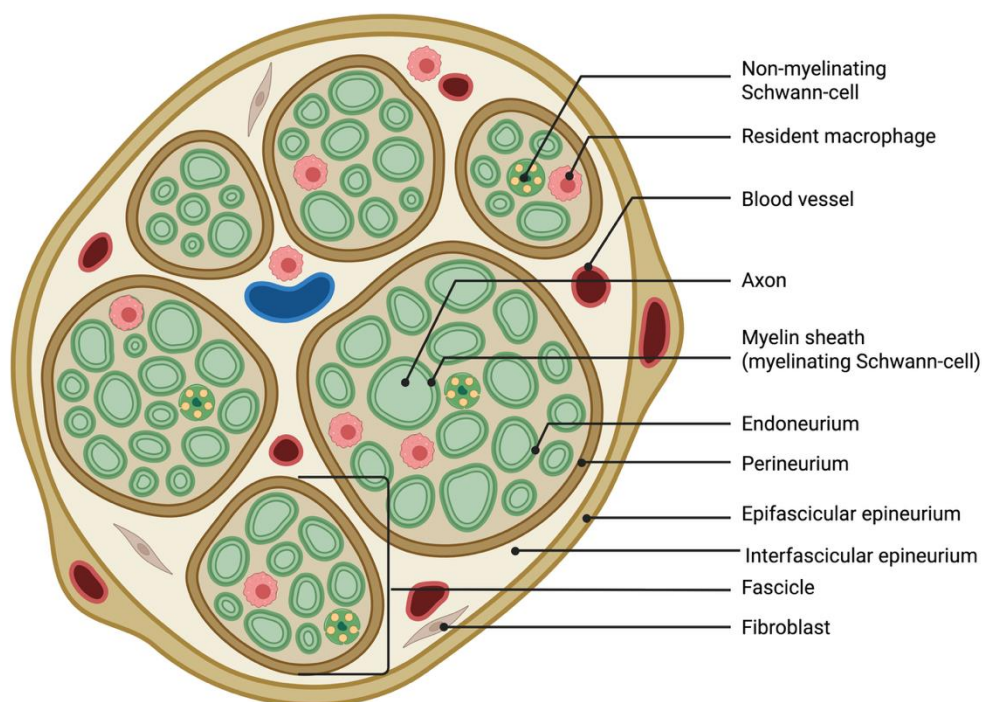


Figure 2 is an illustration of peripheral nerve anatomy, as seen in cross section. It shows the dense outer connective tissue layer of the epifascicular epineurium; the interfascicular epineurium running between the fascicle groups; the perineurium enclosing each fascicle; and adjacent extrinsic vessels. The inner endoneurium is shown housing unmyelinated axons, myelinated nerve fibres, and intrinsic blood vessels. The myelinated axons are wrapped in Schwann cells. Resident macrophages are also shown. This figure was created in <https://BioRender.com>.

When PNI occurs, Schwann cells coordinate the nerve regeneration process by reprogramming into a repair phenotype. The myelin and non-myelin producing Schwann cells distal to nerve injury undergo a large-scale change in gene expression, probably involving thousands of genes, and change function from maintenance of axonal ensheathment and myelin production, to roles supporting nerve regeneration (Jessen and Mirsky, 2016). Wallerian degeneration will first occur distal to the injury site, a process whereby the distal axon stump Schwann cells activate the

innate immune response and chemotaxis of macrophages to the site, via upregulation of cytokines. The recruited macrophages remove dead axon and myelin fragments which may impede axonal growth and promote vascularisation of the healing nerve via further cytokine release (Jessen and Mirsky, 2016) (figure 3).

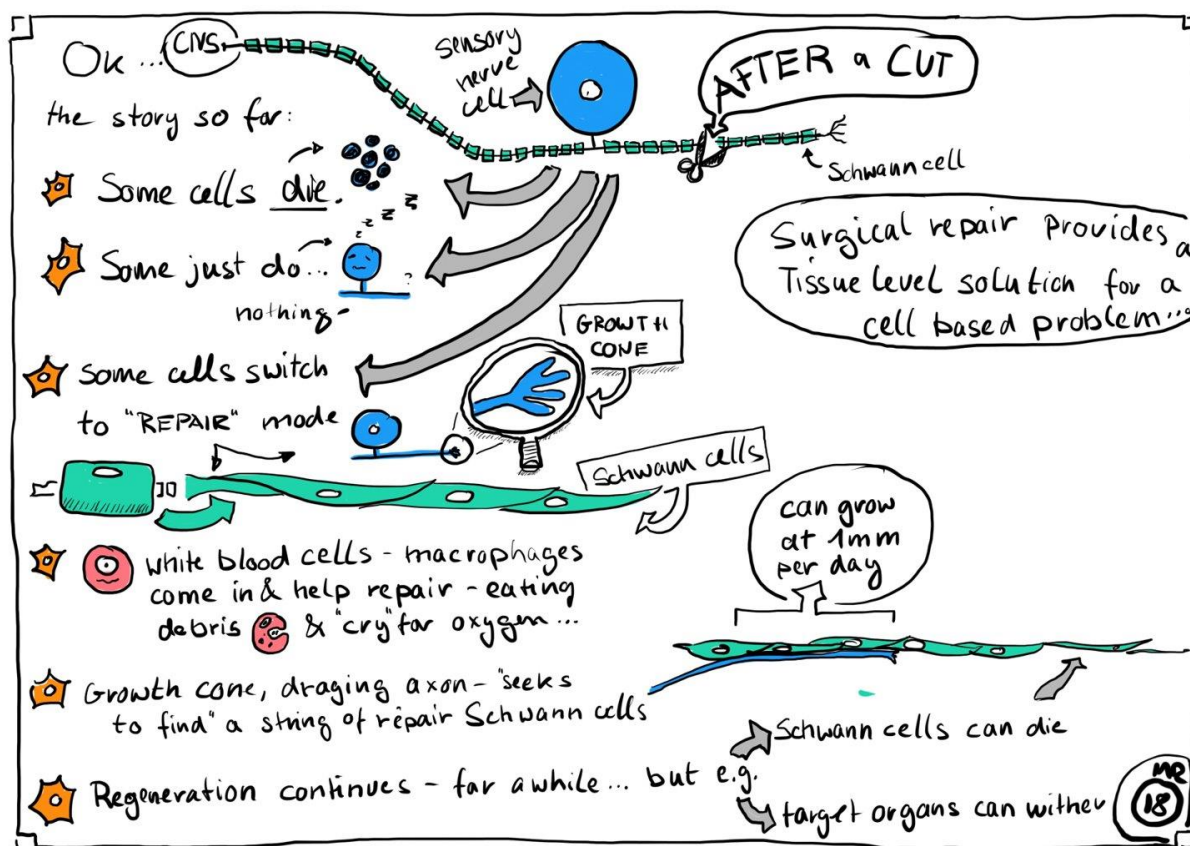


Figure 3 illustrates the natural process of nerve regeneration, including cell death, Schwann cell adoption of a repair phenotype, recruitment of macrophages, and development of the axonal growth cone. From "Mathis Tweeted Progress" 13-18.11.2018 as @sfprocur (www.scienceforprogress.eu) Science Doodle series, with his kind permission.

In adopting their repair phenotype, Schwann cells proliferate and elongate into a spindle shape with bipolar or tripolar extensions. Studies report that this cytoskeletal morphological change

impacts upon Schwann cell proliferation, and migration, and triggers a cascade of cell signalling pathways which are vital for maintenance of the Schwann cell repair phenotype behaviours (Wang et al., 2018, Yu et al., 2009). This includes the alignment of their bodies along the basal lamina to guide axon growth in bands of Bügner and the up-regulation of neurotrophic factors which stimulate axonal regeneration and neuronal survival (Fallon M, 2023). The neurotrophic factors secreted by repair Schwann cells play a pivotal role in guiding and coordinating the process of nerve regeneration. These include glial cell line-derived neurotrophic factor (GDNF), artemin, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), erythropoietin, pleiotrophin, p75NTR and N-cadherin (Jessen and Mirsky, 2016).

If the gap between the proximal (towards the body) and distal (away from the body) axon stumps on either side of the injury is larger than a few millimetres, a tissue forms between the stumps which is known as a nerve bridge. The nerve bridge consists of extracellular matrix consisting of proteins and polysaccharides, fibroblasts, perineural and inflammatory cells. Within this structure, macrophages produce vascular endothelial growth factor (VEGF-A) in response to the hypoxic environment. This starts a chemotactic cascade which guides endothelial cells and blood vessel formation. The blood vessels running through the matrix guide Schwann cell migration, and in turn the Schwann cells guide the axonal growth cone across the nerve bridge (Fornasari et al., 2022).

1.1.2 Neuropathic pain

Pain, temperature, light touch and itch signals are transmitted from the body via A-delta and C fibres. They carry the signal to the dorsal root ganglion, synapse occurs in the dorsal horn of the spine, and then the tract crosses to the ascending pathway on the contralateral (opposite) side where it is conducted up to specific pain centres in the cerebral cortex of the brain (figure 4). The main characteristic feature distinguishing neuropathic pain from other types of pain, is tingling or shooting pain, called paraesthesia. Allodynia is another common manifestation of neuropathic pain, with prevalence reported as 31% in painful polyneuropathy, 75% postherpetic neuralgia

and 79% in postinjury neuropathic pain (Maier et al., 2010). Allodynia is defined as pain arising secondary to non-noxious stimuli (such as light touch) (Cruccu et al., 2018).

Neuropathic pain can become chronic, which is defined as pain that “*lasts for more than three months and ... occurs because of pathological changes or diseases that affect the somatosensory system*” by the International Association of the Study of Pain. Chronic neuropathic pain (CNP) is referred to as a complex clinical syndrome, of unknown specific mechanism. Pain may be constant, require long-term management and is often poorly controlled by standard pain relief medications (Cui et al., 2023). The incidence of CNP has been increasing, and with a prevalence rate of 6.9–10% among the world population it is of global concern (Cui et al., 2023). Therefore, great efforts are made in surgical management and bioengineering research focussed on reducing its occurrence.

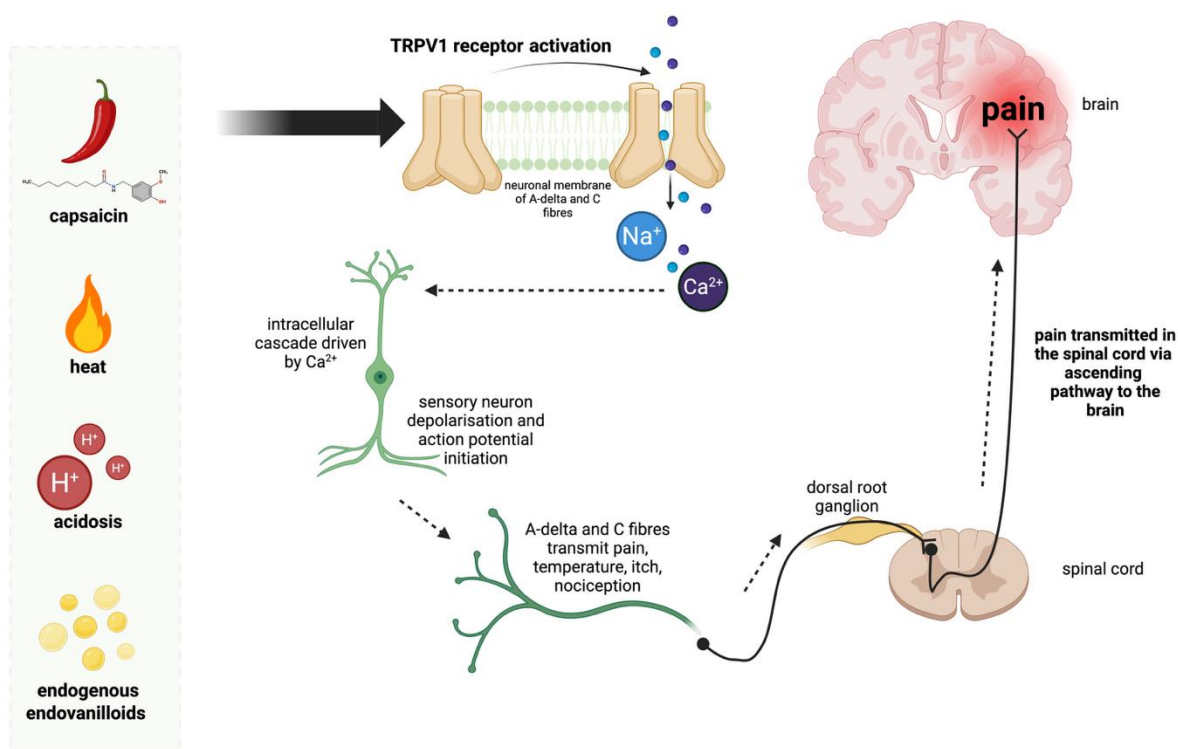


Figure 4 illustrates the mechanism of pain transmission. A-delta and C fibres are the receptors that transmit pain, temperature, light touch and itch information from the peripheral nervous

system to the central nervous system. Both A-delta and C fibres are rich in TRPV1 receptors. They are activated by capsaicin, heat, acidosis and endogenous vanilloids. The TRPV1 receptor undergoes conformational change and allows influx of sodium and calcium into the neuron. The cation influx triggers intracellular cascades, leading to neuronal depolarisation and action potential initiation. The A-delta and C fibres transmit the pain, temperature, or itch signal to the dorsal root ganglion, synapse occurs in the dorsal horn, the tract crosses to the ascending pathway on the contralateral (opposite) side and is conducted to specific pain centres in the brain. This figure was created in <https://BioRender.com>.

1.2 Peripheral nerve repair: where did we come from and where are we going?

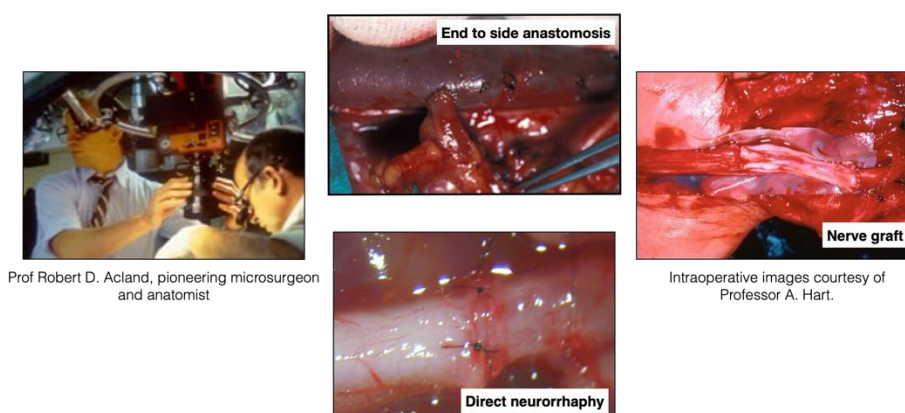
A nerve bridge forms naturally in nerve defects up to ~30 mm wide, which is known as a “critical nerve gap” (Battiston et al., 2005). In defects smaller than 5 mm the nerve stumps may be coapted and sewn together with a 75µm stitch, with a thread finer than a human hair. This procedure, known as neurorrhaphy is performed by the surgeon under loupe magnification, or microscope guidance. The suture and some of the microsurgical instruments necessary for this procedure were invented by Professor Bob Acland in the 1970s, who utilised them to perform Scotland’s first free tissue transfer surgeries (figure 5) (Acland, 1977).

If the gap between the nerve stumps is over 5 mm wide, then coapting them would create tension, which would lead to ischaemia and neuronal death. The first described attempt to bridge a nerve defect to solve this problem seems to have been made by Gluck in 1880, who utilised a piece of bone to bridge the nerve gap. Though unsuccessful, this attempt was followed by experiments by Vanlair et al. (1885), who reported successful nerve fibre regeneration across a 3 cm long tube made of decalcified bone (Gluck, 1880, Vanlair, 1885, Battiston et al., 2005). From there, a host of biological and synthetic materials have been tested, although nothing has been sufficiently proven to improve upon the gold standard of an autologous nerve graft. Extensive research over the past 50 years, has seen development of autographs, allografts, and conduits. Despite these advances, and bioengineered conduits and cadaveric grafts being available on the

market today, the evidence remains too low level (Thomson et al., 2022) to topple the nerve autograft from its gold standard throne, despite its relatively poor outcomes.

“Through his meticulous approach, Bob developed the instruments that moved microsurgery from an experimental procedure to a routine operation that has benefited millions of patients, worldwide.”

Gus McGrouther



R. Acland. A new needle for microvascular surgery. *Surgery*.1972 Jan;71(1):130-1.

Figure 5 is a quote from plastic surgeon Gus McGrouther, which was written in the BMJ obituary of Professor Robert D Acland, and photographs of surgical options for nerve repair. Prof Acland was a pioneering microsurgeon and anatomist who, in the 1970s, invented some of the microsurgical instruments that are still used in the treatment of PNI today. With thanks to Prof Andrew Hart and his patients for the kind permission to use their clinical photographs. Illustrated here are three surgical techniques used to treat peripheral nerve repair, direct neurorrhaphy, end to side anastomosis and autologous nerve graft.

In an ideal world, the patients would be cared for and their injuries assessed, with their nerve injury being identified early. The injury would be immediately explored and cleaned, with direct repair performed for simple injuries, and larger reconstruction planned for complex injuries. In this case, autologous cells could be harvested from the patient during this initial lavage and

assessment, or perhaps allograft donor cells could be used. The therapeutic cells would be cultured in high numbers, promptly, would secrete neurotrophic growth factors, reduce inflammation at the injury site, and would persist long enough at the injury site to relay these benefits meaningfully. From an MRI of the injury a nerve conduit, with millimetre precision, would be 3D printed in a biocompatible and biodegradable material, which would include topography to guide axonal growth and reduce neuroinflammation. This would be seeded with the therapeutic cells before nerve repair performed for the patient. Both the therapeutic cells and the conduit would be radio-opaque and visible via a low ionising imaging modality (MRI) to allow monitoring of nerve regeneration and provide cell tracking to ensure patient safety and inform future treatment advancements. In this ideal scenario, the patient would have less morbidity and would benefit from successful peripheral nerve healing, with improved functional recovery and quality of life.

1.2.1 Biologically active nerve conduits: a combinational approach

A nerve defect larger than 30 mm, represents a critical nerve gap, across which a natural nerve bridge cannot reach. In this instance an autologous nerve graft remains the gold standard for the treatment of these critical nerve gaps due to its unique ability to deliver live autologous Schwann cells (McMorrow et al., 2024). This procedure involves sacrificing a nerve from elsewhere in the body as a donor and moving it into the site of injury (autologous nerve graft). This donor nerve is usually a sensory nerve from the peripheries, such as the sural nerve which supplies sensation to the leg. However, this technique is counterintuitive in that it sacrifices nerve, to treat nerve (Bamba et al., 2021). Autologous nerve graft carries additional morbidity for the patient, with an additional operation site with inherent scar, pain, loss of sensation and risks of infection, wound breakdown, chronic pain and cold sensitivity (Bamba et al., 2021). With the clinical rule of thumb for primary nerve repair that around 50% of the original axons will successfully regenerate through the repair coaptation site, for a nerve graft with two coaptation sites just 25% of the axons are expected to successfully regenerate through the graft (Kim et al., 2020).

Conduits that have been designed as an alternative to autografts strive to overcome this morbidity, provide a protected environment and physical guidance for axonal growth (Fornasari

et al., 2022). Research teams have been investigating a wide range of natural and synthetic materials, as well as composite materials, for creating nerve guidance conduits or scaffolds that facilitate nerve repair and regeneration (Boeckstyns et al., 2013, Åberg et al., 2009, Battiston et al., 2005, Battiston et al., 2000, Bertleff et al., 2005, Den Dunnen et al., 1996, Gould et al., 2013, Heinzl et al., 2021). The addition of bioactive agents like growth factors, live cells and topography into these conduits is expected to boost their efficacy in supporting nerve healing, in the hope of improving outcomes when compared to autologous nerve repair (Ansselin et al., 1997, Lavorato et al., 2021, Zack-Williams et al., 2015, Wan et al., 2024, Thomson et al., 2022, Rodríguez et al., 2000, Palombella et al., 2020, Fairbairn et al., 2015, Kingham et al., 2007). Nanotechnology and 3D printing are allowing for the integration of complex designs, with millimetre precision, and controlled drug release mechanisms (Thomson, 2018, Catherine and Adam, 2003, Higgins and Thomson, 2020, Chen et al., 2024).

1.2.2 Adipose derived stem cells (ADSC) and nerve repair

One of the cells of focus for use in bioengineered conduit design for peripheral nerve repair is the adipose-derived stem cell (ADSC). Harvested from subcutaneous fat, they provide an abundant reservoir, with minimal morbidity and may commonly be accessed from the patient requiring trauma or cancer reconstruction, without additional operative sites. They can also be harvested in large quantities via liposuction (Larocca et al., 2013). Other estimates from systematic reviews suggest that at least 3,000 patients have been treated with ADSCs or stromal vascular fractions across more than 10 different disease areas ADDIN EN.CITE

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 number></record></Cite></EndNote>[OBJ](Larocca et al., 2013)[OBJ].

In the realm of PNI, ADSCs have been shown to improve nerve regeneration through reducing inflammatory cell infiltrate and facilitating axon remyelination (Chen et al., 2023), and by secreting growth factors *in vitro* and *in vivo* animal models (Chu et al., 2019). They also have multipotency and can be differentiated towards osteocyte, chondrocyte, adipocyte or Schwann-

like phenotypes (Kingham et al., 2007). Studies report the dADSC revert back to their stem cell state when the differentiation medium is removed *in vitro* (Faroni et al., 2016), however Thomson (2018) reports the use of microtopography to maintain dADSC phenotype in rat cells. The differentiation protocols often contain toxic or animal derived ingredients which may limit clinical translatability.

1.2.3 The use of non-animal derivatives to improve translatability of stem cell therapeutics

Patient safety is the main factor that dictates the suitability of an experimental therapy for clinical trial and translation potential. Alongside the use of toxic reagents in culture media, the use of animal products may act as a limiting factor to the progression of stem cells from their successful use in animal models into meaningful treatments in human patients. The concerns with the use of animal derivatives include ethical, moral or religious objections including: the risk of exposure to animal derived viral, bacterial or prion infection, inconsistency between batches which affects laboratory reproducibility in the short term, and potential therapeutic reliability in the long term, and the risk of patient immune reactivity (Vaidya et al., 2018).

The standard medium supplement in laboratory cell culture of ADSCs is foetal bovine serum (FBS), as completely serum-free cultures lead to low cell survival. Although FBS does not seem to be required for cell propagation in primary human Schwann cell cultures, it is regularly used to promote their viability (Hörner et al., 2022). Human platelet lysate (HPL) is an alternative to FBS which shows particular promise for use in the treatment of PNI. HPL may be obtained from screened individuals and could potentially be pooled and stored as in other blood products or perhaps obtained autologously from the patient. HPL has higher growth factor content than any other supplement, and in particular relevance to its use in the context of nerve regeneration it contains molecules and growth factors which optimise Schwann cell survival, differentiation, and release of extra-cellular matrix molecules, including neurotrophin 3 (NTF3), nerve growth factor (NGF), brain-derived growth factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) (Lensch et al., 2018, Guiotto et al., 2020).

1.2.4 Fat: a reservoir of Schwann Cells?

It is established in the literature that there are nerves running through fat, and that these nerves are important for the complex physiological roles of adipose tissue in the body (Blaszkiewicz et al., 2019, Stavely et al., 2022, Townsend, 2020, Bartness and Bamshad, 1998, Wang and Ye, 2023, Wang and Ye, 2024, Wang and Ye, 2022). Stavely et al. (2022) described isolation of mouse and human subcutaneous adipose tissue neural stem cells (dedifferentiated Schwann cells) derived from Schwann cells which populate nerve fibre bundles within adipose tissue (Stavely et al., 2022). Chen et al. (2017) described adipose tissue as a potential Schwann cell reservoir in their paper detailing the isolation and identification of Schwann cells from inguinal fat in a rat model. These cells were used in a sciatic nerve conduit repair model in mice, with similar outcomes to nerve derived Schwann cells (Chen et al., 2017). Wang et al. (2017) described extraction of Schwann cells from human eyelid adipose tissue, disposed of during blepharoplasty. These Schwann cells incorporated into a sciatic nerve conduit repair model in mice showed equivalent results to the autograft group (Wang et al., 2017).

A key consideration for culturing Schwann cells *in vitro* is managing competing fibroblast overgrowth. Some protocols include a purification step, which relies on fibroblast tight adherence and Schwann cell light adherence to tissue culture plastic. Using a gentle enzymatic agent such as Collagenase IV or Dispase II to lift the Schwann cells, allows their purification (Wang et al., 2017, Chen and Qi, 2025). Another technique utilised to address the challenge of fibroblast overgrowth in Schwann cell culture was the use of D-valine DMEM reported by Kaewkhaw et al. (2012) (Kaewkhaw et al., 2012). Fibroblasts cannot metabolise D-valine, the enantiomer of essential amino acid L-valine, whereas Schwann cells possess D-amino acid oxidase, an enzyme essential for metabolising the D-valine isomer (Kaewkhaw et al., 2012). These results suggest adipose tissue may act as an abundant reservoir of Schwann cells that may be extracted and utilised to promote the regeneration of injured peripheral nerve. To our knowledge, this has not yet been reported in human abdominal adipose tissue, which would be a particularly clinically relevant site as a potential fat donor site.

1.4 Non-invasive *in vivo* cell tracking

For cells to be safely translated into a therapeutic use in clinical practice, a cell tracking modality is necessary to establish cell migration, proliferation, end differentiation, and function *in vivo*. There are multiple options for cell labelling, but few that meet the following requirements: non-toxicity to the host cell viability and function, specific, sensitive, suitable for detection with low

ionising radiation techniques to minimise patient morbidity, and longevity of action. Stem cells can be genetically engineered to express an ‘imaging reporter’ or fluorescence; however, they pose the risk of stimulating an immunogenic response from the patient, of epigenetic silencing, or malignant transformation and do not have clinical relevance (Cho et al., 2016).

In recent years, superparamagnetic iron oxide nanoparticles (SPIONs) have been recognised as a novel and effective tool for manipulating cell growth and behaviour, and they may also act as a cell tracking agent due to their detection on Magnetic Resonance Imaging (MRI) (and Computed Tomography (CT)) scans (Berry, 2003). SPIONs are metabolised down the haem pathway, with the waste products excreted in urine (Lu et al., 2017). Whilst gadolinium is the standard MRI contrast agent used in the National Health Service (NHS), SPIONs were the first liver-specific contrast agents used and have current licence for use in the NHS as contrast agent for sentinel lymph node biopsy in breast and prostate cancer patients (Berry, Curtis., 2003, NICE, 2022). SPIONs are biocompatible and cost effective, are easy to formulate and functionalise and therefore also have therapeutic potential.

1.5 Aims and hypotheses

It is necessary for scientific innovation to be considered alongside clinical practicality, with a holistic, patient-centred outlook. Investment in research methods that have the potential for therapeutic translatability and collaborating with the people and systems who will be the end users and administrators of that therapy, may advance improvements in outcomes for patients with PNI.

This thesis aims to contribute towards the advancement of translatable interventions for PNI. To gain understanding of the clinical context, this study investigates patient and surgeon experiences of PNI and its treatment, including a case review of patients treated with Qutenza® high dose capsaicin patches and Botox® injections for chronic intractable neuropathic pain, with minimum 6-month follow-up. My hypothesis was that Qutenza® would offer short-term benefit in pain for the patients, before the body becomes tolerant to it, like in TENS therapy (Liebano et al., 2011). In the patients receiving Botox® treatment, I hypothesised that the patients would find at least

initial benefit, secondary either to placebo, or the action of Botox® which is known to be transient for other applications. Surgical procedural placebo is known to be particularly strong (Dettori et al., 2019), and the muscle relaxant and paralysis effects of Botox® are known to be transient and dose dependent (Honndorf et al., 2026). A case report is included in this study, reporting to our knowledge, the first bilateral permanent facial palsy with electrical aetiology. It highlights the importance of timely management of PNI and discusses surgical approaches to improve patient quality of life in the management of their chronic PNI symptoms.

This study reports the findings of a global survey of surgeons asking them why they do or do not use the nerve guidance conduits available on the market for peripheral nerve repair. With the bias of knowing that United Kingdom (UK) surgeons do not use them as standard, and being institutionalised in a system that is government funded, I hypothesised that the majority of surgeons would not be using nerve guidance conduits in their current clinical practice, and that their reasons stated would be lack of evidence in the literature, high financial burden, and inconvenience. In addition, there is evidence of cultural, bureaucratic and historical resistance to medical innovation in even in the presence of high-level evidence (Higgins et al., 2020, Lindsay et al., 2020), including in the introduction of free flap surgery (Fricker, 2016) and indeed in the advent of the creation of the specialty of plastic surgery itself (Andrew, 1994).

Despite an explosion in new knowledge on the molecular mechanisms of disease processes that have been derived from animal models, and the subsequent development of novel technologies, translation into effective treatment for human patients remains slow and sometimes ineffectual. Due diligence and process are absolutely essential to safeguard patients and prevent them from harm (Shah, 2024), but there are several fundamental issues which hamper the closure of the translational gap between what is found in the lab, and treatments in real live patients. First, the “publish or perish” culture puts emphasis on novel and highly significant findings, and negative findings are often not published (Rawat and Meena, 2014). This selectively rewards implausible, low probability observations and high magnitude effects, and under-represents the complexity of biological systems (Frangogiannis, 2022).

As an example of the importance of transparent reporting in the literature, the ‘Ice Maidens’ were the fastest team to ski coast-to-coast across Antarctica. Their team dietician LCpl Rin Cobb explained at the Extreme Medicine Conference (Edinburgh, 2018), that they achieved this great feat by first studying all the attempts that failed. It soon became apparent that previous teams had fatally underestimated the calories that would be required for this journey in extreme conditions. For team Expedition Ice Maidens, pulling all their equipment with no mechanical support vehicles, Cobb calculated that the team members would have to eat continuously for their calorie intake to be sufficient (Taylor et al., 2021). Although pinpointing why an experiment doesn’t work is a difficult task, without sharing vital negative results, the scientific community is setting itself up to be left out in the cold.

In addition, animal models cannot recapitulate the physiological and pathophysiological heterogeneity seen in humans and are unfortunately poor predictors of therapeutic success. This is seen in the translational gap in the study of neuropathic pain and its treatment for instance (Percie du Sert and Rice, 2014). The majority of nerve regeneration modelling is currently occurring in rats, despite the rat being a particularly poor model for the repair of human critical gap defects, due to both their small size and their species-specific neurobiological regenerative profile. This may skew data and lead to inaccurate understanding of the risks and the benefits (Kaplan et al., 2015).

In investigating translatable therapies for the treatment of peripheral nerve injury, this study focuses on clinical findings and lab work in human tissue. The potential for ADSCs to be used therapeutically for peripheral nerve repair was examined, by studying them under the conditions of differentiation down a Schwann-like phenotype, under culture in HPL supplement, and with SPION labelling. This study was inspired from the unique perspective of being a clinical academic research fellow with a foot in both camps, with clinical practice and lab workbench inspiring one other. This work progressed the body of work from our team at the Centre for the Cellular Microenvironment from rat into human tissue *in vitro* models. My hypothesis was that the differentiation protocol for ADSCs (Kingham et al., 2007) was not suitable for translation into humans. I thought HPL seemed a promising replacement for FBS to make cell culture more clinically translatable, especially due to its pro-nerve regenerative ingredients (Palombella et al.,

2020, Escobar and Chaparro, 2016). SPIONs are already used safely in patient care settings, as an imaging modality in the NHS ADDIN EN.CITE

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 number></record></Cite></EndNote>[OBJ] (Berry et al., 2004)[OBJ], and I hypothesised that ADSCs
 would have similar results.

In the final chapter of experimental work, this study discusses Schwann cells derived from
 human abdominal adipose tissue. My hypothesis was that Schwann cells would be extracted
 from this tissue, and that the abdominal fat may act as a useful reservoir of cells for therapeutic
 strategies, and with high translatability. With the anatomical knowledge that nerves and vessels
 run together in neurovascular bundles, I hypothesised that the highest yield of Schwann cells
 might be found in the adipose tissue with the highest vascularity. I also thought that using the D-
 valine DMEM which inherently precludes fibroblast growth (Kaewkhaw et al., 2012) and
 favours Schwann cell proliferation, would serve as the most viable option in terms of reducing
 cell enzymatic damage, increasing cell yield and reducing time in culture.

This work aims to contribute ideas and results to the field of peripheral nerve repair, working
 towards meaningful translational advancements, with the goal of improving clinical outcomes for
 patients suffering from peripheral nerve injury.

Chapter 2 Treatment as good as Gold? Studies of current clinical practice for peripheral nerve injury

2.1 Clinical Case Series of patients treated with Qutenza® for intractable neuropathic pain

2.1.1 Study Acknowledgements

Thank you to the patients for participating in this study. Thanks to Ellen Meland and Ms Lindsay Reid for contributions to data collection and to Prof. Andrew Hart for contributing access to this patient cohort, and clinical intraoperative photographs, for guidance and mentorship.

2.1.2 Introduction

The discovery of the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor was first published in 1997, by Prof. David Julius PhD and team (Caterina et al., 1997a). Julius was curious as to the mechanism by which capsaicin, the active molecule in the chilli pepper, imparts sensations of burning, heat and pain. Their team used a cDNA library from rodent dorsal root ganglia that contained the cell bodies of the capsaicin-activated sensory neurons, to create a functional screen and looked for a gene that confers capsaicin sensitivity to cells that were normally unresponsive. The screen identified a cDNA encoding a novel ion channel (now called TRPV1). Importantly, TRPV1 was shown to be activated by temperatures perceived as painful (above 43°C) (Caterina et al., 1997b). This work advanced the scientific community's understanding of noxious temperature sensation at a molecular level, and Prof. David Julius, PhD went on to become a co-recipient of the Nobel Prize in Physiology or Medicine Award in 2021 (Patrik Ernfors, 2021).

TRPV1 receptors are widely distributed throughout the body and, in addition to capsaicin and heat, they are also activated by acidosis (protons), and a variety of endogenous lipids synthesised in primary sensory neurons and in the brain, termed endovanilloids. This positions TRPV1 as a flexible multi-modal channel that sits at the crossroads of various signalling pathways, allowing

it to integrate and modulate thermal, protein, and chemical signals. Still, the greatest abundance of TRPV1 is found within the peripheral nervous system, in small diameter unmyelinated C-fibres that slowly transmit dull burning pain (Morgan et al., 2019).

A-delta fibres also house TRPV1 receptors. A-delta and C fibres are the primary peripheral afferent nerves in the skin responsible for transmitting pain, temperature, and itch sensations, and play critical roles in nociception and sensory perception. Whilst A-delta fibres have thin myelination and conduct fast, sharp pain, C fibres are unmyelinated and conduct slow, dull or burning pain (these pain transmitting fibres are distinct from myelinated tactile sensors (A-beta fibres) and proprioceptors). Glutamatergic synapses onto second-order neurons occur in the dorsal horn of the spinal cord, where some integration and processing of sensory inputs occurs. From there, the signal is carried by several specific ascending pathways, to distinct sites in the brain. For example, the lateral spinothalamic tract projects multimodal sensory inputs to the lateral thalamus and has been implicated in processing sensory and discriminative aspects of pain. The medial aspect of the spinothalamic tract and the spinoparabrachial tract project to the medial thalamus and limbic structures of the brain and are believed to mediate the emotional and aversive components of pain. The experience of pain is perceived in the cerebral cortex of the brain (Kuner, 2010) (Figure 4).

As introduced, chronic neuropathic pain (CNP) does not refer to a single disease but is instead a complex clinical syndrome. Studies suggest that central and peripheral sensitisation, peripheral, neuroinflammation, dysfunction in descending nociceptive modulatory systems, oxidative stress, activation of glial cells and psychosocial factors may all contribute to its development. Patients often experience spontaneous pain, hyperalgesia (abnormally high sensitivity to pain), stimulus-dependent pain, or abnormal sensation. The main characteristic feature distinguishing neuropathic pain from other types of pain is tingling or shooting pain, called paraesthesia (Cui et al., 2023). Allodynia is another common manifestation of neuropathic pain, with prevalence reported as 31% in painful polyneuropathy, 75% postherpetic neuralgia and 79% in post injury neuropathic pain (Maier et al., 2010). Allodynia is defined as pain arising secondary to non-noxious stimuli (such as light touch) (Cruccu et al., 2018). Studies have indicated that central sensitisation, peripheral sensitisation, neuroinflammation, dysfunction in descending nociceptive modulatory systems, oxidative stress, activation of glial cells and psychosocial factors all may contribute to the development of CNP.

The incidence of CNP has been increasing, and with a prevalence rate of 6.9–10% among the world population, it is of global concern (Cui et al., 2023).

Qutenza® is a high concentration (8%) capsaicin patch that was first approved for the treatment of post-herpetic neuralgia in Europe and the United States in 2008 and gained FDA approval for the treatment of neuropathic pain in Europe in 2009. In NHS Scotland, it is indicated for patients with persistent surface allodynia who have already tried first and second-line therapies including a trial of two of Tricyclic antidepressants (e.g. amitriptyline), an anticonvulsant (e.g. gabapentin), and topical local anaesthetic preparations (e.g. lidocaine patch). Qutenza® is contraindicated in patients with unstable hypertension, or open wounds, and it is unsuitable for use in people who are pregnant, or under 18 years of age (Consortium, 2014).

Capsaicin selectively agonises the Transient Receptor Potential Vanilloid 1 (TRPV1) of the A-delta and C fibre sensory nerves in the epidermis and dermis of the skin, causing TRPV1 receptor conformational change in the neuron cell membranes, with calcium and sodium influx. The TRPV1 activation results in initial sensations of tingling and burning. A cascade of intracellular cation influx, additional activation of TRPV1 channels on the endoplasmic reticulum, and further calcium release (Tsai and Gan, 2026), result in defunctionalisation of these pain transmitting fibres, with subsequent analgesia. With continued exposure, the TRPV1 receptors become less sensitive, leading to their “defunctionalisation” and degeneration, causing them to recede from the epidermis (Cruccu et al., 2018) (figure 6). The strongest capsaicin cream available over the counter is 0.1%, whereas Qutenza 8% patch is available only by prescription and under observation. One topical patch Qutenza®, 30-minute treatment, can lead to analgesic effects lasting for around 90 days and minimum time between treatments is 60 days (Derry et al., 2017).

Armstrong et al. (2025) presented an update on the mechanism of high dose capsaicin, reporting intracellular effects that go beyond activation of the TRPV1 receptor including a net effect of localised denervation of TRPV1-expressing afferent terminals in the epidermis and dermis, and subsequent neuroregeneration. They describe that, following capsaicin induced TRPV1 receptor conformational change, the resulting cytoplasmic calcium overload has multiple effects within

the cell, exerting enzymatic, cytoskeletal, mitochondrial, and osmotic effects, which ultimately lead to localised axonal degeneration and nerve terminal necrosis (Armstrong et al., 2025).

They suggest capsaicin's effects upon mitochondria are central to promoting apoptosis in "hyperactive" nerve endings. Predictably, mitochondrial density is highest within parts of the neuron that are involved in sensory transduction. Here, the excessive calcium levels following TRPV1 activation stimulate opening of the 'permeability transition pore' in the mitochondrial inner membrane. This can lead to pro-apoptotic events including mitochondrial swelling, loss of membrane potential, release of cytochrome c and the generation of reactive oxygen species. The downstream effects include oxidative damage to mitochondrial proteins, lipids, and DNA. Armstrong et al. (2025) further report that high dose capsaicin also directly inhibits mitochondrial respiration independently of TRPV1 activation. After around 1–3 months, there is neuroregeneration, and a further treatment is required (Armstrong et al., 2025).

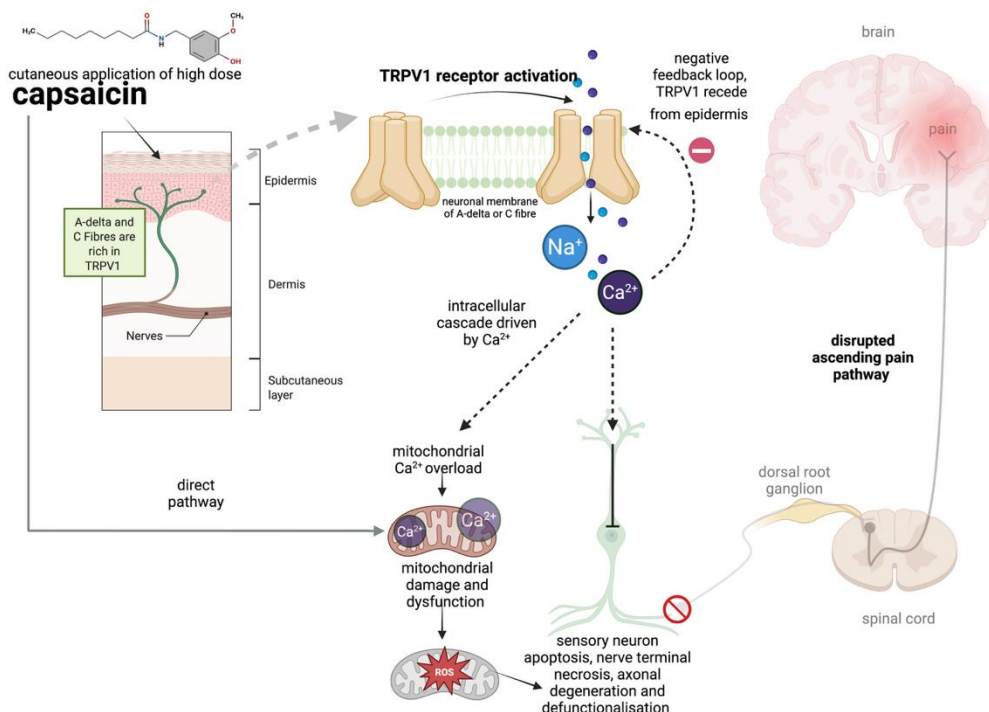


Figure 6 illustrates the mechanism of action of high dose capsaicin therapy in treatment of localised peripheral neuropathic pain. Topical application of capsaicin to skin leads to TRPV1 conformational change and sodium and calcium influx into the neurons of A-delta and C fibres. After high dose (8%) capsaicin is used for 30 minutes (or with repeated exposure to low dose capsaicin) the TRPV1 receptors become “defunctionalised” and recede from the epidermis. Created in <https://BioRender.com>.

2.1.3 Methods

This study is a retrospective clinical audit case review. It includes patients with CNP who were treated with Qutenza® capsaicin 8% patch in the Canniesburn Plastic Surgery and Burns Unit, over a two-year period, with minimum 6 months follow up. Neuropathic pain was confirmed utilising the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) Score (appendix 1). All patients were fully counselled in risks, benefits, alternatives and limitations of Qutenza® as per established consent procedures. Each patient also had physiotherapy and psychology input as standard.

This clinical audit did not require ethics committee approval, as per the National Health Service Health Research Authority Guidance. The patients were anonymised, the study was registered with the local Clinical Governance Lead, and all data are held in accordance with GDPR procedures. Patients provided express written consent for their clinical photographs to be used for research and teaching purposes, on standardised NHS Scotland Greater Glasgow and Clyde consent forms, stored electronically on NHS Clinical Portal.

2.1.4 Results

There were 11 patients: 7 (63%) females and 4 (36%) males in this study group, age ranged between 36-71 years (median 50.5) and a median BMI of 31.33 (range 24-41). The average duration of treatment for neuropathic pain before Qutenza® therapy was 8.3 years (range 1-34 years). Five (45%) patients reported their pain as “moderate”, 6 (55%) reported “extreme” pain, and 7 (64%) patients reported anxiety.

For 100% (11) patients, the pain was at the site of previous surgery: 45% (5) secondary to elective procedure (27% (3) oncological management), and 55% (6) were due to emergency, trauma procedures. The site of the neuropathic pain was anatomically distributed as follows: lower limb (6), upper limb (1), supraclavicular fossa (2), back (2). Six (55%) patients had experienced a primary wound complication; 4 (36%) had primary wound infection; and 2 (18%) had primary wound breakdown. In addition to standard analgesia regimens their pain had shown little improvement with desensitisation massage (6 patients), Botox® (2 patients), steroid injections (2 patients), psychology therapy (2), neurolysis (2), visual feedback (1), TENS (1), and acupuncture (1), prior to Qutenza® therapy.

Treatment was administered intraoperatively for 3 (27%) patients, on the ward for 3 (27%) patients, and in clinic for 7 (64%) patients. Following Qutenza® therapy, 9 (82%) patients reported improved allodynia, 7 (64%) patients perceived improvement in their pain, and 6 (55%) patients stated an improvement in dysaesthesia. Six patients (55%) had a reduction in the dose, or in the number of analgesics in their regimen following Qutenza® therapy, and 2 (18%)

experienced improvements in mobility and their ability to perform tasks of daily living. Seven patients (64%) reported improved quality of life, and one patient (9%) reported improvement in deep neuropathic pain. Two (18%) patients reported no improvement (figure 7).

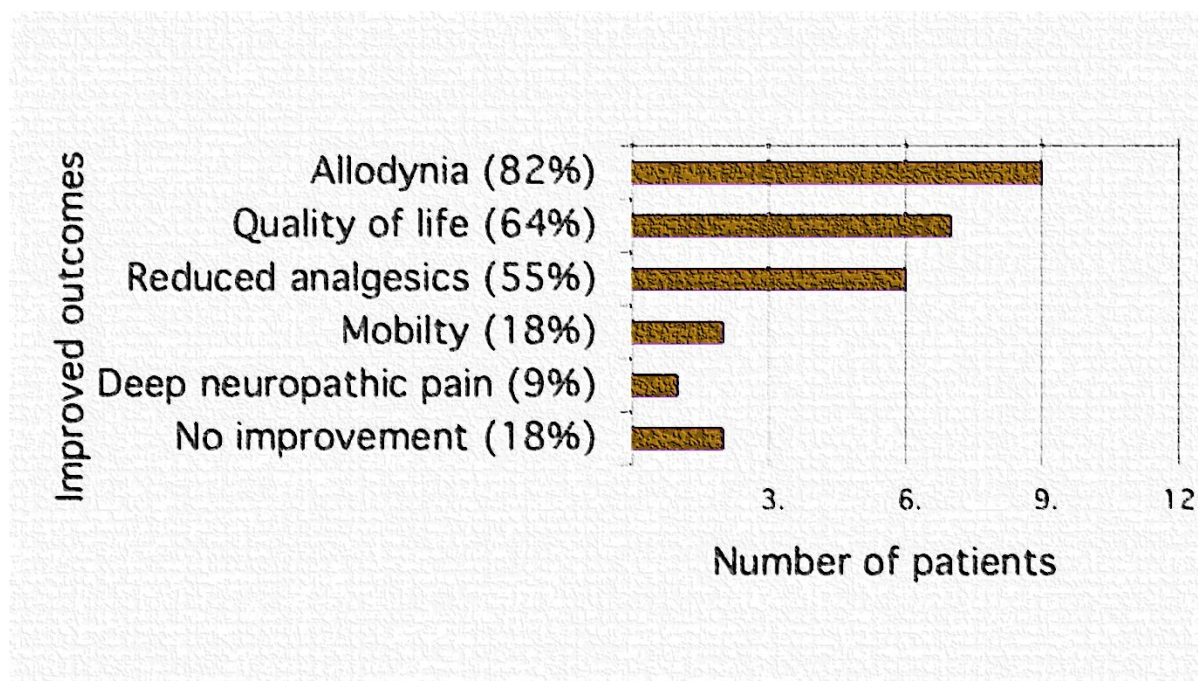


Figure 7 illustrates the patient reported improvement in outcomes following one 30-minute therapeutic intervention with Qutenza® patch application, at minimum 6 months follow up.

Associated complications included wound breakdown (1, 9%) and graft failure (1, 9%), which healed with simple dressings, and one patient touched the patch and then their eye which caused severe pain - after washout of their eye there were no lasting effects. Three (27%) patients required further surgical procedures for pain: neurolysis, neurectomy and fat transfer. Four patients (36%) were successfully discharged from clinic, 1 (9%) patient was referred for further Qutenza® patch therapy, and 1 (9%) patient had further surgical exploration.

2.1.4.1 Case series examples

The following patients kindly gave consent for their cases to be anonymously presented.

Patient A

This lady is a 47-year-old care worker, who smokes. She had wide local excision of a stage 2a melanoma from her right leg in 2012 (figure 8). She subsequently suffered allodynia at the operation site which limited her mobility, and her ability to work, and to sleep. The pain was resistant to trials of neurolysis, Botox®, steroid injection and standard analgesic regimen including gabapentin and tramadol. Following Qutenza® therapy, her pain improved to a level where she stopped her tramadol, improved her exercise tolerance, and returned to work.



Figure 8 is a clinical photograph of Patient A, who has a diagnosis of CNP at the scar site on her right medial leg (arrow), following wide local excision of a melanoma. Clinical photographs courtesy of Prof Andrew Hart and with express consent from the patient.

Patient B

This gentleman is a 38-year-old train driver, who is an ex-smoker. He initially presented with what seemed to be a rapidly growing sarcoma; however, after resection and perforator flap repair, as shown in figure 9, the tumour was found to be a benign neurofibroma. He suffered allodynia at the surgical site, which was resistant to trials of naproxen, lidocaine patches, and desensitisation massage. Following Qutenza® his pain resolved, he stopped all analgesics and returned to work and to playing football.



Figure 9 is a clinical photograph of Patient B, who had a diagnosis of CNP at the scar site on his left lateral leg, after resection and perforator flap repair, for a neurofibroma. Clinical photographs courtesy of Professor Andrew Hart and with express consent from the patient.

Patient C

This lady is one of the two patients in this study who found no benefit from Qutenza® therapy. She had thoracic outlet decompression and neurolysis surgery in 2014, shown in figure 10, following which she had chronic pain at the scar site. This pain was resistant to desensitisation

massage, TENS, steroid injection and acupuncture. She found no benefit from the standard 30-minute trial of Qutenza® therapy application and was then entered into a gabapentin drug trial.



Figure 10 is a clinical photograph of Patient C, who has a diagnosis of CNP at the scar site on her left neck, after thoracic outlet decompression surgery. Clinical photographs courtesy of Professor Andrew Hart and with express consent from the patient.

2.1.5 Discussion

The patients in this study had chronic intractable pain, resistant to standard regimens, but the majority (7, 64%) of these patients perceived benefit in their neuropathic pain and 2 (18%) noted improvement in deep neuropathic pain with Qutenza® therapy. Six (55%) patients had a reduction in the dose, or in the number of analgesics in their regimen, 2 (18%) experienced improvements in mobility and their ability to perform tasks of daily living, and (7, 64%) reported an associated improvement in their quality of life. Derry et al. (2017) updated their Cochrane review in 2017, which included double blind randomised control trials of Qutenza®, encompassing 2488 patients with neuropathic pain (Derry et al., 2017). They found the evidence was low-to-moderate due to small sample sizes. The number needed to treat is defined by NHS Scotland as “a measure used in assessing the effectiveness of a particular intervention. The

number needed to treat is the *average* number of patients who require to be treated for one to benefit compared with a control in a clinical trial. It can be expressed as the reciprocal of the absolute risk reduction.” (Division) Derry et al., 2017) found the “number needed to treat” was 10 to achieve 30% benefit in pain. However, for those who experienced improvement in their pain, the patients also experienced an improvement in their associated symptoms of fatigue, depression and reported improved quality of life (Derry et al., 2017). The capsaicin 8% patch has also been demonstrated to give the same level of pain relief, but with a faster onset of action, fewer systemic side effects and greater patient satisfaction with treatment, when compared with an optimised dose of pregabalin over 8 weeks (Cruccu et al., 2018). The negligible diffusion of capsaicin into the blood over the 30-minute time period also limited effects beyond the skin and, therefore unwanted systemic effects (Armstrong et al., 2025).

In this study, Qutenza® was shown to be particularly effective for the treatment of allodynia (in 82% (9) patients), especially in patients with patchy reinnervation. The results agree with a study by (Cruccu et al., 2018), whereby they conducted a Phase IV, randomised, open-label, head-to-head, 8-week, non-inferiority study involving 465 patients with neuropathic pain, comparing a single capsaicin 8% treatment vs. an optimised regimen of pregabalin for 8 weeks. They found significantly greater reductions in the intensity and area of allodynia and had greater number of patients with resolved symptoms after a single application of the capsaicin 8% patch, compared with optimised dose. In this study Qutenza® also proved to be useful for allowing clinical examination to identify anatomical pathology such as neuroma, this facilitated patients to be identified for surgical intervention to treat their deep neuropathic pain.

