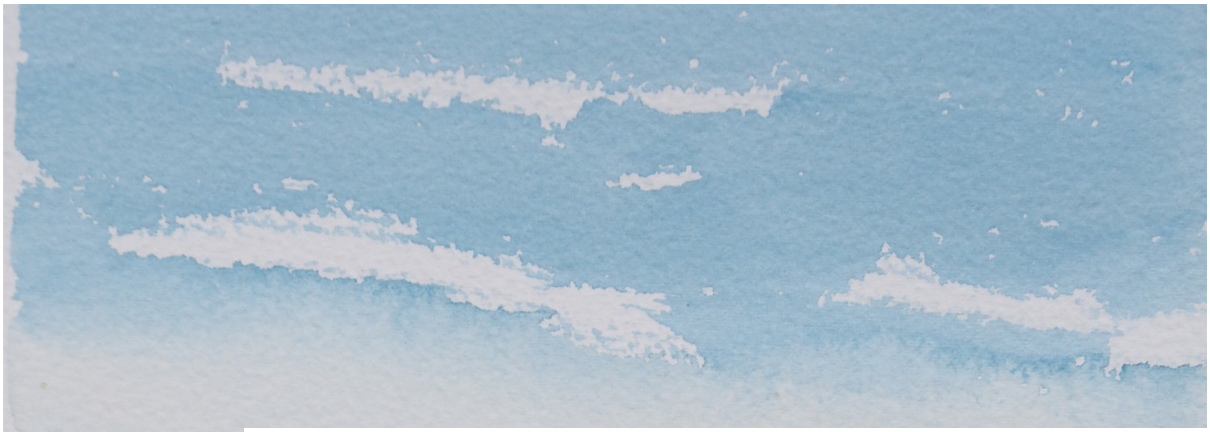
Nerve regeneration in healthy and diabetic rat sciatic nerves

Influence of timing, a novel scaffold and the lactoferrin-derived peptide PXL01

Hazer, Derya Burcu

2024

Document Version:
Early version, also known as pre-print



Nerve regeneration in healthy and diabetic rat sciatic nerves

Influence of timing, a novel scaffold and the lactoferrin-derived peptide PXL01

DERYA BURCU HAZER ROSBERG
DEPARTMENT OF TRANSLATIONAL MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY

A peripheral nerve injury can be devastating for the affected patient, including those patients with a concomitant diabetes. This thesis focuses on nerve regeneration in respect to orchestrated Schwan cell and macrophage response with the signs of neuroprotection revealed by expression of HSP27 and their relation to the axonal outgrowth in nerve injury and different repair and reconstruction models, including delayed and no nerve repair in healthy Wistar and diabetic Goto-Kakizaki rats. Additionally, this thesis presents a promising solution to injured nerve with nerve defect as reconstruction by modified artificial conduit through an inserted membrane with gold and cobalt-oxide nanoparticles which improves nerve regeneration.

This thesis also offers an alternative pharmacological treatment as a local application of PXL01 which is already in clinical use in tendon injuries in hands. It is investigated in primary and autograft reconstructed nerve injury model in healthy and diabetic Goto-Kakizaki rats, indicating its potential use to treat "tethered/scarred" neuroma, even further hypothetical clinical use in nerve reconstruction both in healthy and diabetic patients.

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Translational Medicine
Hand Surgery

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:153
ISBN 978-91-8021-650-0
ISSN 1652-8220

Nerve regeneration in healthy and diabetic rat sciatic nerves

Nerve regeneration in healthy and diabetic rat sciatic nerves

Influence of timing, a novel scaffold and the lactoferrin-derived peptide PXL01

Derya Burcu Hazer Rosberg

LUND
UNIVERSITY

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on December 13th, 2024, at 09.00 in Lilla Aulan, Medicinskt Forskningscentrum, Skåne University Hospital, Malmö

Faculty opponent
Professor Christine Radtke, Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Vienna, Vienna, Austria

Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation **Date of issue** Dec 13th 2024

Author(s): Derya Burcu Hazer Rosberg

Title and subtitle: Nerve regeneration in healthy and diabetic rat sciatic nerves: influence of timing, a novel scaffold and the lactoferrin-derived peptide PXL01

Abstract: Repair or reconstruction after a peripheral nerve injury is a challenge because functional outcome is still unsatisfactory. Treatment in patients with diabetes may be further demanding. Nerve regeneration after nerve injury was investigated, using various repair and reconstruction models, including immediate and delayed repair, bioengineered conduits, or nerve autografts as well as a possibility to stimulate regeneration with the lactoferrin-derived peptide PXL01 in healthy Wistar and diabetic Goto-Kakizaki (GK) rats. I focused on axonal outgrowth in relation to expression of Heat Shock Protein 27 (HSP27) (neuroprotection), Schwann cell response (activated and apoptotic) as well as macrophage response (activated – invading and pro-healing) in sciatic nerve and dorsal root ganglia (DRG). In Paper I, expression of injury-induced HSP27 was investigated with respect to axonal outgrowth after no or immediate nerve repair or repair after a short delay in healthy and diabetic GK rats. A nerve injury, with or without any type of repair, increased HSP27 expression in the injured nerve and in DRG without any impact on axonal outgrowth. A delayed nerve repair had no influence on axonal outgrowth. Paper II focused on nerve regeneration in hollow and modified (inserted membrane with or without gold or gold-cobalt oxide nanoparticles) conduits used to reconstruct a nerve defect in healthy rats. Chambered conduits, especially the modified with inserted membrane with nanoparticles, increased axonal outgrowth and with improvement of other aspects of regeneration (i.e., increased HSP27 expression and activated Schwann cells but less apoptotic Schwann cells). In Paper III, a substance that suppress inflammation - the lactoferrin-derived peptide PXL01 with its sodium hyaluronate carrier - was used to potentially stimulate nerve regeneration after immediate nerve repair in healthy rats. Local application of PXL01, compared to sodium hyaluronate or to saline, had an anti-inflammatory effect (activated CD68 stained macrophages) without affecting pro-healing (CD206 stained) macrophages, but did not influence nerve regeneration. In Paper IV, application of PXL01 was compared to saline regarding nerve regeneration in nerve autografts in healthy and diabetic GK rats. PXL01 increased axonal outgrowth in sciatic nerve, despite a decrease in activated Schwann cells. The treatment increased HSP27 ratio in DRGs. Diabetes decreased axonal outgrowth and impacted activated Schwann cells and sensory neurons in sciatic nerve and DRG, respectively, and had some influence on HSP27 expression and pro-healing macrophages in nerve autografts.

In conclusion, injured sciatic nerves should appropriately be repaired, but a short delay does not influence axonal outgrowth. HSP27 expression in sciatic nerve or in DRG, despite increased after nerve injury with or without a repair, is not associated with axonal outgrowth after any repair or reconstruction procedure. Nerve regeneration can be improved in modified bioengineered nerve conduits, particularly if nanoparticles are added to an inserted membrane. PXL01 decreases inflammation, without affecting pro-healing macrophages, after nerve repair without any effect on nerve regeneration, indicating its potential use to treat “tethered/scarred” neuroma. In hypoxic environments, like nerve autografts, nerve regeneration is improved by application of PXL01. Diabetes increases HSP27 expression in uninjured nerves and decreases axonal outgrowth, independent of repair or reconstruction modality, after injury.

Key words: Nerve injury, repair, reconstruction, diabetes, Goto-Kakizaki rats, HSP27, PXL01, nanoparticles, gold, cobalt oxide, Schwann cell, macrophage, delayed nerve repair, nerve autograft

Lund University, Faculty of Medicine Doctoral Dissertation Series 2024:153

Language English **Number of pages**:126

ISSN: 1652-8220

ISBN: 978-91-8021-650-0

Nerve regeneration in healthy and diabetic rat sciatic nerves

Influence of timing, a novel scaffold and the lactoferrin-derived peptide PXL01

Derya Burcu Hazer Rosberg

LUND
UNIVERSITY

Coverphoto by Derya Burcu Hazer Rosberg

Faculty of Medicine
Department of Translational Medicine - Hand Surgery

Lund University, Faculty of Medicine, Doctoral Dissertation Series 2024:153
ISSN 1652-8220
ISBN 978-91-8021-650-0

Printed in Sweden by Media-Tryck, Lund University
Lund 2024

To my family

Table of Contents

List of Papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Injury-Induced HSP27 Expression in Peripheral Nervous Tissue Is Not Associated with Any Alteration in Axonal Outgrowth after Immediate or Delayed Nerve Repair
Stenberg L, Hazer Rosberg DB, Kohyama S, Suganuma S, Dahlin LB.
Int J Mol Sci. 2021,Aug 11;22(16): 8624. doi:10.3390/ijms22168624

II. Gold and Cobalt Oxide Nanoparticles Modified Poly-Propylene Poly-Ethylene Glycol Membranes in Poly (epsilon-Caprolactone) Conduits Enhance Nerve Regeneration in the Sciatic Nerve of Healthy Rats
Hazer Rosberg DB, Hazer B, Stenberg L, Dahlin LB.
Int J Mol Sci. 2021,Jul 1;2(13):7146. doi:10.3390/ijms22137146

III. PXL01 alters macrophage response with no effect on axonal outgrowth or Schwann cell response after nerve repair in rats
Hazer Rosberg DB, Stenberg L, Mahlapuu M, Dahlin LB.
Regen Med. 2024, Jun 2;19(6):327-343. doi:10.1080/17460751.2024.2361515

IV. Lactoferrin-derived peptide PXL01 impacts nerve regeneration after sciatic nerve reconstruction in healthy and diabetic rats
Hazer Rosberg DB, Mahlapuu M, Perez R, Dahlin LB.
In manuscript

Other publications related to topic of thesis, but not included in the thesis.

Age does not affect the outcome after digital nerve repair in children - A retrospective long term follow up
Rosberg HE, Hazer Rosberg DB, Birkisson I, Dahlin LB.
J Orthop Sci. 2017 Sep;22(5):915-918. doi: 10.1016/j.jos.2017.06.012

Evaluation of small nerve fiber dysfunction in type 2 diabetes
Ekman L, Thrainsdottir S, Englund E, Thomsen N, Rosén I, Hazer Rosberg DB, Petersson J, Eriksson KF, Dahlin LB.
Acta Neurol Scand. 2020 Jan;141(1):38-46. doi: 10.1111/ane.13171.

Author's earlier publications related to topic:

In vivo application of poly-3-hydroxyoctanoate as peripheral nerve graft
Hazer DB, Bal E, Nurlu G, Benli K, Balci S, Öztürk F, Hazer B.
J Zhejiang Univ Sci B. 2013 Nov;14(11):993-1003. doi: 10.1631/jzus.B1300016

The effect of poly(3-hydroxybutyrate-co-3- hydroxyhexanoate) (PHBHHx) and human mesenchymal stem cell (hMSC) on axonal regeneration in experimental sciatic nerve damage
Sakar M, Korkusuz P, Demirbilek M, Cetinkaya DU, Arslan S, Denkbaş EB, Temuçin ÇM, Bilgiç E, Hazer DB, Bozkurt G.
Int J Neurosci. 2014 Sep;124(9):685-96. doi: 10.3109/00207454.2013.876636

Abbreviations

AGE	Advanced glycation end products
ATF3	Activating transcription factor 3
Au	Gold
Ag	Silver
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation
CCl2	Chemokine (C-C motif) ligand 2
Co	Cobalt
CoO	Cobalt-oxide
CNS	Central nervous system
Cu	Copper
CSF-1	Colony stimulating factor 1
DAPI	4′,6-diamino-2-phenylindole
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
ECM	Extracellular matrix
EDS	Energy dispersive X-ray spectroscopy
ERK	Extracellular signal regulated kinase
GAP-43	Growth associated protein 43
GDNF	Glial cell line-derived neurotrophic factor
GK	Goto-Kakizaki
HA	Hyaluronate
HSP	Heat shock protein
HSP27	Heat shock protein 27
IL	Interleukin
IGF	Insulin like growth factor

JNK	Jun amino terminal kinase
LDL	Low density lipoprotein
LIF	Leukemia inhibitory factor
mA	milliampere
MS	Multiple sclerosis
NGF	Nerve growth factor
NP	Nanoparticle
NF	Neurofilament
NT-3	Neurotrophin-3
PAI-1	Plasminogen Activator Inhibitor-1
PA	Plasminogen activators
PBS	Phosphate buffer solution
PCL	Poly (ε-caprolactone)
PEG	Polyethylene glycol
PPEG	Polypropylene-polyethylene glycol
PGA	Polyglycolic acid
PKA	Protein kinase A
PKC	Protein kinase C
PLGA	Poly(l-lactide-co-glycolic acid)
PNA	Processed nerve allograft
PNS	Peripheral nervous system
PHUPNIPAM	Poly(3-hydroxy undecenoate)-poly(N-isopropyl acryl amide)
RAG	Regeneration associated gene
RAGE	Receptor of advanced glycation end products
ROS	Reactive oxygen species
Rho/ROCK	Rho/Rho-associated coiled-coil containing protein kinase
SEM	Scanning electron microscopy
THF	Tetra hydro furan
TGF-1β	Transforming growth factor 1β
TLR	Toll like receptor
TNF	Tumour necrotising factor
tPA	Tissue plasminogen activator
VEGF	Vascular endothelial growth factor
Zn	Zinc
ZnO	Zinc-oxide

Preface

My interest in science and research started when I was a child. My father, Professor Baki Hazer, who is my role model through life, is a chemical engineer and specialized in organic chemistry - especially in polymer science. My fairy tales, or "good night stories", were stories about atoms and electrons or basic physical rules. Our playground, for my sister and myself as children, was dad's laboratory. Distillation tubes were our spaceship. We were full of curiosity and imagination. He showed me the science hidden behind every moment of life - the scientific thinking. From that moment, I decided to walk through the same path of life, the science, like my dad.

The scientific research continued throughout the high school and during the medical faculty years. During my education as a resident in Neurosurgery in Hacettepe University, Faculty of Medicine in Ankara, Turkey and during the years as a specialist, I was interested in patients that had peripheral nerve dysfunction and diseases. At that time, I already conducted experimental projects in peripheral nerve injury and regeneration. As a neurosurgeon, I shifted my clinical practice and experimental research to the spine and peripheral nerve surgery.

After I got my degree as Associate Professor in Neurosurgery in Turkey, I thought I needed to explore and learn more about peripheral nerves. That was how I met Professor Lars B. Dahlin, another "man of science", and then I started my PhD training in Lund, Sweden. The idea of exploring the cellular and molecular mechanism(s) behind repair and reconstruction after nerve injury and the subsequent degeneration and regeneration was amazing. In the meantime, I had the most precious gift in life - my son Axel was born.

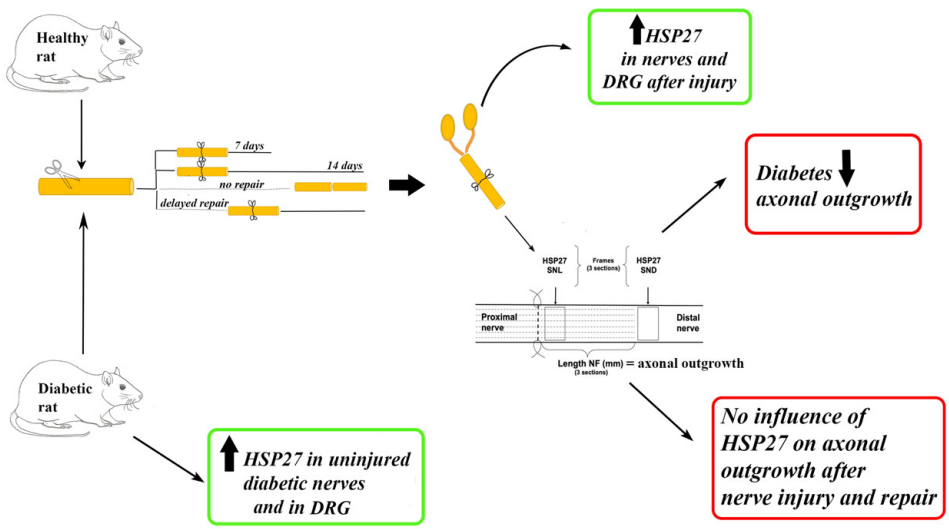
During the time I have worked with my four projects included in the present PhD thesis, I have learned a lot. In this seven years journey, the scientific interactions with colleagues from this and other universities were valuable, but above all the endless support and guidance of my supervisor, Professor Lars B. Dahlin, was precious. From the beginning to the end, during my time as a PhD student, and seeing my son growing up, my curiosity and hunger to better understand the mystery of peripheral nerves grew stronger and stronger. The present thesis, consisting of four different subprojects, is only a section of my endless journey, and hopefully is a guide to even more curiosity in the future.

Thesis at a Glance

Paper I

Injury-induced HSP27 expression in peripheral nervous tissue is not associated with any alteration in axonal outgrowth after immediate or delayed nerve repair

Injury-induced expression of HSP27 was investigated after no, an immediate or a delayed nerve repair after a sciatic nerve transection with 7 or 14 days follow up in healthy and diabetic Goto-Kakizaki (GK) rats. Axonal outgrowth was analysed with neurofilament measures and HSP27 expression was evaluated at site of lesion, in distal nerve end and in DRGs (see image for summary of methods and results).

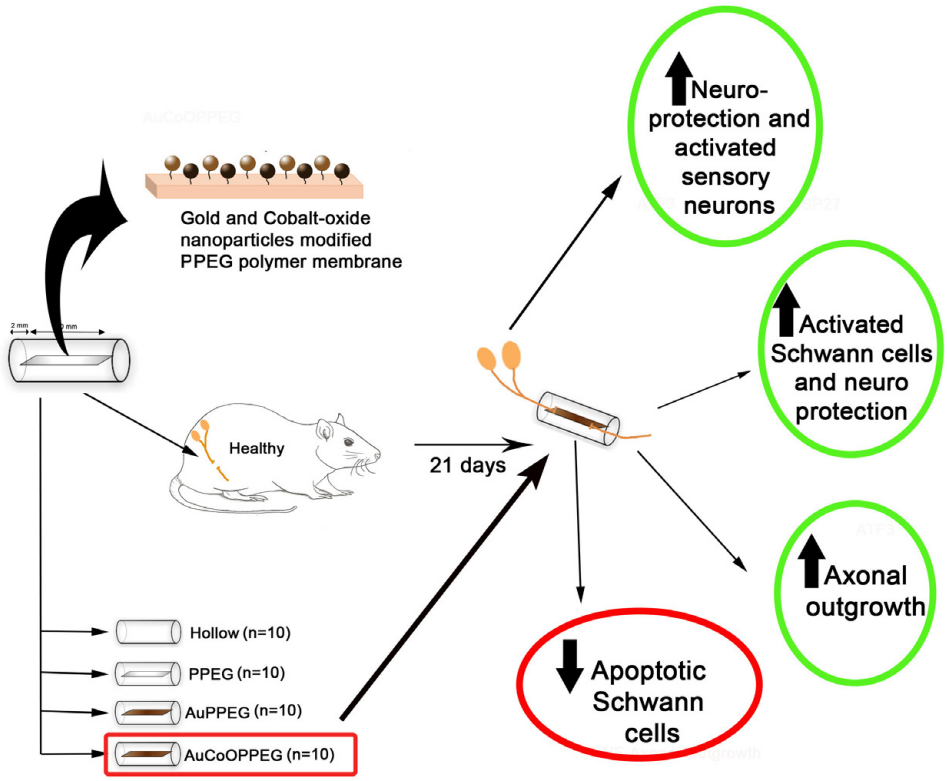


Injured sciatic nerves should appropriately be repaired in healthy and diabetic GK rats, but a short delay does not influence axonal outgrowth. HSP27 expression in sciatic nerve or in DRG, despite an increase after nerve injury with or without a repair, is not associated with any alteration in axonal outgrowth.

Paper II

Gold and cobalt oxide nanoparticle modified poly-propylene poly-ethylene glycol membrane in poly(ε-Caprolactone) conduits enhance nerve regeneration in sciatic nerve of healthy rats.

Regeneration capacity, after bridging a 10 mm nerve defect with either a hollow poly-caprolactone (PCL) conduit or such a conduit with an inserted poly-propylene polyethylene glycol (PPEG) membrane enhanced with gold (Au) or gold-cobalt-oxide (AuCoO) nanoparticles, was investigated 21 days after implantation in healthy Wistar rats (see image for summary).

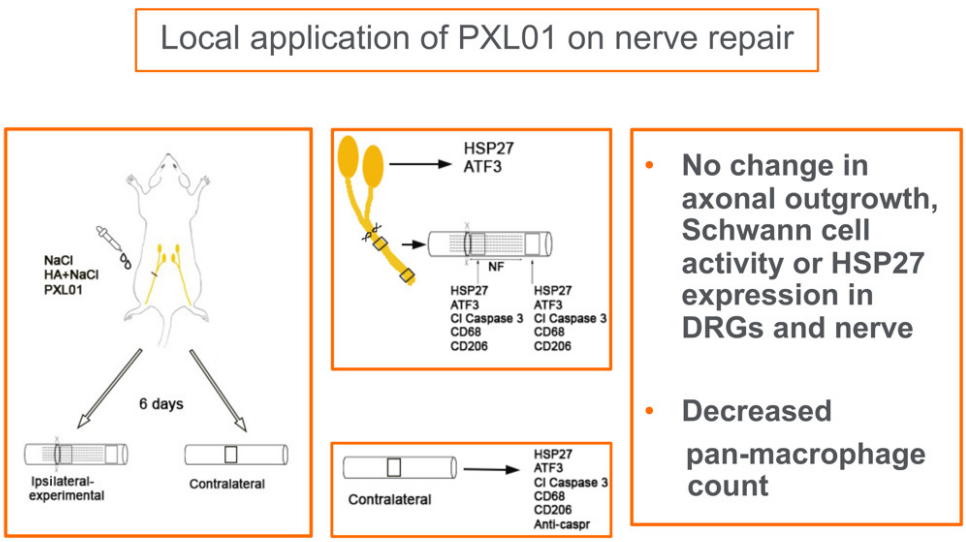


Au and CoO nanoparticle modified membranes in PCL conduits improve axonal outgrowth and increase the regenerative performance after nerve reconstruction.

Paper III

PXL01 alters macrophage response with no effect on axonal outgrowth or Schwann cell response after nerve repair in rats

The potential of PXL01, a synthetic peptide produced from a lactoferrin peptide and clinically used to prevent adhesions, to stimulate nerve regeneration was evaluated. Axonal outgrowth, response of Schwann cells, neuroprotection (HSP27) and expression of macrophages after local application of PXL01 in a sciatic nerve injury and repair model in healthy Wistar rats were investigated. Three different treatment agents - PXL01 in sodium hyaluronate carrier, sodium hyaluronate or sodium chloride solution (0.2 mL) as placebo - were locally applied immediately after nerve repair. Immunohistochemical analyses were done after 6 days.

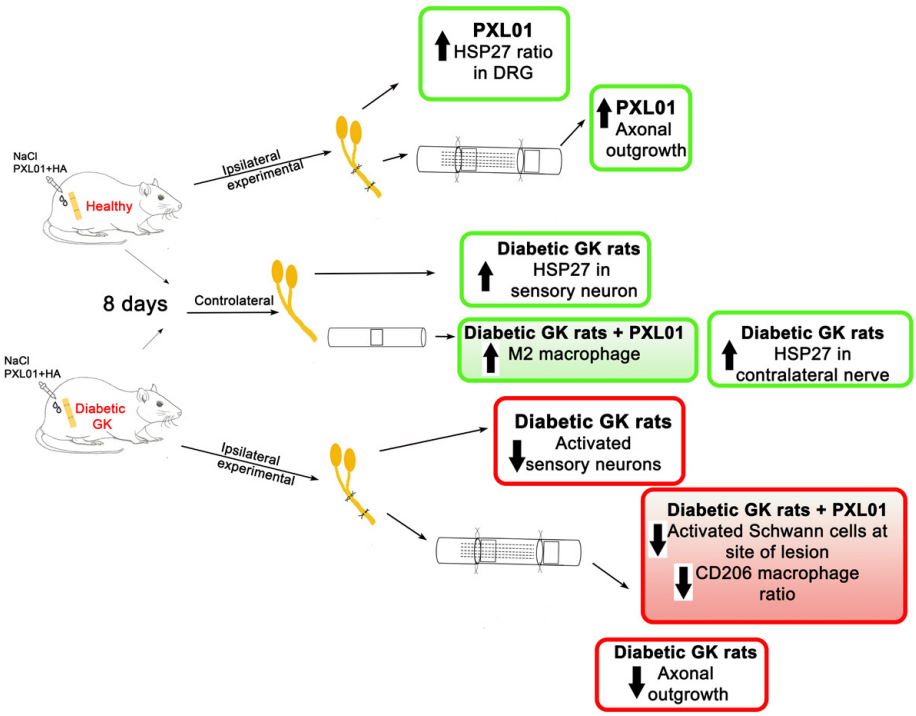


Local application of PLX01 inhibits inflammation (CD68) in transected and immediately repaired rat sciatic nerves without affecting nerve regeneration or pro-healing macrophages (CD206). PLX01 can be used to treat “tethered/scarred” neuroma without affecting nerve regeneration.

Paper IV

Lactoferrin-derived peptide PXL01 impacts nerve regeneration after sciatic nerve reconstruction in healthy and diabetic rats

Application of PXL01, the lactoferrin-derived peptide, around a nerve autograft after reconstruction of a 10 mm long nerve defect in healthy Wistar and in GK rats was evaluated. PXL01 in a sodium hyaluronate carrier was compared to a sodium chloride solution (0.2 mL). At 8 days, axonal outgrowth, Schwann cell activity, HSP27 expression in sciatic nerves and DRGs bilaterally and the macrophage response were analysed immunohistochemically (see image for summary).



PXL01 increases axonal outgrowth in nerve autografts with an impact on activated Schwann cells as well as an increased neuroprotection (HSP27 expression) in DRGs in healthy and diabetic GK rats, indicating that PXL01 is a promising candidate for improvement of nerve regeneration in nerve autografts. Diabetes impacts nerve regeneration in nerve autografts.

Summary in Swedish

Våra händer används dagligen i varierade sammanhang, varför de löper stor risk att skadas av en mångfald orsaker. Av vävnaderna i handen och armen är de perifera nerverna särskilt utsatta. Skador på dessa, liksom på nerverna i benen, är svåra att behandla och slutresultatet kan, trots noggrann behandling, variera beroende på skadans omfattning. Mindre tryckskador medför oftast ett bra slutresultat medan allvarliga skador i form av kompletta avskärningar eller avslitningar av nerven i regel ger kvarstående funktionsbortfall.

Efter en perifer nervskada tillbakabildas nervtrådarna bortom skadan varefter de ersätts av nya som växer ut centralt ifrån. Utväxthastigheten är låg (1-2 mm/dag), vilket bör beaktas om en skada är lokaliserad till över- eller underarm alternativt lår eller underben. Det kan därför krävas lång tid innan hud och muskler åter innerveras. Resultatet kan dessutom försämras genom förtvining (atrofi) av musklerna. Att kunna stimulera nervutväxt kan därför vara av vikt för återkomst av både muskel- och känselfunktion.

Utfallet efter en behandlad perifer nervskada kan påverkas av flera faktorer. En sådan är typ 2 diabetes som idag är en folksjukdom. Orsaken till att den uppstår är okänd, men riskfaktorerna är livsstilsrelaterade, till exempel övervikt, rökning och stress. Det är känt att det karaktäristiskt höga blodsockret efter cirka 10 år leder till diabetesrelaterade nervskador hos många individer. Operation av perifera nervskador hos diabetiker kan ge sämre slutresultatet än hos för övrigt friska, men orsaken är okänd. Det är viktigt att man vid utveckling av nya kirurgiska tekniker, instrument och läkemedel också studerar hur dessa påverkar nervutväxten vid diabetes. Detta är betydelsefullt eftersom fler personer med diabetes kan skada sig i yrkeslivet och för att förstå om det finns andra typer av regenerationsmekanismer involverade vid diabetes. Jag valde att studera nervregeneration i en modell av typ 2 diabetes eftersom många som lever med sjukdomen är i arbetsför ålder och kan drabbas av nervskador.

Vid ”enklare nervavskärningar” kan skadorna kirurgiskt direkt sys ihop genom att ändarna läggs mot varandra och säkras/sys ihop med hjälp av små trådar. Vid slitskador uppstår ofta en defekt mellan de skadade nervändarna, vilket omöjliggör direkt reparation. Andra metoder måste då användas för att överbrygga defekten och den vanligaste är fortfarande att använda en ”mindre viktig” nerv som ”reservdel” – s.k. ”autolog (dvs. från individen själv) nervgraftteknik” – där reservdelen läggs

som en ”bro” mellan de skadade nervändarna. De skadade nervtrådarna kan då använda reservdelen som en brygga för att växa ner till den yttre nervänden och vidare ut till hud och muskler. I de fall defekten är betydligt kortare kan olika typer av ”tuber” (dvs. ”rör”) användas som genom biologiska mekanismer möjliggör ett återskapande av nerven och att med nervtrådar överbrygga gapet. Det finns ett flertal olika kommersiella varianter av tuber att tillgå, men ingen är tillräckligt bra då utformningen fortfarande medför att enbart kortare defekter kan överbryggas. Det finns därför ett behov att utveckla förbättrade alternativ för kliniskt bruk.

Vid nervskador är det inte alltid möjligt att direkt reparera eller rekonstruera skadorna med reservdelar varför kirurgi måste ske med fördröjning. En alltför lång fördröjning kan emellertid medföra ett sämre slutresultat. Det är därför viktigt att förstå exempelvis hur så kallade neuroprotektiva (nervskyddande) mekanismer biologiskt är utvecklade efter nervskador. Skyddsmekanismen har ibland relaterats till uppreglering av ett speciellt ämne - Heat Shock Protein 27 (HSP27) - i den perifera nerven eller i nervtrådarnas cellkroppar i anslutning till ryggmärgen. Hur och när uppregleringen sker efter nervskador i relation till fördröjd kirurgisk åtgärd, särskilt hos individer med diabetes, är inte klarlagt.

Vid behandling av perifera nervskador har man hittills enbart fokuserat på kirurgin, men man har inte tillämpat någon farmakologisk behandling som tillägg för att förbättra slutresultatet. Det finns ett behov av att finna läkemedel som kan förbättra nervtrådsutväxt efter nervskada, men tiden från skadan till den kirurgiska reparationen/rekonstruktionen är också viktig eftersom nervens inneboende mekanismer för läkning riskerar att försämras vad tiden går.

I avhandlingen har jag undersökt:

1) betydelsen av uppreglering i nerverna av det skyddande ämnet HSP27 i samband med omedelbar eller fördröjd nervreparation,

2) hur modifiering av en tub för rekonstruktion av en nervskada kan förbättra nervregenerationen samt

3) hur en farmakologisk substans, som appliceras lokalt i samband med nervkirurgin, skulle kunna förbättra nervutväxt och reparationsmekanismer.

I studierna har jag använt friska råttor och en råttmodell som liknar typ 2 diabetes (så kallade Goto-Kakizaki råttor).

I delarbete ett har jag hos friska och diabetiska råttor studerat förekomst av HSP27 efter olika nervskador som inte reparerats eller som reparerats direkt eller med fördröjning. Studien visar att HSP27 var högre i oskadade nerver hos diabetiska råttor, troligen som en skadereaktion av diabetes, samt att nervfiberutväxten är mer begränsad hos diabetiska råttor efter nervreparationer. HSP27 ökade kraftigt i både den skadade nerven och i nervcellskropparna i anslutning till ryggmärgen som svar på skadan i både friska och diabetiska råttor. Det viktigaste fyndet var att ökningen

av HSP27 inte hade någon påverkan på nervfiberutväxten. En fördröjd nervreparation under kortare tid (få dagar) påverkar inte nervutväxt.

I delarbete två modifierade jag ett speciellt membran med små guld- och koboltoxidpartiklar (så kallade nanopartiklar) som skjutits in i nervtuber för att förbättra nervfiberutväxten. De modifierade tuberna jämfördes med tomma tuber som använts för att överbrygga en kortare nervdefekt hos friska råttor. Resultaten visar att ett membran i tuber medför en längre nervutväxten, dvs. ”bättre”, jämfört med en tom tub. Tillämpning av nanopartiklar i guld och koboltoxid i membranet ökade utväxtlängden ytterligare. Modifierade tuber uppvisade ett bättre svar hos stödjeceller, så kallade Schwannceller, samt ett högre HSP27 svar. Schwanncellerna i den nybildade nerven i tuberna var förknippade med nervfiberväxt, men HSP27 hade ingen påverkan på utväxten. Ett modifierat membran i en tub med guld- eller koboltoxidnanopartiklar kan förbättra nervåterbildningen.

I de två avslutande arbetena har jag använt en farmakologisk substans - PXL01 – som tidigare har använts kliniskt för att minska sammanväxningar i bukhålan efter bukkirurgi och i fingrar efter skador på böjsenor. I studien på böjsenor noterades tecken till bättre återskapad nervfunktion vid samtidigt förekommande nervskada på en känselnerv i ett skadat finger. Jag har undersökt om och hur PXL01 skulle kunna påverka nervfiberutväxten, dels vid direkt nervreparation (som i den kliniska studien), dels vid nervrekonstruktion med hjälp av nervgrafter. I den första studien jämfördes behandling med PXL01 i sin bärarsubstans (sodium hyaluronate) mot användning av enbart hyaluronate och koksalt (sistnämnda s.k. placebo-behandling). PXL01 minskade det inflammatoriska svaret men den hade inte någon effekt på nervfiberutväxt eller på andra celler. PXL01 minskar inflammationen utan att negativt påverka nervreparationsprocessen varför den kan användas som komplement till kirurgi för att behandla nerver som fastnat i ärr utan att den påverkar en eventuell restitution av nervfunktion.

I det sista delarbetet undersöktes effekten av PXL01 på nervfiberutväxt i ett nervgraft på friska och diabetiska råttor. Kortfattat sagt visar det sista arbetet att PXL01 kan öka nervfiberutväxten i nervgrafter efter nervrekonstruktion samt visar tecken till en nervskyddande funktion.

Sammanfattningsvis har min avhandling visat på intressanta neurobiologiska fynd som täcker flera olika aspekter av nervfiberutväxt och andra effekter på celler vid reparation och rekonstruktion av skadad perifer nerv hos friska och diabetiska råttor där resultatet kan direkt tillämpas inom klinisk verksamhet.

Summary in Turkish

Başta kollar ve eller olmak üzere, daha az oranda da bacaklardaki sinirler çeşitli nedenlerle hasar görebilir. Hasarın boyutu, küçük basınç hasarından, sinirin tamamen kesilmesi ve yırtılması şeklinde ciddi hasara kadar değişir. "Daha basit" sinir kopması durumunda, yaralanmalar, uçların birbirine olabildiğince yaklaştırılması ve küçük iplerle sabitlenmesi yoluyla doğrudan cerrahi olarak dikilebilir. Herhangi bir sebepten dolayı, sinir dokusunun kaybı durumunda, hasarlı sinir uçları arasında sıklıkla kusurlar meydana gelir ve bu da doğrudan onarımı imkânsız hale getirir. Sinir doku kaybının gerçekleştiği yaralanmalarda, en yaygın tamir yöntemi "daha az önemli" bir siniri "yedek parça" olarak kullanmaktır ve "Otolog sinir grefti tekniği" olarak adlandırılır. Otolog sinir grefti tekniğinde– (farklı seçenekler vardır), sinir yedek parçası; kopmuş sinirleri uzak sinir ucuna ve daha da aşağıya, deriye ve kaslara taşımak için bir "köprü" olarak kullanılır. Sinir doku kaybının daha kısa olduğu diğer durumlarda, biyolojik mekanizmaların sinirin kendisinin yenilenmesini kolaylaştırdığı farklı türlerde "tüpler" kullanılabilir. Bu tüplerin birkaç farklı ticari çeşidi mevcuttur. Mevcut hiçbir varyant yeterince iyi değildir çünkü tasarımları hâlâ hasarlı sinir uçları arasında yalnızca daha kısa mesafeleri kapatılabilmektedir. Klinik olarak kullanılabilecek iyileştirilmiş alternatiflerin geliştirilmesine ihtiyaç vardır.

Sinir hasarının tipine bağlı olarak, hasarın gecikmeden doğrudan onarılması veya yeniden yapılandırılması her zaman mümkün değildir. Tedavideki gecikme süresinin artması daha kötü sonuçlara yol açabilir. Sinir hasarı sonrası koruyucu (nöroprotektif) mekanizmalar devreye girer, özellikle sinirlerdeki özel bir maddenin – Isı Şoku Proteini 27 (HSP27) – arttığı bildirilmiştir. Yaralanmalardan sonra bu artışın nasıl meydana geldiği, gecikmiş cerrahi müdahaleyle ilişkisi açık değildir.

