

**APORTA DICTAMEN PERICIAL COMPENSAR EPS EXP. 11001400303420210088100****MARIA CATALINA PACHON VALDERRAMA** <MCPACHONV@compensarsalud.com>

Mar 3/10/2023 10:25 AM

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 1 archivos adjuntos (10 MB)

Aporta dictamen pericial.pdf;

Respetados Doctores, buen día.

En mi calidad de apoderada de COMPENSAR EPS, entidad demandada dentro del proceso de responsabilidad médica adelantado por el señor OMAR ORLANDO GALLEGU GONZALEZ y que cursa en el Despacho bajo el radicado 110014003034**20210088100**, conforme a lo dispuesto en audiencia celebrada el pasado 14 de septiembre de 2023, me permito allegar dictamen pericial rendido por la especialidad de ortopedia

De acuerdo con lo señalado en la Ley 2213 de 2022, copio el presente correo a la apoderada del demandante y demás intervinientes.

Cordialmente

**MARÍA CATALINA PACHÓN VALDERRAMA**

Apoderada Compensar EPS

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Doctora

**NELLY ESPERANZA MORALES RODRÍGUEZ**

**JUEZA TREINTA Y CUATRO (34) CIVIL MUNICIPAL DE BOGOTÁ**

E. S. D.

<b>REF.</b>	<b>Allega dictamen pericial de parte rendido por especialista en ortopedia</b>
<b>Radicado:</b>	2021-0881
<b>Proceso:</b>	Verbal de Responsabilidad Médica
<b>Demandante:</b>	Omar Orlando Gallego Gonzalez
<b>Demandados:</b>	Ricardo Andres Becerra Andrade y Caja de Compensación Familiar Compensar – Compensar EPS
<b>Llamado en</b>	
<b>Garantía:</b>	La Equidad Seguros Generales Organismo Cooperativo

**MARÍA CATALINA PACHÓN VALDERRAMA**, mayor de edad, domiciliada en esta ciudad, identificada con la cédula de ciudadanía número 1.019.050.274 de Bogotá D.C., y portadora de la tarjeta profesional número 251.617 del Consejo Superior de la Judicatura, actuando en mi condición de apoderada de la entidad denominada **CAJA DE COMPENSACIÓN FAMILIAR COMPENSAR**, en su programa de entidad promotora de salud - **COMPENSAR EPS**, por medio del presente escrito y en cumplimiento de lo dispuesto por el Despacho en el decreto de pruebas realizado en audiencia del pasado 14 de septiembre de 2023, me permito aportar dictamen pericial rendido por la especialidad de ortopedia.

En este sentido, junto con el presente memorial se adjunta dictamen pericial rendido por el Doctor Fabian Gilberto Gomez Ardila, médico especialista en ortopedia, junto con su hoja de vida y literatura científica en setenta y seis (76) folios.

De la Señora Juez, con todo respeto,



**MARÍA CATALINA PACHÓN VALDERRAMA**

C.C. No. 1.019.050.274 de Bogotá D.C

T.P. No. 251.617 del C.S. de la J.

## **DICTAMEN PERICIAL ESPECIALIDAD DE ORTOPEDIA – MIEMBRO SUPERIOR**

Se lleva a cabo el presente dictamen pericial a solicitud de COMPENSAR EPS para ser aportado dentro del proceso de responsabilidad civil adelantado en su contra bajo el radicado 2021-0881 y que cursa en el Juzgado 34 Civil Municipal de Bogotá

### **A. Identificación del perito**

**Nombre:** Fabian Gilberto Gomez Ardila  
**Cédula:** 79948576  
**Especialidad:** Ortopedia y Traumatología  
**Dirección:** Crr 7ª # 135-78 Torre 4 Apto 903  
**Celular:** 3024574606  
**Email:** drfabiangomez78@gmail.com

### **B. Metodología**

Para llevar a cabo el presente dictamen pericial se procedió a una lectura y estudio detallado de las historias clínicas de las IPS de Compensar correspondientes al señor OMAR ORLANDO GONZÁLEZ GALLEGO sumado a la revisión y conocimiento de artículos científicos, protocolos y guías médicas relacionadas con lesiones neurovasculares.

### **C. Respuestas al cuestionario**

A continuación procederé a dar respuesta a las siguientes preguntas formuladas por COMPENSAR EPS con respecto a la atención médica brindada al paciente OMAR ORLANDO GONZÁLEZ GALLEGO.

1. Sírvasse explicar ¿si la medicina es una ciencia exacta o no? En caso afirmativo o negativo, por favor explicar las razones de su respuesta.

R/ A todas luces la medicina no es una ciencia exacta. Es una ciencia de medios y no de resultados. La medicina busca sobre todas las cosas el bienestar de los pacientes sin poder, en ningún caso, garantizar el resultado pues la respuesta de cada organismo es diferente, en los casos quirúrgicos, los procesos de cicatrización son diferentes, lo que impide tener a ciencia cierta garantía del resultado.

2. De acuerdo con la lectura de la historia clínica del señor OMAR ORLANDO GONZÁLEZ GALLEGO, sírvase señalar los motivos por los cuales este consultó al Doctor RICARDO ANDRES BECERRA ANDRADE el 2 de julio de 2019, precisando si éste tenía antecedentes de intervenciones quirúrgicas previas en la mano izquierda.

R/ Paciente consulto el día 7 de febrero de 2019 por antecedente de cirugía en el antebrazo izquierdo, con posterior imposibilidad para la extensión completa del puño

por probable retracciones de la cicatriz. Fue valorado inicialmente por el servicio de cirugía plástica quienes deciden remisión a manejo por cx de la mano. Posteriormente fue valorado por Ortopedia y traumatología quienes solicitan paraclínicos (rx de puño) para la evaluación objetiva del estado óseo y articular y definir así manejo con "Z" plastia de la cicatriz resultante de su proceso quirúrgico inicial (20años atrás).

Fue valorado en julio de 2019 por el Dr Ricardo Becerra cirujano de mano, donde se propone manejo quirúrgico con "Z" plastias, para corrección de contractura en flexión de la muñeca (condición que resulta de procedimiento quirúrgico realizado 20 años atrás como parte de tratamiento para manejo de flebistis que requirió fasciotomías palmares del antebrazo comprometido)

3. Por favor señale, de acuerdo con las guías y protocolos médicos ¿cuál es el tratamiento descrito en la literatura para la contractura en flexión de la muñeca, precisando si este fue el prescrito al señor OMAR ORLANDO GONZÁLEZ GALLEGO?

R/ La contractura en flexión de la muñeca como resultado de una cicatriz que lleva a la restricción de la movilidad del puño, requiere de manejo quirúrgico con "Z" plastias para corregir la deformidad de las bandas de tensión de la piel, liberando así el tejido en busca de la ganancia de arcos de movimiento. En el caso puntual que nos aqueja, fue el tratamiento que se indicó en día 2 de julio de 2019, y en el cual estoy completamente de acuerdo en su indicación médica.

4. Sírvasse indicar en términos sencillos ¿en que consiste la cirugía de Resección de cicatriz más Z-plastias de muñeca izquierda?

R/ La cirugía consiste en realizar resección de la cicatriz (tejido resultante de procedimiento quirúrgico previo) que está generando la contractura de la articulación y posteriormente diseñar colgajos (en forma de Z) para cambiar las líneas de tensión de la piel, buscando evitar nuevamente contracturas y ganar así movilidad de la articulación comprometida, en este caso el puño izquierdo. El procedimiento busca liberar la articulación del tejido cicatrizal que restringe el arco de movimiento y ganar movilidad con adecuada cobertura de la piel

5. Verificada la historia clínica del paciente, por favor indique al Despacho ¿si el manejo quirúrgico definido el 2 de julio de 2019 por el Doctor RICARDO ANDRES BECERRA ANDRADE fue pertinente y acertado o si, por el contrario, existía otra opción de tratamiento médico teniendo en cuenta el estado de salud del paciente?

R/ Según lo evaluado en la historia clínica, el tratamiento propuesto por el Dr Becerra era el indicado. Como ya lo explicamos anteriormente, la decisión tomada por el cirujano de mano es pertinente y acertada para el tratamiento del paciente. No considero para ese momento otro manejo diferente en aras de mejorar la contractura del puño del paciente.



6. De acuerdo con la descripción quirúrgica de la cirugía practicada el 23 de septiembre de 2019 al señor OMAR ORLANDO GONZÁLEZ GALLEGO, por favor indique ¿si la técnica quirúrgica utilizada en dicha intervención se ajustó a los protocolos y a la *lex artis*?

R/ La descripción del procedimiento quirúrgico realizado al paciente describe adecuadamente la técnica quirúrgica que se realiza para estos casos. También describe la complicación y reparación inmediata de la lesión. Esta técnica se ajusta a los protocolos, a las indicaciones para estos casos, y a lo que la literatura ha probado que tiene mejores resultados en el tratamiento quirúrgico para contracturas articulares por secuelas de cirugías anteriores con cicatrices que comprometen la movilidad articular.

7. En la descripción quirúrgica de la intervención de resección de cicatriz más Z-plastias de muñeca izquierda realizada al señor OMAR ORLANDO GONZÁLEZ GALLEGO, se indican como hallazgos los siguientes: *“Brida cicatrizal longitudinal a través de la cara palmar de la muñeca derecha que genera contractura en flexión de la muñeca con fibrosis sobre los tendones flexores superficiales y profundos y sobre los nervio mediano y cubital. Cicatriz hipertrófica en el eje longitudinal y pliegue palmar de la muñeca izquierda.”* Teniendo en cuenta su experiencia por favor señale ¿si dicho hallazgo genera una mayor complejidad en el procedimiento quirúrgico? Por favor explique su respuesta.

R/ Las cicatrices son per se un tejido diferente al original en el lugar en el que aparecen. Este tejido con características histológicas diferentes puede envolver en su fibrosis otros tejidos circundantes, bien sea musculo, tendón, fascia, nervios, etc. En el lugar donde se generó esta cicatriz, anatómicamente se aumenta la posibilidad de este compromiso por la proximidad de la piel con las estructuras anatómicas profundas, como se explicó claramente en los hallazgos quirúrgicos. Si entendemos que el tejido resultante de su cirugía anterior no es el tejido original, y que medicamente es imposible determinar el grado de afectación del tejido profundo, claramente es una cirugía que genera una mayor complejidad técnica y se incrementa el riesgo de tener complicaciones, las cuales en manos expertas tiene reparo inmediato, como veo que fue en este caso.

8. Sírvasse indicar ¿en qué consiste la lesión parcial del nervio cubital? y si, de acuerdo con su experiencia y la literatura científica, ¿esta corresponde a un riesgo inherente de la cirugía de resección de cicatriz más Z-plastias de muñeca o si, por el contrario, obedece a un error, impericia o mala praxis del cirujano? Explique su respuesta.

R/ La lesión PARCIAL del nervio radial, como su nombre lo indica, es la pérdida parcial de la continuidad de las fibras del nervio comprometido. Como lo dije en la pregunta anterior, es un riesgo al que nos enfrentamos cada vez que llevamos pacientes con estas características a cirugía. A todas luces no es un error médico; en las manos de un cirujano de mano, no es impericia pues el especialista en cuestión tiene suficiente preparación académica y formación de habilidades técnicas, que como resultado le permitieron la identificación de la lesión e inmediata reparación de la misma. Y con esto

explicado no cabe posibilidad a mala praxis pues el cirujano realizo el procedimiento indicado para el caso, teniendo la experiencia suficiente y la formación académica suficiente para realizar el procedimiento quirúrgico.

9. De acuerdo con su respuesta anterior, indique si dicha lesión es prevenible.

R/ Prevenir lesiones como estas en un caso de esta complejidad es muy difícil. Entramos a cirugía con todas esas posibles complicaciones en la cabeza, realizamos cada paso con la mayor precaución posible, pero como ya lo dije, es un tejido tan alterado que no podemos prever el grado de compromiso de estructuras tan sensible como el nervio y la arteria cubital.

10. Sírvasse indicar ¿cuál es el manejo médico descrito en la literatura científica ante la presencia de una lesión de nervio cubital?, precisando si ¿el mismo fue el instaurado por el Doctor RICARDO ANDRES BECERRA ANDRADE?

R/ El manejo indicado para lesiones parciales o totales del nervio radial durante un procedimiento quirúrgico es la identificación de la lesión y la reparación inmediata con neurorrafia. Procedimiento que según lo encontrado en la descripción quirúrgica se realizó inmediatamente, lo que mejora el pronóstico, y para este caso con lesión parcial el pronóstico es favorable.

11. ¿Considera usted que el seguimiento y la evolución post operatoria realizada al paciente por el Doctor RICARDO ANDRES BECERRA ANDRADE fue oportuna, continua y pertinente? Explique su respuesta.

R/ Si. El paciente fue llevado a cirugía el 23 de septiembre, se citó a control el 26 de septiembre y nuevamente el 1 y 17 de octubre lo que evidencia la adecuada periodicidad de los controles, e incluso la remisión a especialidades adicionales para control de dolor, como se evidencia también en la historia clínica con nota del 4 de octubre 2019.

12. Por favor precise si ¿el manejo con terapia física y tens ordenado por el Doctor RICARDO ANDRES BECERRA ANDRADE en el post operatorio, fue adecuado, oportuno y pertinente? Explique su respuesta.

R/ En cuanto a la rehabilitación de este tipo de casos, la terapia física toma un valor incalculable pues es con este tratamiento que se trabaja en la recuperación de la movilidad, se ayuda a disminuir el dolor paulatinamente, y se mejora el tono muscular. El manejo con TENS está indicado para la estimulación del nervio, mejora el dolor neuropático que podría aparecer posterior a la lesión parcial y reparación del nervio. El inicio de la terapia física fue oportuno, y la indicación del tratamiento fue pertinente.

13. De acuerdo con la historia clínica, el 3 de marzo de 2020 se le realizó al señor OMAR ORLANDO GONZÁLEZ GALLEG0 una electromiografía y neuroconducción con los siguientes resultados: *“Neuroconducción n mediano y ulnar bilateral técnica sensitiva antidromica a 14 cm captando en 2do y 5to digito: 1. Latencia sensitivo motora N. mediano normal, 2. Ausencia de potencia sensitivo N. Ulnar, 3. Prolongación latencia motora distal N. Ulnar y DE muy bajo amplitud. Examen de aguja anormal con abundantes signos de denervación en intrínsecos de mano, pobre reclutamiento y sin unidades neuropaticas. Conclusiones: Examen anormal. Lesión axonal severa N. Ulnar izquierdo y sin signos de reinervación”*

13.1. De acuerdo con sus conocimientos y su experiencia, por favor señale ¿si los anteriores resultados implicaban que la lesión del nervio cubital era irreversible o que ya no existía un tratamiento para lograr mejores resultados?

R/ El estudio realizado al paciente nos indica el estado actual del nervio para el momento de la evaluación. Es la interpretación del paso de estímulo eléctrico a través del nervio estudiado. Si bien en ese resultado no había signos de reinervacion, la evaluación clínica en estos casos toma una fuerza predominante pues nos permite evaluar cómo está el nervio y como ha sido la evolución con el pasar de los días. Este resultado no descartar de tajo la posible recuperación del nervio con el pasar de los días.

13.2. Por favor señale si ¿estos resultados paraclínicos eran coincidentes con la evidencia clínica que mostraba el paciente? En caso negativo, sírvase explicar, de acuerdo con su experiencia, a qué se debió esta situación

R/ Como lo indicaba anteriormente, la evaluación clínica nos permite establecer el proceso de recuperación, los hallazgos electromiograficos nos muestran el estado del nervio para ese momento puntual. Es por eso que podemos tener resultados como los vistos en este paciente que clínicamente muestran avances, sin que esto sea una no correlación clínico paraclínico.

13.3. Sírvase indicar si, en su concepto y de acuerdo con su experiencia, ¿este paraclínico se realizó en fecha muy cercana a la lesión, por lo que el mismo no logró evidenciar los resultados del tratamiento de rehabilitación? Por favor explique su respuesta

R/ Estos paraclínicos en los casos de lesión de nervio periférico se toman en varias ocasiones para tener evaluación objetiva del estado de conducción del nervio comprometido. Dependiendo del tiempo de evolución los hallazgos electromiograficos pueden no evidenciar la evolución clínica. Por la proximidad de este estudio al POP pueden evidenciarse estos resultados sin que esto quiera decir que no hay evolución clínica.

14. De acuerdo con la revisión de la historia clínica, por favor indique si ¿desde el 12 de septiembre de 2019 al 23 de junio de 2020 el señor OMAR ORLANDO GONZÁLEZ GALLEGO presentó alguna mejoría en su cuadro clínico, en términos de funcionalidad de la mano izquierda? Explique su respuesta

R/Según lo evidenciado en la historia clínica, y después de haber revisado las notas de terapia física y los hallazgos clínicos reportados por el cirujano tratante, se evidencia recuperación progresiva de su estado clínico

15. Sírvese indicar, de acuerdo con su experiencia y la literatura científica ¿cuál es el tiempo promedio que puede durar la recuperación total de una lesión parcial del nervio cubital?

R/ La tasa de recuperación de un nervio periférico posterior a un lesión, esta cercana a 1mm por día, pero la percepción clínica de la recuperación de una lesión de nervio periférico podría tardar meses e incluso años.

16. El 31 de agosto de 2023 al señor OMAR ORLANDO GONZÁLEZ GALLEGO se le practicó una electromiografía y neuroconducción con los siguientes resultados: *“Las latencias motoras y velocidades de conducción motora correspondientes de los nervios examinados en miembro superior izquierdo son normales. Las latencias sensitivas antidrómicas de los nervios mediano y cubital, izquierdo, son normales. Las latencias sensitivas ortodrómicas de los nervios mediano y cubital no muestran diferencia significativa (mayor de 0.4 msg) al ser comparados ipsilateralmente. La exploración con aguja es normal. Conclusiones: ESTUDIO NORMAL NEGATIVO PARA NEUROÁTIA EN MIEMBRO SUPERIOR IZQUIERDO.”* Por favor señale si, de acuerdo con estos hallazgos, ¿es correcto indicar que el nervio cubital se recuperó totalmente de la lesión?

R/ Como lo explique previamente. La electromiografía evalúa la capacidad del nervio de conducir el estímulo eléctrico a través de sus fibras y a qué velocidad lo logra. Para ese último examen, la respuesta de los nervios periféricos del miembro superior izquierdo tenían una respuesta conductiva normal por lo que se podría considerar como una recuperación de la capacidad del nervio de transmitir el impulso a través de sus fibras.

17. De acuerdo con la lectura de la historia clínica, por favor precise ¿cuál es el estado actual de la mano izquierda del señor OMAR ORLANDO GONZÁLEZ GALLEGO?

R/ La última evaluación encontrada por cirugía de la mano es en junio de 2020, lo que impide conocer por parte del subespecialista su opinión sobre la recuperación. Pero, en nota de agosto de 2023 en evaluación de medicina general se observa en el examen físico que se reporta fuerza 5/5 , e imposibilidad para la abducción del 5to. Lo que nos puede hacer pensar en una recuperación funcional de la mano casi completa, que para

las secuelas de su primera cirugía, sumado a la recuperación de la conductividad del nervio, podríamos decir que el resultado funcional de la mano es muy bueno.

18. ¿Considera que la lesión del nervio cubital documentada durante la cirugía de resección de cicatriz más Z-plastias de muñeca, fue producto de un error médico?

R/ Con todo lo expuesto acá previamente, no considero la lesión del nervio cubital como un error médico sino como una complicación inherente a un procedimiento quirúrgico técnicamente difícil con un grado de complejidad por las secuelas de cirugías anteriores que no era posible de prever, pero con una respuesta médica adecuada al evento y con un resultado electromiografico absolutamente favorable para el caso.

#### **D. Consideraciones**

Manifiesto que no me encuentro incurso en algunas de las causales establecidas en el artículo 50 del C.G.P. y que actualmente no tengo un vínculo con Compensar EPS

En el presente dictamen no he utilizado métodos, experimentos o investigaciones diferentes a las usadas habitualmente en el desarrollo de mi ejercicio profesional o de dictámenes periciales rendidos en otras oportunidades.

A la fecha he rendido mi concepto médico en los siguientes procesos:

Juzgado 7 Civil del Circuito de Cali

Radicado: 2016-0298

Demandante: Karen Fabiana Coronado Artunduaga

Demandado: Comfenalco Valle IPS SAS y otros

Apoderado: Carlos Andres Hernández Escobar

Juzgado 51 Civil del Circuito de Bogotá

Radicado: 2014-0105

Demandante: Joaquín Arevalo Sánchez

Demandado: Compensar EPS

Apoderado: Shirley Lizeth González Lozano

#### **E. Bibliografía**

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**F. Anexos**

Hoja de Vida

Diplomas y títulos académicos



**FABIAN GILBERTO GOMEZ ARDILA**

C.C.79.948.576

RM 871615/06

Escaneado con CamScanner

# Tratamiento de las lesiones de los nervios periféricos. Tendencias actuales del tratamiento quirúrgico

## TREATMENT OF PERIPHERAL NERVES INJURIES. TRENDS IN SURGICAL TREATMENT

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2) Grupo de Ingeniería Tisular. CTS-115. Universidad de Granada

3) Servicio de Neurofisiología Clínica. Hospital Universitario San Cecilio. Granada

### Resumen

Las lesiones nerviosas son habituales en cualquier medio hospitalario. La alta incidencia de estas lesiones y el elevado número de secuelas asociadas a las mismas las hace un problema de salud pública. Proporcionar un tratamiento satisfactorio con un pronóstico funcional aceptable es aún una asignatura pendiente para la medicina actual.