Qutenza® continues to be used as a third line agent. Analysis by the NHS Northern Treatment Advisory group (2017) states that each patch costs £210 and the annual cost of therapy including admission and administration was estimated at about £3000 per patient (Group, 2017). Although topical formulations of low-concentration capsaicin (1%) creams and lotions are available which could be self-administered at home, they have limited medical utility because of the need for frequent applications, the lack of acute pain relief, inconvenience, and frequent associated discomfort, which can result in poor compliance and, therefore, efficacy (Armstrong et al., 2025). The pharmaceutical company Pfizer initially held the patent for the use of pregabalin to

treat pain, under the brand name “Lyrica”. During this time, the NHS cost-effectiveness model found Qutenza® to be more cost effective than pregabalin for treatment of neuropathic pain (Group, 2017). However, pregabalin cost did reduce once the patent eventually ended and ‘generic’ versions of the drug became available (Smyth et al., 2018). Qutenza® will remain patented and therefore generic versions will not be available until approximately March 2030 (Pharsight).

Freitas et al. (2025) published a cost-effectiveness analysis of high-concentration capsaicin patches utilised as per current guidance for patients with neuropathic pain in Scotland; as a third-line add-on to standard drug regimens (Freitas et al., 2025). They found that high-concentration capsaicin patches produce meaningful quality of life gains for patients, at a ‘reasonable’ extra cost. They suggest that when broader economic costs of long-term pain conditions (like time off work) were included, the patch becomes even more cost-effective. Systemic drug relief agents often produce modest pain relief with high number or severity of side effects. This therefore leads to low persistence and frequent switching, which limits real-world effectiveness. By contrast, a high-capsaicin patch offers localised, safe and sustained pain relief, with minimal side effects, making them well tolerated by patients. The high-dose capsaicin patch is one of the few treatments with clinical trials assessing outcomes beyond 13 weeks, and this analysis showed their long-term use does not impact their cost-effectiveness (Freitas et al., 2025).

2.1.6 Conclusion

To conclude, in this study Qutenza® therapy was successful in treating allodynia in 82% (9) of patients and it aided identification of those who would then benefit from surgery to treat anatomical pathology as the source of their neuropathic pain. The majority (64% (7)) perceived benefit with Qutenza®, improved quality of life was reported in 64% (7) and 55% (6) reduced their analgesics after treatment. Four patients (36%) were successfully discharged. Average duration of pain before trial of Qutenza® was 8.3 years. Considering updated evidence supporting its efficacy and cost effectiveness as a therapeutic add-on when pain relief is incomplete with standard oral drug regimens, hopefully patients will be treated, and will experience improvement to their pain profile and quality of life, sooner. Longitudinal study is required to further understand the risks and benefits, and larger scale randomised control trials would increase the quality of evidence available to inform clinical teams and their patients in their decision making.

Chapter 2.2 The utilisation of botulinum toxin A (Botox®) therapy in patients with neuropathic pain

2.2.1 Acknowledgements

Many thanks to the patients for sharing their stories and photographs for research and teaching purposes and to Professor Andrew Hart for providing the patient cohort, clinical images, guidance and mentorship.

2.2.2 Introduction

In 1793, an outbreak of botulism occurred in Bad Wildbad, Germany, due to spoiled sausage. Dr Justinus Kerner, a German physician and poet, conducted animal experiments as well as clinical experiments on himself, and discovered the botulinum toxin. He concluded that the toxin develops under anaerobic conditions, that the toxin blocks neuronal impulses in the autonomic and somatic nervous systems, but doesn't affect sensation or cognition, and that it can be lethal in very small doses. Kerner also suggested that the toxin might have therapeutic potential, by blocking hypersecretion and abnormal movements. The botulinum toxin was named "sausage poisoning" when Kerner published his clinical findings (1817-1822). He is now recognized as the intellectual founder of modern botulinum toxin therapy (Erbguth and Naumann, 1999).

From there, the bacterium *Clostridium botulinum* was isolated and grown, and the mechanism of its toxin was deduced by bacteriologist Professor Emile van Ermengend and his student Robert Koch, following poisoning of 34 attendees after eating ham at a funeral. The toxin was then purified by Herman Sommer and P. Tessmer Snipe. In 1913, a botulism outbreak affecting 12 people at Stanford University, following the consumption of string bean salad, led to Karl Friedrich Meyer and Dr Ernest Dickson's work in the canned food industry, which led to the discovery that botulinum toxin is inactivated by heat. During World War II, the US government reportedly employed Dr Edward Schantz and colleagues to purify botulinum toxin for use in biological warfare. The alleged plan was to give the botulinum toxin capsules to sex workers, who would slip them into the meals of high-ranking Japanese officials. In 1969, more than 100

countries signed the Biological and Toxins Weapons Convention Treaty, and President Nixon ordered for the close of the US biological agent offensive programme laboratories (Patil et al., 2016).

Botulinum toxin A was studied in a pilot, randomised, placebo-controlled trial for the treatment of neuropathic pain, for the first time in 2008 (Ranoux et al. 2008). Doses of up to 200 units were administered at the site of pain, in 29 patients with post-herpetic neuralgia or nerve lesion (trauma, post-surgical) associated with allodynia. This study demonstrated for the first time the efficacy of botulinum toxin type A for the treatment both spontaneous neuropathic pain and allodynia, and was found to be effective at 3 months follow-up (Ranoux et al. 2008). Similar results were subsequently reported in single centre, double-blind, placebo-controlled trials for painful diabetic neuropathy and postherpetic neuralgia (Apalla et al., 2013; Xiao et al., 2010; Yuan et al., 2009). These trials did however have limitations in their study design, and small sample sizes. A meta-analysis of three single-centre, double-blind, placebo-controlled trials conducted by Morra et al. (2016) reported a significant effect of botulinum toxin type A for the treatment of idiopathic trigeminal neuralgia, when compared with placebo.

In contrast, Spagna et al. (2023) identified several unpublished studies which found no effect of subcutaneous or intradermal botulinum toxin A vs placebo for peripheral neuropathic pain in their literature review (2023). They describe the largest such study, which was a multicentre trial including 117 patients with post-herpetic neuralgia, and was supported by Allergan in 2005 (clinicaltrials.gov NCT19152066). However, they suggest that a possible explanation for these negative results was perhaps inclusion of patients who had mild pain intensity, and the inclusion criteria of pain of duration of 3 months, despite the natural history of post-herpetic neuralgia which generally improves spontaneously over this time-period.

Botulinum toxin is classified into eight serotypes, two of which (type A and B) are Food and Drug Administration (FDA) approved for clinical use (Tian et al., 2025). These neurotoxins are structurally similar but serologically and antigenically different, with distinct potencies. Botox® is derived from botulinum toxin A, it was first used in the treatment of strabismus in 1980 and licensed for use in chronic migraine (UK) in 2010. Other licensed indications include cervical

dystonia, primary axillary hyperhidrosis, neurogenic detrusor overactivity and upper limb spasticity (Attal et al., 2016). Examples of off-licence uses in clinical practice include myofascial pain syndrome, chronic low back pain, temporomandibular joint disorders, and neuropathic pain.(Patil et al., 2016) The treatment of neuropathic pain remains a challenge because of the complexity and diversity of its mechanisms (Cui et al., 2023). Most drugs used for the management of neuropathic pain have dose limiting adverse effects, and reportedly only 20–30% of patients specifically respond to these active drugs against placebo, therefore effective drugs without systemic side effects are urgently needed (Attal et al., 2016).

In the active state, the toxin consists of a heavy and a light polypeptide chain, bound by disulfide bonds. The heavy chain gives the toxin the selectivity and irreversible binding to cholinergic receptors, present especially along the presynaptic membrane of motor neurons at the neuromuscular junction. Once bound to the receptor, it is internalised and further separated allowing the light chain to move into the cytoplasm. The light chain connects to SNARE proteins (soluble N-ethylmale-imide-sensitive factor-attachment receptors), which facilitate vesicle fusion and release of acetylcholine from synaptic vesicles (Oh and Chung, 2015, Luvisetto, 2020). By binding to SNARE proteins, the toxin prevents the acetylcholine-filled synaptic vesicles from fusing and being released at the neuromuscular junction, and the neuronal impulse cannot be transmitted across the synaptic cleft to the muscle cell (Patil et al., 2016) (figure 11). At appropriate dosing, a treatment with botulinum toxin leads to a chemical denervation, which results in muscle relaxation for extended periods of time. This effect is temporary with recovery occurring through proximal axonal sprouting and muscle re-innervation to form a new neuromuscular junction (Kermen, 2016).

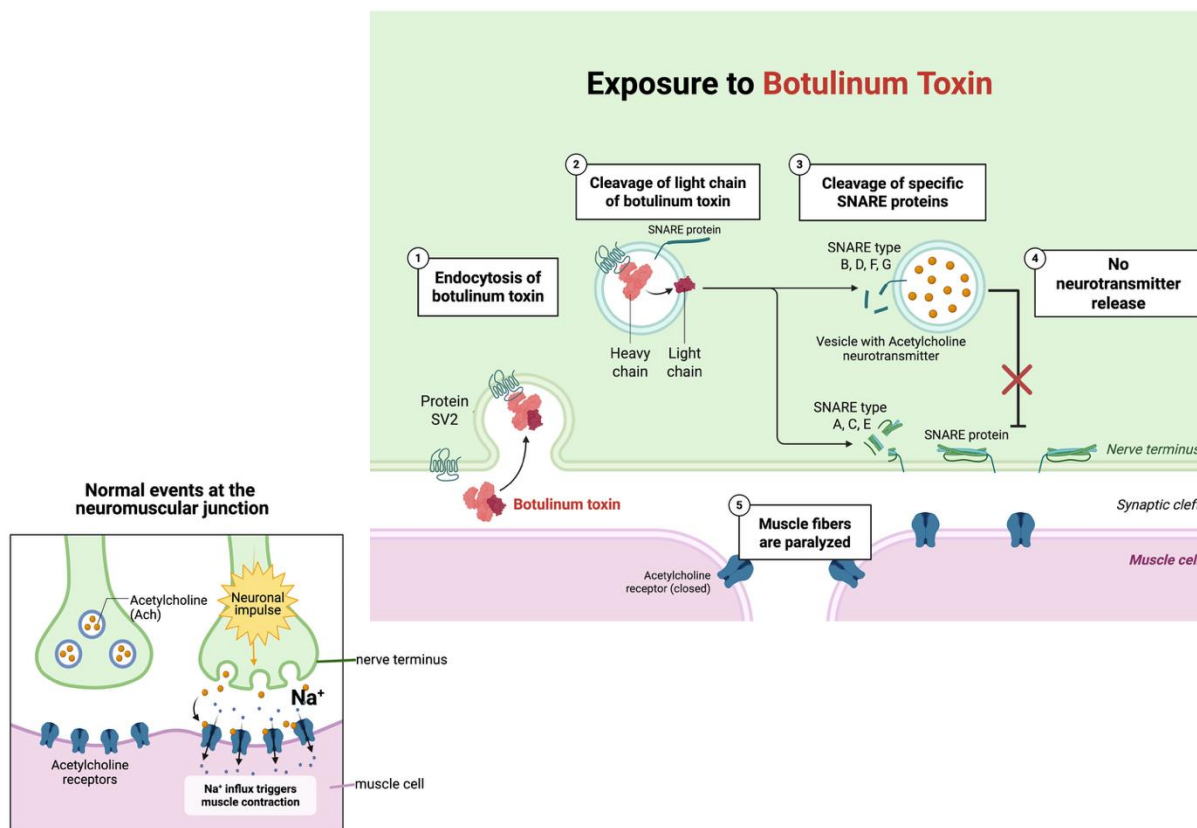


Figure 11 illustrates the mechanism of Botox's muscle paralysing effects.

The toxin is endocytosed by the nerve terminal. It consists of a polypeptide chain with a heavy and light chain. The light chain is cleaved and moves into the cytoplasm to cleave a SNARE protein called SNAP-25 (a synaptosome-associated protein of 25 kDa), which is responsible for acetylcholine vesicles fusing at the neuromuscular junction. This inhibits the neuronal impulse by preventing acetylcholine neurotransmitter release across the synaptic cleft. Without the activation by acetylcholine at the neuromuscular junction, muscle fibres are paralysed. Created in Biorender.com.

Although muscle relaxation in itself may bring analgesic relief, it has been suggested that Botox®'s analgesic effects are multifactorial, involving reduction of neuroinflammation and peripheral sensitization via inhibition of substance P, glutamate, calcitonin gene-related peptide, and vanilloid receptors (Oh and Chung, 2015). Tian et al (2025) propose that microglia

pyroptosis, a newly identified form of inflammatory cell death which is involved in the development of neuropathic pain, is mitigated by botulinum toxin type A (Tian et al., 2025).

2.2.3 Methods and materials

We conducted a retrospective case review of all patients who were treated with Botox® therapy in the Canniesburn Plastic Surgery and Burns Unit, over a two-year period, with minimum 6 months follow up. All patients were fully counselled in risks, benefits, alternative and limitations as per established consent procedures. Each patient also had physiotherapy and psychology input as standard. This clinical audit did not require ethics committee approval, as per the National Health Service Health Research Authority Guidance. The patients were anonymised, the study was registered with the local Clinical Governance Lead, and all data are held in accordance with GDPR procedures. Patients provided express written consent for their clinical photographs to be used for research and teaching purposes, on standardised NHS Scotland Greater Glasgow and Clyde consent forms, stored electronically on NHS Clinical Portal.

2.2.4 Results

Our cohort of 11 patients had 5 (45%) females and 6 (54%) males and had median age of 50 years (range 34-71 years). The patients had a BMI range 18-34 (median 25), 5 were smokers, 2 were ex-smokers, and 4 non-smokers. 54% (6) had pain secondary to traumatic injuries, 36% (4) had pain secondary to systemic sclerosis and 9% (1) had Raynaud's disease. The pain affected the hand or wrist in all but one patient, who had neuropathic pain in their ankle. The patients in the study group had pain symptoms that had been resistant to standard analgesic regimens and complementary therapies such as acupuncture, desensitisation massage and TENS machine, and of a duration of 11.8 years on average (range 3-16 years).

All patients (11) had Botox® intraoperatively and received one single treatment. Botox® was in conjunction with surgical intervention in 73% (8), which included neurolysis 55% (6 patients),

neurectomy 27% (3 patients) and single patients were treated with completion amputation, excision calcinosis, first rib excision and sympathectomy, tenolysis and digital nerve repair with nerve graft reconstruction respectively. Three of the patients (27%) had Botox® injection monotherapy. The dose ranged 30-100units (mean 70 units), 45% (5) injections were intradermal, 18% (2) intraneural, and 36% (4) a combination. There were no complications.

In terms of outcomes, 100% of patients reported benefit to their quality of life, with the majority stating improvement in neuropathic pain (82% (9 patients)) and allodynia (55% (6 patients)), 45% (5 patients) had improved mobility and 36% (4 patients) reduced their analgesic requirements after the post procedural period (figure 12). Five (45%) of the patients were discharged from clinic after treatment, 4/11 (36%) continued routine follow up, 1/11 (9%) was referred for Qutenza® patch therapy and 1/11 (9%) died of other causes before the end of the follow up period.

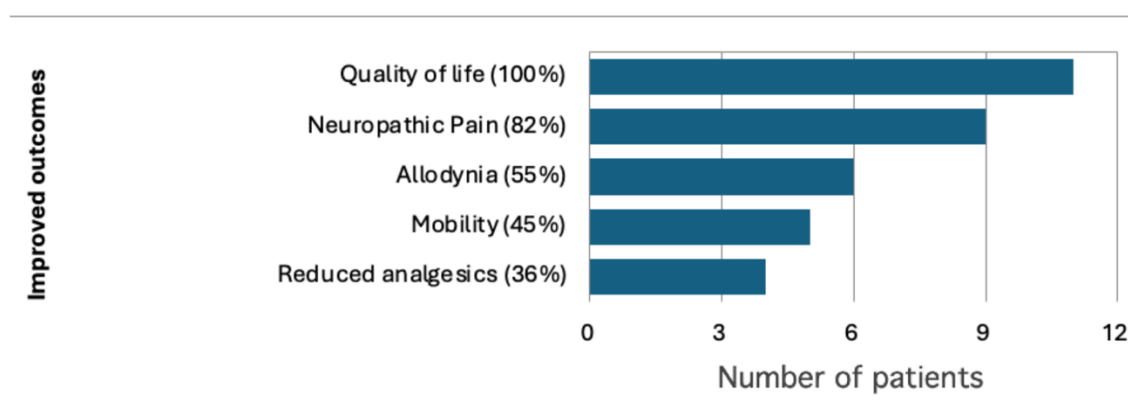


Figure 12 is a graph which illustrates the improved outcomes reported by the patient's following intervention, including a single treatment of Botox®.

2.2.4.1 Case series examples

The following patients kindly gave consent for their cases to be anonymously presented.

Patient 1

This lady is a 55y non-smoker. She has a history of systemic sclerosis with bilateral digital auto necrosis (figure 13) and chronic pain resistant to alprostin, sildenafil, paracetamol and gabapentin regimens. She had trial of Botox® 8 years after her onset of pain, administered to the right hand, which was the worst affected. Eighty units of Botox® was administered to the neurovascular bundle of each digit of her right hand. She also underwent regional sympathectomy, stripping and verapamil to the index to ring finger common digital arteries, and ulnar digital artery to little finger. Post procedure, she reported improved pain, allodynia and mobility in her hands with improved ease of daily living and had no further autoamputation at 2 years follow up.

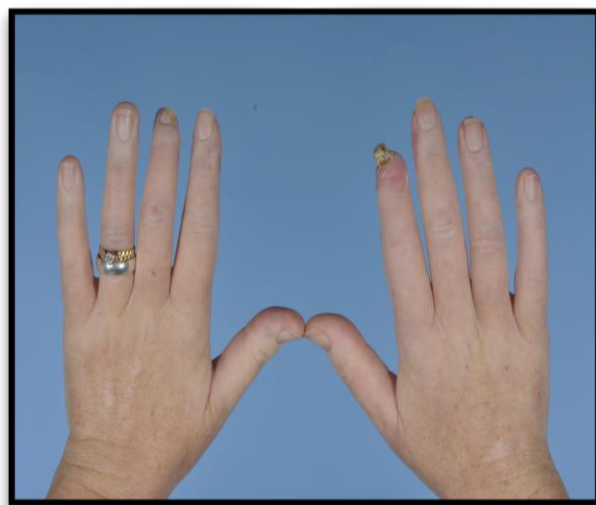


Figure 13 is a clinical photograph of Patient 1, who has a diagnosis of systemic sclerosis with bilateral digital autoamputation. Clinical photographs courtesy of Professor Andrew Hart and with consent from the patient.

Patient 2

The second case is that of a 39-year-old lady who is a smoker. She has a history of alcohol withdrawal seizures and compartment syndrome necessitating fasciotomies and subsequent thoracodorsal artery perforator free flap to her right hand 2014 (figure 14). She also has a history of Raynaud's disease. Her chronic pain was unresponsive to nifedipine, opiates, steroid injection therapy and amitriptyline. She trialed Botox® 3 years after the onset of pain and received 50 units Botox® to each hand with 0.5mls administered to the neurovascular bundle of each digital nerve. She reported improvement in symptoms, allowing amitriptyline to be stopped and she was successfully discharged from clinic.



Figure 14 is a clinical photograph of Patient 2's right hand, illustrating their treatment with a thoracodorsal artery perforator flap. Clinical photographs courtesy of Professor Andrew Hart and with consent from the patient.

Patient 3

The third case is a 71year old, female, non-smoker. She sustained a bimalleolar fracture to her left ankle whilst golfing 2012 (figure 15). This was treated with open reduction and internal fixation and radial forearm flap from her left forearm. This was complicated by infected metalwork in the post-operative period. She suffers chronic neuropathic pain in the peroneal nerve distribution resulting in poor mobility, sleep deprivation and severe allodynia. She had Botox® therapy 5 years after the onset of her pain, with 20 units administered to the superficial

branch of the peroneal nerve. This was at the time of neurectomy of a large terminal neuroma and neurolysis, and a 0.5mg/ml chirocaine catheter was inserted for local anaesthetic postoperative analgesia. The patient reported improvement in her neuropathic pain, but allodynia remained and she was therefore referred for trial of Qutenza® patch therapy.



Figure 15 is a radiograph of patient 3's left ankle, which demonstrates a bimalleolar fracture with metal work in situ.

2.2.5 Discussion

The well-known mechanism by which Botox® inhibits muscle contraction involves the initial uptake of Botox® into the nerve terminal by endocytosis (figure 11). This process typically has a delay of 1 to 4 days, which is why paralysis takes this long to become fully clinically evident. The injection of Botox® for the treatment of patients with chronic ischemic pain, however, has almost immediate and prolonged effects, suggesting a distinct mechanism at play (Neumeister et al., 2009).

Marinelli et al (2010) suggest that botulinum toxin A directly enhances proliferation and maturation of Schwann cells. They report their findings in a murine sciatic nerve ligation model after a single Botox® treatment. They found improved functional outcomes, allodynia and thermal hyperalgesia, along with significantly higher expression of S100 (marker for myelinating and dedifferentiated SC), GFAP (marker for non-myelinating SC and for dedifferentiated cells after injury) and Cdc2 (a cyclin-dependent kinase that regulates the mitotic phase of the cell cycle and the cell migration processes). They suggest botulinum toxin A induces modulation of regenerative processes by Schwann cells (Marinelli et al., 2010).

The effects of Botox® on the improved vascularity of ischemic digits may be mediated sympathetically through a separate neuropathic pathway. Injured C-fibre nociceptors become sensitized and chronic nerve irritation results in an increase in adrenergic receptors on the nerves. Sympathetic stimulation leads to a release of norepinephrine, which acts on the adrenergic receptors to produce both vasoconstriction and pain. The pain is the result of stimulation of the C-polymodal nociceptor, which is known to release the central nervous system neurotransmitter Substance P. Substance P may then act as a self-perpetuating messenger for pain. If Botox® blocks substance P, it may very well decrease or abolish this self-perpetuation cycle of pain and sympathetic stimulation (Neumeister et al., 2009).

There may also be direct or indirect crosstalk between the A and C fibres in the dorsal root ganglion. This method of communication is known as “ephapses”. There are both chemical and anatomical changes that have been observed within the dorsal root ganglion in chronic pain models. Experimental studies have shown that botulinum toxin A inhibits substance P release from cultured embryonic dorsal root ganglion neurons and reduces release of calcitonin gene related peptide (CGRP) from cultured trigeminal ganglia neurons (Brin et al., 2023). It is conceivable that Botox® has more than one mode of pain inhibition, either by neurotransmitter blockade, ectopic Na⁺ channels on chronically irritated nerves, or through its effect on micro vascularity. It has been postulated that the central effects of botulinum toxin A might relate to retrograde transport of the drug along the nerve by axonal trafficking mechanisms like those involved in the transport of other pathogen-related proteins (Neumeister et al., 2009). Attal et al (2016) found that the effects of botulinum toxin A were enhanced in participants with preserved

nociceptive input and suggest this may be explained by the nociceptive fibres transporting the botulinum toxin A to the central nervous system (Attal et al., 2016).

In this study, 82% (9 patients) reported an improvement in neuropathic pain, 55% (6 patients) had less allodynia, 45% (5 patients) had improved mobility and 36% (4 patients) reduced their analgesic requirements after their procedure. This agreed with the findings from Attal et al (2016) randomized, double blind, placebo control trial involving 152 patients. They found that Botox® particularly alleviated mechanical allodynia and hyperalgesia. These effects might be caused by the ability of botulinum toxin A to reduce the proportion of neurons expressing slowly adapting mechanically gated currents, which is linked to mechanonociception (Attal et al., 2016).

Despite systemic drugs, such as pregabalin and duloxetine, having a higher efficacy for sleep and anxiety linked outcomes, all (11) of the patients in this study reported improved quality of life following their treatment. It must be noted however, that Botox® was used as an add-on therapy, alongside surgical intervention in 73% (8) of this cohort. Three of the patients in this study received Botox® monotherapy. A trial by Ranoux et al (2008) randomly assigned patients with localized painful neuropathies and mechanical allodynia to either the botulinum toxin A or placebo groups. They demonstrated that botulinum toxin A had a significant impact on reducing the average level of pain compared to placebo. The pain relief effects became apparent from week 2 and lasted for up to 14 weeks. Decreased allodynia and improved quality of life were reported, and some patients reported complete pain alleviation. However, this study had a small sample size of 29 patients (Ranoux et al., 2008).

2.2.6 Conclusions

This small study agrees with the findings in the literature, that Botox®, as an adjunct, improves patient quality of life, and reduces neuropathic pain and allodynia. These patients had endured chronic neuropathic pain resistant to standard treatment regimens for an average 11.8 years (range 3-16 years). Botox® is also shown to improve chronic pain in those with ischaemic aetiologies. Evidence, however, remains low-level due to small patient cohort sizes. Further study is needed to elucidate long term outcomes.

2.3 Permanent bilateral facial nerve palsy secondary to electrical injury: a case report

2.3.1 Acknowledgements

Thank you to the patient for his time, and permission to share his story and clinical photographs for research and teaching purposes. Thanks to Mr Levi N Ankrah, and Mr Opoku-Ware Ampomah for hosting us in National Reconstructive Plastic Surgery and Burns Centre, Korle-Bu Teaching Hospital, Accra, Ghana, and to Mr Levi Ankrah for editing this paper. Thank you to Mr Stuart Watson for identifying this patient's case of interest, for guidance and mentorship.

2.3.2 Introduction

Electrical injury as a cause of permanent bilateral facial nerve paralysis is extremely rare. Vasquez (1999) reported a case of a 48-year-old gentleman who sustained a transformer arc injury and developed a temporary bilateral facial palsy, but these symptoms resolved over several months (Vasquez et al., 1999). Other temporary unilateral cases have been reported secondary to low voltage electrocution (Ahmed et al., 2007, Ozkiris, 2014) and lightning (Cherington et al., 1995).

Fascial slings provide a viable option in static reconstruction of the oral commissure in the treatment of sialorrhoea, oral incompetence and verbal communication difficulties. The fascia lata sling is associated with short operating time, low donor site morbidity and few complications (Leckenby et al., 2014). In this article, we describe restoration of oral competence using fascia lata sling technique to treat this unusual injury.

2.3.3 Methods

This case report was conducted based on an interview (via interpreter) with the patient during a follow-up consultation with Mr Opoku-Ware Ampomah, and with the use of case file review.

The patient provided written consent for his story and clinical photographs to be shared for research and teaching purposes.

2.3.4 Case report

A 47-year-old Ghanaian male, a maintenance officer with the power distribution company, sustained an electrical injury when a transformer being worked on exploded and a high voltage cable struck him on the scalp. His initial admission was at a military hospital where the significant findings were relatively superficial burns to both upper limbs, subconjunctival haemorrhage, hearing loss, facial oedema and bilateral facial palsy. He had follow-up as an out-patient for over a year, during which there was resolution of his facial swelling and hearing loss, but no return of facial nerve function. He was subsequently lost to follow-up. Three years following the initial injury, he returned to the military hospital and was subsequently referred to Korle Bu Teaching Hospital.

At the time of presentation, he had evidence of bilateral facial palsy with a completely expressionless face and significant wasting of all muscles of the face. There was severe oral incompetence leading to persistent sialorrhoea and difficulties with oral intake and verbal communication. His lower lip was everted under gravity whilst at rest and upon opening the mouth and required manual assistance to achieve closure. There was also sagging of the commissures, lower eyelid ectropion and brow ptosis, bilaterally. All other cranial nerves were grossly intact, but there was some wasting of the temporalis muscle. A CT scan of the head was unremarkable, and nerve conduction studies confirmed that orbicularis oris showed no evidence of electrical activity. A diagnosis of chronic bilateral facial nerve palsy (7th cranial nerve) secondary to high voltage electrical injury was made. He was counselled, consented and planned for a tensor fascia lata sling reconstruction to restore oral competence.

Surgical management

A subdermal tunnel was created from the preauricular region to the lateral aspect of the lower lip bilaterally. This was through a modified facelift incision. The two tunnels were connected through another subdermal tunnel that ran through the entire width of the lower lip. A 25cm x

8cm sheet of fascia lata was harvested from the anterolateral left thigh through a lazy S incision (Figure 16). Due to the prolonged nature of the paralysis and the severity of the commissure depression, a full-thickness wedge of tissue was excised at the lateral aspect of the lower lip bilaterally. The fascia lata graft was then tunneled through the left preauricular incision, through the left cheek, the lower lip, the right cheek, and out the incision on the right preauricular region. With the appropriate tension achieved, the fascia lata sling was anchored to the temperozygomatic arch. The wounds at the lateral aspect of the lower lip were repaired in layers, and the preauricular incisions closed.

The patient's postoperative recovery period was unremarkable, and he was discharged after five days. At follow-up, there were marked improvements in oral competence (Figure 17) and verbal communication. He underwent a further procedure, bilateral permanent tarsorrhaphy, on account of the widened palpebral fissures.



Figure 16 demonstrates the donor site, on the patient's anterolateral left thigh, where the donor fascia lata tissue was harvested.



Figure 17 illustrates the patient's outcome, at two years postop.

2.5.5 Discussion

Electrical injuries are relatively common, accounting for 2.7% of admission to burns units in Ghana (Agbenorku et al., 2014) and up to 8% in Europe (Rai et al., 1999). Neurological sequelae can present as an early or late complication and can affect the central or peripheral nervous system (Ahmed et al., 2007). The pathophysiology of peripheral nerve injury in electrical injury is well described; however, bilateral chronic facial nerve palsy from this mechanism has not previously been reported.

In 1934, Critchley described the neurological effects of lightning and electricity upon the nervous system. In the peripheral nervous system, electrical injury induces infiltration of the epineurium with endothelial cells, ballooning in the myelin sheath due to disintegration and fusion of Schwann cell nuclei and tortuosity with fragmentation of axons. Haemorrhagic changes are commoner in the presence of injury from alternating current whereas vascular tears are more often found in lightning injury (Critchley, 1934). Curiously, neuronal change following electrical

insult tends to be patchy, with healthy and diseased tissue lying in unpredictable proximity and is widely variable, with fatal doses of electrocution leaving no gross cellular alteration of the nervous system in some cases (Critchley, 1934). Factors influencing the degree of damage include the voltage, duration of exposure and resistance to current (Agbenorku et al., 2014). In addition to the damage to the nervous system, electrical burns are associated with soft tissue injury in the form of open entry or exit wounds and deeper damage to muscle and skin (Agbenorku et al., 2014).

In the case of our patient, no open wounds were sustained, and the cutaneous burns were largely superficial, although deep tissue effects are common with high voltage injuries and can be present in the absence of superficial signs (Rai et al., 1999). It is interesting that his initial injuries were largely restricted to two cranial nerves (facial nerve (Cranial Nerve VII) and vestibulo-cochlear (Cranial Nerve VIII) and that this occurred bilaterally. Critchley (1934) noted that in severe injury, the “entire brain and parts of the cord may be swollen, softened and even diffuent”(Critchley, 1934), perhaps a swelling at the common origin of the facial and vestibulo-cochlear nerves at the cerebellopontine angle of the brainstem (located in the junction between the pons and the medulla oblongata) (Acland) would account for the bilateral and symmetrical pattern of his symptoms. The presence of anastomotic connections between the facial and vestibular nerves in the internal auditory canal is also reported to contribute to vestibular disturbance in facial paralysis (Andrews and Reisner, 2017); however, the vestibulocochlear nerve injury in our patient appeared to resolve with time, whilst the facial nerve injury was permanent. Özdogmus et al. (2024) studied the number of fibres and the interconnected relationship of the facial, vestibular and cochlear nerves in their course from the brainstem to their endpoints. Interestingly, they found interconnections between the facial and vestibular nerves but not the facial and cochlear nerves (Ozdoğan et al., 2004). The vestibular nerve bundles showed an increase in the number of nerve fibres from the inner ear end towards the brainstem end of the internal auditory canal, whereas the facial and cochlear nerve bundles showed a reduction in the number of nerve fibres on both sides (Ozdoğan et al., 2004). Perhaps the more robust anatomy of the vestibular component of the nerve protected it, as our patient did not report symptoms of vertigo or imbalance. Many of the secondary pathophysiological effects of electricity are trophic in nature and in the other cases reported in the literature their symptoms

were only transient; hypotheses to explain this phenomenon include an ischaemic aetiology secondary to vasoconstriction following massive autonomic stimulation (Critchley, 1934). It is possible that our patient's nerve palsies were secondary to nerve ischaemia and that the facial nerve insult did not resolve due to persistent facial oedema of a year's duration, rather than the direct electrical insult.

Free functional muscle transfer remains the gold standard to provide spontaneous symmetrical smile in patients with facial paralysis. Leckenby et al. (2013) describe that nerve regeneration and brain plasticity decrease after the first two decades of life and so they offer this procedure between the ages of 5-55 years (Leckenby et al., 2014). Free gracilis muscle transfer, with reinnervation from the contralateral facial nerve or a branch of the trigeminal (usually the nerve to the masseter) is the technique commonly employed (Baccarani et al., 2019). In this patient however, with a bilateral and prolonged paralysis, the options available were largely static procedures. His priorities lay primarily in restoring oral competence and secondarily in the benefits of verbal communication and cosmesis. Furthermore, cost implications of the duration of operating and anaesthetic time and hospital stay, and the technical challenges of performing a bilateral free functional muscle transfer were also considerations. Therefore, fascia lata reconstruction was indicated as a static technique for reconstruction that successfully met his treatment outcome goals, whilst being of low risk and low cost. The main technical challenge was maintaining sufficient tension within the sling to enable oral competence and avoid eversion of the lower lip, while keeping the sling loose enough to allow jaw opening without distortion of the os.

2.3.6 Conclusion

We present the first report of a chronic bilateral facial nerve palsy and severe oral incompetence secondary to high voltage electrical blast injury. Static procedures are an option for patients who present late, with muscle wasting, and provide satisfactory, quick, and cost-effective options to manage severe complications of facial palsy. A carefully planned fascia lata sling was used to restore oral competence, successfully treating sialorrhoea and improving the patient's oral intake, verbal communication and cosmesis.

Chapter 2.4 Do you use nerve guidance conduits for peripheral nerve repair? A global survey.

2.4.1 Acknowledgements

Our sincere thanks to everyone who participated in this survey, for sharing their time and expertise. Thank you to all of those who kindly aided in distributing the survey, including the European Association of Plastic Surgeons (EURAPS) and the British Society for Surgery of the Hand (BSSH). Thank you to Ali Muhammad Sain and Mathis Riehle for contributions to survey design, Ms Suzanne Emma Thomson and Dr Mathis Riehle for contributions to editing, and to Prof Andrew Hart and Ms Suzanne Emma Thomson for assistance with survey distribution.

2.4.2 Introduction

As academic clinicians, it is our pleasure and privilege to bridge the gap between the lab and the clinic, allowing one to inspire and inform the other. It was noted that our colleagues here in Scotland do not use nerve guidance conduits, despite them being available on the market. The authors were aware anecdotally of colleagues using nerve guidance conduits in Turkey and in the US. On review of the literature, there was little evidence detailing the uptake of the use of nerve guidance conduits globally, nor the reasons why. We sought to answer this gap in the literature, to inform our own research group, and that of the wider scientific and clinical community. Our aim is to contribute to the body of knowledge that provides an evidence base for meaningful scientific translation, and improved outcomes for patients with peripheral nerve injury.

2.4.3 Methods and Materials

A survey was designed in Qualtrics XM, with sixteen questions pertaining to the use of nerve guidance conduits for peripheral nerve repair. The questions were in multiple choice or free entry format and underwent ethical approval by the School of Medicine Veterinary and Life Sciences Ethics Committee at University of Glasgow (200240019). Exclusion criteria were stipulated; questions were not regarding allograft, autograft or nerve wrap, but nerve

guidance conduits specifically. The survey was designed for use by the global population of surgeons who undertake peripheral nerve repair work. It was streamlined to take less than three minutes to complete, to maximise completion rates. The survey was distributed through the networks of the authors via word of mouth, email, LinkedIn, Instagram and Whatsapp to local, regional, national and international colleagues, and via the European Association of Plastic Surgeons (EURAPS) and the British Society for Surgery of the Hand (BSSH) via their newsletters and websites. National Hand Surgery Societies were contacted in other countries and continents, but they did not respond.

2.4.4 Results

There were 58 respondents, over an 8month period, practising peripheral nerve repair surgery in 17 different countries (United Kingdom (UK) 15, Turkey 6, Ethiopia 4, Italy 4, Switzerland 3, South Africa 2, Austria 1, Croatia 1, Denmark 1, Germany 1, Ghana 1, Netherlands 1, Romania 1, Spain 1, United Arab Emirates (UAE) 1, United States of America (US) 1, and 13 did not answer) (figure 18).

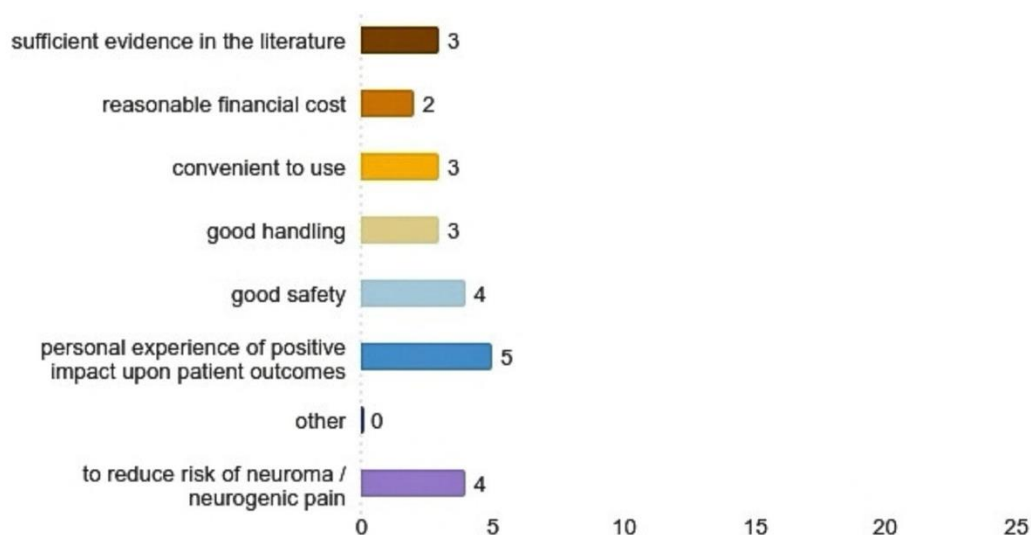


Figure 18 is a graph which illustrates the reasons for using nerve guidance conduits, as given by the surgeon respondents.

The majority of these nations are in Europe (10/17) and have a national health service (6/17), or a national insurance system (8/17) as their dominant mode of funding for healthcare for their populations. Two of the 17 countries from which there were respondents had predominantly private health insurance-based healthcare (table 1).

Table 1 demonstrates the dominant mode of funding for the National Health Care System of the 45 surgeons who stated their country of practice.

Dominant mode of funding for National Health Care System	Tax Based		National Health Insurance		Private Insurance	
Surgeon country of practice (Number of respondents)	UK	15	Turkey	6	UAE	1
	Italy	4	Ethiopia	4	US	1
	Switzerland	3	Austria	1		
	South Africa	2	Croatia	1		
	Denmark	1	Germany	1		
	Spain	1	Ghana	1		
		1	Netherlands	1		
			Romania	1		
Total	27		17		2	

Forty-one of the respondents were plastic surgeons, 4 were orthopaedic surgeons, 1 stated “other” and 12 did not state their specialty. Twenty-five out of the 58 (43%) surgeons were hand surgery specialists, 21 (36%) stated they were not hand surgery specialists, and 12 (20%) did not specify their specialty. Of the 58 respondents, 47 surgeons answered the question pertaining to their use of nerve guidance conduits for peripheral nerve repair; 7/47 surgeons used nerve guidance conduits in their clinical practice, and 40/47 did not.

Of the 7 surgeons who stated that they did use nerve guidance conduits, 5 gave details regarding patient numbers: over the past 12 months 2/5 had treated less than 10 patients, 1/5 had treated

10-20 patients and 2/5 surgeons had treated 40-50 patients with nerve guidance conduits for peripheral nerve injury. When asked for the brands of nerve guidance conduits being used, the responses were as follows: Reaxon (1), Neuragen (2), Axogen (1), and the materials of preference included type 1 collagen (4), polyglycolic acid (1), polycaprolactone (2), polyvinyl alcohol (1) and “other” (2). When asked the maximum size of nerve gap for which a nerve guidance conduit would be used, 6/7 surgeons responded with answers ranging between 4-300mm, and an average of 132.5mm. Six of the 7 surgeons responded about their experience of complications with nerve guidance conduits, 1/6 stated they experienced decreased complication rate, and 5/6 stated that they saw no difference in complication rate when compared to gold standard autologous nerve graft. Of the 7 surgeons who used nerve guidance conduits, 5 answered why: 5/5 stated they had personal experience of positive impact on patient outcomes, 4/5 reported reduced neuroma/neurogenic pain, 4/5 good safety, 3/5 sufficient evidence in the literature, 3/5 good handling, 3/5 convenient to use and 2/5 reasonable financial cost (Figure 19).

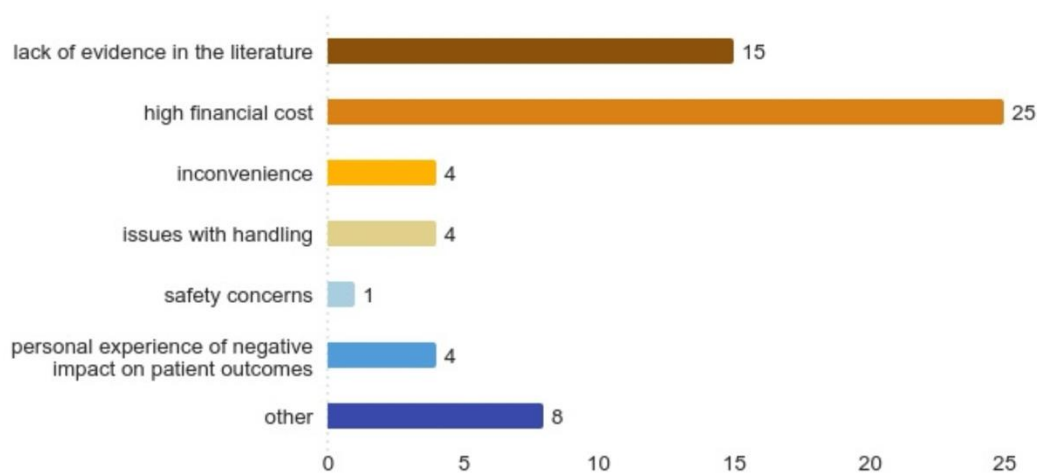


Figure 19 is a graph which illustrates the reasons for not using nerve guidance conduits, as given by 34 of the surgeons

Of the 40 surgeons who stated they did not use nerve guidance conduits for peripheral nerve repair the reasons why were answered by 34 surgeons. Their reasons given were as follows: 25/34 high financial cost, 15/34 lack of evidence in the literature, 8/34 “other”, 4/34

inconvenience, 4/34 issues with handling, 4/34 personal experience of negative impact on patient outcomes, 1/34 safety concerns. When asked about the outcome assessments they routinely use in the follow up of patients who have undergone peripheral nerve repair, 45 surgeons responded. Thirty-two of the 45 used clinical examination of power (eg. British Medical Research Council (BMRC) grading, or direct force measure), 31/45 used touch threshold testing, 29/45 used two-point discrimination testing, 22/45 used nerve conduction studies, 16/45 used ‘any other standardised sensory testing’, 14/45 used cold intolerance testing, 9/45 used Patient reported outcome measures (PROMs), 6/45 stated “other” and 5/45 used a sensorimotor combined assessment tool (eg. integrated functional outcome (eg. Rosen Model Instrument)). Forty-six surgeons informed us for how many years they provided routine follow up for their patients who had been treated with peripheral nerve repair; this ranged from 0-10 years with an average of 3.52 years.

When asked to describe the ideal nerve conduit for their clinical practice the responses included: *“bioabsorbable, non-immunogenic, potential to elute drugs, equivalent outcomes (which matter to patients) to autologous options”*; *“Patient’s own cultured Schwann cells in a nanotubular construct”*; and *“Cost effective, biodegradable, biocompatibility, easily accessible and surgically useable”* (table 2).

Table 2 Depicts answers from the 15 surgeons who gave their opinion about the ideal nerve conduit design for their clinical practice.

Can you describe the ideal nerve guidance conduit for your clinical practice?
Bioabsorbable, non-immunogenic, potential to elute drugs, equivalent outcomes (which matter to patients) to autologous options
Small ie. a few mm diameter, 5-3cm
This would bridge any size of nerve gap, encourage orderly nerve regrowth, minimise localised scarring, be cheap and readily available
Can you describe the ideal nerve guidance conduit for your clinical practice?
Patients own cultured Schwann cells in a nanotubular construct
A conduit that will be clinical as good as autograft for defects of 3cm or longer
Autograft
Autologous nerve graft
NO impact on healing - no neuroma formation - some conduction
Resorb-able, no limit length and accepted cost
Cost effective, biodegradable, biocompatibility, easily accessible and surgically usable
Cost neutral, readily available, no immune response, accessible, reliable, promotion of axonal regeneration and myelination
Easy to use and effective
Available off the shelf, non-immunogenic, biodegradable, additional features to promote nerve regeneration soft but pressure resistant, porous, promoting nerve regeneration
Should be inexpensive or covered by the insurance
Cheap and sure proved to be better than a nerve graft

Two of the surgeons replied that the ideal nerve conduit for their clinical practice was “Autologous nerve graft” (figure 20). When asked if they had any more comments about nerve guidance conduits in their practice for treating peripheral nerve injuries the replies included the following: “*If the right one could be developed it would be very useful*”; “*We use muscle in vein conduits for sensory nerves up to 6cm rather than conduits (results are not convincing in literature)*”¹ (table 3).

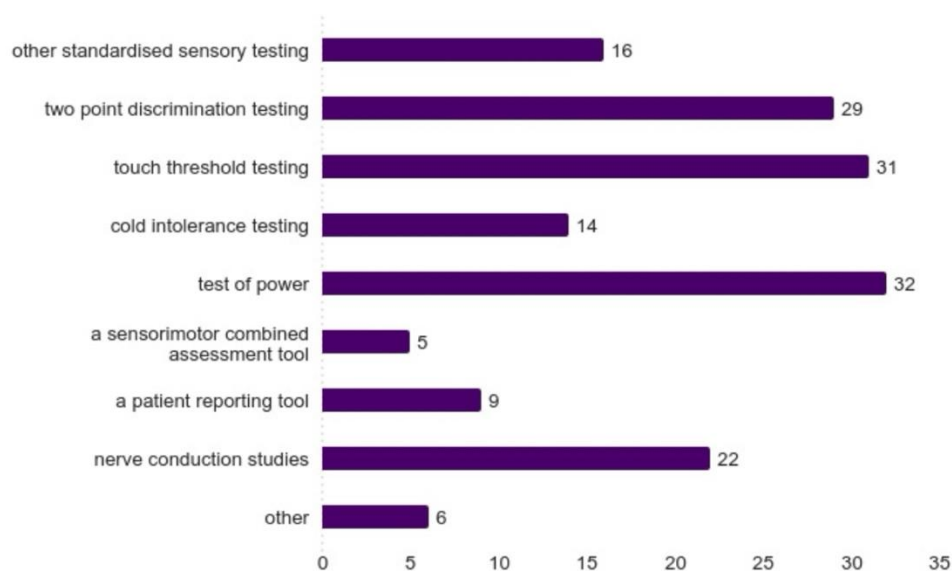


Figure 20 is a graph which depicts the postoperative outcome measures used by 34 of the surgeons

¹ We interpret this to mean that they think the evidence in the literature for the use of nerve guidance conduits is not convincing.

Table 3 Depicts answers from the five surgeons who gave further comments about the use of nerve guidance conduits in their clinical practice

Do you have any other comments regarding the use of nerve guidance conduits in your clinical practice?
Need to be infection resistant, biologically safe, flexible, inexpensive and effective
For digital nerve size conduits, I use the Tos muscle-in-vein technique
We use muscle in vein conduits for sensory nerves up to 6cm rather than conduits (results are not convincing in literature)
If the right one could be developed it would be very useful
To be used with careful technique

2.4.5 Discussion

The cost of nerve guidance conduits is an inhibiting factor to their clinical translation.

Of the 40 surgeons who stated they did not use nerve guidance conduits for peripheral nerve repair the reasons why were answered by 34 surgeons. The commonest stated reason for not using nerve guidance conduits was high financial cost (25/34). The global synthetic nerve conduit market size is calculated at USD 2.78 billion in 2025 and is expected to reach around USD 5.79 billion by 2034, with forecasted growth at a Compound Annual Growth Rate of 8.35%. North America dominated the global synthetic nerve conduit market in 2024, reportedly due to rising prevalence of peripheral nerve injuries and state-of-the-art research and development facilities (S., 28 Jan 2025).

The Food and Drug Administration (FDA) regulates the approval of nerve guidance conduits in the US. As of 2023, the FDA has approved 34 nerve regeneration devices, including nerve

guidance conduits for repairing nerve transection injuries with substantial tissue loss. The average cost of nerve conduits ranges from approximately \$4,000 to \$13,000. This high cost limits the affordability of this therapeutic option globally, especially for people from low and middle-income groups and those who may not have health insurance (S., 28 Jan 2025). Most respondents of this study were working in nations that had a dominant national health service or national health insurance system of healthcare provision (44/46), which likely precluded clinical spending based on resource rationing. In addition to the cost of the conduit, the infrastructure for delivery and storage, microsurgical training, instruments and equipment are overheads to be considered in this treatment pathway. Global healthcare and financial inequalities still preclude the use of microsurgical technique in some nations. Countries such as Brazil, Mexico, Cambodia, Ethiopia, and Rwanda are actively developing their microsurgical capabilities through healthcare infrastructure expansion and global health collaborations. In just one region of Ethiopia, for example, an estimated 120 million people previously lacked access to microsurgery; a gap that new initiatives are working to bridge (Gebremariyam and Weldemichael, 2024).

Lack of evidence in the literature remains a precluding factor to clinical translation of nerve guidance conduits for peripheral nerve repair.

Evidence for the use of any alternatives to the gold standard autologous nerve graft remains of low level due to heterogeneity in study participants, injury pattern, repair timing, outcome measures and follow up intervals (Thomson et al., 2022). In this study, 7 surgeons stated they used nerve guidance conduits for peripheral nerve repair, 3 of the surgeons stated they deemed the evidence for the use of nerve guidance conduits ‘sufficient’. In contrast, of 34 respondents who stated that they did not use nerve guidance conduits in their clinical practice, the second most common reason given was a ‘lack of evidence in the literature’ (15 surgeons). Authors of this study published a Cochrane review summarising the available evidence from parallel group randomised controlled trials (RCTs) and quasi-RCTs on nerve repair in the upper limb using a bioengineered wrap or conduit, with at least 12 months of follow-up (Thomson et al., 2022). Five studies met the criteria for inclusion in their analysis, which evaluated 213 participants with 257 upper limb nerve injuries (Åberg et al., 2009, Bertleff et al., 2005, Boeckstyns et al., 2013, Lundborg et al., 2004, Weber et al., 2000).

Thomson et al. (2022) highlighted evidence suggesting higher adverse events with the use of currently available bioengineered nerve wraps and conduits than with standard repair techniques, and that the need for revision surgery may also be greater (Thomson et al., 2022). In this study, 7 surgeons reported using nerve guidance conduits in their clinical practice and 6 of them gave details. They responded that they were treating patients with nerve guidance conduits for injuries with nerve gaps of 4-300mm, and an average of 132.5mm. Five of the surgeons stated they had personal experience of positive impact upon patient outcomes with the use of nerve guidance conduits, and 4 surgeons stated they think nerve guidance conduits offer 'good safety'. When asked specifically about complications, 1 surgeon stated they experienced decreased complication rate, and 5 stated that they saw no difference in complication rate when compared to gold standard autologous nerve graft. There is a clear need for future randomised multicentre control trials with standardised procedural and logistical design, outcome measures and protocols, and in larger cohorts, to facilitate meta-analysis of data between studies of different devices, and in comparison, with autologous nerve graft controls.

In addition to the evidence in the literature being dominated by small scale studies with differing and therefore non comparable variables, most of this work is done in male rats (Kaplan et al., 2015). The rat is a particularly poor model for the repair of human critical gap defects. As introduced, a critical nerve gap in humans is 30mm whereas in rats, the critical nerve gap is considered 15mm, in rabbits it is 30mm, and in pigs it is 40mm. Rats also have a species specific neurobiological regenerative profile, which doesn't translate to humans. This means that even if longer repairs were possible in rat models, this cannot adequately represent the healing process of a critical gap in humans (Kaplan et al., 2015). Windebank's group (2012) conducted a systematic review of the animal models utilised to study nerve regeneration in tissue engineered scaffolds. They report 74 % of 416 studies were rat models, and 7.5 % in each mouse and rabbit models (Angius et al., 2012).