Periferik sinir hasarının tedavisinde bugüne kadar daha çok cerrahiye odaklanılmış ancak ilaç tedavilerine çok fazla odaklanılmamıştır. Sinir hasarından sonra sonuç hala iyi olmadığından, hasardan sonra sinir lifi büyümesini iyileştirebilecek ilaçların bulunmasına ihtiyaç vardır, ancak hasardan cerrahi onarım/rekonstrüksiyona kadar olan zaman perspektifi de önemlidir. Periferik sinir yaralanmasından sonra, uzakta kalan sinir ucundaki sinir lifleri yenilenir, daha sonra merkezi sinir ucundaki sinir liflerinin, eski bölgelerine kadar büyümeleri gerekmektedir. Büyüme hızı oldukça yavaştır (1-2 mm/gün), ve yaralanmanın uyluk veya alt bacak seviyesinde olması daha kötü sonuçlar doğurabilir. Sinir yaralanmasından sonra derinin ve kasların

tekrar uyarılması uzun zaman alabilir. Kaslarda daha fazla değişiklik (erime) meydana gelebileceğinden sonuç daha da kötüleşebilir. Bu nedenle sinir büyümesini uyarabilmek hem kas hem de duyu fonksiyonunun geri dönüşü açısından önemlidir. Tezimde, acil veya gecikmiş sinir onarımı ile bağlantılı olarak koruyucu HSP27 maddesinin sinirlerdeki artmış salınımının önemini, geliştirilmiş edilmiş sinir tüpünün sinir yenilenmesini nasıl iyileştirebileceğini ve bunun yanında lokal olarak uygulanan farmakolojik bir maddenin (PXL01) sinir yenilenmesi üzerine etkisini araştırdım.

Yeni cerrahi teknikler, cerrahi aletler ve farmakolojik maddeler geliştirilirken, bu yöntemlerin diyabette sinir gelişimini nasıl etkilediğinin de araştırılması önemlidir. Toplumda diyabet hastalığı yaygınlığı hızla artmaktadır ve çalışma hayatındaki diyabetli bireylerde sinir hasarı oluşma riski artmaktadır. Diyabette başka tür yenilenme mekanizmalarının olup olmadığını araştırmak önemlidir. Bu tez kapsamında kendiliğinden diyabet geliştiren ve kan şekerinde hafif artış olan Goto-Kakizaki sıçanlar ile sağlıklı Wistar sıçanlar kullandım.

Çalışmanın ilk bölümünde, hasar anında veya gecikmeli olarak onarılan veya hiç onarılmayan sağlıklı ve diyabetik sıçanlarda farklı takip süresi sonrası periferik sinirdeki HSP27 salınımını inceledim. Çalışma, diyabetik sıçanlarda hasar görmemiş siyatik sinirlerde muhtemelen koruma amacıyla HSP27'nin arttığını, sinir onarımı sonrasında diyabetik sıçanlarda sinir büyümesinin daha sınırlı olduğunu gösterdi. HSP27 hem sağlıklı hem de diyabetik sıçanlarda yaralanmaya yanıt olarak hem yaralı sinirde hem de omuriliğe bitişik sinir hücresi gövdelerinde büyük ölçüde arttı. Ancak en önemli bulgu HSP27'deki artışın sinir lifi büyümesi üzerinde hiçbir etkisinin olmamasıydı. Ayrıca; kısa süreli gecikme sonrası yapılan sinir onarımının sinir büyümesi üzerinde etkisi olmadığı görüldü.

Çalışmanın ikinci bölümünde, sinir büyümesini iyileştirmek için sinir tüplerinin içine altın ve kobalt oksit nanoparçacıkları emdirilen özel bir polimer zar yerleştirilmiş polimer yapıda tüpler kullandım. Bu geliştirilmiş tüpleri, sağlıklı Wistar sıçanlarda sinir doku kaybını kapatmak için kullanılan boş tüplerle karşılaştırdım. Sonuçlar, tüplerdeki zarın, boş tüple karşılaştırıldığında sinir büyümesini arttırdığını, hatta zardaki altın ve kobalt oksit nanoparçacıklarının, büyüme uzunluğunu daha da arttırdığını gösterdi. Geliştirilmiş tüpler aynı zamanda destek hücrelerinde (Schwann hücresi) olumlu tepkiye yol açtı ve HSP27 salınımını da arttırdı. Tüp içinde yeni oluşan sinir dokusundaki Schwann hücrelerinin sinir büyümesi olumlu etkilediği görüldü ancak HSP27 salınımın büyüme üzerinde herhangi bir etki oluşturmadığı tespit edildi. Sonuç olarak, altın veya kobalt oksit nanoparçacıkları içeren zar ile geliştirilmiş sinir tüpünün, sinir büyümesini iyileştirdiği tespit edildi.

Son iki çalışmada, daha önce karın ameliyatlarında ve fleksör tendon yaralanmalarından sonra parmaklarda, yapışıklıkları azaltmak için klinik olarak

kullanılan PXL01'i kullandım. Bu çalışmalarda, doğrudan sinir onarımında (klinik çalışmada olduğu gibi) veya sinir doku köprüsü (otogreft) ile tamir edilmiş sinir hasarında, PXL01'in sinir büyümesini nasıl etkileyebileceğini inceledim. İlk çalışmada, taşıyıcı maddesiyle birlikte (sodyum hiyalüronat) PXL01, tek başına hyaluronat ve tek başına serum fizyolojik kullanımı (plasebo tedavi) karşılaştırıldı. Çalışmada PXL01'in, iltihabi yanıtı azalttığı görüldü ancak sinir lifi büyümesi veya diğer hücreler üzerinde hiçbir etkisi olmadığı tespit edildi. Sonuç olarak, PXL01 tedavisinin sinir onarım sürecini olumsuz etkilemeden iltihabı azalttığı tespit edildi. Bu nedenle, lokal uygulanan PXL01 maddesinin sıkışan sinirleri tedavi etmek için cerrahi müdahalede tamamlayıcı olarak kullanılabileceği öngörüldü. Çalışmanın son bölümünde sağlıklı ve diyabetik sıçanlarda sinir doku köprüsü ile onarılmış sinir hasarında PXL01 tedavisinin sinir lifi büyümesine etkisini araştırdım ve bulgular PXL01'in otograft ile tamir sonrası sinir lifi büyümesini ve nöroprotektif fonksiyon belirtilerini arttırdığını gösterdi.

Özet olarak tezim, sağlıklı ve diyabetik sıçanlarda periferik sinirlerin onarımı ve yeniden yapılandırılması sırasında sinir lifi büyümesinin moleküler yönlerini ve hücreler üzerindeki etkilerini kapsayan; tıbbi klinik uygulamaya doğrudan katkı sağlayabilecek ilginç nörobiyolojik bulgular göstermiştir.

Introduction

The peripheral nervous system (PNS) is “the executive-productive” part of our body, which determines our functionality and daily activities. Any possible injury or disturbance in the PNS results in impaired function with a decreased social activity and productive ability of the individual (Dahlin & Wiberg, 2017; Krishnan et al., 2024). Different disorders, such as “chronic” nerve entrapment disorder, a nerve tumour and a variety of neuropathies, especially diabetic neuropathy are well-known to cause dysfunction of the peripheral nerves (Dahlin & Wiberg, 2017 ; Dillon et al., 2024; Eid et al., 2023; Elafros et al., 2022; Krishnan et al., 2024).

Traumatic nerve injuries, however, is a common cause of nerve injury, resulting in “acute” compression, transection, or laceration of the nerve trunk, with or without a subsequent nerve defect and is often accompanied by an altered functional capacity. Since these injuries and disorders cause impaired function of the extremities, an untreated nerve injury may severely decrease the functional capacity of the individual altogether. Recently, the reported incidence of peripheral nerve injuries in Europe was 11.2/100 000 population per year, and males were found twice more likely to have a nerve injury, and with individuals in the age span 15-45 years being most likely to be affected (Murphy et al., 2023). This in turn results in a high socioeconomic burden to the society (Bergmeister et al., 2020; Krishnan et al., 2024; Reitan et al., 2019; Rosberg et al., 2015; Rosberg et al., 2013).

To overcome the consequences of a peripheral nerve injury, treatment strategies, such as surgical and medical treatments, together with an intense rehabilitation program, play a crucial role and must be optimized in all details. A transection or a laceration injury of a nerve trunk, with or without a nerve defect, always requires a surgical intervention, usually by a primary nerve repair - *a gold standard intervention* - or a reconstruction using nerve autografts (Raza et al., 2020).

Note, that despite the best surgical technique and the enhanced rehabilitation program, functional outcome is overall still unsatisfactory with residual problems, such as decreased mobile functional capacity of the patient (Dahlin & Wiberg, 2017; Lanier et al., 2021; Lin & Jain, 2023; Reitan et al., 2019; Rosberg et al., 2015). This is the reason why it is of outmost importance to understand the mechanisms behind a peripheral nerve injury, as well as the alterations during degeneration as well as the nerve regeneration processes.

Results from clinical studies and trials demonstrate an suboptimal outcome of a peripheral nerve repair or reconstruction, especially regarding the treatment of nerve defects (Atzei et al., 2007; Isaacs et al., 2023; Tan et al., 2023; Thomson et al., 2022) and delayed nerve repairs or reconstructions (Birch & Raji, 1991; Jivan et al., 2009; Kim et al., 2018). However, many of these studies have limitations, such as limited number and heterogenous sampling of patients, presence of concomitant diseases, and a variety of different time aspects of the repair or reconstruction of the nerve injury.

In the present thesis, I have highlighted mainly four important issues that needs to be further addressed in treatment studies on peripheral nerve injuries: (1) the expression of the neuroprotective substance HSP27 in relation to no, immediate or delayed nerve repair or to nerve reconstructions, (2) the concept of using a modified conduit to bridge a nerve defect that needs to be bridged, (3) concomitant diseases like diabetes and (4) the use of pharmacological substances as an adjunct to surgery.

First; the concept of immediate, delayed or no nerve repairs is a major issue, since many patients have a risk of being surgically treated after a time period following a nerve injury for different reasons, e.g., the patient´s or the doctor's delay (Kim et al., 2018), which can have a negative effect on the functional outcome (Dahlin, 2013; Jivan et al., 2009). The diminished functional outcome is probably due to an altered nerve regeneration at the molecular and the cellular levels, especially the Schwann cell response, when treatment is delayed. Hence, attention is deserved to investigate any difference in molecular and cellular response/events, such as the expression of HSP27, between situations when a nerve is not repaired, when it is immediately repaired or repaired with a delay.

The second issue is the extent of the nerve tissue defect itself, because a nerve injury cannot be repaired under tension (Yi & Dahlin, 2010). In such situations, an autologous nerve graft is the best alternative to bridge a nerve defect. However, the limited amount of autologous nerve grafts and the risk of surgical site complications at the donor site have led to the need of using artificial nerve grafts (Isaacs et al., 2023; Thomson et al., 2022) or nerve conduits for short defects (Thomson et al., 2022). Processed nerve allografts (PNA), which are decellularized nerve grafts from dead donors, have been developed and used clinically, but the use has limitations due to biological reasons (Frostadottir et al., 2023). Several biological or bioartificial nerve conduits are also in clinical usage, but clinical trials showed a low overall proof of evidence, and such conduits may be only applicable for short defects (Thomson et al., 2022). Therefore, there is a tremendous amount of documentation with different bioengineered conduit models, including modified with pharmacological agents, to enhance the nerve regeneration.

The third issue in treatment of a peripheral nerve injury is the patients with a concomitant disease, like diabetes, which potentially may impair nerve regeneration. Other laboratories as well as the Dahlin´s laboratory have increased

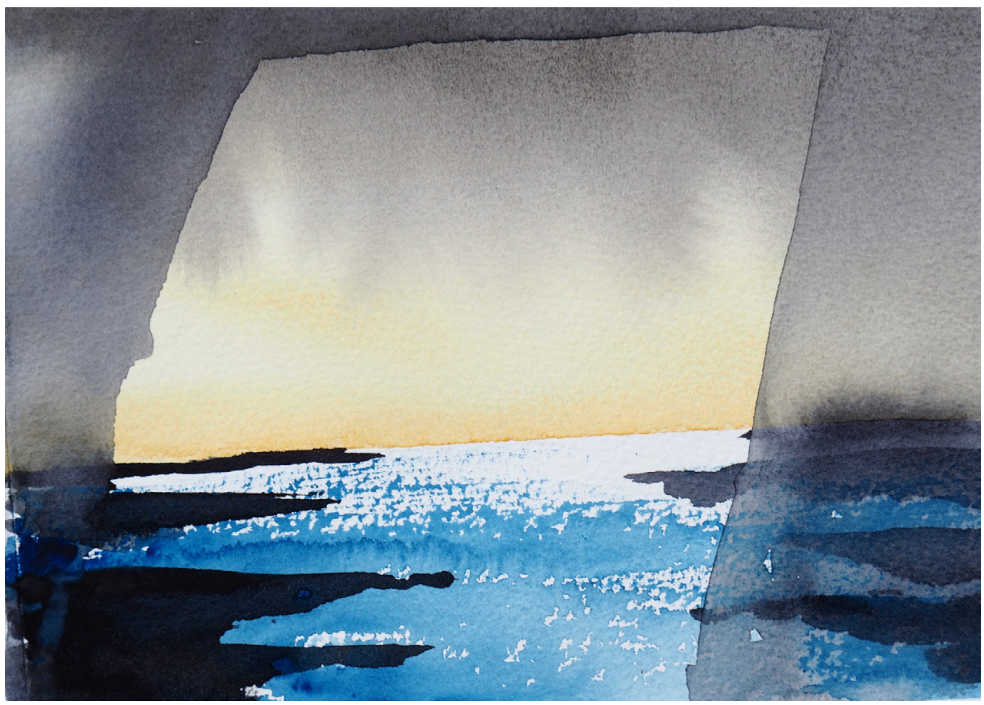
the knowledge about nerve regeneration based on experimental studies on diabetic rats (Stenberg & Dahlin, 2014; Stenberg et al., 2016; Stenberg et al., 2017). However, the knowledge from clinical studies is yet scarce. Patients with diabetes show substantial changes in nerve degeneration and regeneration, particularly in type 1 diabetes (Dahlin, 2023; Mohseni et al., 2017; Osman et al., 2015). In diabetes, the altered "microenvironment" with continuously disturbed blood glucose levels can further affect nerve regeneration in an already disrupted axonal continuity. Several experimental studies are available with particular interest in molecular and cellular mechanisms in nerve regeneration in diabetes. Streptozotocin has been used to induce diabetes in rats, with an induced high blood sugar level, which is not replicating the clinical situation well (Qamar et al., 2023; Salles et al., 2013; Talukdar & Basumatary, 2023). To mimic the clinical setting of diabetes with moderate blood glucose levels, genetically developed Goto-Kakizaki (GK) rats seem to be a more suitable animal model (Dahlin, 2023; Talukdar & Basumatary, 2023). The similarity with the effect on the human beta cells, compared to those cells in other rodent models, is an unique characteristic of GK rats (Stenberg & Dahlin, 2014), making the model valid also for studies on the peripheral nervous system. Thus, it is crucial to examine any novel devices or pharmacological substances also in experimental models using appropriate rat models with diabetes.

Issue four includes the concept that despite the perfected surgical technique and the adequate timing of the nerve repair or reconstruction of the injured nerve, there is still need for an additional medical treatment that enhance nerve regeneration. There have been attempts with different pharmacological agents described in the literature, such as hyaluronic acid (Atzei et al., 2007), and platelet rich plasma (S. L. Wang et al., 2023; Yadav et al., 2022) in both clinical and experimental studies with promising results. PXL01, clinically used in flexor tendon injuries to prevent adhesions, has been evaluated previously in a clinical trial that showed a better sensory nerve function in the patients with a concomitant repaired digital nerve injury (Wiig et al., 2014). This information draws my attention to a hypothesis that application of a substance, like PXL01, directly is beneficial for nerve regeneration after nerve injury and repair.

The ***overall goal*** of present thesis is to contribute to the advancement of the field of peripheral nerve regeneration after injury by addressing three issues as indicated above: ***1) the role of nerve repair and timing of surgery in relation to expression of HSP27; 2) the potential use of a novel and modified scaffold containing nanoparticles and; 3) the capability of the lactoferrin-derived peptide PXL01 to improve nerve regeneration in healthy and in diabetic rats; the latter acting as a rat model of type 2 diabetes.***

The specific aims of my thesis include:

- investigating nerve regeneration after no, immediate, and especially a “delayed” nerve repair with the relation to the potential impact of the neuroprotective substance Heat Shock Protein 27 (HSP27) on axonal outgrowth. This substance is induced in an injured nerve and in DRG in healthy and diabetic rats, and is also important for preservation of nerve function in diabetic neuropathy (Pourhamidi et al., 2011).
- evaluating axonal outgrowth and Schwann cell response after bridging a nerve defect with a modified conduit that is enhanced with an inserted membrane with nanoparticles.
- finally, focus on axonal outgrowth, Schwann cell response, and especially the macrophage reactions after local application of PXL01 in a primary nerve repair model and in a nerve reconstruction model with a nerve autograft; the latter in both healthy rats and in rats with diabetes using the appropriate GK rat model.



Malmö, 2024

Background

The nervous system, known as the “conductor” of the body, is composed of two parts: the central and the peripheral nervous systems (CNS and PNS). The CNS includes the brain and the spinal cord (Geuna et al., 2009; Krishnan et al., 2024). The PNS, as the name implies, extends from the CNS as cranial and spinal nerves, expands throughout the body and the nerve ends reach almost all parts of the body. Although the two systems together form the nervous system, the CNS and PNS have anatomical, histological, functional, cellular, and molecular differences that subsequently lead to distinguished nerve regeneration processes.

Anatomy of the peripheral nerve

The peripheral nerves are the nerve trunks - the extensions of the CNS and formed by the spinal nerves. Peripheral nerves are mainly divided into two types: somatic (sensory, motor, or mixed nerves) and visceral autonomic nerves. The somatic motor nerve fibres originate from the somatic motor neurons in the ventral horn of the spinal cord and perform execution of the voluntary function of the extremities. They reach directly to the target muscle without any synapses, which means that they need to encounter a long distance in the body. The somatic sensory nerve fibres, on the other hand, carry information from the periphery to the central nervous system, and have their neurons in the dorsal root ganglion (DRG) that is located just outside the spinal cord on the designated level (Geuna et al., 2009). However, the visceral autonomic nerves consist of sympathetic and parasympathetic nerve fibres that regulate involuntary physiological processes, including the cardiovascular, respiratory, and gastrointestinal systems. Their neuronal cell bodies are located in the CNS (preganglionic neurons) along the sides of the spinal column (postganglionic sympathetic neurons) and closer to the target tissues (postganglionic parasympathetic neurons) (Waxenbaum et al., 2023).

Independent of type of nerve, there is a constant communication between the nerve cell body and the axon, and their terminals, through proteins transported by mostly anterograde transport, i.e., information carried out from the nerve cell body to the axon, or to a substantial extent by retrograde transport, i.e., from the periphery to the nerve cell body; thus being crucial in initiation of the nerve regeneration process after injury.

The rat sciatic nerve, used in the present system in healthy Wistar and diabetic GK rats, is composed of nerve fibres from the L4, L5 and L6 spinal nerves, but most predominantly the L4 and L5 spinal nerves; L6 spinal nerve contributes only to 10% of the nerve fibres (Delibaş & Kaplan, 2024; Schmalbruch, 1986). It is a mixed nerve, composed of both somatic sensory and motor fibres that coordinates the voluntary movement of the lower extremity (Schmalbruch, 1986). The sensory neurons therefore are located in the DRG of the lumbar 4 to 6, predominantly in the lumbar 4 (L4) and 5 (L5) DRG (Asato et al., 2000). The analysis in this thesis, and in many published studies (Geremia et al., 2010; Z. Wang et al., 2023), therefore focuses on evaluation of events in relation to nerve regeneration in L4 and L5 DRG (Figure 1A).

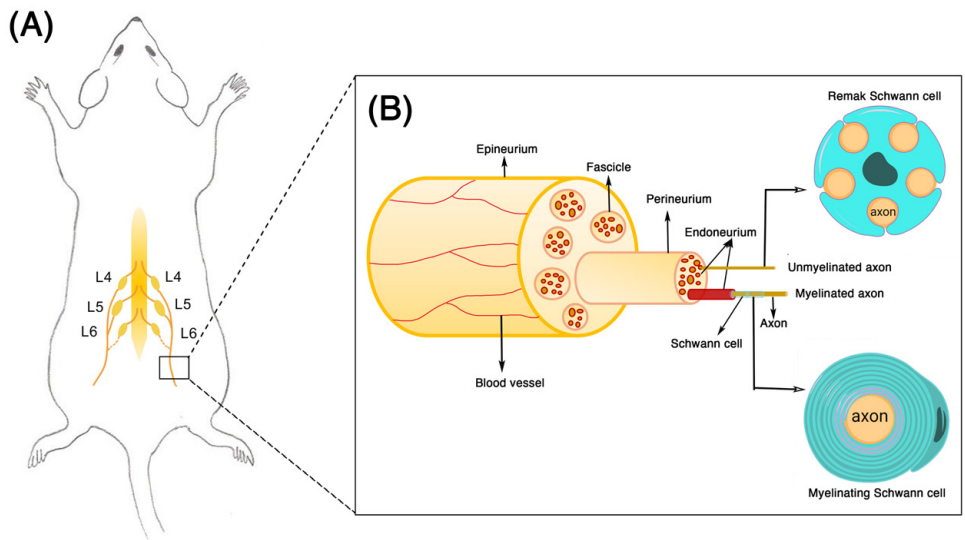


Figure 1. (A) Schematic drawing of the location of used DRGs and the possible DRG variation in rats. L6 spinal nerve contributes only to 10% to the formation of sciatic nerve (showed with dashed line). **(B)** Schematic drawing of the components of the peripheral nerve and the nerve fibre anatomy with myelinated and unmyelinated nerve fibres in relation to the Schwann cells.

A peripheral nerve is composed mainly of a variety in numbers of axons, being myelinated or unmyelinated, that forms the main functional components of the nerve trunk (Dahlin & Wiberg, 2017; Krishnan et al., 2024) (Figure 1B). Myelinated nerve fibres consist of a single axon that is wrapped by a myelin sheet formed by the Schwann cell membrane and cytoplasm (Berthold & Rydmark, 1983). Electrical signalling through the axon is conducted with the help of myelin, and myelin optimizes the velocity of conduction between the nodes of Ranvier. On the other hand, unmyelinated nerve fibres have several axons that are enclosed by one Schwann cell [i.e., Remak cells (Harty & Monk, 2017; Jessen & Mirsky, 2016,

2019)] (Figure 1B). The myelinated and unmyelinated nerve fibres gather around in a connective tissue - so called endoneurium - in the endoneurial space that contains collagen fibres, fibroblasts, macrophages, and mast cells together with a rich network of capillaries. Within this endoneurial space, collagen fibres are grouped around each nerve fibre forming a special endoneurial tube with a basement membrane along its course in the nerve trunk. Several nerve fibres, together with the endoneurial tube and the content of the endoneurial space, form a nerve fascicle together with the surrounding dense connective tissue – perineurium – consisting of flattened cells with certain characteristics (Dahlin & Wiberg, 2017; Krishnan et al., 2024). Several fascicles are then gathered in a loose connective tissue – epineurium – forming at last the nerve trunk. The epineurium contains a rich network of blood vessels where some vessels pierce obliquely through the perineurium to approach the endoneurial capillaries (Figure 1B). Finally, a thin membrane-like layer surrounds the peripheral nerve like the mesentery in the abdominal cavity – the mesoneurium – in which the segmentally approaching blood vessels are located (Sunderland, 1951).

Cells in peripheral nerve of relevance for nerve injury and regeneration

There are several cell types in the peripheral neve trunk that play a key role in nerve injury and nerve regeneration; two of them in particular of interest in the analysis in this thesis. The first cell type is the supporting microglial cell that are called Schwann cells. This cell type has a unique plasticity that enables it to differentiate from a progenitor stage; i.e. the so-called Remak cell to a myelin-producing Schwann cell or to a Schwann cell with repair-promoting properties after an environmental signal, like a nerve injury (Harty & Monk, 2017; Jessen & Mirsky, 2016).

The second cell type is the macrophage, which can be historically divided into two categories: a) resident and b) invading macrophages. Tissue-resident macrophages are present in peripheral nerves under homeostatic conditions and can proliferate, migrate, and produce chemokines following an injury. They are further divided into epineurial and endoneurial macrophages and have different characteristics (Ydens et al., 2020). After axotomy, endoneurial resident macrophages secrete chemo-attractants for the blood born monocytes, and this blood born monocytes then differentiate into invading macrophages that are recruited to the injured nerve. In contrast, epineurial macrophages are not active during the injury or post-injury processes (Cattin & Lloyd, 2016; Giordano & MacDonald, 2021).

The peripheral nerve in diabetes

Diabetes is one of the most common diseases world-wide and is basically divided is type 1 and type 2 diabetes (Feldman et al., 2019; Gregory et al., 2022; Xu et al., 2018). Type 1 diabetes is an autoimmune disease, defined by a total insulin deficiency, whereas type 2 diabetes is characterized by an insulin resistance with other pathophysiological mechanisms involved; both conditions are associated with hyperglycaemia, but a risk of hypoglycaemia may occur as well (Alwafi et al., 2020). Diabetes, overall, is a systemic disease that affects many tissues in the body, such as eyes, kidneys, the cardiovascular system and, as relevant for the present thesis, the PNS. Diabetes targets the entire neuron, particularly the sensory neurons, the Schwann cells and the microvascular system in the PNS, causing neuropathy, i.e., diabetic neuropathy (Feldman et al., 2019). The pathophysiology of diabetic neuropathy, affecting the peripheral nerve particularly in the lower extremity, is complex and differs between type 1 and type 2 diabetes, where hyperglycaemia (particularly crucial in type 1 diabetes) together with hyperlipidaemia/dyslipidaemia (added factors in type 2 diabetes) may induce microangiopathy. Diabetic neuropathy can be "aggressive" in type 1 diabetes, but the condition is often present in around half of the affected individuals of type 2 diabetes at diagnosis. The increased glucose level leads to increased reactive oxygen species (ROS), which subsequently damage mitochondria in Schwann cells and specifically the sensory neurons in DRG (Fernyhough, 2015; Fernyhough & McGavock, 2014). Besides mitochondrial damage, increased glucose levels cause glycation of the structural and functional proteins, which results in production of the advanced glycation end products (AGE). These glycated proteins directly interact with AGE specific receptors, leading to increased proinflammatory molecules and free radicals. In parallel, hyperlipidaemia with excessive free fatty acids causes increased production of ROS that injures Schwann cells. This reaction then activates macrophages to produce cytokines (Padilla et al., 2011), resulting in Schwann cell injury (Goncalves et al., 2017). Furthermore, ROS oxidized-low density lipoproteins (LDL), together with excessive cholesterol, activate signalling cascades and causes nuclear DNA degeneration, which in turn leads to additional ROS accumulation and finally resulting in progressive nerve injury, i.e., the diabetic neuropathy (Cotter & Cameron, 2003; Keller et al., 1999; Vincent et al., 2007).

Diabetes causes microcirculatory dysfunction

A diabetic nerve is depicted by dysfunction of the endothelium with abnormalities of the endoneurial capillaries that has thickened basement membranes with reduced endoneurial perfusion, and therefore a risk for ischemia (Eid et al., 2023). The most characteristic changes in the microcirculation in patients with diabetes is the reduction of the capillary size and thickened basement membrane (Hile & Veves, 2003). This microcirculatory dysfunction leads to reduced endoneurial prefusion

and in the end ischemic changes in the nerve (Feldman et al., 2017). In response to the ischemic changes in the peripheral nerve, endoneurial capillary density increases in the peripheral nerves in diabetes (Kim et al., 2012; Thrainsdottir et al., 2003). In diabetic animal models, there is an impaired endoneurial blood flow due to poor vasodilation of epineurial arterioles (Coppey et al., 2006). Another microvascular feature of diabetic neuropathy is activation of protein kinase C (PKC) that is induced by the hyperglycaemia, which causes a subsequent impact on expression of vascular endothelial growth factor (VEGF), a promotion of vasoconstriction, and induction of hypoxia (Mizukami & Osonoi, 2021). Diabetes can also lower the levels of neurotrophic and angiogenic factors, such as insulin like growth factor (IGF-1), VEGF and nerve growth factor (NGF) (Schratzberger et al., 2001). In an experimental study with diabetic rats, administration of VEGF resulted in an increased nerve conduction velocity and an increased number of small arterioles that supplies the peripheral nerves (Schratzberger et al., 2001).

These molecular and cellular changes in diabetic neuropathy cause complex alterations after an additional peripheral nerve injury and subsequent nerve degeneration and regeneration. It is expected that nerve regeneration is diminished in individuals with diabetes. To understand and explain the mechanisms, several studies have been published. Studies that focus on cellular and molecular response to a nerve injury and subsequent repair or reconstruction, especially related to delayed surgical procedures, are limited, despite recent published studies using the Goto-Kakizaki rat model [see below (Stenberg & Dahlin, 2014; Stenberg et al., 2016; Stenberg et al., 2017)]. The present thesis focuses on the neuronal, axonal and Schwann cell responses, specifically HSP27 expression, to a repaired or reconstructed nerve injury, including a delayed nerve repair, in healthy Wistar rats as well as in diabetic GK rats; the latter having moderately increased glucose levels.

Animal models of diabetes

Diabetes may cause excessive damage to the peripheral nerve, including axons, neurons and Schwann cells together with endothelial damage as discussed above, which leads to a generally irreversible neuropathy. To explore and understand these complex cellular and molecular cascades, access to experimental model(s) that mimics human diabetic neuropathy is essential (M. Yorek, 2022). The hitherto used diabetic rodent models manifest early stages of diabetic neuropathy, and lack more advanced consequences, such as decreased glomerular filtration rate that causes renal dysfunction (Biessels et al., 2014; Talukdar & Basumatary, 2023). Although in theory, it seems impossible to have the exact similar diabetic model to mimic the human situation, several animal models have been successfully used for translational studies (Yorek, 2016; M. A. Yorek, 2022).

There are experimental studies that focus on the cellular and molecular response to nerve injury and models with different repair and reconstruction techniques in

mostly drug-induced diabetes in rats and mice (Talukdar & Basumatary, 2023), but also models with spontaneously developing diabetes with moderate or high blood glucose levels are available (Talukdar & Basumatary, 2023). Here, a description of the diabetic rat models is provided since one of the models is used in the present thesis due to its applicability in peripheral nerve regeneration. Streptozotocin is an alkylating agent and a broad-spectrum antibiotic that causes the destruction of pancreatic β-cells and is hence widely utilized to secondarily induce diabetes that generally resembles type 1 diabetes in rodent models. A single high dose administration of streptozotocin or multiple administrations of a low dose streptozotocin create type 1 diabetes (Yorek, 2016). This former model with a high single dose leads to high blood glucose levels and the rats develop a cachectic condition and therefore needs insulin treatment during the treatment period (Biessels et al., 2014). The multiple dose regime partially damages pancreatic beta cells, causes insulin deficiency, and resembles more of a clinical picture of type 1 diabetes. Due to relative high levels of glucose and the need for insulin treatment, these models are not completely suitable for investigating events after a peripheral nerve injury and subsequent regeneration. Streptozotocin administered with nicotinamide or with high fat diet can produce type 2 diabetes with stable and moderate hyperglycaemia and these models are useful for evaluating anti-diabetic agents (Biessels et al., 2014).

Transgenic Cyp1a1mRen2-Fisher rat is a genetic model that mimics type 1 diabetes with similar conditions (Talukdar & Basumatary, 2023). The bio-breeding rats is another genetic model that mimics untreated diabetes type 1 with high blood glucose levels, which may require daily insulin injections for optimal survival. This condition makes them unsuitable as a peripheral nerve injury and repair or reconstruction model, although there are studies investigating short term cellular responses in a nerve injury and repair model (Stenberg et al., 2012).

Rodent models with moderate blood glucose levels are more suitable for studying nerve regeneration mechanism(s) in relation to surgical procedures in diabetes. Zucker rats, fatty or diabetic or obese, have diabetic type 2 conditions. However, they may have polyphagia, be obese and can even have hypertension, which is another pathophysiological mechanism in peripheral nervous disorders in diabetes. This type of diabetic rodents is more optimal for evaluation of anti-obesity drugs, because the rats have altered lipid levels in the blood (Talukdar & Basumatary, 2023).

Goto-Kakizaki (GK) rats are spontaneous polygenic rats, which develop a diabetes that resemble type 2 diabetes (Janssen et al., 2004; Portha et al., 1991; Talukdar & Basumatary, 2023). The model was developed in Japan by selective breeding of Wistar rats having the highest glucose intolerance in glucose tolerance tests over many generations (Portha et al., 1991). From already an early age (after 4 weeks), they start to exhibit elevated plasma glucose levels and insulin resistance, and especially female rats have a rather stable and moderate high glycaemic level

compared to male rats (Díaz et al., 2019; Östenson et al., 1993). These rats are stated to be a good candidate to study early stages of diabetic neuropathy (Sato et al., 2003). The GK rat develops a moderate hyperglycaemia but does not exhibit hyperlipidaemia, obesity, or hypertension, which makes the model suitable to analyse nerve regeneration since the younger human population that is affected by a nerve injury usually lacks concomitant diseases, such as hyperlipidaemia, obesity and hypertension. Based on these described properties of GK rats, female GK rats were chosen in the present thesis to study the mechanisms, as axonal outgrowth, Schwann cell response, presence of neuroprotective activity as well as macrophage responses, after the various nerve injury and repair or reconstruction models.

Neuropathy in diabetes

The most prevalent complication in diabetes is damage to the peripheral and autonomic nervous systems caused by the focal or diffuse nerve damage that may affect almost half of the diabetic population (Callaghan et al., 2015; Feldman et al., 2019). It is estimated that without proper treatment, among an estimated total population of 9.7 billion individuals worldwide in 2050, approximately one third of the population is expected to get a diagnosis of diabetes with subsequent complications, such as half of them having diabetic neuropathy (Boyle et al., 2010; Imperatore et al., 2012; Lovic et al., 2020). The name of the neuropathies is related to their location, i.e., autonomic neuropathies, such as gastrointestinal neuropathy or cardiac autonomic neuropathy, focal neuropathy, such as mononeuropathy or radiculopathy, or the most common form – the distal symmetric neuropathy, with stocking and glove distribution type of sensory impairment due to involvement of long nerves (Feldman et al., 2019). It is described as a “dying back” phenomena, in which the longest sensory axons are affected with relative preserved cell bodies. As described above, hyperglycaemia and hyperlipidaemia, via the ROS pathway, cause alterations in Schwann-cell-axon transport, alterations in protein expression in DRG and eventually demyelination and degeneration of the nerve fibres. Schwann cells not only lose their capacity to provide energy to axons, but also transfer harmful products to the axons (Eid et al., 2023). In more advanced diabetic neuropathy, axonal damage also triggers cascades in neurons that in turn cause irreversible damage to the nerve (Raghav et al., 2022). The order of damage to the Schwann cells, axons or cell body is not known (Feldman et al., 2019; Feldman et al., 2017).

Today, for nerve damage caused by diabetic neuropathy or even any type of a traumatic injury, the possibility of successful outcome after repair is expected to be low. With the increasing incidence and prevalence of diabetes and the increased living expectancy in the population, the mechanism(s) behind the cellular and molecular changes after nerve injury, degeneration and regeneration have become crucial to understand (Haastert-Talini & Dahlin, 2018), where evaluation of novel surgical and pharmacological treatment strategies have to be investigated also in

diabetic models. Experimental diabetic animal studies show dysfunction of neurons and Schwann cells together with unfavourable distal environment for axonal regeneration with risk for impaired axonal outgrowth depending on the model (Sango et al., 2017). However, with the evolving experimental diabetic animal models and still the lack of definitive treatment for the diabetic neuropathy, there is a need for further studies to understand the cellular interaction in diabetes with concomitant nerve injury and repair or reconstruction. In the present thesis, I have focused on axonal outgrowth, Schwann cell and macrophage responses and HSP27 expression as a sign of neuroprotection in different surgical models in the diabetic GK rats compared to healthy rats.

Peripheral nerve injury and mechanisms in nerve regeneration

A nerve can be injured for many reasons, such as by mechanical, ischemic, thermal, or chemical trauma. Trauma is mostly found in individuals between 15-45 years of age, and males are twice more likely to experience a nerve injury (Murphy et al., 2023). The variety of cells and tissue components in a peripheral nerve can thereby be affected, particularly the most important functional cells – the axons and the Schwann cells. To grade the dysfunction of an affected peripheral nerve, different classification systems have been used over the years. In 1943, Seddon introduced a classification system that categorizes the nerve injuries into three parts according to presence of nerve continuity (Seddon, 1942) (Table 1).

Table 1: Nerve injury classification according to Seddon, Sunderland and MacKinnon.

SEDDON	SUNDERLAND	MACKINNON	FEATURES
Neurapraxia	Type I		With or without myelin damage, but axons and connective tissue are intact
Axonotmesis	Type II		Axonal damage but intact connective tissue.
Axonotmesis	Type III		Axonal damage and injury to endoneurium but intact epineurium.
Axonotmesis	Type IV		Damage to axons, myelin, and the endo- and perineurium. Epineurium is intact.
Neurotmesis	Type V		Complete division of all elements, including epineurium => the nerve is divided.
		Type VI	Combination of any of Sunderland I-V

Neurapraxia consists of a diminished conduction velocity, leaving the axons intact, which in the end recovers without any surgical intervention.