Generalmente, los nervios periféricos se lesionan de forma aguda en el contexto de accidentes de tráfico, heridas por arma de fuego o por arma blanca, amputaciones totales o parciales de cualquier miembro o, simplemente, una herida incisa producida en un ambiente doméstico o laboral. Hoy día las opciones terapéuticas de las que dispone el cirujano para reparar este tipo de lesiones son muy limitadas con una recuperación funcional lenta y exigua en un porcentaje elevado de casos. La progresión de las ciencias médicas y biológicas ha permitido el desarrollo de nuevas técnicas y métodos de Ingeniería Tisular que proyectan avances en la terapia para este tipo de lesiones nerviosas que hasta ahora, se han asociado a una recuperación insatisfactoria, y a veces inexistente.

Palabras clave: lesión nerviosa, conductos nerviosos, ingeniería tisular, microcirugía del nervio periférico.

### Abstract

Nerve damage is common in any hospital environment. The high incidence of these injuries and the high number of aftermath associated makes them a public health problem. To provide a successful treatment with acceptable functional prognosis is still a pending issue for modern medicine.

Generally, peripheral nerves are injured acutely in the context of traffic accidents, gunshot wounds or stab, or partial amputation of any member or simply an incised wound produced at home or business. Today, the therapeutic options available to the surgeon to repair such injuries are very limited with a slow and meager functional recovery in a high percentage of cases. The progression of biological and medical sciences has enabled the development of new techniques and methods Tissue Engineering that project advances in therapy for this type of nerve damage so far, have been associated with an unsatisfactory recovery, and sometimes nonexistent.

Keywords: nerve injury, nerve conduit, tissue engineering, microsurgery of the peripheral nerve.

## 1. Introducción

Las lesiones nerviosas agudas tienen una etiología común en la mayor parte de los casos. Son lesiones graves, que no comprometen la viabilidad de la extremidad afecta, pero sí

pueden comprometer la funcionalidad del miembro afecto y limitar las actividades del sujeto que las sufre, generando en un porcentaje elevado de casos, una minusvalía física, que se asocia a una serie de secuelas psicológicas, no sólo por las limitaciones del sujeto, sino también por el dolor crónico, que a

veces perdura en estos pacientes.

En Estados Unidos, las lesiones del nervio periférico afectan al 2,8% de los pacientes que sufren un traumatismo de cualquier tipo (1), existiendo alrededor de 200.000 personas al año con una lesión nerviosa periférica en miembro superior. En Europa, las cifras son aún mayores, se estima una incidencia de 300.000 casos nuevos por año englobando todos los pacientes de la Unión Europea (2). Todo ello, supone unos 8.648.000 días de baja laboral y 4.916.000 días de ocupación de cama hospitalaria por parte de estos pacientes. Además, a todo ello habría que sumar los problemas derivados de la gran cantidad de secuelas motoras y sensitivas que se asocian a estas lesiones (3). De forma común, los autores consultados expresan que es imposible medir el coste que supone para un sistema de salud (público o privado), pero que de cualquier manera está infravalorado, puesto que el número de pacientes que sufren estas lesiones es elevado y la cronicidad de la mayoría de los procesos hace que la necesidad de tratamientos (médicos y quirúrgicos) se prolongue de forma ilimitada en el tiempo. Además del coste económico, habría que añadir la merma permanente que genera en el paciente y en la familia del paciente este tipo de lesiones, ya que generalmente, son pacientes que asocian trastornos adaptativos ante esta situación de déficit de actividad motora o alteraciones sensitivas.

La alta incidencia de estas lesiones se debe fundamentalmente a que los nervios periféricos son estructuras que se disponen en planos anatómicos superficiales, lo que los hace especialmente vulnerables a agentes externos. Los principales mecanismos lesionales son las heridas incisas, traumatismos cerrados, tracción, isquemia prolongada, quemaduras, congelaciones, radiaciones, lesiones eléctricas, vibración sostenida en el tiempo (4).

Las laceraciones del nervio, producidas por cristales, cuchillos, ventiladores, sierras, etc, son el tipo de lesión más frecuente, que se corresponden con lesiones grado IV o V de Sunderland, y que a veces se asocian a defectos nerviosos que precisan realizar alguna técnica de "puenteo" de este defecto (5). También hay que incluir todas aquellas enfermedades sistémicas que afectan a los nervios periféricos en forma de mononeuritis (por ejemplo la Enfermedad de Churg Strauss) o polineuritis (por ejemplo la Neuropatía Diabética) (6).

La mayor parte de lesiones nerviosas se localizan en el miembro superior, aproximadamente un 75,3% de los casos, además, el nervio que más comúnmente se lesiona es el nervio cubital, bien de forma aislada o bien se combina la lesión con otro nervio, que suele ser el nervio mediano (5). El objetivo de cualquier cirujano en el tratamiento de este tipo de lesiones es permitir y favorecer que el proceso de regeneración axonal se produzca de una manera óptima, por tanto, es fundamental el conocimiento de los procesos biológicos implicados en la regeneración axonal para así favorecer el paso de los brotes axonales a través de la zona de lesión y alcanzar el tejido diana y con ello la recuperación funcional sensitiva y/o motora.

## 2. Clasificación de las lesiones nerviosas

En la actualidad, la clasificación más utilizada en las lesiones nerviosas periféricas es la Sunderland (7), que distingue cinco grados de lesión nerviosa. Esta clasificación tiene una correspondencia con el pronóstico de la lesión, por lo que, a mayor grado, peor pronóstico de recuperación funcional. El grado I de Sunderland es equivalente a la Neuroapraxia de la clasificación de Seddon (8), se trataría de una lesión no estructural del nervio, en la que está alterada la conducción del impulso nervioso a través del cilindroeje. El resto de grados de lesión descritos por Sunderland implican lesión estructural del axón y de las distintas estructuras que envuelven al axón, llegando al grado V de Sunderland, donde se encuentran lesionadas todas las capas del nervio periférico. Este grado V de Sunderland equivale a la neurotmesis de Seddon (Tabla I).

Clasificación de Sunderland	Clasificación de Seddon	Estructuras lesionadas	Estructuras intactas
<u>Grado I</u>	Neuroapraxia	No hay lesión estructural	Todas
<u>Grado II</u>	Axonotmesis	Axones	Endoneuro Perineuro Epineuro
<u>Grado III</u>	Axonotmesis	Axones Endoneuro	Perineuro Epineuro
<u>Grado IV</u>	Axonotmesis	Axones Endoneuro Perineuro	Epineuro
<u>Grado V</u>	Neurotmesis	Todas: Axones Endoneuro Perineuro Epineuro	Ninguna

Tabla I. Clasificación de Sunderland y de Seddon de las lesiones nerviosas periféricas.



El tratamiento de estas lesiones es específico en cada caso. No pueden establecerse unas reglas estrictas de tratamiento que se apliquen de forma generalizada a todos los casos. En algunos casos, como el grado I, el tratamiento consistirá simplemente en adoptar una actitud expectante y esperar a que las alteraciones funcionales del nervio se recuperen para que vuelva a producirse una conducción nerviosa satisfactoria a su través. Por el contrario, en el grado V de Sunderland el tratamiento más aceptado es la revisión quirúrgica del nervio y la reparación del mismo (4). En el resto de grados, el tratamiento depende generalmente de otros factores, como el tipo de nervio lesionado, si es sensitivo o motor, de los músculos inervados por ese nervio, de su localización anatómica, de la presencia de defecto nervioso o no y del tiempo de evolución.

Generalmente, durante el acto quirúrgico no se puede determinar el tipo de lesión nerviosa, salvo el grado V de Sunderland. Para el resto de casos, el grado de lesión nerviosa vendrá determinado por el estudio neurofisiológico realizado posteriormente, preferentemente a partir de la 3ª- 4ª semana desde el momento de la lesión (Figura 1).

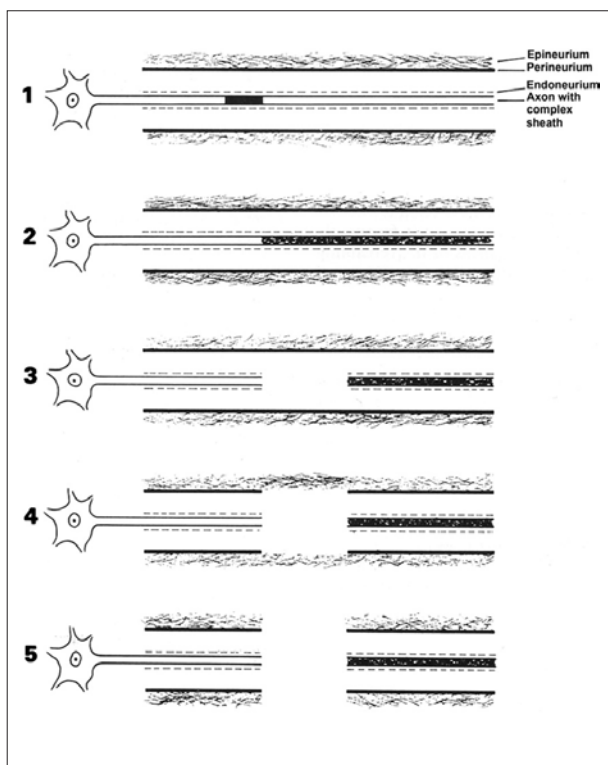


Figura 1. Esquema representativo de las lesiones nerviosas. Grados de Sunderland. Se representan las estructuras histológicas lesionadas en cada tipo de lesión nerviosa.

### 3. Regeneración nerviosa

El proceso de regeneración nerviosa periférica presenta una serie de particularidades que lo diferencian con respecto a otros tejidos en cuanto a la respuesta a la agresión. En aquellas lesiones nerviosas en las que existe una disrupción anatómica de los fascículos axonales o del tronco nervioso completo se produce un proceso de degeneración axonal anterógrada y retrógrada llamada Degeneración Walleriana (9) (Figura 2). Esta degeneración walleriana se debe a la interrupción del flujo axoplásmico desde el soma de la neurona al axón más distal (10). Cualquier parte de una neurona separada de su soma degenera y es destruida por fagocitosis. La degeneración walleriana comporta un conjunto de hallazgos histológicos que ocurren a nivel de los cabos nerviosos donde se produce la lesión y que tiene como objetivo la formación de nuevos brotes axonales que lleguen a contactar con las estructuras axonales del cabo distal. La secuencia de eventos degenerativos incluye cambios moleculares y celulares, y requiere de células de Schwann y macrófagos activos para realizar su función de fagocitar todos los elementos celulares y estructurales del nervio lesionado (11). Por tanto, las células de Schwann también participan en el proceso de desbridamiento, lo cual ocurre los primeros días después de la lesión nerviosa (11); pero al mismo tiempo, también liberan una gran cantidad de citoquinas que actúan como mediadores quimiotácticos que reclutan a macrófagos que circulan en sangre periférica para que actúen también en el proceso de degeneración nerviosa previo a la regeneración (12; 13; 14).

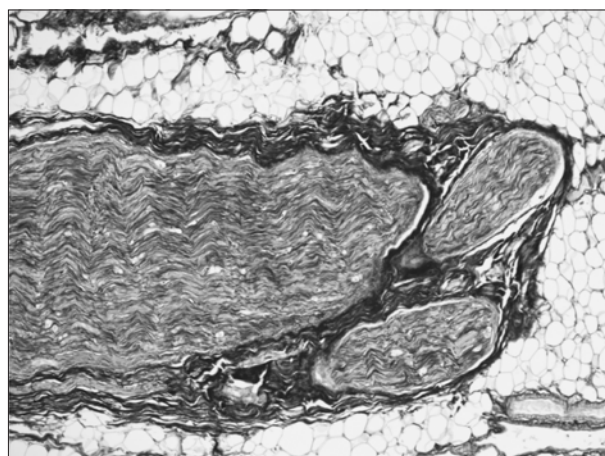


Figura 2. Cambios degenerativos en cabo nervioso distal. Se aprecia una contracción de las estructuras axonales y del tejido de sostén. Infiltración de tejido conectivo denso en el seno del nervio lesionado. Técnica de Picrosirius. 10X.

Generalmente, los primeros días después de la lesión se evidencian un conjunto de cambios morfológicos: el cabo distal se fragmenta, pierde líquido, se contrae y adoptan una morfología más globular. Normalmente, la fragmentación y la contracción de la vaina de mielina ocurren de forma paralela a los cambios degenerativos axonales (Figura 2). Además, los macrófagos infiltran los cabos del nervio con el objetivo de eliminar los restos axonales. Es sabido que la degeneración retrógrada (en el cabo proximal) se prolonga, al menos, un internodo más y que conforme más proximal es la lesión más signos de degeneración se suelen evidenciar (15). En el extremo distal, aunque el axón degenera y desaparece, la membrana basal persiste, generando los llamados tubos endoneurales. Las células de Schwann proliferan y tapizan los tubos formando los llamados Cordones de Büngner, cuyo objetivo es formar una matriz que favorezca el crecimiento de brotes axonales a su través durante el proceso de regeneración axonal (16).

#### 4. Tratamiento quirúrgico de las lesiones nerviosas

El tratamiento quirúrgico de las lesiones nerviosas periféricas implica los principios básicos del tratamiento quirúrgico de cualquier herida, como es el lavado y desbridamiento cuidadoso, eliminación de material extraño y tejido de necrótico bajo anestesia local, regional o general. Se deben de tratar en primer lugar aquellas lesiones coexistentes que puedan poner en peligro la vida del paciente, seguidamente se tratarán las lesiones vasculares, óseas y musculares asociadas a la lesión nerviosa, en caso de que existan, por el orden de prioridad especificado (17). Si la herida es limpia y reciente, el estado general del paciente es satisfactorio y se puede hacer una reparación en un ambiente adecuado, con el personal y el equipo necesarios es preferible realizar una reparación primaria inmediata del nervio en las primeras horas. Si el estado general del paciente no permite la reparación inmediata o si las circunstancias impiden una reparación primaria es preferible la reparación entre 3 y 7 días después de la lesión para descartar la aparición de un proceso séptico en la zona (17). Por otro lado, si existe un defecto segmentario amplio del nervio lesionado se debe suturar los

cabos del nervio lesionado a los tejidos blandos adyacentes con objeto de realizar una reparación diferida e impedir la retracción de los cabos, procedimiento conocido como marcado del nervio (Figura 3).



Figura 3. Lesión del nervio cubital por arma de fuego. Marcado de la lesión nerviosa a los tejidos adyacentes. La zona engrosada central (flecha) corresponde a un neuroma por continuidad del nervio lesionado.

En una fractura cerrada que asocia disfunción del algún nervio periférico es razonable esperar la reinervación y se evitará la exploración quirúrgica inicialmente si esta va a ser tratada de forma incruenta. Se deben valorar periódicamente los progresos funcionales del miembro lesionado mediante electromiogramas periódicos, velocidades de conducción nerviosa y valoración clínica. Si por el contrario, el déficit nervioso es consecuencia de la manipulación o inmovilización con escayola de una fractura cerrada en ausencia de déficit anterior, se recomienda la exploración inicial del nervio (18).

Tratamiento definitivo de las lesiones nerviosas. El objetivo primario de la reparación de una lesión nerviosa es la correcta aproximación de los segmentos nerviosos con la esperanza de alcanzar una reinervación funcional completa de los tejidos (19).

Se han resaltado 4 principios técnicos para la adecuada coaptación de los extremos del nervio lesionado (Figura 4):

Preparación de los muñones del nervio, se realizará con una hoja de bisturí del nº 11 o un bisturí de oftalmólogo. A continuación el cirujano deberá de identificar y separar los fascículos o grupos de fascículos. Recortar con ayuda de un neurótomo los extremos del nervio si están muy lesionados.

Aproximación de los cabos calibrando el grado de tensión entre ambos extremos del nervio, lo cual dependerá del defecto que exista.

Coaptación o neurorrafia de los extremos del nervio. Se debe de prestar especial importancia a la aposición de cada muñón con su extremo correspondiente, a cada fascículo o grupo fascicular con el opuesto para obtener los mejores resultados.

Sutura de los extremos, con el objetivo de mantener la coaptación. Generalmente se realiza con suturas no reabsorbibles, pegamento o adhesivos de fibrina (18).

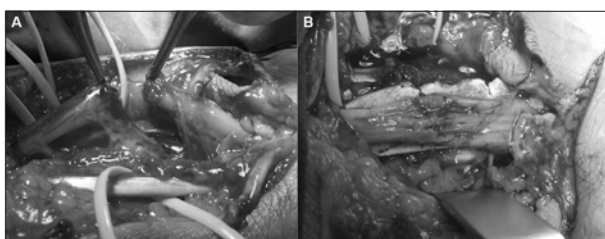


Figura 4. Sección del nervio Mediano a nivel de la muñeca. A. Preparación de los dos cabos nerviosos para llevar a cabo la neurorrafia. B. Resultado final de la lesión nerviosa tras la sutura epineural. Destacar la adecuada orientación de los dos cabos nerviosos, que generalmente viene determinada por la disposición de los vasos epineurales en la superficie del nervio.

La sutura de monofilamento de nylon de 9-0 es considerada la que mejor resiste las fuerzas de distracción. Sin embargo, la de 10-0 se rompe bajo tensión con facilidad y la de 8-0 tiene una tendencia a desgarrar los extremos del nervio reparado (18). Las técnicas microquirúrgicas actualmente utilizadas para la reparación de las lesiones nerviosas fueron descritas por primera vez por Millesi en los años 60 (20). Después de 50 años las técnicas han cambiado, sin embargo, los resultados clínicos de recuperación funcional tras una reparación nerviosa se han mantenido insatisfactorios. Han sido muchos los autores que a lo largo de la historia han intentado revelar la clave del proceso de regeneración y muchos más los que han intentado tratar las lesiones nerviosas mediante el implante de otros tejidos, siendo el resultado bastante pobre en la mayoría de los casos.

#### OPCIONES ACTUALES DE TRATAMIENTO QUIRÚRGICO DE LAS LESIONES NERVIOSAS

Existen distintas opciones de reparación quirúrgica de un nervio lesionado. La utilización de cada una de ellas vendrá determinada por la presencia o no de un defecto nervioso, el periodo de evolución y si ha existido fracaso de una técnica de reparación previa.

Neurolisis externa e interna. Se trata de una técnica de descompresión del nervio periférico. La causa de la compresión puede ser externa al nervio, en cuyo caso bastará la supresión del agente estenosante de forma precoz para la recuperación del nervio (4). A veces a la compresión externa se añade una reacción fibrosa perineural o intrafascicular. En este caso, el tratamiento consiste en la escisión del tejido conectivo interpuesto entre los fascículos con ayuda de microscópio quirúrgico (19).

Neurorrafia terminoterminal. Para lesiones nerviosas completas sin defecto nervioso el tratamiento de elección es la sutura directa entre ambos cabos del nervio lesionado. Este es el tratamiento preferido en todos los casos que sea técnicamente factible (4; 21). El punto clave en la sutura terminoterminal es establecer la continuidad con la alineación rotacional adecuada, es decir, cada fascículo debe estar enfrentado al homónimo del otro extremo del nervio (4; 19).

Además es importante que la sutura realizada entre ambos cabos nerviosos sea una sutura libre de tensión. El aporte vascular de un nervio periférico se produce a partir de un sistema intrínseco y extrínseco que se origina en las arterias locales y regionales, y que penetran en las vainas epineurales y perineurales conectándose entre sí formando un plexo capilar indefinido dentro del endoneuro. El sistema de vascularización más importante del nervio es el intrínseco. Por tanto, una tensión excesiva entre los cabos de un nervio lesionado va a comprometer de forma significativa el aporte vascular intrínseco, lo que va a favorecer la formación en un tejido conectivo denso en sustitución al tejido neural no regenerado.

Existen tres opciones de neurorrafia terminoterminal:

Neurorrafia epineural. Es la opción de elección. La sutura une el epineuro proximal y distal. Se ha demostrado que la ausencia de material de sutura en el interior del tejido de sosten del nervio (perineuro y endoneuro) reduce la formación de neuromas y favorece la regeneración nerviosa (22).

Neurorrafia perineural o fascicular. Se realiza la sutura de cada fascículo de forma individual. Previamente se debe realizar una neurolisis interna con objeto de disecar cada uno de los fascículos. Además se debe añadir la sutura epineural para favorecer el aporte vascular. La sutura intraneural permanente



puede favorecer la fibrosis y de esta forma afectar la recuperación funcional del nervio (23; 24).

Neurorrafia epiperineural. Se trata de realizar una sutura enlazando el epineuro y el perineuro al mismo tiempo.

**INJERTOS NERVIOSOS AUTÓLOGOS.** Es preferible un injerto nervioso cuando existe un defecto nervioso que no puede ser solventado sin tensión mediante tenorrafia terminoterminal. Hoy día los injertos nerviosos son considerados por la mayoría de los autores el gold estándar para el tratamiento de las lesiones nerviosas con defecto que no permiten una sutura terminoterminal (25). Al igual que en la neurorrafia, la sutura que se realiza entre los cabo del nervio lesionado y el nervio injertado debe estar totalmente libre de tensión, dado que la vascularización intrínseca del nervio se podría alterar en caso contrario.

Los axones normalmente entran en los injertos de forma aleatoria. Aquellos axones que interaccionan con una vía emparejada del otro extremo del nervio lesionado es más probable que se produzca la sinapsis con el tejido adecuado. Se produciría el contacto de los conos de crecimiento del nervio lesionado con el tubo endoneural del injerto que estaría “desocupado” de axones, y de esa forma se favorecería la regeneración nerviosa. Aquellos axones que no se hubiesen emparejado satisfactoriamente con el tubo endoneural correspondiente a su fascículo perderían su soporte trófico y se retraerían (26).

Estudios recientes demuestran que un injerto nervioso motor “puro” permite una mejor regeneración que un injerto nervioso sensitivo “puro” apreciándose un mayor número de fibras nerviosas que atraviesan el injerto y de mayor tamaño (27). Sin embargo, el uso de nervios motores para realizar un injerto nervioso resulta impensable debido a la comorbilidad generada, a menos que el injerto proceda de una extremidad insalvable (26). Generalmente se suelen utilizar nervios sensitivos puros para realizar los injertos nerviosos, forzando a los axones a crecer en el interior de unos tubos endoneurales de origen sensitivo hasta que contactan con el cabo distal del nervio. Los axones que atraviesan la sutura distal del injerto serán

los que con más probabilidad sobrevivan, maduren y permitan la recuperación funcional (26).