Muscle-in-vein technique as an alternative to nerve graft

Whilst many bioengineering experimental research efforts have been focussed upon artificial scaffolds for bridging nerve gaps, the use of autologous, non-nervous tissue as a grafting material is a field of interest that is translating into clinical practice (Geuna et al., 2014). When asked in the survey if they had any other comments regarding the use of nerve guidance conduits in their clinical practice, 5 surgeons gave responses. Two of the five advised that for “digital nerve size” defects and “sensory nerves up to 6cm”, they use “muscle-in-vein technique”.

The first application of vein graft for the treatment of peripheral nerve injury seems to have been documented by Wrede in 1909 (Wrede, 1909), where the author reported functional recovery in a patient who was treated for a median nerve injury, using vein to bridge the 45mm gap (Battiston et al., 2005). The efficacy of vein grafts has been confirmed by multiple authors since. However, experimental and clinical studies indicate that vein grafts are consistently effective only for relatively short gaps, of less than 30mm, with vein collapse over longer gaps cited as an issue (Heinzel et al., 2021).

Skeletal muscle scaffolds for nerve reconstruction seem to have been first reported in 1940, by Kraus (Kraus and Reisner, 1940). Their rationale was based on the observation that skeletal muscle has a longitudinally aligned structure that may serve as a guidance scaffold for regenerating axons across a nerve defect, and that skeletal muscle basal laminae resembles the endoneurial tubes of degenerating nerve (Battiston et al., 2005). Pre-degeneration may be used to disrupt the skeletal muscle architecture, to avoid the impediment of regenerating axons. However, RNA expression analysis in a murine model has suggested that the degenerating skeletal muscle fibres in fresh muscle-in-vein combined conduits activate an autotrophic loop based on the neuregulin 1/ErbB system, which in turn may actively support Schwann cell viability (Nicolino et al., 2009).

Combining the above approaches as a muscle-in-vein technique reflects creative problem solving which may overcome the tendency of vein-based nerve grafts to collapse in distances longer than 1–2 cm and reduces the axonal loss that may occur in mono-tissue muscle grafts. It is also a low

cost, low morbidity solution whereby the vein and muscle are harvested from the site of injury without the expense of time, equipment and the risk of complications associated with a second surgical site (Battiston et al., 2000). A meta-analysis of studies comparing muscle-in-vein grafts with autologous nerve graft by Heinzl et al. (2021) advise caution in extrapolating rodent preclinical study data to human clinical contexts for peripheral nerve regeneration, as there are marked differences in aspects of inter-species neurobiology and physiology (Heinzl et al., 2021). Relevant to the context of muscle-in-vein grafts, rodents possess around 1.4x the number of proteases and have markedly increased proteome turnover when compared to humans. Heinzl et al. therefore propose that the muscle fibres in the muscle-in-vein grafts would degrade faster in rodents than in humans, contributing to collapse of the graft before axons have time to traverse it, and inferior results of muscle-in-vein grafts when compared with autologous nerve graft in murine models. They suggest large-animal studies followed by clinical trials as a prerequisite before translation of this technique in clinical practice, to treat patients with peripheral nerve injury (Heinzl et al., 2021).

Battiston et al. (2000) report a clinical case series of 21 peripheral nerve injuries in 20 patients, treated with muscle-in-vein grafting, in Italy, between 1993-1997. The technique was applied for bridging both sensory and mixed nerve gaps, 5- 60mm in length, with minimum follow up of 14 months. They report 'good results' in motor and sensory recovery for 85% of patients, in accordance with the criteria of the Nerve Injuries Committee of the British Medical Research Council, as modified by Mackinnon and Dellon (Battiston et al., 2000). Ederer et al. (2023) report a case series of 43 common palmar digital nerve injuries, in 37 patients from Germany. Primary repair by muscle-in-vein conduits was performed in 22 cases, whereas 21 cases underwent secondary reconstruction, with a median 25-month follow up period. Ederer et al. used American Society for Surgery of the Hand guidelines; 81.4% of their patient's outcomes were classified as excellent or good, with 9.3% rated as fair or poor. The modified Highet and Sander's criteria rated complete clinical recovery in 13 cases; 23 outcomes were regarded as S3+ (recovery of pain and touch sensibility with localisation of the stimulus is good, but recovery of two-point discrimination is imperfect) (Ederer et al., 2022). None of these studies include patient reported outcome measure data.

There remains low uptake of patient reporting tools in the assessment of patient post-peripheral nerve injury.

Measuring the outcome of peripheral nerve surgery is challenging because the spectrum of functional impairment is dependent on the level and severity of the lesion. There are no universally accepted outcome measures both in terms of the parameters to be assessed, and the methods and timing of the assessment; there are also no nerve-specific patient-reported outcome measures (Calcagni and Besmens, 2024). Resultingly, there remains limited clinical trial data in the literature that compares functional outcomes and complication profiles between nerve guidance conduit devices, and with nerve autograft as a control. Thomson et al. (2022) found that the existing evidence for sensory recovery is uncertain, and no data was found for muscle strength at 24 months (Thomson et al., 2022). Standardised assessment must be established globally, between research studies, if metanalysis is to accurately compare surgical approaches and devices.

In a bid to standardise the assessment of peripheral nerve injuries, especially during and after World War II, staff in the Department of Surgery, University of Edinburgh, authored the landmark handbook published by the British Medical Research Council (MRC) '*Aids to examination of Peripheral Nerve Injuries (War memorandum No. 7, 1941)*'. This guidance provided standardised methods and diagrams for examining nerve damage, including the still widely used British MRC muscle strength grading scale. The guidance was revised in 1943 and has been re-printed many times since (Compston, 2010). In this study, 32/45 responding surgeons stated that they examined power in their post-operative assessment of patients who had undergone surgical repair of peripheral nerve injury using the British Medical Research Council (BMRC) grading, or direct force measure. Thirty-one surgeons reported use of touch threshold testing, 16/45 used 'any other standardised sensory testing', and 29/45 used two-point discrimination testing, as per this liminal document. In this study 22/45 surgeons reported using nerve conduction studies in their post-operative assessment of patients treated with peripheral nerve repair. According to the British National Institute for Clinical Excellence (NICE) guidelines (2022), nerve conduction studies should be used in patients where their recovery is not as expected, or if there is no sign of recovery at 6 weeks (guideline NG211, 2022). Forty-six

surgeons informed us for how many years they provide routine follow up for their patients who had been treated with peripheral nerve repair; this ranged from 0-10 years with an average of 3.52 years.

Patient-reported outcome measures (PROMs) and patient-reported experience measures (PREMs) establish the gap between patients' expectations and clinical definitions of 'success'. They offer insight into the patients' experience and perception of the treatment outcome upon their physical, psychological, cognitive and spiritual health, and the subsequent impacts upon their quality of life. The UK Office for Health Improvement and Disparities published guidance (2020) recommending the use of PROMs and PREMs to assess the quality of healthcare experiences from the perspective of the patients. They state this data should then inform healthcare providers, commissioners and other stakeholders to make informed changes to their services, to guide research and design personalised care (Disparities, 2020). In this study, 9/45 surgeons reported the use of PROMs. British Society of Surgery for the Hand (BSSH) guidelines endorse validated PROMs to capture patient experience of patients who undergo treatment for peripheral nerve injury. They advise that since there is currently no single nerve-specific PROM or universal standard, a mix of general (such as PRMOMIS, or DASH) and injury-specific measures must be used to assess function, pain, quality of life, fatigue, and emotional well-being over time (Calcagni and Besmens, 2024).

Nerve guidance conduits and neuroma

Four surgeons in this study reported their experience of nerve guidance conduits was that of "reduced neuroma/neurogenic pain" for their patients. Neuromas are a pathological response to peripheral nerve injury: dense, fibrous, poorly vascularised structures consisting of axons, fibroblasts, and Schwann cells. They form due to collagen remodelling and scar formation during disorganised axonal outgrowth in peripheral nerve regeneration. Neuroma associated pain is reported in up to 30% of patients following peripheral nerve injury (Sisti et al., 2024). The aetiology of neuroma pain remains unconfirmed; it is thought that the extension of axonal fibres from the nerve stump into the matrix of regenerative connective tissue, traction, or pressure on the nerve endings, or abnormal concentrations of nociceptors may be implicated (Sisti et al.,

2024). Treatment options include surgical excision and pharmacological management, aiming to alleviate pain. Several studies have suggested that using nerve conduits or nerve caps for the treatment of peripheral nerve injury may be efficacious in reducing neuroma formation by creating a protective barrier, inhibiting nerve adhesions, and maintaining nerve mobility. In Sisti et al.'s (2024) literature review of published techniques for neuroma prevention and management in patients with peripheral nerve injuries, they found 10 eligible human studies. These reports had mixed results and consisted mainly of low-level evidence, case reports without control groups (Sisti et al., 2024).

The bioengineered conduits being used in the clinical practice of the respondents of this survey include: type 1 collagen (4), polycaprolactone (2), polyglycolic acid (1), polyvinyl alcohol (1) and "other" (2). The use of type I collagen to prevent neuroma in peripheral nerves was studied in 50 patients by Gould et al. (2013), and their study showed promising results, with 85% of the patients showing significant reductions in pain at follow-up (average 36 months)(Gould et al., 2013). De Vrij et al. reported the use of a resorbable tubular conduit composed of a copolymer of lactide and caprolactone in 10 patients post excision of symptomatic neuroma. The patients reported resolved pain and improved disability of the affected upper limb, demonstrated by a decrease in QuickDASH questionnaire scores from 51.6 at baseline, to 14.4 at 12 months(de Vrij et al., 2022). Zukawa et al. (2019) report clinical outcomes and evaluations of in-vivo high resolution ultrasonography assessment of polyglycolic acid-collagen conduits for the treatment of peripheral nerve injury in 4 patients. At 30 months follow up, all 4 patients were very satisfied with pain relief, but recovery of sensory function was insufficient. The ultrasonographic evaluation revealed the PGA outer wall had disappeared by 3 months, postoperatively (Zukawa et al., 2019). No human studies were found when searching for clinical evidence using the key words "polyvinyl alcohol/nerve wrap/neuroma/nerve/human" in Google and Pubmed search engines. Stocco et al. (2024) report a preclinical murine study of 1% oxidised polyvinyl alcohol wraps, and natural leukocyte-fibrin-platelet membrane wraps, compared with the commercially available NeuraWrap. Explant at 12 weeks post-implantation showed no scar tissue or neuroma at the site of injury, in all groups. The natural leukocyte-fibrin-platelet membrane wrap was completely resorbed, while residues of 1% oxidised polyvinyl alcohol wrap and NeuraWrap were observed. Peripheral nerve regeneration was supported in all groups, as seen in histological,

immunohistochemical and morphometric analysis and there was no significant inflammatory infiltrate. Functional analysis showed outcomes comparable to that of the NeuraWrap in both experimental groups (Stocco et al., 2019). Therefore, the use of nerve guidance conduits for peripheral nerve repair remains experimental, until high level evidence becomes available.

Limitations: higher study completion rates and participant numbers are required to draw conclusions.

Meyers et al. (2022) published a systematic review studying surgeon survey completion behaviour, suggesting that web-based surveys have the lowest response rates, compared with in-person, telephone, in-writing or email formats. They report highest uptake of surveys amongst surgeons in Finland, Africa and China and lowest uptake in Belgium, France and the United States (Meyer et al., 2022). Despite the 8-month long, worldwide invitation to participate, and dissemination through one national and one international organisation as well as a multitude of positive social media responses, the total number of surgeons who responded was only 58. The responding surgeons were practising peripheral nerve repair surgery in 17 different countries.

The authors of this study were based in the UK and Turkey, and utilised known contacts at BSSH and EURAPS to request support in disseminating the survey; this appears to have introduced bias in the geographical location of the respondents. Requests for survey completion and dissemination were not granted from known contacts in other geographical locations and were not granted by any individual or organisation that were contacted without a personal connection with the authors. There were no responses from South America, Oceania, or Asia outwith Turkey, and thus our data does not provide a truly global picture. Considering the finding that the responses of this study relied upon personal networks, a global committee where there are authors working in each of the global territories, may improve survey completion rate. Meyers et al. suggest that shorter questions, shorter surveys, personalisation of the questionnaire topic and cover letter, and yes/no questions attributed to higher response and completion rates (Meyer et al., 2022). Despite employing these techniques, only 34 of the 58 returned surveys

were complete, and of the 7 surgeons who stated they did use peripheral nerve guidance conduits, only 5 gave answers in full. Survey design, necessitating completion before submission may improve completion rates, albeit at the cost of response rates. The survey was anonymised at point of entry, as per the ethical approval from the University of Glasgow. It would be valuable for future study, to request the data linking the country of origin to the responses as part of the study design, to increase understanding of geographical location of conduit use, and indeed its accessibility.

In future, increased sample size, with representation from a wider range of countries, perhaps weighted for their dominant mode of healthcare system provision, and an increased rate of complete responses are required to draw accurate conclusions about the global accessibility and uptake of nerve guidance conduits for peripheral nerve repair. This may be achieved by designing a study whereby the authors are an international committee of representative clinicians from a wide range of nations, who can use their personal and professional networks to achieve high quality survey dissemination and subsequent high rates of completion. Based on the knowledge that surveys have higher respondent and completion rates using in-person modes of data collection, a presence at local, regional, national and international conferences as a mode of disseminating the survey may improve the quality and quantity of data. Designing the survey to disallow partial completion may improve survey completion rates, albeit at the risk of reducing overall respondent numbers. Linking the data pertaining to country of clinical practice to the uptake of nerve guidance conduit use could give valuable context to the data, acknowledging the critical numbers required to maintain ethical anonymity.

2.4.6 Conclusion

To conclude, the majority of survey respondents in this study were hand-specialist plastic surgeons, based in Europe. Most of the surveyed surgeons did not use nerve guidance conduits for peripheral nerve repair. The biggest inhibiting factor seemed to be monetary, and it was noted that most of the surgeon respondents work in centres that are predominantly funded by tax or national health insurance.

For the small number of surgeons in this study who did use nerve guidance conduits for peripheral nerve repair, all of them reported personal experience of ‘a positive impact upon patient outcomes’ and ‘no difference’, or ‘improvement’ in complication rates when compared with autologous nerve graft. Their rationale for the use of nerve guidance conduits included ‘good safety’ and ‘reduction in neuroma/pain’. However, the second most common reason given by most surgeons for not utilising nerve guidance conduits was a ‘lack of evidence in the literature’.

The existing evidence regarding nerve guidance conduits for peripheral nerve repair remains low level, due to the small study sizes and the presence of confounding variables, the heterogeneity in the methods and timings of surgical procedures and follow-up assessment which preclude a fair comparison between devices, and the lack of an autologous nerve graft control. Multicentre randomised control trials are required to compare different conduit options versus the gold standard. These trials should be designed to facilitate meta-analysis of data, with large patient cohorts and strict exclusion criteria, standardised procedural protocols and outcome assessment measures, if surgeons are to make informed decisions about the translation of these promising technologies into their routine clinical practice.

Chapter 3 Differentiation of adipose derived stem cells (ADSCs) towards a Schwann cell phenotype (dADSC)

3.1 Introduction

As introduced in Chapter 1, Schwann cells are the architects, engineers and labourers of natural nerve regeneration. They coordinate the process via secretion of neurotrophic factors, they physically guide axons across the zone of injury via bands of Büngner, and they perform remyelination of regenerating axons. However, alternatives to Schwann cells are sought for regenerative medicine solutions to improve outcomes for patients with peripheral nerve injury due to the drawbacks of Schwann cell use, including the donor morbidity of ‘sacrificing nerve to treat nerve’, and the complexities of culturing them *in vitro* in adequate numbers (Fairbairn et al., 2015).

Differentiating another cell into Schwann cell-like phenotype was first demonstrated, for primary rodent neural crest cells, by Stemple and Anderson in 1992 (Hörner et al., 2022). They report sorting for the transmembrane protein p75NGFR and differentiating the neural crest cells towards a Schwann cell phenotype with incubation in forskolin serum-containing medium (Stemple and Anderson, 1992). Further molecular factors influencing glial differentiation were identified in isolated embryonal neural crest cells, laying the foundation for directed differentiation protocols for Schwann-like cells.

Horner et al. (2022) conducted a literature review of *in vitro* Schwann-like differentiation protocols. They concluded that many used the same supplement cocktail, consisting of a short incubation with β -mercaptoethanol and all-trans-retinoic-acid, followed by a combination of Foetal Bovine Serum (FBS), forskolin, Nrg1 (Neuregulin-1 or its specific isoform Glial Growth Factor-2), bFGF (basic Fibroblast Growth Factor), and PDGF (Platelet Derived Growth Factor). They suggest these factors might act through promoting the proliferation and survival of differentiating cells down the Schwann cell lineage (Hörner et al., 2022) (table 8). β -mercaptoethanol can be used as a reducing agent, breaking intramolecular and intermolecular disulfide bonds in proteins, and all-trans-retinoic-acid is a neural morphogen. Schwann cells

express retinoid receptors during normal development and retinoic signalling can have various effects on Schwann cells. These include stimulating migration, or regulation of myelination; however, it must be used with caution as it can also inhibit Schwann cell growth. Forskolin is a cyclic Adenosine Mono Phosphate (cAMP) elevating agent and is the most widely used molecule in Schwann cell differentiation protocols (Hörner et al., 2022). Signalling of the cAMP pathway has been described in Schwann cell development and maturation, as well as myelin formation. In addition, by stimulating cAMP, forskolin seems to synergistically increase the effects of Nrg1, PDGF, or bFGF *in vitro*, possibly through increasing trophic factor receptor levels. In natural nerve regeneration, axons produce Nrg1 (Neuregulin-1), which guides immature Schwann cells to initiate radial sorting bundling, and segregation of larger axons for myelination. bFGF acts as a mitogen and survival factor for Schwann cell precursors and seems to promote transition to immature Schwann cells and their proliferation. PDGF, specifically the isoform PDGF-BB, promotes survival of Schwann cell precursors, as well as of Schwann cells, and acts as Schwann cell mitogen (Hörner et al., 2022). *In vitro* derivation of Schwann cells has now been demonstrated from various human and non-human cell sources, including adipose-derived stem cells (ADSCs) (Zack-Williams et al., 2015).

As described in Chapter 1, human adipose-derived stem cells (ADSCs) are one such cell that may be differentiated into a Schwann-like phenotype (dADSC). They provide an abundant reservoir, with minimal morbidity and may commonly be accessed from the patient requiring trauma or cancer reconstruction, without additional operative sites. dADSCs have shown promising results for promoting neural outgrowth in peripheral nerve injury models *in vitro* and *in vivo*, in animal studies (Zack-Williams et al., 2015, di Summa et al., 2010, Kingham et al., 2007). The current standard differentiation protocols require cells to be either induced into neurospheres using non-adherent culture or, in a monolayer protocol (Kingham et al., 2007, Hörner et al., 2022).

The monolayer protocol as described by Kingham et al. (2007) (Kingham et al., 2007) was chosen for this work on differentiation of human ADSCs as a natural continuation, and progression to increase clinical translatability, of the previous work in our research group in differentiating rat ADSCs. The lab's established techniques, protocols and ethical considerations

were updated and optimised specifically for this work with human ADSCs, and established methods were utilised to determine the efficacy of the protocol in differentiating the human ADSCs to Schwann-like dADSCs (Negro et al., 2022).

Since *in vitro* stem cell-derived Schwann cells usually show an immature phenotype, and as differentiation efficiencies can vary, it is of high importance to thoroughly characterise the cells obtained (Hörner et al., 2022). One method of determining differentiation into a Schwann-like phenotype is a change from a flat, fibroblast-like morphology to a narrow, elongated shape. The cell volume decreases, and the cytoplasm shrinks, with two or three slender processes extending from the cell body. This ‘spindle’ shape is crucial for Schwann cells’ function in repairing peripheral nerve injuries by forming bands of Büngner (Negro et al., 2022).

Another established method of Schwann/Schwann-like cell identification is by immunohistochemistry (Negro et al., 2022). Immunohistochemical markers seen during Schwann cell precursor development include Gap43, MPZ, and S100 β . MPZ and S100 β continue to increase during progression towards immature Schwann cells. The literature suggests that all Schwann cell subtypes continue to express S100 β . Therefore, S100 β is the most used marker for identifying Schwann cells/Schwann-like cells. However, S100 β is not exclusively expressed in Schwann cells; it is also seen on glial and neural cells, melanocytes, adipocytes, and some muscle cell types (Doddrell et al., 2012). As differentiation progresses, markers such as Krox20 start to be expressed, indicating the development into a pre-myelinating state. The marker of maturity and myelinating Schwann cell phenotype is MBP. Whilst expression of Gap43 and p75NGFR persist in the non-myelinating state, cells which become myelinating cease to express them (Hörner et al., 2022). The markers expressed are dependent on other various factors including the induction media, the culture medium, the passage and the time in culture (Zack-Williams et al., 2015). Therefore, it is imperative to combine methods of analysis, and for several markers, to robustly identify Schwann cells.

This study has limitations, including duplicate rather than triplicate biological (patient) repeats for some experiments. It was not possible to compare ADSCs and dADSCs from the same patients. This was partly the result of attempts to keep the passage and time in culture low, as

these factors are known to impact upon the expression of Schwann-like markers (Zack-Williams et al., 2015), and to avoid freeze thaw cycles as a confounding variable.

3.2 Materials and Methods

3.2.1 ADSC extraction from human adipose

The planning and conduct of this study comply with the Human Tissue Act (2004) and it was granted UK Biobank approval (UK Biobank reference number 304/314). Surplus adipose tissue was obtained at the Canniesburn Plastic and Reconstructive Surgery Department, Glasgow Royal Infirmary, from patients who gave informed consent.

Human ADSCs were isolated from the surplus abdominal adipose tissue following breast reconstruction surgery using abdominal autologous flaps (deep inferior epigastric perforator flaps (DIEP)) from the Canniesburn Plastic Surgery Unit, Glasgow Royal Infirmary, Scotland. This followed informed patient consent, and approval from the hospital, and Biobank Biorepository ethics committees, and was in accordance with the Declaration of Helsinki.

All donors were women receiving breast reconstruction by the DIEP method, the majority had undergone mastectomy as treatment for confirmed breast cancer, precancerous change, or were undergoing prophylactic mastectomy in light of genetic predisposition to the disease (eg Breast Cancer genes 1 or 2 (BRCA1, BRCA2)). The patients were identified from the theatre lists, and confirmation was attained from the responsible consultant surgeon's secretary that the patient had not received neo-adjuvant chemotherapy in advance of their procedure, due to the impact this might have upon the stem cells. On the day of the procedure, the consent process was conducted by the operating surgeon, independent to involvement in the study. The collection of the adipose tissue was coordinated with the operating theatre nursing team. The surplus adipose tissue (the half of the DIEP flap on the side of the non-dominant deep inferior epigastric perforator vessel) was kept sterile, on a draped trolley. Following aseptic technique, the skin was

dissected from the adipose tissue, placed in a sterile container, and transported on ice to the laboratory for cell isolation.

ADSCs were isolated utilising collagenase digestion, following an established protocol (Kingham et al., 2007). The adipose tissue was mechanically minced with scissors and then enzymatically dissociated with 0.15% (w/v) collagenase Type II (Gibco) at 37 °C for 1 hour in a water bath. The collagenase reaction was stopped, with one volume of the corresponding complete medium, and centrifuged at 300g for 8 minutes. The solution was passed through a 70µm filter to remove undissociated tissue and further centrifuged at 200g for 5 minutes. The stromal vascular fraction was resuspended in 8 mL of the corresponding complete medium, plated in T75 flasks, and cultured at 37 °C and 5% CO₂ (figure 21). The flasks were washed thrice with HBSS and the medium was changed after 24 hours to remove erythrocytes. The media changes were then conducted every 3–4 days and 0.25% trypsin/versene used as required to maintain cultures of approximately 80% confluency. All experiments were subsequently performed on cells at passages 1–6 (P1–6) to reduce the selection of ADSC subpopulations. The culture medium consisted of Gibco α-MEM (1x)+glutaMAX, (Thermo Fischer Scientific), supplemented with 10% FBS (Sigma Aldrich, Zofingen, Switzerland), and with 1% penicillin/streptomycin (Thermo Fischer Scientific). The extraction protocol was additionally optimised by standardising the volume of adipose tissue by weight, with 48g for every 15mls of 0.15% collagenase solution (as detailed above). Cells showed successful recovery from freezing, storage and recovery after 24 months in liquid nitrogen, and 12 months in -80°C, with use of DMSO and serum.

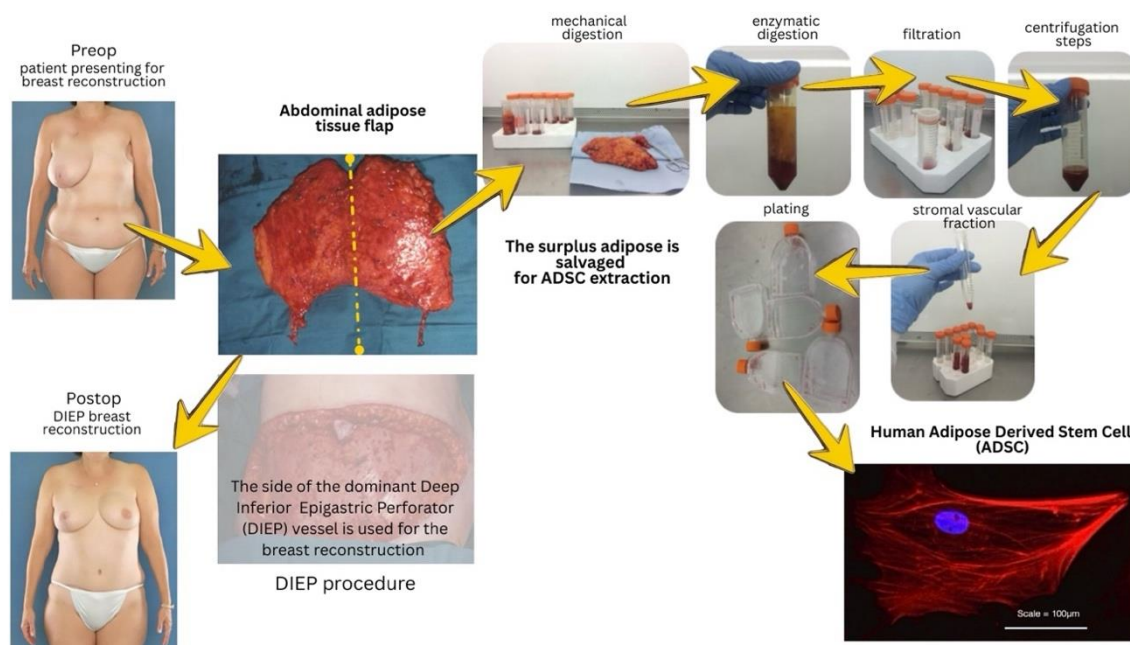


Figure 21 is a flow diagram of the ADSC extraction process. All patients presented for unilateral breast reconstruction by the DIEP technique (pre-op). Once the abdominal adipose tissue flap was removed, the side with the dominant DIEP vessel was dissected and utilised for the reconstruction procedure (leading to post-op result). With the patient's written consent, and in accordance with the named ethical approvals, the surplus abdominal adipose tissue was transported to the lab for the ADSC extraction digest process. Stages include mechanical digestion, enzymatic digestion (collagenase), filtration with 70µm filter, and multiple centrifugation stages, before a stromal vascular fraction was isolated and plated into tissue culture flasks. The ADSCs adhere to the cell culture plastic and become 80% confluent at around 10 days. Primary cells stained for actin (red) and DNA (blue); scale bar 100µm. Clinical photographs provided by Ms Eva Weiller-Mithoff with permission of the patient.

3.2.2 Characterisation of human ADSCs

To confirm the extracted cells were indeed ADSCs, with multipotent potential, the International Society for Cellular Therapy (ISCT) criteria for human mesenchymal stem cells was followed. This includes following established protocols to demonstrate: (1) cell adherence to tissue culture plastic, which was confirmed with expansion of cells in tissue culture plastic flasks; (2) the

expression of the following cluster of differentiation CD markers: CD73, CD90, and CD105 and lack expression of CD14, CD34 and CD45 markers (table 4); and (3) the ability to differentiate down osteocyte, adipocyte and chondrocyte lineages(Dominici et al., 2006).

Table 4 Describes the cluster of differentiation (CD) markers that are utilised in identification of ADSCs due to their presence or absence respectively, and details their function, as per the International Society for Cellular Therapy (ISCT) criteria for human mesenchymal stem cells(Dominici et al., 2006), and the Human Leukocyte Differentiation Antigens (HLDA) workshop BD CD Marker Handbook(Biosciences, 2016).

Cluster of differentiation (CD) marker present on ADSCs	Function	Cluster of differentiation (CD) marker absent on ADSCs	Function
CD73	An ecto-5-prime-nucleotidase, which hydrolyses extracellular nucleotides into membrane-permeable nucleosides	CD14	Mediates the innate immune response to bacterial lipopolysaccharide (LPS)
CD90	Cell adhesion	CD34	Cell adhesion
CD105	Plays a role in haematopoiesis and angiogenesis	CD45	Regulator of T- and B-cell antigen receptor signalling and regulator of cell growth and differentiation

3.2.2.1 Flow cytometry to establish CD markers

Thank you to Dr Euan Ross for his assistance with these flow cytometry experiments. To evaluate immunophenotype, ADSCs (at passage 3) were stained with the following antibodies: specifically anti-CD73 (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labeled), anti-CD90 (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled), anti-CD105 (1:200, IgG1, BD

Biosciences, monoclonal, PE-labelled) as positive mesenchymal stem cell markers; anti-CD34 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled); and anti-CD45 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled), as negative endothelial and hemopoietic markers, respectively. Appropriate isotype controls were used for each fluorophore (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled; 1:50, IgG1, BD Biosciences, monoclonal, PE-labelled; 1:50, IgG2b, BD Biosciences, monoclonal, FITC-labelled). For each antigen, approximately 100,000 cells were resuspended in 100 μ L of antibody diluted in flow cytometry buffer (0.2% FBS in PBS) and incubated for 1 hour, agitated in the dark. The cell surface phenotype was subsequently assessed by BD Accuri C6 apparatus (Erembodegem). Experiments were conducted in technical and biological triplicates (three patient repeats).

3.2.2.2 *Differentiation down multipoetic lineages*

The following experiments were led by Ms Marie Kearns, and I assisted with cell monitoring, media preparation and cell feeding. Three different lineages were tested to establish stemness; following adipogenic, osteogenic and chondrogenic protocols.

3.2.2.2.1 Adipogenic differentiation of ADSCs

ADSCs were seeded at passage 1-2 (P1-2) at a density of $0.5 \times 10^4/\text{cm}^2$, with DMEM/10% FBS and 1% penicillin/streptomycin medium and were maintained until 80% confluent. Adipogenic differentiation was induced using a mesenchymal stem cell adipogenesis kit (Merck). Cells were maintained in either induction media, which consists of DMEM/10% FBS, 1 μ M dexamethasone, 0.5mM IBMX, 6310 μ g/mL insulin, 100 μ M indomethacin and 1% penicillin/streptomycin, or maintenance media (as per above). The induction medium consists of DMEM/10% FBS, 1 μ M dexamethasone, 0.5mM IBMX, 6310 μ g/mL insulin, 100 μ M indomethacin and 1% penicillin/streptomycin. The maintenance medium contains α -MEM/10% FBS, 10 μ g/mL insulin and 1% penicillin/streptomycin. After 21 days of differentiation, medium was aspirated from each well and cells were fixed in 4% paraformaldehyde for 30 minutes. Paraformaldehyde was aspirated and cells rinsed with PBS, 3 thrice. Oil Red O solution, a lysochrome diazo dye, was used in histology to stain neutral triglycerides and lipids bright red/orange. The cells were

washed with water twice and Oil Red O solution (Merck) was added to each well for 50-minute incubation at room temperature. After washing thrice with water, haematoxylin solution (Sigma Aldrich) was added to each well for nuclear staining, with 15-minute incubation. Experiments were conducted in technical biological triplicates.

3.2.2.2.2 Osteogenic differentiation of ADSCs

ADSCs (P1-2) were seeded at a density of $0.5 \times 10^4/\text{cm}^2$ with medium consisting of α -MEM/10% FBS and 1% penicillin/streptomycin and maintained until 80% confluent. Osteogenic differentiation was induced using a mesenchymal stem cell osteogenesis kit (Merck). The induction medium consisted of DMEM/10% FBS with 1% penicillin/streptomycin, 100ug/ml ascorbate, 0.1uM dexamethasone, 10mM β glycerophosphate, for 21 days, with 72 hourly media changes. The cells were fixed in 4% paraformaldehyde for 30 minutes and subsequently washed with PBS and then water. Alizarin Red is a water-soluble anthraquinone dye which stains calcium deposits, creating a bright red, chelated complex. Alizarin Red stain (Sigma Aldrich) was added to each well for a 45-minute incubation, at room temperature. The stain was removed, and the cells washed with distilled water. Experiments were conducted in technical biological triplicates.

3.2.2.2.3 Chondrogenic differentiation of ADSCs

ADSCs (P1-2) were resuspended in StemPro™ Chondrogenesis differentiation medium (Gibco) at a concentration of $5 \times 10^5/\text{ml}$. A pellet culture system, according to the manufacturer's instruction, was formed by adding 500 μ l of the cell suspension into a 15ml falcon tube, with a conical bottom, the lid loosely attached and in upright position. The cells were incubated at 37°C with 5% CO₂ and media was changed 72 hourly by gently aspirating the media and adding fresh differentiation media, without disturbing the cell pellet. After 21 days, media was aspirated from the cell pellet and the pellet was fixed in 4% paraformaldehyde overnight at 4°C. Pellets were subsequently washed thrice in PBS, embedded in OCT embedding compound (Tissue-Tek), frozen with liquid nitrogen and stored at -80°C until further analysis. The pellets were sectioned using a microtome in the Histology Laboratory, by Ms Marie Kearns, with assistance from Mr David Russell. Toluidine Blue is a cationic thiazine dye used in histology to detect acidic tissue components, particularly glycosaminoglycans in cartilage, mast cells, and connective tissues. It

acts via metachromasia, shifting colour from blue (orthochromatic) to purple/reddish-purple (metachromatic) when bound to highly sulphated glycosaminoglycans. Slides were washed with distilled water and stained with Toluidine Blue for 30 minutes. Experiments were conducted in technical biological triplicates.

3.2.3 Differentiation of ADSCs towards Schwann-like phenotype

The ADSC growth medium was supplemented with 1 mM β -mercaptoethanol (Sigma-Aldrich, UK) for 24 hours. After washing, fresh media was then supplemented with 35ng/ml all-trans-retinoic acid (Sigma-Aldrich, UK) for a further 72 hours, before replacement with differentiation medium, comprised of 5ng/ml platelet-derived growth factor (PDGF; Millipore), 10ng/ml basic fibroblast growth factor (bFGF; Bibco Life Tech), 14 μ M forskolin (Sigma-Aldrich) and 252ng/ml GGF-2 (recombinant NRG1-beta, R&D Systems). Cells were then incubated for 2 weeks under standard conditions, with 72 hourly media changes. Experiments were conducted in technical triplicates and biological duplicates.

3.2.4 Immunohistochemistry methods

3.2.4.1 Coverslip coating with Poly-L-lysine (PLL)

Poly-L-lysine (PLL) (Sigma-Aldrich) was stored at a stock solution 4mg/ml, which was filter sterilised (0.22 μ M) into 67 μ l aliquots to avoid freeze/thaw cycles. One 67 μ l aliquot was added per 20ml of milliQ water and filter sterilised. This was added to a petri dish containing ethanol sterilised coverslips and incubated at 37°C for one hour (30 minutes minimum). They were washed x 3 with sterile milliQ water and dried before the laminin coating step.

3.2.4.2 Coverslip coating with laminin

Laminin (Sigma-Aldrich) was stored at stock solution of 1mg/ml with a working solution of 2ng/ml. 40 μ L of the laminin stock solution was added to 20ml PBS and filter sterilised.

The Laminin was added to Petri dish containing the PLL coverslips which had been coated and allowed to dry. Incubated was at 37°C for 1 hour minimum. Coverslips were then washed x3 with sterile PBS.

3.2.4.3 Immunohistochemistry protocol

Cells were seeded at a concentration of 10^4 cells per well in a 24 well plate, on a PLL and laminin coated coverslip (13mm). Cells were fixed once confluent (3 days). Differentiation to Schwann cell phenotype was confirmed by immunocytochemistry for GFAP (anti-GFAP rabbit, Santa Cruz Biotechnology, SC-9065, H0414), S100 (anti-S100 rabbit, abcam, ab868, GR164645-3) and p75 (p75 NGFR goat, Santa Cruz Biotechnology, SC-6188, G0714) neuronal markers and morphology (rhodamine phalloidin, Life Technologies, R415, 1646681) utilising Vectashield mounting medium with DAPI (Vector, H-1200, ZB1130) and fluorescence microscopy (Olympus BX51TF). Experiments were conducted in technical triplicates, with three patient repeats for ADSCs and two patient repeats for dADSCs.

3.2.5 Cell morphology analysis methods

In all conditions, cell morphology was monitored daily under phase-contrast microscope, (10x, Olympus IX81). Cells were fixed with 4% paraformaldehyde for 30 minutes at room temperature and then washed with 1% PBS. Permealising buffer (10.3g sucrose (Fisher BioReagents) + 0.292g NaCl (VWR Chemicals) + 0.06g MgCl₂ (VMR) + 0.476g Hepes (VWR Chemicals) in 100ml 1% PBS, 7.2pH + 0.5ml Triton x) was added for a 5-minute incubation at 4°C, and then a further wash and 5-minute incubation at 37°C with block solution (1% BSA (Fisher BioReagents) in 1% PBS). Staining for the actin cytoskeleton (rhodamine phalloidin, life technologies, R415, 1646681) at 1:500 dilution was conducted, with coverslips mounted with Vectashield DAPI (Vector, H-1200, ZB1130). Experiments were conducted in technical triplicates and biological duplicates.

Imaging and cell labelling ratio analysis was conducted using automated programming, at random, for 100 cells, with high content fluorescence microscopy (Olympus BX51TF). Cell profiler cell image analysis software (4.1.3) pipeline was written and utilised for cell

measurements (minimum 500-1000 cells per condition) (figure 22). Adaptive thresholding of greyscale images identified cells based on DNA staining (DAPI) as 'primary objects', and actin cytoskeleton staining (phalloidin) as 'secondary objects'. The watershed-gradient method was implemented to delineate neighbouring cells, and cells touching the boundary of the images were discarded. Graphpad Prism6 and Microsoft Excel were utilised for data analysis.

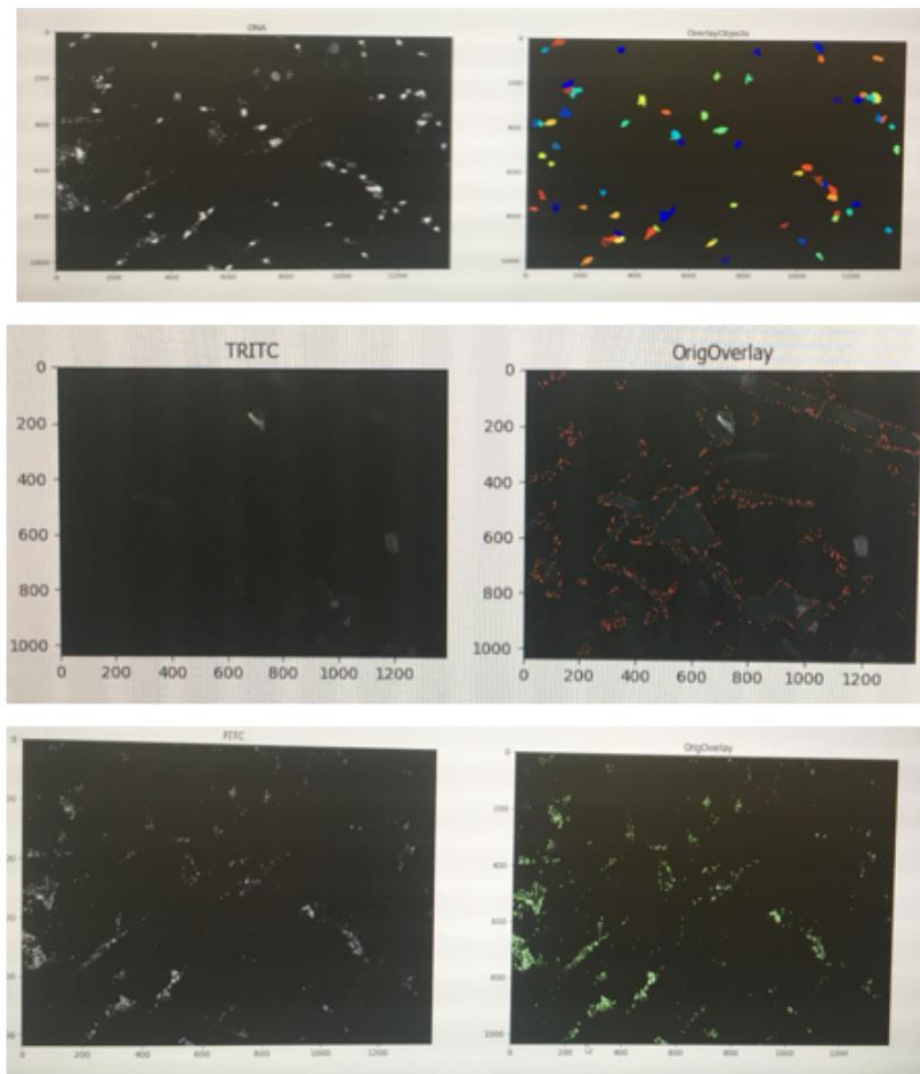


Figure 22 shows three screenshots, which outline the process of the Cell Profiler pipeline being written. To detect individual cells from greyscale microscope photographs, cells were identified by their nuclei as a primary object (DNA), their cytoskeleton as a secondary objects (TRITC) and the SPIONs within their cytoplasm (FITC).

Morphological parameters studied included cell area, cell maximum Feret's diameter, cell eccentricity, nucleus area, nuclei area, nuclei maximum Feret's diameter, nuclei eccentricity, and nuclei solidity. The area refers to the extent of the surface enclosed within the boundaries of that structure. Eccentricity measures how much a shape deviates from a perfect circle, where 0 characterises a perfect circle (figure 23). Maximum Feret's diameter refers to the longest distance between any two parallel lines tangent to the structure's boundary. Nuclei solidity is the ratio of the nucleus area to the area of its convex hull. It is a metric used to quantify the shape irregularity, where a value closer to 1 indicates a smooth, regular shape (circular/ellipsoidal), while lower values indicate irregularity or deformity (Institute., Copyright 2003-2021).

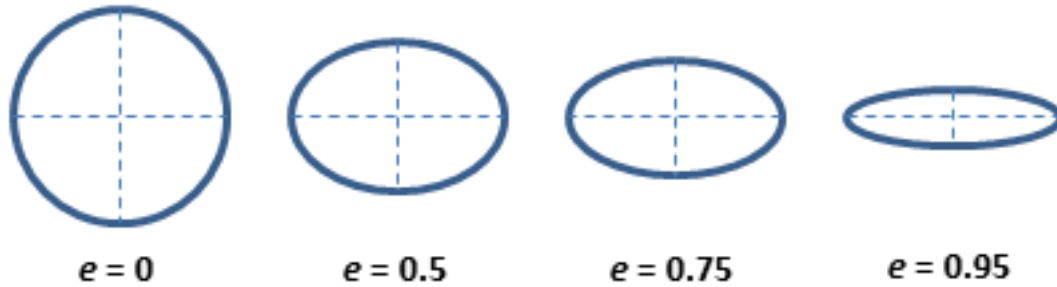


Figure 23 illustrates the eccentricity of an ellipse and is reproduced from Cell Profiler Manual, where eccentricity of a perfect circle=0 (Institute., Copyright 2003-2021).

3.3 Results

3.3.1 Characterisation of human ADSCs

3.3.1.1 CD markers results

The cells isolated using the ADSC extraction protocol(Kingham et al., 2007) were shown to be positive for CD73, CD90 and CD105, and negative for CD45. The cells were positive for CD34 (figure 24) and were not tested for CD14.

In addition, the cells were positive for CD10, CD13, CD29, CD31, CD34, CD44, CD146, CD166, CD271 and HLA-ABC (table 5) and negative for CD38, CD41a, CD140a, CD140b, CD106, HLADR (table 6). This was true for three patient repeats.

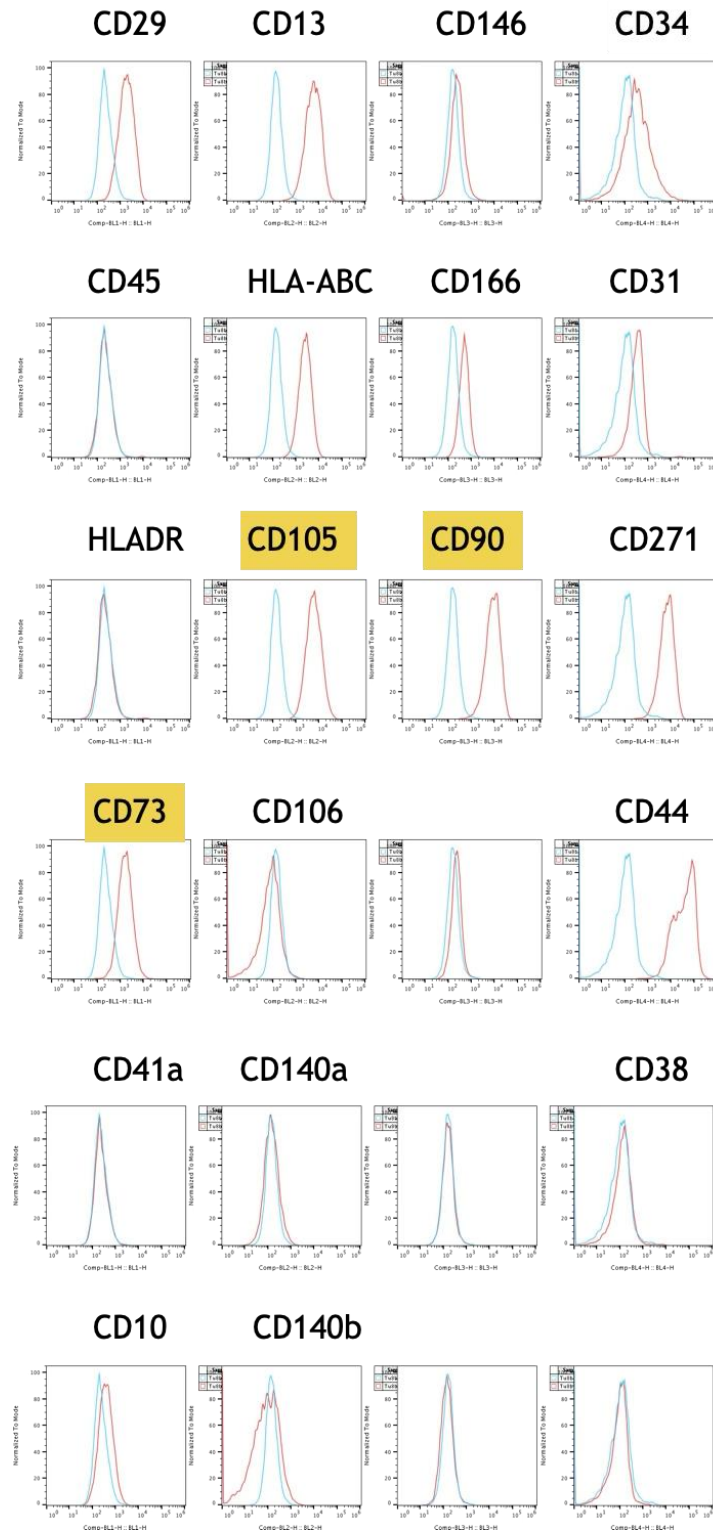


Figure 24 Depicts the Flow Cytometry results as graphs, from BD Accuri C6 apparatus (Erembodegem), where blue is the control, and red is the antibody result.

Table 5 CD markers **present** on the cells isolated following the ADSC extraction process, as tested with flow cytometry, and their reported functions (Biosciences, 2016, Genie).

Cluster of differentiation (CD) markers present	Function
CD10	Endopeptidase that cleaves peptides and inactivates several peptide hormones
CD13	Aminopeptidase (an enzyme that cleaves amino acids from the N-terminus of proteins and peptides)
CD29	Crucial for cell adhesion, migration, and signalling. It plays key roles in tissue development, wound healing, and immune responses
CD31	Cell adhesion, activation, and migration
CD34	A surface marker for haematopoietic stem and progenitor cells, endothelial cells, and vascular tumours; involved in cell adhesion
CD44	A primary receptor for hyaluronic acid to regulate cell interactions, adhesion, migration, and survival
CD73	An ecto-5-prime-nucleotidase, which hydrolyses extracellular nucleotides into membrane-permeable nucleosides
Cluster of differentiation (CD) markers present	Function
CD90	A cell surface glycoprotein, heavily expressed on stem cells, neurons, fibroblasts, and activated endothelial cell and involved in cell adhesion
CD105	Plays a role in haematopoiesis and angiogenesis
CD146 weakly positive	Also known as melanoma cell adhesion molecule; it is a transmembrane glycoprotein cell receptor expressed on endothelial and tumour cells and involved in adhesion, migration, angiogenesis, and immune response
CD166	Mediates cell adhesion; it is involved in T-cell stimulation, neurogenesis, angiogenesis, and tumour metastasis
CD271	Apoptosis, differentiation, neurogenesis
HLA-ABC	Expressed on most nucleated cells, they present endogenous antigens to CD8+ cytotoxic T cells

Table 6 depicts CD markers which were tested for and found **absent** by flow cytometry on the cells isolated following the ADSC extraction process, and their reported functions(Biosciences, 2016, Genie).

Cluster of differentiation (CD) markers absent	Function
CD38	Cell adhesion and signal transduction
CD41a	CD41 plays a crucial role in platelet function and blood coagulation by mediating platelet aggregation
CD140a	Transmembrane receptor tyrosine kinase and member of the PDGFR family, primarily expressed on mesenchymal-derived cells; plays vital roles in wound healing, cell migration, and embryonic development
CD140b	Tyrosine kinase receptor involved in mesenchymal cell proliferation, migration, and angiogenesis. As a key marker for ADSCs and many mesenchymal tumours, it plays a crucial role in atherogenesis, oncogenesis, and fibrotic processes
CD106	Transmembrane glycoprotein that mediates leukocyte-endothelial cell adhesion
HLADR	A cell surface receptor of the MHC class II type, crucial for immune recognition and antigen presentation to CD4 ⁺ T cells
CD45	Surface receptor on white blood cells, which acts as a regulator of the immune system

3.3.1.2 Differentiation down multilineages results

Oil Red O solution, staining for triglycerides and lipids turned bright red/orange, showing a positive result for adipogenic differentiation for approximately 50% of ADSCs treated with the adipogenic differentiation protocol. Alizarin Red staining indicated a positive result with the anthraquinone dye staining calcium deposits bright red after 21 days of osteogenic differentiation protocol. Cell pellets were visible within 48 hours of commencing the chondrogenic differentiation protocol and increased in size throughout time in culture. Cells showed positive staining for glycosaminoglycans with Toluidine Blue stain (figure 25).

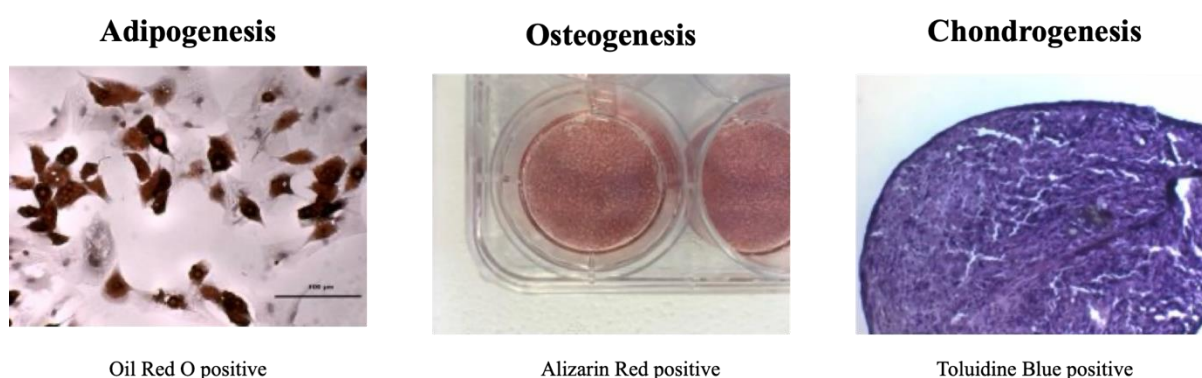


Figure 25 Demonstrates photographs of the ADSCs differentiated down three different cell lines, using the differentiation protocols detailed above. Adipogenesis is demonstrated by red/orange staining using Oil Red O (Scale bar=100µm), Osteogenesis by positive red/orange staining indicating chelation of the anthraquinone dye Alizarin Red with the calcium deposits present in the well plate, and the presence of Chondrogenesis was indicated with positive result for glycosaminoglycans, when the pellets were sectioned using a microtome in the Histology Laboratory and stained using Toluidine Blue.