Axonotmesis is defined as injured axons with loss of its continuity but with intact epi- and perineurium, and endoneurial tube. The function usually recovers naturally through axonal outgrowth along the previous endoneurial tube with its basement membranes.

Neurotmesis is the complete nerve transection that needs repair or reconstruction by surgical intervention.

Sunderland in 1951 (Sunderland, 1951), and with a later modification by MacKinnon et al [type 6; (Mackinnon, 1989)], expanded the classification into five sections based mostly on histological findings (Table 1).

The type I injury represents an ischemic injury that may or may not have segmental myelin damage with intact axons and connective tissue (similar as neurapraxia). In this type of injury there may be a diminished segmental conduction velocity. A type II injury is characterized by an axonal disruption but with intact connective tissue sheaths in which the lesioned axons undergo Wallerian degeneration but eventually fully regenerate (like axonotmesis). A type III injury represents axonal interruption that includes injury to the endoneurium with intact epi- and perineurium. The recovery is quite slow and may need surgical intervention. A type IV degree injury is characterized by damage to axons, myelin, endoneurium and perineurium, leaving only the epineurium intact. This type of injury requires surgical intervention. Finally, a type 5 (neurotmesis) is the nerve injury in which the nerve is transected (injury to axons and all the tissue components). MacKinnon et al, in 1989 (Mackinnon, 1989), introduced the sixth level injury to the Sunderland classification that is the combination of one or two subtypes of previously described nerve injuries (Table 1).

The present thesis focuses on a transection injury, with complete axonal disruption with or without a nerve defect, where different repair and reconstruction models have been applied to answer the presented hypotheses.

Unlike the CNS, the milestones of the nerve injury and nerve regeneration are well documented in the literature and are mostly directed to enhance the nerve regeneration (Cattin & Lloyd, 2016). There are several topics that explains the response to the nerve injury: a) Wallerian degeneration (also known as the distal axonal degeneration), b) cellular reprogramming of the distal nerve end, c) filling the nerve gap irrespective of length (including formation of a nerve bridge), d) changes in the proximal nerve end, and e) neuronal response and sprouting from the injured axon (Krishnan et al., 2024).

Wallerian degeneration and cellular reprogramming in distal nerve end

After a nerve transection, the distal part of the axon in the distal nerve end, which is separated from the proximal nerve end and thereby from the nerve cell body, undergoes Wallerian degeneration; named after the researcher Augustus Waller, who in 1851 transected glossopharyngeal and hypoglossal nerves of the frog (Waller, 1851). Wallerian degeneration involves demyelination and fragmentation of the axon distal to the nerve injury that starts approximately 24 hours after the injury (Krishnan et al., 2024; Zhao et al., 2022). Schwann cells are thought to provide axons with metabolic and trophic support, and they exhibit plasticity, which is of outmost importance in nerve regeneration. Their most important stimuli are the axons (Jessen & Mirsky, 2016, 2019). The Remak and the myelinating Schwann cells in the distal nerve end lose the contact with the axons and the latter change their genetic code from myelin-forming cells to regenerating-repair Schwann cells (Jessen & Mirsky, 2016). These repair Schwann cells rely on the extracellular signal regulated kinase pathway (Gomez-Sanchez et al., 2017; Tiong et al., 2020), which is orchestrated by phosphorylation of c-jun (Gomez-Sanchez et al., 2015). Phosphorylation leads to down-regulation of myelin producing genes and up-regulation of the growth-promoting genes (Gomez-Sanchez et al., 2015; Wang et al., 2015).

The repair cells have several functions, which are basically controlled by a set of repair-supportive characteristics:

i) together with the resident macrophages, the repair Schwann cells phagocyte the axon and myelin debris (Bosch-Queralt et al., 2023; Cattin & Lloyd, 2016);

ii) the repair Schwann cells secrete cytokines, such as Tumour Necrotising Factor-α (TNFα), Interleukin-1α (IL-1α), Interleukin-1ß (IL-1ß), and Leukemia Inhibitory Factor (LIF) (Martini et al., 2008), and recruit immune cells, such as neutrophiles and macrophages from the blood stream;

iii) they promote survival of the injured neurons and axonal elongation by up-regulating factors, such as Glial cell line-Derived Neurotrophic Factor (GDNF), Brain Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT3), and NGF (Martini et al., 2008; Wood & Mackinnon, 2015);

iv) the Schwann cells multiply and reach to three-fold elongation to form bands of Büngner that are essential for guiding the axons to their targets.

An interesting study that planned to block the Wallerian degeneration with the intention to slow down the degeneration process has been performed with Wld^s mice in which the Wallerian degeneration is significantly delayed (Lunn et al., 1989). The authors found that degeneration was prominently delayed with no hinder in nerve regeneration (Lunn et al., 1989) but in the end the axons in these mice still degenerate after 15-25 days (Beirowski et al., 2005). A study from our laboratory

has also shown that axonal outgrowth can be impaired in nerve autografts after nerve reconstruction, where the invasion of cells and macrophages, as well as the myelin breakdown, into the nerve graft before surgery was prevented (Dahlin, 1995).

Regenerative alterations at site of lesion

At the same time as the Wallerian degeneration in the distal nerve end occurs, the proximal end starts to degenerate in somehow backward called retrograde degeneration (Geuna et al., 2009). Axons retract up to the first node of Ranvier and leave some part of the endoneurial tubes empty (i.e., the basal lamina of the Schwann cells) (Geuna et al., 2009). Within hours after injury, the axons in the proximal nerve end produce a great number of collateral and terminal sprouts that advance distally along the tube on the inside of the basal lamina (Cajal, 1928; Dahlin, 1995). With the orchestrated function of Schwann cells, macrophages, and their secretion of various growth factors, and with the crucial impact of the nerve cell body, axonal outgrowth is promoted by the formation of sprouts from the injured axons with their growth cones on the tip that “palpate the environment” through the filopodia. By these structures, axonal outgrowth is supposed to reach to the distal nerve end across the nerve gap, through a formed fibrin matrix between the nerve ends, and down to the target. Axons are known to regenerate at a speed of 1-3 mm/day in humans, which can be higher in rats (Danielsen et al., 1995). The summary of Wallerian degeneration and regenerative alterations are summarized in Figure 2.

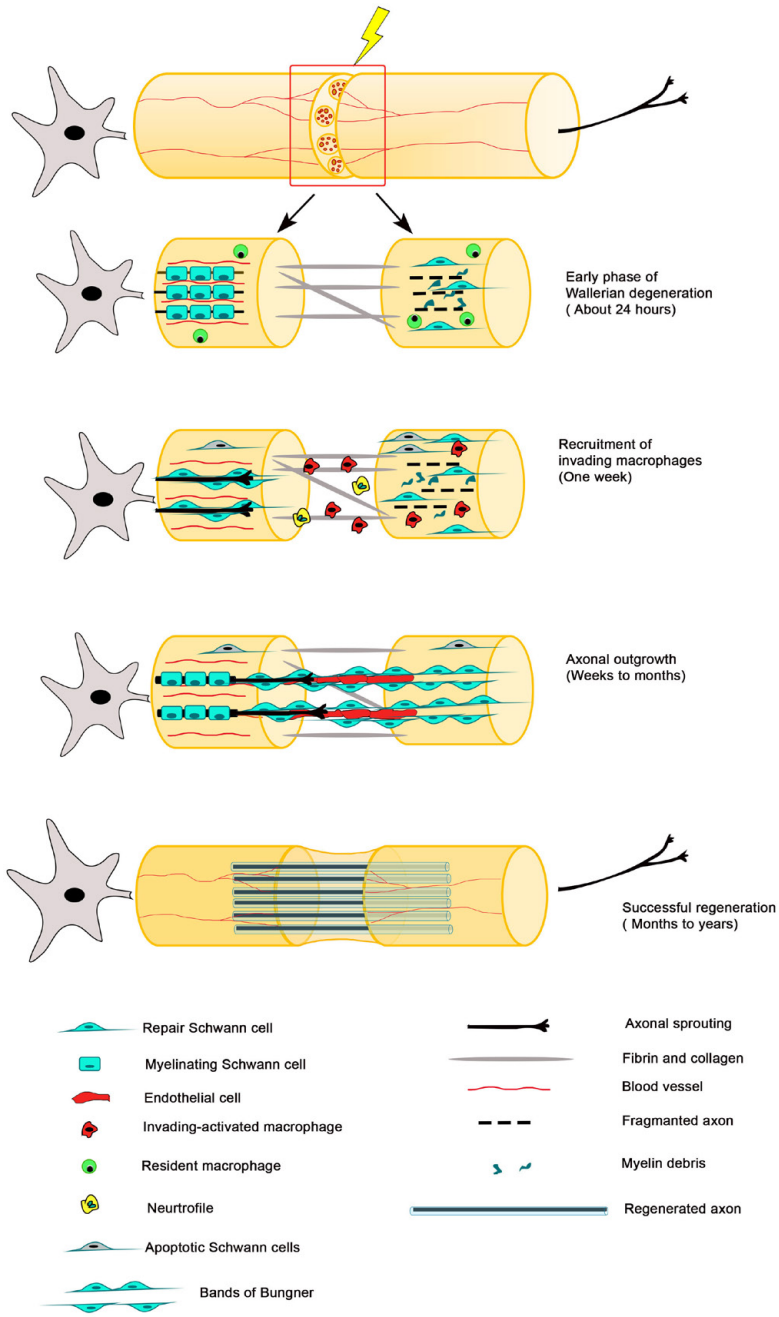


Figure 2: Shematic illustration of nerve degeneration and regeneration and the involved cellular interactions after nerve injury.

Filling the gap between nerve ends and regenerative alterations

After a transection injury to the nerve, the gap between the proximal and distal nerve ends, if not too long (Zhao et al., 1993), is initially filled with a fibrin matrix. First, the tissue resident macrophages are present in this fibrin matrix and then follow the blood recruited macrophages. Because this gap is an hypoxic environment, macrophages secrete angiogenetic factors, such as VEGF, and this in turn facilitate the endothelial cell recruitment and blood vessel formation (Jessen & Mirsky, 2016; Min et al., 2021; Pan et al., 2020). These blood vessels become polarized and play a crucial role for Schwan cell migration and alignment along the axis. In turn, they interact with fibroblasts and form cellular cords, along which axons regenerate (Jessen & Mirsky, 2016; Min et al., 2021; Pan et al., 2020). Then, the new extracellular matrix is formed, containing the growth-promoting substances, like laminin, fibronectin, and galectin-1 as well as specific collagens, such as collagen type I, III and IV (Gonzalez-Perez et al., 2013). The collagen subtypes are produced from the proximal nerve end and thought to be important for the mechanical support for the outgrowing axons (Koopmans et al., 2009). Especially type IV collagen promotes adhesion and migration of Schwann cells and axonal outgrowth (Yu et al., 2023). These Schwann cells proliferate and realign forming the bands of Büngner.

The Schwann cells continue to participate in recruitment of macrophages from the blood stream, which maximizes around 4-7 days after the nerve injury. These macrophages then polarize and form M1 macrophages - so called “pro-inflammatory” that promote degeneration - and M2 macrophages that can be characterized as “anti-inflammatory or pro-regenerative (healing)” that support regeneration. Macrophages are an additional source for growth factors for axons and the non-neuronal cells, i.e., mainly Schwann cells, in the regenerative milieu (Zigmond & Echevarria, 2019). Details about the function of macrophages in nerve degeneration and regeneration are described below. Macrophages are crucial for intraneural blood vessels since they secrete e.g., VEGF, which induces formation of new blood capillaries (Cattin & Lloyd, 2016). These capillaries not only bring blood supply to the milieu but, also by physically aligning with Schwann cells, guide the outgrowing axons to the distal nerve end (Cattin & Lloyd, 2016; Krishnan et al., 2024).

The reaction of the nerve cell body

In the meantime, the axon, and its nerve cell body, rapidly receives information about the injury. After the injury, there is a rapid influx of Ca^{2+} ions into the axons with a stop in transport of proteins within the axons. This information is rapidly transported to the neuronal cell body leading to the up-regulation of a regeneration-associated gene (RAG) program. Neurons that have this program survive but other neurons are at risk and cannot survive the injury. The RAG program activates

regeneration-associated transcription factors, such as the Activating Transcription Factor 3 (ATF3), which is upregulated only in injured-stressed neurons. ATF3 promotes nerve regeneration by Jun amino terminal kinase (JNK)-mediated activation of the induced c-Jun in neurons and in Schwann cells (Hai et al., 1999; Lindwall & Kanje, 2005; Patodia & Raivich, 2012b). ATF3, which is not present in uninjured neurons or Schwann cells, is an excellent marker for "activated" cells; therefore, this marker was used in the present thesis.

Additionally, growth proteins, such as growth associated protein- 43 (GAP-43), BDNF, and galanin, as well as cytoskeleton proteins, like actin and tubulin, are induced (Fu & Gordon, 1997). These factors are then carried out from the neuron anterogradely to the growing axons and to migrating Schwann cells to enhance regeneration. This switch in the neuronal cell body from cell-to-cell communication to survival and regeneration is crucial for the nerve regeneration process (Dahlin, 2023; Patodia & Raivich, 2012a).

Macrophages in nerve degeneration and regeneration

The important macrophages, being mainly of two subtypes, are either present in the epineurium or in the endoneurium [resident - induce inflammation via expression of toll like receptor (TLR), IL-13 and IL-1β (Chen et al., 2015)] or invade the nerve (monocyte driven macrophages recruited the blood stream). The latter are recognized by the marker CD68, but in the literature several used nomenclatures exist. However, it is still unclear exactly which type of macrophages that is specified by CD68 staining (see below) (McLean & Verge, 2016; Mokarram et al., 2012; Potas et al., 2015; Zigmond & Echevarria, 2019). In this thesis, I chose to name the CD68 macrophages as "activated-invading macrophages" (McLean & Verge, 2016; Mokarram et al., 2012; Potas et al., 2015; Zigmond & Echevarria, 2019).

Macrophages show polarization. M1 macrophages, the "proinflammatory", activate inflammation and M2 macrophages, the "anti-inflammatory" or "pro-regenerative"-healing, contribute to tissue remodelling and repair (Roszer, 2015). M1 macrophages express nitric oxide and secrete TNF alfa and IL6 and are considered to promote nerve degeneration (Zigmond & Echevarria, 2019). Some of the surface markers are CD32, the above-mentioned CD68 and CD197 (Liu et al., 2019). In contrast, M2 macrophages express arginase and secrete IL-10, TGF-β and promote nerve regeneration (Mokarram et al., 2012; Roszer, 2015). M1 macrophages are activated by TLR ligands, lipopolysaccharides, TNF alfa, and interferon alfa. M2 macrophages on the other hand is activated by IL-4, IL-13 and TGF-β. M2 macrophages consist also of several subtypes, where M2a and M2c are the subtypes that are specifically of interest in the present thesis. The subtypes stimulate and release factors as IL-10 and are recognized by the surface marker CD206 (Liu et al., 2019). They contribute to cell proliferation and migration, as well as resolution of

inflammation (Liu et al., 2019). Within the scenario of nerve injury and repair or reconstruction, there is a dynamic polarization between the different subtypes of macrophages as a response to the microenvironment at the site of injury and repair or reconstruction area. Thus, M1 macrophages can polarize to M2 over time and M2a subtypes can diverge to the M2c subtypes under the control of different cytokines (Mokarram et al., 2012).

The task of macrophages in degeneration and regeneration locally after a nerve injury is generally described in three sets. First, they clean the remaining "inhibitory" components, such as axons and myelin debris, in the distal nerve end. Second, they stimulate extracellular matrix remodelling by secreting several cytokines and growth factors. Third, they have an active role in facilitating nerve regeneration via regulation of the Schwann cell activity through distinct mechanisms and an active role in DRGs (Chen et al., 2015; Stratton et al., 2018).

At the time of the injury, the resident macrophages, together with the Schwann cells, start to clean axonal and myelin debris from the distal nerve end (Cattin & Lloyd, 2016). This role is previously described in another section. At around the 2nd and 3rd day after nerve injury, infiltration of the "invading" macrophages, by the help of Schwann cells as described above, into the distal nerve end starts. These Schwann cells then secrete chemokine (C-C motif) ligand (CCL2), TNFα, IL-1α, and IL-1β and recruit more invading macrophages into the nerve (Shamash et al., 2002). Attempts to inhibit these factors do not diminish the macrophage recruitment into the injured nerve, which points to that other factors also are crucial in the process (Tofaris et al., 2002). Similarly, C-C chemokine receptor 2 (CCR2) (Chen & Bonaldo, 2013) and TLR2 and 4 (Boivin et al., 2007) are also important in recruiting the macrophages into the injured nerve.

The formed matrix (the details of formation of extracellular matrix were previously described in another section) in the nerve gap between the proximal and distal nerve ends has predominantly macrophages as their major cell type where they exert action (Dahlin, 1995; Dahlin et al., 1996; Jessen & Mirsky, 2016; Min et al., 2021; Pan et al., 2020; Zhao et al., 1993). In this hypoxic environment, as in a nerve autograft or a nerve allograft, the macrophages secrete VEGF that in turn induces angiogenesis and further migration of Schwann cells as described (Cattin & Lloyd, 2016). The extracellular matrix proteins in the formed matrix, that initially contains fibrin, enclose also especially laminin, fibronectin, galectin-1 and collagen type IV and VI that further stimulate macrophage recruitment into the formed matrix. Galectin-1 deficient mice show a significantly decreased amount of macrophage invasion to the site of injury (Gaudet et al., 2009).

Macrophages have also an active role in DRG. The resident macrophages in the DRG proliferate upon stimulation of the colony stimulating factor 1 (CSF-1) (Krishnan et al., 2018). The CCL2 mediated recruitment of macrophages to DRG

also plays a crucial role in the neuronal priming - the neuronal regeneration programming (Niemi et al., 2016).

In this thesis, I was interested in the macrophage response, especially the C68 stained pan-macrophages (activating-invading macrophages) and the CD206 stained anti-inflammatory macrophages, in the surgically repaired or reconstructed nerves as well as in the contralateral sciatic nerves.

Nerve cell death and apoptosis after injury

Besides regeneration-promoting activities at the site of the nerve injury and in the distal nerve end, there is also a programmed cell death as a part of the regeneration process, indicating a delicate balance between regeneration and cell death, particularly related to the Schwann cells but also of the sensory neurons. Following the nerve injury, there is loss of sensory neurons (Welin et al., 2009), with up to 13% at the onset of 3 weeks postinjury, and to a less extent, loss of motor neurons which is more pronounced in a proximal nerve injury (Welin et al., 2009). In addition, the dedifferentiated Schwann cells that lose axonal contact may miss their ability as repair Schwann cells and reprogram themselves. They also go into apoptosis thereby expressing e.g., cleaved caspase 3 as a marker for apoptosis (Krishnan et al., 2024). There is a delicate balance between proliferation and apoptosis of the Schwann cells, as reported by the Dahlin laboratory and others (Saito & Dahlin, 2008; Stenberg et al., 2012; Stenberg et al., 2016; Stenberg et al., 2017). In the present thesis, I decided to use the cleaved caspase 3 antibody as a marker for apoptosis ("apoptotic Schwann cells").

Neuroprotection after nerve injury

Neuroprotective mechanisms are important after a nerve injury as a rescue mechanism (Stetler et al., 2009), which is observed also in diabetic neuropathies (Albers & Pop-Busui, 2014; Feldman et al., 2019; Feldman et al., 2017; Pourhamidi et al., 2014; M. A. Yorek, 2022). Among the described proteins, Heat Shock Proteins (HSPs) are crucial in this context and are reported to be present in human nerve biopsies in healthy subjects and in subjects with type 1 and type 2 diabetes (Ising et al., 2023). Heat Shock Protein 27 (HSP27) exists in neurons and Schwann cell under physiological conditions (Costigan et al., 1998) and is known to be upregulated after stress in sensory neurons following axotomy (Garrido et al., 2001; Jakob et al., 1993; Lindquist & Craig, 1988). After a nerve injury, retrograde axonal transport of Jun amino terminal kinase (JNK) signalling components in neurons contributes to the injury-induced c-Jun phosphorylation and ATF3 induction (Nakagomi et al., 2003). The relation between c-Jun phosphorylation, ATF3 induction, and HSP27 upregulation is also considered to have a major impact on Schwann cells to change their phenotype and become repair cells after injury (Jessen

& Mirsky, 2016). In clinical studies, it is found that decreased blood levels of HSP27, which also is detected in nerve biopsies in healthy subjects and in subjects with type I and 2 diabetes, (Ising et al., 2023), is related to impaired nerve function and presence of neuropathy in subjects with diabetes (Pourhamidi et al., 2014).

In this thesis, I chose to observe the HSP27 expression after different nerve injury and repair or reconstruction models, especially in diabetic GK rats, because it is a suitable marker to investigate neuroprotective mechanisms both in an injured nerve and in the contralateral uninjured nerve as well as in the distant sensory neurons in DRG.

How to repair and reconstruct a nerve injury

The history of nerve repair

The first document in which an author suggested a repair of a nerve injury is by Paulus Aegineta in 600 AD. In 11th century, Avicenna (980-1037 AD) recommended epineurial end-to-end anastomosis with sutures; however, he did not give technical details. After that, in 1275 Saliceto, and in 1296 Guido Lanfranchi, mentioned peripheral nerves in their books. Even in 1363, Guy de Chauliac (Montpellier) mentioned that peripheral nerve surgery to some extent improved the injured limb. The author of Alâim-i Cerrâhîn ("Wonders of Surgeons"), surgeon Ibrahim in Ottoman era in 1505 explains in his book an illustrated case of a peripheral nerve injury of a thief who was stabbed by the horseman while stealing a horse. In that section, it is described that Hippocrates first applied tourniquet to both the proximal and distal sides of the wound and then sutured the nerve ends with a woman's hair instantly, where the patient did not limb afterwards. In other later copies of Alâim-i Cerrâhîn (Wonders of Surgeons), it is stated that Hippocrates together with Galens was among the opponents of nerve repair (Belen et al., 2009; Irisarri, 2024). Gabriella Ferrara in 1543 was first to describe the suturing of the nerve ends with a technique that a special needle with an eye and a very thin thread from a turtle tendon is used.

In the Middle Ages, the peripheral nerve repair practice was stopped due to the belief that the nerves could not regenerate. It accelerated again in eighteenth and nineteenth centuries, and even nerve graft procedures were performed in dogs in 1863 (Dellon & Dellon, 1993; Millesi, 2000). Also, in the late nineteenth century, the first tube was used to bridge a nerve gap. A researcher in Lund, Sweden, John Forssman in 1898, conducted a study with experimental animals on nerve reconstruction with tubes and chambers. A neurologist, Foester, in the beginning of 1900, presented over 700 cases with nerve repair and in some he used nerve grafts. After that, other pioneers, such as Stookey and Bunnell, introduced "cable grafting" (Irisarri, 2024).

Peripheral nerve surgery was developed mainly during the two world wars and with the introduction of the operating microscope (Fugleholm, 2013). One of the pioneers in twentieth century is Professor Hanno Millesi, who helped to improve the nerve surgery techniques and clarify the indications (Millesi, 2000). The possibility of microdissection and the use of microsutures have improved outcome after peripheral nerve surgery. The research group originating from Lund University, Sweden, initiated by Professor Göran Lundborg and continued with Professor Lars Dahlin in collaboration with Professor Martin Kanje, has conducted extensive research on a variety of nerve construction technique based on experimental studies and translation to the clinical practice (Dahlin, 2008; Lundborg, 2000; Lundborg, Rosen, et al., 1997).

Surgical techniques in nerve repair

Even though there has been an accomplishment with the use of the operating microscope in peripheral nerve surgery, the surgical technique to reconstruct a nerve has been almost the same for decades/centuries as indicated above (Grinsell & Keating, 2014; Lin & Jain, 2023; Lundborg, 2000). In the absence of a nerve defect, a direct epineurial end-to-end nerve repair or a group fascicular nerve repair is preferred and performed (Dahlin, 2008). If the proximal nerve end of the injured nerve is not available for suturing, end-to-side neuroraphy may in certain circumstances be a choice (Lundborg, 2000); a technique that will not described further since it is not used or investigated in the present thesis (Bontioti et al., 2005; Cederlund et al., 2009). In the presence of a nerve defect between the nerve ends, nerve reconstruction with an autologous nerve graft, a group fascicular nerve autograft reconstruction or in certain situations use of a nerve allograft can be a possibility. In the absence of available nerve autografts or nerve allografts, and with the patient and surgeons' choice depending on the length of the nerve defect, a nerve reconstruction with a conduit may be applied.

The gold standard technique, which is most often possible in most of the clinical cases after a nerve injury, is the primary nerve repair, defined as end-to-end epineurial nerve suturing (McMorrow et al., 2024). There are several precautions during nerve repair that the surgeon should consider to achieve a better nerve regeneration and a favourable functional recovery (Dahlin, 2008; Lin & Jain, 2023; Lundborg, 2000; Midha & Grochmal, 2019; Yi & Dahlin, 2010). First, tension free nerve repair is crucial, since it is shown that nerve repair under tension results in impaired axonal outgrowth (Yi & Dahlin, 2010). Second, one should try to use a minimum number of stiches/sutures with an appropriate suture material to avoid scarring in the nerve (Dahlin & Wiberg, 2017). If the nerve ends are severed or damaged, trimming of the nerve ends to achieve a clean sight of the fascicles is important. This "manipulation" is shown to result in a superior nerve regeneration with a favourable surgical outcome (Dahlin & Wiberg, 2017; McMorrow et al., 2024).

The other surgical method - group fascicular nerve repair - offers a theoretically good alignment of fascicles but requires more manipulation and therefore causes an increased risk of intraneural scarring (Dahlin, 2008; Lundborg, 2000; McMorrow et al., 2024). In the present thesis, a direct epineurial nerve repair was used to handle the transected rat sciatic nerve in two of the experimental studies (Figure 3).

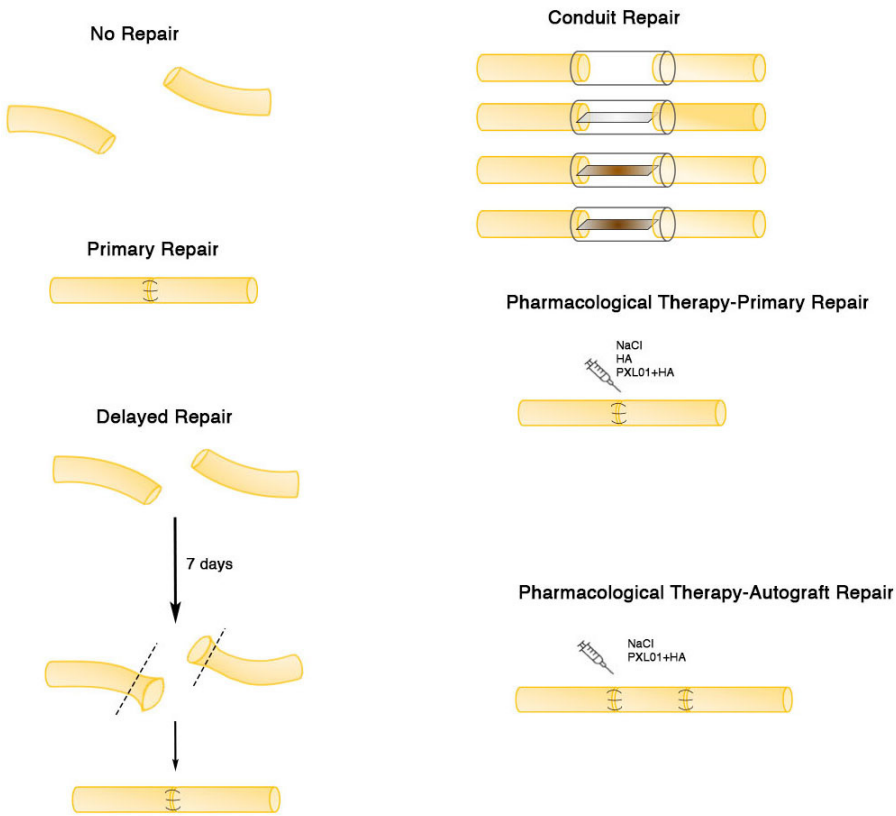


Figure 3: A schematic drawing of the different surgical repair and resconstructions models, including local application of a pharmacological drug, used for the transected sciatic nerve in healthy and diabetic GK rats in the present thesis.

Reconstruction of a nerve defect with nerve autograft

In the clinical setting, a nerve reconstruction with an autologous nerve graft (i.e., nerve autograft), or several nerve autograft cables, is the preferred choice of reconstruction in longer nerve defects (probably >3 cm) in nerve injuries and for certain critical nerves (Grinsell & Keating, 2014; Wu & Chuieng-Yi Lu, 2024).

Autologous nerve autografts can be considered as ideal nerve bridges because they provide suitable scaffolding structures with a ready extracellular matrix filled with aligned endoneurial tubes and Schwann cell supply as well as neurotrophic factors (Siemionow & Brzezicki, 2009). The nerve autograft undergoes remodelling, similar to Wallerian degeneration described above, during the weeks that follow after the nerve graft procedure, including invasion of macrophages, proliferation of the donor graft Schwann cells, digestion of the axon and myelin debris within the graft, and even a robust migration of Schwann cells from the graft to the nerve ends as well as Schwann cells from the proximal and distal nerve ends approaching the nerve graft (Millesi, 1990; Siemionow & Brzezicki, 2009; Tomita et al., 2009). The Schwann cells within the nerve autograft increase their surface cell adhesion molecules and provide a better scaffold with their basement membrane that contains laminin and fibronectin (Fu & Gordon, 1997). The importance of these Schwann cells in the nerve autograft is reported in studies with a pre-degenerated nerve autograft in which the nerve regeneration was superior to the fresh autografts (Danielsen et al., 1995; Kerns et al., 1993). The hypothesis behind these results was introduced as with the pre-degeneration process, and that the Schwann cells proliferate and produce basal lamina proteins, such as laminin and collagen IV. Additionally, there is also a robust invasion of macrophages to the pre-degenerated nerve autograft, which can influence regeneration (Danielsen et al., 1995).

Another important factor with the nerve autograft is the vascularization. At the time of the reconstruction, a nerve autograft is considered as an avascularised structure, which survives by the diffusion from the surrounding tissues and nerve ends. After some time, the nerve autograft is revascularized by the direct capillary ingrowth mainly from the proximal and distal nerve ends but also from the surroundings, where the bed on which the nerve autograft is applied is of crucial importance (Almgren, 1975; Millesi, 2000; Siemionow & Brzezicki, 2009).

However, there are local disadvantages at the donor site of the nerve autograft procedure, such as scarring, neuroma formation and a (low) risk for infection together with a risk for a lack of autograft material if there is need for thicker nerve reconstructions (Hallgren et al., 2013).

Reconstruction of a nerve defect with nerve allograft

To avoid donor site disadvantages and neuroma formation at site of harvest, processed nerve allografts (PNA) can be another option, especially in extensive nerve injuries or if the nerve autograft material is insufficient. A short description of the use of PNA is presented here due to its international topicality. Previously, immunogenic host response due to Schwann cells acting as antigen presenting cells has been reported (Lassner et al., 1995; Lassner et al., 1989). Due to the long duration of immunosuppressive therapy and the side effects of such a treatment, decellularized nerve allografts, i.e., PNA, have become more common. There have been several experimental studies showing outcome of nerve regeneration with nerve allografts. A rat sciatic nerve defect model of 10 mm showed a superior isometric strength with allografts compared to collagen conduits with nerve autografts being superior to both the other treatment options (Giusti et al., 2012). A rat model with a 14 mm nerve defect had higher number of myelinated fibres with nerve allografts compared to conduits, but the nerve autograft was found superior to both modalities (Whitlock et al., 2009). Another study comparing a predegenerated acellular nerve graft to a fresh acellular nerve graft revealed superior regenerative findings supporting the predegenerated acellular nerve grafts (Danielsen et al., 1995). PNAs have been widely used in clinical practice, known as Avance® = nerve graft by Axogen Inc., Fl. USA. However, there are studies in the literature that present different results regarding the outcome of the treatment with allografts: promising (Isaacs et al., 2023; Leckenby et al., 2020) or suboptimal (Huddleston et al., 2021). Thus, the use of PNA is a controversial issue because the lack of appropriate randomised clinical studies in humans (Frostadottir et al., 2023; Jain et al., 2021).

Reconstruction of a nerve defect with conduits

To overcome the disadvantages of the nerve autografts that are described above and due to controversial results reported in literature about PNA, other alternatives as nerve conduits have been developed and used. Nerve conduits are considered as options for nerve defects less than 3 cm in length and can be classified as autologous biological, non-autologous biological or non-biological-artificial-conduits. Biological materials, initially used as nerve conduits, were vein grafts, arterial conduits and muscle and tendon conduits with relative good clinical results compared to sural nerve autografts (Chiu & Strauch, 1990; Konofaos & Ver Halen, 2013; Strauch & Strauch, 2013). Non-autologous biological conduits, such as collagen, are clinically available, with clinical studies showing functional outcome in repair of digital nerves (Bushnell et al., 2008; Lohmeyer et al., 2009) or motor nerves in the forearm (Klein et al., 2016). Silk is another biological conduit, showing promising results in experimental studies (Huang et al., 2012). Artificial conduits are the non-biological conduits, such as bioengineered or polymer-based conduits.

One of the first non-biological conduits was the silicone tube (Dahlin et al., 2007; Nilsson et al., 2005; Zhao et al., 1997) that was tested in clinical trials (Dahlin & Lundborg, 2001; Lundborg, Dahlin, et al., 1997; Lundborg, Rosen, et al., 1997; Zhao et al., 1993), which presented a limited inflammatory response in contrast to the hypothesized increased inflammatory response (Dahlin et al., 2001). However, it was criticized as being not biodegradable and not flexible especially when used near finger joints. Therefore, the search for a more flexible, and bioabsorbable artificial conduit has become the priority.

Polyglycolic acid (PGA) is one of the clinically used bioabsorbable conduits with a market name - Neurotube® (Dahlin & Lundborg, 2001; Dahlin & Wiberg, 2017). It is porous and flexible and allow diffusion of molecules (Kusuhara et al., 2019). Clinical trials with reconstruction of a digital nerve defect showed equal/superior results in nerve regeneration compared to the nerve autograft (Weber et al., 2000) with a disadvantage of extrusion (Battiston et al., 2005). Other types of biodegradable polymer conduits, such as poly (ε-caprolactone) (PCL) (Niu et al., 2014) or chitosan (Haastert-Talini et al., 2013; Stenberg et al., 2016; Stenberg et al., 2017), were also developed and demonstrated successful nerve regeneration in primarily experimental, but also in clinical studies (Neubrech et al., 2016). Bacterial polyesters (Derya Burcu Hazer et al., 2012), such as poly-3-hydroxyoctanoate (Hazer et al., 2013) and poly-3-hydroxybutyrate (Sakar et al., 2014), were also used as conduits presenting axonal regeneration in experimental studies with rats. PCL, one of the biodegradable polymers, is presented as an efficient alternative to nerve autografts (Assaf et al., 2017; Bockelmann et al., 2011; Yu et al., 2011). As being rather transparent and not highly flexible, it is easier to handle and does not collapse easily (Assaf et al., 2017). In this thesis, a bioengineered conduit made of PCL was used to reconstruct a nerve defect of 10 mm in healthy rats. In summary, all nerve graft alternatives, autologous and non-autologous biological nerve grafts together with artificial conduits, are reported to demonstrate almost similar efficacy in terms of nerve regeneration and functional outcome; some in clinical studies but mostly in experimental studies in nerve defects shorter than 3 cm compared to nerve autografts (Grinsell & Keating, 2014; Lin & Jain, 2023). However, nerve autografts are still the gold standard treatment for the nerve defects larger than 3 cm, indicating the need for modifications of the available conduits.

How to influence nerve regeneration in various situations

Conduits are physical tubes that grasp the proximal and distal nerve ends, intentionally hold the secreted fluid between the injured nerve ends and provide guidance of the regenerating axons. The fluid is important for the regenerating axons and contains neurotrophic factors. In the conduit, although differences occur between various conduits, a fibrin matrix is formed that is essential for migration of

important cells in the regeneration process as described in previous sections. Historical achievement from silicone tubes (Lundborg et al., 1982; Lundborg, Rosen, et al., 1997) to modified bioengineered conduits shows that conduits evolve to enhance nerve regeneration (Qian et al., 2021; Rebowe et al., 2018).

Modifications of a conduit

The goal of the use of a nerve conduit is to provide a suitable microenvironment for the outgrowing axons and cells after the nerve injury (Qian et al., 2021). An ideal conduit is described to have the following properties (Phillips et al., 2022; Taylor & Haycock, 2022):

- should be biocompatible and at the same time not induce excessive inflammation,
- being biodegradable to avoid second surgery for removal of the conduit,
- being semipermeable and have adequate porosity to allow exchange of nutrients and interaction of cells and the extracellular matrix,
- should be mechanically stable but at the same time flexible to allow regenerating axons not to be compromised,
- lastly, being easy to fabricate and being comfortable to surgically implant (Rebowe et al., 2018; Taylor & Haycock, 2022; Wong & Chung, 2024).