La morbilidad que se genera en la zona donante es uno de los factores negativos más importantes. Normalmente el nervio utilizado para hacer el injerto debe de cumplir una serie de criterios:

El déficit funcional que se genera de la excisión de ese nervio debe ser aceptable y bien tolerado.

Debe ser accesible, lo contrario conllevaría una disección quirúrgica amplia que generaría más comorbilidad e incremento del tiempo quirúrgico.

El calibre y la longitud del nervio implantado deben ser moderados, no deben implantarse nervios especialmente largos ni gruesos puesto que la vascularización del tejido implantado se producirá a partir de los tejidos circundantes donde se sitúa.

Estas características hacen que el nervio Sural sea el más utilizado para los autoinjertos (Figura 5). Otros nervios que se pueden utilizar son los peroneos, intercostales, y antebraquial cutáneos del antebrazo, entre otros (25).



Figura 5. Izquierda: disección del Nervio Sural en pantorrilla derecha (zona donante). Derecha: interposición del injerto en la zona de defecto (zona receptora). La tijera muestra la zona de contacto entre el injerto y el nervio lesionado a nivel distal.

**Injerto nervioso vascularizado.** Este tipo de injerto nervioso está ganando notoriedad en los últimos años por facilitar la regeneración axonal a través de los injertos que puentean la zona de lesión y por limitar la zona de isquemia central que sufren los injertos de gran calibre y/o de gran longitud (26). Se ha demostrado su eficacia en humanos cuando es preciso un injerto nervioso de gran calibre y longitud que excede los 20 cm tanto en miembro inferior como superior. No sin embargo, en defecto pequeños (28). Los más utilizados en la actualidad son los injertos vascularizados de nervio Radial Superficial y el injerto vascularizado de nervio Femoral Cutáneo

Superficial (29).

**Aloinjertos nerviosos.** Su uso está limitado por la necesidad de agentes inmunosupresores requeridos para que el injerto sea eficaz. A diferencia del trasplante de otros tejidos, el tratamiento inmunosupresor se extenderá hasta que los axones y células de Schwann del propio paciente poblasen el aloinjerto, lo cual se estima en un periodo aproximado de 18 meses (26).

Los aloinjertos y los injertos nerviosos vascularizados están en desuso en la actualidad aunque existen algunos grupos de estudio que lo utilizan en lesiones del plexo braquial y en lesiones de la cola de caballo.

**TRANSFERENCIA NERVIOSA O NEUROTIZACIÓN.** Se trata de una técnica quirúrgica que se realiza de forma casi exclusiva en lesiones proximales, fundamentalmente en lesiones preganglionares del plexo braquial.

Consistiría en transferir fibras nerviosas de un nervio sano a un nervio denervado, con el objetivo de “neurotizar” (inervar) el nervio. Los nervios motores se utilizan para restaurar la función motora y los nervios sensitivos para restablecer la función sensorial. Clásicamente, estas técnicas conllevaban el sacrificio de la función del axón donante, pero con las actuales técnicas terminolaterales no existe tal sacrificio (30). El proceso celular exacto que se produce aún no es bien conocido y es objeto de múltiples estudios. La hipótesis de la regeneración mediante “end to side” (sutura terminolateral) es la invasión desde el muñón proximal seccionado del nervio lesionado, la regeneración desde axones del nervio donante que fue dañado durante la preparación previa del nervio. Al mismo tiempo también se postula que existen brotes axonales colaterales que surgen desde la zona de sutura nerviosa terminolateral y que favorecerían la regeneración del nervio dañado.

En general, son técnicas muy complejas que sólo se realizan en centros especializados en el tratamiento de lesiones nerviosas y, más específicamente en lesiones del plexo braquial.

**INJERTOS NERVIOSOS SINTÉTICOS.** Como alternativa a los injertos nerviosos autólogos para el tratamiento de lesiones nerviosas con defecto existen los injertos nerviosos sintéticos. De esta manera, se utilizan conductos nerviosos como armazón o andamio a través del cual se produce el proceso de regeneración nerviosa (Figura 6).



Figura 6: Colocación de NeuroTube® en fascículos de nervio mediano por neuroma de amputación en una sección parcial producida por un arma blanca que pasó desapercibida en una primera valoración.

Los intentos de tubulización de los nervios periféricos han sido constantes a lo largo de la historia. Desde 1880, año en que Gluck (32) intentó puentear ambos extremos del nervio lesionado mediante matriz ósea desmineralizada, los intentos por puentear la zona de defecto para poner en contacto ambos cabos nerviosos han sido continuos y con unos resultados muy dispares. Otros autores interpusieron arteria braquial, vena safena, fascia muscular, hueso, goma y otros elementos entre los cabos del nervio lesionado con el objetivo de favorecer el crecimiento axonal a su través sin que en ninguno de los casos se obtuviese un resultado satisfactorio.

En el año 1928, Ramón y Cajal (33) hizo importantes aportaciones en el campo de las neurociencias, del que destaca el postulado sobre el concepto de neurotropismo. Este postulado ha permitido determinar qué factores interfieren en el proceso de regeneración nerviosa periférica. Cajal establece que agentes químicos desde el muñón distal del nervio, podrían atraer al muñón proximal, debido a un proceso de regeneración axonal, que estaría mediado por agentes neurotrópicos segregados después de una lesión por los extremos del nervio. Los experimentos realizados por el neuroanatomista Santiago Ramón y Cajal, mostraron que después de una lesión, las fibras dañadas en la médula espinal adulta empiezan a crecer y ramificarse por un cierto tiempo, pero después, éstos brotes se paralizan por los obstáculos insuperables con que se encuentran, hasta que se retraen y desaparecen. Es decir, que la condición traumática es suficiente para sacar a los axones de su “letargo”, ya que las neuronas intentan regenerarse mostrando conos

de crecimiento y arborizaciones. De acuerdo con Cajal, esta condición se frustra, primero, por falta de sustancias capaces de generar una vigorosa capacidad de crecimiento, y segundo, por la ausencia de sustancias capaces de atraer y dirigir a los axones a su destino. (33). En 1911, Tello, llevo a cabo unos experimentos, en los que se cortaba la corteza cerebral y se transplantaba un fragmento de nervio ciático previamente dañado entre 8 y 12 días antes. Se observaba que fibras de varios puntos de la corteza convergían y penetraban en el trasplante a los 12-14 días. Sin embargo, a los 40 días el trasplante disminuía de volumen, se encontraba penetrado por tejido conectivo y en proceso de atrofia y reabsorción, posiblemente, porque las sustancias tróficas habían dejado de secretarse. Por lo tanto, concluyeron que “estos experimentos” confirman que el crecimiento de los axones depende de la presencia de una “comida especial”, la cual es producida en proporciones efectivas únicamente por las células de Schwann de los nervios”, es decir, se demostró que las neuronas pueden crecer si se encuentran en un medio ambiente permisivo (34). Años después, el concepto de neurotropismo de Cajal fue rebatido por Weiss, postulando que era más importante una guía que contactara entre ambos extremos del nervio lesionado, que el neurotropismo, como factor fundamental para la regeneración nerviosa en una lesión de nervio periférico (35).

Fue en los años 80, cuando mediante sofisticados experimentos, se demostró, que ambos factores, neurotropismo (postulado por Cajal) y guía de contacto (postulado por Weiss), eran importantes en la regeneración del nervio periférico, lo que constituye la base para el desarrollo de diferentes técnicas y métodos que se aúnan con el mismo objetivo, favorecer el crecimiento y desarrollo axonal, que va a permitir obtener óptimos resultados clínicos e histológicos en una lesión de nervio periférico (34).

La elaboración de nuevos biomateriales ha permitido la aplicación de los mismos en el tratamiento de las lesiones nerviosas periféricas, de esta forma, algunas marcas han comercializado sus propios conductos sintéticos elaborados con distintos materiales, reabsorbibles y no reabsorbibles, que han sido aprobados por la FDA (*Food and Drugs Administration*) y por la Conformit Europe-Approved.

Conductos nerviosos sintéticos de

colágeno. Se trata de una proteína que ha sido la más comúnmente usada como biomaterial en el sistema nervioso. El colágeno tipo I de la piel bovina y el tipo IV han sido los principales componentes de esas guías nerviosas. El periodo de degradación del conducto nervioso de colágeno oscila entre 1 y 9 meses (36).

Se ha probado que los conductos fabricados con colágeno tipo I constituyen un soporte y una guía tisular para la regeneración nerviosa in vivo. Se han descritos casos de reacción de cuerpo extraño, a pesar de que son estructuras con una baja inmunogenicidad y han demostrado biocompatibilidad in vivo. Este material se ha utilizado para varias aplicaciones biomédicas: piel artificial, apósitos biológicos, desarrollo de fármacos, sustitutos meníngeos y conductos nerviosos (37).

Existen dos marcas comercializadas en España de conductos nerviosos de colágeno:

NeuraGen®  
NeuroMatrix®

Conductos nerviosos sintéticos de poliéster alifático sintético. Es un polímero biodegradable derivado de poliéster alifático sintético como el ácido poliglicólico, láctico y sus copolímeros que se usan como biomateriales en aplicaciones médicas como la fabricación de suturas y fijaciones en cirugía ortopédica (38).

La FDA ha aprobado dos conductos elaborados con este material para la práctica clínica:

NeuroTube® (Ácido poliglicólico)  
NeuroLac® (Poli-DL-Lactico-Caprolactona)

**TRATAMIENTOS PALIATIVOS.** Se realiza en aquellos casos en los que la recuperación espontánea no ha ocurrido o cuando la intervención quirúrgica ha fallado y persiste el déficit funcional que presentaba el paciente previamente (30).

En definitiva el objetivo de los tratamientos paliativos es mejorar la estabilidad articular y permitir el movimiento que los grupos musculares paralizados no ejecutan. (30).

Los procedimientos primarios en la reconstrucción periférica son la artrodesis y las transferencias tendinosas, además han

aparecido técnicas innovadoras como las transferencias de músculo libre llegando a ser otra posible opción. Hay también una parte limitada de amputaciones, osteotomías, liberación de contracturas articulares y musculares. Por otro lado, también se incluyen en este grupo los colgajos sensitivos, que se realizan raramente con el objetivo de mantener la sensibilidad en determinadas zonas anatómicas.

Se han descrito numerosos procedimientos para mejorar la función de miembros superiores y de miembros inferiores. Es fundamental el estudio pormenorizado de cada paciente para determinar cuál de los procedimientos le producirá un mayor beneficio ante distintas posibilidades técnicas (30).

Actualmente, estas técnicas son mucho más utilizadas en miembros superiores, ya que la extremidad superior es mucho más funcional que la inferior y en ella las lesiones nerviosas son mucho más frecuentes. El objetivo, sería por tanto, restaurar la estabilidad y/o movilidad del hombro y además restaurar la flexión del codo y la función de la mano, en el caso del miembro superior (30).

## 5. Innovaciones en el tratamiento de lesiones nerviosas

Es tangible que ninguno de los tratamientos expuestos previamente para resolver las lesiones nerviosas periféricas con defecto es totalmente eficaz. En la actualidad, el *gold estándar* para tratar este tipo de lesiones lo constituye el injerto nervioso autólogo de un nervio sensitivo. Sin embargo, como se ha expuesto previamente, los resultados clínicos y funcionales de los pacientes tratados con autoinjertos no son óptimos ni tampoco homogéneos, a lo que hay que unir la comorbilidad asociada en la zona donante.

Al gran desarrollo de la biomedicina en los últimos años, habría que asociar el conocimiento más profundo de los mecanismos implicados en el proceso de regeneración nerviosa, que nos permiten, además de conocer los elementos celulares que median el proceso y qué factores neurotróficos y neurotrópicos influyen, cómo actúan, cuándo actúan y la manera de mejorar el proceso de regeneración nerviosa que permita el contacto de los brotes

axonales con el cabo distal.

La biotecnología y la ingeniería tisular representan un conjunto de doctrinas científicas multidisciplinares que podrían contribuir a solventar algunos de los problemas médicos de mayor gravedad y más demandados mediante la creación de nuevos tejidos similares a los existentes en los organismos vivos (39) nuevas técnicas que aplican estas doctrinas permiten el desarrollo de nuevos constructos biológicos como alternativas de futuro en la regeneración tisular (39).

La generación de tejidos artificiales empleando células troncales adultas y biomateriales altamente compatibles, es uno de los principales objetivos en la investigación biomédica. Aunque estos tejidos biogenerados mediante ingeniería tisular pudieran ser potencialmente útiles para la sustitución clínica de tejidos dañados, la obtención de células nativas con elevada capacidad de proliferación y diferenciación no es siempre posible (40). Por esta razón, la búsqueda de fuentes celulares alternativas para su utilización como sustitutos de las células nativas es uno de los retos actuales de la medicina regenerativa, ya que permite la elaboración de tejidos y la restitución del órgano dañado (41). En general, algunos autores coinciden en el uso de biomateriales biocompatibles asociados a células madre de origen mesenquimal para el tratamiento de este tipo de lesiones nerviosas. Las células madre empleadas hasta el momento han sido células madre derivadas de la grasa y células madre derivadas de la médula ósea con unos patrones de regeneración nerviosa muy similar entre ambos, sin embargo, al diferenciar ambas estirpes celulares al *células de Schwann like*, los parámetros de regeneración nerviosa mejoran sustancialmente, lo que resalta la especial participación de las células de Schwann en el proceso de regeneración nerviosa (42). Se han realizado experimentos utilizando células madre fetales neurales para el tratamiento de este tipo de lesiones en animales de experimentación obteniendo unos resultados esperanzadores (43).

La regeneración nerviosa se basa fundamentalmente en el crecimiento de los brotes axonales de forma centrífuga desde el



soma de la neurona, que generalmente se ubica en el asta anterior de la médula para motoneuronas o en el ganglio dorsal, en el caso de neuronas sensitivas. De forma que, los brotes axonales se disponen en el tejido conectivo lesionado y crecen en su seno hasta que contactan con la estructura diana (placa motora, receptores sensoriales, músculo liso, etc). A nuestro criterio, después de varias investigaciones y en concordancia con otros autores, el problema fundamental de la regeneración nerviosa radica en la capacidad que tiene el tejido conectivo para proliferar más rápidamente que los brotes axonales, lo que hacen que estos queden incluidos en su seno, provocando lo que se conoce como neuroma en continuidad.

Por tanto, la mayoría de los estudios pretender encontrar la fórmula que permita a los brotes axonales crecer a través de un andamio de distribución tridimensional sin que el crecimiento del tejido conectivo interfiera en la progresión axonal. De esta manera, los distintos investigadores hemos pretendido desarrollar nuevos biomateriales que favorezcan el crecimiento axonal. Tal es el caso de Bunting y colaboradores (44) que desarrollaron conductos nerviosos de fibra de vidrio para facilitar la regeneración axonal en la zona de defecto con unos resultados muy similares al injerto nervioso autólogo.

En conclusión, las nuevas doctrinas como la Ingeniería Tisular y la Medicina Regenerativa permiten el desarrollo de nuevos biomateriales y la aplicación de terapias celulares para el tratamiento de las lesiones nerviosas periféricas, abriendo así la puerta de la esperanza a un número elevado de potenciales pacientes que van a padecer este tipo de lesiones que se asocian a una escasa recuperación funcional en un elevado porcentaje de casos. Por el momento, ninguno de los avances en regeneración nerviosa utilizando técnicas de ingeniería tisular han sido aplicados en pacientes por la ausencia de ensayos clínicos y problemas éticos derivados de este tipo de tratamientos, aunque existen diversos grupos de estudio que pretenden la aplicación de estos modelos para el tratamiento de lesiones nerviosas con defecto.

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## SYMPOSIUM: Peripheral Neuropathies

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# Nerve Injury, Axonal Degeneration and Neural Regeneration: Basic Insights

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**Axotomy or crush of a peripheral nerve leads to degeneration of the distal nerve stump referred to as Wallerian degeneration (WD). During WD a microenvironment is created that allows successful regrowth of nerve fibres from the proximal nerve segment. Schwann cells respond to loss of axons by extrusion of their myelin sheaths, downregulation of myelin genes, dedifferentiation and proliferation. They finally align in tubes (Büngner bands) and express surface molecules that guide regenerating fibres. Hematogenous macrophages are rapidly recruited to the distal stump and remove the vast majority of myelin debris. Molecular changes in the distal stump include upregulation of neurotrophins, neural cell adhesion molecules, cytokines and other soluble factors and their corresponding receptors. Axonal injury not only induces muscle weakness and loss of sensation but also leads to adaptive responses and neuropathic pain. Regrowth of nerve fibres occurs with high specificity with formerly motor fibres preferentially reinnervating muscle. This involves recognition molecules of the L2/HNK-1 family. Nerve regeneration occurs at a rate of 3-4 mm/day after crush and 2-3 mm/day after sectioning a nerve. Nerve regeneration can be fostered pharmacologically. Upon reestablishment of axonal contact Schwann cells remyelinate nerve sprouts and downregulate surface molecules characteristic for precursor/premyelinating or nonmyelinating Schwann cells. At present it is unclear whether axonal regeneration after nerve injury is impeded in neuropathies.**

### Introduction

There are two principal targets of peripheral nerve damage: the axon and the Schwann cells with their myelin sheaths. Attacks on myelin sheaths or myelinating Schwann cells as often seen in inflammatory neuropathies lead to focal demyelination with relative preservation of the axon. Repair mechanisms can fastly restore nerve conduction by remyelination. In contrast, axonal damage by crush, axotomy, ischemia, or inflammation leads to interruption of axonal integrity with ensuing degeneration of nerve fibres distal to the site of insult, a process named Wallerian degeneration (WD) (116). WD begins with prompt degradation of axoplasm and axolemma induced by the activation of axonal proteases and calcium influx (32, 93). To restore function, nerve fibres have to regrow from the site of axonal injury. While both nerve crush and axotomy induce WD, there is an important difference in the probability of successful regeneration. After a crush lesion the continuous basal lamina provides guidance for regenerating axons from the proximal nerve stump to their targets. After axotomy, however, separation of the proximal and distal stumps can impede reinnervation and often leads to the formation of neuroma.

WD sets in motion a machinery of molecular changes in the perikarya as well as in the distal degenerating stump of the injured motor and sensory neurons. Very rapidly nerve fibres from the proximal stump elongate growth cones into and through the distal segment and eventually reinnervate target tissues. The cellular and molecular mechanisms underlying WD and subsequent nerve regeneration are reviewed.

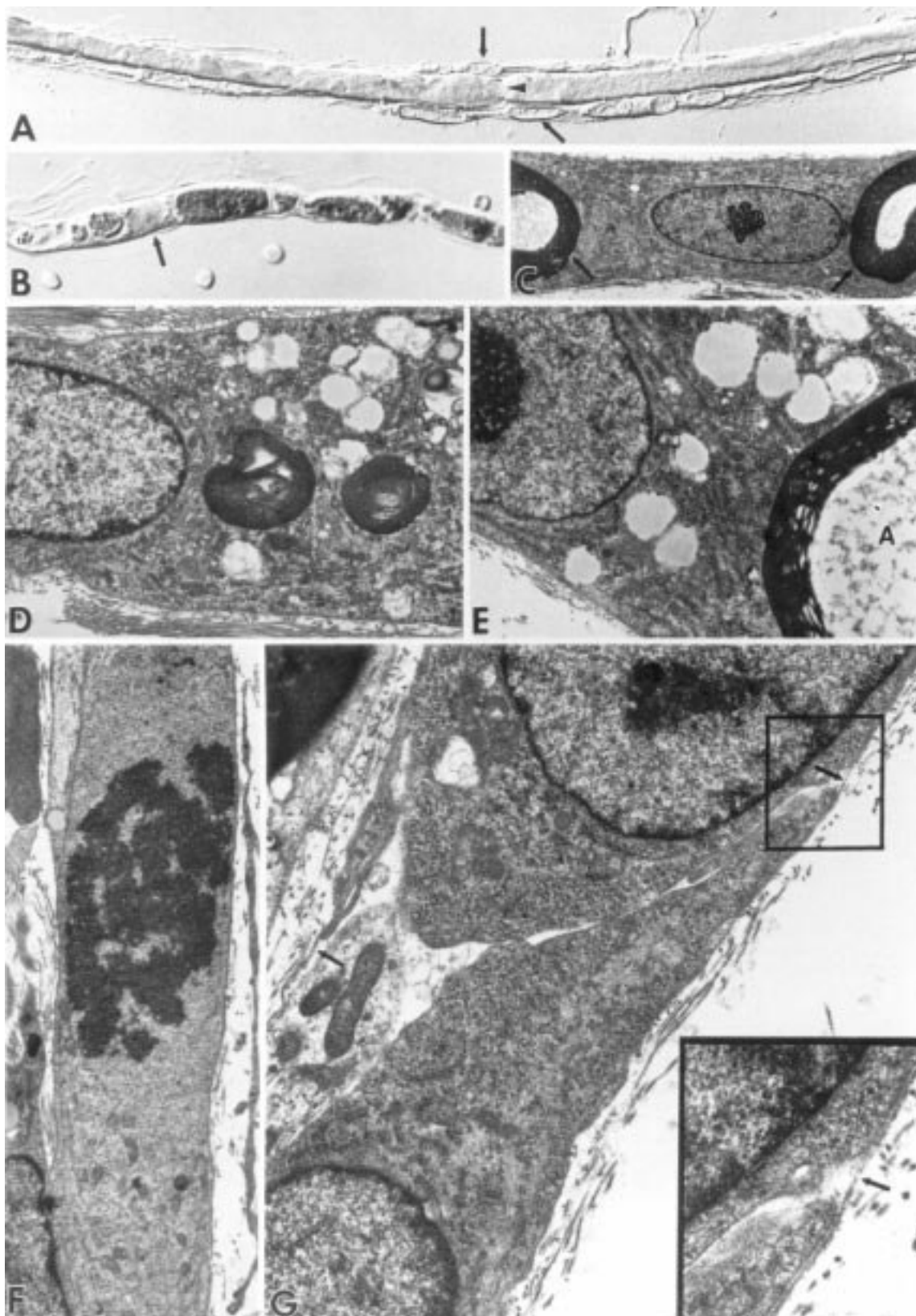
### Cellular responses in the distal stump

As a prompt response to degenerating axons, Schwann cells within two days sequester small whorls of myelin debris and fragment their own myelin sheaths into ovoids (63, 102). Schwann cells phagocytose myelin debris to some extent and form lipid droplets before macrophages enter degenerating nerves (Fig. 1).