3.3.4 Immunohistochemistry results

The human ADSCs were positive for phalloidin staining of the actin cytoskeleton (control) and were positive for GFAP. After the ADSCs were subjected to the 21-day Schwann cell

differentiation protocol (Kingham et al., 2007), these dADSCs were positive for actin (phalloidin), GFAP, and S100 (figure 26). The cells stained for p75NGFR had a contaminant, and results were inconclusive. Each experiment had technical triplicate wells, and the experiments were repeated utilising tissue donated from two different patients for each condition.

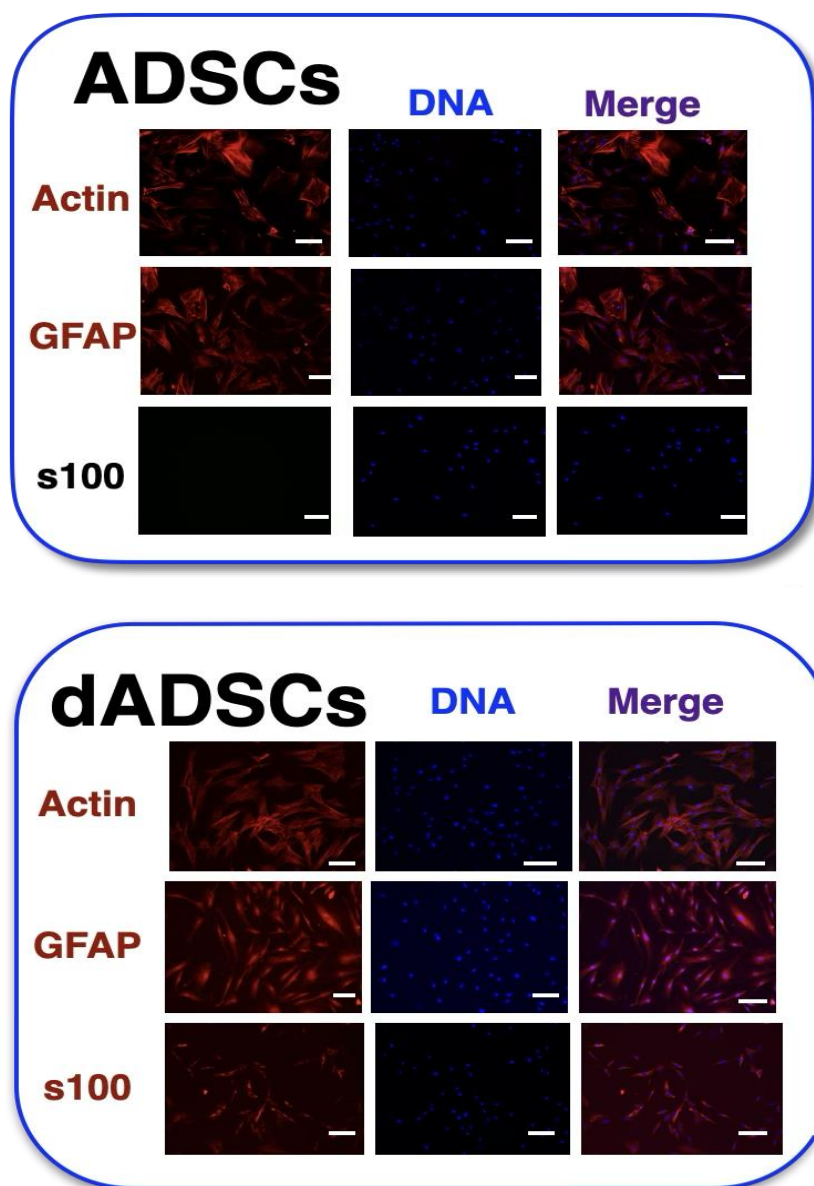


Figure 26 Shows photographs of immunohistochemistry results from the fluorescent microscope (Olympus BX51TF). The ADSC control shows positive staining for phalloidin and GFAP and is negative for S100 (3 patient repeats). After exposure of the ADSC to the Schwann-like

differentiation protocol for 21 days (Kingham et al., 2007), the dADSCs show positivity for phalloidin, GFAP and S100 (2 patient repeats). The antibody stain is shown in red, DAPI staining of the nucleus in blue and the scale bar=100 μ m.

3.3.5 Cell morphology analysis results

Anecdotally, it was noted that rat ADSCs (used in the work of our wider group), and human ADSCs had similar morphology, whereas the differentiated human ADSCs (dADSCs) adopted a more spindle-like morphology (figure27).

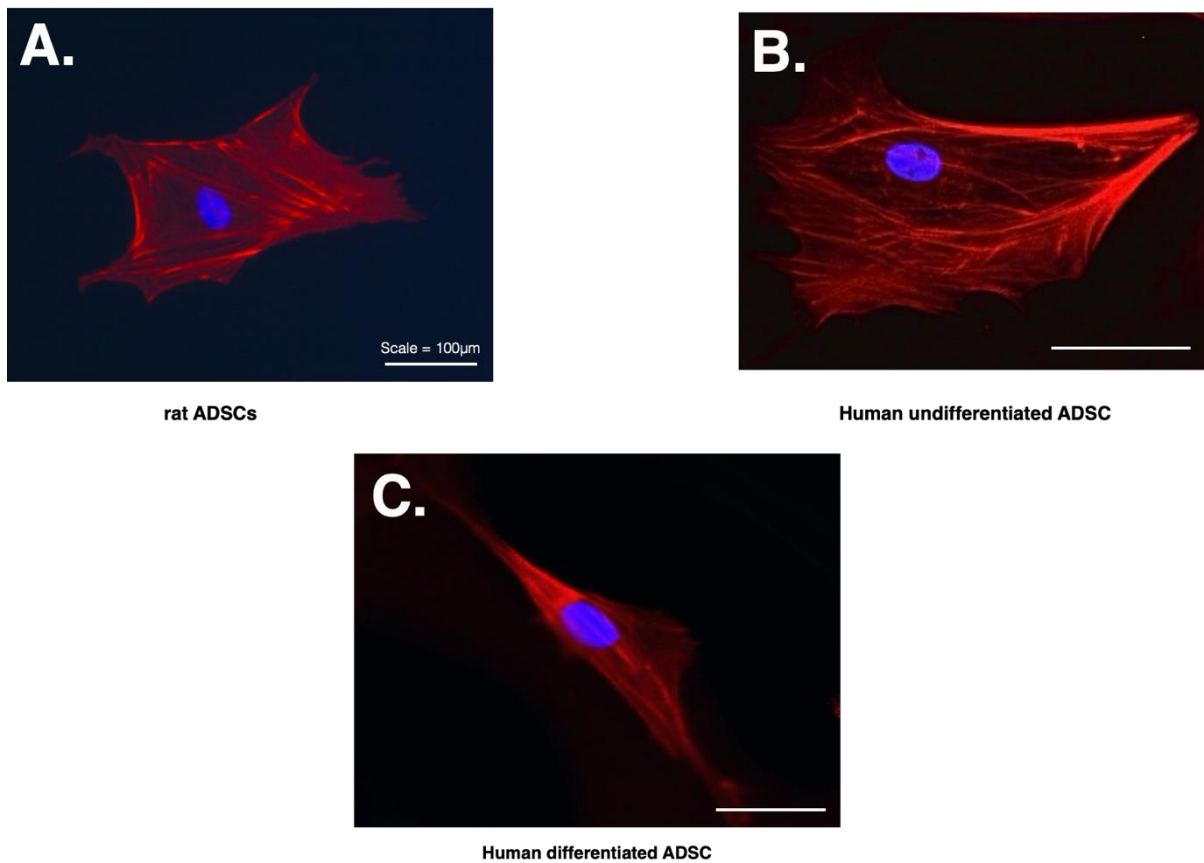


Figure 27 illustrates a rat ADSC (A), a human undifferentiated ADSC (B), and a human ADSC differentiated to a Schwann cell phenotype (referred to here as dADSC) (C), using phalloidin immunostaining of the actin cytoskeleton (red). Scale bar=100 μ m.

Results from two patients were analysed for each condition (ADSC Patients 16 and 3, and dADSC Patients 1 and 5). Analysis was conducted in CellProfiler Cell Image Analysis software, Microsoft Excel and Graphpad Prism5. The dADSCs were found to have a smaller cell area, smaller maximum Feret's diameter and higher eccentricity when compared with ADSCs (Figure 28).

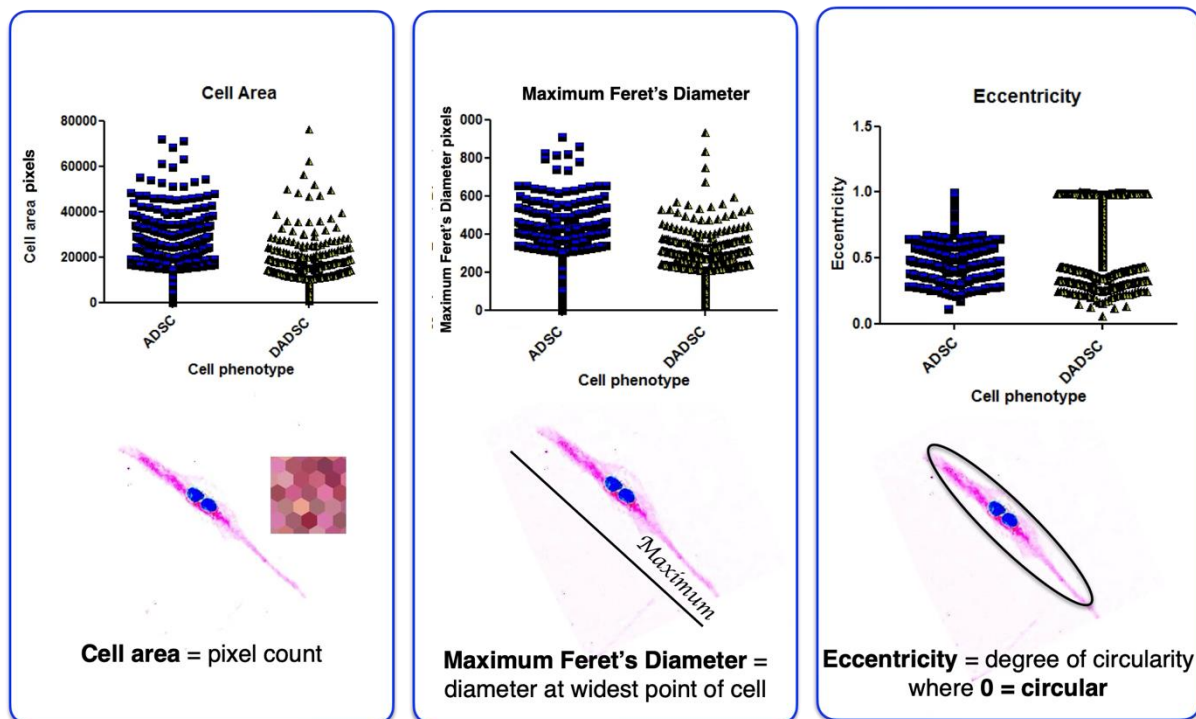


Figure 28 Illustrates that dADSCs adopt a more spindle morphology following differentiation protocol to Schwann-like phenotype. Results from two patients were analysed for each condition, ADSCs and dADSCs respectively, in Cell Profiler and Graphpad Prism5. Cell area refers to the extent of the surface of a cell enclosed within the cell walls. Cell maximum Feret's diameter refers to the longest distance between any two parallel lines tangent to the cell's boundary. Eccentricity measures how much a shape deviates from a perfect circle, where 0 characterises a perfect circle.

Looking at the data for each patient separately (ADSC Patient 16 and Patient 3, dADSC Patient 1 and Patient 5), the cell area data for all the patients is right-skewed, suggesting a group of large

structures pulling the average upward. They also have large standard deviation (Patient 16 ADSC 5,578.61, Patient 3 ADSC 2,896, Patient 1 dADSC 1254, Patient 5 dADSC 4,500) and a wide range, indicating heterogeneity and a wide spread in the cell sizes. The morphology for Patient 1 is in keeping with that expected of spindle morphology; however, Patient 5 data is more in keeping with the flat, larger area, fibroblast-like morphology characteristic of ADSCs (figure 29, table 7).

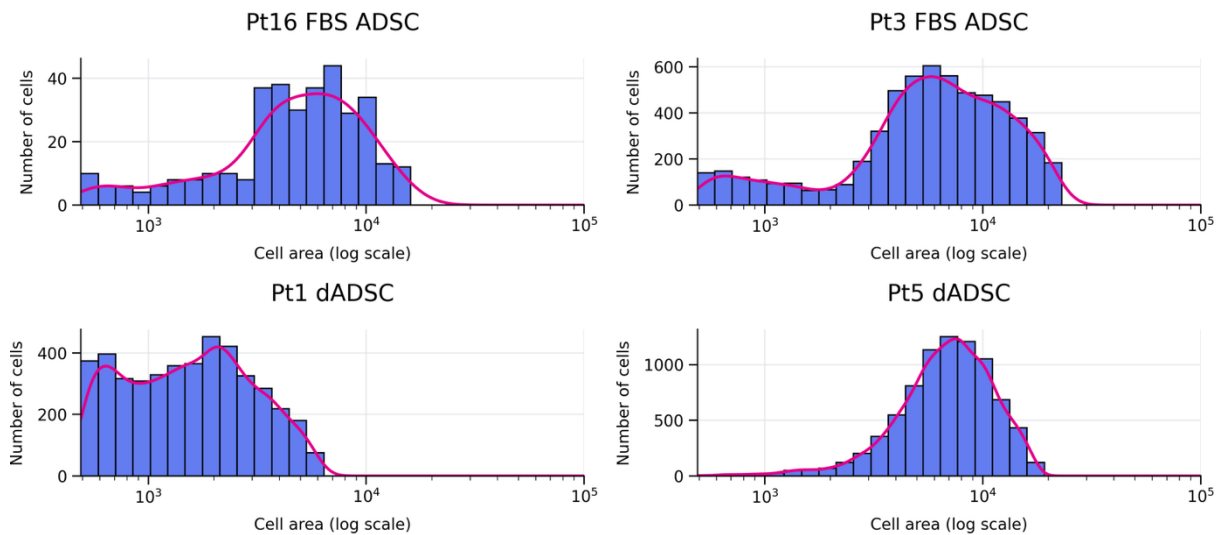


Figure 29 shows histogram (log scale) plots detailing the cell area data and allowing comparison of trends of cell area morphology for Patient 16 ADSC, Patient 3 ADSC, Patient 1 dADSC and Patient 5 dADSC. Log-scaled x-axis ($\log_{10}$) was used due to its suitability for comparing cell area data that spans orders of magnitude. Kernel Density Estimation curves were overlaid to aid understanding of the cell area distribution. It highlights the shift of the curve to the left, indicating spindleform morphology for Patient 1 dADSC, and a change to a bell curve with a shift to the right for Patient 5 dADSC.]

Table 7 Tabulates the individual data for biological (patient) repeats, examining the range, median and mean of the data for the cell area and mean Feret's diameter, for Patient 16 ADSC, Patient 3 ADSC, Patient 1 dADSC and Patient 5 dADSC. It highlights the spindleform morphology findings for Patient1 dADSC, but not for Patient 5 dADSC.

Cell parameter (pixels)	Patient 16 ADSC	Patient 3 ADSC	Patient 1 dADSC	Patient 5 dADSC
Cell area range	491-15,780	493 - 17,403	491-5,976	497-17,303
Cell area median	5,624	6,186	1,891	7,074
Cell area mean	4,958	6,432	1,558	7,514
Maximum Feret's diameter range	32-488	25-397	27 -834	28-715
Maximum Feret's diameter median	188	184	81	181
Maximum Feret's diameter mean	195	186	95	189

3.3.6 Nucleus morphology analysis results

Changes were also seen in the morphology of the cell nuclei following the differentiation protocol, with trends towards smaller nuclei area, with lower maximum Feret's diameter, higher degree of nucleic eccentricity and lower nuclei solidity (figure 30).

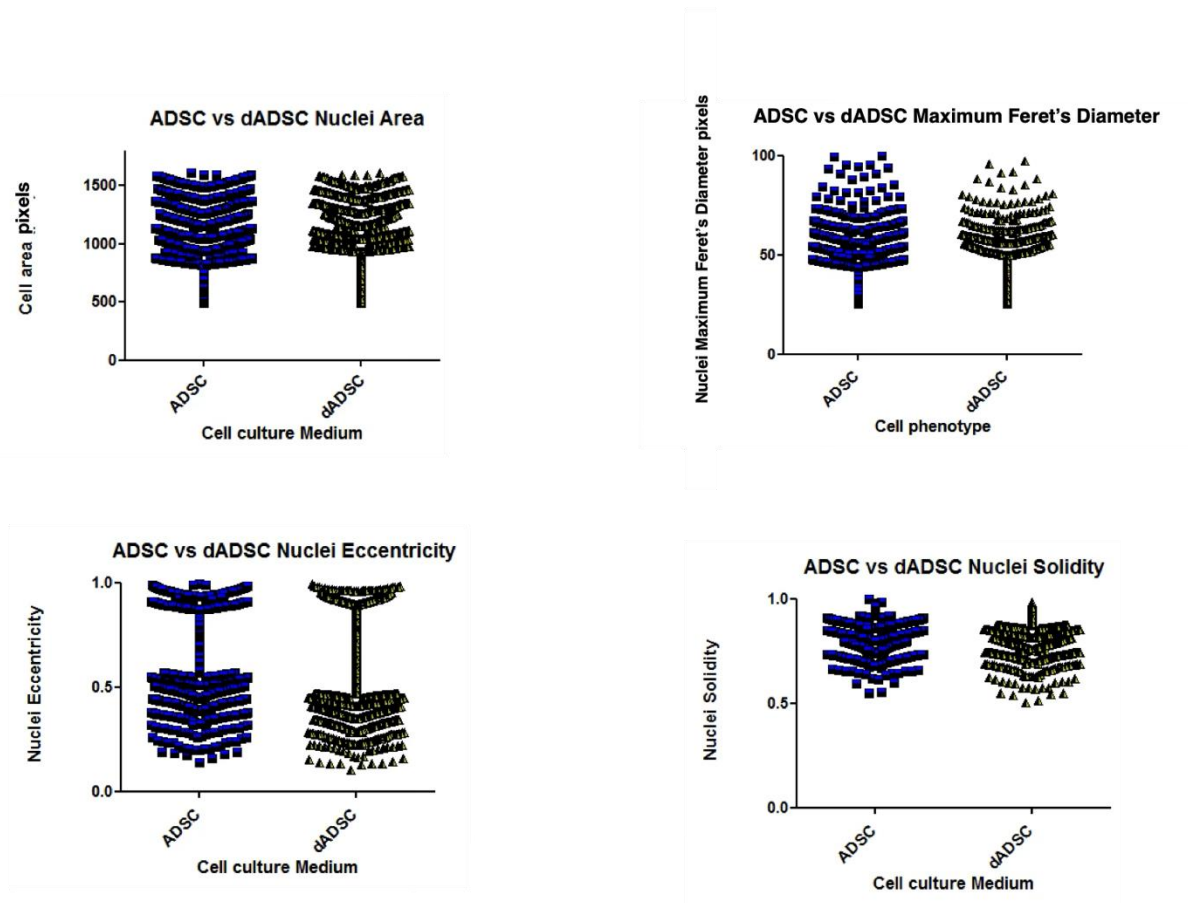


Figure 30 illustrates the analysis of the nuclei following the differentiation protocol to Schwann-like phenotype. Morphology of dADSCs have smaller, more irregular, and rounder nuclei compared with the ADSC control. Data from two patients was analysed for each condition. Nuclei area refers to the extent of the surface of a cell enclosed within the nucleus. Eccentricity measures how much a shape deviates from a perfect circle, where 0 characterises a perfect circle. Nucleus maximum Feret's diameter refers to the longest distance between any two parallel lines tangent to the nucleus' boundary. Nuclei solidity is the ratio of the nucleus area to the area of its convex hull. It is a metric used to quantify the shape irregularity, where a value closer to 1

indicates a smooth, regular shape (circular/ellipsoidal), while lower values indicate irregularity or deformity.

3.3.7 Analysis of the Hazard Statements of the supplements in the differentiation medium

There are 6 supplements added to ADSC medium to create the differentiation medium for the Schwann cell-like differentiation protocol described by Kingham et al. (2007) (Kingham et al., 2007). Studying the manufacturer's instructions for these chemicals, half (3) of these substances have hazard statement warnings about damage to health, and two have long-lasting effects on aquatic life with exposure, necessitating use in a fume hood. The other supplements (3) state that there is "no evidence available indicating acute toxicity". Two of the supplements are derived from bacteria, two are synthetically made, one is plant based and one is now derived from a cell line (Chinese Hamster Ovary cells) but was originally extracted from bovine pituitary.

Table 8 tabulates the supplements in the Kingham et al. (2007) differentiation protocol (Kingham et al., 2007), detailing their function in the process (Hörner et al., 2022), their hazard statements and their origin, as per the manufacturers.

Supplement	Mechanism in differentiation	Hazard Statements	Origin
β -mercapto-ethanol	Breaks intramolecular and intermolecular disulfide bonds in proteins, and prevents high concentration of oxygen radicals in tissue culture media	Regulation (EC) No. 1272/2008 [CLP] H301+H331 - toxic if swallowed or if inhaled H310 - fatal in contact with skin H315 - causes skin irritation H317 - may cause an allergic skin reaction H318 - causes serious eye damage. H361f - suspected of damaging fertility H373 - may cause damage to organs through prolonged or repeated exposure H410 - very toxic to aquatic life with long lasting effects	Synthetic organic chemical produced through the reaction of ethylene oxide and hydrogen sulphide

Supplement	Mechanism in differentiation	Hazard Statements	Origin
Platelet-Derived Growth Factor (PDGF)	Interacts with TGF- β signalling to drive differentiation.	The product contains no substances which at their given concentration, are considered to be hazardous to health There is no evidence available indicating acute toxicity.	produced in <i>E. coli</i> bacteria
Basic Fibroblast Growth Factor (bFGF)	Acts as a potent inducer for mesenchymal stem cell differentiation by binding to FGF receptors, triggering signalling pathways like MAPK/ERK and PI3K-AKT	The product contains no substances which at their given concentration, are hazardous to health. There is no evidence available indicating acute toxicity.	produced in <i>E. coli</i> bacteria
Forskolin	Activates enzyme adenylyl cyclase, triggering cAMP production, triggering PKA/CREB	H312 - acute toxicity (dermal) Category 4, H317 - sensitisation (skin) Category 1	Plant-derived diterpenoid compound (diterpene) isolated from the roots of the Indian

Supplement	Mechanism in differentiation	Hazard Statements	Origin
	pathway signalling pathways that alter gene expression. It promotes neural marker expression (Tuj-1, MAP2) and significant morphological changes, like increased neurite length.		plant <i>Coleus forskohlii</i>
Neuregulin-1 (Ngl1) or its isoform Glial Growth Factor-2 (GGF-2)	Stimulates cellular differentiation and survival by binding to ErbB3 and ErbB4 receptor tyrosine kinases, a potent mitogen for Schwann cells and oligodendrocyte progenitors.	The product contains no substances which at their given concentration, are hazardous to health. There is no evidence available indicating acute toxicity.	It is derived from bovine pituitary extract (originally) and is produced as a recombinant human protein (rhGGF2), typically expressed in stably transfected Chinese Hamster ovary (CHO) cells

3.4 Discussion

Results of the differentiation protocol suggests inter-patient variability and low reproducibility in human tissue

Age and gender of the donor have been identified as significant factors impacting upon differentiation potential, with Selle et al. (2021) reporting a significant decline in proliferative capacity, adipogenic differentiation potential and cell surface expression of SSEA-4, CD146 and CD274 on human bone marrow-derived mesenchymal stem cells from female, but not male, donors with increasing donor age, in a cohort of 175 (Selle et al., 2022). All the patients in this study were females, undergoing breast reconstruction surgery as part of their breast cancer treatment pathway (around 80% of new diagnoses occur in women over 50, and 1 in 3 diagnoses are in women over 70) (NHS). Therefore, the age of donor patients may have contributed to the inter-donor variability seen in the results of this study. It is also important to note, that Kingham et al. (2007) report reproducible results in rat ADSC for a rat sciatic nerve injury model; the translational gap whereby results in animals do not translate into humans (Frangogiannis, 2022) may be at play here (Kearns, 2021).

In both ADSC control groups (Patients 16 and 3), the cells were positive for GFAP. This was curious as ADSC are not typically reported to express GFAP (Zack-Williams et al., 2015). This is perhaps due to culturing the cells on laminin coated glass coverslips. Laminin is known to act through integrin-mediated signalling (such as $\alpha 6 \beta 4$) to enhance GFAP expression in glial progenitor cells (Poitelon et al., 2016). Following the differentiation protocol, the dADSCs maintained this staining, with the addition of positive S100 markers in both Patients 1 and 5. The primary antibody in our protocol (anti-S100 rabbit, abcam, ab868, GR164645-3) stains for both the alpha and beta isoforms and therefore lacks specificity. There was a contaminant in the p75NGFR cultures, which made the results inconclusive. Reid et al. 2011 report a Schwann cell-like phenotype in cells following the (Kingham et al. 2007) protocol, however only approximately 50% of the ‘differentiated’ ADSC showed staining with S100 and GFAP (Reid et al., 2011).

While morphological analysis may indicate culture state and development, human Schwann cell cultures might exhibit heterogeneous and dynamic morphologies that can significantly diverge from the typically reported bipolar or tripolar spindle shape, due to environmental factors such as culture density, growth substrate, or medium composition (Zack-Williams et al., 2015). In cultured Schwann cells, the nuclear location, and thus gene-regulatory activity, is found to vary with cell density, substrate stiffness, and mechanical stretch (Poitelon et al., 2016). In this study dADSCs were found to have a smaller cell area, smaller maximum Feret's diameter and higher eccentricity when compared with ADSCs, with trends towards smaller nuclei area, and lower nuclei solidity. These features suggest confirmation of the observational spindle morphology seen during the differentiation protocol, an accepted Schwann-like phenotype. This was found more so in Patient 1 dADSCs as opposed to Patient 5 dADSCs, again perhaps reflecting inter-patient variability. Further repeats would be required to establish if these trends are statistically significant.

Differentiating adipose-derived stem cells towards a Schwann cell phenotype (dADSC) is currently not compatible with therapeutic translation

Where Kingham et al. (2007) describe a 21-day protocol to differentiate ADSCs (Kingham et al., 2007), Du et al. (2023) report reducing this same protocol down to 2 days by the addition of 50 μ M of sugar analogue Ac5ManNTProp (Du et al., 2023). This reflects a more clinically feasible timeline for nerve repair treatment. They also report achieving significantly upregulated transmembrane protein S100 β and p75NGFR expression and elevated neurotrophic growth factors (Du et al., 2023) compared with the original Kingham protocol. However, the use of potentially carcinogenic compounds in induction stages remains a major barrier to meaningful clinical translation of these cells (table 8), and the use of animal products poses potential for transfer of zoonotic pathogens. Although there have been reports of successful differentiation of ADSCs into a Schwann-like phenotype, there are concerns including the long-term stability and potential for teratogenesis (Xie et al., 2017). Faroni et al. (2016) report dADSCs rapidly reverting to their original stem cell morphology and phenotype, with significantly reduced growth factor expression *in vitro* once the differentiation medium was removed (Faroni et al., 2016). Whilst Thomson et al. (2018) report that differentiation is maintained when rat ADSCs

are cultured on microtopographically patterned substrates (Thomson, 2018). Despite the broad selection of sources and literature available on options that can be used to differentiate Schwann-like cells, and promising results in animal models, no stem cell-derived Schwann cells have yet progressed to clinical trials, largely because of complications around availability, control, safety, and ethical restrictions (Huang et al., 2020).

Study limitations

This study's limitations include duplicate rather than triplicate biological repeats - despite best efforts. Repeated issues with bacterial and fungal contaminants departmentally necessitated destruction of multiple experiments. The microbiological contaminants were not serotyped nor sensitivities gleaned, due to lack of university budget and staffing for microbiological testing, to confirm the infective pathogen. Advice from a microbiologist was sought independently, and it was recommended to add doxycycline or chloramphenicol antibiotic, in addition to the standard 1% pen/strep. It was however not possible to implement this within the scope of this study. It was not possible to compare ADSCs with dADSCs, partly due to the differentiation protocol's 21-day nature. Attempts were made to keep the ADSCs and dADSCs passage and time in culture as low as possible (Zack-Williams et al., 2015), and to avoid freeze-thaw cycles as a confounding variable.

Yang et al. (2018) studied human mesenchymal stem cells (MSCs) and found that cells subjected to extensive *in vitro* passage (passage 8) underwent morphological, phenotypic, and genetic changes. *In vitro* aging led to loss of their typical fibroblast-like spindle morphology and increased morphological abnormality and inhomogeneity. Additionally, with increasing age *in vitro* the MSCs showed reduced cell differentiation potential, particularly for osteogenic differentiation, although adipogenic potential was found to be better preserved. Differentiation down other tissue lineages were not investigated (Yang et al., 2018). They also compared culture mediums (α MEM vs DMEM) as the cells aged *in vitro*. They report that α MEM-based medium may preserve MSC morphology for longer and showed more DNA stability on quantitative polymerase chain reaction (qPCR) testing, and less variation of CD markers over time, than the DMEM cultured cells (Yang et al., 2018). In this study, ADSCs were kept as low passage as

possible (p1-6), which also attempted to mimic a feasibly translatable therapeutic window, and α MEM-based medium was used. Kim et al. (2017) report that human pluripotent stem cells (as opposed to multipotent stem cells like ADSCs) are stable for up to 100 passages (Kim et al., 2017), perhaps a tissue donor bank of pluripotent stem cells would be a more translatable solution than autologous differentiated ADSCs (dADSCs). The addition of 50 μ M of sugar analogue Ac5ManNTProp to the Kingham et al. (2007) protocol, as per the work of Du et al. may also be considered in future to significantly reduce the differentiation protocol length (Du et al., 2023).

3.5 Conclusion

To conclude, the study limitations do not allow for statistical analysis. However, the ADSC extraction protocol was optimised for human abdominal adipose tissue. There were trends towards Schwann-like phenotype, evidenced by immunohistochemistry, and morphological changes, post-differentiation protocol. Further studies into inter-patient variability are required for differentiation down the Schwann cell-like lineage specifically. Although solutions to improve this study have been identified, for as long as there are toxic agents and animal products in the process, the potential for clinical studies and meaningful translation into clinical practice is negligible. Other solutions must be sought that prioritise clinically translatable methodologies.

Chapter 4 Adipose derived stem cells (ADSCs) cultured with human platelet lysate (HPL) supplement

4.1 Introduction

The main factor that dictates the suitability of an experimental therapy for clinical translation is patient safety. Alongside the use of toxic reagents in culture media, the use of animal products may act as a limiting factor to the progression of stem cells from their successful use in animal models into meaningful treatments in human patients. The standard medium supplement in laboratory cell culture of adipose-derived stem cells (ADSCs) is foetal bovine serum (FBS), as completely serum-free cultures lead to low cell survival. Although FBS does not seem to be required for cell propagation in primary human Schwann cell cultures, it is regularly used to promote their viability (Hörner et al., 2022). The concerns with the use of animal derivatives include ethical, moral or religious objections; large variations between batches, which affects laboratory reproducibility in the short term, and potential therapeutic reliability in the long term; the risk of patient immune reactivity; and risk of exposure to animal derived viral, bacterial or prion infection (Vaidya et al., 2018).

Human platelet lysate (HPL) has in fact been shown to have antibacterial properties *in vitro* (Gordon et al., 2021) and *in vivo* (Shariati et al., 2020) whereas immune reactions have been reported as a result of repeated infusion with FBS cultured cells. Selvaggi et al. (1997) report HIV patients receiving syngeneic lymphocyte infusions that were cultured in 5% FBS, with their uninfected identical twins as donors. After the second infusion, 8 of the 12 patients treated developed a Type III hypersensitivity Arthus reaction, with severe localised swelling and tissue damage, which presented as fever above 39 °C, hypotension, rigors, joint pain and swelling, muscle pain, headache, and malaise. They discovered that despite thorough washing of the cells before infusion, these patients had developed antibodies to FBS. These observations suggest that the therapeutic use of human cells cultured in FBS may expose the recipient to immunogenic substances with possible serious clinical sequelae (Selvaggi et al., 1997).

In comparison, HPL may be obtained from screened individuals and could potentially be pooled and stored, as in other therapeutic blood products, or perhaps obtained autologously from the patient. HPL has higher growth factor content than any other supplement, containing neurotrophin 3 (NTF3), nerve growth factor (NGF), brain-derived growth factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF); these molecules influence Schwann cell survival, differentiation and release of extra-cellular matrix molecules (Lensch et al., 2018). Our group (Palombella et al. 2020) report that human ADSCs cultured in HPL show statistically greater secretion of these neurotrophic growth factors than cells cultured in FBS (Palombella et al., 2020) and *in vitro* coculture of HPL ADSCs with primary neurons showed significantly enhanced neural outgrowth compared to the FBS control (Palombella et al., 2020).

In the same study (Palombella et al. 2020), cell culture of ADSCs in completely animal-free culture media was described. They compared mechanical digestion (dissecting the tissue into <0.5cm pieces) and the described collagenase method of ADSC extraction and found no significant differences in terms of cluster of differentiation (CD) marker expression and stem cell plasticity (Palombella et al., 2020). When 5% HPL replaced 10% FBS serum in culture, the expression of CD markers was akin to cells grown in FBS, whilst viable ADSC yield increased and spindle form morphology was adopted (Palombella et al., 2020). Escobar et al. (2016) also report a completely animal-free protocol for ADSC extraction and culture and found that human adipose tissue explants cultured in 5% HPL-supplemented medium show earlier and faster cell proliferation without losing their immunophenotype, when compared with ADSCs cultured in 10% FBS. Osteogenic differentiation of HPL ADSCs was higher than the FBS ADSCs; however, adipogenic differentiation was higher in the FBS ADSCs than the HPL ADSCs (Escobar and Chaparro, 2016).

This study aims to compare FBS ADSCs and ADSCs differentiated down a Schwann-like lineage (dADSCs), with ADSCs cultured in HPL, due to its promising potential for future clinical translation.

4.2 Materials and Methods

4.2.1 Human platelet lysate supplement culture medium

In this study HPL Gold Research Grade (Life Science Group) was used, which was derived from the platelets of 150 consenting, healthy volunteer donors, at certified blood banks in the European Union (21CFR640). All individual donors and complete batches were fully tested for and found non-reactive for anti-HIV-1/2, anti-HCV, HBsAg, syphilis, HIV NAT, HBV NAT and HCV NAT (21CFR610). Each batch was tested for the absence of bacteria, and fungi, PCR tested for mycoplasma and all batches were tested to determine the levels of endotoxins using the limulus amoebocyte lysate test - <0.500 EU/mL. Biological performance of final batches of HPL was assessed by cell culture of mesenchymal stem cells (MSCs)(Production, 03OCT2024).

ADSC culture medium consisted of Gibco α -MEM (1x) +glutaMAX, (Thermo Fischer Scientific) supplemented with 10% FBS (Sigma Aldrich, Zofingen, Switzerland), and with 1% penicillin/streptomycin (Thermo Fischer Scientific). The HPL medium followed the same recipe but replaced the FBS with 5% HPL as per manufacturer instructions (HPL Gold Research Grade, Life Science Group). Filtration of complete media <0.4 μ m was recommended. Recommended storage was -20°C or below, with long term storage -80°C recommended for up to two years. Complete culture medium could be stored at 4°C and was stable for approximately four weeks.

4.2.2 Flow cytometry to establish CD markers

Thank you to Dr Euan Ross for his assistance with these flow cytometry experiments. To assess whether the ADSCs maintained their multipotent potential following incubation in HPL serum, flow cytometry was conducted specifically to investigate the presence of CD73, CD90 and CD105, and the absence of CD34 and CD45 as per the International Society for Cellular Therapy (ISCT) criteria for human MSC guidance(Dominici et al., 2006).

HPL cultured ADSCs and FBS cultured ADSCs were stained with the following antibodies: anti-CD73 (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled); anti-CD90 (1:1000, IgG1,

BD Biosciences, monoclonal, FITC-labelled); anti-CD105 (1:200, IgG1, BD Biosciences, monoclonal, PE-labelled) as positive MSC markers; and anti-CD34 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled); and anti-CD45 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled) as negative endothelial and hemopoietic markers, respectively.

Appropriate isotype controls were used for each fluorophore (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled; 1:50, IgG1, BD Biosciences, monoclonal, PE-labelled; 1:50, IgG2b, BD Biosciences, monoclonal, FITC-labelled). For each antigen, approximately 100,000 cells were resuspended in 100 μ L of antibody diluted in flow cytometry buffer (0.2% FBS in PBS) and incubated for 1 hour, agitated in the dark. The cell surface phenotype was subsequently assessed by BD Accuri C6 apparatus (Erembodegem). Experiments were conducted in technical triplicates and biological duplicates.

4.2.3 Morphology

As previously described in chapters 2 and 3, for all conditions the cell morphology was monitored daily under a phase-contrast microscope (10x, Olympus IX81). Cells were seeded into a 24-well plate at a concentration of 10^4 cells per well. Each well contained a poly-lysine (PLL) and laminin-coated glass coverslip coated as previously described. Once the cells were confluent (3 days) they were fixed with 4% paraformaldehyde for 30 minutes at room temperature and then washed with 1% PBS. Permeabilising buffer (10.3g sucrose (Fisher BioReagents) + 0.292g NaCl (VWR Chemicals) + 0.06g MgCl₂ (VMR) + 0.476g Hepes (VWR Chemicals) in 100ml 1% PBS, 7.2pH + 0.5ml Triton x) was added for a 5-minute incubation at 4°C, and then a further wash and 5-minute incubation at 37°C with block solution (1% bovine serum albumin, (BSA, Fisher BioReagents) in 1% PBS). Staining for the actin cytoskeleton (rhodamine phalloidin, life technologies, R415, 1646681) at 1:500 dilution was conducted, with coverslips mounted with Vectashield DAPI (Vector, H-1200, ZB1130). Experiments were conducted in technical triplicates and biological duplicates.

Imaging and cell labelling ratio analysis was conducted using automated programming, at random, for 100 cells, with high content fluorescence microscopy (Olympus BX51TF). I

developed a Cell Profiler image analysis software (4.1.3) pipeline (Carpenter et al., 2006) and it was utilised for cell measurements (minimum 500-1000 cells per condition) (figure 22, appendix 2). Adaptive thresholding of greyscale images identified cells based on DNA-stained nuclei (DAPI) as ‘primary objects’, and actin cytoskeleton staining (phalloidin) as ‘secondary objects’. The watershed-gradient method was implemented to delineate neighbouring cells, and cells touching the boundary of the images were discarded. Graphpad Prism6 and Microsoft Excel were utilised for data analysis.

4.3 Results

4.3.1 HPL ADSC cluster of Differentiation (CD) markers results

The CD markers upon flow cytometry remained largely unchanged with ADSCs cultured in HPL serum, when compared with ADSCs cultured in FBS. As described in Chapter 3, CD105, CD90 and CD73 were key positive markers indicative of stemness and CD14, CD34 and CD45 were key negative markers indicative of stemness (Table 4) (Dominici et al., 2006) [1]. The ADSCs in the (FBS) control group were found to be positive for CD105, CD90 and CD73, negative for CD45 and weakly positive for CD34 in the FBS cells, and these results were maintained in the HPL cells (Figure 31). CD14 was not tested for.

The cells cultured in HPL perhaps showed increased positivity for CD10, CD140a, and CD140b compared with the FBS cells (Figure 31). CD10 is an endopeptidase that cleaves peptides and inactivates several peptide hormones (Biosciences, 2016); CD140a is a transmembrane platelet-derived growth factor (PDGF) receptor, primarily expressed on mesenchymal-derived cells, which plays vital roles in wound healing, cell migration, and embryonic development; and CD140b is also a transmembrane PDGF receptor that is involved in mesenchymal cell proliferation, migration, and angiogenesis. CD140b is widely expressed on mesenchymal-derived cells, glial cells, and some tumour cells (eg. glioblastoma) (Heldin and Rönstrand, 1990) (table 6). Consistent results were found in two biological repeats comparing Patient 6 and Patient 7 ADSC FBS, and ADSC HPL respectively.

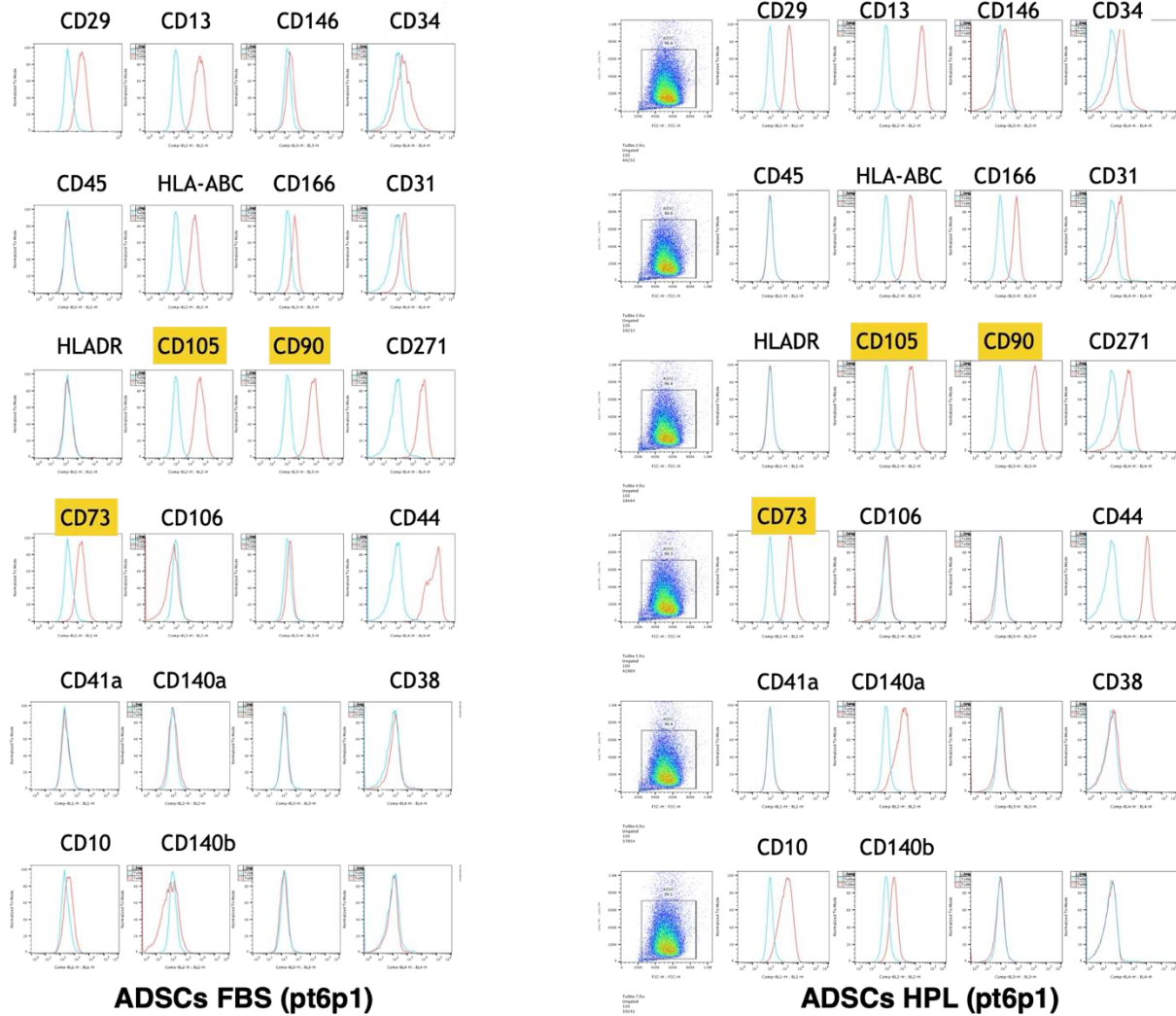


Figure 31 shows CD marker flow cytometry results, where blue=negative control and red=cell condition result. This figure directly compared ADSCs from Patient 6, at passage 1 (pt6p1), with FBS vs HPL serum respectively. CD105, CD90 and CD73 were highlighted as key positive markers indicative of maintained stemness following HPL treatment.

4.3.2 HPL ADSC cell morphology results

Comparing the area, maximum Feret's diameter and eccentricity of FBS to HPL ADSC, these results suggested that the HPL cells adopted a smaller, more spindle-like morphology, with smaller cell area, lower maximum Feret's diameter and higher eccentricity than the FBS cells (figure 32). This data was following 10 days of incubation in the relevant medium.

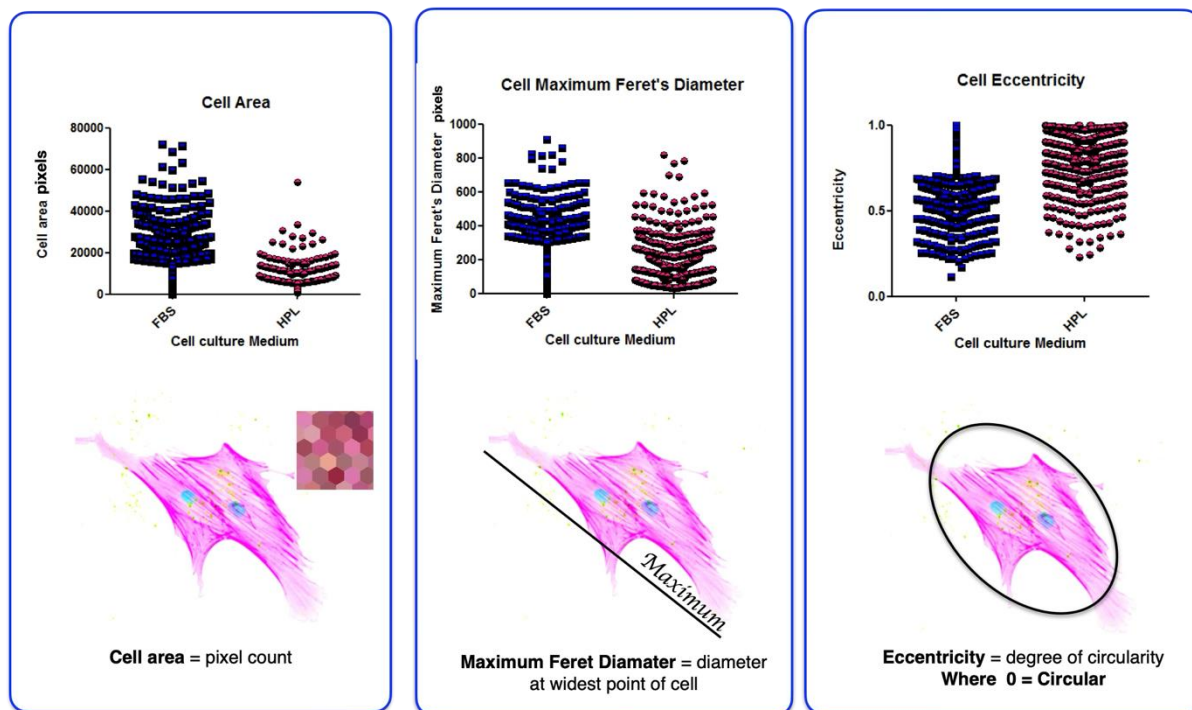


Figure 32 Graphs comparing the area, maximum Feret's diameter and eccentricity of FBS to HPL ADSC following 10 days incubation. These results suggested that the HPL cells were smaller, and more spindle-like than the FBS cells. Cell area referred to the extent of the surface area of the cell as calculated by the area of its cytoskeleton. Cell maximum Feret's diameter referred to the longest distance between any two parallel lines tangent to the cell's boundary. Eccentricity measured how much a shape deviated from a perfect circle, where 0 characterises a perfect circle.

When comparing the FBS ADSCs and the dADSCs with the HPL ADSCs, the FBS ADSCs had the largest cell areas (figure 33). The dADSCs were smaller than FBS ADSCs, with a more

compact phenotype. Their size distribution was tight and consistent, indicating good segmentation and consistency especially in dADSC Patient 1. The HPL ADSCs were the smallest with the most uniform and consistent morphology, which was especially the case in HPL ADSC Patient 7 (figure 33. HPL ADSC Patient 16 showed higher variability, but still much lower than FBS conditions (table 9). Aspect ratio measured the elongation of an object by dividing the major axis length by the minor axis length; the HPL cells showed an aspect ratio of closer to one for more cells than the dADSC group and the FBS group (figure 34).

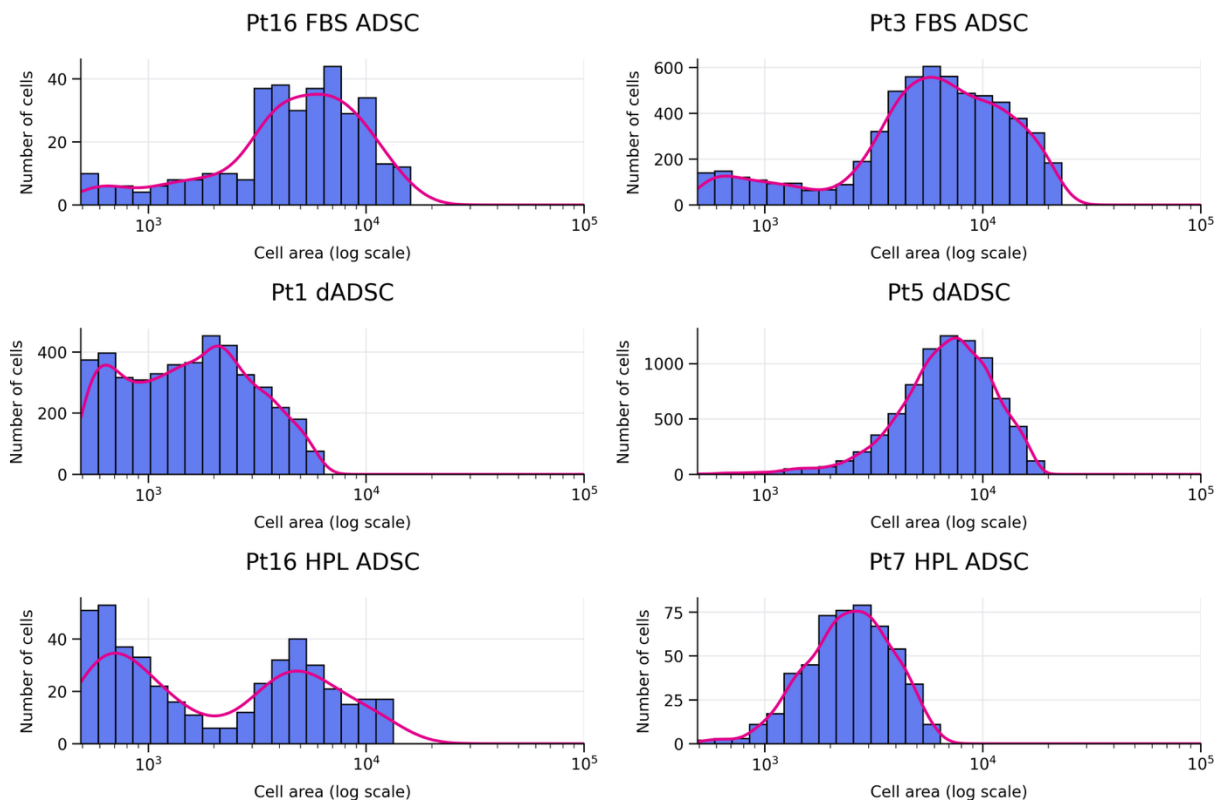


Figure 33 Histogram (log scale) plots detailing the cell area data and allowing comparison of trends of cell area morphology for Patient 16 ADSC, Patient 3 ADSC, Patient 1, Patient 5 dADSC and Patient 16, Patient 7 HPL ADSC, with a minimum of 100 cells analysed per condition. Log-scaled x-axis (log10) was used due to its suitability for comparing cell area data that spans orders of magnitude. Kernel Density Estimation curves were overlaid to aid understanding of the cell area distribution. It highlights the trend for the HPL ADSCs to have the

smallest area. It makes clear the trend for differences between medium groups, and between patients.