However, a hollow conduit was shown to increase the risk of misdirection of the regenerating axons (De Ruiter et al., 2014). With the help of literature, our understanding of the cellular interaction and the physiology of the nerve regeneration have increased, and so the modification and innovative approaches of the nerve conduits. The modifications can be done in several ways.

One can change the material of the conduit, to increase the biocompatibility and at the same time preserve the biodegradable properties, for example collagen as a natural polymer can be blended with poly-caprolactone. Electrospun-polycaprolactone-collagen polymer is reported to support Schwann cell adhesion and proliferation in a 8 mm gap rat model (Yu et al., 2011).

Changing the design of the conduit is another alternative. Since nerves consist of fascicles, adding channels may mimic the microarchitecture of the nerve and therefore possibly enhance nerve regeneration (Gao et al., 2016). This modification was reported to increase the attachment of Schwann cells by five folds compared to hollow conduits (Hadlock et al., 2000). Addition of chambers to chitosan conduits may enhance axonal outgrowth after a delayed nerve reconstruction in rats (Stenberg et al., 2017). However, adding multiple channels does not always have favourable results with respect to functional outcome (Clements et al., 2009).

Grooves and guidance cues are other modifications to increase the inner surface to help attachment and migration of the Schwann cells and regenerating axons (Wong & Chung, 2024). Introducing pores to the conduit is a modification intended to allow transfer of transcription factors and growth factors necessary for cellular communication (Kehoe et al., 2012), but is reported at the same time to decrease the mechanical strength of the conduit (Bell & Haycock, 2012).

Coating the inner surface of the conduit is a further alternative. The use of extracellular matrix, such as collagen and laminin, is common to improve the biocompatibility of the conduit itself (Bell & Haycock, 2012). Axonal outgrowth is significantly better on laminin- and fibronectin-coated polymer films compared to collagen (Armstrong et al., 2007).

Increasing the hydrophilicity of the lumen by modifying the conduit polymer or by coating the lumen of the conduit may also work as a variation. Hydrophilicity can also be increased by modification of the polymer with polyethylene-glycol (PEG) or simply by using PEG itself as coating or as a membrane (Cai et al., 2012; Mokarizadeh et al., 2016).

Biochemical modifications of the conduit polymer can be another alternative by integrating any growth factors or cells into the scaffold itself or by the help of a carrier system. Stromal vascular fraction cells enhanced electrospun poly-L-lactic acid conduits seem to support axonal outgrowth (Frost et al., 2018). Other attempts have been made to enhance nerve regeneration through a conduit by adding embryonal Schwann cells, revealing a better axonal outgrowth compared to acellular conduits (Kalbermatten et al., 2008). In addition, application of growth factors, such as NGF, BDNF, or GDNF, into the conduit is suggested as a further alternative, despite the problems with concentration and timing of the applied factors but seems to improve nerve regeneration within a conduit (Carvalho, Oliveira, et al., 2019; Tuffaha & Lee, 2024). Silk fibre is another alternative for modification of the conduit. Previous in vitro studies with spider dragline silk of *Trichonephila* species showed successful Schwann cell and neuronal cell attachment and axonal growth (Millesi et al., 2021; Roloff et al., 2014). In vivo studies with decellularized veins filled with silk fibres in rats (Allmeling et al., 2008) and in sheep (Kornfeld et al., 2021; Radtke et al., 2011) revealed comparable results with nerve autografts in long distance nerve defects even in up to 6 cm long nerve reconstruction models. Even clinical results were reported with four patients having nerve defects longer than 2 cm that restored part of sensory function and prevented neuroma formation (Vogt et al., 2024).

Another modification can be integrating nanoparticles with which the conduit become sensitive to electrical (graphene) (Assaf et al., 2017), magnetic (iron nanoparticles) (Wang et al., 2020) or light stimulation (gold nanoparticles) (Carvalho-de-Souza et al., 2019). The detailed information about nanoparticle modification of the conduits is discussed in the fallowing section.

Nanoparticles

Nanoparticles (NP), by definition, are the entities whose size ranges from 1 to 100 nm. Old transcripts show that about 4500 years ago, humans utilized natural asbestos nanofibers to develop ceramic. The first synthetic product that consists of nanometre sized glass and quartz was “Egyptian blue” used by Egyptians around third century BC (Jeevanandam et al., 2018). NPs have been a part of a biomedical application mostly in diagnostic imaging, and as drug delivery systems (Peng et al., 2024). In the medical literature, they have been used in research on nerve conduits, particularly to modify nerve conduits as described (Carvalho, Silva-Correia, et al., 2019; Escobar et al., 2022; Sharifi et al., 2023).

Gold (Au) NPs (AuNPs) are attractive in biomedical application due to being inert, easily functionalized, and ease of catalysation. Their sizes ranges from 50-100 nm that is comparable to a large biological molecule (Paviolo & Stoddart, 2017), which is in the range of the particles used in the present thesis; i.e., range 70-100 nm. AuNPs are reported to enhance nerve regeneration by stimulating the outgrowth of axons on AuNP-modified electrospun nanofibers (Baranes et al., 2016). Silk fibres, modified by AuNPs, were observed to facilitate proliferation and adherence of the Schwann cells without any toxic effects as evaluated up to 18 months after a sciatic nerve repair in rats (Das et al., 2015). Furthermore, in vivo experiments using a Poly(l-lactide-co-glycolide) (PLGA) nerve conduit, enhanced with a controlled release of BDNF and AuNPs, show facilitation of the nerve regeneration process in rat sciatic nerves after injury (Jahromi et al., 2020).

Cobalt (Co)-NPs, a metallic NP, are mostly used in cancer treatment as a vehicle for drug delivery or direct as an anti-cancer agent (Huang et al., 2020). Co-NPs act as an apoptotic agent, which stimulates the apoptotic cascade by promoting cleaved caspase 3 activation in leukemic cells after intracellular uptake (Chattopadhyay et al., 2014). Co-doped 3D-polymer scaffold promotes outgrowth of axons and facilitates polarization of pro-inflammatory (M1) macrophages to anti-inflammatory (M2) macrophages (Chen et al., 2021). In this thesis, Co-oxide (CoO)NPs, together with AuNPs, are used for modification of the used PCL nerve conduit.

Administration of NPs in biomedical application is like a two-sided sword due their potential toxicity. Zinc oxide (ZnO) NPs are nephrotoxic, and copper (Cu)-NPs with diameters of 40 nm cause brain toxicity. Silver(Ag)-NPs with a size of 25 - 40 nm seem to cause damage to blood-brain barrier (Barik et al., 2021). Their toxicity is mainly due to intracellular formation of reactive oxygen species, which leads to cell membrane disruption and genotoxicity by DNA damage and finally cell death. (Soderstjerna et al., 2014)

To overcome these toxic effects, NPs should be used in the lowest possible concentration, larger, preferably >40 nm, particles should be applied, and finally

NPs should be integrated to a polymer base or to another structure that can hinder the release and any invasion to systemic circulation (Jeevanandam et al., 2018).

In the present thesis, my choice of NPs is Au- and AuCoO-NPs; a decision which is based on favouring results of a pilot study. I integrated and "anchored" the NPs into a hydrophilic polymer–polyethylene glycol composite to prevent the release of the NPs.

At the end, each modification seems to enhance regeneration within the conduit to some extent, but a balance should be kept in mind. Excessive biodegradability can cause early degradation of the conduit before full regeneration is achieved (Houshyar et al., 2019). Similarly, increased porosity can alter the mechanical strength of the conduit, and additional modification with hydrophilicity in the lumen of the conduit may cause an increased fibrotic cell adhesion besides the increased neural cell affinity (Qian et al., 2021). To overcome these obstacles, the conduit used in the present thesis was chosen to be a biodegradable polymer conduit made of PCL with a membrane coated with a hydrophilic polymer PEG, enhanced with NPs as "locally anchored" to the membrane minimizing the risk for toxicity.

Pharmacological therapy in nerve regeneration

Besides chemical and biological modifications of the environment in nerve regeneration, attempts have been used to facilitate nerve regeneration pharmacologically; both as a systemic treatment as well as a local treatment (Chan et al., 2014; Choi et al., 2022; Lopes et al., 2022; Rayner et al., 2022). A well-known and clinically used drug is dexamethasone, which has been studied in an experimental rat nerve injury model with both systemic and local applications and with positive functional effects (Bolandghamat & Behnam-Rassouli, 2020). Another study, using a sciatic nerve crush model, showed a better function in the walking track analysis with betamethasone administered subcutaneously in short term (Al-Bishri et al., 2005). Another alternative, methyl-prednisolone, is already in clinical use in facial nerve palsy (Yildirim et al., 2015). Experimental studies with intraperitoneal and local application show cellular and functional recovery after a facial nerve injury in rats. In a sciatic nerve crush model, it was also shown to decrease Schwann cell "atrophy" but also increased the perineurial inflammatory cells (Ozturk et al., 2016).

Atorvastatin, which is clinically used to treat hypercholesterolemia, is used as a neuroprotective agent in Alzheimer´s disease and in multiple sclerosis (MS). Such a treatment promotes axonal outgrowth by activating c-Jun and inhibiting extracellular kinase system, such as protein kinase B (AKT), after systemic application in an experimental rat models after nerve injury (Pan et al., 2010).

The other pharmacological agents that have been used in experimental models of nerve injuries are citicoline (a precursor of phosphatidyl-choline), L-carnitine, memantine, and riluzole; all of which show good functional and histological outcome (Bolandghamat & Behnam-Rassouli, 2020). The combination of these agents with stem cells has revealed results that indicate promotion of nerve regeneration. Systemic administration of L-carnitine together with adipose-derived stromal cells seeded on a de-cellularized nerve allograft leads to axonal outgrowth in a 10 mm nerve defect model in rats (Mohammad-Bagher et al., 2019). Systemic administration of L-carnitine has improved topographical and functional outcome after a sciatic nerve crush injury in diabetic rats (Avsar et al., 2014). NeuroHeal®, a mixture of acamprosate and ribavirin, was introduced in experimental studies and showed to reduce the glial scar, to accelerate nerve regeneration by activating the AKT pathway in a delayed rat nerve repair model (Romeo-Guitart et al., 2017) and being favourable for regeneration of sensory neurons (Romeo-Guitart & Casas, 2020); however, further clinical studies were stopped.

Local application of a lactoferrin-derived peptide - PXL01

PXL01 is a synthetic peptide, which is produced from the lactoferrin protein and has been used in a variety of clinical studies to reduce adhesions after surgery, for example after flexor tendon surgery with study participation of our department (Wiig et al., 2014), and to reduce abdominal adhesions (Nilsson et al., 2009) by decreasing secretion of the inflammatory cytokines and enhancing fibrinolysis. The synthetic peptide is applied locally together with its carrier sodium hyaluronate (HA). PXL01 interacts with the plasminogen activating system by reducing the expression of Plasminogen Activator Inhibitor-1 (PAI-1), which can inhibit Schwann cell migration from DRG. Therefore, PXL01 may enhance nerve regeneration through the activation of the plasminogen activator system (Siconolfi & Seeds, 2001).

In the clinical study, investigating the effect of local application of PXL01 on the range of motion in cases with flexor tendon surgery, a better recovery was detected in patients with a repaired concomitant digital nerve injury at 12 weeks (Wiig et al., 2014). That information awakened the question of cellular interaction between outgrowing axons, Schwann cells and macrophages in the regeneration process after a nerve injury. In the present thesis, I searched for the answer to this question by designing two different models of nerve injuries; one with a primary nerve repair and the other with a nerve reconstruction with nerve autografts in both healthy Wistar and diabetic GK rats in which PXL01 was applied locally during surgery.

Pharmacological therapy - tacrolimus (FK506)

FK506, known as tacrolimus, is an immunosuppressive agent that is already in clinical use in kidney and liver as well as in several other tissue transplantations as anti-rejection therapy. Tacrolimus treatment is one of the few clinical drugs that is used for nerve regeneration which is successfully translated from basic experimental research (Daeschler et al., 2023; Marsh et al., 2024; Seixas et al., 2022; Xiao et al., 2024). Rodent and large animal studies with different nerve injury models have demonstrated enhanced nerve regeneration (Gold et al., 1995; Snyder et al., 2006). FK506 is shown to upregulate Heat Shock Proteins (HSPs) and causes upregulation of growth-associated proteins that in turn results in enhanced nerve regeneration (Gold, 1997). It has been shown that even pre-therapy with FK506 increases nerve regeneration in an animal model (Snyder et al., 2006). However, the positive effects of FK506 have been objected in studies from our laboratory (Kvist et al., 2003). The effects of tacrolimus on nerve regeneration are here described to give a full view of the subject.

Immunomodulation by co-stimulation

As presented in previous sections, nerve allografts, have been a possible treatment modality in reconstruction of a nerve defect. However, these nerve allografts that are taken from different living beings can induce a graft-host immune reaction that needs attention (Fansa et al., 2002). A solution to this problem has been introduced as increasing an immune tolerance by co-stimulation blockade. The idea behind this treatment is that several immunosuppressive treatments are used at the same time to achieve the most effective immunosuppression and not just to decrease the immune reaction against the graft but also to enhance cell viability to help nerve regeneration (Tai et al., 2010). Immunomodulation by co-stimulation does not have any beneficial effect on nerve regeneration in short term after nerve allograft reconstruction in mice (Kvist et al., 2007). However, another study has shown that in longer time follow up (three weeks), nerve regeneration in allografts treated with a short course of three doses was equivalent to isograft (Tai et al., 2010). Again, the use of co-stimulation strategy is here described to provide a full view of the subject.

Non-pharmacological therapy with electrical stimulation

Besides pharmacological therapy, electrical stimulation has historically demonstrated a positive effect with greater axonal sprouting, rapid muscle innervation and faster functional recovery (Hoffman & Binet, 1952; Nix & Hopf, 1983), why a short description of the concept is here written. The mechanism(s) behind the treatment is given as the crossing of the axons to the coaptation site with no change in speed of regenerating axon, and also an upregulation of neurotropic

factors (Liu & Fox, 2024). Animal studies, with a reconstructed nerve defect, demonstrate an increased percentage of regenerated axons with no effect on regeneration speed (Witzel et al., 2016), but with functional recovery (Haastert-Talini et al., 2011). There are randomized clinical trials that demonstrate a positive functional outcome with respect to return of two point discrimination as well as grip and key pinch strength (Gordon et al., 2010; Power et al., 2020; Wong et al., 2015; Zhang et al., 2023). With these clinical studies, and with results from animal studies, it is suggested that intraoperative electrical stimulation with duration of 10 minutes and 2-mA current with pulse duration of 100 microseconds delivered just proximal to the injury and repair site for patients operated under general anaesthesia, can give potential benefit for nerve recovery, but its clinical use is not widespread (Liu & Fox, 2024).

Furilden, July,2023

"If you can't explain it simple, you don't understand it well enough."
Albert Einstein

Aim

The overall goal of the present thesis is to improve nerve regeneration and axonal outgrowth with focus on the cellular response in healthy and diabetic GK rats with the specific aims:

- To investigate injury-induced Heat Shock Protein 27 (HSP27) expression and its association to axonal outgrowth in different nerve repair models – i.e., no, immediate, and delayed (7-day delay) nerve repairs with 7- or 14-days follow-up in healthy Wistar and diabetic GK rats.
- To evaluate the regeneration capacity of poly-propylene polyethylene glycol (PPEG) membrane divided poly-caprolactone (PCL) conduits, enhanced with gold (Au) or gold-cobalt-oxide (AuCoO) NPs, in comparison with hollow conduits after 21 days of implantation in healthy Wistar rats.
- To examine the effects of local application of the lactoferrin-derived peptide PXL01 in a sodium hyaluronate carrier system on nerve regeneration with respect to axonal outgrowth, response of Schwann cells and sensory neurons, expression of HSP27 (i.e., neuroprotective function) as well as on the inflammatory response, i.e., presence of pan-macrophages and anti-inflammatory macrophages, after transection and immediate repair of the sciatic nerve in healthy Wistar rats.
- To study the effects of local application of PXL01 in a sodium hyaluronate carrier system in a nerve defect model reconstructed by an autologous nerve graft on nerve regeneration with respect to axonal outgrowth, response of Schwann cells and sensory neurons, expression of HSP27 as well as the inflammatory response in healthy Wistar and diabetic GK rats.

Ravlunda, June, 2021

Methods

A summary of the methods is presented here for the four papers. Please refer to individual articles for more detailed information.

Animal surgery

The experiments were conducted according to the approved ethical permission from the local ethics committee for animal research at Lund University; Animal Ethics Committee of Lund University, Lund, Sweden (protocol code 5.8.18-06842/2019; date of approval 5 June 2019) and carried out according to the European Communities Council's directive regarding care and use of animals for experimental procedures.

Two different species of rats were used in thesis; the Wistar rats as healthy rats, provided from Janvier labs, France (Paper I - IV) and diabetic Goto-Kakizaki (GK) rats provided from the Lund Laboratories via own breeding (Paper I, IV). The Wistar rats represent the healthy rats with normal blood glucose levels, and the GK rats represent and resemble type 2 diabetes with moderately increased blood glucose levels. All used rats were female rats, weighing around 180-300 gr and at the age of around 2 months to 4 months.

In all papers, the sciatic nerve was unilaterally exposed in the midthigh; right or left sciatic nerve according to the preference of the surgeon. In all papers, the nerve was injured by transection and several different nerve repair or reconstruction models were used: nerve transection and no repair (Paper I), nerve transection and immediate repair with epineurial sutures (Paper I, III), nerve transection and delayed nerve repair (7 days) with epineurial sutures (Paper I), a 10 mm nerve segment transection and nerve autograft reconstruction of that segment (Paper IV), a 5 mm nerve segment (Paper II) or a 10 mm nerve segment (Paper II pilot study) transection and bridging by different modified conduits.

Sham operations were included in Paper III and IV in which a pharmacological agent was applied. The nerve was explored in a similar fashion as the experimental model, but no further nerve injury was performed or designated treatment agents were applied; PXL01 in carrier sodium hyaluronate carrier, sodium hyaluronate or sodium chloride solution (0.2 mL).

In all papers, the preoperative preparations were similar. The rats were anaesthetized with an intraperitoneal injection of a mixture of Rompun® (20 mg/mL; Bayer Health Care, Leverkusen, Germany) and Ketalar® (10 mg/mL, Pfizer, Helsinki, Finland) with a dose of a 2 mL Ketalar® and 0.5 mL Rompun® per 100 g body weight. Postoperatively, all rats were treated with the analgesic Temgesic® in a dose of 0.01–0.05 mg/kg (0.3 mg/mL; Schering-Plough Europe, Brussels, Belgium). The animals were kept on a 12 h dark-light cycle with ad libitum feeding.

Poly (ε-caprolactone) (PCL) conduits with modified membranes

The detailed methodology is presented in the Paper II. As a summary, the granule form of PCL was dissolved in Tetra hydro furan (THF) solution and a Kirshner wire was used to dip into the solvent several times to form an even layer of PCL that covers the wire. The obtained PCL conduit was taken out from the stainless-steel rod and cut into 14 mm long (0.5 mm wall thickness, 1 mm inner and 2 mm outer diameter) or 19 mm long (used in the pilot study) conduits.

Polypropylene-polyethylene glycol (PPEG) amphiphilic copolymer was chosen to be the polymer base of the membrane that was used to chamber the conduit. A slightly modified Williamson-ether-synthesis-like reaction was performed for synthesis of the PPEG membrane by the reaction between chlorinated polypropylene and polyethylene glycol. Then, gold nanoparticles, gold and cobalt oxide nanoparticles, and Ag nanoparticles (used in the pilot study) were embedded into the PPEG membranes which were named as AuPPEG, AuCoOPPEG and AgPPEG (used in the pilot study), respectively. Poly(3-hydroxy undecenoate)-poly(N-isopropyl acryl amide) (PHUPNIPAM) amphiphilic copolymer that was used in the pilot study is an unsaturated microbial polyester that is obtained from the fermentation using *Pseudomonas oleovorans* (Erduranlı et al., 2008; Hazer & Steinbuchel, 2007; Kalaycı, 2010; Kocer, 2003; Toraman, 2014). The detailed information of the synthesis of the Au nanoparticle embedded PHUPNIPAM graft copolymer is presented in the Paper II.

From each polymer membrane, 0.1 mm thick, 2 mm width and 10 mm length or 14 mm length rectangular membranes were cut and placed into the conduits. Finally, nano-conduits were formed as follows: 14 mm and 19 mm long PCL conduits with 10 mm and 15 mm long nanocomposite membranes placed parallel to the long axis of the conduit allowing 2 mm on both the proximal and distal ends of the nerve for insertion and suturing of the ends (Table 2).

Table 2. The list of different bioengeneered conduits that were used in the Paper II.

PAPER	CONDUIT	CONDUIT DIMENSIONS	MEMBRANE	NANOPARTICLE	MEMBRANE DIMENSIONS
Main manuscript	Poly(ε-caprolactone) (PCL)	14 x 0.5 x 2 mm	-	-	-
			Poly-propylene poly-ethylene glycol (PPEG)	-	10 x 0.1 x 2 mm
			PPEG	Au	10 x 0.1 x 2 mm
			PPEG	AuCoO	10 x 0.1 x 2 mm
Pilot study	PCL	14 x 0.5 x 2 mm	PPEG	Ag	10 x 0.1 x 2 mm
		19 x 0.5 x 2 mm	PPEG	Ag	14 x 0.1 x 2 mm
	PCL	14 x 0.5 x 2 mm	Poly(3-hydroxy undecenoate)-poly(N-isopropyl acryl amide) (PHU-PNIPAM)	Au	10 x 0.1 x 2 mm
		19 x 0.5 x 2 mm	PHU-PNIPAM	Au	14 x 0.1 x 2 mm
	PCL	14 x 0.5 x 2 mm	PPEG	Au	10 x 0.1 x 2 mm
		19 x 0.5 x 2 mm	PPEG	Au	14 x 0.1 x 2 mm
	PCL	14 x 0.5 x 2 mm	PPEG	AuCoO	10 x 0.1 x 2 mm
		19 x 0.5 x 2 mm	PPEG	AuCoO	14 x 0.1 x 2 mm

Harvesting and processing of tissues

In all papers, the operated nerve, the contralateral uninjured nerve, the DRG on both sides at the L4 and L5 levels were harvested. In Paper II, the spleen and the liver were harvested from the randomly picked animals for the toxicity analysis of the nanoparticles. The processing of the harvested tissues was the same in all papers; fixation was performed in the Stefanini`s solution (i.e., 4% paraformaldehyde and 1.9% picric acid in 0.1 M phosphate buffer, pH 7.2) for 24 h and placed in sucrose solution (20% sucrose in 0.01 M PBS) for 24 hours after washing. Then the specimens were immediately frozen in cryostat and the fresh frozen specimens were sectioned longitudinally with a thickness of 8 μm (Paper I, III, IV) or 6 μm (Paper II) and then mounted on slides.

Immunohistochemistry and macroscopic analysis

The same immunohistochemical procedures were followed in all papers. After washing procedure, the sections were incubated with the primary antigens in PBS over night at 4C, washed again, and incubated with secondary antibody at room temperature. All slides were mounted with Vectashield Mounting Medium with DAPI (4′,6-diamino-2-phenylindole) (Vector Laboratories, Burlingame, CA, USA) and then coverslipped. In all papers, the neurofilament (NF), and Heat Shock Protein 27 (HSP27) analyses were performed. In Paper II, III and IV, activated (ATF3

stained) and apoptotic (cleaved caspase 3 stained) Schwann cells were analysed. The macrophage analyses with pan-macrophage count (CD68) and anti-inflammatory M2 macrophage count (CD206) were performed in Paper III and IV.

To ascertain the Schwann cell identity, the localization of HSP27 expression in sciatic nerve and DRG, and the macrophage identity, double staining procedures were performed with S-100 and neurofilament staining with HSP27 (Paper I, III, IV), ATF3, cleaved caspase 3, CD68 and CD206 staining (Paper III, IV). To see the polarization of macrophage response, several randomly selected sections were double stained with CD68 and CD206 (Paper III). Additionally, to ensure the paranodal staining in the contralateral uninjured nerves, staining with the anti-caspr antibody was performed (Paper III).

In all papers, HSP27 expression in the axons and sensory neurons were analysed in DRG. The activated sensory neurons with ATF3 staining were analysed in Paper II, III and IV. All the primary and secondary antibodies use in thesis is summarized in Table 3.

Table 3. The list of primary and secondary antibodies used in immunohistochemical analysis in the thesis.

	IMMUNHISTOCHEMISTRY	PAPER	PRIMARY ANTIBODY	SECONDARY ANTIBODY
Sciatic nerve	Neurofilament	I,II,III,IV	Monoclonal mouse anti-human neurofilament (1:80; Dako, Glostrup, Denmark)	Alexa Fluor 594- goat anti-mouse IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	Heat Shock Protein 27 (HSP27)	I,III,IV	Polyclonal rabbit anti-HSP27(1:200, ADI-SPA-803, Enzo Life Sciences, Farmingdale, NY, USA)	Alexa Fluor 488-goat anti-rabbit IgG for the HSP27 (1:250, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
		I,II	Polyclonal goat anti-HSP27 (1:200; sc-1048, Santa Cruz Biotechnology, Dallas, TX, USA)	Alexa flour 488-donkey anti-goat IgG antibody (1:500, Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA)
	Activating transcription factor 3 (ATF3)	II,III,IV	Monoclonal mouse anti-ATF3 (1:200, Sc 81189) Santa Cruz Biotechnology, Dallas, TX, USA)	Alexa Fluor 488- goat anti-mouse IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	Cleaved caspase 3	II,III,IV	Monoclonal rabbit anti-cleaved caspase 3 (1:200, Cell signalling Technology, Denvers, MA,USA)	Alexa Fluor 488- goat anti-rabbit IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	CD68	III,IV	Monoclonal mouse anti rat CD68 (ED1) (MCA 341R, 1:400, BioRad, Dallas, TX, USA)	Alexa Fluor 488-goat anti-mouse IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
				Alexa Fluor 594-goat anti-mouse IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	CD206	III,IV	Monoclonal rabbit anti-mannose receptor (CD206) antibody (1:400, ab300621, Abcam, Cambridge, UK)	Alexa Fluor 488- goat anti-rabbit IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	Anti-caspr protein	III	Polyclonal rabbit anti-caspr (anti-caspr) antibody for paranodal staining (1:4500, Abcam, Cambridge, UK)	Alexa Fluor 488- goat anti-rabbit IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
				Alexa Fluor 594- goat anti-rabbit IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
	S-100	I,III,IV	Monoclonal mouse IgG anti S-100 α/β chain (sc-58839, Santa Cruz Biotechnology Inc., Dallas, TX, USA; diluted 1:300)	Alexa fluor 594 goat anti-mouse IgG (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA)
Dorsal root ganglia (DRG)	Heat Shock Protein 27 (HSP27)	I,II,III,IV	Polyclonal rabbit anti-HSP27 (1:200, ADI-SPA-803, Enzo Life Sciences, Farming- dale, NY, USA)	Alexa Fluor 488- goat anti-rabbit IgG (1:250, Invitrogen, Molecular Probes, Eugene, Oregon, USA)
		I	Polyclonal goat anti-HSP27 (1:200; sc-1048, Santa Cruz Biotechnology, Dallas, TX, USA)	Alexa flour 488-donkey anti-goat IgG antibody (1:500, Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA)
	Activating transcription factor 3 (ATF3)	II,III,IV	Monoclonal mouse anti-ATF3 (1:200, Santa Cruz Biotechnology, Dallas, TX, USA)	Alexa Fluor 488- goat anti-mouse IgG (1:500, Invitrogen, Molecular Probes, Eugene, Oregon, USA)

Nanoparticle analysis of polymers and tissue toxicity of nanoparticles

To ensure the presence of the nanoparticle in the polymer membrane, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were performed. After coating procedure (Emitech K550X Sputter Coating Systems, Quorum Technologies, UK) samples were examined under a SEM (JEOL JSM-7600F, Joel Ltd., Tokyo, Japan) with accelerating voltage of 15 kV and elemental analysis was performed with semi quantitative-Inca-energy dispersive X-ray spectroscopy (EDS).

Tissue toxicity of the nanoparticles was analysed with the liver and spleen tissues collected from randomly selected rats in each group at the end of 21 days of implantation. One gram of the tissues was dried and burned. The tissues were frozen and coated with palladium, gold, and carbon. SEM, as described above, was performed and the microscopical images recorded.

Imaging and analysis

All images of the tissue sections in this thesis were taken by a fluorescence microscope (Olympus BX3) with a digital camera (Olympus DP80) and analysed with CellSens Dimension software (Olympus).

The region of interest in the experimental sciatic nerve is described in detail in each paper; the site of lesion is determined as the area just distal to the most proximal suture line in the Paper I, III and IV. In Paper II, the site of the lesion is selected as 3 mm distal to the proximal suture line within the regenerative matrix. The distal nerve end is determined as the area just distal to the point of the longest growing nerve fibres, which in Papers II and IV is the area just distal to the distal suture line.

In Paper II, the macroscopic evaluation of the regenerative matrix was classified according to the thickness of the regenerative matrix; no matrix, loose matrix (<1 mm) and dense matrix (≥ 1 mm). Also, the number of nerve cables (no cable, one full length cable and two cables) was presented.

The immunohistochemical analysis is described in detail in each paper. As a summary, the images of the HSP27 staining were analysed with ImageJ program (U.S. National Institutes of Health, Bethesda, Maryland, USA). Axonal outgrowth was measured as the longest neurofilament protein staining starting from the proximal suture line. ATF3 and cleaved caspase 3 stained Schwann cells together with the CD68 and CD206 stained macrophages were counted manually and were expressed as percentage of total number of DAPI-positive cells within the selected area. DAPI cells were counted manually in Paper I, and with a ready plug-in

program in Image J in Paper II, III, and IV. ATF3 stained sensory neurons were also counted manually in the whole DRG section. For all immunofluorescence analyses, except for the macrophage analysis in Paper III, three random slides were selected from each rat and analysed, and a mean value was calculated.

Statistical analyses

The results in all papers are presented as median values with 25th and 75th percentiles and nonparametric tests - Kruskal Wallis test - were used to analyse the difference between experimental groups with the Mann–Whitney (MW) test used as a post hoc analysis. In Paper I, Fisher's method for independent samples were used to identify difference between different surgical methods and health status of the rats.

Wilcoxon signed rank test was used to detect any difference between the experimental and contralateral sides as well as between the proximal and distal lesion sites. Intra-rater reliability was conducted to ensure the analyses of the HSP27 expression in two different measurements by one observer.

To have a consistent analysis of the DAPI cell count, Blant Altman statistical analysis was performed for the data taken from 50 slides in which the DAPI positive cells were counted manually or with the previously described plug-in in Image J.

Spearman's correlation test was used to see the relation between variables, where a r-value >0.30 (i.e., >0.30–0.7 = moderate correlation; >0.70 = strong correlation) was set as relevant.

Linear regression analyses were performed to evaluate the influence of predictive factors, such as health status in Paper I, the expression of HSP27 in sciatic nerve and in DRG (Paper I, II, III), the Schwann cell activity in the sciatic nerve (Paper II, III) and the macrophage activity (Paper III) on length of axonal outgrowth (i.e., neurofilament; dependent factor); represented as unstandardized Beta [95% CI] and the p-value. A 2x2 factorial ANOVA was conducted (Paper IV) to examine the effect of the health status and treatment on same parameters. A p-value of < 0.05 was considered significant.

Ethical Considerations

Peripheral nerve injuries are still a major problem, a surgical challenge and the injuries give the patient a lot of suffering with decreased sensation and muscle strength as well as a high risk of pain and cold sensitivity.

The surgical repair or reconstruction procedures are not always successful, giving the individual extensive problems but also cause huge economical costs for the society while these serious nerve injuries mainly affect young working individuals.

To gain new knowledge how cells and nerves react to an injury and repair, or reconstruction, is of major importance to be able to develop new surgical nerve repair and reconstruction methods. This can make it possible to improve the life situation for those men and women, with or without diabetes, who will get a peripheral nerve injury. It may also be possible to shorten the rehabilitation process and therefore may decrease the costs for both the individual and the society. To highlight these problems, I have conducted this thesis, which is composed of four different projects with subprojects involving different nerve injuries and repair or reconstruction models with different time of follow up. Besides different surgical methods that are used, new pharmacological agents, like PXL01, and nanoparticles are also introduced within these projects. I decided to conduct these projects in living subjects, and I chose to study rats. When there is a project that is conducted in a living being, the question that immediately arises, is why I chose to study nerve regeneration in living beings, in this situation rats. One should always explain the reason for the choice according to ethical guidelines and rules. Ethical considerations play an important role when conducting an experimental study, where the 3R concept - Replace, Reduce and Refine - must be met (Lee et al., 2020; Ogden et al., 2016; Taylor & Haycock, 2022).

With the Replacement concept, one should try to replace a living being with the lowest possible species that allows the scientific study to answer all the questions. To be able to understand healing mechanisms, and to improve surgical methods, it is necessary to be able to study different types of injuries to a major nerve trunk in an animal limb. In vitro experiments cannot give answers to all scientific questions since the injury and surgical procedure cannot be performed in a test tube. It is also not possible to study the complex balance between different cells in a nerve trunk after an injury and in connection with diabetes in vitro. Cultured cells from animals, which are studied in a test tube model, are not behaving in the same way as in the living species.

To Reduce is to decrease the number of living individuals included in the study to the lowest amount. By planning the experiments thoroughly with correct comparisons between different rats with various injuries, meticulous statistical calculation based on earlier research, it will be possible to reduce the number of rats and keep them to a minimal and adequate number.

The Refinement is the concept that tries to alleviate or minimal pain and distress as well as to enhance wellbeing for the study. The rat has one major nerve, the sciatic nerve, in the hind leg that has the same size as a digital nerve in a human finger, which make the rat sciatic nerve model suitable for the present experiment. The healthy Wistar rat is suitable when the healing process can be compared with the

diabetic GK rats which spontaneously develop diabetic with a relative low blood sugar level, resembling type 2 diabetes in humans. Very careful controls of the rats are done daily by both researchers and animal personal after the surgery to be able to ensure the rats general health after the injury and the surgical repair or reconstruction, including provision of analgesics postoperatively. The follow-up times are kept as short as possible. The nerve injury in the rat is the same as in humans. The sciatic nerve injury in rats causes a disturbed sensation in the injured nerve distribution area and a decreased muscle function. This gives the rat an altered walking pattern after the injury. However, the rat usually adapts to this situation. The return of function is followed carefully. The pharmacological substances PXL01 and NPs have been used and studied in experimental and clinical studies earlier without detecting any negative reactions. The substances have not been found to be toxic to animals or humans. This is reasonable safe for the rats to examine if the substances have a positive effect on the healing mechanism after a nerve injury.

The ethical consent for this thesis was approved as described above.

"Science never solves a problem without creating ten more."
George Bernard Shaw

Results

The results are summarised here, and for detailed information the reader is referred to the individual papers.

Paper I. Injury-induced HSP27 expression in peripheral nervous tissue is not associated with any alteration in axonal outgrowth after immediate or delayed nerve repair.

- In healthy and diabetic rats, various types of nerve injury and repair models increase HSP27 expression in the sciatic nerve and in DRG.
- Diabetes is associated with an increased HSP27 expression in nerves and in DRG at the uninjured side.
- Diabetes is associated with a decreased axonal outgrowth after injury in the different surgical models.
- Immediate nerve repair has a positive effect on axonal outgrowth compared to an unrepaired nerve injury, but a nerve repair with a short delay has no influence on axonal outgrowth compared to immediate nerve repair.
- The increased expression of HSP27 in the injured sciatic nerve or in the DRG at the injured side has no impact on axonal outgrowth.

Healthy rat
Diabetic rat
7 days
14 days
no repair
delayed repair
HSP27 in uninjured diabetic nerves and in DRG
HSP27 in nerves and DRG after injury
Diabetes axonal outgrowth
No influence of HSP27 on axonal outgrowth after nerve injury and repair
HSP27 SNL
Frames (3 sections)
HSP27 SND
Proximal nerve
Distal nerve
Length NF (mm) = axonal outgrowth (3 sections)

Paper II. Gold and cobalt oxide nanoparticle modified poly-propylene poly-ethylene glycol membrane in poly(ε-Caprolactone) conduits enhance nerve regeneration in sciatic nerve of healthy rats.

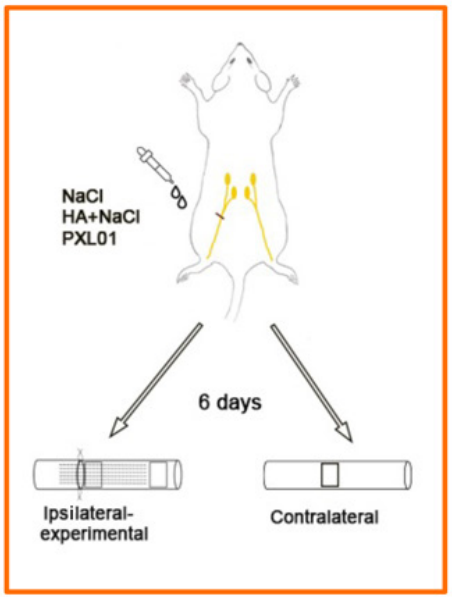
- Modification of the used conduits, either with a membrane or with a NP modified membrane, used to bridge a nerve defect demonstrates longer axonal outgrowth.
- HSP27 expression in the formed regenerative matrix in the conduits was higher in NP membrane conduits compared to hollow conduits.
- Chambered conduits contain a higher number of activated Schwann cells in the formed regenerative matrix compared to hollow conduits.
- Chambered conduits, used to bridge a nerve defect, have higher expression of HSP27 in the ipsilateral DRG compared to hollow conduit.
- HSP27 expression in the regenerative matrix and in DRG has no influence on axonal outgrowth.
- More activated Schwann cells in the regenerative matrix is associated with longer axonal outgrowth, whereas a higher number of apoptotic Schwann cells in the regenerative matrix has a negative impact on axonal outgrowth.