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After the initial extrusion of myelin sheaths, Schwann cells divide with a maximum at day 3 (Fig. 1) (18, 102) and line up within the basal lamina tube to form bands of Büngner, that later provide guidance cues for regenerating nerve fibres. Beginning on day 2 and with a maximum between days 4 to 7, hematogenous macrophages enter the distal stump and migrate to the ovoids (Fig. 2). Within two weeks macrophages completely clear myelin debris (13, 63, 102). The molecular mechanisms involved in macrophage-mediated myelin clearance have recently been reviewed (13). Briefly, at least two surface molecules are involved in myelin recognition and uptake. Complement receptor type 3 (CR3) is constitutively expressed on macrophages. The corresponding complement component C3 is detectable at the surface of degenerating myelin sheaths by immunoelectronmicroscopy, indicating that myelin is opsonized by complement during WD (14). In support of a functional role of CR3, *in vivo*-application of the antibody Mac-1 against rat CR3 after nerve transection caused a significant reduction of myelin phagocytosis (15). The galactose-specific lectin MAC-2 is induced on both phagocytosing macrophages and Schwann cells during WD (86). Functionally, application of galactose and lactose which bind to MAC-2 also block myelin clearance during WD. Moreover, very slow progression of Wallerian degeneration in C57Bl/Ola mice (64) is associated with reduced upregulation of MAC-2 (86). MAC-2 expression on Schwann cells and macrophages can be induced by granulocyte macrophage colony stimulating factor (GM-CSF) (89). Fibroblasts but not Schwann cell and macrophages produce GM-CSF after nerve injury indicating a significant role of fibroblasts in the cascade of molecular events during WD.

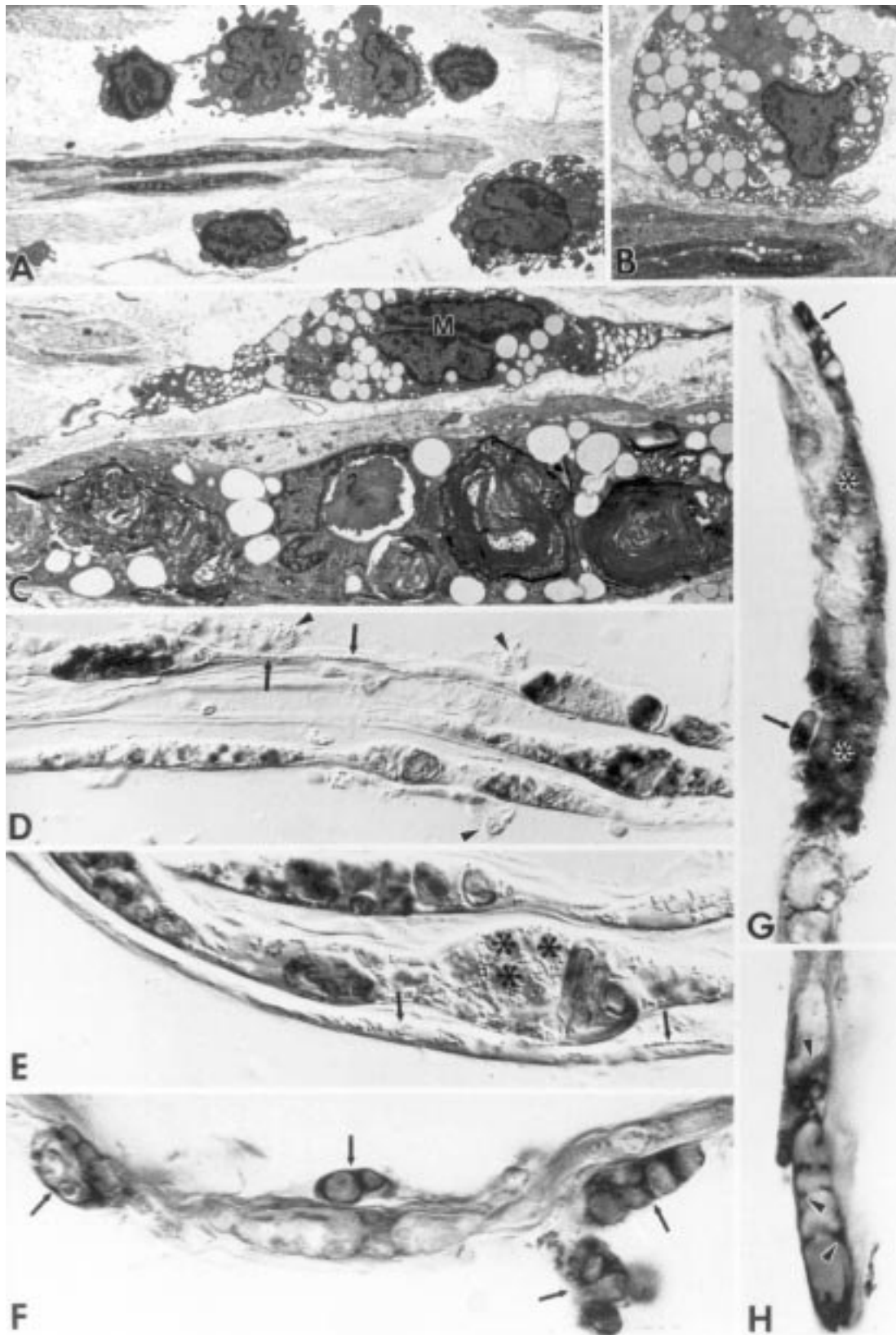
There have been longstanding controversies to what extent Schwann cells versus macrophages and to what extent resident endoneurial (42) versus hematogenous macrophages are involved in myelin clearance (reviewed in 13). Brück and colleagues (16) depleted

hematogenous macrophages by application of dichloromethylene diphosphonate containing liposomes. After nerve transection, the number of phagocytes within the degenerating nerves was significantly reduced in the macrophage depleted rats, but macrophage depletion did not completely abolish myelin degradation. This study further substantiated the predominant role of hematogenous macrophages in myelin clearance and, on the other hand, supported the notion that resident cells in the peripheral nervous system participate in myelin removal and can partly replace hematogenous macrophages. Accordingly, Schwann cells in macrophage depleted animals contained more myelin debris than in sham treated controls after axotomy (16), and whole body irradiation moderately decreased myelin clearance after axotomy (81). Moreover, *in vitro* Schwann cells are capable to carry out degradation of short myelin segments without the assistance of macrophages (28). In summary, there is overwhelming evidence that under normal conditions hematogenous macrophages remove the vast majority of myelin debris.

The mechanism of recruitment of hematogenous macrophages in WD is still unclear. There is conflicting evidence on the putative role of cellular adhesion molecules. Tissue inflammation involves multiple cell adhesion steps between invading leukocytes and endothelial cells (reviewed in 100). Macrophages bear the CD11a/CD18 (LFA-1), the very late antigen-4 (VLA-4) and the CD11b/CD18 (Mac-1; complement type 3 receptor) complex on their surface. The corresponding endothelial counterreceptors are intercellular adhesion molecule-1 (ICAM-1) for LFA-1/Mac-1 and vascular cellular adhesion molecule-1 (VCAM-1) for VLA-4. Usually, ICAM-1 and VCAM-1 are expressed at low levels on endothelial cells, but are strongly upregulated in inflammatory diseases by cytokines. While one study described ICAM-1 and VCAM-1 expression during WD in mice (19), we did not find upregulation of ICAM-1 at

**Figure 1.** (Left) Teased fibres and electron micrographs illustrating early stages of Wallerian degeneration. **(A)** In this bundle of three teased fibres 24h after transection, the small fibres (arrows) have already undergone segmentation of myelin into early ovoids. The large myelinated fibre with its node (arrowhead) appears normal at this time. x 720. **(B)** By 72 h the Schwann cell perikaryon (arrow) occupies the whole cross-sectional area of the nerve fibre and myelin fragmentation into early ovoids is well underway. x 790. **(C)** Electron micrograph of a longitudinal section illustrating the Schwann cell perikaryon 48h after nerve transection; note the myelin sheath remnants at either side of the perikaryon (arrows). x 2930. **(D)** At 3 days, small whorls of myelin debris are sequestered within the Schwann cell cytoplasm and lipid droplets can be seen. x 6170. **(E)** Note numerous lipid droplets adjacent to the Schwann cell nucleus, and clearly within Schwann cell cytoplasm. Note also that residual myelin remains but the axoplasm (A) has been replaced by granular and amorphous debris. x 8250. **(F)** An example of frequent mitotic figures found in Schwann cells on day 3. x 6350. **(G)** Post-mitotic Schwann cells (day 3) separate longitudinally, leaving interdigitating processes. Note that both Schwann cells are covered by the original basal lamina (arrows), while apposing Schwann cell surfaces are not covered by basal lamina. x 13 500. Inset shows boxed region at higher power. x 27 800. Reprinted from Stoll et al. (1989) J Neurocytol 18:671-683 with permission.





the maximum of macrophage infiltration at day 4 in the rat (103). As shown by Brown *et al.* (10) application of antibodies against ICAM-1 had no influence on macrophage entry into the distal nerve segment. On the other hand, Vougioukas *et al.* (113) described significantly lower numbers of macrophages in transgenic ICAM-1 deficient mice after axotomy. Based on these conflicting data, the role of the LFA-1/Mac-1 complex and endothelial ICAM-1 expression in macrophage recruitment in WD awaits further clarification. The VLA-4 / VCAM-1 pathway appears not to be involved in cellular infiltration after nerve injury. We did not see VCAM-1 mRNA expression in the distal stump of degenerating nerves (50) and application of anti-VCAM-1 antibodies had no influence on macrophage infiltration (10). Interestingly, T cell inflammation is lacking in WD (33). In contrast to other nonimmune lesion paradigms of the nervous system such as cerebral ischemia (94), neutrophils are only transiently present during the first few hours after peripheral nerve injury.

### Molecular responses in the distal stump

Transection or crush of a peripheral nerve sets in motion a dramatic change in the molecular composition of the distal nerve segments (25, 34, 36). Thereby a microenvironment develops that supports axonal regeneration in the PNS and, moreover, allows elongation of usually nonregenerating transected CNS fibre tracts into grafted PNS nerve segments (2). Upon loss of axonal contact myelinating Schwann cells downregulate steady state mRNA levels of the myelin components myelin basic protein (MBP), myelin associated glycoprotein (MAG), protein zero (P<sub>0</sub>), peripheral myelin protein-22 (PMP22) and periaxin within two days after injury (61, 92, 98). Formerly myelinating Schwann cells dedifferentiate and acquire the phenotype of pre/nonmyelinating Schwann cells by expression of p75 low affinity nerve growth factor receptors (NGF-R), glial fibrillary acidic protein (GFAP), glial maturation factor- $\beta$ , the cell adhe-

sion molecule L1 and neural cell adhesion molecule (NCAM) (8, 52, 68). Transcription factors Pax3, SCIP, c-jun and Krox-20 are involved in the regulation of Schwann cell de- and redifferentiation (54, 123). Denervated Schwann cells strongly reexpress Pax3 and c-jun, but downregulate Krox-20. Pax3, a paired-domain transcription factor, is expressed in embryonic Schwann cells, persists in non-myelinating Schwann cells in the adult, but is downregulated in myelin-forming Schwann cells (53). Functionally, Pax3 represses transcription of myelin genes (53). SCIP is a POU-domain transcription factor. SCIP is expressed in early myelinating Schwann cells for a short period and is downregulated in Schwann cells that maintain a myelin sheath (72, 91, 123). *in vitro*, SCIP acts as a repressor of the genes encoding P<sub>0</sub> and MBP (72). Transgenic mice lacking SCIP show a delay in Schwann cell differentiation, but myelinate normally (49). Krox-20 is an immediate early gene that belongs to a class of transcription factors with zinc-finger motifs. Krox-20 expression in the adult peripheral nerve is restricted to myelin forming Schwann cells (123). C-jun is expressed only by non-myelinating Schwann cells in normal nerve, but reexpressed by denervated formerly myelinating Schwann cells after axotomy (97). When axons regenerate into the distal stump, the expression of c-jun declines as Schwann cells remyelinate axons. Functionally, C-jun seems not to directly affect myelin-specific gene expression in Schwann cells (97).

As a response to axonal loss Schwann cells divide during WD. Glial growth factors (GGF) are potent Schwann cell mitogens *in vitro* and have been implicated in Schwann cell proliferation *in vivo*. Induction of mRNAs encoding the GGF subfamily of neuregulins occurs in nerves beginning 3 days postaxotomy and thus coincides with the onset of Schwann cell DNA synthesis and proliferation (18). GGF expression is accompanied by upregulation of GGF-receptors, the erbB membrane tyrosine kinases 2 and 3. Further studies showed

**Figure 2.** (Left) Electron micrographs and teased fibres illustrating macrophages in nerves during mid-stages of Wallerian degeneration. (A) A collection of mononuclear cells within the endoneurial space 4 days after transection. x 2520. (B) Foamy macrophage near a blood vessel 14 days after transection. x 3990. (C), Electron micrograph of longitudinal section of a nerve illustrating an ovoid with an overlying lipid-filled macrophage (M) outside the nerve fibre (14 days). x 4000. (D,E) Teased nerve fibres (14 days) visualized with Nomarski optics. (D) Note the segregation of myelin debris into discrete ovoids connected by attenuated Schwann cell bands containing lipid droplets (arrows). Note also that cells containing lipid droplets are present outside the nerve fibres (arrowheads); as demonstrated below, these cells are macrophages. x 610. (E) In the region of the myelin masses there are numerous nuclei (asterisks). Note also the widely distributed lipid droplets, including lipid droplets within attenuated Schwann cells bands (arrows). x 1100. (F) Teased fibre (14 days) stained with anti-ED1 antibody, an immunocytochemical marker for macrophages. Note that around these ovoids there are multiple ED1-positive macrophages (arrows). x 1370. (G) In this 14-day teased fibre, ED1-positive macrophages are present outside (arrows) and inside the fibre (Asterisks). x 1050. (H) In these ovoids, ED1 immunoreactivity surrounds the myelin masses within the nerve fibre (arrowheads). x 940. Reprinted from Stoll et al. (1989) J Neurocytol 18:671-683 with permission.

that phosphorylation of erbB coincides with Schwann cells proliferation (59). Although Schwann cell proliferation rapidly ceases GGF expression persists into late stages of WD.

Nerve injury leads to upregulation of neurotrophic factors and their receptors in the degenerating nerve segment (6, 31, 52). The functional role of neurotrophins in the peripheral nervous system will be described in detail in an accompanying chapter in this issue. Briefly, within hours after axonal damage bioactivity as well as mRNA levels of nerve growth factor (NGF) increase dramatically and show a second peak of expression 2-3 days after injury (31, 46). Moreover, there is a continuous slow increase of brain derived neurotrophic factor (BDNF) mRNA starting after day 3 post-lesion and reaching maximal levels 3-4 weeks later (71). In contrast to the above mentioned neurotrophins, expression of ciliary neurotrophic factor (CNTF) requires intact axon-Schwann cell interactions and is reduced both on the mRNA and protein level in WD (29, 96). Schwann cells produce NGF and BDNF after nerve injury. Changes in neurotrophin expression are accompanied by the upregulation of the corresponding receptors (31). Insulin-like growth factor-I (IGF-I) is another neurotrophic factor produced by Schwann cells in the early stages of WD. Schwann cells also express the corresponding receptor. Interestingly, after postlesion day 7 infiltrating macrophages become the predominant source of IGF-I in the distal stump (22) suggesting that inflammatory cells also provide neurotrophic support.

In WD transcripts and protein levels for pro- and antiinflammatory cytokines are strongly upregulated (7, 9, 24, 35, 85, 88). Cytokine induction occurs in the absence of T cell inflammation. Cytokines play an important role in the pathogenesis of T cell-mediated autoimmune diseases of the nervous system where they orchestrate immune responses (reviewed in 3, 44). The fundamental role of cytokines in neural development (reviewed in 70) and nerve repair after injury have only recently widely been appreciated (35, 62, 108). Within 24 hours after nerve crush IL1 $\beta$ -mRNA levels are increased and remain at high levels throughout the first week (35). IL1 protein could be extracted from distal nerve segments of transected peripheral nerves (88). It has been shown that IL1 induces synthesis of NGF in Schwann cells (62). In support of an important role of IL1 in nerve regeneration, application of interleukin-1 receptor antagonist, which binds to and antagonizes IL1, impedes peripheral nerve regeneration (43). Simultaneously with the induction of IL1, increased levels of IL6- and IL10-mRNA and protein are detectable

within 1 day after nerve crush (7, 9, 35, 85). Schwann cells express IL10-mRNA (51). IL6 protein has been localized in Schwann cells, fibroblasts and macrophages (7, 85). With a delay of few days significant mRNA induction for the proinflammatory cytokines IFN $\gamma$  and IL12 has been found after nerve crush. IL12-mRNA expression peaks between days 7 and 14 (35), when myelin phagocytosis by macrophages is at maximum (102). The functional roles of IFN $\gamma$  and IL12 in nerve degeneration and regeneration are unknown at present. Schwann cells express TNF-immunoreactivity in normal and injured sciatic nerves (115). In addition, after nerve injury infiltrating macrophages express strong TNF immunoreactivity (104). TNF $\alpha$  has been implicated in neuropathic pain during WD (114; see below). In normal adult sciatic nerve TGF-beta 1, -beta 2, and beta-3 are expressed in the cytoplasm of Schwann cells (90). Axotomy leads to an increase in TGF-beta 1 mRNA levels, while TGF-beta 3 mRNA falls. Although the roles of cytokines in nerve regeneration have to be further elucidated, these studies show that a significant cytokine induction can occur in the peripheral nervous system despite the absence of a T cell response.

Leukemia inhibitory factor (LIF) is another important cytokine that shows an increased expression after axotomy (24, 58). The cellular source of LIF in WD is unknown. Embryonic Schwann cells express high levels of LIF mRNA in culture (58). When applied to the site of nerve transection LIF enhances the survival of sensory and motor neurons in neonatal rats (20, 21). LIF is retrogradely transported in sensory, motor and sympathetic neurons in adult animals (24, 45). Tham and colleagues (108) cut sciatic nerves of adult rats and bridged proximal and distal nerve stumps by a silicone cuff containing LIF. At 12 weeks after transection LIF treatment significantly increased the conduction velocity of the newly regenerated nerve, the diameter and number of regenerated myelinated axons, the force of contraction of the reinnervated muscle, and the muscle mass.

In Wallerian degeneration myelin-derived lipids are reutilized for regeneration and remyelination. Apolipoproteins D and E (ApoD, ApoE) are lipid binding proteins which accumulate in the distal stump after axotomy (74, 99). ApoE is produced by infiltrating macrophages (105) while ApoD is simultaneously expressed by endoneurial fibroblasts (99). Functional studies showed that lipoproteins are taken up by neuritic growth cones and Schwann cells (48, 87). However, nerve regeneration and cholesterol reutilization may also occur in the absence of apolipoproteins E and A-I as shown in transgenic mice (40).

### **Wallerian degeneration and hyperalgesia**

Axotomy of sciatic nerve not only leads to muscle weakness and loss of sensation but also to adaptive responses with ensuing neuropathic pain. Chronic loose constriction of the sciatic nerve produces mechanoallodynia and thermal hyperalgesia in rats and mice during the time in which the nerve distal to the ligature site undergoes WD (79, 84). C57Bl/WLD mice, showing delayed Wallerian degeneration after axotomy, concomitantly exhibit reduced hyperalgesia temporally associated with reduced numbers of phagocytic cells in injured nerve. The pathomechanism of lesion-induced hyperalgesia still needs to be elucidated, although there is evidence that the cytokine TNF $\alpha$  and sprouting of sympathetic fibres are involved. As described above, infiltrating macrophages in Wallerian degeneration express TNF $\alpha$  immunoreactivity (104) and intraneural injection of TNF $\alpha$  into nerves induces neuropathic pain (114). Sympathetic axons invade the DRG following a peripheral nerve lesion and form baskets around large DRG neurons (84). This sympathetic sprouting is markedly delayed in C57Bl/WLD mice. Moreover, nerve injury produces a long-lasting rearrangement in the organisation of primary afferent central terminals (120). Usually low-threshold mechanoreceptors terminate in laminae III and IV and unmyelinated C fibres, most of which are nociceptors, terminate predominantly in lamina II in the dorsal horn of the spinal cord. After peripheral nerve injury the central terminals of axotomized myelinated afferents, including large A beta fibres, sprout into lamina II that normally receives only C-fibre input with the consequence of hyperalgesia. Interestingly, even intact myelinated primary afferents have the capacity for collateral sprouting (26). Two weeks after cutting the posterior cutaneous nerve and leaving the adjacent saphenous nerve intact, fibres from the saphenous nerve sprouted into an area of lamina II that is normally innervated exclusively by the adjacent posterior cutaneous nerve. This sprouting process could explain the observed sensory hypersensitivity at the edges of denervated skin. Taken together these studies provided strong evidence that a peripheral nerve lesion may induce structural reorganisation in the adult central nervous system.