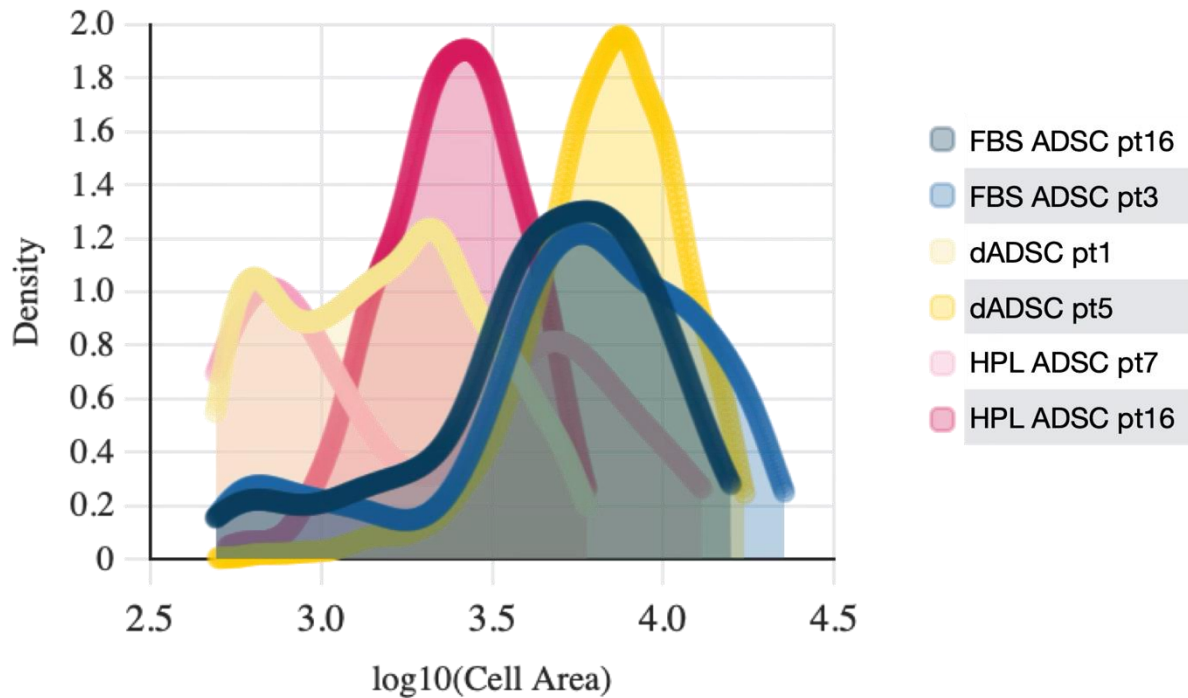


Figure 34 is a Kernel Distribution Estimation (KDE) plot, allowing comparison of trends of the cell area data (pixels), of Patient 16 FBS ADSC (dark blue), Patient 3 FBS ADSC (light blue), Patient 1 dADSC (light yellow) and Patient 5 dADSC (dark yellow), Patient 16 HPL ADSC (dark pink) and Patient 7 HPL ADSC (light pink). HPL ADSCs appeared to have the smallest cell area, followed by DADSC and FBS ADSC. The KDE was used to account for the right-skewed nature of cell size distributions and to facilitate visualisation of proportional differences across the full range of values. It highlights the presence of morphological distinctions between patient donors and medium groups. Cell area values were plotted on a logarithmic x-axis to allow for comparison between large data sets, illustrating the typical and extremes of values and allowing the general trends to be more easily interpreted. The density reflects the cell area probability and distribution shape, normalising the data and making plots comparable across the heterogenic samples.

Table 9 is an adaptation of table 7, with the addition of the data for HPL ADSCs to allow for comparison of cell and maximum Feret's diameter range, mean and median values. It highlights a trend towards spindle-form morphology for Patient1 dADSC, Patient 16 HPL ADSC and Patient 7 HPL ADSC, with smaller area, and an increase of maximum Feret's diameter upper range for Patients 1 and 5 dADSC and Patients 16 and 7 HPL ADSC cells, when compared to the FBS ADSC control group.

Cell parameter (pixels)	Patient16 ADSC FBS	Patient3 ADSC FBS	Patient1 dADSC	Patient5 dADSC	Patient16 ADSC HPL	Patient7 ADSC HPL
Cell area range	491- 15,780	493 - 17,403	491- 5,976	497- 17,303	491-12,996	532- 5978
Cell area median	5,624	6,186	1,891	7,074	1610	2481
Cell area mean	4,958	6,432	1,558	7,514	3,336	2,661
Maximum Feret's diameter range	32-488	25-397	27 -834	28-715	30-687	30-687
Maximum Feret's diameter Median	188	184	81	181	131	130
Maximum Feret's diameter mean	195	186	95	189	142	141

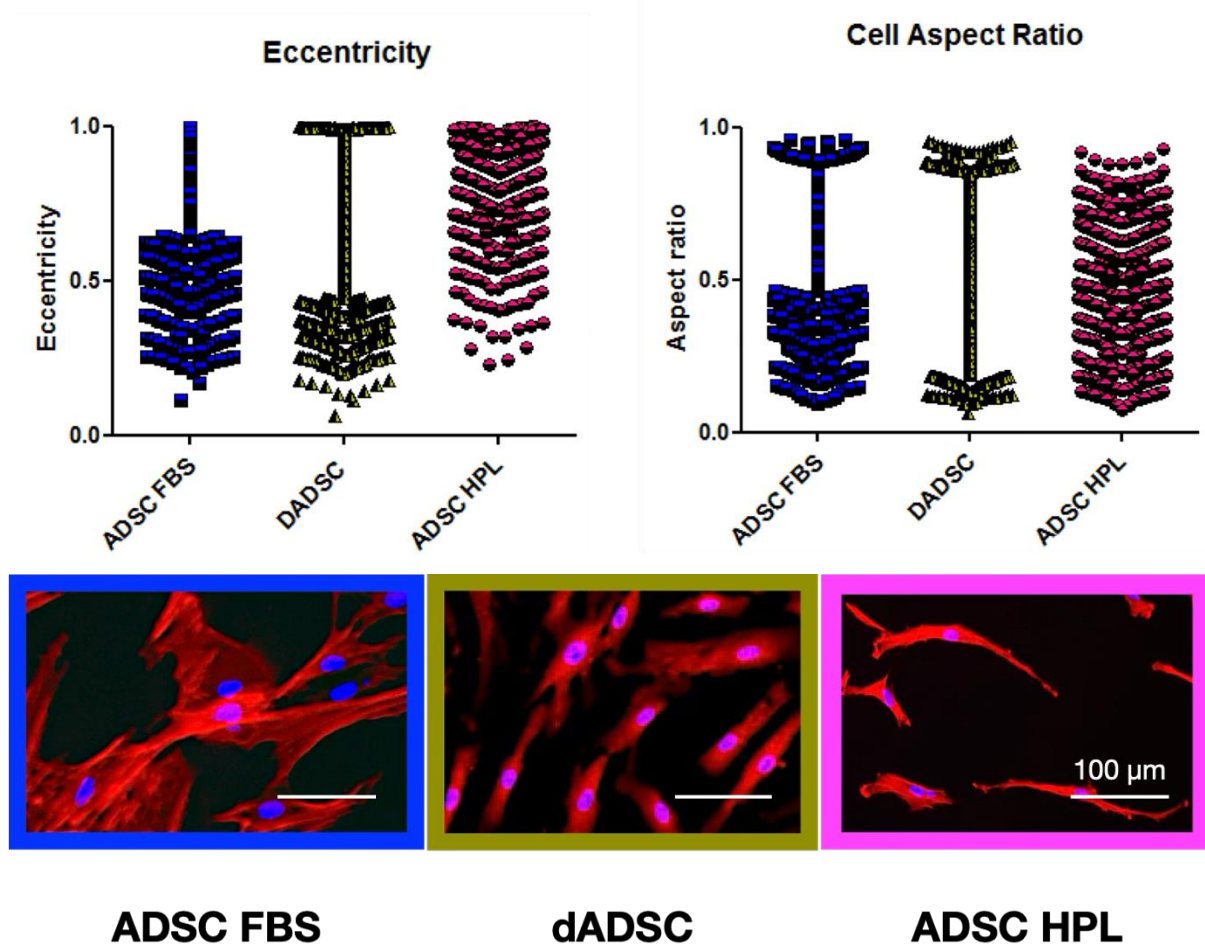


Figure 35 shows graphs and photographs (scale bar=100 μ m) comparing the morphology of FBS ADSCs, dADSCs and HPL ADSCs. Actin (phalloidin, red) and DNA (DAPI, blue) stained samples of cells on day 3, with seeding at concentration of 10^4 cells per cm^2 cultured on laminin coated glass. These images suggest that the ADSCs cultured in HPL were smaller, and more spindle-like, as measured by the eccentricity and aspect ratio, than the ADSCs cultured in FBS, and dADSCs. Eccentricity measures how much a shape deviates from a perfect circle, where 0 characterises a perfect circle. Aspect ratio measures the elongation of an object by dividing the major axis length by the minor axis length (Institute., Copyright 2003-2021).

4.3.3 HPL ADSC nuclear morphology results

Differences in nucleic morphology between the FBS and HPL ADSC were also analysed. The nuclei of the HPL cultured cells were found to have smaller area (pixels) and maximum Feret's diameter (pixels), rounder eccentricity, and had greater solidity than the FBS cultured cells (figure 36).

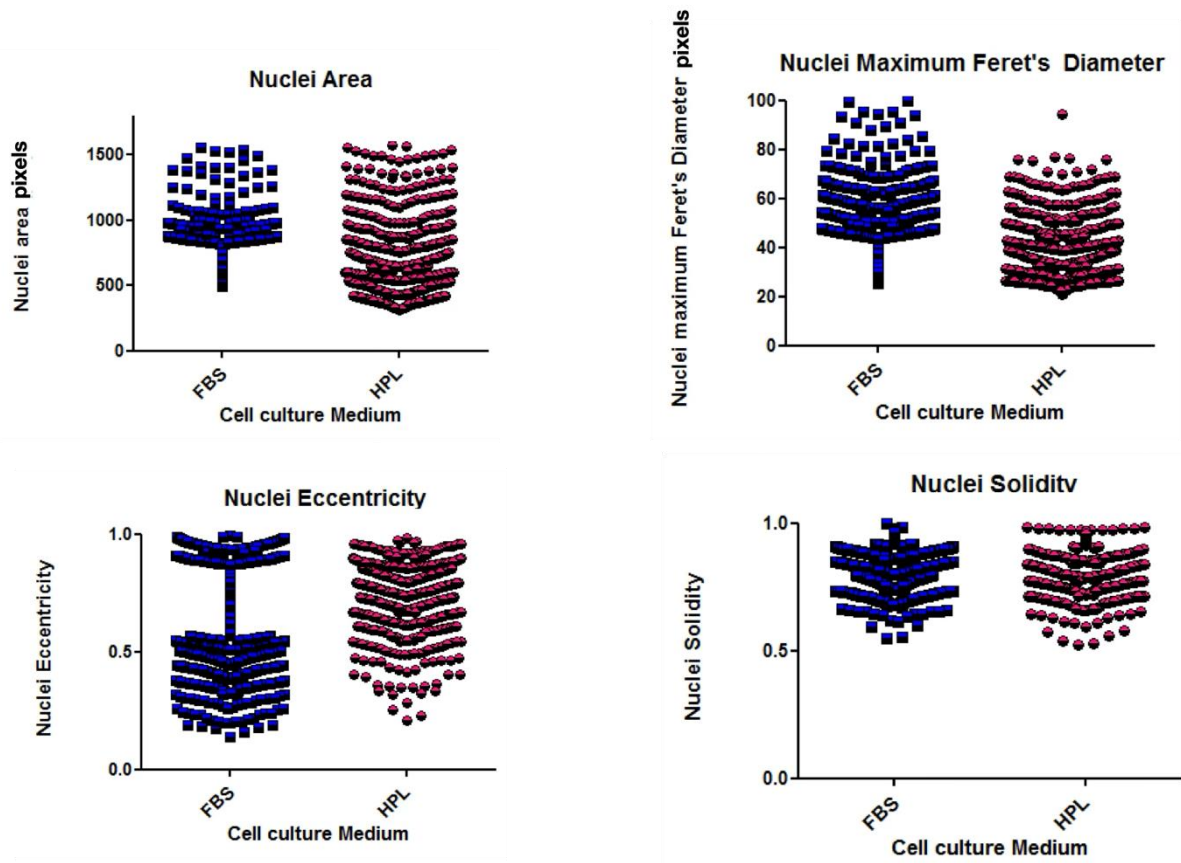


Figure 36 illustrates the analysis of the nuclei between the FBS and HPL cultured ADSCs. Morphology of HPL cells had smaller, more irregular, and rounder nuclei compared with the ADSC control. Data from two patients was analysed for each condition. Nuclei area refers to the extent of the surface of a cell enclosed within the nucleus. Eccentricity measures how much a shape deviates from a perfect circle, where 0 characterises a perfect circle. Nucleus maximum Feret's diameter refers to the longest distance between any two parallel lines tangent to the

nucleus boundary. Nuclei solidity was the ratio of the nucleus area to the area of its convex hull. It is a metric used to quantify the shape irregularity, where a value closer to 1 indicates a smooth, regular shape (circular/ellipsoidal), while lower values indicate irregularity or deformity.

4.3.4 Cell culture contaminations

There were issues with contamination during the study period, and instances of disposal of entire incubators worth of work, and for several month's work from every incubator in the department, in attempts to contain infection. Infections occurred and reoccurred, despite most cultures being maintained with pen/strep 1%, with antibiotic-free cultures being housed in separate incubators, and laboratory procedures and strict aseptic technique being adhered to throughout. The infections increased in frequency and severity after moving into a new lab building in 2022. It was noted in this study that in 6 of the cases of contamination only the FBS cultured cells were affected, whilst the HPL cells did not show signs of infection. The infection in the FBS cultured cells was with fungus in 3 of the cases (Figure 37), and bacteria in 3 of the cases. In one case, the FBS supplement aliquot showed fungal growth upon incubation; However, the source of infection was unclear in the other cases.



Figure 37 is a photograph showing an example T75 culture flask containing FBS-cultured ADSCs, which is contaminated with fungus.

4.4 Discussion

HPL cultured ADSCs showed trends towards increased spindle morphology compared with FBS ADSCs and dADSCs

The spindle-form morphology of Schwann cells is thought to be important in their structural role in guiding regenerating axons, by forming Bands of Bügner (Doddrell et al., 2012). Although a further biological repeat is necessary to conduct statistical analysis, this study suggested a trend towards a shift to the left in the cell area distribution curve for HPL-cultured ADSCs, quantifying the observation that HPL cells adopted a smaller and spindle-cell morphology. This is found in the literature, for instance Attaelmanan et al. (2025) compared umbilical cord-derived MSCs cultured in differing concentrations of HPL, with FBS control. They observed more organised colonies and individually smaller, thinner, shinier, and a more pronounced spindle shape in the HPL cells. They report spindle morphology being observed in 3 days for 10% concentration HPL, 5 days for 7% HPL and 7 days for 5% HPL (Attaelmanan and Khalil, 2025). Our experiments were conducted at day 10 from extraction, with 5% concentration HPL.

This data supports distinct phenotypes across media and donor conditions

This study suggested that the HPL ADSCs were the smallest, with the least spread of the study groups. The FBS ADSCs were the largest, flattest and with the most cytoskeletal engagement. The cells cultured in HPL perhaps showed increased positivity for CD10, CD140a, and CD140b compared with the FBS cells (Figure 31). CD140a and CD140b are both transmembrane PDGF receptors (Guiotto et al., 2020, Mohamed et al., 2020). CD140a is primarily expressed on mesenchymal-derived cells, whilst CD140b is involved in mesenchymal cell proliferation, migration, and angiogenesis. Of note, HPL is known to contain significantly more PDGF than FBS. The dADSCs and HPL group showed distinct distribution curves between patients, whereas the findings in the FBS group were more consistent between patients. This might indicate donor-related differences or cell state dependent phenotypic subpopulations (Doddrell et al., 2012). Further repeats would be required for statistical analysis. As discussed in Chapter 3, inter-donor

variability has been reported in the differentiation potential in mesenchymal stem cells, with age and sex being determining factors(Selle et al., 2022).

Recurrent sample infections of unknown origins occurred throughout the study

To reduce the risk of postoperative infection, the donor patients were administered a routine dose of prophylactic Coamoxiclav IV (if allergic, then an alternative, such as gentamicin was given) and betadine (povidone-iodine) surgical prep was used as standard before knife to skin, therefore making the patient-derived adipose tissue a seemingly unlikely nidus of infection. However, Szabłowska-Gadomska et al. (2023) studied the rate and nature of microorganism contaminations in lipoaspirate obtained from 100 patients under clinical conditions for a clinical trial. They found contamination in 41/100 samples with 13 different microorganisms identified, the most commonly occurring bacteria were *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Staphylococcus capitis*, and *Bacillus* spp, namely physiological flora normally associated with human skin. This led to their conclusion that the starting material for ADSC-based medicinal products should be considered contaminated even if it is harvested under the strictest regimen. They successfully implemented decontamination steps as routine procedure, with repeated rinsing of the starting tissue in an antibiotic and antimycotic solution(Szabłowska-Gadomska et al., 2023).

During cell digests, the sample was kept sterile in dissection and transport; in the laboratory, sterile gloves and scissors were utilised as additional measures to guard against contamination. Technique was observed by lab supervisor Dr Mathis Riehle in case of identifying possible infective introductions by the author, and nothing was noted apart from caution to ensure lids of adipose tissue containing flasks were tightly closed during digest incubation at 37°C in the water bath. Whilst the distilled water used in the water bath is a low-nutrient environment, some types of bacteria (known as “oligotrophs”, such as *Pseudomonas aeruginosa* and *Mycoplasma* spp.) can still grow(Hirsch, 1986). Perhaps a trial of adding Chemgene to the water bath might be a future intervention in an attempt to lower potential contaminants. Chemgene is effective against

bacteria, viruses and fungi, by quickly penetrating the cell outer membrane or viral envelope, denaturing DNA or RNA and inducing apoptosis (Byotrol, 20205).

Lab protocols were strictly adhered to; all equipment and materials were misted with 70% isopropyl alcohol (IPA) before entry into the safety cabinet, and Class II Biological Safety Cabinets with high-efficiency particulate air (HEPA) filtration were used throughout. The standard cleaning regimen consisted of wiping the cabinet with 70% ethanol, which was allowed to evaporate. On the advent of departmental infections, all lab members were administered additional decontamination training. Towards the end of the study period, departmental measures introduced 30 minutes of UV treatment before every safety cabinet use (rather than overnight UV treatment only) and an additional cleaning step with Chemgene before the alcohol cleaning. Cleaning of the primary cell culture labs (where human tissues were used) had to be done by immunised individuals (for Hepatitis B); therefore, this was left to academic staff and students. It was noted that the infections increased to more regular and severe occurrences after moving into a new lab building in 2022.

This work initially took place in the Joseph Black building at University of Glasgow and then moved to the Advanced Research Centre (ARC) at University of Glasgow in 2022. In the United Kingdom (UK), biological safety is governed by the Advisory Committee on Dangerous Pathogens, who make recommendations to the UK government Health and Safety Executive (HSE), and health ministers on hazards, risks, and containment measures for pathogens. They issue guidance that informs the legal requirements under the Control of Substances Hazardous to Health Regulations 2002 (COSHH) (Government, 2002). These guidelines advise primary cell culture labs should have negative air pressure relative to corridors, in order to contain the escape of infectious aerosols from potentially infected cell samples into the rest of the building, in accordance with 'containment level 3' (Government, 2002). However, as an observation, the floor design in the ARC is open plan; therefore, adjacent to the cell-culture lab is not an isolated corridor from which air is drawn, but the high traffic area of a large and busy (>100 people) open lab, and outside the lab office spaces, making the cell culture rooms accumulators for dust.

Of note, Multiplex were the construction company who built both the Advanced Research Centre (Multiplex, 2026a) and the Queen Elizabeth University Hospital (QEUPH) (Multiplex,

2026b). Since 2015, problems with the standard of ventilation systems meant to purify the air, and contamination of water at QEUH, have required the relocation of patients for their safety(Peters, 2024). An independent review recently concluded that 84 children were infected with rare bacteria, linked to inadequacies of the hospital building(Brooks, 2026). There is an ongoing public inquiry and police criminal investigation into whether the deaths of 7 immunocompromised patients (including adults and children, many of whom were being treated for cancer) were preventable, and secondary to the hospital environment(Gillies, 2026).

It would be interesting to ascertain whether active air sampling (volumetric sampling), passive air sampling (settle plates), surface sampling (contact plates) and comprehensive water testing have been conducted to establish and regularly audit the adequacy of the built environment including the ventilation system, the air changes, and the water supply at the ARC. It has been reported that most bacteria sampled from Grade A and B cleanrooms and clean zones are Gram-positive. Gram-positive rods (eg. *Bacillus*) were found to be transferred to cleanrooms on equipment or dust. Gram-positive cocci (eg. *Staphylococcus* spp. and *Micrococcus* spp.) were associated with normal human microbiota and were even found in sites with hazmat suits, hood and mask personal protective equipment (PPE). Areas identified as the source of contaminating microorganisms included the windows, doors, doorhandles, the floor areas around essential equipment such as the centrifuge and incubator, and the walls(Szabłowska-Gadomska et al., 2023). From their experience of eliminating persistent infection in ADSC culture and adipose cell-derived medical products, Szabłowska-Gadomska et al. (2023) recommended that continuous monitoring of the environment, sampling at 5 stages of the culture process, with active improvement of the procedures used including the cleanroom sanitation process being required(Szabłowska-Gadomska et al., 2023). In the event of further issues with infection in the ARC, a proactive step might include the investment in microbiologist expertise to identify contaminants and advise antibiotic specificity and sensitivity, to increase understanding of source and reduce risk of future recurrences. Reduced contaminations and discarded experimental work would save taxpayer and charity funding, resources with environmental impacts, and the finite time and precious energy of students and staff.

The use of HPL seems protective against fungal and bacterial contamination

Infections occurred in this lab work, despite the majority of cultures in the lab being maintained with pen/step 1%. It was noted that use of HPL in cell culture seemed to be protective against both bacterial and fungal infections *in vitro*; in 6 of the cases of contamination, only the FBS cultured cells were affected (3 fungal, and 3 bacterial) whilst the HPL cells did not show signs of infection upon daily observation under x40 magnification. In one case the FBS supplement aliquot showed fungal growth upon incubation; however, the source of infection was unclear in the other cases. Whilst anti-fungal agents can be used in cell culture, they are not typically included in routine, long-term media for several reasons, primarily related to cell toxicity, the potential to mask contamination, and the promotion of resistant strains. The preferred method to control fungal contamination is strict adherence to aseptic techniques, not prophylactic drug use.

The development of drug-resistant “superbugs” puts many gains of modern medicine at risk and has been named “one of the top global public health and development threats” by the World Health Organisation (Organisation, 2023). In a study by Murray et al. (2022) they concluded that on the basis of their predictive statistical models, there were an estimated 4.95 million (3.62–6.57) deaths globally in the year 2019 alone that were associated with bacterial antimicrobial resistance (Murray et al., 2022). Overuse of antimicrobials is one of the main driving factors (Chandrasekhar et al., 2024) and finding alternatives to antimicrobial-drugs is an urgent need for human, animal and environmental health (Gordon et al., 2021). In addition to the aforementioned benefits of the use of HPL in cell culture, *in vitro* and *in vivo* nerve regeneration animal models, if HPL can confer protection from infection and reduce the use of antimicrobials, this would be extremely clinically relevant.

Gordon et al. (2021) found that platelets showed a strong antimicrobial effect and investigated the mechanisms of action (Gordon et al., 2021). They reported that platelets contained alpha granules, which released chemotactic factors involved in activating the host innate immune response. Platelets also facilitated the antibody-dependent immune reaction and produced bacteria-killing free radicals. Interestingly, they found that equine platelet lysate (ePL) had different effects on different strains of bacteria *in vitro*; ePL delayed the exponential growth curve of *P. aeruginosa* and *E. coli* in a concentration-dependent manner, whilst ePL’s effect on

E. faecalis and *S. aureus* growth was not concentration-dependent. For all strains, 20% ePL caused a significant reduction in bacterial yield and growth rate, and they concluded that pooled ePL, from multiple donors, is more effective at suppressing cell-mediated inflammation than ePL obtained from an individual horse, possibly because it contained a more consistent balance of effector proteins (Gordon et al., 2021). Shariati et al. (2020) even reported platelet-derived biomaterials having an anti-bacterial effect on Methicillin-resistant *Streptococcus aureus* (MRSA), as measured using diffusion discs, and time-kill assays. They reported human-derived platelet-rich plasma subjected to 4 freeze-thaw cycles (to disrupt the platelet membranes and release their contents) was effective against MRSA and *Pneumonia aeruginosa* within 4 hours of treatment. In their model using 48 rats, they concluded that platelets had potential for use as a topical antimicrobial for infected burn wounds as evidenced by significantly reduced necrotic and inflammatory tissue upon histopathological analysis of the treatment group (Shariati et al., 2020). Huang et al. (2021) also reported use of topical HPL for a corneal abrasion model in mice, and suggested HPL supported wound healing, and corneal nerve regeneration *in vivo* (Huang et al., 2021).

4.5 Conclusion

HPL does show potential for clinical translation in nerve regeneration therapies. It has been used for completely animal-free ADSC culture *in vitro*, whilst maintaining the necessary cell functionality and even showing reported benefits compared with the current standard. In clinical settings, it has feasibility as a pooled blood derived product, stored by the blood bank. The literature reports improved neuronal outgrowth in *in vitro* and *in vivo* studies, increased proliferation, increased cell viability, significantly greater secretion of neurotrophic growth factors than FBS-cultured ADSCs and adoption of a spindle-form morphology. This study added the analysis of morphological differences, which included comparison to FBS ADSCs and ADSCs differentiated down a Schwann cell-like phenotype (dADSCs), suggesting that HPL-cultured ADSCs were more consistent, and more spindle-form. Further replicates and *in vivo* nerve repair models are required to draw conclusions. The possibility for the use of human platelet-derived biomaterials for wound healing and antimicrobial applications is exciting. For the translation of any cell therapy to be feasible, a well-designed contamination control strategy must be in place, and must be regularly audited and actioned.

Chapter 5 Super Paramagnetic Iron Oxide Nanoparticle (SPION) labelling: a strategy for non-invasive *in vivo* cell tracking

5.1 Introduction

To optimise patient safety in the translation of novel cell therapies into clinical practice, cell tracking is needed to establish cell migration, proliferation, end differentiation, and function *in vivo*. The cell labelling modality should be non-invasive, non-toxic to the host cell viability and function, specific to the labelled host cell, visualised using low ionising radiation techniques to minimise morbidity to the patient and have longevity enough to allow for appropriate follow-up. There are multiple options for cell labelling, but few that meet all of these requirements.

Stem cells can be genetically engineered to express an ‘imaging reporter’; these include enzymes, receptors or iron-chelating proteins. These approaches have the benefit of longevity and specificity of cell tracking and have proved useful in animal models. However, due to the nature of their action through gene modification, they pose the risk of stimulating an immunogenic response from the patient and of epigenetic silencing or malignant transformation and therefore do not have clinical relevance (Cho et al., 2016).

In recent years, superparamagnetic iron oxide nanoparticles (SPIONs) have been recognised as a novel and effective tool for manipulating cell growth and behaviour. The magnetic characteristics of the nanoparticles can be tailored according to their size, shape, functional groups, and conjugated cargos, thus determining the interactions between the nanoparticles and the cells (Wei et al., 2021). SPIONs are a cell labelling option which have the benefit of being detectable by magnetic resonance imaging (MRI), an imaging modality with lower morbidity and greater sensitivity over other imaging modalities, with capabilities of deep tissue penetration and high resolution anatomical, structural, and molecular level imaging (Rahman, 2023). Once phagocytosed, the SPIONs are metabolised down the haem pathway, with the waste products excreted in urine (Lu et al., 2017). While gadolinium is the standard MRI contrast agent used in the National Health Service (NHS), SPIONs were the first liver-specific contrast agents used,

and alternatives are essential in some patients, particularly in the presence of comorbidities such as kidney disease or renal failure where there is risk of gadolinium induced renal fibrosis (Berry, 2003). Iron oxide nanoparticles are currently widely used as a contrast medium option for MRI, as they are easy to formulate, functionalise, are biocompatible and cost effective. For instance, Sienna+® are 60nm SPIONs that are currently used in the NHS, with National Institute for Health and Care Excellence (NICE (2022)) approval, for sentinel lymph node biopsies in breast and prostate cancer patients (NICE, 2022). Clinical trials have demonstrated that SPION techniques are non-inferior to Technetium-99m radioisotope and blue dye methods in detecting sentinel lymph nodes, with some studies showing better detection rates in a study of breast cancer patients (Karakatsanis et al., 2016). Compared with traditional Technetium-99m, SPIONs are not radioactive, which removes the need for nuclear medicine facilities, specialised waste disposal, and radiation exposure safety protocols. The tracer can be injected days before surgery because it stays in the lymph nodes, allowing for flexible surgical scheduling, with optional preoperative MRI identification of sentinel lymph nodes, and intraoperative handheld magnetometer detection of the sentinel lymph nodes (NICE, 2022).

In addition to cell tracking and MRI contrast utilities, SPIONs are clinically relevant due to their suitability for functionalisation, with therapeutic benefits. The superparamagnetic properties of SPIONs can be utilised to hone the site of treatment delivery, with research advances seen in treatments such as administration of intracellular hyperthermia to induce cell death (i.e. in cancer cells) (Rahman, 2023, Berry et al., 2004). Another such example is drug delivery, which is used in both research and current clinical practice applications for cancer, cardiovascular disease, neurological disorders, autoimmune disease, and infectious disease treatments (Rahman, 2023). SPIONs have high magnetic susceptibility and do not retain residual magnetism once the magnetic field is removed, thus avoiding potential clogging of blood vessels due to magnetic aggregation (Vangijzegem et al., 2023). Of note, a SPION formulation named Ferumoxytol, which was used to treat iron deficiency anaemia was recalled in the UK and Europe in 2015 due to adverse hypersensitivity, including fatal anaphylactic reactions. It is still in use with Food and Drug Administration (FDA) approval in the US, with additional monitoring in place, for applications such as malignant nodal metastasis and brain tumour imaging (Dr Rebecca Curtis, 2015). This highlights the importance of rigorous research, comprehensive trials and thorough monitoring of novel therapies, to allow for safe clinical translation.

Specific to the treatment of peripheral nerve injury, Lu et al. (2017) described magnetic nanoparticle labelling and the application of a magnetic field inducing a neural-like phenotype in human Mesenchymal Stem Cells (MSC). Their results suggest greater proliferation and significantly greater gene expression and protein synthesis of neurotrophic growth factors BDNF, GDNF, NT-3, and VEGF in MSCs, when labelled with 10% magnetic nanoparticles and exposed to magnetic fields with adjustable magnetic induction of 0–20 mT and frequency of 0–100 Hz *in vitro* (Lu et al., 2017). Liu et al. (2015) described the use of magnetic nanoparticles in Schwann cells, whereby magnetic nanocomposites were shown to support cell adhesion, cell spreading, and promote proliferation of Schwann cells and their secretion of neurotrophic growth factors whilst under magnetic field exposure. These effects were significantly improved by administering the magnetic nanoparticles via an impregnated chitosan–glycerophosphate polymer scaffold (Liu et al., 2015b), highlighting the benefits of combinational approaches that incorporate bioengineered materials with therapeutic cells.

Work by Suzanne Thomson used primary rat ADSCs labelled with 200nm green fluorescing SPIONs (Chemicell) (Thomson, 2018). The labelled cells were detectable on MicroCT, with resolution better than 5µm, in a bioengineered conduit *ex vivo* at 7 days post-implantation. These were autologous rat ADSCs labelled with SPIONs at 0.1mg/ml concentration and seeded in a micro-textured polycaprolactone conduit, inside a rat sciatic nerve repair model (Figure 1 SPION) (Higgins and Thomson, 2020) (figure 38). In the following, I progress this work into human cells, and compare labelled and unlabelled FBS ADSCs with HPL ADSCs, in efforts to move closer towards meaningful clinical translation of this body of work.

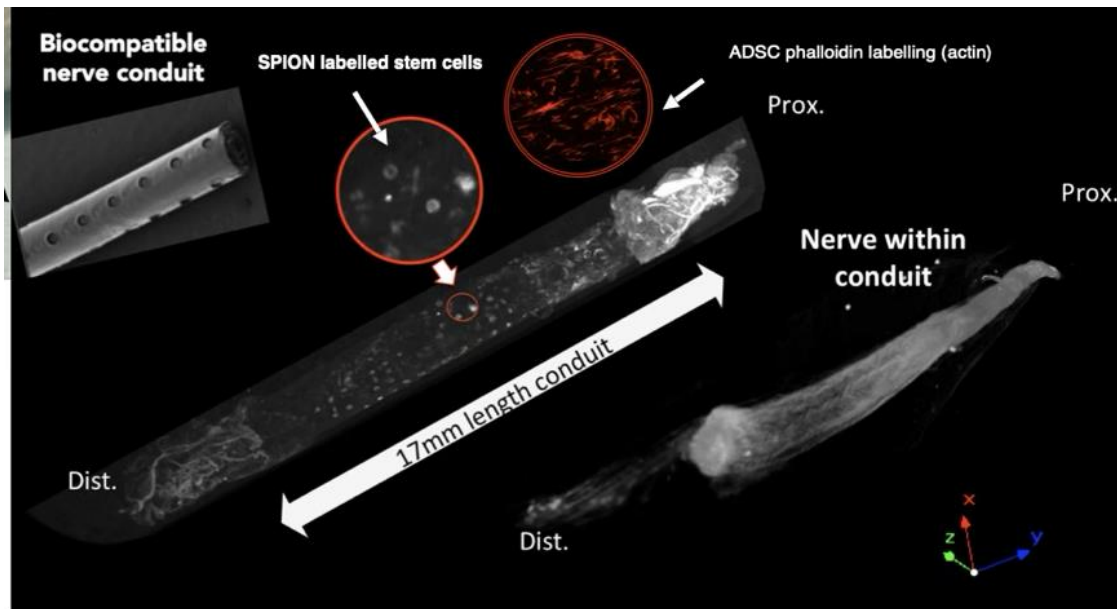


Figure 38 is a micro CT image of a biocompatible, biodegradable polycaprolactone conduit with custom topography, and seeded rat ADSCs which are labelled with 0.1mg/ml SPIONs *in situ*. This conduit had been used to repair a sciatic nerve injury in a rat model and scanned *ex vivo* 7 days after implantation. This image is used with kind permission of Ms Suzanne Emma Thomson (Thomson, 2018).

5.2 Materials and Methods

5.2.1 Labelling protocol

Cells (FBS ADSCs, HPL ADSCs and dADSCs) were cultured in T75 flasks until 80% confluent and then seeded into a 24-well plate at a concentration of 10^4 cells per well (day1). Each well contained a poly-lysine (PLL) and laminin-coated glass coverslip (as below). On day 2 of culture, the cells were labelled with SPIONs - green fluorescing (490nm emission), 200nm nano-screenMAG-PEA SPIONs (Chemicell). These have a magnetite core and poly-dimethylamin-co-epichlorhydrin-co-ethylendiamin matrix and an amine functional group. Uptake into cells occurred via endocytosis, when incubated for 30 minutes with a 280mT static magnetic field utilising a MagnetoFACTOR-24 device (Chemicell). Following SPION incubation, samples

were washed thrice with HBSS to remove external SPIONs and incubated with fresh medium. SPION concentrations of 0.01mg/ml and 0.1mg/ml were compared, with unlabelled controls. Fluorescence microscopy (Olympus) was utilised to confirm that the 200nm green fluorescent SPIONs (Chemicell) were *in situ* within the cell cytoplasm. This added to work from our group using TEM to locate the SPIONs within the cytoplasm of rat ADSCs(Thomson et al., 2014).



Figure 39 illustrates the experimental setup of SPION-labelling cells in a 24-well plate with a MagnetoFACTOR-24 device (Chemicell).

5.2.2 Immunohistochemistry

5.2.2.1 Coverslip coating with Poly-L-lysine (PLL)

Poly-L-lysine (PLL) (Sigma-Aldrich) was stored at a stock solution 4mg/ml, which was filter sterilised (0.22 μ M) into 67 μ l aliquots to avoid freeze/thaw cycles. One 67 μ l aliquot was added per 20ml of milliQ water and filter sterilised. This was added to a petri dish containing ethanol sterilised coverslips and incubated at 37°C for one hour (30 minutes minimum). They were washed x 3 with sterile milliQ water and dried before the laminin coating step.

5.2.2.2 Coverslip coating with laminin

Laminin (Sigma-Aldrich) was stored at stock solution of 1mg/ml with a working solution of 2ng/ml. 40 μ L of the laminin stock solution was added to 20ml PBS and filter sterilised.

The Laminin was added to Petri dish containing the PLL coverslips which had been coated and allowed to dry. Incubated was at 37°C for 1 hour minimum. Coverslips were then washed x3 with sterile PBS.

5.2.2.3 Immunohistochemistry protocol

At day 3 post-SPION labelling, cell phenotype was investigated using immunocytochemistry. Staining was undertaken for GFAP (anti-GFAP rabbit, Santa Cruz Biotechnology, SC-9065, H0414), S100 (anti-S100 rabbit, abcam, ab868, GR164645-3) and p75 (p75 NGFR goat, Santa Cruz Biotechnology, SC-6188, G0714) neuronal markers and morphology (rhodamine phalloidin, Life Technologies, R415, 1646681) utilising Vectashield mounting medium with DAPI (Vector, H-1200, ZB1130) and fluorescence microscopy (Olympus BX51TF).

Experiments were conducted in technical triplicates, with three patient repeats for FBS ADSCs, and two patient repeats for dADSCs.

5.2.3 Flow Cytometry

As per previous chapters, thank you to Dr Euan Ross for his assistance with these flow cytometry experiments. To evaluate immunophenotype, ADSCs were stained with the following antibodies: specifically anti-CD73 (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled), anti-CD90 (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled), anti-CD105 (1:200, IgG1, BD Biosciences, monoclonal, PE-labelled) as positive MSC markers; anti-CD34 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled); and anti-CD45 (1:66, IgG1, BD Biosciences, monoclonal, FITC-labelled), as negative endothelial and hemopoietic markers, respectively. Appropriate isotype controls were used for each fluorophore (1:1000, IgG1, BD Biosciences, monoclonal, FITC-labelled; 1:50, IgG1, BD Biosciences, monoclonal, PE-labelled; 1:50, IgG2b, BD Biosciences, monoclonal, FITC-labelled). For each antigen, approximately 100,000 cells were resuspended in 100 µL of antibody diluted in flow cytometry buffer (0.2% FBS in PBS) and incubated for 1 hour, agitated in the dark. The cell surface phenotype was subsequently assessed by BD Accuri C6 apparatus (Erembodegem). Experiments were conducted in technical and biological triplicates (three patient repeats) for SPION-labelled FBS ADSCs at 0.01mg/ml and 0.1mg/ml concentration.

5.2.4 Morphological analysis

The cellular response to the nanoparticles, manifested as changes in cell morphology, were studied as follows. Cells were seeded into a 24-well plate at a concentration of 10^4 cells per well, labelled with SPIONs on day 2, and fixed with formaldehyde on day 5. Cells were fixed with 4% paraformaldehyde for 30 minutes at room temperature and then washed with 1% PBS.

Permealising buffer (10.3g sucrose, Fisher BioReagents) + 0.292g NaCl (VWR Chemicals) + 0.06g $MgCl_2$ (VMR) + 0.476g Hepes (VWR Chemicals) in 100ml 1% PBS, 7.2pH + 0.5ml Triton x) was added for a 5-minute incubation at 4°C, and then a further wash and 5-minute incubation at 37°C with block solution (1% BSA (Fisher BioReagents) in 1% PBS). Staining for the actin cytoskeleton (rhodamine phalloidin, life technologies, R415, 1646681) at 1:500 dilution was conducted, with coverslips mounted with Vectashield DAPI (Vector, H-1200, ZB1130). Experiments were conducted in technical triplicates and biological duplicates.

Imaging and cell labelling ratio analysis was conducted using automated programming, at random, for 100 cells, with high content fluorescence microscopy (Olympus BX51TF). I wrote a Cell Profiler cell image analysis software (4.1.3) pipeline (Carpenter et al., 2006), and utilised it for cell measurements (minimum 500-1000 cells per condition) (Appendix 2). Adaptive thresholding of greyscale images identified cells based on DNA staining (DAPI) as ‘primary objects’, and actin cytoskeleton staining (phalloidin) as ‘secondary objects’. The watershed-gradient method was implemented to delineate neighbouring cells, and cells touching the boundary of the images were discarded (Institute., Copyright 2003-2021). Graphpad Prism6 and Microsoft Excel were utilised for statistical analysis.

5.2.5 SPION labelling live/dead viability analysis

To investigate the viability of HPL vs FBS ADSC cultures and the potential toxicity of SPIONs (Chemicell) for these cells at 0.01mg/ml and 0.1mg/ml concentrations, a live-dead assay was conducted in accordance with the manufacturer’s instructions (BioVison Inc). Briefly, cells were incubated with SPIONs as described, and live dead assay performed at 72 hours; ADSCs were washed with HBSS, and the staining solution introduced to each sample, followed by incubation

at 37°C for 15 minutes. Cell viability was then observed by fluorescence microscopy (Olympus BX51TF); living cells were labelled green and dead cells were labelled red with a minimum of 1,000 measured per condition. Dead cells vs live cells were counted manually, and data analysed in Graphpad prism 6.

5.2.6 Growth curve analysis

To analyse HPL and FBS ADSCs labelled with 0.01mg/ml and 0.1mg/ml SPIONs, 24-well plates were setup as previously described, but seeded at a concentration of 5,000 cells per well to allow for longer period of study. Daily for 5 days, plates were removed and fixed with 4% paraformaldehyde for 30 minutes at room temperature. Cells at ten randomly automated points on each cover slip (Olympus BX51TF) were hand counted with a clicker at 10x magnification. Data was analysed in Microsoft Excel, with the median number of cells plotted for each condition, for each day. This experiment was conducted with Patient 16 cells for FBS and HPL conditions.

5.3 Results

5.3.1 ADSC and SPION labelling results

This work was the progression from previous studies in our group, in rat ADSCs. A minimum of 100 human cells were analysed per condition, 98% human ADSCs and 96% human dADSCs exhibited labelling respectively (ADSCs for 3 patients and dADSCs 2 patient repeats) (figure 40).

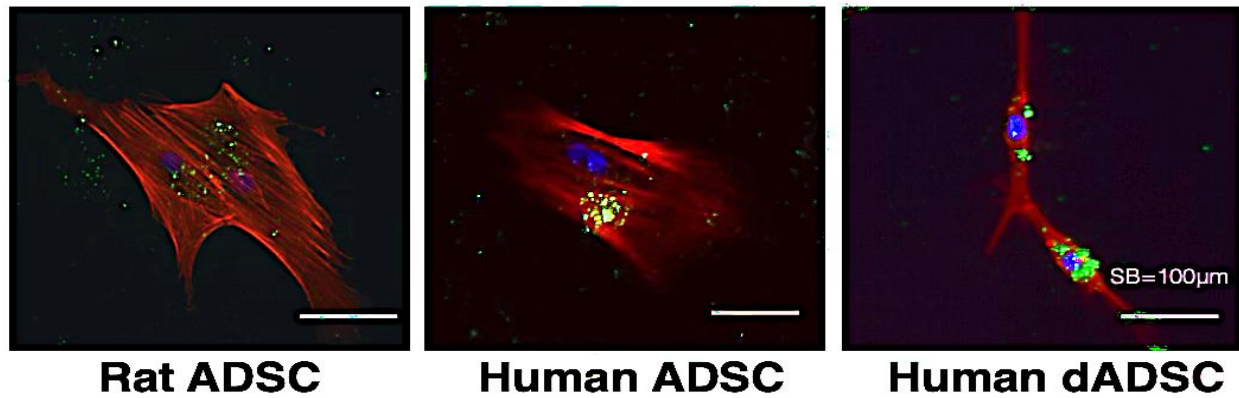


Figure 40 shows photographs from the Olympus BX51TF fluorescence microscope. Red=actin cytoskeleton (phalloidin), blue=DNA (DAPI), green=SPIONs at 0.1mg/ml concentration.

5.3.2 SPION immunohistochemistry results

Both ADSCs and dADSCs maintained their immunohistochemical markers after labelling with 0.1mg/ml SPIONs (Figure 41). Analysis of micrographs (Olympus BX51TF) demonstrated that ADSCs stain positive for GFAP; actin and DNA staining helped with image analysis of cell shape. The dADSCs showed positive staining for GFAP and s100 before and after SPION treatment (figure 41). Technical repeats were in triplicate, 3 biological (patient) repeats for ADSCs and 2 patient repeats for dADSCs. Analysis was conducted on day 3 post SPION labelling (day 5 in culture). All cells were passage 2-6. The scale bar=100µm.

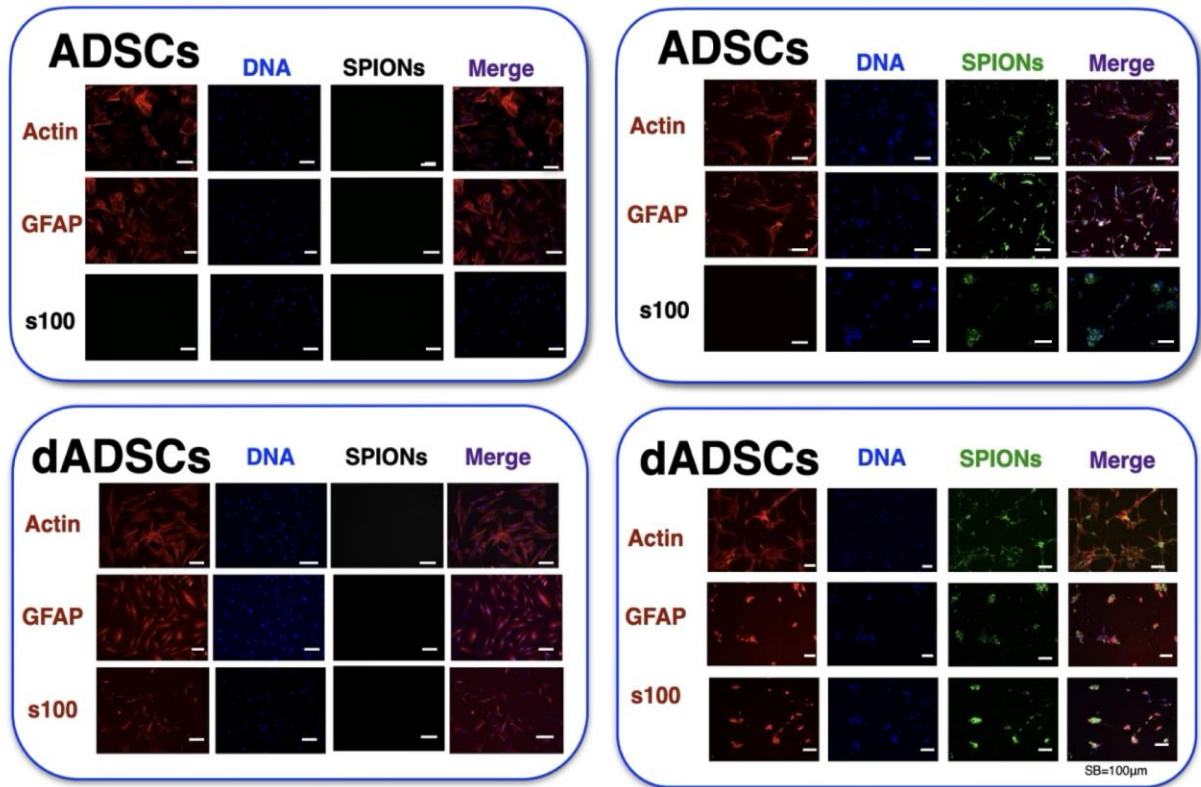


Figure 41 Select fluorescence micrographs of immunohistochemistry, demonstrating that ADSCs' and dADSCs' immunohistochemistry is unchanged in the presence of SPION labelling at 0.1mg/ml. Top panels: ADSCs without (left) or with (right) SPIONs. ADSCs stained for either actin or GFAP or S100 (red), and DNA (blue), and SPIONS (green). ADSCs positive for GFAP and negative for S100. Bottom panels: dADSCs without (left) or with (right) SPIONs. dADSCs stained for either actin or GFAP or S100 (red), and DNA (blue), and SPIONS (green). dADSC positive for both GFAP and S100. The scale bar=100µm.

5.3.3 SPION flow cytometry results

Flow cytometry shows that the ADSCs maintain their Cluster Differentiation (CD) markers post SPION labelling. CD90, CD105, and CD73 were expressed after labelling with 0.01mg/ml and 0.1mg/ml concentrations (figure 42).

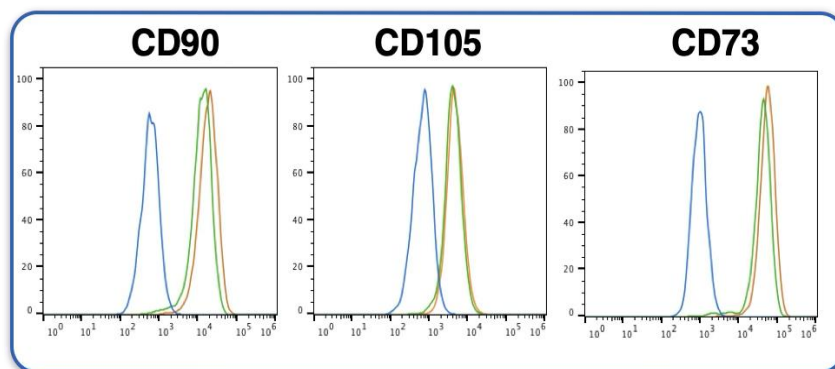


Figure 42 flow cytometry results confirming that the ADSCs maintained their CD markers post-labelling, with CD90, CD105, and CD73 expressed after labelling with 0.01mg/ml (orange) and 0.1mg/ml (green) concentrations blue=negative control). This was conducted with 100,000 cells per condition and repeated in biological triplicate.

5.3.4 Cell morphology results

5.3.4 SPION cell morphology results

Analysing ADSC morphology using greyscale images of ADSCs at day 5 in culture (3 days post labelling), in a specifically designed Cell Profiler software pipeline, showed morphological changes that were concentration dependent, with spindle-like morphology adopted by the low concentration (0.01mg/ml) labelled cells and smaller, rounder morphology demonstrated with high concentration (0.1mg/ml) SPIONs (Figure 43). Comparing the morphology of HPL-cultured cells, with FBS-labelled cells; the HPL cells have the smallest cell area, the HPL cells and FBS-labelled cells (0.01mg/ml and 0.1mg/ml concentration) have lower maximum Feret's diameter

than non-labelled FBS cells, and the degree of eccentricity is similarly high between HPL and 0.01mg/ml concentration labelled cells (Figure 44). Statistical analysis is not possible due to the number of biological repeats.

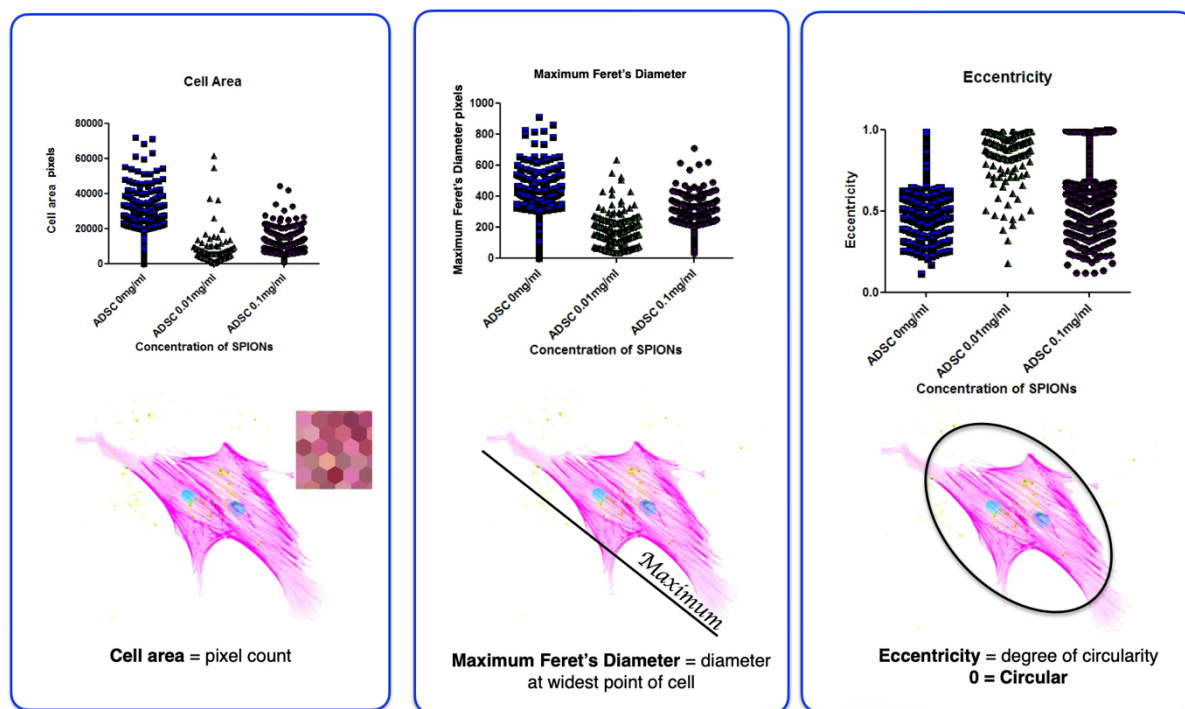


Figure 43 shows graphs comparing the cell area, maximum Feret's diameter (widest diameter of the cell) and eccentricity (degree of circularity where 0=circular) for cells cultured in FBS serum, without or with concentrations of either 0.01mg/ml or 0.1mg/ml SPIONs. These data had technical repeats in triplicate, with one biological patient sample for the labelled cells.