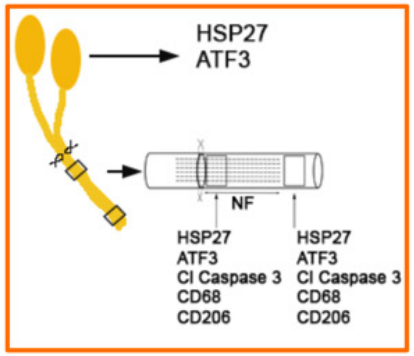
Neuro-protection and activated sensory neurons
Activated Schwann cells and neuro protection
Axonal outgrowth
Apoptotic Schwann cells
21 days
Gold and Cobalt-oxide nanoparticles modified PPEG polymer membrane
Healthy
Hollow (n=10)
PPEG (n=10)
AuPPEG (n=10)
AuCoOPPEG (n=10)
2 mm
5 mm

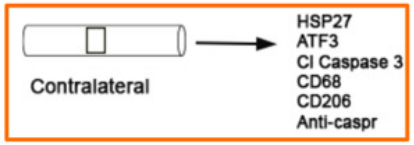
Paper III. PXL01 alters macrophage response with no effect on axonal outgrowth or Schwann cell response after nerve repair in rats.

- PXL01 treatment causes fewer CD68 stained macrophages at the site of lesion and in the distal nerve end compared to treatment with the carrier solution (sodium hyaluronate) or to placebo (saline) but does not alter the anti-inflammatory CD206 stained macrophage count.
- The number of CD68 stained macrophages is higher at site of lesion compared to the distal nerve end in all treatment groups.
- Axonal outgrowth, HSP27 expression as well as activated and apoptotic Schwann cell counts do not differ between treatment groups.
- A nerve injury and subsequent repair cause an increase in HSP27 expression and in the anti-inflammatory CD206 macrophage count in the operated sciatic nerve compared to contralateral side.
- The percentage of activated sensory neurons and the HSP27 expression in the sensory neurons in DRG do not differ between the treatment groups.
- An increased apoptotic Schwann cell count in the distal nerve end has a positive impact on axonal outgrowth whereas presence of CD68 stained macrophages in sciatic nerve does not influence axonal outgrowth.

Local application of PXL01 on nerve repair







- **No change in axonal outgrowth, Schwann cell activity or HSP27 expression in DRGs and nerve**
- **Decreased pan-macrophage count**

Paper IV. Lactoferrin-derived peptide PXL01 impacts nerve regeneration after sciatic nerve reconstruction in healthy and diabetic rats

- Reconstruction of a nerve defect with a nerve autograft is associated with an increased expression of HSP27 in the operated sciatic nerve compared to the contralateral nerve in both treatment modalities (PXL01 or saline) and health status (healthy and diabetes).
- Activated Schwann cell count is higher in the sciatic nerve (both at lesion site and in distal nerve end) in healthy rats compared to diabetic rats in each treatment group.
- Factorial ANOVA analyses show that both health status (being diabetes) and treatment (PXL01 treatment) influence axonal outgrowth, activated Schwann cells at the site of lesion, CD206 macrophage count in the unoperated contralateral sciatic nerve and the HSP27 expression in the contralateral DRG.
- Local treatment with PXL01 of a nerve autograft reconstructed sciatic nerve injury improves axonal outgrowth together with an increase in the HSP27 ratios in the DRG both in healthy and diabetic GK rats.
- Diabetes decreases axonal outgrowth and number of activated Schwann cells at site of lesion and in distal nerve end. Diabetes also decreases number of activated sensory neurons in DRG but increases HSP27 expression in uninjured DRG and sciatic nerves with some effects on the pro-inflammatory and pro-healing macrophages in the sciatic nerve.

PXL01
HSP27 ratio in DRG
PXL01
Axonal outgrowth
Diabetic GK rats
HSP27 in sensory neuron
Diabetic GK rats + PXL01
M2 macrophage
Diabetic GK rats
HSP27 in contralateral nerve
Diabetic GK rats
Activated sensory neurons
Diabetic GK rats + PXL01
Activated Schwann cells at site of lesion
CD206 macrophage ratio
Diabetic GK rats
Axonal outgrowth
Ipsilateral experimental
Controlateral
Ipsilateral experimental
8 days
Healthy
Diabetic GK
NaCl
PXL01+HA
NaCl
PXL01+HA

Discussion

The present thesis focuses on axonal outgrowth and the cellular responses after sciatic nerve injuries and various surgical procedures, particularly on Schwann cells and macrophages, together with expression of one of the Heat Shock Proteins, HSP27, belonging to the family of chaperones that are involved in protein folding, assembly, translocation and degradation in intracellular processes. The nerve regeneration processes were investigated after no nerve repair, immediate nerve repair, delayed nerve repair, reconstruction of a nerve defect with nerve autografts or with modified bioengineered conduits, and finally after local application of a pharmacological agent, PXL01, which potentially can stimulate axonal outgrowth in both healthy and diabetic GK rats. I focused on early regenerative events after nerve injury and repair or reconstruction. The achieved results will be discussed related to the time factor of nerve repair, including if a repair should be done at all, possibilities to improve nerve regeneration concerning the "tube concept" with modification of membranes, the option of using a locally applied pharmacological peptide to stimulate nerve regeneration after nerve repair or nerve reconstruction and finally discussing the impact of diabetes in some of the regeneration models.

Time schedule for repair of a nerve injury and relation to HSP27 expression

One focus, especially in Paper I, was the expression of HSP27 and its association with axonal outgrowth on different timelines after injury and with or without immediate nerve repair, or a delayed repair, in healthy Wistar and diabetic GK rats. HSP27, a member of heat shock protein family, already exists in CNS, spinal cord and in primary sensory neurons in physiological conditions as previously observed in experimental studies (Costigan et al., 1998). In addition, HSP27, is observed in human nerve biopsies from the forearm from healthy and diabetes type 1 and 2 human subjects, indicating its clinical relevance (Ising et al., 2023). Blood samples from type 1 and type 2 diabetic patients have shown that higher levels of serum HSP27 are related to better nerve function and fewer signs of neuropathy (Pourhamidi et al., 2011; Pourhamidi et al., 2014), which points out the neuroprotective effect of HSP27 rather than directly interacting with axonal outgrowth as in the present thesis. Experimental studies have also demonstrated that

HSP27 is increased in sensory neurons after axotomy (Costigan et al., 1998). It is antero- and retrogradely transported and is present in Schwann cells in accordance with previous (Hirata et al., 2003) and present findings. This is further indicated by the higher ratio of HSP27 at the site of lesion compared to the distal nerve end, indicating the presence of HSP27 where outgrowing axons interact with Schwann cells.

Experimental and clinical studies point to a neuroprotective and a regeneration supportive act of HSP27 expression in general (Benn et al., 2002; Nakagomi et al., 2003; Pourhamidi et al., 2011; Read & Gorman, 2009). However, there is a lack of information about HSP27 expression at the site of lesion and in the distal nerve end as well as in the DRG in relation to different nerve injury and repair or reconstruction models. I aimed to highlight these issues in the present thesis, such as a delayed nerve repair (Paper I), as well as the interaction of HSP27 with Schwann cell activity (Paper II, III, IV) and presence of macrophages (Paper III, IV) in nerve reconstruction with nerve autografts and conduits. In Paper I, III and IV, I confirmed by double staining with S100 and neurofilament that HSP27 exists in both axons and Schwann cells. HSP27 was differently expressed at the site of lesion and in the distal nerve end in Paper I and II, which could be explained by the different interaction between axons and Schwann cells in the nerve and in the regenerative matrix.

In the current thesis, I did not see any association with axonal outgrowth after sciatic nerve injury and immediate or delayed nerve repair as well as in conduit or nerve autograft reconstructed models to the expression of HSP27 in healthy or diabetic GK rats, despite its profound upregulation after the nerve injury. Comparison of the 7 days follow up after an immediate nerve repair or a delayed nerve repair of 7 days revealed no difference in axonal outgrowth despite a higher expression of HSP27 at site of lesion after a delayed nerve repair. In addition, comparison of the 14 days follow up with no to immediate nerve repair revealed, as expected, a longer axonal outgrowth after immediate nerve repair as well as a higher HSP27 after no nerve repair at site of lesion; the latter possibly as a response to the injury.

The nerve repair model with a delay of 7 days can be considered as an early-delayed nerve repair model. A previous publication has reported an impaired axonal outgrowth after a delayed nerve repair (14 days), which may be due to a decreased presence of p-ERK1/2 in Schwann cells as well as other changes with decreased activation and an increased apoptosis of Schwann cell in the distal nerve end (Tsuda et al., 2011), thereby with a diminished Schwann cell support for the outgrowing axons. In addition, a decrease in activated and an increase in apoptotic Schwann cells have been detected after a delayed nerve repair of 30 days (Saito & Dahlin, 2008). In an experimental study with different timelines of a delayed nerve repair (1, 4, 6, 8 or 12 weeks) that was compared with immediate nerve repair using nerve action potential as an outcome, the threshold for a delay was presented to be 4 weeks and should not be more than that (Wu et al., 2013), which is supported by the

experimental studies of activated and apoptotic Schwann cells (Saito et al., 2009). Other authors have in experimental studies evaluated longer periods of a delayed nerve repair with analyses of muscle atrophy and Schwann cell count, indicating that the critical time point for a poor outcome is 3 months in rats (Jonsson et al., 2013). Hypothetically, one should also expect an altered expression of HSP27 in a delayed nerve repair, which may affect the axonal outgrowth. In the present thesis, I detected an increased HSP27 expression at the site of lesion in sciatic nerve and in DRG after a delayed nerve repair compared to immediate repair, but the increase in HSP27 expression did not influence axonal outgrowth (Paper I).

Together, these data suggest that a delayed nerve repair alters the cellular and molecular response in the nerves with induction of HSP27. However, an impaired axonal outgrowth was observed if no repair (i.e., just leave the nerve ends) was performed. The data supports an opinion that an injured nerve should be repaired clinically (Jain et al., 2019) and that, according to the discussion above, a "best before date " of not more than 4 weeks is at least recommended; probably shorter for technical reasons. However, based on surgical and technical reasons, a nerve injury should promptly be repaired. Even though there are cases in literature that show recovered sensory function after an unrepaired injured digital nerve, the risk of neuroma formation after the injury remains unresolved (Poppler et al., 2018) and a high risk for persistent pain problems, despite further surgery, exists (Dahlin, Gudinge, et al., 2023; Dahlin, Zimmerman, et al., 2023). To summarize, in all the studies presented in this thesis I found no association between axonal outgrowth and the levels of HSP27 expression.

Why does a nerve repair or a nerve reconstruction fail?

Despite reports in various studies, stating that an effective nerve repair can be performed with the aid of an operating microscope, outcome after nerve injury and repair or reconstruction is insufficient (Grinsell & Keating, 2014; Midha & Grochmal, 2019). Several factors explain an unsatisfactory outcome, such as that i) the proximal nerve end cannot appropriately support axonal growth; ii) not all axons grow through the suture gap or the bridged defect and reach the distal nerve end; iii) the outgrowth of the axons is misdirected; iv) the speed of the outgrowth is relatively slow in comparison to the degeneration process in the distal nerve end (Jessen & Mirsky, 2019; Krishnan et al., 2024).

Axonal outgrowth is calculated to 1-3 mm/day in humans but may be higher in rat sciatic nerves (Danielsen et al., 1995; Kerns et al., 1993). Chronic denervation with a resulting slow outgrowth of axons to the targets causes target atrophy, and with a risk that some specific neurons also gradually die and cannot longer support the outgrowing axons (Hoke, 2006; Sulaiman & Gordon, 2013). As a part of these points, the intention of Paper III and IV was to improve the outgrowth of the axons

based on data from the clinical study of flexor tendon surgery with the sub-analyses of the injured digital nerve injuries (Wiig et al., 2014). However, as the outcome in Paper III showed, no positive effect on axonal outgrowth was observed in short term despite a clear effect on the treatment with a diminished response of the CD68 stained macrophages. In contrast, a positive effect of the PXL01 treatment was observed in nerve autografts (Paper IV), where data indicated an improved length of axonal outgrowth at the investigated time point. The mechanism(s) of this effect cannot clearly be defined, although the treatment showed an effect on the HSP27 expression ratio in DRG. However, as indicated in Paper I, HSP27 expression is not related to axonal outgrowth, but may be neuroprotective.

Another important factor that controls the success of nerve regeneration is the distal nerve end, which gradually loses its ability to support the outgrowing axons through the reduction of the Schwann cell population or by the fading of the "repair Schwann cells" (Jessen & Mirsky, 2019). Unfortunately, the speed of axons cannot meet the rate of degeneration and alterations of Schwann cells, therefore the regeneration fails. The interaction between the Schwann cells and the neuron and its axon is crucial. Schwann cells need the axonal contact to hold their "repair" status, why their ability may be reduced over time if regeneration fails. New formation of capillaries, for example by stimulation of the macrophages, helps to align the Schwann cells thereby forming guidance cues for the outgrowing axons (Krishnan et al., 2024). The outgrowth of axons may therefore be associated to the microcirculation and formation of capillaries as well as to the proliferation and migration of Schwann cells in the distal nerve end. Therefore, models that evaluate nerve regeneration after a delayed nerve repair should be included in research to better understand failure mechanisms in such situations.

In Paper I, I evaluated axonal outgrowth and HSP27 expression in different surgical nerve repair models with focus on any failure mechanism(s) related to no nerve repair (such as in a situation where the nerve is not repaired at all for different reasons), immediate nerve repair (an optimal situation) and a delayed nerve repair (a "suboptimal" situation depending on the time after injury). Immediate nerve repair was superior with axonal outgrowth compared to no nerve repair, but interestingly a shortly (i.e., 7 days) delayed nerve repair had no negative impact on axonal outgrowth, indicating similar axonal outgrowth as after the immediate nerve repair despite the mentioned increased HSP27 expression. Again, whether a delayed nerve repair of 7 days has any influence on nerve regeneration in a clinical situation yet needs to be clarified (Jain et al., 2019; Kim et al., 2018; Kusuhara et al., 2019; Weber & Isaacs, 2023). Nerve reconstruction with a conduit also lacks an endoneurial structure that is vital for Schwann cells and outgrowing axons (Cattin & Lloyd, 2016; McMorrow et al., 2024; Pan et al., 2020), which is another factor that diminish the nerve regeneration process in conduits if the distance in the conduit is too long. Therefore, I included models with membranes with different nanoparticles to improve the nerve regeneration process in conduits (Paper II).

Sprouting of outgrowing axons responds to guidance cues in the regenerating environment and Schwann cells help to attract or to repel outgrowing axons with chemical substances, such as VEGF-1 (attracts motor axons), BDNF, NT-3, NGF, and GDNF (attracts sensory axons) (Bolívar et al., 2020). Thus, that was also the attempt to modify the conduits with inserted membranes with "cues" of nanoparticles in Paper II, a procedure that had a positive effect on nerve regeneration. Still, even a modified conduit has a limited effect on nerve regeneration in shorter nerve defects.

In reconstruction of a nerve defect with a conduit, a nerve autograft or a PNA, the problem of hypoxia must be considered. The formed regenerative matrix within a conduit as well as the environment in the PNA or in the nerve autograft are all initially avascular; thus, a hypoxic environment for the outgrowing axons and the surviving, migrating and proliferating Schwann cells. Even if angiogenesis starts early, interestingly through the macrophages, by secretion of substances, such as VEGF (Cattin et al., 2015), it takes time until new capillaries are formed, basically from the proximal and distal ends of the conduit or graft and provides blood supply to the outgrowing axons and the Schwann cells. The outcome of hypoxia is a diminished axonal outgrowth, which is probably more pronounced in conduits than in nerve autografts and in particular compared to an immediate nerve repair in which the distal nerve end is well vascularized (Pan et al., 2020). As a part of these points, the intention of Paper IV was to improve the outgrowth of the axons in an autograft reconstructed model. Paper III with primary repair model showed no positive effect on axonal outgrowth in short term despite a clear effect of the local PXL01 treatment with a diminished response of the CD68 stained macrophages. In contrast, a positive effect of the PXL01 treatment was observed in nerve autografts (Paper IV), in which a hypoxic environment is present initially, and data indicated an improved length of axonal outgrowth at the investigated time point. The mechanism(s) of PXL01 treatment effect, especially in the nerve autograft reconstruction model, will be discussed in a later section.

Attempts to overcome failures of nerve regeneration

Modification of conduits in nerve regeneration

Different methods of modification of conduits have been discussed. One modification is to increase the luminal surface by diving the conduit into a variety of small or larger "chambers" as in Paper II (Johansson & Dahlin, 2014). Division of the lumen may help to guide the outgrowing axons and the migration of Schwann cells. Previous studies have shown that dividing a conduit into two chambers by a membrane gives a promising result regarding axonal outgrowth and functional

outcome almost reaching the level of functional outcome of nerve autografts (Meyer et al., 2016). Another study indicated a better axonal outgrowth and an improved functional outcome with one inserted film compared to three films (Clements et al., 2009). In accordance, a superiority, with respect to function and histochemistry of one channel in a NeuraGen® conduit, bridging a 10 mm nerve defect in rats, compared to two to four channels, have been reported (Yao et al., 2010).

I designed a PCL conduit and modified it with one membrane that was made of Poly-propylene-polyethylene glycol (PPEG) and additionally enhanced it with gold and gold-cobalt-oxide NPs (Paper II). In accordance with reports in the literature, the chambered conduit with a PPEG membrane led to a longer axonal outgrowth at the investigated time point of 21 days. I chose PCL as a material for the conduit because of its highly hydrophobic properties, its slow degradation rate, its rigidity and being transparent which makes it easier to suture (Assaf et al., 2017; Taylor & Haycock, 2022; Ulery et al., 2011). A co-polymer of PCL, known as Neurolac®, has been used in a rat study with a 15 mm sciatic nerve defect. At follow up at 2 years after surgery, data showed axonal outgrowth but still with observed remnants of the conduit and a foreign body reaction present (Meek & Jansen, 2009). A clinical study using Neurolac® to bridge up to 20 mm nerve defects is reported to successfully support nerve regeneration and with a recovery of sensibility as good as after a primary nerve repair up to one year after surgery (Bertleff et al., 2005; Kehoe et al., 2012). Later, PCL conduits have been modified by carbon and graphene NPs that seem to speed up degradation rate (Assaf et al., 2017). In addition, Reid et al reported nearly a similar axonal outgrowth compared to nerve autografts in rats 18 weeks after implantation with hydroxide treated PCL conduits (Reid et al., 2013) but with lighter muscle weight compared to the nerve autograft, indicating a diminished functional outcome. Based on these data, PCL was used in Paper II with the intention to modify it and to further improve nerve regeneration. Previous publications, together with the present data (Paper II), indicate that a mechanical modification may improve nerve regeneration, but functional outcome may still be insufficient. Thus, there is a need for further enhancement of the design of conduits, such as increasing the hydrophilicity and increasing the cellular adhesions.

Initially, I conducted a pilot study with different polymer membranes and NPs to analyse how different nanoparticles (i.e., silver, gold and gold cobalt oxide) on a polymer composite membrane (PPEG or PHUPNIPAM) with a 10 mm or a 15 mm nerve defect model may affect nerve regeneration. The best result with the longest axonal outgrowth was observed in the 10 mm nerve defect model with a PPEG membrane and gold and gold-cobalt oxide nanoparticles (Paper II, supplementary file). Therefore, I chose to divide the present conduit into two chambers with a membrane made by PPEG, which is a hydrophilic and biocompatible polymer and a good carrier for integrating NPs (Hazer et al., 2011; D. B. Hazer et al., 2012; Hazer et al., 2016). Polyethylene glycol (PEG) can be neuroprotective by silencing

oxidative stress on neurons (Papastefanaki et al., 2015) and can also facilitate axonal outgrowth as a conduit (Mokarizadeh et al., 2016). Recent clinical (Bamba et al., 2016) and experimental studies (Paskal et al., 2019) with PEG fusion may enable rapid fusion of nerve ends with promising outcome with respect to axonal regeneration and prevention of Wallerian degeneration.

I detected a longer axonal outgrowth in the chambered conduits compared to the hollow conduits. In almost half of the rats, I detected two full-length cables, and the second cable revealed axonal outgrowth and Schwann cell response as well as HSP27 expression that mimic the main regenerated cable at the follow up of 21 days. This phenomenon can be explained by a high hydrophilicity of the PPEG membrane, supporting cellular attachment and permitting molecular communication in between chambers (Qian et al., 2021). Immunohistochemical analysis showed that the membrane chambered conduits, compared to hollow conduits, attracted more activated (i.e., ATF3 stained) Schwann cells to the regenerative matrix and even induced a higher HSP27 expression in the distal nerve end compared to hollow conduit, highlighting a neuroprotective effect. However, like in Paper I, there was no direct impact of HSP27 expression in the regenerated matrix or in DRG on axonal outgrowth (Paper II). Again, this information suggests that HSP27 expression in axons and in Schwann cells, together with the sensory neurons and surroundings in DRG, acts mainly as a neuroprotective protein rather than a protein that stimulates axonal outgrowth.

Additional modification of the membrane with nanoparticles – with gold and gold - cobalt-oxide – improved the length of axonal outgrowth at the investigated time point. AuNPs were a good candidate for biomedical applications due to its inert and non-immunogenic character, its good biocompatibility and its relatively low toxicity (Paviolo & Stoddart, 2017). Several studies have shown an enhanced nerve regeneration, such as successful axonal outgrowth on electrospun nanofibers coated with Au nanoparticles (Baranes et al., 2016), silk fibres coated with Au NPs attracting and facilitating adhesion of Schwann cells without toxicity at 18 months (Das et al., 2015), and even stem cell modified conduits with continued secretion of gold nanoparticles (Jahromi et al., 2020). On the other hand, a magnetic nanoparticle, cobalt oxide, is used as an anticancer agent (Huang et al., 2020), being most active intracellularly by increasing cleaved caspase 3 activity in leukemic cells (Chattopadhyay et al., 2014). Although there is no published information concerning application of cobalt-oxide nanoparticles in peripheral nerve research, the results in Paper II indicate that modification of the nanoparticle-embedded membranes attracted more activated Schwann cells to the regenerative matrix together with a higher HSP27 expression with the former probably be the factor that drives the significantly longer axonal outgrowth compared to hollow and non-modified PPEG membrane chambered conduits. Additionally, CoO nanoparticles in the PPEG membrane (Paper II) decreased the amount of cleaved caspase 3 stained Schwann cells in the regenerative matrix and this low apoptotic Schwann cell count

had a direct positive influence on axonal outgrowth. Hypothetically this can be explained as: NPs applied on a suitable membrane may form nano-islands, inducing a change on the surface of the membrane, which facilitates attachment of Schwann cells. This may help the Schwann cells to stay in an activated state rather than an apoptotic state leading to an enhanced axonal outgrowth. There may be negative effects of using NPs in conduits. Toxicity of NPs is one disadvantage of the biomedical application (Paviolo & Stoddart, 2017; Soderstjerna et al., 2014). Metal NPs in high concentrations, especially silver nanoparticles, cause oxidative stress on cells and production of reactive oxygen species, leading to disruption of the cell membrane, DNA damage and cell death (see Background). In Paper II, I used gold and cobalt-oxide NPs, because gold NPs are inert metal NPs. However, cobalt oxide, which can activate apoptosis and oxidative stress mechanisms itself, causes cellular toxicity why it is used in cancer treatment. Two factors can be involved in the mechanism(s) of toxicity of metal NPs. First, the intranuclear activity of the NP and second, the degree of the release of the NPs from the nanocomplex (Chattopadhyay et al., 2014; Paviolo & Stoddart, 2017). In Paper II, the size of the gold and cobalt-oxide nanoparticles were greater than 50 nm (i.e., around 70–100 nm), and they were embedded into the PPEG membranes, which probably increased their stability. Furthermore, I did not detect any traces of nanoparticles systemically at 21 days of the implantation, indicating a low risk for a general toxicity.

Pharmacological treatment to improve nerve regeneration

As I have mentioned in the background section, there have been several agents used, both clinically and experimentally, to influence nerve regeneration (Rayner et al., 2022). Unfortunately, usage of some of all mentioned substances may be limited due to systemic side effects (Rayner et al., 2022). These adverse systemic effects could be due to a rather slow axonal outgrowth that needs to be met with long term treatment periods. Therefore, to choose a local administration method could be a solution to the problem. I used local application of PXL01 in the present study; a substance that has been used clinically without any adverse effects and with indications of an improved axonal outgrowth after a digital nerve injury in humans (Wiig et al., 2014). In the future, one may develop a carrier system, such as a biodegradable polymer, that releases a drug at a rate that cover the regeneration time.

Nerve repair model

PXL01, the lactoferrin-derived peptide, shows an improvement of sensory nerve function in patients with a digital nerve injury (Wiig et al., 2014). Its anti-adhesive effect is due to an indirect increased expression of lubricin, which in turn decreases mRNA expression of IL-1, IL-6 and IL-8 observed in the tendon sheaths in flexor tendon surgery (Edsfeldt et al., 2017). PXL01 also activates the plasminogen

activating system (Siconolfi & Seeds, 2001). Tissue plasminogen activator (tPA) enhances Schwann cell migration and axonal regrowth by degrading the extracellular matrix (Klimovich et al., 2021). In cell culture, growth cones of regenerating axons secrete proteases, specifically plasminogen activators (PAs), which are believed to facilitate growth cone movement by digesting extracellular matrices and cell adhesion molecules (Klimovich et al., 2021). After a nerve crush injury, PA activity is detected hours after the injury and stays active until day 7 within the regenerating axons (Siconolfi & Seeds, 2001). I designed two studies with the intention to evaluate effects of PXL01 - an immediate nerve repair model in healthy rats (Paper III) and a nerve autograft model in healthy and diabetic rats (Paper IV), focusing on a variety of cellular responses. In the nerve repair model, PXL01 had no impact on axonal outgrowth, Schwann cells response or HSP27 expression, neither in the sciatic nerve nor in DRG. However, it inhibited the recruitment of activated-invading (i.e., CD68 stained) macrophages at 6 days without any alteration in the number of M2 pro-healing macrophages (i.e., CD206 stained). Recruited macrophages reach a peak within a week after injury, which is crucial for proper axonal regrowth by the cleaning of the debris in Wallerian degeneration (Cattin & Lloyd, 2016). The decrease in invading macrophages at site of injury without their polarization to pro-healing macrophages can potentially be explained by PXL01 preferentially inhibits production of inflammatory cytokines, such as IL-1 and IL-6 (Edsfeldt et al., 2017), but has no effect on IL-10 that contributes to the polarization of M2 macrophages (Liu et al., 2019). However, this macrophage inhibition did not directly have any negative effect on axonal outgrowth at 6 days after the repair of the nerve injury. This may be due to PXL01 not altering the Schwann cell activity and HSP27 expression in the operated sciatic nerve and points that the unaltered population of M2 macrophages has somehow a protective effect. Maybe, a longer time is needed to see a change in axonal outgrowth. The inhibitory effect of macrophages brings another curiosity that PXL01 may behave in another way in a different nerve injury model, such as reconstruction with a nerve autograft or a scaffold in a nerve defect model (i.e., Paper IV).

Nerve reconstruction model

The regenerative events in a nerve autograft used to reconstruct a nerve defect are different in which there is a non-vascularized section, which creates an initial hypoxic environment for the present and possibly migrating and proliferating Schwann cells as well as the outgrowing axons (see Background). The sprouting axons, with their growth cones, need to pass through this hypoxic environment in close interaction with the Schwann cells that remain after the surgical graft procedure. During this process, macrophages again play a crucial role by invading the area and secreting several substances, like VEGF, which help angiogenesis (Cattin & Lloyd, 2016). Additionally, newly formed endothelial cell cords serve as a track that also helps to physically align the Schwann cells and the outgrowing axons during the nerve regeneration process (Cattin & Lloyd, 2016; Krishnan et al.,

2024). One may speculate that PXL01 can influence the macrophages in another way in the nerve autografts. Surprisingly, I did not find any differences in numbers of CD68 or CD206 stained macrophages in the nerve autografts with or without PXL01 treatment (Paper IV). An inhibition of numbers of activated Schwann cells by PXL01 was detected. Schwann cells need oxygen to survive before the angiogenesis starts. Such revascularization starts approximately 3 days after an autologous nerve reconstruction in rabbits (Penkert et al., 1988; Tiam M. Saffari et al., 2020). Hypothetically, PXL01 may affect the angiogenesis of the nerve autografts, which may subsequently delay that process. Therefore, the activated Schwann cells may not proliferate until the time that new vessels are formed. Surprisingly, the decreased Schwann cell activity did not have any negative impact on axonal outgrowth; instead PXL01 facilitated axonal outgrowth which may be due to the activation of the PA system. However, I did not analyse the plasminogen activating system in sciatic nerves in this thesis. Facilitated axonal outgrowth in nerve autografts by PXL01, but not in a nerve repair model, supports the findings that PXL01 may be more important in a hypoxic microenvironment. This also may suggest that PXL01 could be more effective in conduits used to reconstruct nerve defects.

Additionally, PXL01 treatment increased M2 macrophage activity in the contralateral nerve and this increase affected the ratio of M2 macrophages in the distal nerve end which caused a decrease by the PXL01 treatment. Together with the data that PXL01 decreases HSP27 expression in the contralateral DRG, again an increase in HSP27 ratio in the DRG was observed, indicating somehow a neuroprotective effect in the nerve autograft reconstructed model (Read & Gorman, 2009). If the distant neuroprotective effect present in DRG, and in the contralateral sciatic nerve as pro-healing macrophages, are processes that have any impact on the improved axonal outgrowth by PXl01 is not known; probably being less likely. I could not detect any similar effect in the model with nerve repair in the healthy rats, which again implies that PXl01 reacts more efficiently in a hypoxic environment.

Diabetic rat models for nerve regeneration

As the prevalence of diabetes increases in the world (Feldman et al., 2017; Gregory et al., 2022; Sun et al., 2022), it is important to understand the cellular and molecular interaction of nerve regeneration in diabetes. Subjects with diabetes are active and have a professional life with a risk of a peripheral nerve injury. In addition, the understanding of the degeneration and regeneration processes in diabetes, particularly in the presence of neuropathy, is crucial (Albers & Pop-Busui, 2014; Dahlin, 2023; Eid et al., 2023).

In the literature, most experimental models with a nerve injury is performed in rats with streptozotocin-induced diabetes (Lee et al., 2015; Nishida et al., 2013), which in review articles considered to mimic mostly type 1 diabetes, with a toxic

destruction of the beta cell islands in the pancreas, causing high blood glucose levels and a katabolic condition (Biessels et al., 2014; Talukdar & Basumatary, 2023; M. Yorek, 2022; Yorek, 2016). "Multi-low dosing" streptozotocin-induced diabetes is more often used as a diabetes model, causing more stable clinical condition without cachexia. There is generally criticism against this streptozotocin-induced diabetes model because of the presence of non-specific effects on kidney and nerve tissue (Yorek, 2016). However, there are reports on nerve regeneration after a nerve injury with this model that presents an impaired axonal outgrowth over time (Nishida et al., 2013; Sango et al., 2017; Terada et al., 1998). Another alternative of a diabetic rat model is the genetic model with spontaneously developed diabetes in Biobreeding (BB)-rats. They exhibit even higher blood glucose levels and in short-term nerve injury models it is considered to also mimic diabetes type 1 (Talukdar & Basumatary, 2023; M. A. Yorek, 2022). However, for longer time periods, there is need for insulin injections, which makes it more difficult to judge the effect of diabetes on nerve regeneration. A nerve transection injury with an immediate nerve repair model in BB rats shows a similar axonal outgrowth as in healthy rats despite a significantly higher number of activated and apoptotic Schwann cells both at site of lesion and in the distal nerve end compared to healthy rats (Stenberg et al., 2012).

I used the genetically modified rats – the GK rat model - to imitate diabetes (Paper I, IV), which more resembles type 2 diabetes with relatively moderate blood glucose levels in humans, thereby not requiring additional insulin treatment despite a long follow up. The used rats were around 8 weeks old, with a weight of around 200 gr, and therefore at a relative young adult age in the rat´s life span (Sengupta, 2013). From the age of 2 weeks, they start to have a glucose intolerance and by 4 weeks hyperglycaemia is found and later early stages of diabetic neuropathy are reported in adult rats (Talukdar & Basumatary, 2023). Thus, the used diabetic rat model is a suitable model to investigate nerve regeneration in conjunction with different techniques to stimulate such a process as previously reported (Stenberg et al., 2016; Stenberg et al., 2017).

Nerve regeneration in the diabetic Goto-Kakizaki model

Axonal outgrowth

Diabetes was associated with a decreased axonal outgrowth after immediate nerve repair (Paper I). In addition, probably as a response to a subclinical neuropathy, HSP27 expression was higher in the contralateral uninjured nerve and in DRG among the diabetic rats compared to the healthy ones, indicating that diabetic rats may be a suitable model to investigate the effects of diabetes on nerve regeneration with a clinical implication (Paper I). Previous data from our research group, evaluating nerve regeneration up to 21 days in nerve reconstruction using hollow chitosan conduits compared to reconstruction with nerve autografts, revealed that

not only autologous nerve grafts were superior to hollow chitosan conduits, but that diabetic GK rats showed a longer axonal outgrowth compared to healthy rats (Stenberg et al., 2016). Even a delayed nerve reconstruction in a 15 mm long nerve defect that is reconstructed by a chitosan conduit, reinforced with a porous membrane, shows a better nerve regeneration process in GK rats, where the nerve defect is bridged by a nerve autograft or a chitosan conduit (Meyer et al., 2016; Stenberg et al., 2017). In both studies, the follow-up time was longer than the time I have analysed in Paper I and IV. This fact could be reason why there was a shorter axonal outgrowth in diabetic rats in the present thesis, especially in delayed nerve repair. Overall, this indicates that it is important to investigate nerve regeneration in different rat models, such as diabetic rat models, for a better overview of novel nerve repair and nerve reconstruction methods as indicated in the studies of PLX01 treatment (Paper III, IV). No effect of PLX01 was observed after immediate repair of a sciatic nerve injury in healthy rats (Paper III), but an improved axonal outgrowth was seen in the nerve autografts after treatment with PLX01 where both healthy and diabetic GK rats were used (Paper IV).

In summary, axonal outgrowth in nerve autografts after nerve reconstruction in diabetic rats in short term (8 days), with and without treatment with PXL01, is shorter that healthy rats, which is opposite to data where diabetic rats are followed for longer time (21 days) (Stenberg et al., 2016). Current data may indicate that the time required to prepare the environment of the autograft in diabetes takes longer, where the nerve regeneration process initially is impaired in diabetic rats but over time improves (Stenberg et al., 2016).

HSP27 expression

Diabetic rats showed a higher HSP27 expression in the uninjured nerves at the 7 days follow up and an even higher expression at the site of lesion at 7- and 14-days follow up. The former finding was later supported in Paper IV with higher detectable HSP27 expression in the contralateral uninjured sciatic nerve. The question is if this reaction comes from "the physiological" state of expression that already is in the body or that there is also a reaction to the injury in the contralateral uninjured nerve. To answer this question, I conducted a pilot study with sham operated and naïve rats (for details see Method section) in healthy and diabetic rats. The results showed that in sham operated healthy and diabetic rats there was HSP27 expression in both sides of the sciatic nerves (sham operated side and unoperated side) and of DRG. Statistical analyses were not conducted but HSP27 expression was similar in both sides in both sciatic nerve and DRG. In naïve rats, HSP27 expression was also detected in sciatic nerve and in DRG. The intensity of the HSP27 expression in naïve rats was similar to sham operated rats (statistical analysis not conducted). When HSP27 expression in sciatic nerve and in DRG of sham operated and naïve rats were compared to the contralateral-uninjured side of the pharmacologically treated nerve injury-repaired rats, sham operated, and naïve rats revealed a trend to less HSP27

expression (again no statistical analysis was conducted). There was not enough data to analyse any difference between healthy and diabetic rats with respect to HSP27 expression in sham operated or naïve rats. This entity about the sensing of the injury by the contralateral side has also been reported by others with detection of higher levels of transcription factors and increased growth-related gene expression together with increased axonal growth capacity in the contralateral or distant DRG after injury or in sciatic nerve (Hashimoto-Torii et al., 2018; Verge et al., 2020). The authors point out the importance of including naïve and sham operated individuals in the project planning (Hashimoto-Torii et al., 2018; Verge et al., 2020). The relative higher expression of HSP27 in the diabetic GK rats is in accordance with a clinical study postulating that decreased serum levels of HSP27 relates to impaired nerve function (Pourhamidi et al., 2014). A slight, but insignificant, increase in HSP27 is also seen in human nerve biopsies in type 1 diabetes compared to healthy subjects and subjects with type 2 diabetes (Ising et al., 2023). HSP27 is probably active in "prevention" or "compensation" of the mechanism(s) involved in diabetic neuropathy in humans (Pourhamidi et al., 2011). The contralateral effect on HSP27 expression in diabetic GK rats can still be interpreted as a reaction to a probable onset of subclinical diabetic neuropathy (Murakawa et al., 2002). Diabetes is reported to correlate and be associated with a diminished axonal outgrowth by other (Kennedy & Zochodne, 2005; Stenberg et al., 2012) and present (Paper I) studies, but HSP27 expression does not have any impact on axonal outgrowth (Paper I, IV).