### **Responses of dorsal root ganglia and motor neurons to peripheral nerve injury**

The early response of the perikaryon to axonal injury includes chromatolysis (41) and upregulation of the transcription factor Jun which persists until regeneration of the peripheral nerve is completed (60). Growth-asso-

ciated protein (GAP)-43/B50 and the intermediate filament protein peripherin were upregulated within the first day after an axonal lesion and have been implicated in axon elongation (111, 119). The three neurofilament genes NF-L, NF-M, and NF-H are downregulated (76, 119) while class II and III  $\beta$ -tubulin mRNAs and proteins increase after axotomy (47,73).

In sensory neurons axonal injury leads to additional changes in neuropeptide and cytokine expression. IL6 mRNA and protein appear within 1 day in large and medium-sized ganglia after sciatic nerve transection with a maximal expression after 2 and 4 days and a decrease below threshold of detection within 1 week (78). Moreover, IL1 $\beta$  and TNF $\alpha$  mRNAs are upregulated, but the cellular sources have not yet been defined. There is evidence that the cytokine LIF is involved in triggering part of the cell body reaction in sensory neurons (23, 107). Following axonal damage the neuropeptide galanin which plays a tonic inhibitory role in the mediation of spinal cord excitability (118) is upregulated. In transgenic mice in which the gene for the cytokine LIF had been knocked out, galanin expression in DRG after sciatic nerve lesion was reduced (23, 107). Furthermore, axonal damage induces a marked upregulation of nitric oxide synthase in primary sensory neurons (112, 122).

Axotomized motoneurons respond by increased NGF-R mRNA and protein (55), as well as trkB mRNA expression, which is the receptor for BDNF and NT-4 (82). Increase is maximal at day 3 and returns to normal within three weeks. Additional changes include modification of glutamate receptor expression (83) and a transient increase in calcitonin gene-related peptide immunoreactivity (5).

### **Regeneration of nerve fibres**

After axonal injury peripheral nerve fibres regenerate from the proximal stump. This process can be examined electrophysiologically. By using implanted electrodes in the cat, Fugleholm and colleagues (30) could follow the speed of axonal regrowth and found that the rate of elongation was 3-4mm/day after crushing a nerve, but only 2.5mm/d after sectioning. Regenerating axons preferentially grew inside internal structures of endoneurial Schwann cell tubes. Depletion of Schwann cells did not influence axonal elongation when the basal lamina remained in continuity suggesting a critical role of extracellular matrix proteins in axon regeneration (30).

In the distal stump a number of neurite outgrowth promoting molecules are upregulated (reviewed in 67).



From postlesional day 4 onwards increasing numbers of previously myelin forming Schwann cells expressed the cell surface molecules L1 and N-CAM (68). *In vitro*, L1 and to a lesser degree N-CAM are involved in Schwann cell-mediated neurite extension (95). Ninjurin (nerve injury-induced protein) is another homophilic adhesion molecule that is upregulated after axotomy (4). Ninjurin is located on the cellular surfaces of axons and Schwann cells and promotes neurite extension of dorsal root ganglion neurons *in vitro*. Among the extracellular matrix components, laminin is one of the most effective promoters of neurite extension *in vitro*. Surprisingly, laminin B chain mRNA steady state levels are decreased in the distal stump and gradually increase to baseline levels as regenerating nerves arrive at distal segments and reestablish normal axon-Schwann cell contact (27). At axon-Schwann cell contacts, however, laminin accumulates (57). To assess the functional role of laminin in nerve regeneration, Agius and Cochard (1) used a tissue section culture in which embryonic chick sensory neurons were grown on a denervated peripheral nerve substrate. Anti-laminin-2 (merosin) antibodies drastically reduced the percentage of growing neurons and the total length of neurites on the denervated nerve sections. Similarly, application of anti-laminin antibodies impeded axonal regeneration in Schwann cell depleted nerve implants *in vivo* (117).

In injured nerves axonal regeneration leads to Schwann cell redifferentiation which occurs in a proximal-to-distal direction relative to the site of injury. When Schwann cells are recontacted by regrowing axons, myelin-gene repressing transcription factor Pax3 and c-jun are downregulated, while SCIP is transiently upregulated indicating a premyelinating Schwann cell stage (54, 123). Downregulation of SCIP is followed by reinduction of myelin specific genes (61) and persistent expression the transcription factor Krox-20 (123). Krox-20 is critically involved in the completion of myelination (109). During early stages of myelination Schwann cell mesaxon membranes containing MAG are converted to compact myelin lamellae. This is accompanied by removal of MAG from and insertion of MBP, PMP22 and P<sub>0</sub> into mesaxon membranes (110). Transgenic Krox-20 *-/-* mice are able to produce MAG, but lack MBP and P<sub>0</sub> suggesting a functional role of Krox-20 in the induction of late myelin components (109). Upon remyelination of regenerated axons, Schwann cells loose dedifferentiation markers L1, N-CAM, GFAP and p75 NGF-R characteristic for a nonmyelinating stage (67, 91). The regeneration-induced molecular Schwann cell program leads to rapid remyelination of nerve

sprouts and exactly recapitulates myelination during nerve development with respect to the sequence and timing of gene-induction.

Upon appropriate length of axonal regrowth target organs are finally reached and muscle and skin are reinnervated. This occurs with an extraordinary high specificity even from injured mixed sensory-motor nerves. Brushart (17) showed that motor axons preferentially reinnervated motor pathways. Subsequent studies revealed that guidance molecules of the L2/HNK-1 family were involved in this process (69). The L2/HNK-1 carbohydrate epitope was originally described as a cell surface component of human natural killer cells and is common in a large family of recognition molecules (reviewed in 67). L2/HNK-1 is detectable in association with Schwann cells in ventral spinal roots and motor axon-related Schwann cells of muscle nerves, but not in dorsal roots and sensory cutaneous nerves (69). After nerve transection myelinating Schwann cells previously associated with motor neurons differ from those Schwann cells that had myelinated sensory axons by their ability to express L2 when contacted by motor axons. L2/HNK-1 expression during critical stages of reinnervation provides an advantage for motor axons regenerating into the appropriate muscle pathways over those regenerating into the inappropriate sensory pathways (69). In regenerating femoral nerve of rat, motor axons seem to explore possible pathways by sending out collateral branches into both appropriate and inappropriate nerve branches. Pathway specificity is subsequently gained by pruning off those collaterals which have grown into the inappropriate nerve branch (17). Recently, anatomical evidence for specificity was also provided during regeneration of sensory afferent projections to muscle (66). The accuracy of sensory afferent regeneration was highly correlated with the accuracy of motor regeneration suggesting that two distinct neuronal populations that project to muscle respond in parallel to specific guidance factors during the regeneration process. The accuracy of pathway finding of sensory fibres to skin and the signal molecules involved have not yet been addressed directly. Zelena and Zacharova (121), however, could show that pacinian corpuscles are reinnervated after sciatic nerve crush.

Successful regeneration seems to depend tightly on the appropriate timing of the cellular and molecular degeneration program. In C57Bl/Ola mice Wallerian degeneration following a nerve lesion is very slow due to an axonal defect (37, 64). Concomitantly, recruitment of macrophages is delayed and the dramatic increase in mRNA levels for both NGF and low affinity NGF recep-

tor (p75) as observed in wild type mice is lacking. These changes lead to impairment of sensory axon regeneration (11, 12). In contrast, motor nerves regenerate at normal velocity even through uncleared myelin debris (11, 12, 75). At present it is unclear whether the rate of nerve regeneration is altered in neuropathies. In a chronic inflammatory neuropathy model in rabbits, antibodies against the myelin component galactocerebroside had no influence on the speed and efficiency of axonal regeneration and remyelination (106).

There have been several attempts to increase the rate of regeneration pharmacologically. Gold and colleagues (39) showed that subcutaneous application of the immunosuppressant FK506 increases regeneration of sensory fibres in the rat by 16% after nerve crush. *In vitro* FK506 promotes neurite outgrowth in PC12 cells and sensory ganglia (101). FK506 is inactive by itself and requires binding to an FK506-binding protein-12 (FKBP-12), a member of the immunophilin family (38). Following sciatic nerve crush FKBP-12 mRNA strongly increases in lumbar motor neurons and dorsal root ganglia neurons (65). The FK506/FKBP-12 complex inhibits the activity of the calcium activated phosphatase, calcineurin, which itself activates GAP-43 and nitric oxide synthase. It turned out, however, that the neurotrophic effects of FK506 are independent of calcineurin inactivation and not related to its immunosuppressive properties (38, 101). Prosaposin and the above described cytokine LIF are further compounds that significantly increase the number of regenerating nerve fibres *in vivo* (56, 108). Prosaposin is the precursor of saposins which activate sphingolipid hydrolases. Functionally, prosaposin stimulates neuritogenesis in neuroblastoma cells (80). It is significantly upregulated after peripheral nerve injury (34). When added to collagen-filled nerve guides after nerve transection prosaposin dramatically increased the number of regenerating nerve fibres within the guide (56). The potential role of exogenous application of neurotrophins in facilitating nerve regeneration is reviewed in an accompanying chapter in this volume. Recent evidence suggests that neurotrophins may reverse axotomy-induced changes in adult motor and sensory neurons (77). The physiologically lower nerve conduction velocity in the proximal part of transected nerve fibres could be increased by local application of neurotrophins 3 and 4/5.

### Limitations and Perspectives

During the past decade enormous progress has been made in the understanding of the cellular events and molecular changes during degeneration and regenera-

tion of peripheral nerves. However, our knowledge of the regulatory mechanisms and signaling cascades underlying the complex molecular regeneration program is still very limited. This is not surprising. Systematic differential hybridization screening approaches using cDNA libraries of normal and regenerating rat sciatic nerve revealed that the majority of the cloned sequences encode novel genes or known genes that were not previously shown to be expressed in the nervous system (25, 34). Thus, the majority of the molecular players involved in nerve regeneration await identification. Moreover, with respect to neuropathies it is unclear whether peripheral nerve disorders can interfere with this regeneration program and impede regenerative responses. This is of particular interest in chronic disease stages such as diabetic, chronic inflammatory and hereditary neuropathies with axonal loss. Isolation and characterization of further regulatory genes that trigger and control the genetic program is probably the most critical step towards a better understanding of the physiology and pathophysiology of nerve regeneration.

### Acknowledgements

Work from the authors' laboratories was supported by the Deutsche Forschungsgemeinschaft and the Gemeinnützige Hertie-Stiftung. G.S. holds a Hermann- and Lilly-Schilling professorship.

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Article in *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* · September 2002

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# 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

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Injury to peripheral nerves results in the infiltration of immune cells, which remove axonal- and myelin-derived material. Schwann cells could play a key role in this process by regulating macrophage infiltration. We show here that medium conditioned by primary denervated Schwann cells or the Schwannoma cell line RN22 produces chemotactic activity for macrophages. The presence of blocking antibodies to macrophage chemoattractant protein-1 (MCP-1) or leukemia inhibitory factor (LIF) reduced this activity to ~35 and 65% of control levels, respectively, and only 15% remained in the presence of both antibodies. The presence of chemotactic LIF in Schwann cell-conditioned medium was confirmed by using cells from *lilf*<sup>−/−</sup> mice. Although interleukin-6 (IL-6) is not itself a chemotactic factor, we found that medium from

*il-6*<sup>−/−</sup> nerves showed only 40% of the activity secreted by wild-type nerves. Furthermore, IL-6 rapidly induced LIF mRNA in primary Schwann cells, and LIF rapidly induced MCP-1 mRNA expression. Treatment of RN22 Schwannoma cells with IL-6 or LIF enhanced the secretion of the chemotactic activity of these cells.

These observations show that Schwann cells attract macrophages by secreting MCP-1 and LIF. They also provide evidence for an autocrine-signaling cascade involving IL-6, LIF, and MCP-1, which amplifies the Schwann cell-derived chemotactic signals gradually, in agreement with the delayed entry of macrophages to injured nerves.

**Key words:** chemotaxis; macrophage; leukemia inhibitory factor; interleukin 6; regeneration; neuropathy

Injury to peripheral nerve initiates a complex cascade of signals involving neurons, glia, and cells of the immune system that leads to Wallerian degeneration (for review, see Scherer and Salzer, 2001). An important component of this process is the invasion of macrophages. For many years, there have been uncertainties regarding the role of Schwann cells in regulating this macrophage recruitment (Beuche and Friede, 1984; Scheidt and Friede, 1987; Stoll et al., 1989). Two features of the myelomonocytic response in damaged peripheral nerves distinguish it from that seen in non-neuronal tissues (Perry and Brown, 1992): (1) after cut or crush injury, only small numbers of neutrophils are found in the distal segment, and (2) there is a delay of 2 or 3 d before a major influx of macrophages (Ramon y Cajal, 1928; Beuche and Friede, 1984; Crang and Blakemore, 1986; Perry et al., 1987; Stoll et al., 1989). This delay is consistent with the idea that the signals that most effectively attract macrophages are generated by a relatively slow-moving signaling cascade. This cascade is probably triggered by factors arising in proliferating dedifferentiating Schwann cells, as highlighted by observations on C57BL/Ola mice. Nerve injury in these mice does not induce acute Schwann cell dedifferentia-

tion and myelin breakdown, and macrophage invasion is sparse and slow (Lunn et al., 1989; Perry et al., 1990; Glass et al., 1993; Mack et al., 2001). This is attributable to protection offered by a chimeric gene containing an N-terminal fragment of ubiquitination factor E4B fused to nicotinamide mononucleotide adenylyl-transferase (Mack et al., 2001).

Cytokines induced in Schwann cells after peripheral nerve injury could play a key role in the interactions between Schwann cells and macrophages. The neurotrophic cytokines leukemia inhibitory factor (LIF) and interleukin-6 (IL-6) are both involved in the neuronal and immune responses to injury (Patterson, 1994; Gadiant and Patterson, 1999). Schwann cells in transected nerves upregulate expression of LIF and IL-6 (Banner and Patterson, 1994; Curtis et al., 1994; Bolin et al., 1995; Bourde et al., 1996; Kurek et al., 1996). Moreover, LIF (but not IL-6) induces chemotaxis of peritoneal macrophages, and macrophage infiltration into injured sciatic nerve is delayed in LIF knock-out mice (Sugiura et al., 2000). Another potential Schwann cell-derived macrophage attractant, monocyte chemoattractant protein-1 (MCP-1), attracts macrophages in other systems and is induced in Schwann cells by nerve transection with a time course that lags behind that of LIF and IL-6 (Murphy, 1994; Baggiolini, 1998; Toews et al., 1998; Siebert et al., 2000). Schwann cells also produce a number of other cytokines, including IL-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and IL-8 (Bergsteinsdottir et al., 1991; Wagner and Myers, 1996; Rutkowski et al., 1999).

Thus, although Schwann cells deprived of axonal contact in transected nerves express several signals that might act on macrophages, it has not been shown directly that these Schwann cells produce macrophage chemotactic activity. Moreover, although there are many studies on cytokine induction after peripheral

Received Dec. 26, 2001; revised May 6, 2002; accepted May 24, 2002.

This work was supported by awards to G.K.T. from the Wolfson Foundation, the Howard Hughes Medical Institute through a California Institute of Technology (Caltech) Summer Undergraduate Research Fellowship, SmithKline Beecham Pharmaceuticals, and Trinity College (Cambridge, UK). R.M. and K.R.J. are supported by the Wellcome Trust. The work at Caltech was supported by a grant to P.H.P. from the National Institute of Neurological Disorders and Stroke. We thank Soheila Sharghi Namini and Sarah Dickinson for technical assistance with conditioned medium preparation and Doreen McDowell and Debbie Bartram for administrative assistance.

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nerve injury, little is known about the mechanisms that regulate their expression. A recent paper pinpoints TNF- $\alpha$  as an inducer of MCP-1 in Schwann cells after nerve injury, particularly at the relatively late time of 4 d (Subang and Richardson, 2001). Here, we have examined signals that might contribute to Schwann cell-derived chemotactic activity at earlier time points and studied how their expression is regulated. We have directly measured macrophage chemotactic activity generated by normal Schwann cells, RN22 Schwannoma cells, and cut nerves taken from LIF and IL-6 knock-out animals. We find that LIF and MCP-1 are important components of the secreted signals that attract macrophages. We also provide evidence for an autocrine-signaling cascade involving IL-6, LIF, and MCP-1 in Schwann cells that could result in a gradual amplification of the macrophage attractant activity secreted by these cells.

## MATERIALS AND METHODS

**Defined medium.** For all conditioned media, we used a supplemented defined medium identical to that used in previous studies (Jessen et al., 1994). It consists of a 1:1 mixture of DMEM and Ham's F-12 supplemented with insulin (5  $\mu$ g/ml), transferrin (100  $\mu$ g/ml), glutamine (1 mM), progesterone (60 ng/ml), putrescine (16  $\mu$ g/ml), selenium (160 ng/ml), T4 (400 ng/ml), T3 (10.1 ng/ml), bovine serum albumin (BSA) (0.035%), penicillin (100 IU/ml), and streptomycin (100 IU/ml). For all conditioned media, 20  $\mu$ M leupeptin was added to the defined medium to prevent proteolysis. Sources of the reagents used have been detailed in previous papers (Jessen et al., 1994; Meier et al., 1999).

**Schwann cell cultures and conditioned medium preparation.** Sciatic nerves from 4-d-old rats were dissociated, and Schwann cells were purified by immunopanning to remove contaminating cells, essentially as described previously (Jessen et al., 1990, 1994; Lee et al., 1997). Schwann cells were resuspended in defined medium, counted, and plated onto laminin-coated coverslips for immunostaining with anti-S100 antibodies as described previously (Meier et al., 1999). This confirmed that Schwann cells are 99.5  $\pm$  0.5% pure after immunopanning. For conditioned medium preparation, Schwann cells were plated onto 35-mm-diameter poly-L-lysine and laminin-coated tissue culture plastic dishes (4.5–7  $\times$  10<sup>5</sup> cells total). After 24 hr of incubation, the conditioned medium was collected, centrifuged for 10 min at 1000 rpm, and stored in BSA (3 mg/ml)-coated cryotubes at –80°C until further use. For LIF- or IL-6-treated primary Schwann cells, recombinant mouse LIF or recombinant rat IL-6 (R & D Systems, Oxon, UK) was added to the cultures at two different concentrations, 2 and 20 ng/ml, always accompanied by a control with no treatment. Cells were incubated under standard conditions (37°C, 5% CO<sub>2</sub>) for 1 and 3 hr (LIF) and for 1, 3, 6, 10.5, and 24 hr (IL-6).

**RN22 Schwannoma cell-conditioned medium.** Cells were grown in Roswell Park Memorial Institute (RPMI)-1640 (Sigma-Aldrich, Poole, UK) containing 10% fetal calf serum (Sigma-Aldrich) until they reached 70% confluence. Subsequently, they were changed to defined medium and left for 24 hr to adapt to the new conditions. Fresh medium was then added, and the cell line was treated with 20 ng/ml LIF or IL-6. After a 3 hr incubation in 37°C/5% CO<sub>2</sub>, the cells were washed three times, and fresh defined medium was added. After an additional 24 hr incubation, the conditioned medium was removed, centrifuged for 10 min at 1000 rpm, and stored at –80°C until further use.

**Mouse sciatic nerve conditioned medium.** The strain of LIF knock-out (*lif*<sup>–/–</sup>) mice used in this work was that of Stewart et al. (1992), which has been intermittently back-crossed with C57BL/6 to maintain fertility and viability. The IL-6 knock-out strain (*il-6*<sup>–/–</sup>) was produced by Kopf et al. (1994) and was purchased from The Jackson Laboratory (Bar Harbor, MA). Sciatic nerves were excised from wild-type, *lif*<sup>–/–</sup>, *il-6*<sup>–/–</sup>, as well as LIF/IL-6 double knock-out (*lif/il-6*<sup>–/–</sup>) mice. The nerves were cleaned of debris, cut in 2 mm pieces, and cultured in 24 well plates for 24 and 48 hr. The supernatant was aliquoted for storage as described above.

**Peritoneal macrophages and chemotaxis assay.** BALB/c mice between 8 and 10 weeks of age were injected intraperitoneally with 2 ml of 10% protease peptone (Difco, Detroit, MI). Four days later, peritoneal exudate cells were collected by lavage of the peritoneal cavity with 5 ml of ice-cold PBS. After washing with PBS, the peritoneal cells were resuspended at a concentration of 10<sup>6</sup>/ml in RPMI-1640 containing 0.1% BSA. Chemotactic activity was assayed in a multiwell microchamber

AP48 (Neuroprobe, Gaithersburg, MD) (Falk et al., 1980) after optimal chemotaxis conditions were established (Sugiura et al., 2000). This method of measuring chemotaxis is now widely used and is thought to minimize complications associated with earlier assays (Wilkinson, 1982; Bignold, 1988). Briefly, 25  $\mu$ l of chemoattractant was added to the bottom wells. A polycarbonate filter sheet (25  $\times$  80 mm, 8  $\mu$ m pores; Nucleopore Corp., Pleasanton, CA), without polyvinylpyrrolidone coating to prevent migrated cells from falling off (Harvath et al., 1980), was placed on top of the wells in the bottom plate. The gasket and top plate were fixed in place, and the upper wells were carefully loaded with 50  $\mu$ l of cell suspension (5  $\times$  10<sup>4</sup> cells). The assembly was incubated for 100 min at 37°C with 5% CO<sub>2</sub> in humidified air. After incubation, the top plate, gasket, and filter were removed; cells on the top of the filter that had not migrated through were wiped off; and the filter was fixed and stained with Hema color (Harleco, Gibbstown, NJ). All cells that had migrated were counted under light microscopy at 400 $\times$  magnification. Data are presented as the chemotactic index, which is defined as the number of cells that migrated in the presence of a test protein or conditioned medium divided by the number of cells that migrated in the presence of medium alone (Sugiura et al., 2000). In each experiment, the efficiency of migration was monitored using recombinant MCP-1 as a positive control. Experiments in which the chemotactic index obtained with MCP-1 at 10 ng/ml was <3 were discarded.