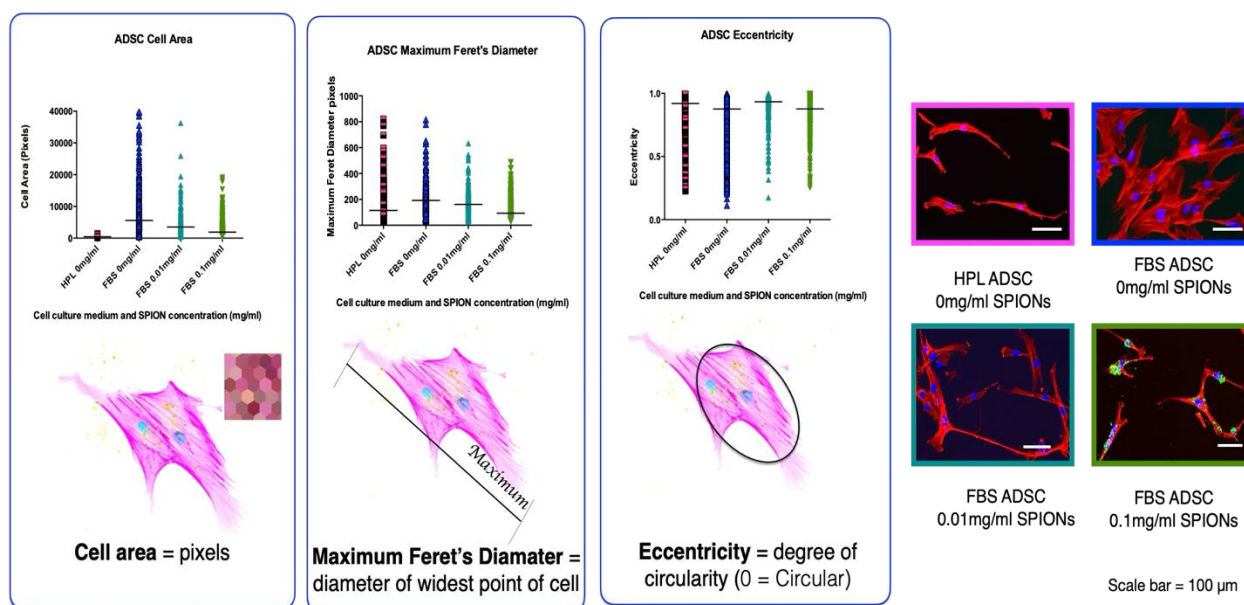


Figure 44 shows graphs and photographs comparing the morphology in terms of cell area, maximum Feret's diameter (widest diameter of the cell) and eccentricity (degree of circularity where 0=circular) for cells cultured in HPL serum and FBS serum, with concentrations of 0mg/ml, 0.01mg/ml and 0.1mg/ml SPIONs. These data had technical repeats in triplicate, with two biological repeats for the HPL and FBS non-labelled cells and one biological patient sample for the labelled cells. They show a trend of HPL having the smallest cell area, the HPL and high concentration labelled cells having the lowest maximum Feret's diameter and the HPL and low concentration cells having the highest degree of eccentricity. Bars indicate the mean results; red=actin cytoskeleton (phalloidin), blue=DNA (DAPI), green= SPIONs. The scale bar=100 μ m.

5.3.5 SPION nuclear morphology results

Compared to control ADSCs, the FBS-cultured ADSCs' nuclei became larger, rounder and more 'solid' with the higher concentration (0.1mg/ml) of SPIONs; with the lower concentration of SPIONs (0.01mg/ml) the nuclei became smaller, more eccentric (aspect ratio) and presented as more solid (figure 45). Cell profiler defined nuclear solidity as the ratio of the area of the nucleus to the area of its convex hull. This measure was a valuable metric for analysing nuclear deformation, such as identifying irregularly shaped, indented, or blebbing nuclei, which are often markers of disease, senescence, or DNA damage. A nuclear solidity of 1 indicates a perfectly

smooth nucleus with no indentations, with a solidity of <1 indicating an irregular nucleic border (Institute., Copyright 2003-2021).

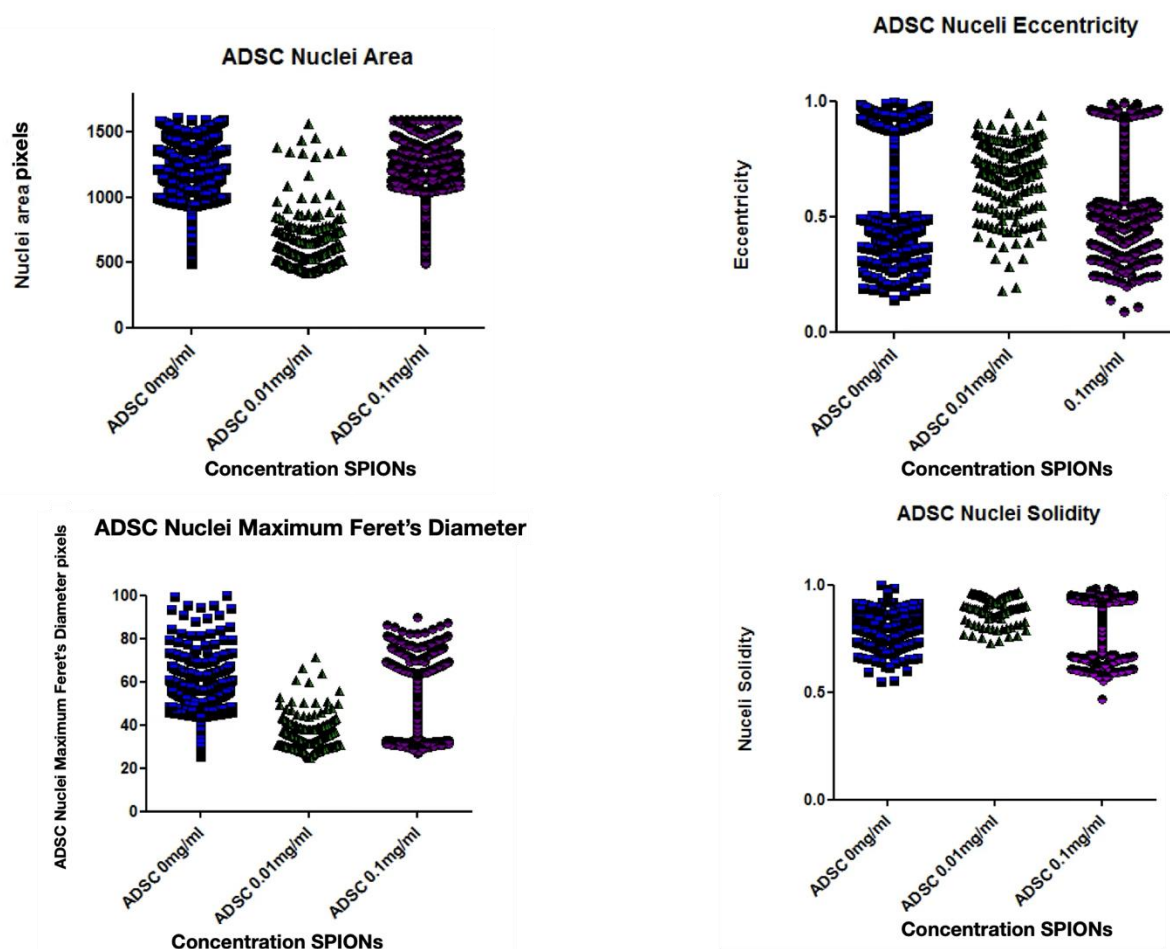


Figure 45 shows graphs comparing the nuclei area, eccentricity (the ratio of the distance between the foci of an ellipse and its major axis length measuring the degree of circularity, where 0=circular), maximum Feret's diameter (widest diameter of the cell) and solidity (degree of irregularity of the nucleic border)(Institute., Copyright 2003-2021) for cells cultured in FBS serum, with concentrations of 0mg/ml, 0.01mg/ml and 0.1mg/ml SPIONs. The ADSCs nuclei became larger, rounder and more solid (less irregular) with high concentration (0.1mg/ml) of SPIONs, and smaller, more eccentric and more solid with low concentration SPION labelling (0.01mg/ml).

5.3.6 SPION labelling live/dead viability analysis results

Live dead survival assay (BioVison Inc.) indicated similar percentage survival between FBS and HPL-cultured cells; 98% and 96% respectively (Figure 46). The percentage of viable cells reduced in a dose-dependent manner in both groups, with higher cell death occurring with high concentration (0.1mg/ml) SPION labelling in the FBS and HPL cells (Figure 47). The FBS cells showed 95% viability with 0.01mg/ml concentrations SPIONs, and 93% viability with 0.1mg/ml concentration labelling. The viability of the HPL cells had a consistent trend, dropping 10% from baseline, to 86% with low dose (0.01mg/ml) labelling, and 84% with high dose (0.1mg/ml) labelling.

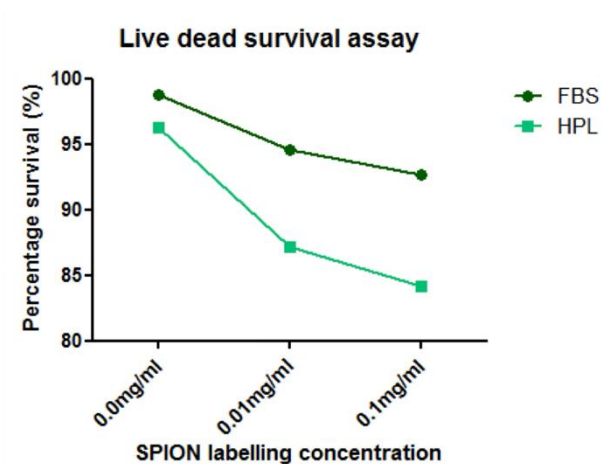


Figure 46 shows the results of live dead survival assay, comparing FBS and HPL-cultured cells with low (0.01mg/ml) and high (0.1mg/ml) concentration of SPION labelling. The trend indicates a dose-dependent reduction in viability, and lower viability in HPL compared with FBS cells, in one biological (patient) sample. More repeats are needed for statistical analysis.

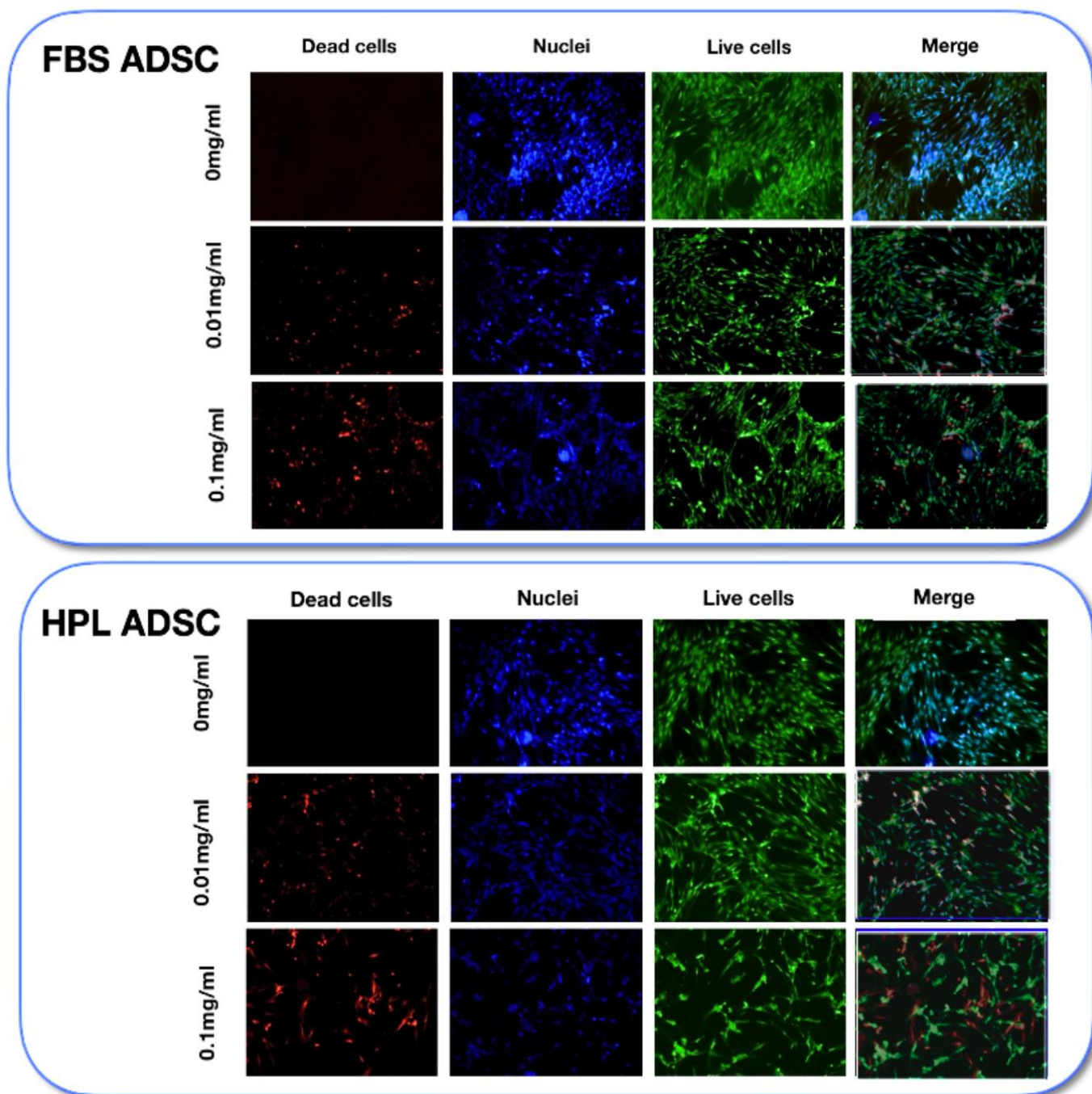


Figure 47 shows examples of the viability results (live cells in green) of the FBS and HPL cells at 0mg/ml, 0.01mg/ml and 0.1mg/ml concentration of SPION labelling (Olympus). Higher numbers of dead ADSCs (in red) are seen in the high dose (0.1mg/ml) group, higher number of viable (in green) ADSCs are seen in the non-labelled and low-dose (0.01mg/ml) group. The nucleic DNA is in blue (DAPI).

5.3.7 Growth curve analysis results

Growth curves were studied to investigate the impact of HPL serum, and SPION labelling upon ADSCs, with non-labelled FBS ADSCs as a control (figure 48). For the FBS and HPL ADSCs, proliferation was greatest in the unlabelled (0mg/ml SPION concentration) group until day 3, after which the low dose (0.01mg/ml SPIONs) ADSCs had a higher number of cells. The high dose (0.1mg/ml SPIONs) ADSC had the lowest proliferation throughout for FBS cells, but the unlabelled HPL cells had the lowest numbers each day. The FBS growth curve dropped off by day 5, whereas the HPL curve continued climbing. The greatest number of cells was seen in the HPL ADSCs with low-dose (0.01mg/ml) SPIONs. This study quantified observed anecdotal findings that cell proliferation was greater (and time to confluence shorter) with HPL cells and with low-dose (0.01mg/ml) SPIONs. The same patient's cells, at the same passage (pt16p1) were utilised for this study. Repeats utilising ADSCs from other biological donors is required to confirm trends and perform statistical analysis.

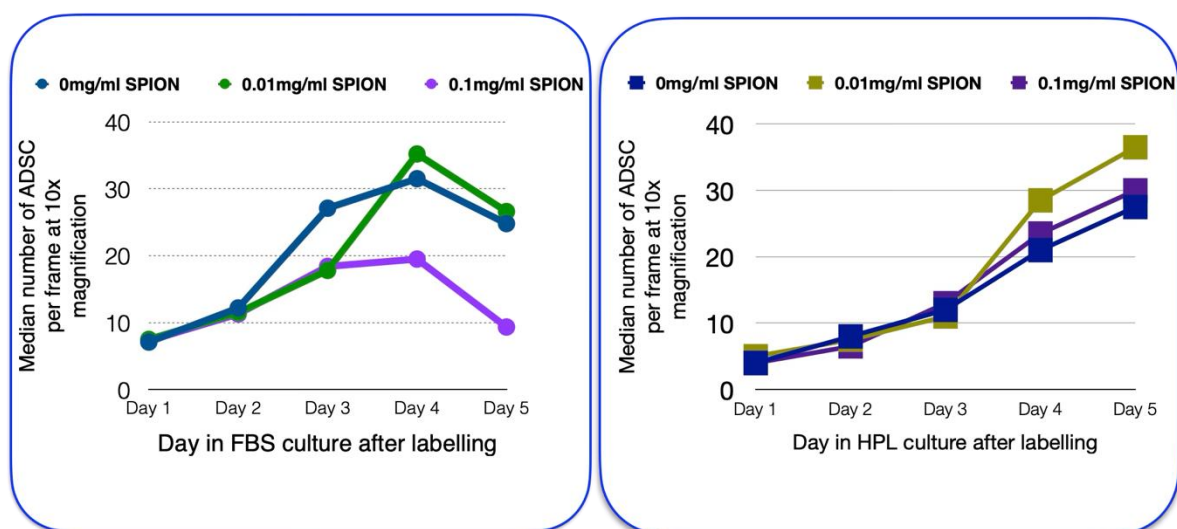


Figure 48 compares graphs of the median number of ADSCs (pt16p1) cultured with either FBS or HPL. Data are ADSCs counted per frame at 10x magnification (from 10 analysed images) for 5 days after initial ADSC seeding at a density of 5,000 cells per well in a 24-well plate. On the left, growth curve shows ADSCs grown with FBS without (blue), or with added SPIONs at

either 0.01mg/ml (green) or 0.1mg/ml (purple). On the right, growth curve shows ADSCs grown with HPL (squares) without (dark blue), or with added SPIONs at 0.01mg/ml (ochre) and 0.1mg/ml (dark purple). For both FBS and HPL cells, proliferation was greatest in the unlabelled group until day 3, after which the low-dose (0.01mg/ml SPIONs) ADSC proliferation was greatest. ADSCs with the high dose of 0.1mg/ml SPIONs had the lowest proliferation throughout for FBS cells, but in the HPL cells, the unlabelled cells had the lowest numbers each day compared with the HPL-labelled cells. The FBS growth curve dropped off by day 5, whereas the HPL curve continued climbing. The highest number of cells was seen in the HPL cells, at 0.01mg/ml concentration on day 5. No biological repeats.

5.4 Discussion

Trends suggests ADSCs have a reduced viability with increasing SPION labelling

Many studies have shown that the composition, size and surface functionalisation of SPIONs were the key factors in determining their toxicity (Wei et al., 2021, Berry et al., 2004, Berry et al., 2002). Zhang et al. (2014) demonstrated that the viability and apoptosis of rabbit MSCs did not alter after labelling with uncoated SPIONs (Zhang et al., 2014). Similar results by Lee et al. (2012) suggested that there was no influence on the viability of human MSCs treated with SPIONs with unfractionated heparin coating (Lee et al., 2012). Berry et al. (2004) however, in their study of human fibroblasts, found that the unlabelled control cells were 100% viable, with the plain nanoparticle labelled cells having 79% and the dextran-coated nanoparticle labelled cells had 83% viability. In this study, the trend suggests that SPIONs reduced cell viability in a dose-dependent manner; however, all ADSCs maintained viability above 86%, even in the presence of high dose (0.1mg/ml) labelling. The cells had a viability of 98% and 96% for the unlabelled FBS and HPL ADSCs respectively, with viability reducing to 95% (FBS) and 86% (HPL) viability with 0.01mg/ml labelling, and a further 93% (FBS) and 84% (HPL) with 0.1mg/ml concentration SPIONs. The SPIONs (Chemicell) used here were larger than the aforementioned studies, at 200nm, and were unconjugated, they had an amine functional group, a magnetite core, and a poly-dimethylamin-co-epichlorhydrin-co-ethylendiamin matrix. Further repeats are required to conduct statistical analysis.

Studies in animal models have analysed the toxicity of SPIONs at a histological level, via pathological examination of organ tissue sections to assess tissue damage, inflammation or pathology after administration of SPION-labelled cells. Ledda et al. (2020) showed that sub-5nm silica-coated superparamagnetic iron oxide nanoparticles in human stem cells, which had been intravenously injected in experimental mice, immediately accumulated in the kidneys, followed by the liver and lungs; the iron level in the spleen and stomach was not significantly affected post-nanoparticle injection. The SPIONs were completely cleared from the kidneys at day 7 post-injection but accumulated in the liver and the lungs over the 7-day period. On histological analysis, no major organs showed lesions or necrosis, and no sign of tissue toxicity was found (at 7 weeks) (Lee et al., 2012). Geburek et al. (2016) reported longevity of autologous MSCs labelled with 45-60nm SPIONs coated with carboxydextran, injected in an equine tendinopathy model. The MSCs were still detectable on MRI, and although their concentration had decreased over time, a high concentration of labelled MSCs persisted at the healed injury site upon post-mortem of (4) culled animals at 9 weeks (Geburek et al., 2016). A dilution study is required to understand how the SPIONs are passed to daughter cells upon replication, and their persistence *in vivo* to confirm that they persist long enough for clinically relevant stages of post-operative follow up, to ensure the cell therapy safety profile and inform clinical decision making.

ADSCs develop a spindle-like morphology with low dose 0.01mg/ml SPION labelling, but do not stain positive for s100 neuronal marker

It is reported in the literature that animal and human stem cells labelled with SPIONs maintain their differentiation potential and can still be differentiated down a Schwann-like phenotype (Lu et al., 2017, Zhang et al., 2014). In this study, it was confirmed that the FBS ADSCs maintained their CD markers indicative of differentiation potential (CD90, CD105, CD75) with 0.01mg/ml and 0.1mg/ml concentration SPIONs. Interestingly, FBS ADSCs in this study adopted a spindle-like morphology with low-dose (0.01mg/ml) SPION (Chemicell) labelling, in the absence of differentiation medium or any other additional variables that might induce a Schwann cell phenotype (such as topographical cues (Thomson et al., 2014)). However, they did not show immunohistochemistry positivity for s100, which would indicate adoption of a Schwann-like

phenotype. At high-dose SPION labelling (0.1mg/ml) the cells became less elongated, with smaller cell area compared with the low dose (0.01mg/ml) FBS ADSCs and they had lower viability. Berry et al. (2004) studied Infinity™ Telomerase immortalised primary human fibroblasts labelled with 10nm SPIONs with a magnetite core and either no coating (plain) or dextran coating. They described smaller cell morphology, with actin appearing condensed and less organised in the fibroblasts labelled with the plain nanoparticles; meanwhile, the cells incubated with the dextran coated nanoparticles exhibited clear actin fibres and tubulin radiating through the cell and appeared to localise in large ring structures at the cell lamella (Berry et al., 2004).

Further repeats are required to establish statistical significance in this study, and further study is required to ascertain links between this change in morphology and any impacts upon functional characteristics. It has been reported that undifferentiated ADSCs promote *in vitro* dorsal root ganglion (DRG) outgrowth by means of secreting growth factors and inhibiting reactive oxidative species, and other studies have indicated that bone marrow-derived MSCs promote Schwann cell proliferation and survival and enhance neurotrophic factor expression (Reid et al., 2011). It would be interesting to determine if the changes towards a spindle-like morphology might confer any additional beneficial impacts of ADSCs upon nerve regeneration, from FBS ADSCs labelled with SPIONs (Chemicell) at a concentration of 0.01mg/ml and in HPL-cultured ADSCs.

In response to nerve injury, Schwann cells are reprogrammed to a series of specialised repair-promoting phenotypes, known as repair Schwann cells, which play a pivotal role in nerve regeneration. It is known that Schwann cells become more spindle form when they adopt this repair phenotype during natural nerve healing, and that it is a cytoskeletal change from G-actin to F-actin that is responsible. In turn, this change in morphological constitution triggers a number of cell signalling pathways which maintain the repair behaviours of the cell, including proliferation, migration and secretion of neurotrophic factors (Yu et al., 2009, Jessen and Mirsky, 2016, Wang et al., 2018). In Schwann cells during their change to repair phenotype, their nuclei undergo profound transcriptional and morphological changes with a notable shift in the location and structure of their nucleus (Jessen and Mirsky, 2016). This process is governed by c-Jun

expression, a transcription factor known to regulate gene expression for regulation of the cell cycle, cell survival and cell apoptosis (Wisdom et al., 1999). During their bipolar and tripolar elongation, the nucleus of the Schwann cells often moves to the leading front of the cell during migration (Jessen and Mirsky, 2016). Of note, there were nucleic changes seen in both the SPION (figure 45) and HPL groups (figure 36). The nucleus of the lower concentration of SPIONs (0.01mg/ml) the nuclei became smaller, more eccentric (aspect ratio) and presented as more solid. The ADSC with high dose SPION labelling (0.1mg/ml) became larger, rounder and more solid with the higher concentration of SPIONs, whilst HPL SPION labelled cells (see chapter 4) were smaller and rounder.

It is normal for the nuclear envelope to adapt to changes in its environment, and the nuclear lamina is mechanoresponsive. The lamina is interconnected with the cell cytoskeleton by intermediate proteins, and this allows sensing and rapid cellular response via complex post-translational control of its proteins. In turn the lamina is intimately connected with epigenetic modifiers. Therefore, changes in nuclear morphology are found with changes in cell differentiation and migration, however they can also be indicators of disease. A reduction in cell solidity (an increase in the irregularity of the nuclear border in the form of blebbing, or invaginations) can be indicative of aging, mutation, or cancer. Further studies at genomic and molecular level are required to elucidate the downstream effects of the cytoskeletal and nucleic morphological changes seen in this study, to illuminate if these morphological transfigurations equate to functional changes, and whether these changes are useful (or not) for peripheral nerve repair.

Transience of the Schwann cell repair phenotype state is one of the key reasons for nerve regeneration failure in humans. Liu et al. (2022) described the use of 50nm rhodamine functionalised and polydopamine coated SPIONs to maintain rat Schwann cells in their repair phenotype, in a rat sciatic nerve repair model using a magnetic field of 6 T/m. Upregulation of Schwann cell neurotrophins, immune-related cytokines, and transcription factor expression specific to Schwann cell repair phenotype, in addition to Schwann cell spindle-form elongation and branching were induced by the application of the SPION-mediated magnetic field(Liu et al., 2022). Liu et al. (2026) further described specific SPION targeting of F-actin in the Schwann cell

cytoskeleton, to direct magnetically actuated mechanical stimulation. This is based on the principle that Schwann cells are highly sensitive to the external mechanical environment and transduce mechanical cues into intracellular biochemical signals, with subsequent modulation in their functional state. They described this magnetic induced mechanical force eliciting adoption of spindle-like morphology via F-actin filament re-organisation and polymerisation leading to Piezo1 ion channel upregulation and gating, transmembrane Ca^{2+} signal driven transcription reprogramming, and ultimately Schwann cell adoption of their repair state phenotype (Liu et al., 2026).

The growth study suggests ADSCs with HPL serum, and ADSC labelled with 0.01mg/ml concentration SPIONs, seem to have greatest proliferation by day 5

It is clear in the literature that the size and coating of the SPIONs are crucial to their impact upon cells (Wei et al., 2021, Berry et al., 2004, Berry et al., 2002). This study suggests the additional impact of dose, with 0.1mg/ml SPION dosing reducing the proliferation of both the FBS and HPL cultured ADSC by day 3. Berry et al. (2002) demonstrated that human fibroblasts labelled with 20nm elastin-coated had greater proliferation than fibroblasts labelled with 520nm elastin-coated SPIONs, when compared with plain particles (Berry et al., 2002). Huang et al. (2009) reported that the use of Ferucarbotran, (45-60nm SPIONs, with a magnetite core, and carboxydextran coating) is non-toxic to human MSCs, and promotes MSC proliferation. They proposed that the cell growth is promoted via the ability of Ferucarbotran to diminish intracellular H_2O_2 through intrinsic peroxidase-like activity. It is known that free iron plays an important role in cell cycle progression, cell proliferation and apoptosis. Ferucarbotran is metabolised to free iron by lysosomal degradation, and this is thought to accelerate cell cycle progression via protein regulators. HPL's effect of increasing human ADSC proliferation is well documented (Palombella et al., 2020, Guiotto et al., 2020, Escobar and Chaparro, 2016, Mohamed et al., 2020). Since nerve injury has a narrow and critical therapeutic window (McMorrow et al., 2024), methods that safely expedite delivery of cell therapies may be relevant to their potential for clinical translation.

5.5 Conclusion

The size and coating of nanoparticles are known to be crucial in determining their impact upon cells. The trends in the results of this *in vitro* study suggest that low dose (0.01mg/ml) green fluorescing (490nm emission), 200nm, magnetite core, poly-dimethylamin-co-epichlorhydrin-co-ethylendiamin matrix, amine functional group, nano-screenMAG-PEA SPIONs (Chemicell) did show some potential for possible future translation into clinical practice as a cell-tracking modality for human ADSCs. The ADSCs maintained their CD markers of differentiation potential, and immunohistological staining after labelling at low (0.01mg/ml and high dose (0.1mg/ml) SPIONs. ADSCs developed a spindle-like morphology with low dose 0.01mg/ml SPION labelling; further study is necessary to establish the significance. Trends suggest ADSCs had a reduced viability with increasing SPION-labelling concentration, but all cells maintained above 86% viability. The growth study suggests ADSC with HPL serum, and ADSC labelled with 0.01mg/ml concentration SPIONs, seemed to have greatest proliferation by day 5. Since nerve injury has a narrow and critical therapeutic window, means to safely expedite delivery of cell therapies may be clinically meaningful.

Chapter 6 Schwann cell extraction from Human Abdominal Adipose Tissue

6.1 Introduction

As established, Schwann cells are the conductors of nerve regeneration. Their mechanical guidance forms bands of Büngner, which act as the train tracks that axons follow in order to traverse the injury towards their injured counterpart on the other side. They secrete essential neurotrophic growth factors, chemokines, and extracellular matrix, key to coordinating the stages of the nerve healing journey (Resch et al., 2018). The current clinical gold standard of nerve repair treatment is to sew the ends of the nerve back together, under a microscope, using a stitch that's finer than a human hair. Or, if there is a defect in the nerve and the ends would only meet under harmful tension, then a donor nerve is sought (usually a sensory nerve such as the sural nerve) and transferred from another part of the body to bridge the gap, as an autologous graft. However, both of these solutions often lead to an incomplete recovery (Chen et al., 2017), which is unsurprising considering that they do not address the neurobiology of the injury. The graft treatment also leads to additional morbidity (harm) to the patient in terms of an additional surgical site, scar, pain, and functional impairment with loss of sensation after sural nerve harvest (Bamba et al., 2021).

In response to this, the scientific community have developed alternatives to nerve grafts. Bioengineered nerve conduits have been designed in efforts to reduce patient morbidity of peripheral nerve repair treatment, whilst simultaneously improving the outcomes. Nerve grafts of different materials (Boeckstyns et al., 2013, Thomson, 2018), with topography known to transiently increase expression of mTORC2 (mechanistic target of rapamycin complex 2), leading to promotion of peripheral nerve repair (Thomson et al., 2017). Conduits may also be impregnated with neurotrophic factors, or drugs (McMorrow et al., 2024, Zhu et al., 2022). Seeding the conduits with cells is another mode of improving the nerve regeneration process (Fairbairn et al., 2015, Zack-Williams et al., 2015). Schwann cells are seldom used as a therapeutic cell in nerve guidance conduits, due to the counterintuitive nature of sacrificing a nerve to obtain them, their slow and difficult *in vitro* culture and low yield from nerve tissue

(Palombella et al., 2020). Instead, adipose-derived stem cells (ADSC) have been widely investigated as an option that has low morbidity in the harvesting of the tissue of origin. A search of ClinicalTrials.gov for "adipose derived stem cells" indicates that over 394 clinical trials have been conducted worldwide, with roughly 132 of those already completed (2026). Other estimates from systematic reviews suggest that at least 3,000 patients have been treated with ADSCs or stromal vascular fractions across more than 10 different disease areas (Skrypnyk, 2025). Upon considering the potential of the donor Deep Inferior Epigastric Perforator (DIEP) abdominal flap tissue, it occurred to me that skin is incredibly well innervated and vascularised, and that there are vessels and nerves running through the underlying fat to reach the skin. There are accounts in the literature that confirm findings of nerves, and Schwann cells in adipose tissue (Stavely et al., 2022, Andersen et al., 2016, Chen et al., 2017). A hypothesis emerged that perhaps Schwann cells could be derived from this human abdominal adipose tissue.

On reviewing the literature, Massier et al. (2023) used single cell and transcriptomic maps of white adipose tissues, identifying over 60 subpopulations of cells (Massier et al., 2023). White adipose tissue, once thought of as bland and amorphous, is now recognised as being a complex organ, home to a wide array of important biological processes including energy homeostasis, glucose and lipid balance, blood pressure control and immune defence (Emont et al., 2022). Initially believed to be controlled hormonally, emerging evidence suggests that adipose tissue contains its own complex nervous system consisting of sympathetic and sensory innervation (Townsend, 2020). There are reports of Schwann cells being extracted from adipose tissue in rat and in human models, and of these cells being utilised in peripheral nerve repair models. Stavely et al. (2022) described isolation of mouse and human subcutaneous adipose tissue neural stem cells (dedifferentiated Schwann cells), derived from Schwann cells which populate nerve fibre bundles within the adipose tissue (Stavely et al., 2022). Chen et al. (2017) described fat tissue as a potential Schwann cell reservoir in their paper detailing the isolation and identification of Schwann cells from inguinal fat in a rat model. These cells were used in a sciatic nerve conduit repair model in mice, with similar outcomes to nerve-derived Schwann cells (Chen et al., 2017). Wang et al. (2017) described extraction of Schwann cells from human eyelid adipose tissue, disposed of during blepharoplasty. These Schwann cells incorporated into a sciatic nerve conduit repair model in mice showed equivalent results to the autograft group. These results suggest

human adipose can promote the regeneration of injured peripheral nerves and show promise for providing an abundant, easily accessible supply of Schwann cells for peripheral nerve repair bioengineered therapies. To my knowledge extraction of Schwann cells from human abdominal adipose tissue has not yet been described.

As introduced, the adipose tissue in this study is obtained from consenting donors undergoing DIEP breast reconstruction procedures as part of their breast cancer treatment or prophylactic care. Half of an abdominal flap equates to one breast reconstruction, and therefore in patients undergoing unilateral breast reconstruction procedure, the side with the dominant blood vessel (deep inferior epigastric perforator (DIEP)) is used and the other side is discarded. Therefore, the side of the flap with which we conduct this study is the side with the non-dominant perforator. Originally described by Schefflan and Dinner in 1983, in 1992 Hartrampf et al. introduced the distinction of the lower abdominal flap in terms of 4 “zones”, based on the anatomy of the dominant perforator, with zone I being closest to the dominant perforator and therefore having the richest blood supply, and zone IV being furthest from the dominant perforator (Hartrampf, 1992). The system saw refinements by Holm et al. (2006) which swapped the position of Hartrampf’s zones II and III to better reflect the lateral row perforators of the deep inferior epigastric artery, based on intraoperative testing of the blood flow and tissue perfusion using laser-induced fluorescence of indocyanine green, in a study of 15 patients undergoing DIEP flap breast reconstruction (Holm et al., 2006) (figure 49) knowledge and nomenclature help clinicians predict flap viability when planning the procedure.

Therefore, according to Holm’s perfusion zones, the abdominal adipose of the DIEP flap, on the side of the dominant perforator is known as zone I (closest to the perforator) and zone III (lateral to zone I). Whereas the abdominal adipose of the DIEP flap on the side of the non-dominant perforator is classified as zone II medially, and zone IV laterally. The half of the DIEP flap with the dominant perforator and vascular system (zone I and III) is used for the patient’s breast reconstruction, and in cases of unilateral reconstruction, the side of the non-dominant perforator (zones II and IV) is usually discarded in the clinical waste. In this study we retrieved the surplus adipose tissue from the half of the flap supplied by the non-dominant perforator, the equivalent of flap perfusion zones II and IV (which will be referred to as zone II or Zone IV henceforth).

This surplus abdominal adipose tissue was collected with ethical and patient consent. This study hypothesised that nerves would run with vessels, and therefore the more vascularised the area of abdominal adipose, the more Schwann cells would be extracted. I therefore took separate samples from zone II and zone IV for each donor to investigate if there were any discernible differences in Schwann cell yield which might reflect this anatomy.

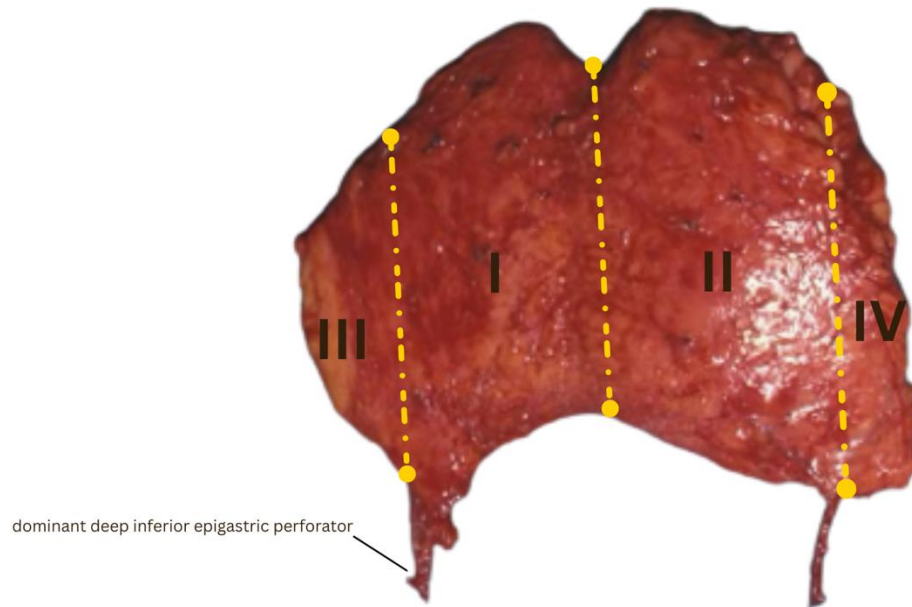


Figure 49 delineates the Holms' zones I-IV based on the blood supply and tissue perfusion from highest (I) to lowest (IV) as predicated by the anatomical position of the dominant deep inferior epigastric perforator (DIEP)(Holm et al., 2006). With thanks to the patient for their consent, and Ms Eva Wheeler-Mothoff for use of their clinical photographs.

6.2 Materials and methods

6.2.1 True cut anatomical analysis of adipose tissue

As a positive control, the following protocol was conducted upon one patient sample of ulnar nerve, in addition to the abdominal adipose tissue samples from four different patients. As per

the protocol for collection and processing of surplus abdominal adipose from consenting donors undergoing DIEP breast reconstruction surgery, the following was conducted in accordance with NHSGGC BioBank Biorepository ethical approval (number 314/344), and informed consent obtained from the patients, on standardised NHS consent forms, by the operating surgeon.

A 2x2x2cm sample of the adipose tissue was taken from the surplus DIEP flap and fixed in buffered 4% paraformaldehyde. Patient 1 adipose was a test of the stains for calibration. In Patients 2, 3 and 4 zone II and zone IV were specifically sampled for each patient to investigate if there was a gross difference in Schwann cell yield. The samples were safely transported to the University of Glasgow Veterinary School Histopathology Technician Mr Frazer Bell, who conducted the following protocol, with thanks.

The abdominal adipose tissue was placed into 10% neutral buffered formalin then processed through the following cycle: 70% ethanol, 60 minutes at 35°C, 100% Ethanol 60,60,60,90,120 minutes at 35°C, Xylene 60,60,90 minutes at 35°C, Wax 120,120,120 minutes at 62°C under vacuum. Embedding was conducted with Thermo Scientific Histostar. To prepare the sections for staining, a Microtome (HM340E semi-automated, EpreDia) was used to cut tissue sections at a thickness of 3µm. The sections for immunohistochemistry were then mounted on Slidemate Plus Adhesion Microscope Slides (EpreDia), sections for H&E staining were mounted on ClariTex Microscope Slides (CellPath), deparaffinised in several changes of Histo-Clear Histology Clearing Agent (National Diagnostics) and re-hydrated in ethanol solutions of decreasing concentration. The sections were then rinsed in water and stained as follows.

6.2.1.1 H&E staining

The sections were automatically stained using a Gemini AS automated stainer (EpreDia). Slides were dewaxed through three changes of HistoClear, two changes of 100% ethanol, one change of 70% ethanol, and then rinsed in running tap water. They were stained in Gill's haematoxylin for 5 minutes, rinsed in water, differentiated in 1% hydrochloric acid in 100% ethanol, rinsed in water, blued in Scott's tap water substitute, and rinsed in water. Then they were put in Putts eosin stain for 5 minutes, rinsed in water, dehydrated through one change of 70% ethanol, two

changes of 100% ethanol then cleared through three changes of HistoClear. Slides were automatically coverslipped using ClearVue coverslipper (Epredia).

6.2.1.2 Antigen retrieval

Antigen retrieval was performed using heat-induced epitope retrieval (HIER), sections were treated at full pressure with Access Retrieval Unit (Menarini) in sodium citrate buffer (pH 6) for NF200 (neurofilament protein 200kDa, present in mature axons) and negative control for 90 seconds at 124°C. The sections were then rinsed in Tris Tween buffer (pH 7.5). S100 didn't require any antigen retrieval.

6.2.1.3 Immunohistochemistry

The sections were treated for 5 minutes at room temperature with 3% hydrogen peroxide in phosphate buffered saline to quench endogenous peroxidase activity. After washing twice with TRIS Tween buffer (pH 7.5), sections were incubated for 30 minutes at room temperature with the primary antibody NF200 (Abcam, ab8135) at 1:100 and S100 (Dako, Z0311) 1:350, then washed with TRIS Tween buffer (pH 7.5). For primary antibody detection, the sections were incubated with EnVision+ System HRP Labelled Polymer Anti-Rabbit Secondary Antibody (Dako) at room temperature. They were washed with TRIS Tween buffer, followed by two 5-minute incubations with 3,3'-diaminobenzidine (DAB) substrate-chromogen (EnVision+ System, Dako). Sections were then rinsed twice with distilled water for 5 minutes. Tissues were counterstained using Gill's haematoxylin, slides were automatically coverslipped using ClearVue coverslipper (Epredia). Following staining, all slides were scanned on the Motic EasyScan 60 at x20 magnification.

I conducted image assessment (Dudek, 2008) in Microsoft PowerPoint, with second opinion and detailed annotated analysis by and with thanks to pathologist Dr Angelika Frances, University of Glasgow, School of Veterinary Medicine.

6.2.2 Extraction of Schwann cells from human abdominal adipose

6.2.2.1 Development of the extraction protocol

There is a wealth of literature pertaining to Schwann cell (SC) culture, a search in Pubmed search engine using words “Schwann cell, *in vitro*, human” yielded 1,960 results, whereas “adipose derived Schwann cells” yielded 2 results (21st April 2026)(Chen et al., 2017, Chen and Qi, 2025). Trends were noted in the ingredients of Schwann cell media reported in the literature, whether there were pre-degeneration, dissociation or purification steps, and the model species. Seven papers were chosen for detailed analysis, based on their differing approaches, and hypotheses were formed based on their relevance in terms of appropriateness for potential clinical translatability.

6.2.2.2 Schwann cell extraction from human abdominal adipose protocol

Petri dishes were coated in PLL (Sigma-Aldrich) 24 hours in advance and laminin (Sigma-Aldrich) coated on the day of the experiment (as previously described in Chapters 3-5). Adipose tissue was weighed for future calculation of cell yield and then dissected into 1–2 mm³ pieces, washed twice with PBS and then enzymatically digested for 120 minutes in 37°C water bath, utilising 0.2% (0.27 U/ml) Dispase II (Thermofischer) in HBSS supplemented with 1% penicillin/streptomycin. After centrifugation at 1000 g for 5 minutes, cell pellets were resuspended and cultured in two different Schwann cell mediums, to allow for comparison and optimisation of protocols.

The first was “standard Schwann cell medium”, composed of DMEM (Thermo-Fischer) medium supplemented with 10% FBS, 1% penicillin/streptomycin (Invitrogen), 10 ng/ml heregulin- β -1 (Invitrogen, UK) and 2 μ M forskolin (Sigma-Aldrich). Cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C. After 48 hours of culture the purification step was as follows: culture medium was replaced with 0.2% (0.27 U/ml) Dispase II (Thermofischer) in DMEM (Thermofischer) supplemented with 10% FBS, 1% penicillin/streptomycin for 20 minutes at 37°C incubation. The flasks were kept shaking horizontally for 1 minute to release lightly adhered Schwann cells, purifying them from the tightly adhered fibroblasts. The suspended cells

were then collected into a 50 ml conical tube (Corning) and centrifuged at 600 g for 3 minutes. The cell pellet was resuspended in medium and replated in T75 flasks. The second media was composed of DMEM with D-valine (Cell Culture Technologies) which was made up from powder, with sterilised MilliQ™ water and sodium bicarbonate to adjust to pH7.2-7.4. It was also supplemented with 10% FBS, 1% penicillin/streptomycin (Invitrogen), 10 ng/ml heregulin- β -1 (Invitrogen) and 2 μ M forskolin (Sigma-Aldrich).

6.3 Results

6.3.1 True cut anatomical analysis of adipose tissue

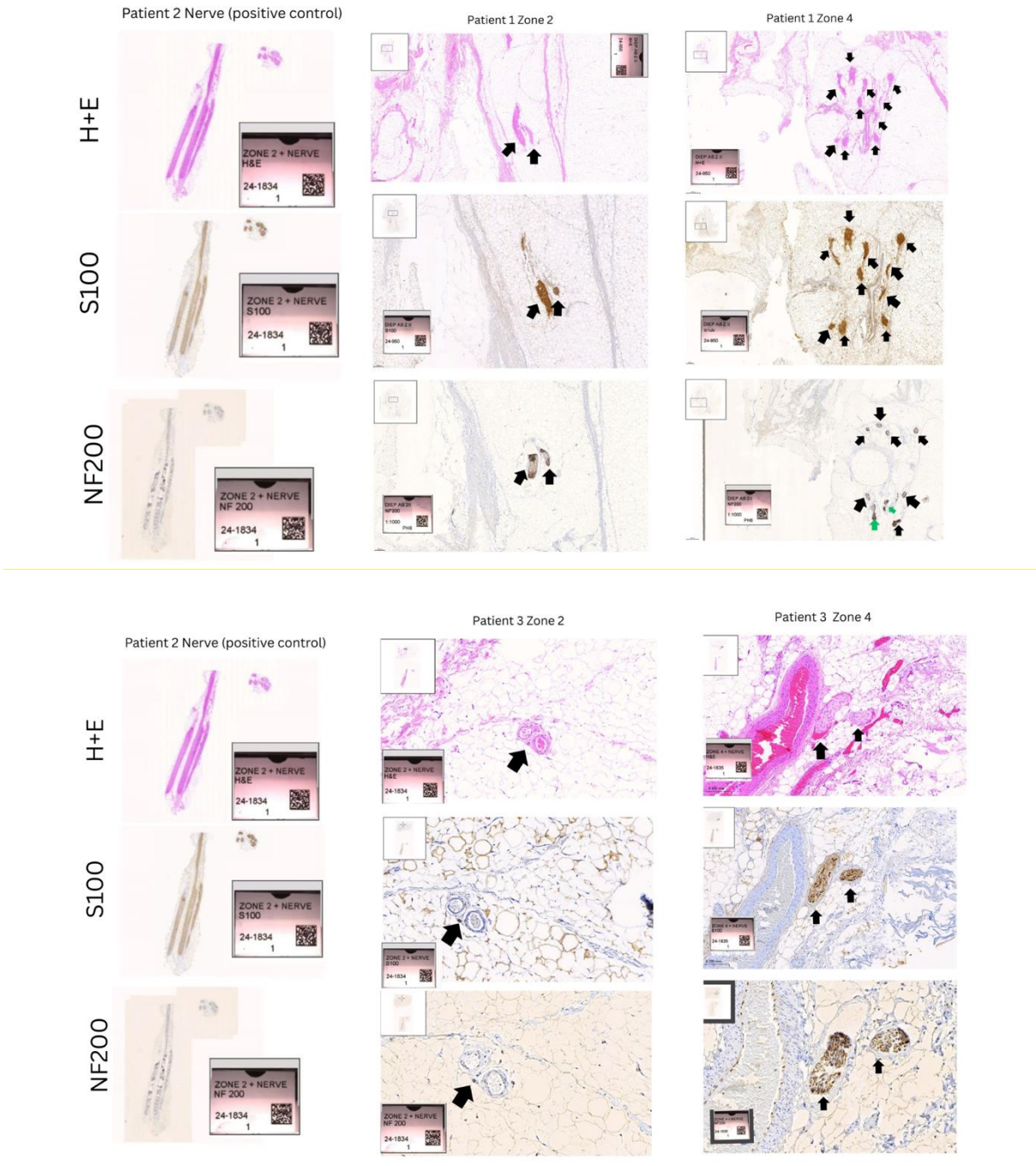
The abdominal adipose samples from 3 donors were delineated with H+E and tested for NF200 and s100. All of the fat samples contained mature nerve and Schwann cells with positive staining for NF200 and S100, respectively. An ulnar nerve sample (discarded nerve secondary to trauma) from an additional consented donor acted as a positive control for the staining (table 10, figure 50). There were nerves confirmed running through the adipose tissue (figure 50). The biggest nerves, with brightest staining for Schwann cells, were found in neurovascular bundles, running with the vasculature. There were also smaller nerves identified running independently through the adipose tissue. There was also positive staining for NF200 and s100 identified in the walls of the vasculature, perhaps highlighting the non-myelinating Schwann cells and nerves within the blood vessel walls (*nervi vasorum*). On comparison of zone II and zone IV adipose, there was no notable difference in the patterns of staining between the zones in the samples investigated here. The only sample in which there was no staining for s100 (there was positive staining for NF200 however) came from zone II, in Patient 4. A specific and meticulously designed study is necessary to investigate this further, and to explore whether there is a difference in the concentration of nerves and therefore Schwann cell yield between different zones of the abdomen.

Table 10 shows complementary analysis performed by and with thanks to pathologist Dr Angelika Frances, confirming the presence of Schwann cells staining for NF200 and S100, and their presence in nerves running independently through the adipose tissue, nerves running in neurovascular bundles. A sample of nerve was stained as a positive control. The DIEP flap, described according to Holm's perfusion zones II and IV (figure 49), did not have clear distinctions in terms of the abundance or size of nerves; a specifically designed study is necessary to investigate this hypothesis further.