Schwann cell activity

Diabetes, with a systemic high blood glucose level, leads to an increased oxidative stress and an increase in free radicals (Albers & Pop-Busui, 2014), which in turn causes damage to the peripheral nerve, resulting in neuropathy. It is speculated that Schwann cells in a diabetic nerve lose their capacity to provide energy to the axons by a mechanism that hyperglycaemia causes Schwann cells mitochondria to produce high amount of ROS. This in turn causes damage to mitochondria DNA, resulting in insufficient energy generation and mitochondrial apoptosis (Fernyhough, 2015; Li et al., 2023; Wu et al., 2024). This pathophysiological mechanism may be especially important in the nerve autograft reconstructed model after a nerve injury. Again, in such a condition there is already an hypoxic environment initially after the reconstruction, and the Schwann cells and the outgrowing axons rely mostly on diffusion from the environment for energy supply (Pan et al., 2020; T. M. Saffari et al., 2020). Thus, it is important to apply nerve autografts in a suitable bed, such as in a healthy muscle tissue with the intention to provide the nerve autograft with an optimal oxygen tension. The hypoxic state can be one explanation why a lower percentage of activated Schwann cells, both at site of lesion and in the distal nerve end, in diabetic rats, independent of the treatment modality (Paper IV), is present. This finding is an important factor that causes a diminished axonal outgrowth in diabetic rats in the nerve autograft reconstruction model early point after the surgical procedure. Previous studies with nerve autograft reconstruction models in diabetic

rats (Stenberg et al., 2016) and nerve repair in the streptozotocin-induced diabetic rat model (Stenberg et al., 2012) show that numbers of apoptotic Schwann cells are high in the sciatic nerve, which is contrast to the present nerve autograft reconstruction model (Paper IV). In Paper IV, with the nerve autograft reconstruction model, I also detected fewer activated sensory neurons in DRG in diabetic rats, which may contribute to the impaired axonal outgrowth. The data indicates that in diabetes there is a delicate balance in Schwann cell activity that regulates the axonal outgrowth.

Macrophages

An important evaluation when investigating the potential effect of PLX01 is evaluation of the macrophage response also in diabetic rats. Macrophages were hypothesized to be affected in diabetic individuals after a nerve injury. Upon an acute peripheral nerve injury, RAGE [receptor of advanced glycation end products (AGEs)] are expressed in activated Schwann cells and in infiltrating mononuclear phagocytes (Sorci et al., 2013). In a nerve crush model in diabetic wild type mice, there is macrophage polarization, an increased amount of pro-inflammatory M1 macrophages and fewer pro-regenerative M2 macrophages compared to healthy and RAGE deficient ones and this result is explained to be due to RAGE activity (Juranek et al., 2013). After a nerve injury, activated Schwann cells and recruited macrophages have more AGE receptors on their surface that interact not only with AGE products but also with several other pro-inflammatory and regulatory molecules (Sango et al., 2017). An impaired axonal outgrowth may be due to RAGE dependent alterations in the macrophage polarization, favouring the M1 pro-inflammatory macrophages (Sango et al., 2017).

In Paper IV, in contrast to the literature, I detected a decrease in the activated-invading macrophage count (CD68 stained) at the site of lesion in diabetic rats together with a decrease in CD206 stained macrophage ratio in the distal nerve end. However, there was a prominent increase in the M2 (CD206 stained) macrophage count in the contralateral uninjured nerves among the diabetic GK rats. The contralateral increase in the M2 macrophage count could be due to an increased neuroprotective response that previously was explained by an increased HSP27. The decreased count of CD68 stained macrophages, especially at the site of lesion, could be due to a decrease in activated Schwann cell count present in the diabetic rats that are already responsible to recruit macrophages to the injury site but fail to do so.

Diabetes, sex and nerve regeneration

I only investigate female rats (Paper I, III, IV) in relation to HSP27 expression and the effects of PXL01 treatment, but one may consider in the future to use also male rats since sex differences have been reported (Stenberg & Dahlin, 2014). In both healthy and diabetic GK rats, male rats show a longer axonal outgrowth than female

rats (Stenberg & Dahlin, 2014). As earlier pointed out, the blood sugar levels may be more unstable in male rats compared to female rats, which may be a strong argument to use female rats in the present projects (See Background).

Funäsdalen, July, 2020

Strength and limitations

In this thesis, axonal outgrowth, the Schwann cell response, and the distant reaction of sensory neurons in DRG were investigated after application of different, and clinically relevant, surgical methods - from primary and immediate nerve repair to the nerve autograft or conduit reconstructions. Addition of the macrophage response to this cellular response was a complement to understand the orchestrated cellular interaction.

However, there are several aspects that one can consider as drawbacks. First, the observation period in the immunohistochemical analysis was rather short (longest time was 21 days in Paper II). These days, i.e. 6-8 and 14 days, were chosen with respect to the repair and the reconstruction methods. The reason is that I was especially interested in the cellular response at the time where the outgrowing axons should reach the nerve autograft or conduit and passing the suture line after nerve repair, but not too far allowing analysing the response in the distal nerve end without the presence of outgrowing axons.

Another aspect is that only female rats were used. There are some sex differences with respect to nerve regeneration, in favour of male sex having a longer axonal outgrowth at the presently used time points for evaluation (Stenberg & Dahlin, 2014). However, in research, female rats are the principal rat type to investigate impact of drugs and stimulations, and by not using the male rats, one can also avoid the unstable blood sugar levels that potentially occur in male diabetic rats (Stenberg & Dahlin, 2014).

In Paper II, I used conduits to reconstruct a nerve defect, but I did not include any nerve autograft reconstruction as a control group. Additionally, there was no additional experimental group of PCL conduit with a PPEG membrane modified by only cobalt oxide NPs. A future project should include a nerve autograft model as a control group together with investigation of cobalt oxide in nerve regeneration.

Also, in Paper III and IV, I applied a new pharmacological drug locally to the operated nerve. One question could be how much of this drug stays in the operative field and more of that how much of that remains on the nerve. I could only evaluate the presence of the drug macroscopically and did not perform any analyses. As far as I could observe, there was a milky solution, not so thick as initially, that stayed on the nerve after 6 and 8 days compared to a clear nerve tissue in the saline group, suggesting that there is still active compound at the observation time.

The last, but not the least, I did not use any methods to investigate functional outcome in long-term in the different surgical methods. The functional outcome is more relevant in longer follow up periods, especially when a new pharmacological treatment or a new bioengineered conduit is evaluated. In studies using PXL01, I already knew from the clinical study that indicated a better functional outcome in repaired human injured digital nerves, but the question was what may happen at the

cellular levels (Wiig et al., 2014). The bioengineered conduit study was planned in the first place to see first the biocompatibility and the cellular response, and evaluation of the functional outcome is planned in the future.

Future perspectives

Despite the achievement of understanding the multicellular interaction and knowledge about molecular pathways presented in the literature after a peripheral nerve injury and subsequent regeneration, the outcome of nerve repair or reconstruction is still insufficient. Those cases are still a challenge in clinical practice. Different surgical techniques, addition of pharmacological agents, as well as introduction and modification of different bioengineered conduits, some enhanced with stem cells or growth factors, have been reported in medical literature with a variety of regenerative properties and outcome.

In this thesis, I have gathered four studies in which I have analysed the cellular aspects with potential interactions in various nerve repair and reconstruction models, including neuroprotection mirrored by HSP27 expression, in healthy and diabetic rats. I aimed to introduce a new bioengineered conduit, chambered with a membrane that was modified with gold and gold-cobalt oxide nanoparticles, which may be an aspect that can further be developed in the future, e.g., longer nerve defects, and other time points for follow up etc. The most important obstacle in clinical practice is the nerve injury cases with large nerve defects, which future studies should focus on. Utilization of growth factors or stem cells to bioengineered conduits have not yielded any significant improvement and create some challenges, particularly using stem cells. Whether the present conduit can be used in models with longer nerve defects is not known and a specific focus is needed to overcome the discussed hypoxic microenvironment. Enhancement of the conduit lumen with other filling material in the lumen, such as spider silk fibres (Radtke et al., 2011; Vogt et al., 2024) or with extracellular matrix proteins, can be a solution to overcome this obstacle.

When peripheral nerve injury and repair and reconstruction models, that are clinically relevant, are introduced, one should strongly apply these models in rat models where a concomitant disease, like diabetes, cardiovascular disease etc., is present. Novel methods should be applicable also in such individuals as well as also be potentially suitable for all types of peripheral nerves, such as digital and major nerve trunks in the upper and lower limbs. The complexity of neuroprotective mechanism(s) must further be analysed both in health and disease. A deeper knowledge may be achieved from various nerve biopsies after nerve injuries in relation to time after the injury.

Finally, introduction of a new pharmacological agents, such as PXL01, which is already established in clinically practice as an antiadhesive agent in hand surgery, is strongly advised to further facilitate nerve regeneration beyond what can be done by surgery. Interestingly, PXL01 can decrease inflammation through altering the pan (CD68) macrophage response without interfering with the pro-healing (CD206) macrophages or impairing axonal outgrowth. Whether PXL01, or other similar substances, may be applied as an adjunct in surgical treatment of a specific type of neuroma, i.e., scarred, or tethered neuroma, is not known but must be elucidated clinically. The question is if substances, like PXL01, may be an additional clinical tool in immediate or in delayed nerve reconstruction is also most relevant.

Fårö, Gotland , July, 2023

"*Yesterday I was clever, so I wanted to change the world.*
Today I am wise, so I am changing myself."
Mevlânâ Celâleddin-i Rûmî

Conclusions

The main conclusions, based on the present four included papers, are that:

- A nerve injury extensively increases expression of HSP27 ipsilaterally in an injured sciatic nerve and in DRG in healthy and diabetic Goto-Kakizaki (GK) rats but HSP27 has no impact on axonal outgrowth.
- A nerve injury should appropriately be repaired, but a short (7 days) delay of the nerve repair does not influence axonal outgrowth.
- Conduits with an inserted poly-propylene poly-ethylene glycol (PPEG) membrane – i.e., "chambered conduits" – show an improved nerve regeneration compared to hollow conduits. Modification of such a membrane with gold or gold-cobalt-oxide NPs promotes the nerve regeneration process even more.
- Treatment with PLX01 locally around a nerve repair inhibits inflammation through pan (CD68)-macrophages without affecting the pro (CD206)-healing macrophages but has no impact on nerve regeneration in such a short-term nerve repair model.
- Local application of PXL01 in a short-term nerve autograft reconstruction model may improve axonal outgrowth and has an impact on Schwann cell activation as well as on HSP27 expression in DRG in healthy and diabetic GK rats.
- Diabetes has a negative impact on nerve regeneration after nerve injury and repair or reconstruction in short-term as well as is associated with an increased expression of HSP27 in the sciatic nerve and in DRG on the uninjured side.

Acknowledgement

I wish to express my sincere gratitude to **Lund University**, for all the support during the doctoral education period and everyone who contributed to this work, to all my friends and colleagues, with special thanks to:

Professor Lars B. Dahlin, my main supervisor, for your timeless and "zoneless" support and guidance, not only through the dissertation but also my journey about medical profession in Sweden.

Lena Stenberg, Ph.D., my co-supervisor, for your patience having me in the lab and teaching me literally from the scratch. Thank you for the invaluable discussions in front of the microscope and thank you for just being there for me.

Associate Professor Ulrica Englund Johansson, my co-supervisor, for your support during the project and in the final phase.

Margit Mahlapuu, co-author, and a colleague from Göteborg University, thank you for your endless support and help, especially with technical and logistic support to provide the pharmacological agent.

Raquel Perez, co-author, for your support in statistical analysis and "very simple" explanation for the data.

Professor Baki Hazer, my father and co-author from Kapadokya University, Department of Aircraft Airframe Engine Maintenance, Ürgüp, for your lifetime guidance, encouragement and being my mentor.

Sho Kohyama and **Seigo Suganuma**, co-authors from Japan, thank you for your collaborations.

Tina Folker, research administrator in the Department of Hand Surgery, not just for all the administrative support through the years, but also being my friend, and for making the life easy for me in Sweden.

Consultant Emma Svensdotter, the chief of the Neurosurgery department, and the former chief **Nils Ståhl**, and to all colleagues in Neurosurgery clinic in Lund for the support and giving me time to finish my thesis parallel to my clinical work.

Professor Mansur Harmandar, former rector of Muğla Sıtkı Koçman University, for making it possible for me to start and go on with my PhD education in Sweden.

Professor Nevres Armağan, former dean of Medical Faculty in Muğla Sıtkı Koçman University, for your full support and being by my side in my scientific journey in Sweden even though I was full time employee in neurosurgery clinic in Muğla Sıtkı Koçman University in Turkey.

Professor Gürsel Leblebicioğlu, colleague from Hacettepe University, Department of Hand Surgery, Ankara for introducing me to the great area of hand surgery, especially peripheral nerve surgery. You are the reason I met Lars in the first place.

Professor Selçuk Palaoğlu, former supervisor and colleague from Hacettepe University, Department of Neurosurgery, Ankara for your scientific support and especially your guidance for choosing the endless peripheral nerve ocean as my scientific path.

Hans Eric Rosberg, my partner in life, and colleague in hand surgery, for just being there for me. Thank you for being a problem solver and allow me to live with my thesis 24/7. I could not do it without you.

My mother **Kadriye** and my sister **Ebru**, for being there for me by heart.

Axel, my son, my joy of life, you make every moment worth it.

The papers included in this thesis were supported by grants from the Swedish Research Council (2021-01942), Lund University, Elly Olsson's Foundation, Region Skåne and Skåne University Hospital, Sydvästra Skånes Diabetesförening as well as the Swedish Diabetes Foundation (DIA2020-492).

References

Al-Bishri, A., Dahlin, L., Sunzel, B., & Rosenquist, J. (2005). Systemic Betamethasone Accelerates Functional Recovery After a Crush Injury to Rat Sciatic Nerve. *Journal of Oral and Maxillofacial Surgery*, *63*(7), 973-977. https://doi.org/https://doi.org/10.1016/j.joms.2004.10.010

Albers, J. W., & Pop-Busui, R. (2014). Diabetic Neuropathy: Mechanisms, Emerging Treatments, and Subtypes. *Current Neurology and Neuroscience Reports*, *14*(8), 473. https://doi.org/10.1007/s11910-014-0473-5

Allmeling, C., Jokuszies, A., Reimers, K., Kall, S., Choi, C. Y., Brandes, G., Kasper, C., Scheper, T., Guggenheim, M., & Vogt, P. M. (2008). Spider silk fibres in artificial nerve constructs promote peripheral nerve regeneration. *Cell Prolif*, *41*(3), 408-420. https://doi.org/10.1111/j.1365-2184.2008.00534.x

Almgren, K. G. (1975). Revascularization of free pheripheral nerve grafts. An experimental study in the rabbit. *Acta Orthop Scand Suppl*, *154*, 1-104. https://doi.org/10.3109/ort.1974.45.suppl-154.01

Alwafi, H., Alsharif, A. A., Wei, L., Langan, D., Naser, A. Y., Mongkhon, P., Bell, J. S., Ilomaki, J., Al Metwazi, M. S., Man, K. K. C., Fang, G., & Wong, I. C. K. (2020). Incidence and prevalence of hypoglycaemia in type 1 and type 2 diabetes individuals: A systematic review and meta-analysis. *Diabetes Research and Clinical Practice*, *170*, 108522. https://doi.org/https://doi.org/10.1016/j.diabres.2020.108522

Armstrong, S. J., Wiberg, M., Terenghi, G., & Kingham, P. J. (2007). ECM molecules mediate both Schwann cell proliferation and activation to enhance neurite outgrowth. *Tissue Eng*, *13*(12), 2863-2870. https://doi.org/10.1089/ten.2007.0055

Asato, F., Butler, M., Blomberg, H., & Gordh, T. (2000). Variation in rat sciatic nerve anatomy: implications for a rat model of neuropathic pain. *J Peripher Nerv Syst*, *5*(1), 19-21. https://doi.org/10.1046/j.1529-8027.2000.00155.x

Assaf, K., Leal, C. V., Derami, M. S., de Rezende Duek, E. A., Ceragioli, H. J., & de Oliveira, A. L. R. (2017). Sciatic nerve repair using poly(epsilon-caprolactone) tubular prosthesis associated with nanoparticles of carbon and graphene. *Brain Behav*, *7*(8), e00755. https://doi.org/10.1002/brb3.755

Atzei, A., Calcagni, M., Breda, B., Fasolo, G., Pajardi, G., & Cugola, L. (2007). Clinical evaluation of a hyaluronan-based gel following microsurgical reconstruction of peripheral nerves of the hand. *Microsurgery*, *27*(1), 2-7. https://doi.org/10.1002/micr.20299

Avsar, Ü. Z., Avsar, U., Aydin, A., Yayla, M., Ozturkkaragoz, B., Un, H., Saritemur, M., & Mercantepe, T. (2014). L-carnitine alleviates sciatic nerve crush injury in rats: functional and electron microscopy assessments. *Neural Regeneration Research*, *9*(10). https://journals.lww.com/nrronline/fulltext/2014/09100/l_carnitine_alleviates_sciatic_nerve_crush_injury.5.aspx

Bamba, R., Waitayawinyu, T., Nookala, R., Riley, D. C., Boyer, R. B., Sexton, K. W., Boonyasirikool, C., Niempoog, S., Kelm, N. D., Does, M. D., Dortch, R. D., Shack, R. B., & Thayer, W. P. (2016). A novel therapy to promote axonal fusion in human digital nerves. *J Trauma Acute Care Surg*, *81*(5 Suppl 2 Proceedings of the 2015 Military Health System Research Symposium), S177-S183. https://doi.org/10.1097/TA.0000000000001203

Baranes, K., Shevach, M., Shefi, O., & Dvir, T. (2016). Gold Nanoparticle-Decorated Scaffolds Promote Neuronal Differentiation and Maturation. *Nano Lett*, *16*(5), 2916-2920. https://doi.org/10.1021/acs.nanolett.5b04033

Barik, T. K., Maity, G. C., Gupta, P., Mohan, L., & Santra, T. S. (2021). Nanomaterials: An Introduction. In T. S. Santra & L. Mohan (Eds.), *Nanomaterials and Their Biomedical Applications* (pp. 1-27). Springer Singapore. https://doi.org/10.1007/978-981-33-6252-9_1

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*(4), 258-267. https://doi.org/10.1002/micr.20127

Beirowski, B., Adalbert, R., Wagner, D., Grumme, D. S., Addicks, K., Ribchester, R. R., & Coleman, M. P. (2005). The progressive nature of Wallerian degeneration in wild-type and slow Wallerian degeneration (WldS) nerves. *BMC Neuroscience*, *6*(1), 6. https://doi.org/10.1186/1471-2202-6-6

Belen, D., Aciduman, A., & Er, U. (2009). History of peripheral nerve repair: may the procedure have been practiced in Hippocratic School? *Surg Neurol*, *72*(2), 190-193; discussion 193-194. https://doi.org/10.1016/j.surneu.2008.03.030

Bell, J. H., & Haycock, J. W. (2012). Next generation nerve guides: materials, fabrication, growth factors, and cell delivery. *Tissue Eng Part B Rev*, *18*(2), 116-128. https://doi.org/10.1089/ten.TEB.2011.0498

Benn, S. C., Perrelet, D., Kato, A. C., Scholz, J., Decosterd, I., Mannion, R. J., Bakowska, J. C., & Woolf, C. J. (2002). Hsp27 Upregulation and Phosphorylation Is Required for Injured Sensory and Motor Neuron Survival. *Neuron*, *36*(1), 45-56. https://doi.org/10.1016/S0896-6273(02)00941-8

Bergmeister, K. D., Große-Hartlage, L., Daeschler, S. C., Rhodius, P., Böcker, A., Beyersdorff, M., Kern, A. O., Kneser, U., & Harhaus, L. (2020). Acute and long-term costs of 268 peripheral nerve injuries in the upper extremity. *PLoS One*, *15*(4), e0229530. https://doi.org/10.1371/journal.pone.0229530

Berthold, C. H., & Rydmark, M. (1983). Electrophysiology and morphology of myelinated nerve fibers. VI. Anatomy of the paranode-node-paranode region in the cat. *Experientia*, *39*(9), 964-976. https://doi.org/10.1007/bf01989761

Bertleff, M. J. O. E., 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*(3), 513-518. https://doi.org/https://doi.org/10.1016/j.jhsa.2004.12.009

Biessels, G. J., Bril, V., Calcutt, N. A., Cameron, N. E., Cotter, M. A., Dobrowsky, R., Feldman, E. L., Fernyhough, P., Jakobsen, J., Malik, R. A., Mizisin, A. P., Oates, P. J., Obrosova, I. G., Pop-Busui, R., Russell, J. W., Sima, A. A., Stevens, M. J., Schmidt, R. E., Tesfaye, S., . . . Zochodne, D. W. (2014). Phenotyping animal models of diabetic neuropathy: a consensus statement of the diabetic neuropathy study group of the EASD (Neurodiab). *J Peripher Nerv Syst*, *19*(2), 77-87. https://doi.org/10.1111/jns5.12072

Birch, R., & Raji, A. R. (1991). Repair of median and ulnar nerves. Primary suture is best. *J Bone Joint Surg Br*, *73*(1), 154-157. https://doi.org/10.1302/0301-620x.73b1.1991753

Bockelmann, J., Klinkhammer, K., von Holst, A., Seiler, N., Faissner, A., Brook, G. A., Klee, D., & Mey, J. (2011). Functionalization of electrospun poly(epsilon-caprolactone) fibers with the extracellular matrix-derived peptide GRGDS improves guidance of schwann cell migration and axonal growth. *Tissue Eng Part A*, *17*(3-4), 475-486. https://doi.org/10.1089/ten.TEA.2010.0369

Boivin, A., Pineau, I., Barrette, B., Filali, M., Vallières, N., Rivest, S., & Lacroix, S. (2007). Toll-like receptor signaling is critical for Wallerian degeneration and functional recovery after peripheral nerve injury. *J Neurosci*, *27*(46), 12565-12576. https://doi.org/10.1523/jneurosci.3027-07.2007

Bolandghamat, S., & Behnam-Rassouli, M. (2020). Recent Findings on the Effects of Pharmacological Agents on the Nerve Regeneration after Peripheral Nerve Injury. *Curr Neuropharmacol*, *18*(11), 1154-1163. https://doi.org/10.2174/1570159X18666200507084024

Bolívar, S., Navarro, X., & Udina, E. (2020). Schwann Cell Role in Selectivity of Nerve Regeneration. *Cells*, *9*(9), 2131. https://www.mdpi.com/2073-4409/9/9/2131

Bontioti, E., Kanje, M., Lundborg, G., & Dahlin, L. B. (2005). End-to-side nerve repair in the upper extremity of rat. *J Peripher Nerv Syst*, *10*(1), 58-68. https://doi.org/10.1111/j.1085-9489.2005.10109.x

Bosch-Queralt, M., Fledrich, R., & Stassart, R. M. (2023). Schwann cell functions in peripheral nerve development and repair. *Neurobiol Dis*, *176*, 105952. https://doi.org/10.1016/j.nbd.2022.105952

Boyle, J. P., Thompson, T. J., Gregg, E. W., Barker, L. E., & Williamson, D. F. (2010). Projection of the year 2050 burden of diabetes in the US adult population: dynamic modeling of incidence, mortality, and prediabetes prevalence. *Popul Health Metr*, *8*, 29. https://doi.org/10.1186/1478-7954-8-29

Bushnell, B. D., McWilliams, A. D., Whitener, G. B., & Messer, T. M. (2008). Early clinical experience with collagen nerve tubes in digital nerve repair. *J Hand Surg Am*, *33*(7), 1081-1087. https://doi.org/10.1016/j.jhsa.2008.03.015

Cai, L., Lu, J., Sheen, V., & Wang, S. (2012). Lubricated biodegradable polymer networks for regulating nerve cell behavior and fabricating nerve conduits with a compositional gradient. *Biomacromolecules*, *13*(2), 358-368. https://doi.org/10.1021/bm201372u

Cajal, S. R. y. (1928). *Degeneration and regeneration of the nervous system.* .

Callaghan, B. C., Price, R. S., Chen, K. S., & Feldman, E. L. (2015). The Importance of Rare Subtypes in Diagnosis and Treatment of Peripheral Neuropathy: A Review. *JAMA Neurol*, *72*(12), 1510-1518. https://doi.org/10.1001/jamaneurol.2015.2347

Carvalho, C. R., Oliveira, J. M., & Reis, R. L. (2019). Modern Trends for Peripheral Nerve Repair and Regeneration: Beyond the Hollow Nerve Guidance Conduit. *Front Bioeng Biotechnol*, *7*, 337. https://doi.org/10.3389/fbioe.2019.00337

Carvalho, C. R., Silva-Correia, J., Oliveira, J. M., & Reis, R. L. (2019). Nanotechnology in peripheral nerve repair and reconstruction. *Adv Drug Deliv Rev*, *148*, 308-343. https://doi.org/10.1016/j.addr.2019.01.006

Carvalho-de-Souza, J. L., Nag, O. K., Oh, E., Huston, A. L., Vurgaftman, I., Pepperberg, D. R., Bezanilla, F., & Delehanty, J. B. (2019). Cholesterol Functionalization of Gold Nanoparticles Enhances Photoactivation of Neural Activity. *ACS Chemical Neuroscience*, *10*(3), 1478-1487. https://doi.org/10.1021/acschemneuro.8b00486

Cattin, A. L., Burden, J. J., Van Emmenis, L., Mackenzie, F. E., Hoving, J. J., Garcia Calavia, N., Guo, Y., McLaughlin, M., Rosenberg, L. H., Quereda, V., Jamecna, D., Napoli, I., Parrinello, S., Enver, T., Ruhrberg, C., & Lloyd, A. C. (2015). Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves. *Cell*, *162*(5), 1127-1139. https://doi.org/10.1016/j.cell.2015.07.021

Cattin, A. L., & Lloyd, A. C. (2016). The multicellular complexity of peripheral nerve regeneration. *Curr Opin Neurobiol*, *39*, 38-46. https://doi.org/10.1016/j.conb.2016.04.005

Cederlund, R. I., Thomsen, N., Thrainsdottir, S., Eriksson, K. F., Sundkvist, G., & Dahlin, L. B. (2009). Hand disorders, hand function, and activities of daily living in elderly men with type 2 diabetes. *J Diabetes Complications*, *23*(1), 32-39. https://doi.org/10.1016/j.jdiacomp.2007.09.002

Chan, K. M., Gordon, T., Zochodne, D. W., & Power, H. A. (2014). Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets. *Exp Neurol*, *261*, 826-835. https://doi.org/https://doi.org/10.1016/j.expneurol.2014.09.006

Chattopadhyay, S., Dash, S. K., Kar Mahapatra, S., Tripathy, S., Ghosh, T., Das, B., Das, D., Pramanik, P., & Roy, S. (2014). Chitosan-modified cobalt oxide nanoparticles stimulate TNF-alpha-mediated apoptosis in human leukemic cells. *J Biol Inorg Chem*, *19*(3), 399-414. https://doi.org/10.1007/s00775-013-1085-2

Chen, P., & Bonaldo, P. (2013). Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. *Int Rev Cell Mol Biol*, *301*, 1-35. https://doi.org/10.1016/b978-0-12-407704-1.00001-4

Chen, P., Piao, X., & Bonaldo, P. (2015). Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol*, *130*(5), 605-618. https://doi.org/10.1007/s00401-015-1482-4

Chen, Y., Long, X., Lin, W., Du, B., Yin, H., Lan, W., Zhao, D., Li, Z., Li, J., Luo, F., & Tan, H. (2021). Bioactive 3D porous cobalt-doped alginate/waterborne polyurethane scaffolds with a coral reef-like rough surface for nerve tissue engineering application [10.1039/D0TB02347G]. *Journal of Materials Chemistry B*, *9*(2), 322-335. https://doi.org/10.1039/D0TB02347G

Chiu, D. T., & Strauch, B. (1990). A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. *Plast Reconstr Surg*, *86*(5), 928-934. https://doi.org/10.1097/00006534-199011000-00015

Choi, S. Y., Kim, J. M., Jung, J., Park, D. C., Yoo, M. C., Kim, S. S., Kim, S. H., & Yeo, S. G. (2022). Review of Drug Therapy for Peripheral Facial Nerve Regeneration That Can Be Used in Actual Clinical Practice. *Biomedicines*, *10*(7). https://doi.org/10.3390/biomedicines10071678

Clements, I. P., Kim, Y. T., English, A. W., Lu, X., Chung, A., & Bellamkonda, R. V. (2009). Thin-film enhanced nerve guidance channels for peripheral nerve repair. *Biomaterials*, *30*(23-24), 3834-3846. https://doi.org/10.1016/j.biomaterials.2009.04.022

Coppey, L. J., Davidson, E. P., Rinehart, T. W., Gellett, J. S., Oltman, C. L., Lund, D. D., & Yorek, M. A. (2006). ACE Inhibitor or Angiotensin II Receptor Antagonist Attenuates Diabetic Neuropathy in Streptozotocin-Induced Diabetic Rats. *Diabetes*, *55*(2), 341-348. https://doi.org/10.2337/diabetes.55.02.06.db05-0885

Costigan, M., Mannion, R. J., Kendall, G., Lewis, S. E., Campagna, J. A., Coggeshall, R. E., Meridith-Middleton, J., Tate, S., & Woolf, C. J. (1998). Heat Shock Protein 27: Developmental Regulation and Expression after Peripheral Nerve Injury. *The Journal of Neuroscience*, *18*(15), 5891-5900. https://doi.org/10.1523/jneurosci.18-15-05891.1998

Cotter, M. A., & Cameron, N. E. (2003). Effect of the NAD(P)H oxidase inhibitor, apocynin, on peripheral nerve perfusion and function in diabetic rats. *Life Sci*, *73*(14), 1813-1824. https://doi.org/10.1016/s0024-3205(03)00508-3

Daeschler, S. C., Feinberg, K., Harhaus, L., Kneser, U., Gordon, T., & Borschel, G. H. (2023). Advancing Nerve Regeneration: Translational Perspectives of Tacrolimus (FK506). *Int J Mol Sci*, *24*(16). https://doi.org/10.3390/ijms241612771

Dahlin, E., Gudinge, H., Dahlin, L. B., & Nyman, E. (2023). Neuromas cause severe residual problems at long-term despite surgery. *Sci Rep*, *13*(1), 15693. https://doi.org/10.1038/s41598-023-42245-4

Dahlin, E., Zimmerman, M., & Nyman, E. (2023). Patient reported symptoms and disabilities before and after neuroma surgery: a register-based study. *Sci Rep*, *13*(1), 17226. https://doi.org/10.1038/s41598-023-44027-4

Dahlin, L., Brandt, J., Nilsson, A., Lundborg, G., & Kanje, M. (2007). Schwann cells, acutely dissociated from a predegenerated nerve trunk, can be applied into a matrix used to bridge nerve defects in rats. *Acta Neurochir Suppl*, *100*, 57-59. https://doi.org/10.1007/978-3-211-72958-8_12

Dahlin, L., & Lundborg, G. (2001). The use of silicone tubing in the late repair of the median and ulnar nerves in the forearm. *J Hand Surg Br*, *26*(4), 393-394. https://doi.org/10.1054/jhsb.2001.0561

Dahlin, L. B. (1995). Prevention of macrophage invasion impairs regeneration in nerve grafts. *Brain Res*, *679*(2), 274-280. https://doi.org/10.1016/0006-8993(95)00249-p

Dahlin, L. B. (2008). Techniques of peripheral nerve repair. *Scand J Surg*, *97*(4), 310-316. https://doi.org/10.1177/145749690809700407

Dahlin, L. B. (2013). The role of timing in nerve reconstruction. *Int Rev Neurobiol*, *109*, 151-164. https://doi.org/10.1016/B978-0-12-420045-6.00007-9

Dahlin, L. B. (2023). The Dynamics of Nerve Degeneration and Regeneration in a Healthy Milieu and in Diabetes. *Int J Mol Sci*, *24*(20). https://doi.org/10.3390/ijms242015241

Dahlin, L. B., Anagnostaki, L., & Lundborg, G. (2001). Tissue response to silicone tubes used to repair human median and ulnar nerves. *Scand J Plast Reconstr Surg Hand Surg*, *35*(1), 29-34. https://doi.org/10.1080/02844310151032510

Dahlin, L. B., Miyauchi, A., Thomsen, P., Danielsen, N., & Kanje, M. (1996). Stimulation of nerve regeneration by macrophages in granulation tissue. *Restor Neurol Neurosci*, *9*(3), 141-149. https://doi.org/10.3233/RNN-1996-9302

Dahlin, L. B., & Wiberg, M. (2017). Nerve injuries of the upper extremity and hand. *EFORT Open Rev*, *2*(5), 158-170. https://doi.org/10.1302/2058-5241.2.160071

Danielsen, N., Kerns, J. M., Holmquist, B., Zhao, Q., Lundborg, G., & Kanje, M. (1995). Predegeneration enhances regeneration into acellular nerve grafts. *Brain Research*, *681*(1), 105-108. https://doi.org/https://doi.org/10.1016/0006-8993(95)00300-F

Das, S., Sharma, M., Saharia, D., Sarma, K. K., Sarma, M. G., Borthakur, B. B., & Bora, U. (2015). In vivo studies of silk based gold nano-composite conduits for functional peripheral nerve regeneration. *Biomaterials*, *62*, 66-75. https://doi.org/10.1016/j.biomaterials.2015.04.047

De Ruiter, G. C. W., Spinner, R. J., Verhaagen, J., & Maless, M. J. A. (2014). Misdirection and guidance of regenerating axons after experimental nerve injury and repair: A review [Review]. *Journal of Neurosurgery*, *120*(2), 493-501. https://doi.org/10.3171/2013.8.JNS122300

Delibaş, B., & Kaplan, S. (2024). The histomorphological and stereological assessment of rat dorsal root ganglion tissues after various types of sciatic nerve injury. *Histochem Cell Biol*, *161*(2), 145-163. https://doi.org/10.1007/s00418-023-02242-0

Dellon, E. S., & Dellon, A. L. (1993). The first nerve graft, Vulpian, and the nineteenth century neural regeneration controversy. *J Hand Surg Am*, *18*(2), 369-372. https://doi.org/10.1016/0363-5023(93)90378-G

Díaz, A., López-Grueso, R., Gambini, J., Monleón, D., Mas-Bargues, C., Abdelaziz, K. M., Viña, J., & Borrás, C. (2019). Sex Differences in Age-Associated Type 2 Diabetes in Rats-Role of Estrogens and Oxidative Stress. *Oxid Med Cell Longev*, *2019*, 6734836. https://doi.org/10.1155/2019/6734836

Dillon, B. R., Ang, L., & Pop-Busui, R. (2024). Spectrum of Diabetic Neuropathy: New Insights in Diagnosis and Treatment. *Annu Rev Med*, *75*, 293-306. https://doi.org/10.1146/annurev-med-043021-033114

Edsfeldt, S., Holm, B., Mahlapuu, M., Reno, C., Hart, D. A., & Wiig, M. (2017). PXL01 in sodium hyaluronate results in increased PRG4 expression: a potential mechanism for anti-adhesion. *Ups J Med Sci*, *122*(1), 28-34. https://doi.org/10.1080/03009734.2016.1230157

Eid, S. A., Rumora, A. E., Beirowski, B., Bennett, D. L., Hur, J., Savelieff, M. G., & Feldman, E. L. (2023). New perspectives in diabetic neuropathy. *Neuron*, *111*(17), 2623-2641. https://doi.org/10.1016/j.neuron.2023.05.003

Elafros, M. A., Andersen, H., Bennett, D. L., Savelieff, M. G., Viswanathan, V., Callaghan, B. C., & Feldman, E. L. (2022). Towards prevention of diabetic peripheral neuropathy: clinical presentation, pathogenesis, and new treatments. *Lancet Neurol*, *21*(10), 922-936. https://doi.org/10.1016/s1474-4422(22)00188-0

Erduranlı, H., Hazer, B., & Borcaklı, M. (2008). Post Polymerization of Saturated and Unsaturated Poly (3-hydroxy alkanoate) s. Macromolecular symposia,

Escobar, A., Reis, R. L., & Oliveira, J. M. (2022). Nanoparticles for neurotrophic factor delivery in nerve guidance conduits for peripheral nerve repair. *Nanomedicine (Lond)*, *17*(7), 477-494. https://doi.org/10.2217/nnm-2021-0413