**Antibody blocking.** Anti-LIF and anti-MCP-1 antibodies (R & D Systems) were used at a concentration of 50  $\mu$ g/ml. In control experiments, we confirmed that antibodies to MCP-1 block the activity of murine MCP-1. We also confirmed the specificity of the blocking experiments by showing that blocking antibodies for neurotrophin-3 (NT-3) (2.5  $\mu$ g/ml), a factor that is secreted into Schwann cell-conditioned medium (Meier et al., 1999), or hepatocyte growth factor (5  $\mu$ g/ml), a known chemoattractant (Galimi et al., 2001), do not inhibit the chemotactic activity.

**Isolation of RNA and first-strand cDNA synthesis.** Total RNA was prepared using Ultraspec reagent (Biotecx, Houston, TX) or TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturers' instructions. Total RNA from immunopanned Schwann cells and from intact nerves was quantified by measuring the optical density at 260 and 280 nm and analyzed for integrity by agarose gel electrophoresis under denaturing conditions.

**Semiquantitative reverse transcriptase-PCR.** One microgram (LIF-treated Schwann cell cultures), 2  $\mu$ g (intact nerve, Schwann cell cultures, and IL-6-treated Schwann cell cultures) total RNA was reverse-transcribed into cDNA in a 50  $\mu$ l reaction containing 50 mM Tris-HCl, pH 8.3, 75 mM MgCl<sub>2</sub>, 10 mM DTT, 0.5–1.5 mM deoxyNTPs (dNTPs), and either 100–300 ng of random hexamers or 500 ng of oligo-dT<sub>17–18</sub> as primer and 200–300 U of Superscript II reverse transcriptase (RT) (Invitrogen). The reaction was incubated for 90 min at 42°C, followed by 10 min at 70°C. The remaining RNA was denatured by adding 1  $\mu$ l of RNase A (10 mg/ml; Boehringer Mannheim, East Sussex, UK) and incubating for 30 min at 37°C. The relative amount of cDNA synthesized from each sample was determined by PCR amplification using specific primers for 18S or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer pairs were designed as follows (product size in parentheses): MCP-1 forward primer, 5'-ctggcctgtgtgttcacagtgc-3'; MCP-1 reverse primer, 5'-gttggtgaggtctgtgaagacatc-3' (380 bp) (Sun et al., 1997); macrophage inhibitory protein-1 $\alpha$  (MIP-1 $\alpha$ ) forward primer, 5'-gaaggtctccaccactgccttgc-3'; MIP-1 $\alpha$  reverse primer, 5'-gactcgacctgtactacggact-3' (350 bp) (Sun et al., 1997); LIF forward primer, 5'-ccgtgtcacggcaacctatgaaccagatc-3'; LIF reverse primer, 5'-ggggacacagggcactccacatggccac-3' (395 bp) (Patterson and Fann, 1997); inducible nitric oxide synthase (iNOS) forward primer, 5'-tgatgtgctgctctgtgtct-3'; iNOS reverse primer, 5'-acttctccaggatgtgtta-3' (350 bp) (Bonmann et al., 1997); 18S forward primer, 5'-cctcgaagaagctcctgta-3'; 18S reverse primer, 5'-gggaacgcgtgcatttat-3' (350 bp) (Blanchard et al., 1996); GAPDH forward primer, 5'-ttccagatgactctaccc-3'; and GAPDH reverse primer, 5'-atggactgtgtcatgagccc-3' (398 bp) (Brown et al., 1997). One microliter of cDNA from each sample was amplified in a 50  $\mu$ l PCR, containing 1 $\times$  reaction buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, and 0.1% Triton X-100); 1–2 mM MgCl<sub>2</sub>, 0.2 mM dATP, dGTP, dTTP, and dCTP; a 0.5 mM concentration of each primer listed above; and 1.5 U of Taq DNA polymerase (Invitrogen). The cDNA was amplified after determining the optimal number of cycles and annealing temperature for each primer: 18S, 21 cycles; MCP-1, 25 cycles when oligo-dT<sub>17–18</sub> primers were used or 35 cycles when random hexamers were used for reverse transcription; MIP-1 $\alpha$  and iNOS, 35 cycles; and LIF, 35 cycles. For MCP-1 and MIP-1 $\alpha$ , PCRs were performed under a hot start program (94°C for 4.5

min). Cycling conditions were as follows: the mixture was initially incubated once for 3 or 5 min at 94°C; denatured at 94°C for 30 sec or 1 min; and annealed at 50°C (MCP-1), 55°C (18S), 57°C (LIF and GAPDH), or 60°C (iNOS and MIP-1 $\alpha$ ) for 1 min, followed by 72°C for 30 sec or 1 min with an extension of 5 min. The number of cycles used for semiquantitative PCR was in the linear part of the amplification profile.

**Relative quantification of RT-PCR products.** The intensity of the PCR products was measured using densitometry (Scion Image 1.62c software; Scion Corp., Frederick, MD), and the ratio of the intensity of MCP-1 and LIF signal to 18S and/or GAPDH was calculated for each sample. These ratios were compared at the indicated time points to obtain a numerical estimate of the changes in the cDNA of interest after treatment with either IL-6 or LIF.

**Statistical analysis.** All results are presented as mean  $\pm$  SEM. The statistical significance of differences in macrophage migration toward putative stimuli versus medium controls was analyzed by Student's *t* test. All experiments were performed three times, four replicates each, unless otherwise stated.

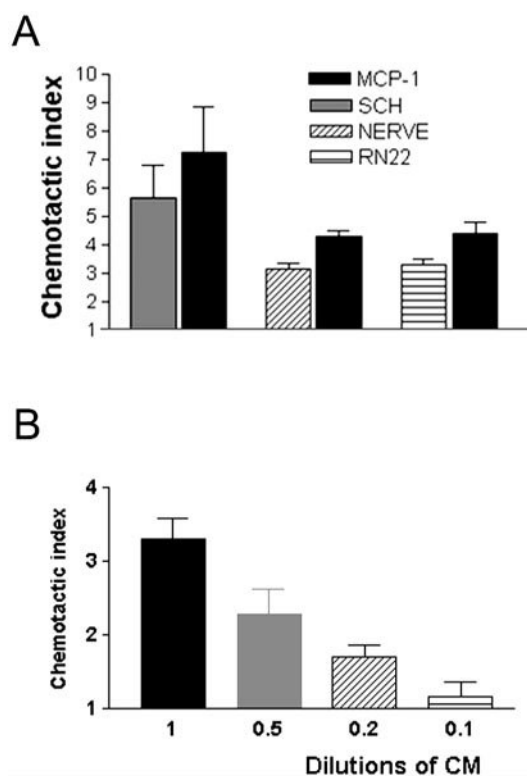
## RESULTS

### Primary Schwann cells secrete chemotactic activity

Although *in situ* hybridization and other studies of transected nerves show that injury induces cytokine and chemokine mRNAs in Schwann cells (see the introductory remarks), it has not been shown directly that Schwann cells secrete macrophage chemotactic activity. To test this, we used cultures of primary Schwann cells as a model of denervated Schwann cells in the distal stump of cut nerves. This is because there is little evidence for a major qualitative difference in molecular expression between Schwann cells in distal nerve stumps and Schwann cells cultured *in vitro* in the absence of neurons, with the possible exception of nerve growth factor (Mirsky and Jessen, 1990). For comparison, we also used segments cut from mouse sciatic nerves placed in culture without dissociation and the Schwannoma cell line RN22. If Schwann cells secrete chemotactic signals, medium conditioned by these tissues should induce macrophage chemotaxis. We tested this using the AP48 microchamber assay. We found that defined medium conditioned for 24 hr by dense cultures of immunopanned Schwann cells from nerves of 4-d-old rats, nerve segments, or the RN22 cell line all contained significant chemotactic activity (Fig. 1*A*). The chemotactic index for all three media was similar when compared with the migration stimulated by the well established macrophage attractant MCP-1 (10 ng/ml) in parallel experiments on sister cultures. The Schwann cell-conditioned medium was shown to act in a dose-dependent manner (Fig. 1*B*).

### MCP-1 and LIF are components of the Schwann cell-derived chemotactic activity

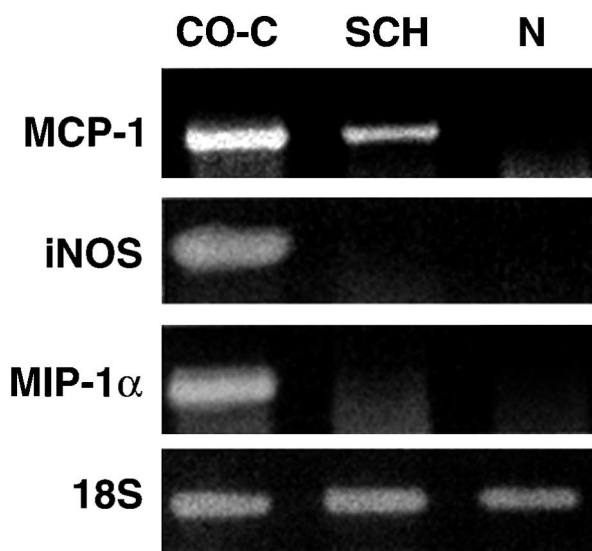
Concerning the identity of the Schwann cell-derived chemotactic signals, we initially considered MCP-1, in view of its potent chemotactic and activating properties in macrophage migration assays and in view of other evidence that MCP-1 is a component of the regulatory cascades that attract macrophages into cut nerves *in vivo* (Toews et al., 1998; Siebert et al., 2000; Subang and Richardson, 2001). First, we tested whether MCP-1 is upregulated when Schwann cells are removed from axonal contact and cultured under conditions used to generate the conditioned medium. RT-PCR was used to compare mRNA from freshly isolated intact nerves representing Schwann cells in normal contact with axons both with mRNA from immunopurified Schwann cells after a 24 hr period *in vitro* and with mRNA from unpurified cultures of dissociated nerve containing both Schwann cells and fibroblasts isolated after a 24 hr period *in vitro*. In addition to MCP-1 mRNA, we also examined mRNA for the related  $\beta$ -chemokine MIP-1 $\alpha$  and for iNOS, an enzyme upregulated in Schwann cells



**Figure 1.** Schwann cells secrete macrophage chemoattractant activity. *A*, Immunopurified cells (SCH), a Schwann cell line (RN22), and nerve segments (NERVE) secrete macrophage chemoattractant activity. All media were conditioned for 24 hr and used undiluted (see Materials and Methods). For each of these three determinations, MCP-1 (10 ng/ml) served as a positive control in a parallel assay as shown. Defined medium served as negative control. In this and all subsequent illustrations of migration assays, the results are expressed as chemotactic index (see Materials and Methods). *B*, The Schwann cell-derived macrophage chemotactic activity acts in a dose-dependent manner. Conditioned medium (CM) from immunopurified Schwann cells was used undiluted and at the dilutions indicated.

in response to treatment with the inflammatory cytokines interferon- $\gamma$  or TNF- $\alpha$  (Gold et al., 1996). This comparison showed that whereas intact nerves expressed undetectable levels of these molecules, purified primary Schwann cells selectively upregulated MCP-1. In contrast, mixed cultures containing both Schwann cells and fibroblasts expressed all signals tested (i.e., MCP-1, MIP-1 $\alpha$ , and iNOS) (Fig. 2).

Therefore, we tested whether the chemotactic activity present in medium conditioned by purified Schwann cells could be inhibited by antibodies that selectively neutralize MCP-1. We extended these experiments to include antibodies that neutralize LIF, because this cytokine is induced in Schwann cells by sciatic nerve injury *in vivo* and also has macrophage chemotactic activity *in vitro* (Banner and Patterson, 1994; Sugiura et al., 2000). We found that neutralizing antibodies against either MCP-1 or LIF could inhibit the chemotactic activity in the conditioned medium (Fig. 3*A*). The MCP-1 antibody blocked 60–70% of the activity ( $p < 0.0001$ ), whereas the anti-LIF antibody blocked 30–40% of the activity ( $p < 0.006$ ). When the conditioned medium was incubated with both antibodies, only 15% of the chemotactic activity remained ( $p < 0.0001$ ). We have shown previously that the RN22 Schwann cell line, like Schwann cells, secreted a macrophage chemoattractant (Fig. 1*A*). Supporting a major role for



**Figure 2.** Schwann cells upregulate MCP-1 mRNA when deprived of axonal contact. mRNA levels in freshly isolated nerves (*N*) are compared with levels in unpurified cultures of dissociated nerve (*CO-C*) and purified Schwann cells (*SCH*) after overnight incubation. RT-PCR results are shown for MCP-1-, iNOS-, MIP-1 $\alpha$ -, and 18S-specific primers. 18S control samples were used to demonstrate equal loading in all tracks. These results show that purified Schwann cells upregulate MCP-1 mRNA but not MIP-1 $\alpha$  or iNOS mRNA when they are deprived of axonal contact *in vitro*, whereas mixed cultures (containing Schwann cells and fibroblasts) also upregulate iNOS and MIP-1 $\alpha$ .

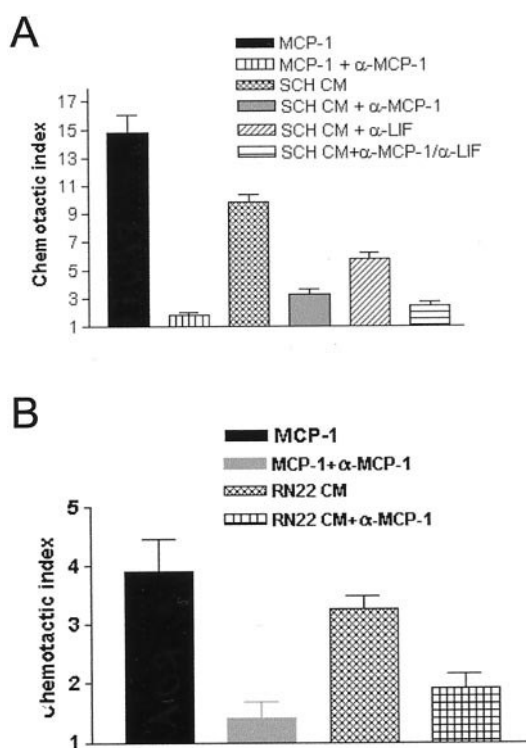
MCP-1 as a glial cell-derived chemotactic signal, we found that neutralizing MCP-1 antibodies also blocked ~60% of the RN22-derived activity (Fig. 3*B*).

If LIF acts as a Schwann cell-derived chemoattractant, conditioned medium from nerves of mice in which LIF has been genetically inactivated should be relatively ineffective in attracting macrophages. We tested this by comparing macrophage chemotactic activity in media conditioned by nerve segments from wild-type or *lif*<sup>−/−</sup> mice (Fig. 4*A*). We found that medium from *lif*<sup>−/−</sup> nerves contained only 40% of the activity found in media from wild-type nerves ( $p < 0.013$ ), in agreement with the experiments above using blocking antibodies.

Together, these experiments strongly indicate that MCP-1 and LIF are the principal factors for macrophage chemotactic activity directly secreted by Schwann cells.

#### Autocrine circuits involving IL-6 and LIF regulate the secretion of chemotactic activity from Schwann cells

We then considered how the secretion of chemotactic agents such as MCP-1 and LIF might be regulated. After axotomy *in vivo*, LIF mRNA levels peak at 24 hr, whereas mRNA for the major macrophage attractant MCP-1 does not reach peak levels until 48 hr after cutting (Banner and Patterson, 1994; Carroll and Frohnert, 1998; Toews et al., 1998; Subang and Richardson, 2001). The relatively slow time course of MCP-1 induction suggests the existence of earlier regulatory cascades triggered by nerve transection. A number of factors point to IL-6 as a favorable candidate for such a role: (1) IL-6 mRNA is rapidly induced by axotomy, reaching a peak at 12 hr (Bolin et al., 1995; Bourde et al., 1996; Kurek et al., 1996), (2) IL-6 has been implicated previously in neuronal and immune responses to injury (Gadient and Patterson, 1999), and (3) although IL-6 does not itself attract macrophages (Sugiura et al., 2000), cut nerves in *il-6*<sup>−/−</sup> mice



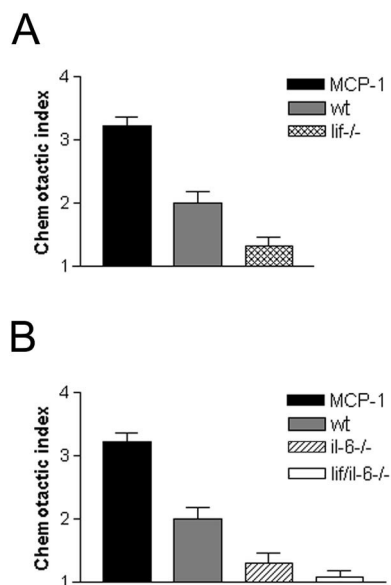
**Figure 3.** Schwann cells secrete chemotactic activity that is blocked by anti-MCP-1 and/or anti-LIF antibodies. *A*, Conditioned medium (CM) from primary rat Schwann cells (*SCH*) contains chemotactic activity that can be blocked by anti-MCP-1 ( $\alpha$ -MCP-1) and/or anti-LIF ( $\alpha$ -LIF)-neutralizing antibodies. The MCP-1 antibody blocks 60–70% of this activity ( $p < 0.0001$ ), whereas the LIF antibody blocks 30–40% of this activity ( $p < 0.006$ ). When the conditioned medium is incubated with both antibodies, only 15% of the chemotactic activity remains ( $p < 0.0001$ ). *B*, Neutralizing MCP-1 antibodies reduce chemotactic activity in medium conditioned by RN22 cells by 60% ( $p < 0.002$ ) in accordance with findings using conditioned medium from primary Schwann cells. *A*, *B*, MCP-1 was used as a positive control, and MCP-1 in combination with the anti-MCP-1 antibody was used to confirm the effectiveness of the blocking antibody.

show reduced macrophage recruitment (Klein et al., 1997), suggesting that IL-6 attracts macrophages to nerves through an indirect mechanism.

To test the involvement of IL-6, we compared the chemotactic activity of medium conditioned by nerves from *il-6*<sup>−/−</sup> mice with conditioned medium from segments of wild-type nerves (Fig. 4*B*). We found that, although IL-6 does not attract macrophages (above), the medium conditioned by *il-6*<sup>−/−</sup> cells contained only 40% of the activity present in medium from normal nerves ( $p < 0.013$ ), a figure similar to that obtained with medium from *lif*<sup>−/−</sup> nerves (Fig. 4*A*). In these experiments, we also examined medium conditioned by nerves lacking both IL-6 and LIF. Although the medium from these *lif/il-6*<sup>−/−</sup> nerves showed a strong reduction in macrophage recruiting activity when compared with normal nerves ( $p < 0.0004$ ), the difference between media from the double knock-out nerves and media from the corresponding single knock-out nerves was not statistically significant. This indicates that both LIF and IL-6 are essential for nerves to generate maximum chemotactic activity.

The low chemotactic activity in medium from *il-6*<sup>−/−</sup> nerves could be explained if the role of IL-6 was to act as a positive regulator for the generation of downstream chemotactic factors. First we tested whether IL-6 regulated the expression of mRNAs for LIF in immunopurified Schwann cells using RT-PCR (Fig.



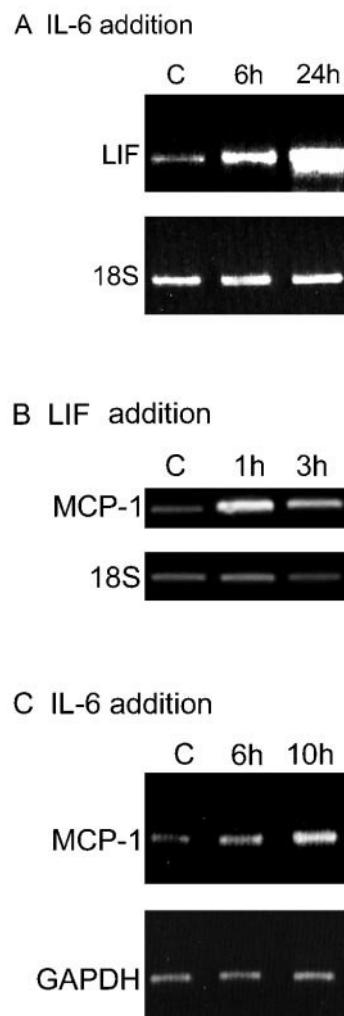


**Figure 4.** Conditioned medium from nerves of *lif*<sup>-/-</sup> mice and *il-6*<sup>-/-</sup> mice shows less chemotactic activity than medium from normal wild-type nerves (wt). *A*, Medium conditioned for 24 hr by nerve segments from *lif*<sup>-/-</sup> mice contains significantly less chemotactic activity than media conditioned by nerves from wild-type mice. *B*, Media conditioned for 24 hr by nerve segments from *il-6*<sup>-/-</sup> mice or by nerves from mice lacking both IL-6 and LIF (*lif/il-6*<sup>-/-</sup>) contain significantly less chemotactic activity than media from wild-type nerves. The difference between media from *il-6*<sup>-/-</sup> and *lif/il-6*<sup>-/-</sup> nerves is not statistically significant. *A*, *B*, MCP-1 (10 ng/ml) was used as a positive control.

5A). We found that IL-6 strongly increased the abundance of LIF mRNA from 6 hr onward (significant elevation was seen already at 1 hr; data not shown) (Fig. 5A). We then tested whether LIF, being rapidly and therefore presumably directly induced by IL-6, would, in turn, rapidly induce MCP-1. Using RT-PCR, we determined that already at 1 and 3 hr, exposure to LIF (20 ng/ml) clearly increased the levels of MCP-1 mRNA (Fig. 5B). Therefore, these results suggest the existence of a signaling cascade in which IL-6 induces LIF, which then induces MCP-1, a model that is consistent with the sequential activation of these genes in cut nerves (for references, see above) and our finding that medium conditioned by *il-6*<sup>-/-</sup> cells lacks chemotactic activity despite the fact that IL-6 is not a chemoattractant.