Table 10 complementary analysis of Histochemistry of DIEP sections with pathologist Dr Angelika Frances

Tissue Sample	H&E	NF200	S100
Patient 1 Adipose machine calibration	yes	yes	yes (both machines)
Patient 1 Adipose (zone II)	yes	yes, looks as if staining endothelial cells in muscle sections	yes, lot of background (including muscles)
Patient 1 Adipose (zone IV)	yes	yes, looks as if staining endothelial cells in muscle sections	yes, lot of background (including muscles)
Patient 2 Nerve	yes	yes	yes
Patient 3 Adipose (zone II)	yes, tiny	yes, tiny staining endothelial cells	adipocytes, collagen
Patient 3 Adipose (zone IV)	yes, one near large vessels	yes, one near large vessels, endothelial cells	yes, one near large vessels, intravascular material
Patient 4 Adipose (zone II)	Many tiny	yes, endothelial cells and tiny aggregates with vessels	yes, corresponding to H&E and NF200

Patient 4 Adipose (zone IV)	yes, two tiny	yes, two tiny, endothelial cells	yes, a few more, but all tiny
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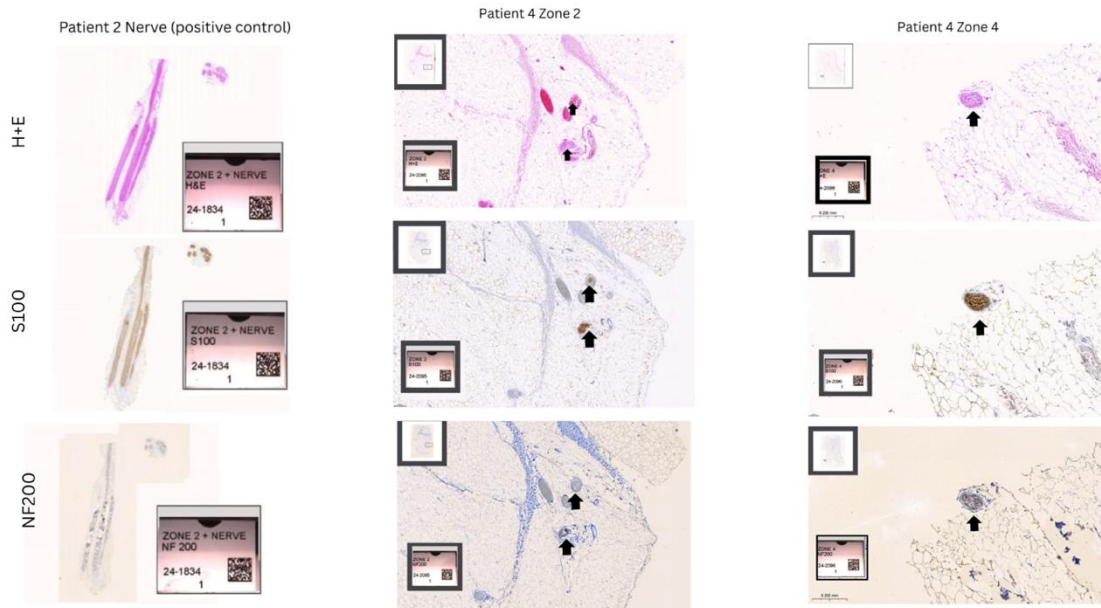


Figure 50 shows micrographs of adipose tissue sections with positive staining for Schwann cells S100 and NF200, with H+E staining as a control and with nerve as a positive control. Black arrows highlight positively stained nerves (NF200) and Schwann cells (s100), where green arrows highlight positive staining that was not replicated in the other stain, which may demonstrate the difference in the anatomy at the section of the adipose as is determined by this technique. The findings of S100 and NF200 positivity were reproducible in 3 biological patient adipose tissue repeats, with multiple samples per zone from each donor. Zones II and IV did not have clear distinctions in terms of the abundance or size of nerves, a specifically designed study is necessary to investigate this hypothesis further.

6.3.2 Development of the extraction protocol

It is well established that fibroblast overgrowth prohibits Schwann cell culture *in vitro* (Andersen et al., 2016, Chen et al., 2017, Kaewkhaw et al., 2012, Niapour et al., 2016, Resch et al., 2018). To combat this, Kaewkhaw et al. (2012) report purposeful substitution of L-valine with its enantiomer D-valine in the culture of rat Schwann cells. Fibroblasts cannot metabolise the D-enantiomer of the amino acid and therefore cannot survive in L-valine-free media (Kaewkhaw et al., 2012), favouring establishment of Schwann cells in culture. In contrast, the Schwann cells express the enzyme D-amino acid oxidase, which is essential for metabolising the D-valine amino acid isomer. I hypothesised that the D-valine DMEM would promote highest yield of Schwann cells. In addition, the exclusion of an enzymatic purification step was hypothesised to reduce the risk of damage to, or loss of Schwann cells, and it reduces the time in culture - both of which would be clinically relevant for translatability of the technique.

This D-valine DMEM was compared to “standard Schwann cell medium” which seemed to be the most reported in the literature; DMEM with 10% foetal bovine serum (FBS), 10 nM neuregulin and 2 μ M forskolin (Chen et al., 2017, Niapour et al., 2016, Wang et al., 2017) (table 11). Neuregulin peptide (heregulin- β -1 is a potent neuregulin isoform) mimics the action of axon-bound neuregulin, which is the natural mitogenic (encouraging cell replication) factor for Schwann cells (Aldrich, 2026c). Forskolin directly activates adenylyl cyclase and induces increased intracellular cAMP and is required to both synergistically enhance neuregulin-dependent Schwann cell proliferation (Aldrich, 2026a). It is reported in the literature that neuregulin growth factors require co-stimulation with a cyclic adenosine monophosphate-elevating agent, such as forskolin, to produce maximal cell proliferation.

Liu et al. (2015) and Niapour et al. (2016) reported the use of Dulbecco's Modified Eagle's Medium nutrient mixture F-12 (Gibco), which is a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 nutrient mixture (Liu et al., 2015a, Niapour et al., 2016). It combines DMEM's high glucose and amino acid levels with the rich, complex components of F-12, which adds 21 amino acids, 10 vitamins, glucose, iron, zinc, and often HEPES buffer to the medium. Dulbecco's Modified Eagle's Medium nutrient mixture F-12 (Gibco) is designed to

support the growth of a broad range of mammalian cells, including fibroblasts and epithelial cells (Thermofischer, 2026).

Other authors added specific ingredients to the “standard Schwann cell medium”, for instance Resch et al. (2018) included sodium pyruvate, which they purported served as an antioxidant ROS-scavenger(Resch et al., 2018). Like Kaewkhaw et al. (2012), they also included N2 supplement (which contains human transferrin, insulin, progesterone, putrescine and selenite (Invitrogen) (Kaewkhaw et al., 2012, Resch et al., 2018). According to the manufacturer, N2 supplement is specifically formulated to support the growth, viability, and differentiation of stem cells towards neural phenotypes. Insulin promotes cell survival, transferrin transports iron, progesterone modulates cellular function, putrescine promotes cell proliferation and selenite is an essential antioxidant(scientific, 2026). Kaewkhaw et al. (2012) and Liu et al. (2015) added bovine pituitary extract (BPE) to their Schwann cell medium. BPE is routinely used as a mitogenic supplement in serum-free growth medium. In addition to its mitogenic activity, BPE contains a variety of growth factors (such as fibroblast growth factors) and hormones with reported antioxidant activity, which makes it useful for optimising conditions for delicate cells (Kaewkhaw et al., 2012, Liu et al., 2015a). It is noted by the manufacturer (Sigma-Aldrich) that it is intended for research use only and not for human or veterinary diagnostic or therapeutic use (Kent and Bomser, 2003).

L-glutamine was included in the Schwann cell medium used by Kaewkhaw et al. (2012) and Niapour et al. (2016), among others (Kaewkhaw et al., 2012, Niapour et al., 2016). L-glutamine is the most abundant amino acid in the human body, and in all mammalian blood and tissues. A glutamine enantiomer, it serves as a primary energy source in rapidly dividing cells. It is a key substrate for the synthesis of proteins and nucleotides, supporting cell growth and division. L-glutamine is also crucial for maintaining redox balance within cells by contributing to the synthesis of glutathione, a major antioxidant (Aldrich, 2026b).

Various growth factors are included in Schwann cell medium by different authors, invariably a combination of bFGF (basic fibroblast growth factor) (Niapour et al., 2016, Resch et al., 2018) and EGF (epidermal growth factor) (Resch et al., 2018), which are common mitogens for almost

all kinds of cells, including Schwann cells. PDGF-AA (platelet-derived growth factor-AA) is an inducer of protein synthesis and used in the protocol of Resch et al. (2018)(Resch et al., 2018). B27 (neurobasal) supplement was added to Schwann cell medium reported by Niapour et al. (2016) (Niapour et al., 2016). B27 is a supplement designed by Brewer et al. (1993) to optimise serum-free medium for the growth of neural tissue. They reported systematically adjusting the concentration of 20 components of previously published serum-free media to optimise survival of rat hippocampal neurons. They also reported that high survival was achieved with osmolarity lower than found in Dulbecco's Modified Eagle's Medium (DMEM), by reducing cysteine and glutamine concentrations and by the elimination of toxic ferrous sulphate found in DMEM/F12 (Brewer et al., 1993).

Niapour et al. published a protocol (2016) that included a 10-day predegeneration step, and a 20-hour dissociation step, before introduction of a Schwann cell maintenance media. Their protocol described the process of extracting Schwann cells from rat peripheral nerve. The predegeneration step was designed to take advantage of Wallerian degeneration to obtain a higher number of Schwann cells in an “activated” state, and involves incubating rat sciatic nerves in DMEM, FBS, L-glutamine and pen/strep antibiotic, before a 20-hour enzymatic ‘dissociation’ stage using both collagenase NB4 and Dispase II (Niapour et al., 2016). In protocols that use an enzymatic purification step using the detachment method, collagenase NB4, Dispase II or a combination of both were used. These proteolytic enzymes have in common their ability to achieve “gentle” cell detachment from culture tissue plastic, whilst maintaining high cell viability (Wang et al., 2017, Chen et al., 2017).

All of the protocol examples discussed here (table 11) included penicillin/streptomycin antibiotics at a concentration of 1% sol. Kaewkhaw et al. (2012) also included Amphotericin B, a broad spectrum anti-fungal (Kaewkhaw et al., 2012).

To reduce variables, and due to financial restraints, I determined that the only difference between experimental groups for this proof-of-concept study would be the DMEM vs D-valine DMEM (with bicarbonate to balance the pH). “Standard Schwann cell ingredients” of 10% FBS, 10nM heregulin- β -1 and 2 μ M forskolin were included, alongside 1% penicillin/streptomycin antibiotic

in both groups. Pragmatically, Dispase II was used as the enzyme for both extraction digest and purification step in the standard Schwann cell medium group. The coverslips were PLL and laminin coated as it is documented in signalling studies that laminin is essential for Schwann cell proliferation, and for cytoskeletal arrangement in the spindle morphology, which is vital for cell signalling pathways, including those involved in myelination (Yu et al., 2009).

Table 11 details examples of different Schwann cell medium reported in the literature, and the rationale for their ingredients as per the authors, and the manufacturer's instructions. The citation is noted, followed by the medium recipe, rationale and the animal model, for comparison (Andersen et al., 2016, Chen et al., 2017, Kaewkhaw et al., 2012, Liu et al., 2015a, Niapour et al., 2016, Resch et al., 2018, Wang et al., 2017).

Model	Rationale	Medium	Citation
rat	-neuregulin peptide mimics the action of axon-bound neuregulin, which is the natural mitogenic factor for developing SCs. -Forskolin directly activates adenylyl cyclase and induces intracellular cAMP. It is required to both synergistically enhance neuregulin-dependent SC proliferation and reduce fibroblast contamination.	Schwann cell medium: -DMEM -10% foetal bovine serum (FBS) -10 nM neuregulin -2 μM forskolin	Andersen, N.D., Srinivas, S., Piñero, G. and Monje, P.V., 2016. A rapid and versatile method for the isolation, purification and cryogenic storage of Schwann cells from adult rodent nerves. Scientific Reports, 6(1), p.31781.
rat	-the non-essential amino acid D-valine, instead of L-valine, can selectively inhibit fibroblast proliferation as fibroblasts do not have the enzyme DAAO necessary to convert D-valine into L-valine. Therefore, fibroblasts die from amino acid depletion after a few days. -Bovine pituitary extract is rich in growth factors (such as fibroblast growth factors) and is often used to optimise culture conditions of delicate cells. -Amphotericin B is a broad spectrum anti-fungal	Schwann cell medium: -DMEM–D-valine (4.5 mg/ml glucose (PAA Laboratories) -2 mM Glutamine (Sigma-Aldrich) -10% FBS -1% N2 Supplement (100$\times$: 1 mM human transferrin; 0.0861 mM insulin, recombinant; 0.002 mM progesterone; 10.01 mM putrescine and 0.00301 mM selenite) (Invitrogen) -20 μg/ml bovine pituitary extract (Sigma-Aldrich) -5 μM Forskolin (Sigma-Aldrich) -100 U/ml penicillin/streptomycin - 0.25 μg/ml amphotericin B	Kaewkhaw, R., Scutt, A.M. and Haycock, J.W., 2012. Integrated culture and purification of rat Schwann cells from freshly isolated adult tissue. Nature protocols, 7(11), pp.1996-2004.

Model	Rationale	Medium	Citation
rat	-Peripheral nerve predegeneration takes advantage of Wallerian degeneration to obtain more and activated SCs in culture comparing to intact nerve culture -2.5% knock out serum replacement for optimal Schwann cell proliferation and hindering of fibroblast expansion -bFGF and EGF are common mitogens for almost all kinds of cells, including Schwann Cells. -Neuregulin is a Schwann cell specific mitogen.	Predegeneration medium for 10 days: -DMEM (Invitrogen) -10% FBS (Invitrogen) -2 mM L-glutamine (Invitrogen) -100 U/ml penicillin/streptomycin (Invitrogen) Dissociation medium 20h: -DMEM -15 % FBS -0.125 % collagenase NB4 (Invitrogen) -1.25 U/ml dispase (Invitrogen) Schwann cell expansion medium: -DMEM/F12 (Invitrogen) -2.5% knock out serum replacement (Invitrogen) -2microM forskolin (Calbiochem) -10nM nueregulin (Sigma) -20 ng/ml bFGF (Sigma) -20 ng/ml EGF (Sigma) - 2 % B27 (Invitrogen)	Niapour, N., Mohammadi-Ghalehbin, B., Golmohammadi, M.G., Gholami, M.R., Amani, M. and Niapour, A., 2016. An efficient system for selection and culture of Schwann cells from adult rat peripheral nerves. <i>Cytotechnology</i>, 68(4), pp.629-636.
mouse	-After 48h of cultivation, cells were purified with 0.2% Disasell, using the differing adhesions of fibroblasts (tightly adherent) and Schwann cells (lightly adherent) to suspend and re-plate the concentrated Schwann cells. -heregulin-β-1 is a potent neuregulin isoform	Schwann cell medium: -DMEM -10% FBS -10 ng/ml heregulin-β-1 (Pepro Tech) -2 μM forskolin (Sigma) Detachment method after 48hrs: (50 μl per segment) -Collagenase NB4 (Serva, Germany) -Disasell (Serva) into DMEM at a concentration of 0.2% (0.27 U/ml).	Chen, L., Jin, Y., Yang, X., Liu, Z., Wang, Y., Wang, G., Qi, Z. and Shen, Z., 2017. Fat tissue, a potential Schwann cell reservoir: isolation and identification of adipose-derived Schwann cells. <i>American journal of translational research</i>, 9(5), p.2579.

Model	Rationale	Medium	Citation
human	-The tightly adhered cells (fibroblast-like cells) and lightly adhered cells (Schwann cells) were isolated based on their characteristics of adhesion to the culture dish, using Collagenase NB4. -Conduct two rounds of purification to acquire higher purity of Schwann cells.	Schwann cell medium: -DMEM -10% FBS (Hyclone) -1% penicillin/streptomycin (Invitrogen) -10 ng/ml heregulin-β-1 (PeproTech) -2 μM forskolin (Sigma) Detachment method after 48 hrs: -DMEM -0.05% (0.07 U/ml) Collagenase NB4 -10% FBS -1% penicillin/streptomycin	Wang, G., Cao, L., Wang, Y., Hua, Y., Cai, Z., Chen, J., Chen, L., Jin, Y., Niu, L., Shen, H. and Lu, Y., 2017. Human eyelid adipose tissue-derived Schwann cells promote regeneration of a transected sciatic nerve. Scientific Reports, 7(1), p.43248.
human	-Sodium pyruvate is added in high glucose culture; pyruvate serves as an antioxidant scavenger, helping to mitigate the enhanced reactive oxygen species production generated under high-glucose conditions. -PDGF-AA (platelet-derived growth factor-AA) is an inducer of protein synthesis.	-2% FBS -1% Penicillin/Streptomycin -0.5% Na⁺ Pyruvate -2 μM Forskolin -10 ng/mL hFGF -10 ng/mL heregulin-β-1 -5 ng/mL PDGF-AA -0.5% N2 supplement	Resch, A., Wolf, S., Mann, A., Weiss, T., Stetco, A.L. and Radtke, C., 2018. Co-culturing human adipose derived stem cells and Schwann cells on spider silk—a new approach as prerequisite for enhanced nerve regeneration. International journal of molecular sciences, 20(1), p.71.

Model	Rationale	Medium	Citation
rat		Schwann cell maintenance Medium: -Dulbecco's Modified Eagle's Medium nutrient mixture F-12 (DMEM/F12, Gibco) -10% FBS (Gibco) -antibiotics (100 UI/mL penicillin and 100 µg/mL streptomycin solution) -20 µg/mL bovine pituitary extract (Biomedical Technologies) -2 µM/mL forskolin (Sigma-Aldrich)	Liu, Z., Huang, L., Liu, L., Luo, B., Liang, M., Sun, Z., Zhu, S., Quan, X., Yang, Y., Ma, T. and Huang, J., 2015. Activation of Schwann cells in vitro by magnetic nanocomposites via applied magnetic field. International journal of nanomedicine, 6pp.43, .

6.3.3 Extraction protocol results

Qualitatively it appears that the hypothesis, that the D-valine medium would prevent fibroblast overgrowth and allow Schwann cells growth, may be correct. These cells appeared fusiform, with a phase-refractile bipolar or tripolar morphology and a small cytoplasm to nucleus ratio, in keeping with Schwann cell morphology (figure 51A). Upon Dispase II purification step of the Schwann cell standard media group at 48 hours, the lifted, lightly adhered cells, appeared to have flat and spread morphology akin to stem cells such as ADSC, at day 5 (figure 51C). The tightly adhered cells are phenotypically in-keeping with copious fibroblasts, as expected, with flat, poorly refractive polygonal morphology (figure 51B). This is in-keeping with the observations of these cell types in the literature(Wang et al., 2017). These findings could not be quantified before the occurrence of bacterial contamination which necessitated experiment disposal. Further repeats, with multi-method phenotyping is required to draw conclusions.

Figure 51

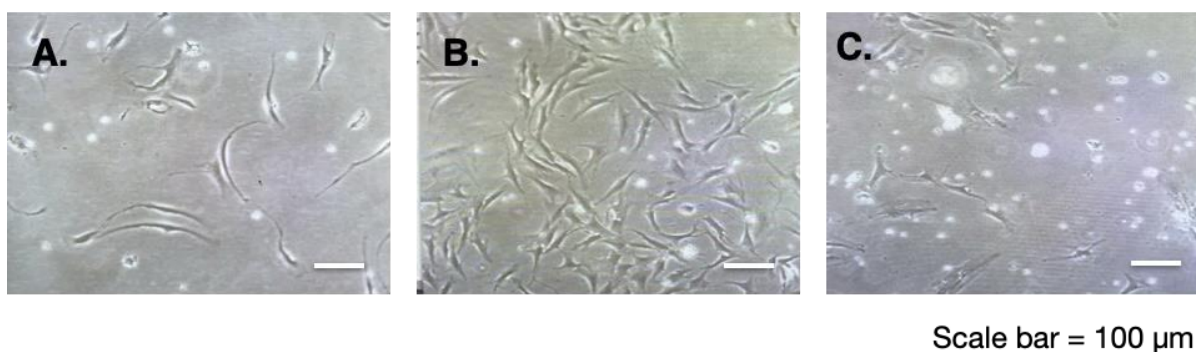


Figure 51 Greyscale photographs of different conditions following Schwann cell extraction protocol from abdominal adipose tissue, at day 5 in culture. A=D-valine Schwann cell medium, B=residual adhered cells in standard Schwann cell medium, following the purification step with Dispase II, and C=purified cell group in standard Schwann cell medium. This experiment was repeated once. The bright white dots are indicative of bacterial infection (which appeared to be cocci). Scale bar=100 μm . A. seem to be spindleform cells, suggestive of the possibility that these are Schwann cells. B. are in-keeping with fibroblasts. C. appear more spread out, akin to

stem cell or ADSC morphology. Further repeats and cell identification is required to draw conclusions.

6.4 Discussion

The presence of nerves in the adipose tissue was confirmed; the largest nerves were found in neuromuscular bundles, small independent nerves were seen in the adipose, and Schwann cells were also identified in the walls of the vasculature

As per my hypothesis, there were nerves running with blood vessels traversing through the fat samples in neurovascular bundles, which are commonly surrounded by collagen. The best yield of Schwann cells was found adjacent to largest vessels in the adipose. On comparison of zone II and zone IV, with the hypothesis that the more vascular zone II might contain a higher abundance of Schwann cells, there was no notable difference in the patterns of staining between the zones in the samples investigated here. In fact, zone II in Patient 4 was the only sample in which there was no staining for s100 (Schwann cells) but there was positive staining for NF200 (mature axons) however. A specific and meticulously designed study is necessary to investigate this hypothesis further. There were also nerves identified running independently through the adipose tissue. Interestingly, there was positive staining identified in the walls of the vasculature, perhaps highlighting the non-myelinating Schwann cells of the nerves within the blood vessel walls (*nervi vasorum*). Vital et al. reported the finding of unmyelinated C fibres in the media and adventitia layers of the walls of varicose veins (Vital et al., 2010). Herbst et al. reported a strong S100 immunostaining of nerve fibres along the wall of the vasa vasorum and in the media of the saphenous vein in humans (Herbst et al., 1992). These findings suggest the vasculature may pose a source of Schwann cells, and perhaps also supports the hypothesis that well-vascularised tissue will house a higher number of Schwann cells upon digestion of the adipose.

There are already reports in the literature of using FBS alternatives for Schwann cell culture

Niapour et al. (2016) also reported using KnockOut™ serum instead of FBS for their Schwann cell maintenance medium. KnockOut™ serum is a specialised formulation which is designed to

support the growth and pluripotency of stem cells while minimising unwanted differentiation. They do not explain their rationale for using it here for Schwann cell culture derived from rat peripheral nerve, but it may have been used to reduce the number of fibroblasts, as KnockOut™ serum replacement formulations don't provide as many adhesion factors as FBS, which can reduce the attachment or slow proliferation in some types of fibroblast. It may also have been to reduce the variability in batches seen in FBS, to reduce the risk of zoonosis, or due to its high levels of proteins, amino acids, growth factors, trace elements and vitamin antioxidants(Thermofischer). Once confirmation of Schwann cell extraction from human abdominal adipose is obtained, a next step would include trialling HPL as the serum supplement. As discussed, many authors added growth factors to the “standard Schwann cell medium”, including bFGF, EGF and PDGF. It is known that HPL is rich in all of these growth factors, and more besides, including TGF- β (transforming growth factor-beta), VEGF (vascular endothelial growth factor), and IGF-1 (insulin like growth factor-1) (Palombella et al., 2020, Guiotto et al., 2020). To our knowledge the use of HPL in Schwann cell culture has not yet been reported.

Methods to reduce the risk of contamination

In our protocol, the adipose tissue was washed twice in sterile PBS before the digest, this was in accordance with the procedure from Wang et al. (2017) in their report of extracting Schwann cells from human eyelid adipose (Wang et al., 2017). Chen et al. (2017) in their report of obtaining adipose-derived Schwann cells from rat inguinal adipose did not include a wash step at all (Chen et al., 2017). However, multiple different approaches were reported in the literature to reduce the risk of the contamination in Schwann cell culture. In their model of extracting Schwann cells from human nerves surplus to free flap surgery, Resch et al. (2018) first washed the tissue specimens in PBS 1% antibiotic/antimycotic(Resch et al., 2018). Kaewkhaw et al. (2012) reported including amphotericin B, a broad-spectrum antifungal, in their Schwann cell medium (Kaewkhaw et al., 2012). In light of the issues with contamination experienced in this study, additional wash steps and additional antimycotic prophylaxis would be prudent.

6.5 Conclusion

To conclude, Schwann cells are essential for peripheral nerve regeneration. Currently the “sacrificing nerve to treat nerve” paradox of obtaining Schwann cells from nerve samples, the long time in culture, and the low cell yield, act as barriers to their use in bioengineered solutions. It has been reported in rat and human studies that Schwann cells can be extracted from adipose tissue (Chen et al., 2017, Wang et al., 2017). To our knowledge this has not yet been demonstrated in human abdominal adipose tissue, which may act as an abundant autologous reservoir of therapeutic cells, with low patient morbidity in their extraction and therefore, have a promising potential for clinical translation. The addition of growth factors and growth factor rich serum is known to increase Schwann cell proliferation and may therefore reduce the timescale from injury to incubation, to therapy provision in a clinical context. This proof-of-concept study confirms the presence of NF200 and s100 positive staining in nerves present in the adipose: in neurovascular bundles, in independent smaller nerves, and in the walls of the vasculature respectively. To inform the site of Schwann cell harvest in a clinical setting, it was hypothesised that the abdominal adipose tissue from the anatomical location which corresponds to Holm’s perfusion zone II (the medial aspect of the half of the DIEP flap which is supplied by the non-dominant perforator), would yield more Schwann cells than that of zone IV (the lateral aspect half of the half of the DIEP flap which is supplied by the non-dominant perforator), due to its greater vascularisation. This could not be determined based on the small sample size of this study. Further meticulously designed study is required to investigate this hypothesis further. Observationally, following adipose digestion, Schwann cell maintenance media which uses D-valine DMEM appears to eliminate fibroblast overgrowth and allow for the culture of Schwann cells. Further experiments, with multimodal cell identification methods, are required to quantify and confirm this finding.

7 Conclusions and future directions

7.1 Conclusions

In summary, this study sought to contribute clinical and laboratory insights into therapies for peripheral nerve injury (PNI), focusing on areas that have potential for meaningful clinical translation. This study aims to fill gaps in the knowledge about the patient reported outcomes for newly introduced therapies, by reporting findings in patients with chronic neuropathic pain (CNP) treated with Qutenza® and Botox®, in our plastic surgery and burns unit. This study also contributes the first report of permanent bilateral facial nerve palsy from electrical injury and discusses the treatment of the sequelae of PNI. The surgeon's perspective and uptake of nerve guidance conduits for the treatment of PNI was lacking from the literature; this study hopes to garner clinical insights and user experience to inform work in the laboratory. The laboratory work in this study aims to contribute experimental evidence in cell therapy techniques which might have potential for translation into clinical settings.

The majority of patients in our study reported that a single treatment with Qutenza® 8% capsaicin patch or Botox® (botulinum toxin A) therapy did improve their chronic and treatment resistant neuropathic pain symptoms. They reported improvements in allodynia, a reduction in their load of analgesics, and improved quality of life. Patients treated with Botox® also reported improvement in their deep neuropathic pain, which was seen less in the Qutenza® patients. The improved allodynia was useful in allowing patients to tolerate clinical examination, facilitating their surgeon in identifying and treating deeper anatomical pathology, such as neuroma. Not only do these local treatments have lower side effect profiles than standard regimens with systemic analgesics, emerging evidence suggests that these novel therapies may also treat underlying CNP pathophysiology via promoting neuroregeneration. Armstrong et al. (2025) report high dose capsaicin's effects on overstimulating and downregulating the TRPV1 receptor, with the net effect of localised denervation of TRPV1-expressing afferent terminals in the epidermis and dermis, and subsequent neuroregeneration. Whilst Marinelli et al. (2010) suggest that botulinum toxin A induces promotion of regenerative processes by Schwann cells. Average duration of chronic pain before these patients had trials of Qutenza® and Botox®, was 8.3 and 11.8 years

respectively. Hopefully as evidence increases for these therapies, the time from diagnosis to treatment reduces for patients.

I hypothesised that pain relief with these therapies would be transient, however for Qutenza® the patients who reduced their analgesics did not increase them again within our follow-up window (minimum 6 months), and 4/11 patients were discharged from clinic. One patient was rereferred for another patch. Most of the patients receiving Botox® did so as an adjunct, alongside surgical intervention, however benefit was also reported from the 3 patients who received it as monotherapy. Longitudinal study is required to further understand the risks and benefits, and larger scale randomised control trials would increase the quality of evidence available to inform clinical teams and their patients in their decision making.

In presenting the first report of a chronic bilateral facial nerve palsy and severe oral incompetence secondary to high voltage electrical blast injury, we demonstrated the importance of prompt identification, treatment planning, intervention and consistent follow up for patient's suffering from PNI. We report that static reconstructive techniques such as the fascia lata sling, are an option for patients who present late with muscle wasting, and that this treatment provided a satisfactory, quick, and cost-effective option to manage the severe complications of facial palsy. The procedure restored oral competence for the patient, successfully treating sialorrhoea and improving his oral intake, verbal communication and cosmesis. Further study of patient perceived outcomes, which utilise validated patient reported outcome measures, is vital to ensure that patient and clinician opinion of "successful results" align, and that scientific advancements remain patient centered. Efforts to make technological advancements affordable is essential to prevent healthcare inequalities, and to establish people-centered justice in healthcare provision, globally.

Upon distributing a survey to surgeons globally, seeking their experience and opinion of the use of nerve guidance conduits in the treatment of peripheral nerve surgery, the findings were interesting. The majority of survey respondents in this study were hand-specialist plastic surgeons based in Europe, and they did not use nerve guidance conduits. In keeping with my hypothesis, the main reasons given were a lack of evidence in the literature, financial burden and inconvenience. I expected the lack of high-level evidence available to be the predominant reason

given, however financial burden was cited most often by the respondents. It was noted that most of the surgeons work in centres that are predominantly funded by tax or national health insurance. For the small number of surgeons in this study who did use nerve guidance conduits for peripheral nerve repair, all of them reported personal experience of ‘a positive impact upon patient outcomes’ and ‘no difference’, or ‘improvement’ in complication rates when compared with autologous nerve graft treatment for PNI. Their rationale for the use of nerve guidance conduits included ‘good safety’ and ‘reduction in neuroma/pain’. Further study of surgeon perspectives and practice is required to tailor scientific advancements towards practicably translatable solutions. Study design to increase network of survey distribution by collaborating with global authors who then report their local findings may improve survey uptake numbers and completion rates.

Meticulously designed multicentre, comparable randomised control trials are required to allow meta-analysis comparing different conduit options versus the gold standard for PNI. The lab work limitations of this study included the number of patient tissue repeats, that often fell short of allowing statistical analysis to be performed, despite best efforts. However, these *in vitro* preliminary results do show a number of interesting trends. Protocols were optimised for human tissue and are now published here for future use and development by the scientific community.

The adipose derived stem cell (ADSC) extraction protocol was optimised for human abdominal adipose tissue. Nerves were positively identified in human abdominal adipose tissue, and Schwann cells did seem to be extracted from this source using d-valine DMEM medium to reduce fibroblast contamination. To our knowledge extraction of Schwann cells from human abdominal adipose tissue has not yet been reported. The findings of this study suggest that abdominal adipose may act as an abundant autologous reservoir of cells suitable for clinical applications, with low associated patient morbidity in their extraction, and therefore abdominal adipose derived Schwann cells may have a promising potential for clinical translation.

Following the ADSC differentiation protocol down the Schwann cell lineage (dADSC), there were trends towards Schwann-like phenotype, evidenced by immunohistochemistry positive staining for s100. Interestingly, morphological changes to a spindle form morphology were

quantified in dADSC, ADSC with 0.01mg/ml superparamagnetic iron oxide nanoparticle (SPION) labelling and the human platelet lysate (HPL) ADSC. It is known that Schwann cells become more spindle form when they adopt a repair phenotype during natural nerve healing, and that this cytoskeletal change from G-actin to F-actin triggers a number of cell signalling pathways which maintain the repair behaviours of the cell, including proliferation, migration and secretion of neurotrophic factors (Yu et al., 2009, Jessen and Mirsky, 2016, Wang et al., 2018). The dADSC, ADSC with 0.01mg/ml labelling and the HPL ADSC also showed greater proliferation than the Foetal Bovine Serum (FBS) ADSC group. Further studies at genomic and molecular level are required to elucidate the downstream effects, if this morphological transfiguration equates to functional changes, and whether these changes are useful (or not) for peripheral nerve repair.

I concluded that dADSC do not have potential for clinical translation, unless key components of the differentiation process can be replaced with non-toxic alternatives. The work of Du et al. (2023) expedited the differentiation process of ADSC towards Schwann cell phenotype from 21 days (Kingham et al., 2007) to a 2-day protocol, which was a step forward from the bench towards the bedside. However, this study and others have found the Kingham protocol to be unreliable in human tissue (Kearns, 2021). In contrast, HPL does show potential for clinical translation in the field of nerve regeneration. Pooled donor HPL as a Bloodbank product may be most clinically relevant to reduce inter batch variability, for greater concentration of growth factors and convenience (Guiotto et al., 2020). HPL also appears to provide microbiological resistance to infection, which is highly clinically relevant. Trends suggest ADSCs had a reduced viability with increasing SPION-labelling concentration, but all cells maintained above 86% viability. The growth study results suggested HPL 0.01mg/ml SPION ADSC seemed to have greatest proliferation by day 5. Since nerve injury has a narrow and critical therapeutic window, means to safely expedite delivery of cell therapies may be clinically meaningful.

The size and coating of nanoparticles are known to be crucial in determining their impact upon cells (Berry et al., 2002, Berry, 2003, Berry et al., 2004, Lewis et al., 2015). This study highlights the importance of SPION concentration, with higher dose correlating with lower proliferation and viability rates, and a smaller, rounder morphology. The trends in these results

suggest dose 0.01mg/ml concentration green fluorescing (490nm emission), 200nm, magnetite core, poly-dimethylamin-co-epichlorhydrin-co-ethylendiamin matrix, amine functional group, nano-screenMAG-PEA SPIONs (Chemicell) did show some potential for possible future translation into clinical practice as a cell-tracking modality for human ADSCs. The ADSCs maintained their CD markers of differentiation potential, and immunohistological staining after labelling. Further study is required.

To conclude, this study contributes new low-level evidence in the field of peripheral nerve injury and repair. It highlights the importance of communication and collaboration between patients, scientist and clinicians to ensure that time, resources and energy are focussed upon strategies for peripheral nerve regeneration that may result in clinically feasible solutions. Currently the literature is lacking in patient reported outcome data, and is dominated by small studies, with non-standardised materials, methods and outcome measures, which utilise toxic and animal derived materials, and in animal models which don't translate into humans. These factors inhibit comprehensive metanalysis and production of the high-level evidence that is necessary for the progression of good ideas into clinical trials and therapeutic interventions for patients.

Most studies of peripheral nerve injury, repair and nerve guidance conduits are in rat models, which do not translate into humans for multiple reasons. A nerve guidance conduit is required clinically to treat the critical nerve gap (>30mm), so testing them on 10-15mm gaps in rats is suboptimal (Kaplan et al., 2015). The sciatic nerve is the commonest animal models for PNI due to its relatively large size, surgical accessibility, and mixed sensorimotor function, which resemble human peripheral nerves. However, rats have species-specific neuroregeneration which differs from human nerve healing. In addition, rat models often use mixed (motor and sensory) grafts when sensory autograft (often sural nerve) is standard for treatment in patients (Kaplan et al., 2015). Considerations for the scientific acumen and animal welfare of animal models include the species and the subspecies of the animal. Rats have high established rates of self-mutilation (autotomy) secondary to PNI modelling, with reported rates of as high as 91% of autotomy, necessitating cull of the experimental animals (Carr et al., 1992, Weber et al., 1993). Rates of autotomy in female Sprague Dawley rats were found to be significantly lower than in male rats and a rate of 0% has been reported in the study of Lewis rats (Zhou et al., 2026), therefore Lewis

rats may be subspecies of choice where rat PNI models are necessary, appropriate and clinically relevant. Legislation as per the European Union Directive, Recital 13, states that study design should “require the use of species with the lowest capacity to experience pain, suffering, distress or lasting harm, that are optimal for extrapolation into the target species”. Recital 17 states “the use of non-human primates should be permitted only in those biomedical areas essential for the benefit of human beings, for which no alternative replacement methods are yet available” (The European Parliament, 2010). It can be posited that hundreds of rat models which cannot meaningfully advance science into patient treatment causes more harm to sentient beings than a small number of high quality studies in non-human primate animals, and that just because a species looks less like us, and shares less of our DNA, that does not mean that they experience less suffering (Panksepp and Lahvis, 2011).

It can be acknowledged that the cost and workload of keeping large numbers of animals is more practicable in a small animal species, rat anatomy is outlined, and protocols are established. However, it feels like continuing to use a square wheel, when reinventing one that’s round may prove more efficacious, and a more ethical use of finite planetary resources, and animal life. Animal models do not represent the clinical condition of PNI in many ways; results from young and healthy, genetically homogenous rats that have strong regenerative potential and rapid healing rates provide a very different regenerative paradigm to that of diverse human patients with PNI (Tos et al., 2009, Kaplan et al., 2015). Although there is no perfect model, the quality of animal studies and the translatability of their results may be improved through delineating how animal models correspond to human disease, and with the use of study design that excludes irrelevant animals, that balance for gender, split litter mates among experimental groups, and track genes.

This study is in keeping with the past 50 years of work from the National Centre for the replacement, refinement and reduction (“the NC3Rs”) of animals in research. They advise that animal studies should be replaced by human tissues and cells, established cell lines mathematical and computer models, or human volunteers whenever possible. There is a growing body evidence that animal free *in vitro* culture methods are successful in the field of PNI (Escobar and Chaparro, 2016, Lensch et al., 2018, Tos et al., 2009). Where animal studies are deemed

necessary, the NC3Rs advise studies should be designed and analysed to ensure robust and reproducible results.

This study highlights the vital importance of inter and intra-organisational communication and cross pollination between the scientific, surgical and patient communities. This was the pleasure and privilege of my experience since starting my laboratory training in the field of peripheral nerve injury and repair, and clinical work in plastic and burns surgery as a senior clinical research fellow in 2017, and as Reconstruction Surgery Trials Network (RSTN) representative for Scotland (2020-2021). RSTN is UK wide research collaborative that aims to develop multicentre studies in hand and plastic surgery. It was established with funding from the British Society for Surgery of the Hand (BSSH) and the British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) and is part of the Royal College of Surgeons Clinical Trials Initiative. In the UK the NHS can quickly and efficiently generate very large datasets compared to other centres and countries and therefore provides opportunity for high quality research output.

Moving forward as a community, if research teams could set aside rivalry culture and meet in open collaboration, perhaps workforces and budgets could be pooled to facilitate meaningful largescale standardised work, in more applicable animal models, in more appropriate animals. Sharing and standardising protocols would facilitate the publication of the high-level evidence that is required for clinical studies to occur. Collaborative trial networks illustrate intentional design to provide high quality, multicentre evidence. Interventions such as these may facilitate safe translation of the innovation, passion and dedication seen in our labs, into benefits for our patients in future.

7.2 Future directions

As any researcher knows, each answer begets at least 5 new questions. Initial future work arising from this study is to complete an adequate number of biological and technical repeats to allow for statistical analysis to be made, and further meticulous investigation of variables to optimise the Schwann cell extraction protocol. Following these adjacent steps, some other questions which arose during this study are as follows.

7.2.1 Are HPL ADSC and SPION labelled ADSC impacted by gender of the adipose donor?

It is common knowledge that there is a sex bias which is still present in the majority of research disciplines, where male subjects (cells, animals, patients) predominate pre-clinical studies and drug trials, leaving gaps in knowledge about female biology, physiology, and health (Karastergiou et al., 2012). In fact, the anatomy and innervation of the clitoris was only accurately described in the literature for the first time in 2005 (by consultant urologist Ms Helen O'Connell) (O'CONNELL et al., 2005), 36 years after the first man walked on the moon, and 20 years after the advent of the mobile phone. Sex differences in mechanisms underlying basic biological processes have now been elucidated- including in pain signalling and drug metabolism. Legislative changes were made in the US grant agencies, and the European research programme (Horizon 2020(Innovation, 2020)) to address these issues, as gender bias has detrimental consequences for quality of science and the health of the population.

Of note, all adipose donors in this study were female patients attending for DIEP breast reconstruction, as part of their prophylactic or therapeutic breast cancer care and therefore it is important to observe these results as only half of the picture. *In vitro* studies of differences in the adipose tissue of males and females suggest that subcutaneous adipocytes and the adipose tissue from women show higher lipoprotein lipase activity, (an enzyme essential for fat metabolism), lipid synthesis, and insulin-stimulated glucose uptake compared to males. The central obesity more typical in males is known to have deleterious metabolic and cardiovascular consequences

(Karastergiou et al., 2012). Bianconi et al. (2020) studied sex specific transcriptome differences in human ADSC and report differences in chromosomal segments and gene expression of ADSC between the sexes. These differences related to processes including inflammation, adipogenic and neurogenic differentiation, and cell communication (Bianconi et al., 2020). PNI is commoner in males (Kim et al., 2020), making further study to clarify the trends identified in this study with gender parity particularly pertinent.

7.2.2 What are the genomic, metabolic and molecular effects of the adoption of spindle morphology seen in dADSC, HPL ADSC and 0.01mg/ml SPION ADSC?

In adopting their repair phenotype, Schwann cells adopt an elongated spindle morphology with bipolar, or tripolar elongations. Studies report that this cytoskeletal change impacts cell signalling pathways relating to Schwann cell proliferation, and migration, and triggers a cascade of cell signalling pathways which are vital for maintenance of the Schwann cell repair phenotype behaviours (Wang et al., 2018, Yu et al., 2009). This includes the alignment of their bodies along the basal lamina to guide axon growth in “bands of Bügner” and the up-regulation of neurotrophic factors which stimulate axonal regeneration and neuronal survival. Changes were also seen in the morphology of the nuclei of dADSC, HPL ADSC and SPION labelled ADSC. Next steps from this study would be to ascertain at a genomic, metabolic and molecular level what (if any) effects these morphological configuration changes have upon cell signalling and function in dADSC, HPL ADSC and 0.01mg/ml SPION ADSC.

7.2.3 What is the dilution of the SPIONs as they proliferate and does this longevity impact upon their clinical relevance as a tracking modality?

When a cell labelled with SPIONs proliferates, the SPIONs may not be divided evenly between the daughter cells. After several cell cycles the labelling can be diluted below detectable levels. Proliferation rates are high in the cells being investigated for application in bioengineered solutions for peripheral nerve repair, and this is usually viewed favourably in light of the narrow therapeutic window in PNI. SPIONs may also be removed by the cells by exocytosis, and smaller particles are exocytosed at a higher rate (Li et al., 2013). The coating on SPIONs may

also influence exocytosis, Xu et al. (2012) report that a change in the SPION coating (with polylactic-co-glycolic acid) increased the longevity of labelling threefold (Xu et al., 2012). High iron loading is also reported to be of importance for cellular MRI due to reduced iron content over time (Li et al., 2013). The addition of a timelapse study would be useful to better understand the dilution pattern and longevity of these SPIONs in these cells as they proliferate.

7.2.4 Is there higher yield of Schwann cells from abdominal adipose with the greatest vascular supply?

Identifying the area of abdominal adipose which might yield the highest number of Schwann cells would be highly clinically relevant, in terms of the translatability of their use for the treatment of peripheral nerve injury. Holm et al. (2006) report the vascularity of the lower abdominal adipose (and skin) flap in four zones, with zone 1 being best perfused and 4 being the least perfused (Holm et al., 2006) (Figure 49). This study confirms that the source of the largest nerves in the human abdominal adipose lie within neurovascular bundles, and that there are also Schwann cells in the walls of the blood vessels themselves, this is in-keeping with the literature pertaining to other anatomical locations (Herbst et al., 1992, Dudek, 2008). As such, it seems likely that the most vascular areas of the abdominal adipose tissue would yield the highest number of abdominal adipose derived Schwann cells. This could be established by systematic true cut study of slices of an entire abdominal flap, although this would be painstaking and expensive work. In addition, abdominal adipose could be digested, with cell yield from each equivalent Holm's perfusion zone quantified, as cell count/gram of adipose tissue. In addition, Chen et al. (2019) report MRI techniques which can visualise nerve fascicles in high definition (Chen et al., 2019), so perhaps MRI mapping may delineate the anatomical innervation of human abdominal adipose.

7.2.5 Would Schwann cells cultured in HPL be of repair phenotype?

The neurotrophic factors secreted by repair Schwann cells play a pivotal role in guiding and coordinating the process of nerve regeneration, and also in the maintenance of Schwann cells in their repair phenotype; their proliferation, migration, release of extracellular matrix molecules

and their survival. These include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), artemin, vascular endothelial growth factor (VEGF), erythropoietin, pleiotrophin, p75NTR and N-cadherin (Jessen and Mirsky, 2016). Whilst HPL has higher growth factor content than any other supplement, and in particular relevance to its use in the context of nerve regeneration it contains brain-derived growth factor (BDNF), neurotrophin 3 (NTF3), nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF) (Lensch et al., 2018). If this were the case, in addition to HPLs useful antimicrobial properties, this would be very relevant for clinical translation of cell therapy for peripheral nerve repair.

References

- ÅBERG, M., LJUNGBERG, C., EDIN, E., MILLQVIST, H., NORDH, E., THEORIN, A., TERENGHI, G. & WIBERG, M. 2009. Clinical evaluation of a resorbable wrap-around implant as an alternative to nerve repair: a prospective, assessor-blinded, randomised clinical study of sensory, motor and functional recovery after peripheral nerve repair. *Journal of plastic, reconstructive & aesthetic surgery*, 62, 1503-1509.
- ACLAND, R. 4.8.10 *Facial and vestibulo-cochlear nerves (cranial nerves VII, VIII)* [Online]. Wolters Kluwer. Available: <https://aclandanatomy.com/multimediaplayer.aspx?multimediaId=10528513> [Accessed 1st March 2026].
- ACLAND, R. D. 1977. Instrumentation for microsurgery. *The Orthopedic clinics of North America*, 8 2, 281-94.
- AGBENORKU, P., AGBENORKU, E., AKPALOO, J., OBENG, G. & AGBLEY, D. 2014. Electrical burns: The trend and risk factors in the Ghanaian population. *Ann Burns Fire Disasters*, 27, 176-83.
- AHMED, I., FARHAN, W. & DURHAM, L. 2007. Unilateral facial nerve paralysis after electrocution injury. *The Journal of Laryngology & Otology*, 121, 494-496.
- ALDRICH, S. 2026a. *Forskolin, Products, Bioactive small molecules, F6886* [Online]. Available: https://www.sigmaaldrich.com/GB/en/product/sigma/f6886?utm_source=google&utm_medium=cpc&utm_campaign=22199337529&utm_content=178316452310&gad_source=1&gad_campaignid=22199337529&gbraid=0AAAAAD8kLQRyJVtbUM-msCWBAZKQUUHJ0&gclid=CjwKCAjwwJzPBhBREiwAJfHRnenA06ni-bbMQ7-DS94qtR9zwy9_nxuh9e8UpM1lrXDne_-zltdWyBoCPTQQA_vD_BwE [Accessed 21st April 2026].
- ALDRICH, S. 2026b. *L-Glutamine G8540* [Online]. Merck. Available: <https://www.sigmaaldrich.com/GB/en/product/sigma/g8540> [Accessed 21st April 2026].
- ALDRICH, S. 2026c. *Neuregulin, Products, Animal Component Free Culture Reagents, SRP3055* [Online]. Merck. Available: <https://www.sigmaaldrich.com/GB/en/product/sigma/srp3055> [Accessed 21st April 2026].
- ANDERSEN, N. D., SRINIVAS, S., PIÑERO, G. & MONJE, P. V. 2016. A rapid and versatile method for the isolation, purification and cryogenic storage of Schwann cells from adult rodent nerves. *Sci Rep*, 6, 31781.
- ANDREW, D. R. 1994. The Guinea Pig Club. *Aviat Space Environ Med*, 65, 428-33.
- ANDREWS, C. J. & REISNER, A. D. 2017. Neurological and neuropsychological consequences of electrical and lightning shock: review and theories of causation. *Neural Regen Res*, 12, 677-686.

- ANGIUS, D., WANG, H., SPINNER, R. J., GUTIERREZ-COTTO, Y., YASZEMSKI, M. J. & WINDEBANK, A. J. 2012. A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials*, 33, 8034-8039.
- ANSSELIN, A., FINK, T. & DAVEY, D. 1997. Peripheral nerve regeneration through nerve guides seeded with adult Schwann cells. *Neuropathology and applied neurobiology*, 23, 387-398.
- APALLA Z, SOTIRIOU, E., LALLAS, A., LAZARIDOU, E., IOANNIDES, D., 2013. Botulinum toxin A in postherpetic neuralgia: a parallel, randomized, double-blind, single-dose, placebo- controlled trial. *Clin. J. Pain* 29, 857–864.
- ARMSTRONG, D. G., BLEY, K., SIMPSON, D. M., STAATS, P., ALLEN, S., CARNEVALE, A. & MARCONDES, L. 2025. Diabetic Peripheral Neuropathy: Pathophysiology and New Insights into the Mechanism of Action of High-Concentration Topical Capsaicin. *J Exp Pharmacol*, 17, 651-665.
- ATTAELEMANAN, G. A. & KHALIL, H. B. 2025. Assessment of Umbilical Cord Mesenchymal Stem Cell Cultivation Using Fetal Bovine Serum or Platelet Lysate. *Cureus*, 17, e78044.
- ATTAL, N., DE ANDRADE, D. C., ADAM, F., RANOUX, D., TEIXEIRA, M. J., GALHARDONI, R., RAICHER, I., ÜÇEYLER, N., SOMMER, C. & BOUHASSIRA, D. 2016. Safety and efficacy of repeated injections of botulinum toxin A in peripheral neuropathic pain (BOTNEP): a randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*, 15, 555-565.
- BACCARANI, A., STARNONI, M., ZACCARIA, G., ANESI, A., BENANTI, E., SPAGGIARI, A. & DE SANTIS, G. 2019. Obturator Nerve Split for Gracilis Free-flap Double Reinnervation in Facial Paralysis. *Plast Reconstr Surg Glob Open*, 7, e2106.
- BAMBA, R., LOEWENSTEIN, S. N. & ADKINSON, J. M. 2021. Donor site morbidity after sural nerve grafting: A systematic review. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 74, 3055-3060.
- BARTNESS, T. J. & BAMSHAD, M. 1998. Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*.
- BATTISTON, B., GEUNA, S., FERRERO, M. & TOS, P. 2005. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. *Microsurgery*, 25, 258-267.
- BATTISTON, B., TOS, P., CUSHWAY, T. R. & GEUNA, S. 2000. Nerve repair by means of vein filled with muscle grafts I. Clinical results. *Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery*, 20, 32-36.
- BERRY, C. C., RUDERSHAUSEN, S., TELLER, J. & CURTIS, A. S. G. 2002. The influence of elastin-coated 520-nm- and 20-nm-diameter nanoparticles on human fibroblasts in vitro. *IEEE Transactions on NanoBioscience*, 1, 105-109.
- BERRY, C. C., WELLS, S., CHARLES, S., AITCHISON, G. & CURTIS, A. S. 2004. Cell response to dextran-derivatised iron oxide nanoparticles post internalisation. *Biomaterials*, 25, 5405-13.
- BERRY, C. C. A. C., A.S. 2003. Functionalisation of magnetic nanoparticles for applications in biomedicine. *Journal of Physics D: Applied Physics*, 36, R198.

- BERTLEFF, M. J., MEEK, M. F. & NICOLAI, J.-P. A. 2005. A prospective clinical evaluation of biodegradable neurolac nerve guides for sensory nerve repair in the hand. *The Journal of hand surgery*, 30, 513-518.
- BIANCONI, E., CASADEI, R., FRABETTI, F., VENTURA, C., FACCHIN, F. & CANAIDER, S. 2020. Sex-Specific Transcriptome Differences in Human Adipose Mesenchymal Stem Cells. *Genes (Basel)*, 11.
- BIOSCIENCES, B. 2016. *Human and Mouse CD Marker Handbook* [Online]. BD Life Sciences, Becton, Dickinson and Company. Available: http://bdbiosciences.com/content/dam/bdb/marketing-documents/cd_marker_handbook.pdf [Accessed 30th March 26].
- BLASZKIEWICZ, M., WILLOWS, J. W., JOHNSON, C. P. & TOWNSEND, K. L. 2019. The Importance of Peripheral Nerves in Adipose Tissue for the Regulation of Energy Balance. *Biology (Basel)*, 8.
- BOECKSTYNS, M. E., SØRENSEN, A. I., VIÑETA, J. F., ROSÉN, B., NAVARRO, X., ARCHIBALD, S. J., VALSS-SOLÉ, J., MOLDOVAN, M. & KRARUP, C. 2013. Collagen conduit versus microsurgical neurorrhaphy: 2-year follow-up of a prospective, blinded clinical and electrophysiological multicenter randomized, controlled trial. *The Journal of hand surgery*, 38, 2405-2411.
- BREWER, G. J., TORRICELLI, J. R., EVEGE, E. K. & PRICE, P. J. 1993. Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *J Neurosci Res*, 35, 567-76.
- BRIN MF, BURSTEIN R. Botox (onabotulinumtoxinA) mechanism of action. *Medicine (Baltimore)*. 2023 Jul 1;102(S1):e32372. doi: 10.1097/MD.00000000000032372. PMID: 37499078; PMCID: PMC10374191.
- BROOKS, S. C. L. 2026. *Deaths of two more patients at Glasgow hospital under investigation* [Online]. The Guardian. Available: <https://www.theguardian.com/uk-news/2026/feb/01/death-patients-glasgow-queen-elizabeth-university-hospital-investigated> [Accessed 3rd April 2026].
- BRUSHART, T. M. 2011. *Nerve Repair*, Oxford, UK, Oxford University Press.
- BYOTROL. 20205. *Introducing CHEMGEN Medlab: meeting modern standards with enhanced efficacy and compliance* [Online]. Available: <https://byotrol.com/introducing-chemgene-medlab-meeting-modern-standards-with-enhanced-efficacy-and-compliance/#:~:text=CHEMGENE%20MedLab%20was%20developed%20to,completed%20against%20each%20product%20formulation>. [Accessed 3rd April 2026].
- CALCAGNI, M. & BESMENS, I. S. 2024. The role of patient-reported outcomes in peripheral nerve surgery. *Journal of Hand Surgery (European Volume)*, 49, 681-686.
- CARPENTER, A. E., JONES, T. R., LAMPRECHT, M. R., CLARKE, C., KANG, I. H., FRIMAN, O., GUERTIN, D. A., CHANG, J. H., LINDQUIST, R. A., MOFFAT, J., GOLLAND, P. & SABATINI, D. M. 2006. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. *Genome Biology*, 7, R100.
- CARR, M. M., BEST, T. J., MACKINNON, S. E. & EVANS, P. J. 1992. Strain differences in autotomy in rats undergoing sciatic nerve transection or repair. *Ann Plast Surg*, 28, 538-44.
- CATERINA, M. J., SCHUMACHER, M. A., TOMINAGA, M., ROSEN, T. A., LEVINE, J. D. & JULIUS, D. 1997a. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 389, 816-824.