Fansa, H., Schneider, W., Wolf, G., & Keilhoff, G. (2002). Host responses after acellular muscle basal lamina allografting used as a matrix for tissue engineered nerve grafts1. *Transplantation*, *74*(3), 381-387. https://doi.org/10.1097/00007890-200208150-00015

Feldman, E. L., Callaghan, B. C., Pop-Busui, R., Zochodne, D. W., Wright, D. E., Bennett, D. L., Bril, V., Russell, J. W., & Viswanathan, V. (2019). Diabetic neuropathy. *Nat Rev Dis Primers*, *5*(1), 42. https://doi.org/10.1038/s41572-019-0097-9

Feldman, E. L., Nave, K. A., Jensen, T. S., & Bennett, D. L. H. (2017). New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. *Neuron*, *93*(6), 1296-1313. https://doi.org/10.1016/j.neuron.2017.02.005

Fernyhough, P. (2015). Mitochondrial dysfunction in diabetic neuropathy: a series of unfortunate metabolic events. *Curr Diab Rep*, *15*(11), 89. https://doi.org/10.1007/s11892-015-0671-9

Fernyhough, P., & McGavock, J. (2014). Mechanisms of disease: Mitochondrial dysfunction in sensory neuropathy and other complications in diabetes. *Handb Clin Neurol*, *126*, 353-377. https://doi.org/10.1016/b978-0-444-53480-4.00027-8

Frost, H. K., Andersson, T., Johansson, S., Englund-Johansson, U., Ekstrom, P., Dahlin, L. B., & Johansson, F. (2018). Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo. *Sci Rep*, *8*(1), 16716. https://doi.org/10.1038/s41598-018-34699-8

Frostadottir, D., Chemnitz, A., Johansson Ot, L. J., Holst, J., & Dahlin, L. B. (2023). Evaluation of Processed Nerve Allograft in Peripheral Nerve Surgery: A Systematic Review and Critical Appraisal. *Plast Reconstr Surg Glob Open*, *11*(6), e5088. https://doi.org/10.1097/gox.0000000000005088

Fu, S. Y., & Gordon, T. (1997). The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol*, *14*(1-2), 67-116. https://doi.org/10.1007/bf02740621

Fugleholm, K. (2013). Chapter 45 - The surgery of peripheral nerves (including tumors). In G. Said & C. Krarup (Eds.), *Handbook of Clinical Neurology* (Vol. 115, pp. 781-802). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-444-52902-2.00045-X

Gao, M., Lu, P., Lynam, D., Bednark, B., Campana, W. M., Sakamoto, J., & Tuszynski, M. (2016). BDNF gene delivery within and beyond templated agarose multi-channel guidance scaffolds enhances peripheral nerve regeneration. *J Neural Eng*, *13*(6), 066011. https://doi.org/10.1088/1741-2560/13/6/066011

Garrido, C., Gurbuxani, S., Ravagnan, L., & Kroemer, G. (2001). Heat Shock Proteins: Endogenous Modulators of Apoptotic Cell Death. *Biochemical and Biophysical Research Communications*, *286*(3), 433-442. https://doi.org/https://doi.org/10.1006/bbrc.2001.5427

Gaudet, A. D., Leung, M., Poirier, F., Kadoya, T., Horie, H., & Ramer, M. S. (2009). A role for galectin-1 in the immune response to peripheral nerve injury. *Exp Neurol*, *220*(2), 320-327. https://doi.org/10.1016/j.expneurol.2009.09.007

Geremia, N. M., Pettersson, L. M., Hasmatali, J. C., Hryciw, T., Danielsen, N., Schreyer, D. J., & Verge, V. M. (2010). Endogenous BDNF regulates induction of intrinsic neuronal growth programs in injured sensory neurons. *Exp Neurol*, *223*(1), 128-142. https://doi.org/10.1016/j.expneurol.2009.07.022

Geuna, S., Raimondo, S., Ronchi, G., Di Scipio, F., Tos, P., Czaja, K., & Fornaro, M. (2009). Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. *Int Rev Neurobiol*, *87*, 27-46. https://doi.org/10.1016/S0074-7742(09)87003-7

Giordano, T., & MacDonald, W. (2021). Thoracic endometriosis presenting as recurrent pleural effusions. *Radiol Case Rep*, *16*(2), 250-253. https://doi.org/10.1016/j.radcr.2020.11.025

Giusti, G., Willems, W. F., Kremer, T., Friedrich, P. F., Bishop, A. T., & Shin, A. Y. (2012). Return of motor function after segmental nerve loss in a rat model: comparison of autogenous nerve graft, collagen conduit, and processed allograft (AxoGen). *J Bone Joint Surg Am*, *94*(5), 410-417. https://doi.org/10.2106/JBJS.K.00253

Gold, B., Katoh, K., & Storm-Dickerson, T. (1995). The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. *The Journal of Neuroscience*, *15*(11), 7509-7516. https://doi.org/10.1523/jneurosci.15-11-07509.1995

Gold, B. G. (1997). FK506 and the role of immunophilins in nerve regeneration [Article]. *Molecular Neurobiology*, *15*(3), 285-306. https://doi.org/10.1007/BF02740664

Gomez-Sanchez, J. A., Carty, L., Iruarrizaga-Lejarreta, M., Palomo-Irigoyen, M., Varela-Rey, M., Griffith, M., Hantke, J., Macias-Camara, N., Azkargorta, M., Aurrekoetxea, I., De Juan, V. G., Jefferies, H. B. J., Aspichueta, P., Elortza, F., Aransay, A. M., Martínez-Chantar, M. L., Baas, F., Mato, J. M., Mirsky, R., . . . Jessen, K. R. (2015). Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves. *Journal of Cell Biology*, *210*(1), 153-168. https://doi.org/10.1083/jcb.201503019

Gomez-Sanchez, J. A., Pilch, K. S., van der Lans, M., Fazal, S. V., Benito, C., Wagstaff, L. J., Mirsky, R., & Jessen, K. R. (2017). After Nerve Injury, Lineage Tracing Shows That Myelin and Remak Schwann Cells Elongate Extensively and Branch to Form Repair Schwann Cells, Which Shorten Radically on Remyelination. *The Journal of Neuroscience*, *37*(37), 9086-9099. https://doi.org/10.1523/jneurosci.1453-17.2017

Goncalves, N. P., Vaegter, C. B., Andersen, H., Ostergaard, L., Calcutt, N. A., & Jensen, T. S. (2017). Schwann cell interactions with axons and microvessels in diabetic neuropathy. *Nat Rev Neurol*, *13*(3), 135-147. https://doi.org/10.1038/nrneurol.2016.201

Gonzalez-Perez, F., Udina, E., & Navarro, X. (2013). Chapter Ten - Extracellular Matrix Components in Peripheral Nerve Regeneration. In S. Geuna, I. Perroteau, P. Tos, & B. Battiston (Eds.), *International Review of Neurobiology* (Vol. 108, pp. 257-275). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-410499-0.00010-1

Gordon, T., Amirjani, N., Edwards, D. C., & Chan, K. M. (2010). Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol*, *223*(1), 192-202. https://doi.org/https://doi.org/10.1016/j.expneurol.2009.09.020

Gregory, G. A., Robinson, T. I. G., Linklater, S. E., Wang, F., Colagiuri, S., de Beaufort, C., Donaghue, K. C., International Diabetes Federation Diabetes Atlas Type 1 Diabetes in Adults Special Interest, G., Magliano, D. J., Maniam, J., Orchard, T. J., Rai, P., & Ogle, G. D. (2022). Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. *Lancet Diabetes Endocrinol*, *10*(10), 741-760. https://doi.org/10.1016/S2213-8587(22)00218-2

Grinsell, D., & Keating, C. P. (2014). Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int*, *2014*, 698256. https://doi.org/10.1155/2014/698256

Haastert-Talini, K., & Dahlin, L. B. (2018). Diabetes, its impact on peripheral nerve regeneration: lessons from pre-clinical rat models towards nerve repair and reconstruction. *Neural Regen Res*, *13*(1), 65-66. https://doi.org/10.4103/1673-5374.224370

Haastert-Talini, K., Geuna, S., Dahlin, L. B., Meyer, C., Stenberg, L., Freier, T., Heimann, C., Barwig, C., Pinto, L. F., Raimondo, S., Gambarotta, G., Samy, S. R., Sousa, N., Salgado, A. J., Ratzka, A., Wrobel, S., & Grothe, C. (2013). Chitosan tubes of varying degrees of acetylation for bridging peripheral nerve defects. *Biomaterials*, *34*(38), 9886-9904. https://doi.org/10.1016/j.biomaterials.2013.08.074

Haastert-Talini, K., Schmitte, R., Korte, N., Klode, D., Ratzka, A., & Grothe, C. (2011). Electrical Stimulation Accelerates Axonal and Functional Peripheral Nerve Regeneration across Long Gaps. *Journal of Neurotrauma*, *28*(4), 661-674. https://doi.org/10.1089/neu.2010.1637

Hadlock, T., Sundback, C., Hunter, D., Cheney, M., & Vacanti, J. P. (2000). A Polymer Foam Conduit Seeded with Schwann Cells Promotes Guided Peripheral Nerve Regeneration. *Tissue Engineering*, *6*(2), 119-127. https://doi.org/10.1089/107632700320748

Hai, T., Wolfgang, C. D., Marsee, D. K., Allen, A. E., & Sivaprasad, U. (1999). ATF3 and stress responses. *Gene Expr*, *7*(4-6), 321-335.

Hallgren, A., Björkman, A., Chemnitz, A., & Dahlin, L. B. (2013). Subjective outcome related to donor site morbidity after sural nerve graft harvesting: a survey in 41 patients. *BMC Surg*, *13*, 39. https://doi.org/10.1186/1471-2482-13-39

Harty, B. L., & Monk, K. R. (2017). Unwrapping the unappreciated: recent progress in Remak Schwann cell biology. *Curr Opin Neurobiol*, *47*, 131-137. https://doi.org/10.1016/j.conb.2017.10.003

Hashimoto-Torii, K., Sasaki, M., Chang, Y. W., Hwang, H., Waxman, S. G., Kocsis, J. D., Rakic, P., & Torii, M. (2018). Detection of local and remote cellular damage caused by spinal cord and peripheral nerve injury using a heat shock signaling reporter system. *IBRO Rep*, *5*, 91-98. https://doi.org/10.1016/j.ibror.2018.11.003

Hazer, B., & Steinbuchel, A. (2007). Increased diversification of polyhydroxyalkanoates by modification reactions for industrial and medical applications. *Appl Microbiol Biotechnol*, *74*(1), 1-12. https://doi.org/10.1007/s00253-006-0732-8

Hazer, D. B., Bal, E., Nurlu, G., Benli, K., Balci, S., Ozturk, F., & Hazer, B. (2013). In vivo application of poly-3-hydroxyoctanoate as peripheral nerve graft. *J Zhejiang Univ Sci B*, *14*(11), 993-1003. https://doi.org/10.1631/jzus.B1300016

Hazer, D. B., Hazer, B., & Dincer, N. (2011). Soft tissue response to the presence of polypropylene-G-poly(ethylene glycol) comb-type graft copolymers containing gold nanoparticles. *J Biomed Biotechnol*, *2011*, 956169. https://doi.org/10.1155/2011/956169

Hazer, D. B., Kılıçay, E., & Hazer, B. (2012). Poly(3-hydroxyalkanoate)s: Diversification and biomedical applications: A state of the art review. *Materials Science and Engineering: C*, *32*(4), 637-647. https://doi.org/https://doi.org/10.1016/j.msec.2012.01.021

Hazer, D. B., Mut, M., Dincer, N., Saribas, Z., Hazer, B., & Ozgen, T. (2012). The efficacy of silver-embedded polypropylene-grafted polyethylene glycol-coated ventricular catheters on prevention of shunt catheter infection in rats. *Childs Nerv Syst*, *28*(6), 839-846. https://doi.org/10.1007/s00381-012-1729-5

Hazer, D. B., Sakar, M., Dere, Y., Altinkanat, G., Ziyal, M. I., & Hazer, B. (2016). Antimicrobial Effect of Polymer-Based Silver Nanoparticle Coated Pedicle Screws: Experimental Research on Biofilm Inhibition in Rabbits. *Spine (Phila Pa 1976)*, *41*(6), E323-329. https://doi.org/10.1097/BRS.0000000000001223

Hile, C., & Veves, A. (2003). Diabetic neuropathy and microcirculation. *Curr Diab Rep*, *3*(6), 446-451. https://doi.org/10.1007/s11892-003-0006-0

Hirata, K., He, J., Hirakawa, Y., Liu, W., Wang, S., & Kawabuchi, M. (2003). HSP27 is markedly induced in Schwann cell columns and associated regenerating axons. *Glia*, *42*(1), 1-11. https://doi.org/https://doi.org/10.1002/glia.10105

Hoffman, H., & Binet, F. (1952). ACCELERATION AND RETARDATTON OF THE PROCESS OF AXON-SPROUTTNG IN PARTIALLY DENERVATED MUSCLES. *Australian Journal of Experimental Biology and Medical Science*, *30*(6), 541-566. https://doi.org/https://doi.org/10.1038/icb.1952.52

Hoke, A. (2006). Mechanisms of Disease: what factors limit the success of peripheral nerve regeneration in humans? *Nat Clin Pract Neurol*, *2*(8), 448-454. https://doi.org/10.1038/ncpneuro0262

Houshyar, S., Bhattacharyya, A., & Shanks, R. (2019). Peripheral Nerve Conduit: Materials and Structures. *ACS Chemical Neuroscience*, *10*(8), 3349-3365. https://doi.org/https://doi.org/10.1021/acschemneuro.9b00203

Huang, W., Begum, R., Barber, T., Ibba, V., Tee, N. C., Hussain, M., Arastoo, M., Yang, Q., Robson, L. G., Lesage, S., Gheysens, T., Skaer, N. J., Knight, D. P., & Priestley, J. V. (2012). Regenerative potential of silk conduits in repair of peripheral nerve injury in adult rats. *Biomaterials*, *33*(1), 59-71. https://doi.org/10.1016/j.biomaterials.2011.09.030

Huang, X., Cai, H., Zhou, H., Li, T., Jin, H., Evans, C. E., Cai, J., & Pi, J. (2020). Cobalt oxide nanoparticle-synergized protein degradation and phototherapy for enhanced anticancer therapeutics. *Acta Biomater*. https://doi.org/10.1016/j.actbio.2020.11.036

Huddleston, H. P., Kurtzman, J. S., Connors, K. M., & Koehler, S. M. (2021). A Retrospective Case Series of Peripheral Mixed Nerve Reconstruction Failures Using Processed Nerve Allografts. *Plast Reconstr Surg Glob Open*, *9*(12), e3983. https://doi.org/10.1097/GOX.0000000000003983

Imperatore, G., Boyle, J. P., Thompson, T. J., Case, D., Dabelea, D., Hamman, R. F., Lawrence, J. M., Liese, A. D., Liu, L. L., Mayer-Davis, E. J., Rodriguez, B. L., & Standiford, D. (2012). Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2050: dynamic modeling of incidence, mortality, and population growth. *Diabetes Care*, *35*(12), 2515-2520. https://doi.org/10.2337/dc12-0669

Irisarri, C. (2024). History of peripheral nerve injuries. *J Hand Surg Eur Vol*, *49*(6), 812-823. https://doi.org/10.1177/17531934231198455

Isaacs, J., Nydick, J. A., Means, K. R., Merrell, G. A., Ilyas, A., Levin, L. S., & Group, R. S. (2023). A Multicenter Prospective Randomized Comparison of Conduits Versus Decellularized Nerve Allograft for Digital Nerve Repairs. *J Hand Surg Am*, *48*(9), 904-913. https://doi.org/10.1016/j.jhsa.2023.05.020

Ising, E., Ahrman, E., Thomsen, N. O. B., Akesson, A., Malmstrom, J., & Dahlin, L. B. (2023). Quantification of heat shock proteins in the posterior interosseous nerve among subjects with type 1 and type 2 diabetes compared to healthy controls. *Front Neurosci*, *17*, 1227557. https://doi.org/10.3389/fnins.2023.1227557

Jahromi, M., Razavi, S., Seyedebrahimi, R., Reisi, P., & Kazemi, M. (2020). Regeneration of Rat Sciatic Nerve Using PLGA Conduit Containing Rat ADSCs with Controlled Release of BDNF and Gold Nanoparticles. *J Mol Neurosci*. https://doi.org/10.1007/s12031-020-01694-6

Jain, A., Dunlop, R., Hems, T., & Tang, J. B. (2019). Outcomes of surgical repair of a single digital nerve in adults. *Journal of Hand Surgery (European Volume)*, *44*(6), 560-565. https://doi.org/10.1177/1753193419846761

Jain, S. A., Nydick, J., Leversedge, F., Power, D., Styron, J., Safa, B., & Buncke, G. (2021). Clinical Outcomes of Symptomatic Neuroma Resection and Reconstruction with Processed Nerve Allograft. *Plast Reconstr Surg Glob Open*, *9*(10), e3832. https://doi.org/10.1097/GOX.0000000000003832

Jakob, U., Gaestel, M., Engel, K., & Buchner, J. (1993). Small heat shock proteins are molecular chaperones. *J Biol Chem*, *268*(3), 1517-1520. https://www.ncbi.nlm.nih.gov/pubmed/8093612

Janssen, U., Vassiliadou, A., Riley, S. G., Phillips, A. O., & Floege, J. (2004). The quest for a model of type II diabetes with nephropathy: the Goto Kakizaki rat. *J Nephrol*, *17*(6), 769-773.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology*, *9*, 1050-1074. https://doi.org/10.3762/bjnano.9.98

Jessen, K. R., & Mirsky, R. (2016). The repair Schwann cell and its function in regenerating nerves. *J Physiol*, *594*(13), 3521-3531. https://doi.org/10.1113/JP270874

Jessen, K. R., & Mirsky, R. (2019). The Success and Failure of the Schwann Cell Response to Nerve Injury. *Front Cell Neurosci*, *13*, 33. https://doi.org/10.3389/fncel.2019.00033

Jivan, S., Kumar, N., Wiberg, M., & Kay, S. (2009). The influence of pre-surgical delay on functional outcome after reconstruction of brachial plexus injuries. *J Plast Reconstr Aesthet Surg*, *62*(4), 472-479. https://doi.org/10.1016/j.bjps.2007.11.027

Johansson, F., & Dahlin, L. B. (2014). The multiple silicone tube device, "tubes within a tube," for multiplication in nerve reconstruction. *Biomed Res Int*, *2014*, 689127. https://doi.org/10.1155/2014/689127

Jonsson, S., Wiberg, R., McGrath, A. M., Novikov, L. N., Wiberg, M., Novikova, L. N., & Kingham, P. J. (2013). Effect of delayed peripheral nerve repair on nerve regeneration, Schwann cell function and target muscle recovery. *PLoS One*, *8*(2), e56484. https://doi.org/10.1371/journal.pone.0056484

Juranek, J. K., Geddis, M. S., Song, F., Zhang, J., Garcia, J., Rosario, R., Yan, S. F., Brannagan, T. H., & Schmidt, A. M. (2013). RAGE Deficiency Improves Postinjury Sciatic Nerve Regeneration in Type 1 Diabetic Mice. *Diabetes*, *62*(3), 931-943. https://doi.org/10.2337/db12-0632

Kalaycı, Ö. A., Cömert, F.B., Hazer, B. (2010). Synthesis, characterization, and antibacterial activity of metal nanoparticles embedded into amphiphilic comb-type graft copolymers. *Polymer Bulletin*, *65*, 215-226.

Kalbermatten, D. F., Erba, P., Mahay, D., Wiberg, M., Pierer, G., & Terenghi, G. (2008). Schwann cell strip for peripheral nerve repair. *J Hand Surg Eur Vol*, *33*(5), 587-594. https://doi.org/10.1177/1753193408090755

Kehoe, S., Zhang, X. F., & Boyd, D. (2012). FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury*, *43*(5), 553-572. https://doi.org/10.1016/j.injury.2010.12.030

Keller, J. N., Hanni, K. B., & Markesbery, W. R. (1999). Oxidized low-density lipoprotein induces neuronal death: implications for calcium, reactive oxygen species, and caspases. *J Neurochem*, *72*(6), 2601-2609. https://doi.org/10.1046/j.1471-4159.1999.0722601.x

Kennedy, J. M., & Zochodne, D. W. (2005). Impaired peripheral nerve regeneration in diabetes mellitus. *Journal of the Peripheral Nervous System*, *10*(2), 144-157. https://doi.org/https://doi.org/10.1111/j.1085-9489.2005.0010205.x

Kerns, J. M., Danielsen, N., Holmquist, B., Kanje, M., & Lundborg, G. (1993). The influence of predegeneration on regeneration through peripheral nerve grafts in the rat. *Exp Neurol*, *122*(1), 28-36. https://doi.org/10.1006/exnr.1993.1104

Kim, H., Kim, J. J., & Yoon, Y. S. (2012). Emerging therapy for diabetic neuropathy: cell therapy targeting vessels and nerves. *Endocr Metab Immune Disord Drug Targets*, *12*(2), 168-178. https://doi.org/10.2174/187153012800493486

Kim, J. S., Bonsu, N. Y., Leland, H. A., Carey, J. N., Patel, K. M., & Seruya, M. (2018). A Systematic Review of Prognostic Factors for Sensory Recovery After Digital Nerve Reconstruction. *Ann Plast Surg*, *80*(5S Suppl 5), S311-s316. https://doi.org/10.1097/sap.0000000000001440

Klein, S., Vykoukal, J., Felthaus, O., Dienstknecht, T., & Prantl, L. (2016). Collagen Type I Conduits for the Regeneration of Nerve Defects. *Materials (Basel)*, *9*(4). https://doi.org/10.3390/ma9040219

Klimovich, P., Rubina, K., Sysoeva, V., & Semina, E. (2021). New Frontiers in Peripheral Nerve Regeneration: Concerns and Remedies. *International Journal of Molecular Sciences*, *22*(24), 13380. https://www.mdpi.com/1422-0067/22/24/13380

Kocer, H., Borcakli, M., Ilter, S., Hazer, B. (2003). Production of bacterial polyesters from some various new substrates by Alcaligenes eutrophus and Pseudomonas oleovorans. *Tr. J. Chem*, *27*, 365-373.

Konofaos, P., & Ver Halen, J. P. (2013). Nerve repair by means of tubulization: past, present, future. *J Reconstr Microsurg*, *29*(3), 149-164. https://doi.org/10.1055/s-0032-1333316

Koopmans, G., Hasse, B., & Sinis, N. (2009). Chapter 19 The Role of Collagen in Peripheral Nerve Repair. In *International Review of Neurobiology* (Vol. 87, pp. 363-379). Academic Press. https://doi.org/https://doi.org/10.1016/S0074-7742(09)87019-0

Kornfeld, T., Nessler, J., Helmer, C., Hannemann, R., Waldmann, K. H., Peck, C. T., Hoffmann, P., Brandes, G., Vogt, P. M., & Radtke, C. (2021). Spider silk nerve graft promotes axonal regeneration on long distance nerve defect in a sheep model. *Biomaterials*, *271*, 120692. https://doi.org/10.1016/j.biomaterials.2021.120692

Krishnan, A., Bhavanam, S., & Zochodne, D. (2018). An Intimate Role for Adult Dorsal Root Ganglia Resident Cycling Cells in the Generation of Local Macrophages and Satellite Glial Cells. *Journal of Neuropathology & Experimental Neurology*, *77*(10), 929-941. https://doi.org/10.1093/jnen/nly072

Krishnan, A., Verge, V. M. K., & Zochodne, D. W. (2024). Hallmarks of peripheral nerve injury and regeneration. *Handb Clin Neurol*, *201*, 1-17. https://doi.org/10.1016/B978-0-323-90108-6.00014-4

Kusuhara, H., Hirase, Y., Isogai, N., & Sueyoshi, Y. (2019). A clinical multi-center registry study on digital nerve repair using a biodegradable nerve conduit of PGA with external and internal collagen scaffolding. *Microsurgery*, *39*(5), 395-399. https://doi.org/10.1002/micr.30417

Kvist, M., Danielsen, N., & Dahlin, L. B. (2003). Effects of FK506 on regeneration and macrophages in injured rat sciatic nerve. *J Peripher Nerv Syst*, *8*(4), 251-259. https://doi.org/10.1111/j.1085-9489.2003.03021.x

Kvist, M., Lemplesis, V., Kanje, M., Ekberg, H., Corbascio, M., & Dahlin, L. B. (2007). Immunomodulation by costimulation blockade inhibits rejection of nerve allografts. *J Peripher Nerv Syst*, *12*(2), 83-90. https://doi.org/10.1111/j.1529-8027.2007.00126.x

Lanier, S. T., Hill, J. R., Dy, C. J., & Brogan, D. M. (2021). Evolving Techniques in Peripheral Nerve Regeneration. *J Hand Surg Am*, *46*(8), 695-701. https://doi.org/10.1016/j.jhsa.2021.04.019

Lassner, F., Becker, M., & Berger, A. (1995). Degeneration and regeneration in nerve autografts and allografts. *Microsurgery*, *16*(1), 4-8. https://doi.org/10.1002/micr.1920160104

Lassner, F., Schaller, E., Steinhoff, G., Wonigeit, K., Walter, G. F., & Berger, A. (1989). Cellular mechanisms of rejection and regeneration in peripheral nerve allografts. *Transplantation*, *48*(3), 386-392. https://doi.org/10.1097/00007890-198909000-00006

Leckenby, J. I., Furrer, C., Haug, L., Juon Personeni, B., & Vögelin, E. (2020). A Retrospective Case Series Reporting the Outcomes of Avance Nerve Allografts in the Treatment of Peripheral Nerve Injuries. *Plast Reconstr Surg*, *145*(2), 368e-381e. https://doi.org/10.1097/prs.0000000000006485

Lee, H. C., Hsu, Y. M., Tsai, C. C., Ke, C. J., Yao, C. H., & Chen, Y. S. (2015). Improved peripheral nerve regeneration in streptozotocin-induced diabetic rats by oral lumbrokinase. *Am J Chin Med*, *43*(2), 215-230. https://doi.org/10.1142/s0192415x15500147

Lee, K., Lee, D., & Kang, B. (2020). The 'R' principles in laboratory animal experiments. *Laboratory Animal Research*, *36*. https://doi.org/10.1186/s42826-020-00078-6

Li, J., Guan, R., & Pan, L. (2023). Mechanism of Schwann cells in diabetic peripheral neuropathy: A review. *Medicine (Baltimore)*, *102*(1), e32653. https://doi.org/10.1097/md.0000000000032653

Lin, J. S., & Jain, S. A. (2023). Challenges in Nerve Repair and Reconstruction. *Hand Clin*, *39*(3), 403-415. https://doi.org/10.1016/j.hcl.2023.05.001

Lindquist, S., & Craig, E. A. (1988). THE HEAT-SHOCK PROTEINS. *Annual Review of Genetics*, *22*(Volume 22, 1988), 631-677. https://doi.org/https://doi.org/10.1146/annurev.ge.22.120188.003215

Lindwall, C., & Kanje, M. (2005). Retrograde axonal transport of JNK signaling molecules influence injury induced nuclear changes in p-c-Jun and ATF3 in adult rat sensory neurons. *Molecular and Cellular Neuroscience*, *29*(2), 269-282. https://doi.org/https://doi.org/10.1016/j.mcn.2005.03.002

Liu, P., Peng, J., Han, G. H., Ding, X., Wei, S., Gao, G., Huang, K., Chang, F., & Wang, Y. (2019). Role of macrophages in peripheral nerve injury and repair. *Neural Regen Res*, *14*(8), 1335-1342. https://doi.org/10.4103/1673-5374.253510

Liu, Y., & Fox, P. M. (2024). The Role of Electrical Stimulation in Peripheral Nerve Regeneration: Current Evidence and Future Directions. *Journal of Hand Surgery Global Online*, *6*(5), 718-721. https://doi.org/https://doi.org/10.1016/j.jhsg.2024.01.022

Lohmeyer, J. A., Siemers, F., Machens, H. G., & Mailander, P. (2009). The clinical use of artificial nerve conduits for digital nerve repair: a prospective cohort study and literature review. *J Reconstr Microsurg*, *25*(1), 55-61. https://doi.org/10.1055/s-0028-1103505

Lopes, B., Sousa, P., Alvites, R., Branquinho, M., Sousa, A. C., Mendonça, C., Atayde, L. M., Luís, A. L., Varejão, A. S. P., & Maurício, A. C. (2022). Peripheral Nerve Injury Treatments and Advances: One Health Perspective. *Int J Mol Sci*, *23*(2). https://doi.org/10.3390/ijms23020918

Lovic, D., Piperidou, A., Zografou, I., Grassos, H., Pittaras, A., & Manolis, A. (2020). The Growing Epidemic of Diabetes Mellitus. *Curr Vasc Pharmacol*, *18*(2), 104-109. https://doi.org/10.2174/1570161117666190405165911

Lundborg, G. (2000). A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J Hand Surg Am*, *25*(3), 391-414. https://doi.org/10.1053/jhsu.2000.4165

Lundborg, G., Dahlin, L., Dohi, D., Kanje, M., & Terada, N. (1997). A new type of "bioartificial" nerve graft for bridging extended defects in nerves. *J Hand Surg Br*, *22*(3), 299-303. https://doi.org/10.1016/s0266-7681(97)80390-7

Lundborg, G., Dahlin, L. B., Danielsen, N., Gelberman, R. H., Longo, F. M., Powell, H. C., & Varon, S. (1982). Nerve regeneration in silicone chambers: Influence of gap length and of distal stump components. *Exp Neurol*, *76*(2), 361-375. https://doi.org/https://doi.org/10.1016/0014-4886(82)90215-1

Lundborg, G., Rosen, B., Dahlin, L., Danielsen, N., & Holmberg, J. (1997). Tubular versus conventional repair of median and ulnar nerves in the human forearm: early results from a prospective, randomized, clinical study. *J Hand Surg Am*, *22*(1), 99-106. https://doi.org/10.1016/S0363-5023(05)80188-1

Lunn, E. R., Perry, V. H., Brown, M. C., Rosen, H., & Gordon, S. (1989). Absence of Wallerian Degeneration does not Hinder Regeneration in Peripheral Nerve. *European Journal of Neuroscience*, *1*(1), 27-33. https://doi.org/https://doi.org/10.1111/j.1460-9568.1989.tb00771.x

Mackinnon, S. E. (1989). New directions in peripheral nerve surgery. *Ann Plast Surg*, *22*(3), 257-273. https://doi.org/10.1097/00000637-198903000-00013

Marsh, E. B., Snyder-Warwick, A. K., Mackinnon, S. E., & Wood, M. D. (2024). Interpretation of Data from Translational Rodent Nerve Injury and Repair Models. *Hand Clinics*, *40*(3), 429-440. https://doi.org/https://doi.org/10.1016/j.hcl.2024.03.004

Martini, R., Fischer, S., López-Vales, R., & David, S. (2008). Interactions between Schwann cells and macrophages in injury and inherited demyelinating disease. *Glia*, *56*(14), 1566-1577. https://doi.org/10.1002/glia.20766

McLean, N. A., & Verge, V. M. (2016). Dynamic impact of brief electrical nerve stimulation on the neural immune axis-polarization of macrophages toward a pro-repair phenotype in demyelinated peripheral nerve. *Glia*, *64*(9), 1546-1561. https://doi.org/10.1002/glia.23021

McMorrow, L. A., Czarnecki, P., Reid, A. J., & Tos, P. (2024). Current perspectives on peripheral nerve repair and management of the nerve gap. *J Hand Surg Eur Vol*, 17531934241242002. https://doi.org/10.1177/17531934241242002

Meek, M. F., & Jansen, K. (2009). Two years after in vivo implantation of poly(DL-lactide-ε-caprolactone) nerve guides: Has the material finally resorbed? *Journal of Biomedical Materials Research Part A*, *89A*(3), 734-738. https://doi.org/https://doi.org/10.1002/jbm.a.32024

Meyer, C., Stenberg, L., Gonzalez-Perez, F., Wrobel, S., Ronchi, G., Udina, E., Suganuma, S., Geuna, S., Navarro, X., Dahlin, L. B., Grothe, C., & Haastert-Talini, K. (2016). Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves. *Biomaterials*, *76*, 33-51. https://doi.org/10.1016/j.biomaterials.2015.10.040

Midha, R., & Grochmal, J. (2019). Surgery for nerve injury: current and future perspectives. *J Neurosurg*, *130*(3), 675-685. https://doi.org/10.3171/2018.11.Jns181520

Millesi, F., Weiss, T., Mann, A., Haertinger, M., Semmler, L., Supper, P., Pils, D., Naghilou, A., & Radtke, C. (2021). Defining the regenerative effects of native spider silk fibers on primary Schwann cells, sensory neurons, and nerve-associated fibroblasts. *The FASEB Journal*, *35*(2), e21196. https://doi.org/https://doi.org/10.1096/fj.202001447R

Millesi, H. (1990). Progress in peripheral nerve reconstruction. *World J Surg*, *14*(6), 733-747. https://doi.org/10.1007/bf01670520

Millesi, H. (2000). Techniques for nerve grafting. *Hand Clin*, *16*(1), 73-91, viii. https://www.ncbi.nlm.nih.gov/pubmed/10696578

Min, Q., Parkinson, D. B., & Dun, X.-P. (2021). Migrating Schwann cells direct axon regeneration within the peripheral nerve bridge. *Glia*, *69*(2), 235-254. https://doi.org/https://doi.org/10.1002/glia.23892

Mizukami, H., & Osonoi, S. (2021). Collateral Glucose-Utlizing Pathwaya in Diabetic Polyneuropathy. *International Journal of Molecular Sciences*, *22*(1), 94. https://www.mdpi.com/1422-0067/22/1/94

Mohammad-Bagher, G., Arash, A., Morteza, B. R., Naser, M. S., & Ali, M. (2019). Synergistic Effects of Acetyl-l-Carnitine and Adipose-Derived Stromal Cells on Improving Regenerative Capacity of Acellular Nerve Allograft in Sciatic Nerve Defect. *J Pharmacol Exp Ther*, *368*(3), 490-502. https://doi.org/10.1124/jpet.118.254540

Mohseni, S., Badii, M., Kylhammar, A., Thomsen, N. O. B., Eriksson, K. F., Malik, R. A., Rosen, I., & Dahlin, L. B. (2017). Longitudinal study of neuropathy, microangiopathy, and autophagy in sural nerve: Implications for diabetic neuropathy. *Brain Behav*, *7*(8), e00763. https://doi.org/10.1002/brb3.763

Mokarizadeh, A., Mehrshad, A., & Mohammadi, R. (2016). Local Polyethylene Glycol in Combination with Chitosan Based Hybrid Nanofiber Conduit Accelerates Transected Peripheral Nerve Regeneration. *J Invest Surg*, *29*(3), 167-174. https://doi.org/10.3109/08941939.2015.1098758

Mokarram, N., Merchant, A., Mukhatyar, V., Patel, G., & Bellamkonda, R. V. (2012). Effect of modulating macrophage phenotype on peripheral nerve repair. *Biomaterials*, *33*(34), 8793-8801. https://doi.org/10.1016/j.biomaterials.2012.08.050

Murakawa, Y., Zhang, W., Pierson, C. R., Brismar, T., Ostenson, C. G., Efendic, S., & Sima, A. A. (2002). Impaired glucose tolerance and insulinopenia in the GK-rat causes peripheral neuropathy. *Diabetes Metab Res Rev*, *18*(6), 473-483. https://doi.org/10.1002/dmrr.326

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). *J Plast Reconstr Aesthet Surg*, *80*, 75-85. https://doi.org/10.1016/j.bjps.2023.02.017

Nakagomi, S., Suzuki, Y., Namikawa, K., Kiryu-Seo, S., & Kiyama, H. (2003). Expression of the activating transcription factor 3 prevents c-Jun N-terminal kinase-induced neuronal death by promoting heat shock protein 27 expression and Akt activation. *J Neurosci*, *23*(12), 5187-5196. https://doi.org/10.1523/JNEUROSCI.23-12-05187.2003

Neubrech, F., Heider, S., Harhaus, L., Bickert, B., Kneser, U., & Kremer, T. (2016). Chitosan nerve tube for primary repair of traumatic sensory nerve lesions of the hand without a gap: study protocol for a randomized controlled trial. *Trials*, *17*, 48. https://doi.org/10.1186/s13063-015-1148-5

Niemi, J. P., DeFrancesco-Lisowitz, A., Cregg, J. M., Howarth, M., & Zigmond, R. E. (2016). Overexpression of the monocyte chemokine CCL2 in dorsal root ganglion neurons causes a conditioning-like increase in neurite outgrowth and does so via a STAT3 dependent mechanism. *Exp Neurol*, *275 Pt 1*(0 1), 25-37. https://doi.org/10.1016/j.expneurol.2015.09.018

Nilsson, A., Dahlin, L., Lundborg, G., & Kanje, M. (2005). Graft repair of a peripheral nerve without the sacrifice of a healthy donor nerve by the use of acutely dissociated autologous Schwann cells. *Scand J Plast Reconstr Surg Hand Surg*, *39*(1), 1-6. https://doi.org/10.1080/02844310410017979

Nilsson, E., Björn, C., Sjöstrand, V., Lindgren, K., Münnich, M., Mattsby-Baltzer, I., Ivarsson, M. L., Olmarker, K., & Mahlapuu, M. (2009). A novel polypeptide derived from human lactoferrin in sodium hyaluronate prevents postsurgical adhesion formation in the rat. *Ann Surg*, *250*(6), 1021-1028. https://doi.org/10.1097/sla.0b013e3181b246a7

Nishida, N., Yamagishi, S., Mizukami, H., & Yagihashi, S. (2013). Impaired nerve fiber regeneration in axotomized peripheral nerves in streptozotocin-diabetic rats. *J Diabetes Investig*, *4*(6), 533-539. https://doi.org/10.1111/jdi.12115

Niu, Y., Chen, K. C., He, T., Yu, W., Huang, S., & Xu, K. (2014). Scaffolds from block polyurethanes based on poly(ε-caprolactone) (PCL) and poly(ethylene glycol) (PEG) for peripheral nerve regeneration. *Biomaterials*, *35*(14), 4266-4277. https://doi.org/10.1016/j.biomaterials.2014.02.013

Nix, W. A., & Hopf, H. C. (1983). Electrical stimulation of regenerating nerve and its effect on motor recovery. *Brain Research*, *272*(1), 21-25. https://doi.org/https://doi.org/10.1016/0006-8993(83)90360-8

Ogden, B. E., Pang William, W., Agui, T., & Lee, B. H. (2016). Laboratory Animal Laws, Regulations, Guidelines and Standards in China Mainland, Japan, and Korea. *ILAR J*, *57*(3), 301-311. https://doi.org/10.1093/ilar/ilw018

Osman, A. A., Dahlin, L. B., Thomsen, N. O., & Mohseni, S. (2015). Autophagy in the posterior interosseous nerve of patients with type 1 and type 2 diabetes mellitus: an ultrastructural study. *Diabetologia*, *58*(3), 625-632. https://doi.org/10.1007/s00125-014-3477-4

Östenson, C. G., Khan, A., Abdel-Halim, S. M., Guenifi, A., Suzuki, K., Goto, Y., & Efendic, S. (1993). Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. *Diabetologia*, *36*(1), 3-8. https://doi.org/10.1007/BF00399086

Ozturk, O., Tezcan, A. H., Adali, Y., Yıldırım, C. H., Aksoy, O., Yagmurdur, H., & Bilge, A. (2016). Effect of ozone and methylprednisolone treatment following crush type sciatic nerve injury. *Acta Cirúrgica Brasileira*, *31*.