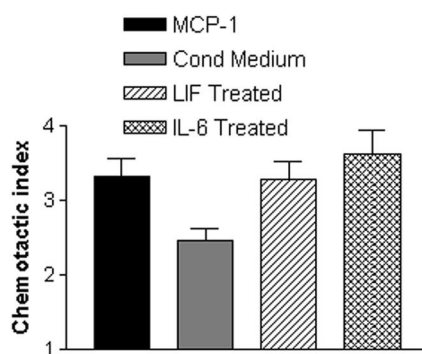
This idea was tested in two additional sets of experiments. First, it predicts that it might be possible to demonstrate induction of MCP-1 when IL-6 is added to Schwann cell cultures, although this effect might be small because it would depend on a sufficient concentration of LIF building up in the culture dish. Furthermore, because this effect should be indirect and mediated by LIF, the MCP-1 induction should take place with a delay when compared with the induction of LIF by IL-6. Therefore, we used RT-PCR to monitor MCP-1 mRNA in purified Schwann cell cultures after addition of IL-6 (Fig. 5C). Elevation of MCP-1 mRNA was observed, although a significant response was not seen until at the 10 hr time point. This finding is in agreement with previous observations that IL-6 does not elevate MCP-1 mRNA in Schwann cells 3 hr after application (Subang and Richardson, 2001).

Second, if the IL-6-stimulated LIF expression and the LIF-stimulated MCP-1 expression shown above were functionally significant, cells exposed to either IL-6 or LIF should generate



**Figure 5.** IL-6 enhances cytokine expression in Schwann cells. *A*, IL-6 enhances expression of LIF in purified Schwann cells. The results are from RT-PCR assays of untreated Schwann cells [controls (C)] and Schwann cells treated with 20 ng/ml IL-6 for 6 and 24 hr as indicated. Note that the elevation of LIF mRNA is already clear at 6 hr. 18S control samples were run as shown to control for loading in all tracks. Densitometric comparison of the LIF signals with the corresponding 18S signals shows that LIF elevation is threefold at 6 hr and sevenfold at 24 hr (see Materials and Methods). *B*, LIF enhances expression of MCP-1 in purified Schwann cells. The results show RT-PCR assays of untreated cells (C) and Schwann cells treated with 20 ng/ml LIF for 1 and 3 hr as indicated. Note that the elevation of MCP-1 mRNA is already clear at 1 hr. 18S control samples were run as shown to control for loading in all tracks. Densitometric comparison of the LIF signals with the corresponding 18S signals shows that MCP-1 elevation is fourfold at 1 hr and fivefold at 3 hr. *C*, IL-6 enhances expression of MCP-1 in purified Schwann cells. The results show RT-PCR assays of untreated cells (C) and cells treated with 20 ng/ml IL-6 for 6 and 10 hr as indicated. The elevation of MCP-1 mRNA is not unambiguous until the 10 hr point. This delay is consistent with the idea that IL-6 controls MCP-1 levels indirectly by activating LIF (Fig. 7). GAPDH PCR was run as shown to control for loading in all tracks. Densitometric comparison of the LIF signals with the corresponding GAPDH signals shows that MCP-1 elevation is 1.4-fold at 6 hr and threefold at 10 hr.

more chemotactic activity than unstimulated control cells. To test this possibility, the RN22 Schwann cell line was treated for 3 hr with LIF or IL-6 (both at 20 ng/ml). The cells were then washed extensively to ensure that any added factors were removed from the wells. Conditioned medium was collected from the cells after



**Figure 6.** Exogenous LIF or IL-6 induces chemotactic activity in the RN22 Schwann cell line. RN22 cells were treated for 3 hr with 20 ng/ml LIF or IL-6 and then washed extensively. Conditioned medium (*Cond Medium*) was collected from the cells after an additional 24 hr incubation and tested in the migration assay. LIF and IL-6 increased the level of chemotactic activity in conditioned medium by 34% ( $p < 0.02$ ) and 44% ( $p < 0.005$ ), respectively.

an additional 24 hr incubation in defined medium. We found that preincubation with either LIF or IL-6 increased macrophage chemotactic activity by 34% ( $p < 0.02$ ) and 44% ( $p < 0.005$ ), respectively (Fig. 6).

## DISCUSSION

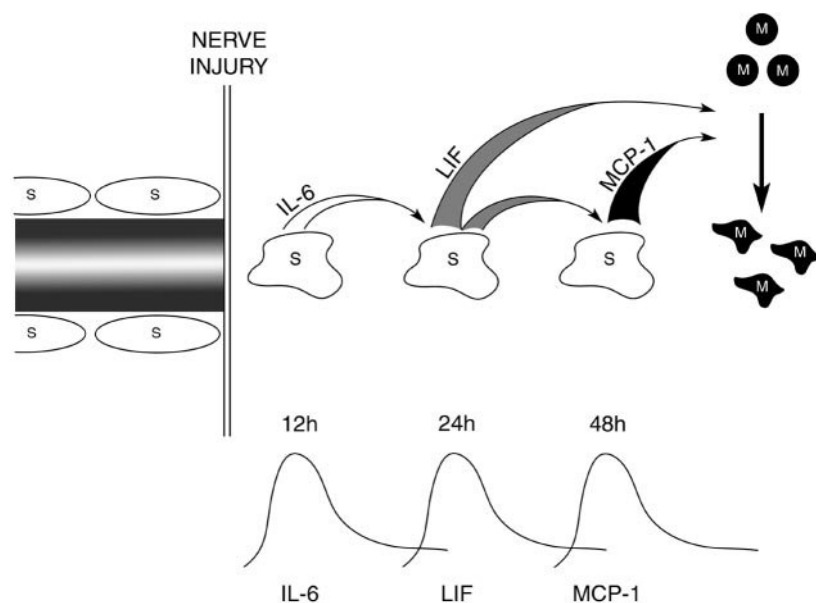
Together, the experiments reported here are consistent with the notions that: (1) Schwann cells directly attract macrophages by secretion of MCP-1 and LIF and (2) induction of these chemoattractants in denervated Schwann cells is regulated by autocrine circuits involving IL-6 and LIF (Fig. 7). The existence of such a signaling cascade and the consequent late elevation of the major macrophage attractant MCP-1 is in line with the delayed entry of macrophages into cut nerves *in vivo* (see the introductory remarks).

In a functional blocking assay, we show that both MCP-1 and LIF are present in Schwann cell-conditioned medium, and that they are the principal mediators of the Schwann cell-derived chemotactic activity. In our experiments, MCP-1 accounted for 60–70% of Schwann cell chemotactic activity, whereas LIF ac-

counted for 30–40%. In agreement with this, recombinant LIF has direct chemotactic activity on macrophages in the micro-chamber assay, with a maximum chemotaxis index that is one-half that of recombinant MCP-1 (Sugiura et al., 2000). MCP-1 also accounted for 60% of the chemotactic activity secreted by RN22, a cell line that closely mimics primary Schwann cells in other aspects of injury and repair responses (Varon et al., 1981; Longo et al., 1982; Hill, 1987). The role of LIF in chemotaxis is also supported by two findings. The addition of LIF to primary Schwann cell cultures induced MCP-1 mRNA, whereas conditioned medium from *lif*<sup>−/−</sup> mouse sciatic nerves contained significantly less chemotactic activity than wild-type controls. Our data are also consistent with *in vivo* evidence that macrophage recruitment to peripheral nerves is reduced in mice lacking the main receptor for MCP-1 or in *lif*<sup>−/−</sup> mice after sciatic nerve and/or brain injury (Siebert et al., 2000; Sugiura et al., 2000).

Because both LIF and IL-6 are known to induce cascades of inflammatory mediators in other cell types (Villiger et al., 1992; Hartner et al., 1997; Shimon et al., 1997; Marin et al., 2001), we investigated whether they could regulate Schwann cell chemotactic activity in a similar manner. IL-6 is not directly chemotactic for macrophages (Sugiura et al., 2000), but nerve injury in *il-6*<sup>−/−</sup> mice results in reduced macrophage recruitment (Klein et al., 1997). Our finding that chemotactic activity secreted by cut nerves from *il-6*<sup>−/−</sup> mice is also reduced compared with controls suggests that this is the reason for the reduced influx of macrophages. Treatment of primary Schwann cells with IL-6 induces LIF at both early (1 and 3 hr) (our unpublished observations) and more prolonged time points. Furthermore, treatment of RN22 cells with either LIF or IL-6 enhances the chemotactic activity secreted in the conditioned medium. When combined with evidence from others that IL-6 does not directly induce MCP-1 in Schwann cells within 3 hr (Subang and Richardson, 2001), our data suggest that induction of MCP-1 in Schwann cells may be dependent on previous induction of LIF or other factors that can induce MCP-1 directly.

Therefore, Schwann cell-derived IL-6 and LIF could induce the expression of downstream chemotactic signals in Schwann cells themselves. After nerve transection *in vivo*, IL-6 mRNA is



**Figure 7.** A tentative model of a cytokine-signaling cascade controlling macrophage (*M*) entry to damaged nerves. An autocrine cascade of IL-6 and LIF enhances Schwann cell (*S*) secretion of LIF and MCP-1, both of which directly attract macrophages. This indirect regulation of the major macrophage attractant MCP-1 is in agreement with observed delay in macrophage recruitment to transected nerves *in vivo*.

strongly but transiently induced, reaching peak levels at 12 hr (Bolin et al., 1995; Bourde et al., 1996; Kurek et al., 1996); LIF mRNA expression peaks at 24 hr and declines at 3 d postoperatively (Banner and Patterson, 1994; Curtis et al., 1994; Kurek et al., 1996), whereas MCP-1 mRNA reaches peak levels at 48 hr after axotomy (Chien et al., 1997; Toews et al., 1998). These observations, together with our present findings, allow us to propose that *in vivo*, early induced cytokines such as IL-6 and weaker chemoattractants such as LIF may be essential Schwann cell-derived autocrine factors for the expression of stronger downstream chemotactic signals such as MCP-1 (Fig. 7). In support of this, axonal breakdown appears to be an important regulator of IL-6 but not MCP-1 production by Schwann cells (Rutkowski et al., 1999). It is also of interest that although there is a very significant deficit in chemotactic activity in the conditioned medium from both *lif*<sup>−/−</sup> and *il-6*<sup>−/−</sup> nerves, the activity did not drop significantly further in conditioned medium from double knock-out nerves. A related observation was that in response to cortical injury, the astroglial and microglial responses to injury are significantly reduced in the *lif*<sup>−/−</sup> and *il-6*<sup>−/−</sup> mice, but the double knock-out mice show no further reduction in these responses (Sugiura et al., 2000). Therefore, in both the CNS and the PNS, these two cytokines seem to operate in series rather than in parallel, forming one injury response pathway. Supporting the notion of a single pathway is a recent finding that LIF regulates IL-6 expression in the complete Freund's adjuvant model of neurogenic cutaneous inflammation (Zhu et al., 2001). The reverse appears to be true in the sciatic nerve, because IL-6 induces LIF mRNA in Schwann cells and after nerve cut IL-6 is induced before LIF (Kurek et al., 1996; Toews et al., 1998). The model proposed here is also consistent with the observation that after peripheral nerve damage, the major influx of macrophages begins 2–3 d after injury (Ramon y Cajal, 1928; Beuche and Friede, 1984; Crang and Blakemore, 1986; Perry et al., 1987; Clemence et al., 1989; Stoll et al., 1989). The high potency of MCP-1 and the good correlation of its time course of induction with peak macrophage recruitment suggest that the proposed cascade model may represent an important window into the inflammatory response.

Although MCP-1 and LIF account very well for the chemotactic activity in Schwann cell-conditioned medium *in vivo*, other chemotactic factors or mechanisms are likely to contribute. For example, cultured Schwann cells produce IL-1 $\beta$  (Bergsteinsdottir et al., 1991). This cytokine induces both LIF and MCP-1 mRNA synthesis under some conditions, although it does not induce MCP-1 in cultured Schwann cells (Carlson et al., 1996; Schwarz et al., 1997; Subang and Richardson, 2001). TNF- $\alpha$ , which is expressed by Schwann cells 7 d after injury (Wagner and Myers, 1996), induces MCP-1 mRNA when added to Schwann cells, and although TNF- $\alpha$  receptor-null mice do not show lower MCP-1 mRNA levels than wild-type mice in the distal stump at early time points, they do at 4 d after transection (Subang and Richardson, 2001). In some systems, IL-6 acts in concert with its soluble receptor to promote leukocyte recruitment and could function similarly in nerves (Hurst et al., 2001; Marin et al., 2001).

The present observations point to Schwann cells as an important target for therapeutic intervention in peripheral nerve disease. For example, in experimental allergic neuritis, an animal model for human demyelinating polyneuritis (Guillain Barre syndrome), upregulation of MCP-1 mRNA precedes the clinical onset of disease (Fujioka et al., 1999). It remains unclear what first triggers the upregulation of MCP-1 before overt mononuclear cell infiltration. If this situation parallels that of nerve injury

described here, activation of autocrine IL-6/LIF circuits in Schwann cells by myelin breakdown, in turn promoted by early recruitment of neuritogenic T cells, could be the mechanism that stimulates MCP-1 production.

Previous studies have led to the suggestion that a disturbance in the axon–myelin–Schwann cell unit is sufficient to induce macrophage recruitment, and it is widely accepted that this is the initiating signal for the inflammatory reaction in peripheral nerve injury. Axonal breakdown is undoubtedly a key event in Wallerian degeneration, and neuron-derived diffusible molecules may regulate Schwann cell gene expression (Bruck et al., 1995, 1997). Our findings raise the possibility that Schwann cells are also active regulators of the early inflammatory response, rather than simply passive targets of extrinsic signals. This active regulation could be achieved in part by autocrine circuits, which enhance the selectivity and potency of the chemotactic activity appropriately throughout the injury response. It has been shown previously that Schwann cells can establish autocrine circuits mediated by insulin-like growth factor, NT-3, and platelet-derived growth factor-BB, which are sufficient to prevent Schwann cell death in the absence of axons (Jessen and Mirsky, 1999; Meier et al., 1999), and that LIF, in combination with other factors, is an autocrine survival factor for Schwann cells (Dowsing et al., 1999). Furthermore, axonal contact suppresses LIF mRNA expression in Schwann cells (Matsuoka et al., 1997). The latter could represent a physiological mechanism by which regenerating axons restrict the action of such autocrine circuits during repair. Together with our present data, these findings suggest that expression of autocrine loops might be a major mechanism by which Schwann cells regulate gene expression and possibly phenotypic changes when they are deprived of axonal contact.

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# Tissue Engineering Strategies for Peripheral Nerve Regeneration

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### Specialty section:

This article was submitted to  
Experimental Therapeutics,  
a section of the journal  
Frontiers in Neurology

Received: 31 August 2021

Accepted: 15 October 2021

Published: 16 November 2021

### Citation:

Li Y, Ma Z, Ren Y, Lu D, Li T, Li W,  
Wang J, Ma H and Zhao J (2021)  
Tissue Engineering Strategies for  
Peripheral Nerve Regeneration.  
Front. Neurol. 12:768267.  
doi: 10.3389/fneur.2021.768267

A peripheral nerve injury (PNI) has severe and profound effects on the life of a patient. The therapeutic approach remains one of the most challenging clinical problems. In recent years, many constructive nerve regeneration schemes are proposed at home and abroad. Nerve tissue engineering plays an important role. It develops an ideal nerve substitute called artificial nerve. Given the complexity of nerve regeneration, this review summarizes the pathophysiology and tissue-engineered repairing strategies of the PNI. Moreover, we discussed the scaffolds and seed cells for neural tissue engineering. Furthermore, we have emphasized the role of 3D printing in tissue engineering.

**Keywords:** peripheral nerve regeneration, nerve tissue engineering, pathophysiology, scaffolds, cells, 3D printing

## INTRODUCTION

A peripheral nerve injury (PNI) is a medical problem mainly caused by external trauma after stretching, tearing, or extrusion of peripheral nerves. (1) It has attracted social attention because of its enormous social and economic pressure. In the United States, the economic loss caused by nerve injury exceeds \$150 billion annually, and the treatment cost exceeds billions of dollars every year. People often underestimate the incidence of PNI, which seriously affects the quality of life of the patients (1–3).

The peripheral nervous system can self-regenerate and repair itself after injury. However, the repair is often slow and incomplete because axon extension depends on the synthesis and transportation of the intracellular substances, and the regeneration speed is similar to that of axon transportation, about 1–3 mm/day (4). In general, the stretch or crush injury results are better than those of transection. The recovery of the distal injury is better than that of the proximal injury, because axon growth only needs a short distance to reach the distal stump.

In the previous studies, substantial functional recovery of mild and moderate nerve injury can be achieved by surgical operation (such as, nerve suture and nerve transplantation) or non-surgical operation (such as, magnetic field, electric field, He-Ne Laser, and traditional Chinese Medicine) (3). The nerve suture and nerve grafts are extensively used for surgical nerve repair in the experiment and the clinic. An end-to-end nerve suture requires that the nerve stumps are aligned to achieve the best repair effect, and only the short nerve gap (<5 mm) can be used (5). When the damage gap is more significant (more than 5 mm), it is not possibly repaired by the meticulous microscopic surgery, and nerve autograft is regarded as the gold standard (3, 5). However, this damages the healthy nerves, and the number of donors is limited (6).

With the rapid development of cell biology and materials science, a new discipline, tissue engineering, is established to construct a different method of peripheral nerve repair. The core



of nerve tissue engineering is to build a three-dimensional complex composed of cells and biomaterials and make the nerve guiding catheters (NGCs). The catheters are active scaffolds that can effectively guide axon regeneration. They contain the essential cells and neurotrophic factors that support axon regeneration. Implantation of the NGCs in the injured site can simulate the neural structure after Wallerian degeneration and avoid immune reactions. Even in some injury models, the clinical effect is similar to that of autologous nerve transplantation. The ideal NGCs should have excellent mechanical strength, good biocompatibility, biodegradability, and permeability (7).

In recent years, the research on neural tissue engineering has become more in-depth, and various NGCs have emerged one after another. To find a more suitable clinical treatment, the researchers need to pay attention to the regeneration effect of axons and focus on the transformation of basic research to clinical. Here, we aimed to review the pathophysiology and repairing strategies for PNI, focusing on the latest advances in nerve tissue engineering.

## **PATHOPHYSIOLOGY**

### **Anatomical Physiology**

The periphery nerve system consists of all the nerves from the three main categories: the spine nerve, cranial nerve, visceral nerve, and their associated ganglia (8). The first two types of nerve fibers with myelin sheath are Schwann cells (SCs) wrapped in several layers, while the third nerve fibers are generally non-myelinated. The nerve bundles include the nerve fibers and connective tissue, which constitute the nerves as mentioned above. A connective tissue can be divided into three layers: epineurium, perineurium, and endoneurium. It contains the blood vessels in the epineurium, which provide the nerve fibers with trophic support. In the endoneurium, there are different types of cells except for connective tissue and nerve fibers (9, 10). For example, SCs produce neurotrophins and extracellular matrix (ECM), thus providing the nerve-growth microenvironment. In addition, it plays a crucial role in nerve regeneration. The macrophages are the essential cells in the inflammatory response, generally divided into two phenotypes and play different roles in nerve degeneration and regeneration (11).

### **Injury Types**

Many factors contributed to the PNI (12). The basic injury types seen in the clinical practice are three main kinds (Table 1). The most common type is stretch-related injury. As we all know, after an external force pulls the spring, the spring will stretch in the direction of the power, and when the intensity exceeds a specific limit, plastic deformation will occur. Similarly, an injury occurs when traction forces exceed the stretch limit strength of the nerves, whose properties are similar to the properties of spring (15). For example, excessive traction caused by an overextension of the neck at birth leads to a particular type of birth paralysis limited to

the fifth and sixth cervical nerves, which is also called Erb's palsy (13).

The laceration is another essential type of PNI, accounting for about 30% of the serious injuries (12). In this case, these can be complete transection of the nerve, and hence the process of nerve degeneration and regeneration can be observed clearly. Compression is the third injury type. There are two mechanisms of the damaged nerve, ischemia and extrusion.

### **Injury Scale (IS)**

In the clinical diagnosis and treatment of PNI, the first step is to clarify the degree of injury. The most classic injury classifications were proposed by Seddon and Sunderland and are still in use today (Figure 1) (15–17).

The nerve injuries are divided into three categories by Seddon (16). In neurapraxia, the nerve fiber is slightly squeezed, but the axon is not broken, and there is no morphological change, only temporary functional changes. In axonotmesis, the nerve fibers are severely crushed, the axons are broken, and their functions temporarily disappear, accompanied by muscle atrophy, but the myelin and SCs still exist. In neurotmesis, the nerve bundle is broken due to severe trauma, which is difficult to recover. Sometimes it can be recovered from surgery, such as severe laceration. On this basis, Sunderland subdivided the nerve injury classification into five levels (15). The first-degree injury corresponds to Seddon's classification of neuropraxia. From first degree to fifth degree, the severity of the injury increases. The second, third, and fourth-degree injuries correspond to the endoneurium, perineurium, and epineurium lesions. The fifth degree of injury corresponds with the neurotmesis of Seddon, which is irrecoverable damage (3, 12).