- CATERINA, M. J., SCHUMACHER, M. A., TOMINAGA, M., ROSEN, T. A., LEVINE, J. D. & JULIUS, D. 1997b. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 389, 816-24.
- CHANDRASEKHAR, D., JOSEPH, C. M., MURALI, S. & YAHYA, M. 2024. Superbugs: An invincible threat in post antibiotic era. *Clinical Epidemiology and Global Health*, 28, 101499.
- CHEN, L., JIN, Y., YANG, X., LIU, Z., WANG, Y., WANG, G., QI, Z. & SHEN, Z. 2017. Fat tissue, a potential Schwann cell reservoir: isolation and identification of adipose-derived Schwann cells. *Am J Transl Res*, 9, 2579-2594.
- CHEN, L. & QI, Z. 2025. Analysis of the Regeneration Ability of Adipose-Derived Schwann Cells for Sciatic Nerve Defects. *Ann Plast Surg*, 95, 63-69.
- CHEN, S.-H., WU, C.-C., TSENG, W.-L., LU, F.-I., LIU, Y.-H., LIN, S.-P., LIN, S.-C. & HSUEH, Y.-Y. 2023. Adipose-derived stem cells modulate neuroinflammation and improve functional recovery in chronic constriction injury of the rat sciatic nerve. *Frontiers in Neuroscience*, 17, 1172740.
- CHEN, S. H., WANG, H. W., YANG, P. C., CHEN, S. S., HO, C. H., YANG, P. C., KAO, Y. C., LIU, S. W., CHIU, H., LIN, Y. J., CHUANG, E. Y., HUANG, J. H., KAO, H. K. & HUANG, C. C. 2024. Schwann cells acquire a repair phenotype after assembling into spheroids and show enhanced in vivo therapeutic potential for promoting peripheral nerve repair. *Bioeng Transl Med*, 9, e10635.
- CHEN, Y., HAACKE, E. M. & LI, J. 2019. Peripheral nerve magnetic resonance imaging. *F1000Res*, 8.
- CHERINGTON, M., YARNELL, P. R. & LONDON, S. F. 1995. Neurologic complications of lightning injuries. *Western journal of medicine*, 162, 413.
- ClinicalTrials.gov [Online]. National Library of Medicine. 2026. Available: <https://clinicaltrials.gov/> [Accessed 30th March 2026].
- CHO, I. K., WANG, S., MAO, H. & CHAN, A. W. 2016. Genetic engineered molecular imaging probes for applications in cell therapy: emphasis on MRI approach. *Am J Nucl Med Mol Imaging*, 6, 234-261.
- CHU, D.-T., NGUYEN THI PHUONG, T., TIEN, N. L. B., TRAN, D. K., MINH, L. B., THANH, V. V., GIA ANH, P., PHAM, V. H. & THI NGA, V. 2019. Adipose tissue stem cells for therapy: an update on the progress of isolation, culture, storage, and clinical application. *Journal of Clinical Medicine*, 8, 917.
- COMPSTON, A. 2010. Aids to the investigation of peripheral nerve injuries. Medical Research Council: Nerve Injuries Research Committee. His Majesty's Stationery Office: 1942; pp. 48 (iii) and 74 figures and 7 diagrams; with aids to the examination of the peripheral nervous system. By Michael O'Brien for the Guarantors of Brain. Saunders Elsevier: 2010; pp.[8] 64 and 94 Figures. *Brain*, 133, 2838-2844.
- CONSORTIUM, S. M. 2014. *Capsaicin Re-submission* [Online]. Health Improvement Scotland. Available: <https://scottishmedicines.org.uk/medicines-advice/capsaicin-qutenza-resubmission-67311/> [Accessed 6th March 2026].
- CRITCHLEY, M. 1934. NEUROLOGICAL EFFECTS OF LIGHTNING AND OF ELECTRICITY. *The Lancet*, 223, 68-72.
- CRUCCU, G., NURMIKKO, T. J., ERNAULT, E., RIAZ, F. K., MCBRIDE, W. T. & HAANPÄÄ, M. 2018. Superiority of capsaicin 8% patch versus oral pregabalin on

- dynamic mechanical allodynia in patients with peripheral neuropathic pain. *Eur J Pain*, 22, 700-706.
- CUI, C.-X., LIU, H.-Y., YUE, N., DU, Y.-R., CHE, L.-M. & YU, J.-S. 2023. Research progress on the mechanism of chronic neuropathic pain. *IBRO Neuroscience Reports*, 14, 80-85.
- DE VRIJ, E. L., SCHÄFER, T. R., VAN MULKEN, T. & BERTLEFF, M. J. 2022. Surgical treatment of symptomatic neuromas: a feasibility study using the NEUROCAP® bioresorbable nerve capping device. *Journal of Hand Surgery (European Volume)*, 47, 212-214.
- DEN DUNNEN, W., VAN DER LEI, B., SCHAKENRAAD, J., STOKROOS, I., BLAAUW, E., BARTELS, H., PENNING, A. & ROBINSON, P. 1996. Poly (DL-lactide-ε-caprolactone) nerve guides perform better than autologous nerve grafts. *Microsurgery: Official Journal of the International Microsurgical Society and the European Federation of Societies for Microsurgery*, 17, 348-357.
- DERRY, S., RICE, A. S., COLE, P., TAN, T. & MOORE, R. A. 2017. Topical capsaicin (high concentration) for chronic neuropathic pain in adults. *Cochrane Database Syst Rev*, 1, Cd007393.
- DETTORI, J. R., NORVELL, D. C. & CHAPMAN, J. R. 2019. The Art of Surgery: The Strange World of the Placebo Response. *Global Spine J*, 9, 680-683.
- DI SUMMA, P. G., KINGHAM, P. J., RAFFOUL, W., WIBERG, M., TERENGHI, G. & KALBERMATTEN, D. F. 2010. Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg*, 63, 1544-52.
- DISPARITIES, O. F. H. I. A. 2020. *Guidance Patient-reported outcomes and experiences study* [Online]. United Kingdom: © Crown copyright. Available: <https://www.gov.uk/guidance/patient-reported-outcomes-and-experiences-study> [Accessed 12th February 2026 2026].
- DIVISION, S. G. E. P. T. *Understanding drug efficacy and numbers needed to treat (NNT)* [Online]. NHS Scotland. Available: <https://www.rightdecisions.scot.nhs.uk/polypharmacy-guidance-realistic-prescribing/principles/understanding-drug-efficacy-and-numbers-needed-to-treat-nnt/> [Accessed 6th March 2026].
- DODDRELL, R. D., DUN, X. P., MOATE, R. M., JESSEN, K. R., MIRSKY, R. & PARKINSON, D. B. 2012. Regulation of Schwann cell differentiation and proliferation by the Pax-3 transcription factor. *Glia*, 60, 1269-1278.
- DOMINICI, M., LE BLANC, K., MUELLER, I., SLAPER-CORTENBACH, I., MARINI, F. C., KRAUSE, D. S., DEANS, R. J., KEATING, A., PROCKOP, D. J. & HORWITZ, E. M. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, 8, 315-317.
- DR REBECCA CURTIS, M. D. 2015. *Rienso Ferumoxytol withdrawal* [Online]. Takeda. Available: https://assets.publishing.service.gov.uk/media/55159aa7ed915d142400005f/Rienso_DHP_C_sent_13_March_2015.pdf [Accessed 12th April 2026].
- DU, J., WANG, Z., LIU, X., HU, C., YAREMA, K. J. & JIA, X. 2023. Improving Schwann Cell Differentiation from Human Adipose Stem Cells with Metabolic Glycoengineering. *Cells*, 12, 1190.
- DUDEK, R. W. 2008. *High-yield Histopathology*, Lippincott Williams & Wilkins.

- EDERER, I. A., KOLBENSCHLAG, J., DAIGELER, A. & WAHLER, T. 2022. Muscle-in-vein conduits for the treatment of symptomatic Neuroma of sensory digital nerves. *Journal of Personalized Medicine*, 12, 1514.
- EMONT, M. P., JACOBS, C., ESSENE, A. L., PANT, D., TENEN, D., COLLELUORI, G., DI VINCENZO, A., JØRGENSEN, A. M., DASHTI, H. & STEFEK, A. 2022. A single-cell atlas of human and mouse white adipose tissue. *Nature*, 603, 926-933.
- ERBGUTH, F. J. & NAUMANN, M. 1999. Historical aspects of botulinum toxin: Justinus Kerner (1786-1862) and the "sausage poison". *Neurology*, 53, 1850-3.
- ESCOBAR, C. H. & CHAPARRO, O. 2016. Xeno-Free Extraction, Culture, and Cryopreservation of Human Adipose-Derived Mesenchymal Stem Cells. *Stem Cells Transl Med*, 5, 358-65.
- FAIRBAIRN, N. G., MEPPELINK, A. M., NG-GLAZIER, J., RANDOLPH, M. A. & WINOGRAD, J. M. 2015. Augmenting peripheral nerve regeneration using stem cells: A review of current opinion. *World J Stem Cells*, 7, 11-26.
- FALLON M, T. P. 2023. *Histology, Schwann Cells* [Online]. StatPearls [Internet]. Treasure Island. StatPearls Publishing. Available: <https://www.ncbi.nlm.nih.gov/books/NBK544316/> [Accessed 28th April 2026].
- FARONI, A., SMITH, R. J., LU, L. & REID, A. J. 2016. Human Schwann-like cells derived from adipose-derived mesenchymal stem cells rapidly de-differentiate in the absence of stimulating medium. *Eur J Neurosci*, 43, 417-30.
- FORNASARI, B. E., ZEN, F., NATO, G., FOGLI, M., LUZZATI, F., RONCHI, G., RAIMONDO, S. & GAMBAROTTA, G. 2022. Blood Vessels: The Pathway Used by Schwann Cells to Colonize Nerve Conduits. *Int J Mol Sci*, 23.
- FRANGOGIANNIS, N. G. 2022. Why animal model studies are lost in translation. *J Cardiovasc Aging*, 2.
- FREITAS, R., VAN KEEP, M., KANDASWAMY, P., PRÜFERT, A., AGER, M. & EERDEKENS, M. 2025. Cost-Effectiveness of a High-Concentration (179 mg) Capsaicin Patch for the Treatment of Patients with Peripheral Neuropathic Pain in Scotland. *Pain and Therapy*, 14, 1573-1595.
- FRICKER, J. 2016. Robert Acland OBITUARY. BMJ PUBLISHING GROUP BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H
- GEBREMARIYAM, Z. T. & WELDEMICHAEL, N. A. 2024. Pioneering microsurgery services in low-income countries: insights from a plastic surgeon's experience in Ethiopia. *Plastic and Reconstructive Surgery-Global Open*, 12, e5705.
- GEBUREK, F., MUNDLE, K., CONRAD, S., HELDIGE, M., WALLISER, U., VAN SCHIE, H. T., VAN WEEREN, R., SKUTELLA, T. & STADLER, P. M. 2016. Tracking of autologous adipose tissue-derived mesenchymal stromal cells with in vivo magnetic resonance imaging and histology after intralesional treatment of artificial equine tendon lesions--a pilot study. *Stem Cell Res Ther*, 7, 21.
- GENIE, A. *Ultimate Guide: CD Markers List* [Online]. Available: <https://www.assaygenie.com/cd-markers-list?srsId=AfmBOoo691K7x5Q1EDJnxLidJl1OnRtSJ-9WK5gaSTGZCfakAQtgroyM> [Accessed 18th February 2026].
- GEUNA, S., TOS, P., TITOLO, P., CICLAMINI, D., BENINGO, T. & BATTISTON, B. 2014. Update on nerve repair by biological tubulization. *Journal of brachial plexus and peripheral nerve injury*, 9, e16-e21.

- GILLIES, S. C. C. 2026. *Fresh Concerns at Queen Elisabeth University Hospital as mould and water ingress closes parts of cancer ward* [Online]. UK: Sky News. Available: <https://news.sky.com/story/fresh-concerns-at-glasgows-queen-elizabeth-university-hospital-as-mould-and-water-ingress-closes-parts-of-cancer-ward-13513891#:~:text='We%20must%20stop%20operations%20here,%22aware%20of%20an%20incident%22>. [Accessed 3rd April 2026].
- GLUCK, T. 1880. Ueber neuroplastik auf dem wege der transplantation. *Arch Klin Chir*, 25, 6.
- GORDON, J., ÁLVAREZ-NARVÁEZ, S. & PERONI, J. F. 2021. Antimicrobial Effects of Equine Platelet Lysate. *Frontiers in Veterinary Science*, Volume 8 - 2021.
- GOULD, J. S., NARANJE, S. M., MCGWIN JR, G., FLORENCE, M. & CHEPPALLI, S. 2013. Use of collagen conduits in management of painful neuromas of the foot and ankle. *Foot & ankle international*, 34, 932-940.
- GOVERNMENT, U. 2002. *Control of Substances Hazardous to Health Regulations 2002 (COSHH)* [Online]. Available: <https://www.legislation.gov.uk/ukxi/2002/2677/regulation/7> [Accessed].
- GROUP, N. N. T. A. 2017. *Qutenza® (capsaicin) cutaneous patch for neuropathic pain Treatment Appraisal: Decision Summary* [Online]. Available: <https://ntag.nhs.uk/wp-content/uploads/2021/10/NTAG-Decision-Summary-Qutenza-updated-June-2017.pdf> [Accessed 28th February 2026].
- GUIDELINE NG211, N. 2022. Rehabilitation after traumatic injury.
- GUIOTTO, M., RAFFOUL, W., HART, A. M., RIEHLE, M. O. & DI SUMMA, P. G. 2020. Human platelet lysate to substitute fetal bovine serum in hMSC expansion for translational applications: a systematic review. *J Transl Med*, 18, 351.
- HARTRAMPF, C. R. J. 1992. Hartrampf's Breast Reconstruction with Living Tissue. *Plastic and Reconstructive Surgery*, 89, 151-152.
- HEINZEL, J. C., QUYEN NGUYEN, M., KEFALIANAKIS, L., PRAHM, C., DAIGELER, A., HERCHER, D. & KOLBENSCHLAG, J. 2021. A systematic review and meta-analysis of studies comparing muscle-in-vein conduits with autologous nerve grafts for nerve reconstruction. *Scientific reports*, 11, 11691.
- HELDIN, C.-H. & RÖNNSTRAND, L. 1990. Platelet-Derived Growth Factor B Type Receptor. In: LITWACK, G. (ed.) *Receptor Purification: Volume 1 Receptors for CNS Agents, Growth Factors, Hormones, and Related Substances*. Totowa, NJ: Humana Press.
- HERBST, W., EBERLE, K., OZEN, Y. & HORNSTEIN, O. 1992. The innervation of the great saphenous vein: an immunohistochemical study with special regard to regulatory peptides. *Vasa. Zeitschrift für Gefasskrankheiten*, 21, 253-257.
- HIGGINS, G. C., ROBERTSON, E., HORSELY, C., MCLEAN, N. & DOUGLAS, J. 2020. FFP3 reusable respirators for COVID-19; adequate and suitable in the healthcare setting. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 73, 1357-1404.
- HIGGINS, G. C. & THOMSON, S. E. 2020. Peripheral nerve regeneration: The resolution revolution. *Journal of plastic, reconstructive & aesthetic surgery : JPRAS*.
- HIRSCH, P. 1986. Microbial life at extremely low nutrient levels. *Adv Space Res*, 6, 287-98.
- HOLM, C., MAYR, M., HÖFTER, E. & NINKOVIC, M. 2006. Perfusion zones of the DIEP flap revisited: a clinical study. *Plast Reconstr Surg*, 117, 37-43.
- HONNDORF, S., KÜHBACH, K., EISELE, K. H., SHOKUROVA, A., BUCH, P., JATZKE, C., TAYLOR, H. V. & FINK, K. 2026. Comparative Analytics and Pharmacodynamics of

- the Complex Protein-Free Botulinum Toxin Type A Formulations DaxibotulinumtoxinA, IncobotulinumtoxinA and RelabotulinumtoxinA. *Toxins (Basel)*, 18.
- HUANG, C. T., CHU, H. S., HUNG, K. C., CHEN, L. W., CHEN, M. Y., HU, F. R. & CHEN, W. L. 2021. The effect of human platelet lysate on corneal nerve regeneration. *Br J Ophthalmol*, 105, 884-890.
- HUANG, Z., POWELL, R., PHILLIPS, J. B. & HAASTERT-TALINI, K. 2020. Perspective on Schwann Cells Derived from Induced Pluripotent Stem Cells in Peripheral Nerve Tissue Engineering. *Cells*, 9.
- HÖRNER, S. J., COUTURIER, N., GUEIBER, D. C., HAFNER, M. & RUDOLF, R. 2022. Development and In Vitro Differentiation of Schwann Cells. *Cells*, 11.
- INNOVATION, D. G. F. R. A. 2020. *Horizon 2020* [Online]. Horizon 2020, European Union. Available: https://research-and-innovation.ec.europa.eu/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-2020_en [Accessed 30th April 2026].
- INSTITUTE., B. Copyright 2003-2021. *Cell Profiler Manual* [Online]. Available: <https://cellprofiler-manual.s3.amazonaws.com/CellProfiler-3.0.0/modules/measurement.html> [Accessed 18th February 2026].
- JESSEN, K. R. & MIRSKY, R. 2016. The repair Schwann cell and its function in regenerating nerves. *J Physiol*, 594, 3521-31.
- KAWEKHAW, R., SCUTT, A. M. & HAYCOCK, J. W. 2012. Integrated culture and purification of rat Schwann cells from freshly isolated adult tissue. *Nature protocols*, 7, 1996-2004.
- KAPLAN, H. M., MISHRA, P. & KOHN, J. 2015. The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med*, 26, 226.
- KARAKATSANIS, A., CHRISTIANSEN, P. M., FISCHER, L., HEDIN, C., PISTIOLI, L., SUND, M., RASMUSSEN, N. R., JØRNSGÅRD, H., TEGNELIUS, D., ERIKSSON, S., DASKALAKIS, K., WÄRNBERG, F., MARKOPOULOS, C. J. & BERGKVIST, L. 2016. The Nordic SentiMag trial: a comparison of super paramagnetic iron oxide (SPIO) nanoparticles versus Tc(99) and patent blue in the detection of sentinel node (SN) in patients with breast cancer and a meta-analysis of earlier studies. *Breast Cancer Res Treat*, 157, 281-294.
- KARASTERGIOU, K., SMITH, S. R., GREENBERG, A. S. & FRIED, S. K. 2012. Sex differences in human adipose tissues - the biology of pear shape. *Biol Sex Differ*, 3, 13.
- KEARNS, M. 2021. *Increasing the Neurotrophic Potential of Human Adipose Derived Stem Cells in the Setting of Peripheral Nerve Injury*. MD, University of Glasgow.
- KENT, K. D. & BOMSER, J. A. 2003. Bovine pituitary extract provides remarkable protection against oxidative stress in human prostate epithelial cells. *In Vitro Cell Dev Biol Anim*, 39, 388-94.
- KERMEN, R. 2016. Botulinum toxin for chronic pain conditions. *Disease-a-Month*, 62, 353-357.
- KIM, H.-S., LEE, J., LEE, D. Y., KIM, Y.-D., KIM, J. Y., LIM, H. J., LIM, S. & CHO, Y. S. 2017. Schwann cell precursors from human pluripotent stem cells as a potential therapeutic target for myelin repair. *Stem cell reports*, 8, 1714-1726.

- KIM, J., CHOI, Y. E., KIM, J. H., LEE, S. H., OH, S. & KIM, S. H. 2020. Nerve Repair and Orthodromic and Antidromic Nerve Grafts: An Experimental Comparative Study in Rabbit. *Biomed Res Int*, 2020, 5046832.
- KINGHAM, P. J., KALBERMATTEN, D. F., MAHAY, D., ARMSTRONG, S. J., WIBERG, M. & TERENGHI, G. 2007. Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. *Exp Neurol*, 207, 267-74.
- KRAUS, H. & REISNER, H. 1940. Behandlungsergebnisse von Verletzungen pripherer Nerven mit besonderer Berücksichtigung der Schussverletzungen del Jahre 1919, 1927, und 1934. *Arch Klin Chir*, 199, 318-336.
- KUNER, R. 2010. Central mechanisms of pathological pain. *Nat Med*, 16, 1258-66.
- LAROCCA, R. A., MORAES-VIEIRA, P. M., BASSI, E. J., SEMEDO, P., DE ALMEIDA, D. C., DA SILVA, M. B., THORNLEY, T., PACHECO-SILVA, A. & CÂMARA, N. O. 2013. Adipose tissue-derived mesenchymal stem cells increase skin allograft survival and inhibit Th-17 immune response. *PLoS One*, 8, e76396.
- LAVORATO, A., RAIMONDO, S., BOIDO, M., MURATORI, L., DURANTE, G., COFANO, F., VINCITORIO, F., PETRONE, S., TITOLO, P. & TARTARA, F. 2021. Mesenchymal stem cell treatment perspectives in peripheral nerve regeneration: systematic review. *International journal of molecular sciences*, 22, 572.
- LECKENBY, J. I., HARRISON, D. H. & GROBBELAAR, A. O. 2014. Static support in the facial palsy patient: a case series of 51 patients using tensor fascia lata slings as the sole treatment for correcting the position of the mouth. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 67, 350-357.
- LEE, J.-H., JUNG, M. J., HWANG, Y. H., LEE, Y. J., LEE, S., LEE, D. Y. & SHIN, H. 2012. Heparin-coated superparamagnetic iron oxide for in vivo MR imaging of human MSCs. *Biomaterials*, 33, 4861-4871.
- LENSCH, M., MUISE, A., WHITE, L., BADOWSKI, M. & HARRIS, D. 2018. Comparison of Synthetic Media Designed for Expansion of Adipose-Derived Mesenchymal Stromal Cells. *Biomedicines*, 6.
- LEWIS, E. E., CHILD, H. W., HURSTHOUSE, A., STIRLING, D., MCCULLY, M., PATERSON, D., MULLIN, M. & BERRY, C. C. 2015. The influence of particle size and static magnetic fields on the uptake of magnetic nanoparticles into three dimensional cell-seeded collagen gel cultures. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 103, 1294-1301.
- LI, L., JIANG, W., LUO, K., SONG, H., LAN, F., WU, Y. & GU, Z. 2013. Superparamagnetic Iron Oxide Nanoparticles as MRI contrast agents for Non-invasive Stem Cell Labeling and Tracking. *Theranostics*, 3, 595-615.
- LIEBANO, R. E., RAKEL, B., VANCE, C. G. T., WALSH, D. M. & SLUKA, K. A. 2011. An investigation of the development of analgesic tolerance to TENS in humans. *Pain*, 152, 335-342.
- LINDSAY, K. J., LEONARD, D. A., HIGGINS, G. C., ROBERTSON, E. & PERKS, G. 2020. Invited editorial from the social media editor of JPRAS; Leadership in the time of COVID-19. *J Plast Reconstr Aesthet Surg*, 73, 1405-1408.
- LIU, T., WANG, Y., LU, L. & LIU, Y. 2022. SPIONs mediated magnetic actuation promotes nerve regeneration by inducing and maintaining repair-supportive phenotypes in Schwann cells. *Journal of Nanobiotechnology*, 20, 159.

- LIU, T., YANG, M., TIAN, W., REN, J., LU, L. & WANG, Y. 2026. f-SPION-mediated magnetic stimulation induces reparative Schwann cell reprogramming via cytoskeletal dynamics-gated activation of Piezo1. *Journal of Nanobiotechnology*.
- LIU, Z., HUANG, L., LIU, L., LUO, B., LIANG, M., SUN, Z., ZHU, S., QUAN, X., YANG, Y. & MA, T. 2015a. Activation of Schwann cells in vitro by magnetic nanocomposites via applied magnetic field. *International journal of nanomedicine*, 43-61.
- LIU, Z., HUANG, L., LIU, L., LUO, B., LIANG, M., SUN, Z., ZHU, S., QUAN, X., YANG, Y., MA, T., HUANG, J. & LUO, Z. 2015b. Activation of Schwann cells in vitro by magnetic nanocomposites via applied magnetic field. *Int J Nanomedicine*, 10, 43-61.
- LU, C.-W., HSIAO, J.-K., LIU, H.-M. & WU, C.-H. 2017. Characterization of an iron oxide nanoparticle labelling and MRI-based protocol for inducing human mesenchymal stem cells into neural-like cells. *Scientific Reports*, 7, 3587.
- LUNDBORG, G., ROSEN, B., DAHLIN, L., HOLMBERG, J. & ROSÉN, I. 2004. Tubular repair of the median or ulnar nerve in the human forearm: a 5-year follow-up. *Journal of hand surgery*, 29, 100-107.
- LUVISETTO, S. 2020. Botulinum Toxin and Neuronal Regeneration after Traumatic Injury of Central and Peripheral Nervous System. *Toxins (Basel)*, 12.
- MACKAY, B. J., COX, C. T., VALERIO, I. L., GREENBERG, J. A., BUNCKE, G. M., EVANS, P. J., MERCER, D. M., MCKEE, D. M. & DUCIC, I. 2021. Evidence-Based Approach to Timing of Nerve Surgery: A Review. *Ann Plast Surg*, 87, e1-e21.
- MAIER, C., BARON, R., TOELLE, T., BINDER, A., BIRBAUMER, N., BIRKLEIN, F., GIERTHMÜHLEN, J., FLOR, H., GEBER, C., HUGE, V., ENAX-KRUMOVA, E., LANDWEHRMEYER, G. B., MAGERL, W., MAIHÖFNER, C., RICHTER, H., ROLKE, R., SCHERENS, A., SCHWARZ, A., SOMMER, C. & TREEDE, R. D. 2010. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. *Pain*, 150, 439-50.
- MARINELLI, S., LUVISETTO, S., COBIANCHI, S., MAKUCH, W., OBARA, I., MEZZAROMA, E., CARUSO, M., STRAFACE, E., PRZEWLOCKA, B. & PAVONE, F. 2010. Botulinum neurotoxin type A counteracts neuropathic pain and facilitates functional recovery after peripheral nerve injury in animal models. *Neuroscience*, 171, 316-28.
- MASSIER, L., JALKANEN, J., ELMASTAS, M., ZHONG, J., WANG, T., NONO NANKAM, P. A., FREND-CUMBO, S., BÄCKDAHL, J., SUBRAMANIAN, N. & SEKINE, T. 2023. An integrated single cell and spatial transcriptomic map of human white adipose tissue. *Nature communications*, 14, 1438.
- MCMORROW, L. A., CZARNECKI, P., REID, A. J. & TOS, P. 2024. Current perspectives on peripheral nerve repair and management of the nerve gap. *Journal of Hand Surgery (European Volume)*, 49, 698-711.
- MEYER, V. M., BENJAMENS, S., EL MOUMNI, M., LANGE, J. F. & POL, R. A. 2022. Global overview of response rates in patient and health care professional surveys in surgery: a systematic review. *Annals of surgery*, 275, e75-e81.
- MOHAMED, H. E., ASKER, M. E., KOTB, N. S. & EL HABAB, A. M. 2020. Human platelet lysate efficiency, stability, and optimal heparin concentration required in culture of mammalian cells. *Blood research*, 55, 35.

- MORGAN, M., NENCINI, S., THAI, J. & IVANUSIC, J. J. 2019. TRPV1 activation alters the function of A δ and C fiber sensory neurons that innervate bone. *Bone*, 123, 168-175.
- MULTIPLEX. 2026a. *ARC* [Online]. Available: <https://www.multiplex.global/projects/the-advanced-research-centre-arc-university-of-glasgow/> [Accessed 3rd April 2026].
- MULTIPLEX. 2026b. *QEUH* [Online]. Available: <https://www.multiplex.global/projects/the-queen-elizabeth-university-hospital-the-royal-hospital-for-children/> [Accessed 3rd April 2026].
- MURPHY, R. N. A., DE SCHOULEPNIKOFF, C., CHEN, J. H. C., COLUMB, M. O., BEDFORD, J., WONG, J. K. & REID, A. J. 2023. The incidence and management of peripheral nerve injury in England (2005–2020). *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 80, 75-85.
- MURRAY, C. J., IKUTA, K. S., SHARARA, F., SWETSCHINSKI, L., AGUILAR, G. R., GRAY, A., HAN, C., BISIGNANO, C., RAO, P. & WOOL, E. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, 399, 629-655.
- NADI M, D. W., DAS JM. . 2026. *Peripheral Nerve Injury*. [Online]. StatPearls Publishing StatPearls [Internet]. Available: <https://www.ncbi.nlm.nih.gov/books/NBK549848/> [Accessed 28th April 2026].
- NEGRO, S., PIRAZZINI, M. & RIGONI, M. 2022. Models and methods to study Schwann cells. *J Anat*, 241, 1235-1258.
- NEUMEISTER, M. W., CHAMBERS, C. B., HERRON, M. S., WEBB, K., WIETFELDT, J., GILLESPIE, J. N., BUENO JR, R. A. & COONEY, C. M. 2009. Botox therapy for ischemic digits. *Plastic and reconstructive surgery*, 124, 191-201.
- NHS. *Be Clear on Cancer* [Online]. Public Health England. Available: <https://www.england.nhs.uk/south/wp-content/uploads/sites/6/2017/03/Large-print-breast-cancer-leaflet.pdf> [Accessed 2nd April 2026].
- NIAPOUR, N., MOHAMMADI-GHALEHBIN, B., GOLMOHAMMADI, M. G., GHOLAMI, M. R., AMANI, M. & NIAPOUR, A. 2016. An efficient system for selection and culture of Schwann cells from adult rat peripheral nerves. *Cytotechnology*, 68, 629-636.
- NICE, N. I. F. H. A. C. E. 2022. *MagTrace and SentiMag for locating sentinel lymph nodes for breast cancer* [Online]. Available: <https://www.nice.org.uk/guidance/htg642/resources/magtrace-and-sentimag-for-locating-sentinel-lymph-nodes-for-breast-cancer-pdf-14578097696197#:~:text=These%20are%20called%20sentinel%20lymph,sentinel%20lymph%20node%20biopsy%20procedure.&text=Is%20this%20treatment%20right%20for,making%20decisions%20about%20your%20care.&text=How%20well%20does%20it%20work%20compared%20with%20other%20treatments?&text=What%20are%20the%20risks%20or,How%20likely%20are%20they?&text=How%20will%20the%20treatment%20affect%20my%20day%2Dto%2Dday%20life?&text=What%20happens%20if%20the%20treatment%20does%20not%20work?&text=What%20happens%20if%20I%20do.Are%20there%20other%20treatments%20available?> [Accessed 12th April 2026].
- NICOLINO, S., PANETTO, A., RAIMONDO, S., GAMBAROTTA, G., GUZZINI, M., FORNARO, M., BATTISTON, B., TOS, P., GEUNA, S. & PERROTEAU, I. 2009. Denervation and reinnervation of adult skeletal muscle modulate mRNA expression of neuregulin-1 and ErbB receptors. *Microsurgery: Official Journal of the International*

- Microsurgical Society and the European Federation of Societies for Microsurgery*, 29, 464-472.
- O'CONNELL, H. E., SANJEEVAN, K. V. & HUTSON, J. M. 2005. Anatomy of the clitoris. *The Journal of urology*, 174, 1189-1195.
- OH, H.-M. & CHUNG, M. E. 2015. Botulinum toxin for neuropathic pain: a review of the literature. *Toxins*, 7, 3127-3154.
- ORGANISATION, W. H. 2023. *Antimicrobial resistance* [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance#:~:text=The%20global%20rise%20in%20antibiotic,infections%20that%20can not%20be%20treated>. [Accessed 3rd April 2026].
- OZDOĞMUŞ, O., SEZEN, O., KUBILAY, U., SAKA, E., DUMAN, U., SAN, T. & CAVDAR, S. 2004. Connections between the facial, vestibular and cochlear nerve bundles within the internal auditory canal. *J Anat*, 205, 65-75.
- OZKIRIS, M. 2014. Unilateral sensorineural hearing loss and facial nerve paralysis associated with low-voltage electrical shock. *Ear Nose Throat J*, 93, 62-6.
- PALOMBELLA, S., GUIOTTO, M., HIGGINS, G. C., APPELEGATE, L. L., RAFFOUL, W., CHERUBINO, M., HART, A., RIEHLE, M. O. & DI SUMMA, P. G. 2020. Human platelet lysate as a potential clinical-translatable supplement to support the neurotrophic properties of human adipose-derived stem cells. *Stem Cell Res Ther*, 11, 432.
- PANKSEPP, J. B. & LAHVIS, G. P. 2011. Rodent empathy and affective neuroscience. *Neurosci Biobehav Rev*, 35, 1864-75.
- PATIL, S., WILLETT, O., THOMPSON, T., HERMANN, R., RAMANATHAN, S., CORNETT, E. M., FOX, C. J. & KAYE, A. D. 2016. Botulinum Toxin: Pharmacology and Therapeutic Roles in Pain States. *Curr Pain Headache Rep*, 20, 15.
- PATRIK ERNFORS, A. E. M., PER SVENNINGSSON. 2021. *Discoveries of receptors for temperature and touch* [Online]. Available: <https://www.nobelprize.org/prizes/medicine/2021/advanced-information/> [Accessed 25th February 2026].
- PERCIE DU SERT, N. & RICE, A. S. 2014. Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain. *Br J Pharmacol*, 171, 2951-63.
- PETERS, D. C. 2024. *Post Oral Evidence Statement - Dr Christine Peters - 22.10.2024* [Online]. Scottish Hospitals Inquiry. Available: <https://www.hospitalsinquiry.scot/inquiry-document/post-oral-evidence-statement-dr-christine-peters-22102024> [Accessed 3rd April 2026].
- PHARSIGHT. *Qutenza Patent Expiration* [Online]. Available: <https://pharsight.greyb.com/drug/qutenza-patent-expiration> [Accessed 8th March 2026].
- POITELON, Y., LOPEZ-ANIDO, C., CATIGNAS, K., BERTI, C., PALMISANO, M., WILLIAMSON, C., AMEROSO, D., ABIKO, K., HWANG, Y., GREGORIEFF, A., WRANA, J. L., ASMANI, M., ZHAO, R., SIM, F. J., WRABETZ, L., SVAREN, J. & FELTRI, M. L. 2016. YAP and TAZ control peripheral myelination and the expression of laminin receptors in Schwann cells. *Nature Neuroscience*, 19, 879-887.
- PRODUCTION, L. S. 03OCT2024. *HPL Gold Research Grade Product Sheet* [Online]. Available: <https://lifesciencegroup.co.uk/wp-content/uploads/2025/10/hPL-Gold-Research-Grade-Product-Information-Sheet-PI115.pdf> [Accessed 18th February 2026].
- RAHMAN, M. 2023. Magnetic Resonance Imaging and Iron-oxide Nanoparticles in the era of Personalized Medicine. *Nanotheranostics*, 7, 424-449.

- RAI, J., JESCHKE, M. G., BARROW, R. E. & HERNDON, D. N. 1999. Electrical injuries: a 30-year review. *J Trauma*, 46, 933-6.
- RANOUX, D., ATTAL, N., MORAIN, F. & BOUHASSIRA, D. 2008. Botulinum toxin type A induces direct analgesic effects in chronic neuropathic pain. *Ann Neurol*, 64, 274-83.
- RAWAT, S. & MEENA, S. 2014. Publish or perish: Where are we heading? *J Res Med Sci*, 19, 87-9.
- REID, A. J., SUN, M., WIBERG, M., DOWNES, S., TERENGHI, G. & KINGHAM, P. J. 2011. Nerve repair with adipose-derived stem cells protects dorsal root ganglia neurons from apoptosis. *Neuroscience*, 199, 515-522.
- RESCH, A., WOLF, S., MANN, A., WEISS, T., STETCO, A.-L. & RADTKE, C. 2018. Co-culturing human adipose derived stem cells and Schwann cells on spider silk—a new approach as prerequisite for enhanced nerve regeneration. *International journal of molecular sciences*, 20, 71.
- RODRÍGUEZ, F. J., VERDÚ, E., CEBALLOS, D. & NAVARRO, X. 2000. Nerve guides seeded with autologous Schwann cells improve nerve regeneration. *Experimental neurology*, 161, 571-584.
- S., G. 28 Jan 2025. *Nerve Repair and Regeneration Market Size, Share, and Trends 2025 to 2034* [Online]. [Accessed Report Code : 2335].
- SCIENTIFIC, F. 2026. *Gibco N2 supplement* [Online]. Thermofischer. Available: [https://www.thermofischer.com/order/catalog/product/17502001/fags#:~:text=Which%20supplement%20can%20I%20add,5%20Supplement%20can%20be%20used](https://www.thermofisher.com/order/catalog/product/17502001/fags#:~:text=Which%20supplement%20can%20I%20add,5%20Supplement%20can%20be%20used). [Accessed 18th February 2026].
- SELLE, M., KOCH, J. D., ONGSIEK, A., ULBRICH, L., YE, W., JIANG, Z., KRETTEK, C., NEUNABER, C. & NOACK, S. 2022. Influence of age on stem cells depends on the sex of the bone marrow donor. *Journal of Cellular and Molecular Medicine*, 26, 1594-1605.
- SELVAGGI, T. A., WALKER, R. E. & FLEISHER, T. A. 1997. Development of antibodies to fetal calf serum with arthus-like reactions in human immunodeficiency virus–infected patients given syngeneic lymphocyte infusions. *Blood, The Journal of the American Society of Hematology*, 89, 776-779.
- SHAH, A. M. 2024. Artificial trachea transplant: Tale of two stories. *Artif Organs*, 48, 575-576.
- SHARIATI, A., MORADABADI, A., AZIMI, T. & GHAZNAVI-RAD, E. 2020. Wound healing properties and antimicrobial activity of platelet-derived biomaterials. *Scientific Reports*, 10, 1032.
- SISTI, A., UYGUR, S., LOPEZ-SCHULTZ, S. D. & KONOFAOS, P. 2024. Nerve capping techniques for neuroma management: a comprehensive literature review. *Annals of plastic surgery*, 92, 106-119.
- SKRYPNYK, M. 2025. Current progress and limitations of research regarding the therapeutic use of adipose-derived stem cells: literature review. *Journal of Umm Al-Qura University for Applied Sciences*, 11, 63-75.
- SMYTH, D., CROKER, R. & GOLDACRE, B. 2018. Pregabalin: what the patent litigation means for doctors and drug companies. *BMJ*, 361, k2318.
- SPAGNA A, ATTAL N. Botulinum toxin A and neuropathic pain: An update. *Toxicon*. 2023 Aug 15;232:107208. doi: 10.1016/j.toxicon.2023.107208. Epub 2023 Jun 27. PMID: 37385479.
- STAVELY, R., HOTTA, R., PICARD, N., RAHMAN, A. A., PAN, W., BHAVE, S., OMER, M., HO, W. L. N., GUYER, R. A. & GOLDSTEIN, A. M. 2022. Schwann cells in the

- subcutaneous adipose tissue have neurogenic potential and can be used for regenerative therapies. *Sci Transl Med*, 14, eabl8753.
- STEMPLE, D. L. & ANDERSON, D. J. 1992. Isolation of a stem cell for neurons and glia from the mammalian neural crest. *Cell*, 71, 973-85.
- STOCCO, E., BARBON, S., MACCHI, V., TIENGO, C., PETRELLI, L., RAMBALDO, A., BOREAN, A., CAPELLI, S., FILIPPI, A. & ROMANATO, F. 2019. New bioresorbable wraps based on oxidized polyvinyl alcohol and leukocyte-fibrin-platelet membrane to support peripheral nerve neuroorrhaphy: Preclinical comparison versus NeuraWrap. *Scientific Reports*, 9, 17193.
- SZABŁOWSKA-GADOMSKA, I., HUMIEŃKA, M., BRZEZICKA, J., CHRÓŚCICKA, A., PŁACZKOWSKA, J., OŁDAK, T. & LEWANDOWSKA-SZUMIEL, M. 2023. Microbiological Aspects of Pharmaceutical Manufacturing of Adipose-Derived Stem Cell-Based Medicinal Products. *Cells*, 12.
- TAYLOR, N., GIFFORD, R. M., COBB, R., WARDLE, S. L., JONES, S., BLACKADDER-WEINSTEIN, J., HATTERSLEY, J., WILSON, A., IMRAY, C., GREEVES, J. P., REYNOLDS, R. & WOODS, D. R. 2021. Experience from the selection and nutritional preparation for Expedition ICE MAIDEN: the first successful all-female unassisted Antarctic traverse. *BMJ Mil Health*, 167, 27-32.
- THE EUROPEAN PARLIAMENT, C. O. T. P. O. A. U. F. S. P. 2010. *Directive 2010/63/EU* [Online]. Official Journal of the European Union. Available: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF> [Accessed 5th May 2026].
- THERMOFISCHER. *Knockout Serum 10828028* [Online]. Thermo Fichser Scientific. Available: [https://www.thermofisher.com/order/catalog/product/10828028#:~:text=KnockOut%E2%84%A2%20Serum%20Replacement.%20Knockout%20SR%20is%20a,maintain%20undifferentiated%20ES%20cells%20in%20culture%20\(1\)](https://www.thermofisher.com/order/catalog/product/10828028#:~:text=KnockOut%E2%84%A2%20Serum%20Replacement.%20Knockout%20SR%20is%20a,maintain%20undifferentiated%20ES%20cells%20in%20culture%20(1)). [Accessed 22nd April 2026].
- THERMOFISCHER. 2026. *Dulbecco's Modified Eagle Medium F12 (DMEM F12) cell culture media* [Online]. Available: <https://www.thermofisher.com/uk/en/home/life-science/cell-culture/mammalian-cell-culture/cell-culture-media/dmem-fl12.html> [Accessed 21st April 2026].
- THOMSON, S. E. 2018. *Translational development of a three-dimensional bioactive conduit for peripheral nerve repair, through the application of topographical cues & stem cell support*. [Online]. Available: <https://theses.gla.ac.uk/id/eprint/8747> [Accessed 25th March 2026 2026].
- THOMSON, S. E., CHARALAMBOUS, C., SMITH, C.-A., TSIMBOURI, P. M., DÉJARDIN, T., KINGHAM, P. J., HART, A. & RIEHLE, M. O. 2017. Microtopographical cues promote peripheral nerve regeneration via transient mTORC2 activation. *Acta Biomaterialia*, 60, 220 - 231.
- THOMSON, S. E., DÉJARDIN, T., TSIMBOURI, M. P., HART, A. M. & RIEHLE, M. O. 2014. Combinational approaches to improve outcomes following peripheral nerve repair. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, 67, 1465.
- THOMSON, S. E., NG, N. Y., RIEHLE, M. O., KINGHAM, P. J., DAHLIN, L. B., WIBERG, M. & HART, A. M. 2022. Bioengineered nerve conduits and wraps for peripheral nerve repair of the upper limb. *Cochrane Database of Systematic Reviews*.

- TIAN, S., WU, L., ZHENG, H., CHENG, Z., LIU, J., LIU, M., YU, X., TU, J. & WU, W. 2025. Botulinum toxin type A inhibits microglia pyroptosis by suppressing Cblb-mediated degradation of Pdlim1 to attenuate neuropathic pain. *J Headache Pain*, 26, 165.
- TOS, P., RONCHI, G., PAPALIA, I., SALLEN, V., LEGAGNEUX, J., GEUNA, S. & GIACOBINI-ROBECCHI, M. G. 2009. Chapter 4: Methods and protocols in peripheral nerve regeneration experimental research: part I-experimental models. *Int Rev Neurobiol*, 87, 47-79.
- TOWNSEND, K. L. 2020. The re-emergence of adipose innervation as a research focus. *Nature Reviews Endocrinology*, 16, 127-128.
- TSAI, E. H. & GAN, T. J. 2026. TRPV1 Receptor Modulators: Emerging Therapies for Neuropathic, Osteoarthritic, and Perioperative Pain. *CNS Drugs*.
- VAIDYA, V., DHERE, R., AGNIHOTRI, S., MULEY, R., PATIL, S. & PAWAR, A. 2018. Ultraviolet-C irradiation for inactivation of viruses in foetal bovine serum. *Vaccine*, 36, 4215-4221.
- VANGIJZEGEM, T., LECOMTE, V., TERNAD, I., VAN LEUVEN, L., MULLER, R. N., STANICKI, D. & LAURENT, S. 2023. Superparamagnetic Iron Oxide Nanoparticles (SPION): From Fundamentals to State-of-the-Art Innovative Applications for Cancer Therapy. *Pharmaceutics*, 15, 236.
- VANLAIR, C. 1885. *Nouvelles recherches expérimentales sur la régénération des nerfs*, Librairie Clemm.
- VASQUEZ, J. C., SHUSTERMAN, E. M. & HANSBROUGH, J. F. 1999. Bilateral Facial Nerve Paralysis After High Voltage Electrical Injury. *The Journal of Burn Care & Rehabilitation*, 20, 307-308.
- VITAL, A., CARLES, D., SERISE, J. M. & BOISSEAU, M. R. 2010. Evidence for unmyelinated C fibres and inflammatory cells in human varicose saphenous vein. *Int J Angiol*, 19, e73-7.
- WAN, T., LI, Q.-C., QIN, M.-Y., WANG, Y.-L., ZHANG, F.-S., ZHANG, X.-M., ZHANG, Y.-C. & ZHANG, P.-X. 2024. Strategies for treating traumatic neuromas with tissue-engineered materials. *Biomolecules*, 14, 484.
- WANG, G., CAO, L., WANG, Y., HUA, Y., CAI, Z., CHEN, J., CHEN, L., JIN, Y., NIU, L. & SHEN, H. 2017. Human eyelid adipose tissue-derived Schwann cells promote regeneration of a transected sciatic nerve. *Scientific Reports*, 7, 43248.
- WANG, Y., SHAN, Q., PAN, J. & YI, S. 2018. Actin Cytoskeleton Affects Schwann Cell Migration and Peripheral Nerve Regeneration. *Front Physiol*, 9, 23.
- WANG, Y. & YE, L. 2022. Sensory nerves regulate fat functions. NATURE PORTFOLIO HEIDELBERGER PLATZ 3, BERLIN, 14197, GERMANY.
- WANG, Y. & YE, L. 2023. Somatosensory innervation of adipose tissues. *Physiology & behavior*, 265, 114174.
- WANG, Y. & YE, L. 2024. The afferent function of adipose innervation. *Diabetes*, 73, 348-354.
- WEBER, R. A., BREIDENBACH, W. C., BROWN, R. E., JABALEY, M. E. & MASS, D. P. 2000. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plastic and reconstructive surgery*, 106, 1036-1045.
- WEBER, R. A., PROCTOR, W. H., WARNER, M. R. & VERHEYDEN, C. N. 1993. Autotomy and the sciatic functional index. *Microsurgery*, 14, 323-327.

- WEI, H., HU, Y., WANG, J., GAO, X., QIAN, X. & TANG, M. 2021. Superparamagnetic Iron Oxide Nanoparticles: Cytotoxicity, Metabolism, and Cellular Behavior in Biomedicine Applications. *Int J Nanomedicine*, 16, 6097-6113.
- WISDOM, R., JOHNSON, R. S. & MOORE, C. 1999. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *Embo j*, 18, 188-97.
- WREDE, L. 1909. Uberbrueckung eines nervendefektes mittels seidennaht und leben venenstueckes. *Dt Med Wschr*, 35, 1125-1146.
- XIAO, L., MACKEY, S., HUI, H., XONG, D., ZHANG, Q., ZHANG, D., 2010. Subcutaneous injection of botulinum toxin a is beneficial in postherpetic neuralgia. *Pain Med*. Malden Mass 11, 1827–1833.
- XIE, S., LU, F., HAN, J., TAO, K., WANG, H., SIMENTAL, A., HU, D. & YANG, H. 2017. Efficient generation of functional Schwann cells from adipose-derived stem cells in defined conditions. *Cell Cycle*, 16, 841-851.
- XU, C., MIRANDA-NIEVES, D., ANKRUM, J. A., MATTHIESEN, M. E., PHILLIPS, J. A., ROES, I., WOJTKIEWICZ, G. R., JUNEJA, V., KULTIMA, J. R. & ZHAO, W. 2012. Tracking mesenchymal stem cells with iron oxide nanoparticle loaded poly (lactide-co-glycolide) microparticles. *Nano letters*, 12, 4131-4139.
- YANG, Y.-H. K., OGANDO, C. R., WANG SEE, C., CHANG, T.-Y. & BARABINO, G. A. 2018. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. *Stem Cell Research & Therapy*, 9, 131.
- YUAN, R.Y., SHEU, J.J., YU, J.M., CHEN, W.T., TSENG, I.J., CHANG, H.H., HU, C.J., 2009. Botulinum toxin for diabetic neuropathic pain: a randomized double-blind crossover trial. *Neurology* 72, 1473–1478.
- YU, W. M., CHEN, Z. L., NORTH, A. J. & STRICKLAND, S. 2009. Laminin is required for Schwann cell morphogenesis. *J Cell Sci*, 122, 929-36.
- ZACK-WILLIAMS, S. D., BUTLER, P. E. & KALASKAR, D. M. 2015. Current progress in use of adipose derived stem cells in peripheral nerve regeneration. *World Journal of Stem Cells*, 7, 51.
- ZHANG, R., LI, J., LI, J. & XIE, J. 2014. Efficient In vitro labeling rabbit bone marrow-derived mesenchymal stem cells with SPIO and differentiating into neural-like cells. *Molecules and cells*, 37, 650-655.
- ZHOU, L., HUO, T., ZHANG, W., HAN, N., WEN, Y. & ZHANG, P. 2023. New techniques and methods for prevention and treatment of symptomatic traumatic neuroma: A systematic review. *Front Neurol*, 14, 1086806.
- ZHOU, L., YANG, C. Z. & BITTNER, G. D. 2026. PEG-Fusion Repair After Peripheral Nerve Injuries Enhances Behavioral Recovery and Reduces Self-Mutilation in Rat Models. *Neurology International*, 18, 83.
- ZHU, J., FU, Q., SONG, L., LIU, L., ZHENG, Z., XU, Y. & ZHANG, Z. 2022. Advances in peripheral nerve injury repair with the application of nanomaterials. *Journal of Nanomaterials*, 2022, 7619884.
- ZUKAWA, M., OSADA, R. & KIMURA, T. 2019. Clinical outcome and ultrasonographic evaluation of treatment using polyglycolic acid-collagen tube for chronic neuropathic pain after peripheral nerve injury. *Journal of Orthopaedic Science*, 24, 1064-1067.

Appendices

Appendix 1

Hunter Integrated Pain Service
Brief Pain Inventory

Disc 2006

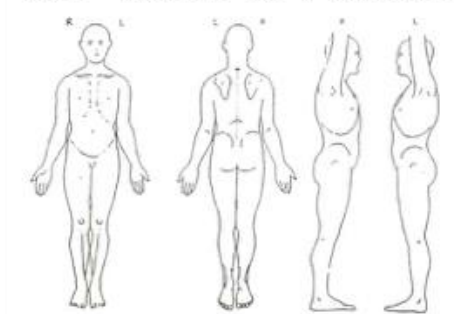
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Office Use Only
Date of Analysis: _____
MRN: _____
Pain Severity: _____
Pain Interference: _____

Date: _____

Name: _____

1. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts most.



2. Please rate your pain by circling the one number that best describes your pain at its worst in the last week.

0 1 2 3 4 5 6 7 8 9 10

No pain Pain as bad as you can imagine

3. Please rate your pain by circling the one number that best describes your pain at its least in the last week.

[illegible]

4. Please rate your pain by circling the one number that best describes your pain on average.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

5. Please rate your pain by circling the one number that tells how much pain you have right now.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

6. What treatments or medications are you receiving for your pain?

Page 1 of 2

Appendix 2

Appendix 2 details the stages of building the pipeline in Cell profiler cell image analysis software (4.1.3). Adaptive thresholding of greyscale images identified cells based on DNA staining (DAPI) as ‘primary objects’, and actin cytoskeleton staining (phalloidin) as ‘secondary

objects'. The watershed-gradient method was implemented to delineate neighbouring cells, and cells touching the boundary of the images were discarded. The pipeline was written and utilised for cell measurements (minimum 500-1000 cells per condition).