Padilla, A., Descorbeth, M., Almeyda, A. L., Payne, K., & De Leon, M. (2011). Hyperglycemia magnifies Schwann cell dysfunction and cell death triggered by PA-induced lipotoxicity. *Brain Res*, *1370*, 64-79. https://doi.org/10.1016/j.brainres.2010.11.013

Pan, D., Mackinnon, S. E., & Wood, M. D. (2020). Advances in the repair of segmental nerve injuries and trends in reconstruction. *Muscle Nerve*, *61*(6), 726-739. https://doi.org/10.1002/mus.26797

Pan, H.-C., Yang, D.-Y., Ou, Y.-C., Ho, S.-P., Cheng, F.-C., & Chen, C.-J. (2010). Neuroprotective Effect of Atorvastatin in an Experimental Model of Nerve Crush Injury. *Neurosurgery*, *67*(2). https://journals.lww.com/neurosurgery/fulltext/2010/08000/neuroprotective_effect_of_atorvastatin_in_an.28.aspx

Papastefanaki, F., Jakovcevski, I., Poulia, N., Djogo, N., Schulz, F., Martinovic, T., Ciric, D., Loers, G., Vossmeyer, T., Weller, H., Schachner, M., & Matsas, R. (2015). Intraspinal Delivery of Polyethylene Glycol-coated Gold Nanoparticles Promotes Functional Recovery After Spinal Cord Injury. *Mol Ther*, *23*(6), 993-1002. https://doi.org/10.1038/mt.2015.50

Paskal, A. M., Paskal, W., Pietruski, P., & Wlodarski, P. K. (2019). Polyethylene Glycol: The Future of Posttraumatic Nerve Repair? Systemic Review. *Int J Mol Sci*, *20*(6). https://doi.org/10.3390/ijms20061478

Patodia, S., & Raivich, G. (2012a). Downstream effector molecules in successful peripheral nerve regeneration. *Cell Tissue Res*, *349*(1), 15-26. https://doi.org/10.1007/s00441-012-1416-6

Patodia, S., & Raivich, G. (2012b). Role of transcription factors in peripheral nerve regeneration. *Front Mol Neurosci*, *5*, 8. https://doi.org/10.3389/fnmol.2012.00008

Paviolo, C., & Stoddart, P. R. (2017). Gold Nanoparticles for Modulating Neuronal Behavior. *Nanomaterials (Basel)*, *7*(4). https://doi.org/10.3390/nano7040092

Peng, X., Fang, J., Lou, C., Yang, L., Shan, S., Wang, Z., Chen, Y., Li, H., & Li, X. (2024). Engineered nanoparticles for precise targeted drug delivery and enhanced therapeutic efficacy in cancer immunotherapy. *Acta Pharm Sin B*, *14*(8), 3432-3456. https://doi.org/10.1016/j.apsb.2024.05.010

Penkert, G., Bini, W., & Samii, M. (1988). Revascularization of nerve grafts: an experimental study. *Journal of Reconstructive Microsurgery*, *4*(4), 319-325. https://doi.org/10.1055/s-2007-1006938

Phillips, J. B., Hercher, D., & Hausner, T. (2022). *Peripheral Nerve Tissue Engineering and Regeneration*. Springer.

Poppler, L. H., Parikh, R. P., Bichanich, M. J., Rebehn, K., Bettlach, C. R., Mackinnon, S. E., & Moore, A. M. (2018). Surgical interventions for the treatment of painful neuroma: a comparative meta-analysis. *Pain*, *159*(2). https://journals.lww.com/pain/fulltext/2018/02000/surgical_interventions_for_the_treatment_of.5.aspx

Portha, B., Serradas, P., Bailbé, D., Suzuki, K.-I., Goto, Y., & Giroix, M.-H. (1991). β-Cell Insensitivity to Glucose in the GK Rat, a Spontaneous Nonobese Model for Type II Diabetes. *Diabetes*, *40*(4), 486-491. https://doi.org/10.2337/diab.40.4.486

Potas, J. R., Haque, F., Maclean, F. L., & Nisbet, D. R. (2015). Interleukin-10 conjugated electrospun polycaprolactone (PCL) nanofibre scaffolds for promoting alternatively activated (M2) macrophages around the peripheral nerve in vivo. *J Immunol Methods*, *420*, 38-49. https://doi.org/10.1016/j.jim.2015.03.013

Pourhamidi, K., Dahlin, L. B., Boman, K., & Rolandsson, O. (2011). Heat shock protein 27 is associated with better nerve function and fewer signs of neuropathy. *Diabetologia*, *54*(12), 3143-3149. https://doi.org/10.1007/s00125-011-2303-5

Pourhamidi, K., Skarstrand, H., Dahlin, L. B., & Rolandsson, O. (2014). HSP27 concentrations are lower in patients with type 1 diabetes and correlate with large nerve fiber dysfunction. *Diabetes Care*, *37*(3), e49-50. https://doi.org/10.2337/dc13-1780

Power, H. A., Morhart, M. J., Olson, J. L., & Chan, K. M. (2020). Postsurgical Electrical Stimulation Enhances Recovery Following Surgery for Severe Cubital Tunnel Syndrome: A Double-Blind Randomized Controlled Trial. *Neurosurgery*, *86*(6). https://journals.lww.com/neurosurgery/fulltext/2020/06000/postsurgical_electrical_stimulation_enhances.3.aspx

Qamar, F., Sultana, S., & Sharma, M. (2023). Animal models for induction of diabetes and its complications. *J Diabetes Metab Disord*, *22*(2), 1021-1028. https://doi.org/10.1007/s40200-023-01277-3

Qian, Y., Lin, H., Yan, Z., Shi, J., & Fan, C. (2021). Functional nanomaterials in peripheral nerve regeneration: Scaffold design, chemical principles and microenvironmental remodeling. *Materials Today*, *51*, 165-187. https://doi.org/https://doi.org/10.1016/j.mattod.2021.09.014

Radtke, C., Allmeling, C., Waldmann, K. H., Reimers, K., Thies, K., Schenk, H. C., Hillmer, A., Guggenheim, M., Brandes, G., & Vogt, P. M. (2011). Spider silk constructs enhance axonal regeneration and remyelination in long nerve defects in sheep. *PLoS One*, *6*(2), e16990. https://doi.org/10.1371/journal.pone.0016990

Raghav, A., Singh, M., Jeong, G. B., Giri, R., Agarwal, S., & Kala, S. (2022). New horizons of biomaterials in treatment of nerve damage in diabetes mellitus: A translational prospective review. *Front Endocrinol (Lausanne)*, *13*, 1036220. https://doi.org/10.3389/fendo.2022.1036220

Rayner, M. L. D., Healy, J., & Phillips, J. B. (2022). Drug Therapies for Peripheral Nerve Injuries. In J. B. Phillips, D. Hercher, & T. Hausner (Eds.), *Peripheral Nerve Tissue Engineering and Regeneration* (pp. 437-463). Springer International Publishing. https://doi.org/10.1007/978-3-030-21052-6_16

Raza, C., Riaz, H. A., Anjum, R., & Shakeel, N. U. A. (2020). Repair strategies for injured peripheral nerve: Review. *Life Sci*, *243*, 117308. https://doi.org/10.1016/j.lfs.2020.117308

Read, D. E., & Gorman, A. M. (2009). Heat shock protein 27 in neuronal survival and neurite outgrowth. *Biochem Biophys Res Commun*, *382*(1), 6-8. https://doi.org/10.1016/j.bbrc.2009.02.114

Rebowe, R., Rogers, A., Yang, X., Kundu, S. C., Smith, T. L., & Li, Z. (2018). Nerve Repair with Nerve Conduits: Problems, Solutions, and Future Directions. *J Hand Microsurg*, *10*(2), 61-65. https://doi.org/10.1055/s-0038-1626687

Reid, A. J., de Luca, A. C., Faroni, A., Downes, S., Sun, M., Terenghi, G., & Kingham, P. J. (2013). Long term peripheral nerve regeneration using a novel PCL nerve conduit. *Neurosci Lett*, *544*, 125-130. https://doi.org/10.1016/j.neulet.2013.04.001

Reitan, I., Dahlin, L. B., & Rosberg, H. E. (2019). Patient-reported quality of life and hand disability in elderly patients after a traumatic hand injury - a retrospective study. *Health Qual Life Outcomes*, *17*(1), 148. https://doi.org/10.1186/s12955-019-1215-9

Roloff, F., Strauß, S., Vogt, P. M., Bicker, G., & Radtke, C. (2014). Spider Silk as Guiding Biomaterial for Human Model Neurons. *BioMed Research International*, *2014*(1), 906819. https://doi.org/https://doi.org/10.1155/2014/906819

Romeo-Guitart, D., & Casas, C. (2020). NeuroHeal Treatment Alleviates Neuropathic Pain and Enhances Sensory Axon Regeneration. *Cells*, *9*(4). https://doi.org/10.3390/cells9040808

Romeo-Guitart, D., Forés, J., Navarro, X., & Casas, C. (2017). Boosted Regeneration and Reduced Denervated Muscle Atrophy by NeuroHeal in a Pre-clinical Model of Lumbar Root Avulsion with Delayed Reimplantation. *Scientific Reports*, *7*(1), 12028. https://doi.org/10.1038/s41598-017-11086-3

Rosberg, H.-E., Hazer, D. B., & Dahlin, L. B. (2015). Outcome of Repaired Digital Nerve Injuries in Children – Influence of Age in a Retrospective Long-Term Follow-Up. *The Journal of Hand Surgery*, *40*(9). https://doi.org/10.1016/j.jhsa.2015.06.094

Rosberg, H. E., Carlsson, K. S., Cederlund, R. I., Ramel, E., & Dahlin, L. B. (2013). Costs and outcome for serious hand and arm injuries during the first year after trauma - a prospective study. *BMC Public Health*, *13*, 501. https://doi.org/10.1186/1471-2458-13-501

Roszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. *Mediators Inflamm*, *2015*, 816460. https://doi.org/10.1155/2015/816460

Saffari, T. M., Bedar, M., Hundepool, C. A., Bishop, A. T., & Shin, A. Y. (2020). The role of vascularization in nerve regeneration of nerve graft. *Neural Regen Res*, *15*(9), 1573-1579. https://doi.org/10.4103/1673-5374.276327

Saffari, T. M., Mathot, F., Friedrich, P. F., Bishop, A. T., & Shin, A. Y. (2020). Revascularization patterns of nerve allografts in a rat sciatic nerve defect model. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, *73*(3), 460-468. https://doi.org/https://doi.org/10.1016/j.bjps.2019.11.048

Saito, H., & Dahlin, L. B. (2008). Expression of ATF3 and axonal outgrowth are impaired after delayed nerve repair. *BMC Neurosci*, *9*, 88. https://doi.org/10.1186/1471-2202-9-88

Saito, H., Kanje, M., & Dahlin, L. B. (2009). Delayed nerve repair increases number of caspase 3 stained Schwann cells. *Neurosci Lett*, *456*(1), 30-33. https://doi.org/10.1016/j.neulet.2009.03.075

Sakar, M., Korkusuz, P., Demirbilek, M., Cetinkaya, D. U., Arslan, S., Denkbas, E. B., Temucin, C. M., Bilgic, E., Hazer, D. B., & Bozkurt, G. (2014). The effect of poly(3-hydroxybutyrate-co-3- hydroxyhexanoate) (PHBHHx) and human mesenchymal stem cell (hMSC) on axonal regeneration in experimental sciatic nerve damage. *Int J Neurosci*, *124*(9), 685-696. https://doi.org/10.3109/00207454.2013.876636

Salles, G. S., Jr., Faria, J. C., Busnardo, F. F., Gemperli, R., & Ferreira, M. C. (2013). Evaluation of nerve regeneration in diabetic rats. *Acta Cir Bras*, *28*(7), 509-517. https://doi.org/10.1590/s0102-86502013000700006

Sango, K., Mizukami, H., Horie, H., & Yagihashi, S. (2017). Impaired Axonal Regeneration in Diabetes. Perspective on the Underlying Mechanism from In Vivo and In Vitro Experimental Studies [Review]. *Frontiers in Endocrinology*, *8*. https://doi.org/10.3389/fendo.2017.00012

Sato, N., Komatsu, K., & Kurumatani, H. (2003). Late Onset of Diabetic Nephropathy in Spontaneously Diabetic GK Rats. *American Journal of Nephrology*, *23*(5), 334-342. https://doi.org/10.1159/000072915

Schmalbruch, H. (1986). Fiber composition of the rat sciatic nerve. *Anat Rec*, *215*(1), 71-81. https://doi.org/10.1002/ar.1092150111

Schratzberger, P., Walter, D. H., Rittig, K., Bahlmann, F. H., Pola, R., Curry, C., Silver, M., Krainin, J. G., Weinberg, D. H., Ropper, A. H., & Isner, J. M. (2001). Reversal of experimental diabetic neuropathy by VEGF gene transfer. *J Clin Invest*, *107*(9), 1083-1092. https://doi.org/10.1172/jci12188

Seddon, H. J. (1942). A Classification of Nerve Injuries. *Br Med J*, *2*(4260), 237-239. https://doi.org/10.1136/bmj.2.4260.237

Seixas, S. F., Forte, G. C., Magnus, G. A., Stanham, V., Mattiello, R., & Silva, J. B. (2022). Effect of Tacrolimus and Cyclosporine Immunosuppressants on Peripheral Nerve Regeneration: Systematic Review and Meta-analysis. *Rev Bras Ortop (Sao Paulo)*, *57*(2), 207-213. https://doi.org/10.1055/s-0041-1736467

Sengupta, P. (2013). The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med*, *4*(6), 624-630.

Shamash, S., Reichert, F., & Rotshenker, S. (2002). The cytokine network of Wallerian degeneration: tumor necrosis factor-alpha, interleukin-1alpha, and interleukin-1beta. *J Neurosci*, *22*(8), 3052-3060. https://doi.org/10.1523/jneurosci.22-08-03052.2002

Sharifi, M., Farahani, M. K., Salehi, M., Atashi, A., Alizadeh, M., Kheradmandi, R., & Molzemi, S. (2023). Exploring the Physicochemical, Electroactive, and Biodelivery Properties of Metal Nanoparticles on Peripheral Nerve Regeneration. *ACS Biomater Sci Eng*, *9*(1), 106-138. https://doi.org/10.1021/acsbiomaterials.2c01216

Siconolfi, L. B., & Seeds, N. W. (2001). Induction of the plasminogen activator system accompanies peripheral nerve regeneration after sciatic nerve crush. *J Neurosci*, *21*(12), 4336-4347. https://doi.org/10.1523/JNEUROSCI.21-12-04336.2001

Siemionow, M., & Brzezicki, G. (2009). Chapter 8: Current techniques and concepts in peripheral nerve repair. *Int Rev Neurobiol*, *87*, 141-172. https://doi.org/10.1016/S0074-7742(09)87008-6

Snyder, A. K., Fox, I. K., Nichols, C. M., Rickman, S. R., Hunter, D. A., Tung, T. H. H., & Mackinnon, S. E. (2006). Neuroregenerative Effects of Preinjury FK-506 Administration. *Plastic and Reconstructive Surgery*, *118*(2). https://journals.lww.com/plasreconsurg/fulltext/2006/08000/neuroregenerative_effects_of_preinjury_fk_506.13.aspx

Soderstjerna, E., Bauer, P., Cedervall, T., Abdshill, H., Johansson, F., & Johansson, U. E. (2014). Silver and gold nanoparticles exposure to in vitro cultured retina--studies on nanoparticle internalization, apoptosis, oxidative stress, glial- and microglial activity. *PLoS One*, *9*(8), e105359. https://doi.org/10.1371/journal.pone.0105359

Sorci, G., Riuzzi, F., Giambanco, I., & Donato, R. (2013). RAGE in tissue homeostasis, repair and regeneration. *Biochim Biophys Acta*, *1833*(1), 101-109. https://doi.org/10.1016/j.bbamcr.2012.10.021

Stenberg, L., & Dahlin, L. B. (2014). Gender differences in nerve regeneration after sciatic nerve injury and repair in healthy and in type 2 diabetic Goto-Kakizaki rats. *BMC Neurosci*, *15*, 107. https://doi.org/10.1186/1471-2202-15-107

Stenberg, L., Kanje, M., Dolezal, K., & Dahlin, L. B. (2012). Expression of Activating Transcription Factor 3 (ATF 3) and caspase 3 in Schwann cells and axonal outgrowth after sciatic nerve repair in diabetic BB rats. *Neuroscience Letters*, *515*(1), 34-38. https://doi.org/https://doi.org/10.1016/j.neulet.2012.03.011

Stenberg, L., Kodama, A., Lindwall-Blom, C., & Dahlin, L. B. (2016). Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats. *Eur J Neurosci*, *43*(3), 463-473. https://doi.org/10.1111/ejn.13068

Stenberg, L., Stossel, M., Ronchi, G., Geuna, S., Yin, Y., Mommert, S., Martensson, L., Metzen, J., Grothe, C., Dahlin, L. B., & Haastert-Talini, K. (2017). Regeneration of long-distance peripheral nerve defects after delayed reconstruction in healthy and diabetic rats is supported by immunomodulatory chitosan nerve guides. *BMC Neurosci*, *18*(1), 53. https://doi.org/10.1186/s12868-017-0374-z

Stetler, R. A., Gao, Y., Signore, A. P., Cao, G., & Chen, J. (2009). HSP27: mechanisms of cellular protection against neuronal injury. *Curr Mol Med*, *9*(7), 863-872. https://doi.org/10.2174/156652409789105561

Stratton, J. A., Holmes, A., Rosin, N. L., Sinha, S., Vohra, M., Burma, N. E., Trang, T., Midha, R., & Biernaskie, J. (2018). Macrophages Regulate Schwann Cell Maturation after Nerve Injury. *Cell Rep*, *24*(10), 2561-2572.e2566. https://doi.org/10.1016/j.celrep.2018.08.004

Strauch, R. J., & Strauch, B. (2013). Nerve conduits: an update on tubular nerve repair and reconstruction. *J Hand Surg Am*, *38*(6), 1252-1255; quiz 1255. https://doi.org/10.1016/j.jhsa.2013.02.034

Sulaiman, W., & Gordon, T. (2013). Neurobiology of peripheral nerve injury, regeneration, and functional recovery: from bench top research to bedside application. *Ochsner J*, *13*(1), 100-108. https://www.ncbi.nlm.nih.gov/pubmed/23531634

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*, *183*, 109119. https://doi.org/10.1016/j.diabres.2021.109119

Sunderland, S. (1951). A classification of peripheral nerve injuries producing loss of function. *Brain*, *74*(4), 491-516. https://doi.org/10.1093/brain/74.4.491

Tai, C. Y., Weber, R. V., Mackinnon, S. E., & Tung, T. H. (2010). Multiple costimulatory blockade in the peripheral nerve allograft. *Neurol Res*, *32*(3), 332-336. https://doi.org/10.1179/174313209x385635

Talukdar, A., & Basumatary, M. (2023). Rodent models to study type 1 and type 2 diabetes induced human diabetic nephropathy. *Mol Biol Rep*, *50*(9), 7759-7782. https://doi.org/10.1007/s11033-023-08621-z

Tan, R. E. S., Jeyaratnam, S., & Lim, A. Y. T. (2023). Updates in peripheral nerve surgery of the upper extremity: diagnosis and treatment options. *Ann Transl Med*, *11*(11), 391. https://doi.org/10.21037/atm-23-1500

Taylor, C. S., & Haycock, J. W. (2022). Biomaterials and Scaffolds for Repair of the Peripheral Nervous System. In J. B. Phillips, D. Hercher, & T. Hausner (Eds.), *Peripheral Nerve Tissue Engineering and Regeneration* (pp. 245-279). Springer International Publishing. https://doi.org/10.1007/978-3-030-21052-6_3

Terada, M., Yasuda, H., & Kikkawa, R. (1998). Delayed Wallerian degeneration and increased neurofilament phosphorylation in sciatic nerves of rats with streptozocin-induced diabetes. *Journal of the Neurological Sciences*, *155*(1), 23-30. https://doi.org/10.1016/S0022-510X(97)00269-4

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 Syst Rev*, *12*(12), CD012574. https://doi.org/10.1002/14651858.CD012574.pub2

Thrainsdottir, S., Malik, R. A., Dahlin, L. B., Wiksell, P., Eriksson, K. F., Rosén, I., Petersson, J., Greene, D. A., & Sundkvist, G. (2003). Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. *Diabetes*, *52*(10), 2615-2622. https://doi.org/10.2337/diabetes.52.10.2615

Tiong, Y. L., Ng, K. Y., Koh, R. Y., Ponnudurai, G., & Chye, S. M. (2020). Melatonin promotes Schwann cell dedifferentiation and proliferation through the Ras/Raf/ERK and MAPK pathways, and glial cell-derived neurotrophic factor expression. *Exp Ther Med*, *20*(5), 16. https://doi.org/10.3892/etm.2020.9143

Tofaris, G. K., Patterson, P. H., Jessen, K. R., & Mirsky, R. (2002). Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J Neurosci*, *22*(15), 6696-6703. https://doi.org/10.1523/jneurosci.22-15-06696.2002

Tomita, K., Hata, Y., Kubo, T., Fujiwara, T., Yano, K., & Hosokawa, K. (2009). Effects of the in vivo predegenerated nerve graft on early Schwann cell migration: quantitative analysis using S100-GFP mice. *Neurosci Lett*, *461*(1), 36-40. https://doi.org/10.1016/j.neulet.2009.05.075

Toraman, T. H., B. (2014). Synthesis and Characterization of the Novel Thermoresponsive Conjugates Based on Poly(3-hydroxy alkanoates). *Journal of polymers and the environment*, *22*, 159-166. https://doi.org/https://doi.org/10.1007/s10924-014-0646-y

Tsuda, Y., Kanje, M., & Dahlin, L. B. (2011). Axonal outgrowth is associated with increased ERK 1/2 activation but decreased caspase 3 linked cell death in Schwann cells after immediate nerve repair in rats. *BMC Neurosci*, *12*, 12. https://doi.org/10.1186/1471-2202-12-12

Tuffaha, S., & Lee, E. B. (2024). Growth Factors to Enhance Nerve Regeneration: Approaching Clinical Translation. *Hand Clinics*, *40*(3), 399-408. https://doi.org/https://doi.org/10.1016/j.hcl.2024.04.002

Ulery, B. D., Nair, L. S., & Laurencin, C. T. (2011). Biomedical Applications of Biodegradable Polymers. *J Polym Sci B Polym Phys*, *49*(12), 832-864. https://doi.org/10.1002/polb.22259

Verge, V. M. K., Hasmatali, J. C. D., & Misra, V. (2020). When the left side knows something happened to the right - sensing injury in neurons contralateral and remote to injury. *Neural Regen Res*, *15*(10), 1854-1855. https://doi.org/10.4103/1673-5374.280316

Vincent, A. M., Perrone, L., Sullivan, K. A., Backus, C., Sastry, A. M., Lastoskie, C., & Feldman, E. L. (2007). Receptor for advanced glycation end products activation injures primary sensory neurons via oxidative stress. *Endocrinology*, *148*(2), 548-558. https://doi.org/10.1210/en.2006-0073

Vogt, P. M., Radtke, C., Krezdorn, N., Kollewe, K., Liebsch, C., Dastagir, K., & Strauß, S. (2024). Biological conduits based on spider silk for reconstruction of extended nerve defects. *Innovative Surgical Sciences*, *9*(3), 133-142. https://doi.org/doi:10.1515/iss-2023-0050

Waller, A. (1851). Experiments on the Section of the Glosso-Pharyngeal and Hypoglossal Nerves of the Frog, and Observations of the Alterations Produced Thereby in the Structure of Their Primitive Fibres. *Edinb Med Surg J*, *76*(189), 369-376.

Wang, L., Yuan, D., Zhang, D., Zhang, W., Liu, C., Cheng, H., Song, Y., & Tan, Q. (2015). Ginsenoside Re Promotes Nerve Regeneration by Facilitating the Proliferation, Differentiation and Migration of Schwann Cells via the ERK- and JNK-Dependent Pathway in Rat Model of Sciatic Nerve Crush Injury. *Cellular and Molecular Neurobiology*, *35*(6), 827-840. https://doi.org/10.1007/s10571-015-0177-7

Wang, S. L., Liu, X. L., Kang, Z. C., & Wang, Y. S. (2023). Platelet-rich plasma promotes peripheral nerve regeneration after sciatic nerve injury. *Neural Regen Res*, *18*(2), 375-381. https://doi.org/10.4103/1673-5374.346461

Wang, Y., Li, B., Xu, H., Du, S., Liu, T., Ren, J., Zhang, J., Zhang, H., Liu, Y., & Lu, L. (2020). Growth and elongation of axons through mechanical tension mediated by fluorescent-magnetic bifunctional Fe3O4·Rhodamine 6G@PDA superparticles. *Journal of Nanobiotechnology*, *18*(1), 64. https://doi.org/10.1186/s12951-020-00621-6

Wang, Z., Yi, X. Z., & Yu, A. X. (2023). Regenerative peripheral nerve interface prevents neuroma formation after peripheral nerve transection. *Neural Regen Res*, *18*(4), 814-818. https://doi.org/10.4103/1673-5374.353498

Waxenbaum, J. A., Reddy, V., & Varacallo, M. (2023). *Anatomy, Autonomic Nervous System*. StatPearls Publishing, Treasure Island (FL). http://europepmc.org/abstract/MED/30969667

http://europepmc.org/books/NBK539845

https://www.ncbi.nlm.nih.gov/books/NBK539845

Weber, M. B., & Isaacs, J. E. (2023). Digital Nerve Injury: Assessment and Treatment. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons*, *31*(15), 802-812. https://doi.org/10.5435/jaaos-d-23-00255

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. *Plast Reconstr Surg*, *106*(5), 1036-1045; discussion 1046-1038. https://doi.org/10.1097/00006534-200010000-00013

Welin, D., Novikova, L. N., Wiberg, M., Kellerth, J. O., & Novikov, L. N. (2009). Effects of N-acetyl-cysteine on the survival and regeneration of sural sensory neurons in adult rats. *Brain Res*, *1287*, 58-66. https://doi.org/10.1016/j.brainres.2009.06.038

Whitlock, E. L., Tuffaha, S. H., Luciano, J. P., Yan, Y., Hunter, D. A., Magill, C. K., Moore, A. M., Tong, A. Y., Mackinnon, S. E., & Borschel, G. H. (2009). Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve*, *39*(6), 787-799. https://doi.org/10.1002/mus.21220

Wiig, M. E., Dahlin, L. B., Friden, J., Hagberg, L., Larsen, S. E., Wiklund, K., & Mahlapuu, M. (2014). PXL01 in sodium hyaluronate for improvement of hand recovery after flexor tendon repair surgery: randomized controlled trial. *PLoS One*, *9*(10), e110735. https://doi.org/10.1371/journal.pone.0110735

Witzel, C., Brushart, T. M., Koulaxouzidis, G., & Infanger, M. (2016). Electrical Nerve Stimulation Enhances Perilesional Branching after Nerve Grafting but Fails to Increase Regeneration Speed in a Murine Model [Article]. *Journal of Reconstructive Microsurgery*, *32*(6), 491-497. https://doi.org/10.1055/s-0036-1579540

Wong, G. C., & Chung, K. C. (2024). Bioengineered Nerve Conduits and Wraps. *Hand Clinics*, *40*(3), 379-387. https://doi.org/https://doi.org/10.1016/j.hcl.2024.03.003

Wong, J. N., Olson, J. L., Morhart, M. J., & Chan, K. M. (2015). Electrical stimulation enhances sensory recovery: A randomized controlled trial. *Annals of Neurology*, *77*(6), 996-1006. https://doi.org/https://doi.org/10.1002/ana.24397

Wood, M. D., & Mackinnon, S. E. (2015). Pathways regulating modality-specific axonal regeneration in peripheral nerve. *Exp Neurol*, *265*, 171-175. https://doi.org/https://doi.org/10.1016/j.expneurol.2015.02.001

Wu, L., Wang, X. J., Luo, X., Zhang, J., Zhao, X., & Chen, Q. (2024). Diabetic peripheral neuropathy based on Schwann cell injury: mechanisms of cell death regulation and therapeutic perspectives [Review]. *Frontiers in Endocrinology*, *15*. https://doi.org/10.3389/fendo.2024.1427679

Wu, P., Spinner, R. J., Gu, Y., Yaszemski, M. J., Windebank, A. J., & Wang, H. (2013). Delayed repair of the peripheral nerve: A novel model in the rat sciatic nerve. *Journal of Neuroscience Methods*, *214*(1), 37-44. https://doi.org/https://doi.org/10.1016/j.jneumeth.2013.01.003

Wu, R. T., & Chuieng-Yi Lu, J. (2024). Nerve Autograft: Preservation of a Lost Art. *Hand Clinics*, *40*(3), 347-356. https://doi.org/https://doi.org/10.1016/j.hcl.2024.04.004

Xiao, B., Feturi, F., Su, A. A., Van der Merwe, Y., Barnett, J. M., Jabbari, K., Khatter, N. J., Li, B., Katzel, E. B., Venkataramanan, R., Solari, M. G., Wagner, W. R., Steketee, M. B., Simons, D. J., & Washington, K. M. (2024). Nerve Wrap for Local Delivery of FK506/Tacrolimus Accelerates Nerve Regeneration. *Int J Mol Sci*, *25*(2). https://doi.org/10.3390/ijms25020847

Xu, G., Liu, B., Sun, Y., Du, Y., Snetselaar, L. G., Hu, F. B., & Bao, W. (2018). Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ*, *362*, k1497. https://doi.org/10.1136/bmj.k1497

Yadav, A., Ramasamy, T. S., Lin, S. C., Chen, S. H., Lu, J., Liu, Y. H., Lu, F. I., Hsueh, Y. Y., Lin, S. P., & Wu, C. C. (2022). Autologous Platelet-Rich Growth Factor Reduces M1 Macrophages and Modulates Inflammatory Microenvironments to Promote Sciatic Nerve Regeneration. *Biomedicines*, *10*(8). https://doi.org/10.3390/biomedicines10081991

Yao, L., de Ruiter, G. C., Wang, H., Knight, A. M., Spinner, R. J., Yaszemski, M. J., Windebank, A. J., & Pandit, A. (2010). Controlling dispersion of axonal regeneration using a multichannel collagen nerve conduit. *Biomaterials*, *31*(22), 5789-5797. https://doi.org/10.1016/j.biomaterials.2010.03.081

Ydens, E., Amann, L., Asselbergh, B., Scott, C. L., Martens, L., Sichien, D., Mossad, O., Blank, T., De Prijck, S., Low, D., Masuda, T., Saeys, Y., Timmerman, V., Stumm, R., Ginhoux, F., Prinz, M., Janssens, S., & Guilliams, M. (2020). Profiling peripheral nerve macrophages reveals two macrophage subsets with distinct localization, transcriptome and response to injury. *Nat Neurosci*, *23*(5), 676-689. https://doi.org/10.1038/s41593-020-0618-6

Yi, C., & Dahlin, L. B. (2010). Impaired nerve regeneration and Schwann cell activation after repair with tension. *Neuroreport*, *21*(14), 958-962. https://doi.org/10.1097/WNR.0b013e32833e787f

Yildirim, M. A., Karlidag, T., Akpolat, N., Kaygusuz, I., Keles, E., Yalcin, S., & Akyigit, A. (2015). The Effect of Methylprednisolone on Facial Nerve Paralysis With Different Etiologies. *Journal of Craniofacial Surgery*, *26*(3). https://journals.lww.com/jcraniofacialsurgery/fulltext/2015/05000/the_effect_of_methylprednisolone_on_facial_nerve.48.aspx

Yorek, M. (2022). Treatment for Diabetic Peripheral Neuropathy: What have we Learned from Animal Models? *Curr Diabetes Rev*, *18*(5), e040521193121. https://doi.org/10.2174/1573399817666210504101609

Yorek, M. A. (2016). Alternatives to the Streptozotocin-Diabetic Rodent. *Int Rev Neurobiol*, *127*, 89-112. https://doi.org/10.1016/bs.irn.2016.03.002

Yorek, M. A. (2022). Diabetic Neuropathy: In Search of a Treatment. *Curr Diabetes Rev*, *18*(5), e070422203248. https://doi.org/10.2174/1573399818052204071046O6

Yu, P., Zhang, G., Hou, B., Song, E., Wen, J., Ba, Y., Zhu, D., Wang, G., & Qin, F. (2023). Effects of ECM proteins (laminin, fibronectin, and type IV collagen) on the biological behavior of Schwann cells and their roles in the process of remyelination after peripheral nerve injury. *Front Bioeng Biotechnol*, *11*, 1133718. https://doi.org/10.3389/fbioe.2023.1133718

Yu, W., Zhao, W., Zhu, C., Zhang, X., Ye, D., Zhang, W., Zhou, Y., Jiang, X., & Zhang, Z. (2011). Sciatic nerve regeneration in rats by a promising electrospun collagen/poly(epsilon-caprolactone) nerve conduit with tailored degradation rate. *BMC Neurosci*, *12*, 68. https://doi.org/10.1186/1471-2202-12-68

Zhang, X., Xiu, X., Wang, P., Han, Y., Chang, W., & Zhao, J. (2023). Intraoperative electrical stimulation promotes the short-term recovery of patients with cubital tunnel syndrome after surgery. *Journal of Orthopaedic Surgery and Research*, *18*(1), 270.

Zhao, Q., Dahlin, L. B., Kanje, M., & Lundborg, G. (1993). Repair of the transected rat sciatic nerve: matrix formation within implanted silicone tubes. *Restor Neurol Neurosci*, *5*(3), 197-204. https://doi.org/10.3233/rnn-1993-5304

Zhao, Q., Drott, J., Laurell, T., Wallman, L., Lindström, K., Bjursten, L. M., Lundborg, G., Montelius, L., & Danielsen, N. (1997). Rat sciatic nerve regeneration through a micromachined silicon chip. *Biomaterials*, *18*(1), 75-80. https://doi.org/10.1016/s0142-9612(96)00112-3

Zhao, X. F., Huffman, L. D., Hafner, H., Athaiya, M., Finneran, M. C., Kalinski, A. L., Kohen, R., Flynn, C., Passino, R., Johnson, C. N., Kohrman, D., Kawaguchi, R., Yang, L. J. S., Twiss, J. L., Geschwind, D. H., Corfas, G., & Giger, R. J. (2022). The injured sciatic nerve atlas (iSNAT), insights into the cellular and molecular basis of neural tissue degeneration and regeneration. *Elife*, *11*. https://doi.org/10.7554/eLife.80881

Zigmond, R. E., & Echevarria, F. D. (2019). Macrophage biology in the peripheral nervous system after injury. *Prog Neurobiol*, *173*, 102-121. https://doi.org/10.1016/j.pneurobio.2018.12.001