## **RECOVERY OF FUNCTION AFTER TRAUMA**

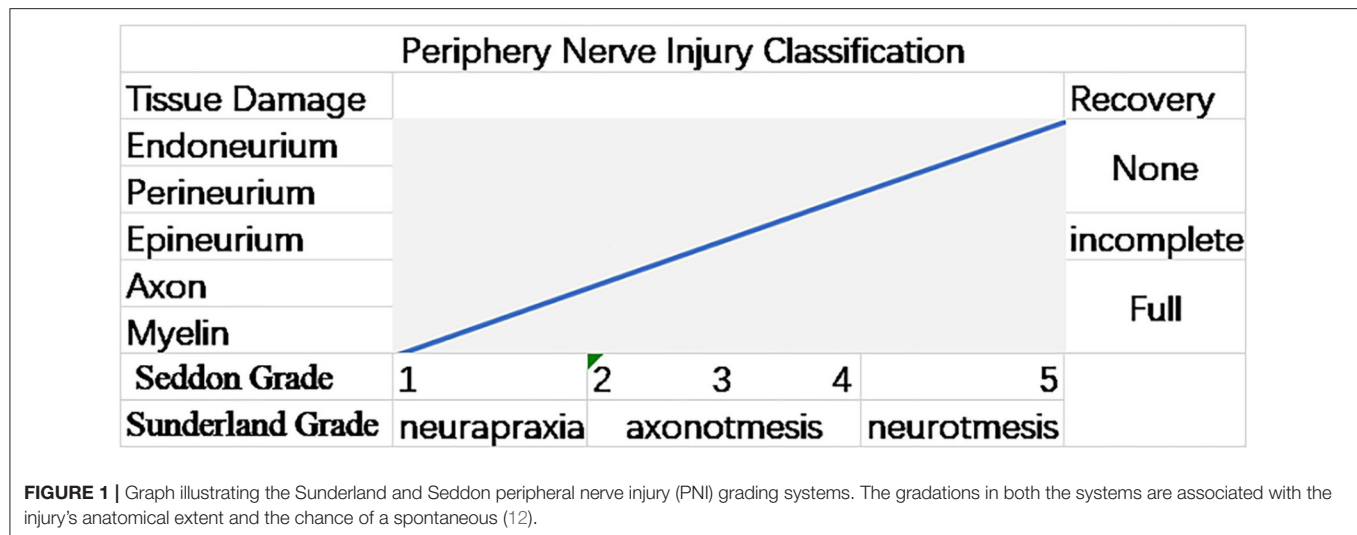
### **Pathological Mechanism**

The normal surrounding myelinated nerve is wrapped by a Schwann cell membrane and forms an onion skin-like myelin sheath. This remarkable structure ensures the transmission of specific electrical signals and maintains the normal neurophysiological functions. When an external force acts to stretch, compress, or tear the peripheral nerve, it will cause the distal nerve stump to degenerate, called Wallerian degeneration. At the site of nerve injury, the axon is broken and disintegrated by an external force, forming an inflammatory environment. The myelin sheath is also broken down and lost, and the macrophages are recruited to the injury site to swallow most myelin debris and lipid droplets.

Schwann cells are the most important cells for peripheral nerve regeneration. Due to the transection of the nerve fibers, they lose physical contact with the neuronal cells. Under the combined action of various cytokines (such as transcription factor c-Jun, and histone deacetylase 1 and 2), they reversely differentiate into repairing the phenotype cells. From the proximal stump to the distal stump, the Büngner band is formed, which plays a vital role in the peripheral nerve regeneration (18, 19). Furthermore, every Schwann cell has a set of genes.

**TABLE 1** | Common types of nerve damage.

Injury type	Continuity	Injury mechanism	Recovery speed	Example	References
Stretch-related injury	Generally keep	Stretching force exceeds the elastic limit of the nerve membrane.		Erb's palsy	(13)
Laceration	Mostly keep	The nerve membrane is wholly or partially ruptured.	2–3 mm/day	Common, such as knife wounds	(12)
Compression	Generally keep	Nerve compression or prolonged ischemia (>8 h).	3–4 mm/day	Radial neuropathy	(14)



These genes can encode proteins or biologically active substances that promote nerve regeneration and prevent cell apoptosis, such as laminin, type V collagen, and nerve growth factor (NGF). Nerve regeneration is precise. Generally, the speed of nerve regeneration is 3–4 mm/day after compression and 2–3 mm/day after laceration. The regenerated nerve fibers can dominate the muscle preferentially (20).

## Traditional Repairing Strategies

With the development of medical technology and equipment, the treatment of peripheral nerve injury has made significant progress. Various strategies for peripheral nerve repair are summarized here. Briefly, there are two kinds of common repair strategies: surgical repair (such as neurorrhaphy, nerve transfer, nerve grafts, and tubes) and non-surgical repair (such as magnetic and electric field, He–Ne laser, electro photoluminescence, and traditional Chinese medicine) (Table 2) (3, 22).

A nerve suture is a standard method for the surgical treatment of nerve injury. The specific process is to remove the necrotic tissue and then suture the nerve end to end. The repair effect depends on the perfection of the repair technology and the distance from the injury site to the target tissue. If the space is too long, even if the nerve stumps are aligned, the time for axons to regenerate and connect to the corresponding part exceeds a time window, which will impair the effect of peripheral nerve repair (3). Nerve suture relies on the axon to produce collateral

sprouting. During the operation, part of the axon of the nerve of the donor was artificially destroyed, causing its terminal to germinate, refilling the gap between the nerve stump (29).

A nerve transplantation is another surgical method, although autologous nerve transplantation is the gold standard for treating the long gap nerve injury. In large peripheral nerve lesions, a vascularized nerve transplant can provide blood to the nerve stump, prevent hypoxia, and provide nutrients (3). However, the neuronal process requires a complete nerve donor typically taken from the autologous sensory nerve, which will damage the original healthy tissue (21). With the development of decellularization technology, the allogeneic nerve transplantation can replace the autologous nerve transplantation, which reduces the damage to the autologous nerve and weakens the immune response to achieve the purpose of repair. Zhang et al. completely demyelinated and cellularized the heterogeneous nerves, leaving natural NGF and ECM, integrated them with the autologous nerve-differentiated adipose-derived mesenchymal stem cells (MSCs) to form a nerve conduit. The conduit was implanted in the rats to repair 10 mm sciatic nerve defects (30).

When there is no graft, another intervention may be used, i.e., nerve transfer. The proximal nerve strain is directly connected to the muscled abdomen (22). It can effectively restore the early damage caused by the loss of function, but it requires strict monitoring of the physiological state of the donor nerve by electromyography. The principle is that the healthy nerve bundles will be redirected to the distal part of the injured

TABLE 2 | Repair strategies of peripheral nerve injury (PNI).

Repair strategy	Name	Specific method	Features (including advantages and disadvantages)	Animal and preclinical studies (References)
Surgical repair	Neurorrhaphy	First, remove dead tissue, then the nerve suture end to end.	Commonly used in the case of minor damage, the recovery effect depends on the distance from the damaged site to the target organ.	(3)
	Nerve transfer	Connect the proximal nerve stump directly to the muscled abdomen.	Alternative therapies for nerve transplantation reduce proximal nerve damage and a long surgical treatment window (~4 months).	(21)
	Nerve grafts	Take the autologous nerve and transplant it to the nerve defect site.	It is the gold standard for treating long gap nerve injury. Still, it has several disadvantages, such as the limited availability of donor tissue, the sacrifice of functional nerves, and the potential formation of a neuroma.	(22)
	Tubes	Nerve conduits (simulating the physiological structure of healthy nerves, providing physical protection for peripheral nerve regeneration, and stimulating endogenous nerve regeneration potential), essential elements: scaffold, seed cells, cytokines.	The reparability depends on the type of injury, the material of the catheter, etc. It will not damage the self-healthy nerves.	(23)
Non-surgical repair	Magnetic and electric field	Examples include therapeutic ultrasound, low-intensity laser therapy (LLLT), transcutaneous electrical nerve stimulation (TENS), and pulsed electromagnetic field therapy (PEMF).	Adjuvant treatments, various methods have different therapeutic effects. For example, LLLT may be beneficial in patients with rotator cuff disease.	(24)
	He-Ne laser	780 nm laser irradiation treatment.	A specific wavelength of light can improve peripheral nerve function.	(25)
	Electro photoluminescence	Electric acupuncture stimulation down-regulate mir-1b, promote Schwann cell proliferation and nerve repair.	Auxiliary means for peripheral nerve repair.	(26)
	Traditional Chinese medicine	Such as internal and external application, acupuncture, massage, bloodletting, etc.	The methods are diverse and influential. For example, Astragalus (inhibit the expression of caspase-1 and caspase-3, reduce neuronal apoptosis) and some treatment principles are still under study.	(27, 28)

nerve (near the neuromuscular junction) to replace the role of the original nerve to achieve functional motor recovery. For the PNIs that cannot be regenerated entirely within a time window, nerve transfer helps protect and preserve the distal motor endplates until the natural axons are wholly regenerated (30). Different from the first three methods, the principle of “tubes” repair of nerve injury is to simulate the physiological structure of the healthy nerve, provide physical protection for peripheral nerve regeneration, and stimulate endogenous nerve regeneration potential. The preceding “tubes” were often natural products, such as muscle bundles, amniotic tubes, and venous tubes. The therapeutic effect is unsatisfying.

Over the past few years, the nerve-guided catheters are developed consisting of scaffolding and cells, which have opened a new world for peripheral nerve repair strategy. Of course, the role of non-surgical treatment in the repair of the peripheral nerves is not negligible. In a randomized controlled trial of the patients with incomplete long-term PNI, 780 nm laser irradiation treatment found that a specific wavelength of light can gradually improve the peripheral nerve function, leading to significant functional recovery (25). In addition, electro-photoluminescence has the potential to affect the peripheral

nerve regeneration. A study found that after electroacupuncture stimulation of the injured site, mir-1b in the injured local nerve was regulated downward. *In vitro*, it was confirmed that the overexpression of mir-1b could inhibit the expression of brain-derived neurotrophic factor (BDNF) in the rat Schwann cell line. The research may indicate that the electroacupuncture might promote Schwann cell proliferation and recovery of sciatic nerve function by downregulating mir-1b (26).

A traditional Chinese medicine can also reduce neuronal mortality and promote the peripheral nerve regeneration. The treatment methods typically include internal and external application, acupuncture, massage, and bloodletting. The studies have demonstrated that the active principles of the traditional Chinese medicine have effects on anti-apoptosis and neurogenesis (27). Taking the example of Buyang Huanwu decoction, it contains four active ingredients, namely, alkaloid, glycoside, polysaccharide, and aglycone. It was found that they may reduce the production of inflammatory cytokines and reduce the neuronal apoptosis by inhibiting the expression of caspase-1 and caspase-3 (30). As well, Zhong et al. used a preparation of Astragalus (refined from the active ingredients of Astragalus extract) as an inducer and found that it may regulate

the differentiation of MSCs into the neuron-like cells through the Wnt signaling pathways. This discovery provides an idea for using the preparation of Astragalus in the treatment of neurodegenerative diseases (28). Li et al. studied that Astragalus polysaccharides can protect mitochondria and anti-aging by inhibiting mitochondrial damage and recovery reactive oxygen species (ROS), meaning that they can be used to repair peripheral nerve damage (31).

## Repair Strategies for Nerve Tissue Engineering

After nerve suture, it took a long time to appreciate the importance of the nerve injury repair strategies and mechanisms. Until medical research entered the microcosmic domain, the neural microenvironment, the assessment of nerve injury, and the cellular and molecular mechanisms involved, and the clinical therapeutic principle for peripheral nerve injury were gradually understood. The methods of nerve tissue engineering for peripheral nerve injuries were also developed.

The core of nerve tissue engineering is to establish the composition of a three-dimensional complex with the cells and biological materials, and the key is manufactured scaffolds, selecting the seeding cells, and adding the neurotrophic factors (32). Compared with other treatment methods, it has taken another route, which develops nerve guidance conduits (NGCs) together with the nerve regeneration cells and small biological molecules to replace the traditional nerve transplantation. The applications of NGCs effectively avoid an immune response, and even in some of the injury scales, the clinical treatment effect is equivalent to the nerve autografts (33, 34).

Nerve repairing with the catheter is a part of the repair of PNI by tissue engineering, while recovering the function of the target organ is another part, especially rehabilitation treatment of muscle atrophy (35). That is because the nerve injury causes double damage. After PNI, muscle denervation causes atrophy and loss of original function.

The strategy of nerve tissue engineering to repair the PNI combines the advantages of the former two methods, changes the original repair ideas, and transforms the so-called “replace the damaged nerve” repair approach into “guide and promote the axon regeneration of the damaged part.” The purpose of nerve tissue engineering is to establish NGCs, which can accurately guide the reconnection of nerve stumps. In some long nerve gaps, it shows nerve recovery ability comparable with the autologous nerve transplantation (32, 35, 36). In the following chapters, the composition, development, and recent research results of NGCs will be described (Table 3). It can be seen from the table that excellent NGCs generally meet the following characteristics: absorbable/degradable, compound SCs/cytokine/cell affinity, topographic clues inside the scaffold conform to cell cord growth, and good elasticity/ductility. These NGCs can adapt to the repair of peripheral nerves with longer gaps (15 mm gap) and effectively promote nerve regeneration. Among them, the NGCs of the composite biomaterials have superior biocompatibility, biodegradability, excellent mechanical properties, good mechanical properties, and cell affinity. They are

superior to the natural polymers and non-biosynthetic polymers in repairing the nerves (39, 44).

## Scaffold

Scaffold fabrication is the cornerstone of NGCs. An ideal scaffold should be passive physical support connecting the proximal and distal stump and positively meeting the needs of multiple nerve regeneration. The appropriate scaffolds should provide a place for the growth factors to gather and SCs to grow, protect, and guide the proximal stump to extend to the distal stump, like a bridge to connect the distal nerve (45). At the same time, it can isolate the newborn axons from the external environment, avoid many inflammatory cells infiltrating into the injured area, help accumulate high concentration neurotrophic factors in it, and reduce scar formation and neuroma (46). In addition, the effects of topographic cues, electrical conductivity, and biodegradability on the nerve regeneration cannot be ignored (47). Although there is no ideal technology reported so far, there are many research reports on the design, materials, and fabrication methods of scaffolds (33, 36, 38).

In general, the scaffold should maintain a tubular or linear structure to adapt to the natural bundle structure of axons in biological design. Its primary characteristics include good mechanical properties, well-biocompatibility, biodegradability, and enough permeability (7). The collagen fiber, fibrin, poly (lactic-co-glycolic-acid) (PLGA), polylactic acid or polylactide (PLA), poly (propylene fumarate) (PPF), and agarose can be used as the scaffold materials, and there are pros and cons associated with each process (34).

In 1982, it was reported that the non-degradable silicone tube was used to wrap the severed sciatic nerve of the rats to repair the 6 mm gap *in vivo* system (37). The semipermeable collagen catheter could also repair the PNI *in vitro* (48). However, these scaffolds are unstable and inactive *in vivo*, so they are gradually eliminated. With the progress of materials science, more and more high-quality polymer materials are studied. The new scaffolds can simulate the physicochemical and mechanical properties of the biological tissues, have muscular mechanical strength, and their degradation pattern is based on simple hydrolysis (49).

There was a composite hydrogel catheter used for nerve regeneration, which was made from different volumes of polyacrylamide, graphene oxide, gelatin, and sodium alginate (PAM/GO/Gel/SA, and PGGS). The conduit had good elasticity and good mechanical properties. The catheter was found to have high mechanical stress, which adapted to the unexpected changes of nerve tissue during exercise. It promoted the regeneration of the sciatic nerve in the rats (36).

The agarose-based biomaterials have also achieved success in *in vitro* experiments. It is a polysaccharide extracted from agar found in red algae, which has biocompatibility and non-immunogenicity (50). The template agarose scaffolds made of the fiber bundles enhanced the structural stability and promoted the growth and extension of regenerated axon tissue in the gap of more than 10 mm (51). Chávez-Delgado et al. (52) used a chitosan prosthesis containing neurosteroids (progesterone [PROG] and pregnenolone [PREG]) to bridge

**TABLE 3 |** Material, design, and applications of currently available nerve guidance conduits (NGCs).

Material	Composition, design	Animal,Nerve	Injury	Outcome	References
Silicone	Non-degradable silicone tube	Rat, sciatic nerve	6 mm gap	The nerve trunk regenerates in the first few weeks, bridging the gap between the proximal and distal stumps. And in each case, the regenerative nerve appears as a cord-like structure surrounded by transparent liquid.	(37)
Collagen	Combination of absorbable collagen catheter and autologous SCs (200,000 cells/ $\mu$ l)	Rat, sciatic nerve	13 mm gap (critical size defect)	After 4 weeks, the addition of Schwann cells can enhance the regeneration of myelinated axons. After 16 weeks, the regeneration effect is similar to the reversed autograft.	(38)
Composite hydrogel	Catheters composed of different volumes of polyacrylamide, graphene oxide, gelatin, and sodium alginate (PAM/GO/Gel/SA, PGGS), inner diameter 2 mm, diameter 6 mm	Rat, sciatic nerve	3 mm gap	The catheter has good elasticity, flexibility, and mechanical properties. HE staining, Masson's trichrome staining, and immunohistochemistry have confirmed that it has a good effect in repairing sciatic nerve injury.	(34)
Poly (L-lactic acid) (PLLA)/soy protein isolate (SPI)	Highly oriented poly (L-lactic acid) (PLLA)/soy protein isolate (SPI) nanofiber nerve catheter	Rat, sciatic nerve	10 mm gap	Three months later, the motor function recovery of the experimental group was better than that of the autograft group. The density of regenerated myelinated nerve fibers in the BMSC-(BDNF + GDNF) group was 16,940.5/mm <sup>2</sup> , higher than the autograft group (16,206.4/mm <sup>2</sup> ).	(39)
Collagen, chitosan	Chitosan tubes bind aligned extracellular matrix (proteins and cells)	Rat, sciatic nerve	15 mm gap (critical size defect)	Four months later, SC alignment bracket 15 mm nerve defect that the regeneration success rate was 100%.	(40)
Peripheral epineurium	Embryonic stem cell-derived neural progenitor cells are implanted into the gap between nerve stumps, and the peripheral epineurium serves as a natural conduit.	Rat, sciatic nerve	10 mm gap	Three months after nerve transection, H & E staining examination showed significant apparent transected nerve regeneration and nerve reconnection.	(41)
Heterogenous fibrin	Heterologous fibrin sealant scaffold	Rat, sciatic nerve	5 mm gap	Catwalk and von Frey's functional recovery tests showed the regeneration of sensory fibers and active recovery.	(42)
Poly ( $\epsilon$ -caprolactone) (PCL), nanofibers	IL-10 Conjugated electrospun poly ( $\epsilon$ -caprolactone) (PCL) nanofiber	Rat, sciatic nerve	10 mm gap	The scaffold can significantly change the phenotype of macrophages in the body and affect peripheral nerve regeneration.	(43)
Hydrogels, nanoparticles	3D printed nerve catheters with an inner diameter of 1.5 mm, an outer diameter of 2.5 mm, and a length of 13 mm	Rat, sciatic nerve	10 mm gap	The nerve in the experimental group recovered well, and the nerve conduction velocity was 30.4 m/s after 3 months, which was not much different from the autograft group (33.9 m/s).	(44)
Human fibroblasts	Bio3D catheter with an inner diameter of 2 mm and a wall thickness of 500 microns	Rat, sciatic nerve	5 mm gap	At 24 weeks after surgery, no tumors were observed in any rats in the Bio3D group. They showed apparent nerve regeneration, which was not significantly different from the autologous nerve transplantation group.	(33)

the 10 mm gap in the facial nerve of a rabbit. The chitosan scaffold has good biocompatibility and slow degradation rate, and can be used as a guide channel for the axon growth. In addition, the chitosan scaffold can act as an *in-situ* neurosteroid delivery device, a long-term release neurosteroid carrier. After 45 days, the regenerated tissue showed myelinated nerve fibers of different sizes and shapes. The statistical methods showed that there was a significant difference between the experimental

group and the control group, and the PROG-loaded chitosan prosthesis produced the best-guided nerve regeneration and recovery. The chitosan scaffolds containing PROG are used to bridge the 10 mm gap in the facial nerves of a rabbit, which can promote the regeneration of the injured peripheral nerves, indicating that the function of damaged nerves may be improved by PROG and other neurotrophic substances (53). A chitosan nerve conduit impregnated with neurosteroid PROG



was used to induce regeneration of the sciatic nerve in the adult female dogs repairing a 15 mm defect. The histological analysis and electron microscopy studies showed that the damaged distal nerve segment showed a structure similar to that of a normal nerve (54).

Of course, it is too broad to rely solely on the scaffolds to promote peripheral nerve regeneration. With the development of research on the mechanism of nerve injury and repair, the role of nerve scaffolds in the nerve regeneration has become more diverse. Zhang et al. found that the effect of peripheral nerve regeneration with multiple factors (cells, growth factors, and scaffolds) is higher than that of a single element and can be compared with the effect of autologous nerve transplantation. The benefit of multiple factors is higher than that of a single factor and can be compared with the impact of nerve autografts. The bone mesenchymal stem cells (BMSCs) were introduced into the highly oriented poly (L-lactic acid) (PLLA)/soy protein isolate (SPI) nanofiber nerve conduits as the seed cells to repair the 10-mm sciatic nerve defects in the rats, and showed promising results and superior to the autografts group in some aspects (39).

The fabrication of scaffolds is not only simple stacking or cutting. The most common manufacturing methods include solvent casting (with or without salt leaching), gas foaming (with or without the leaching), phase separation, freeze-drying, and electrospinning (55). In addition, the topographic cues and fillers inside the scaffolds also increase the difficulty of production. Therefore, given these requirements, the traditional process is modified, and some unique manufacturing methods are adopted, such as micro-patterning, injection molding, unidirectional freezing, and electrospinning (9).

## Cell-Based Therapy

The process of nerve regeneration could be promoted by the cells. This is a rather complex and highly coordinated cell–cell interaction process in which the cellular, molecular pathways are not yet clarified (56). Therefore, the effect of the single-cell pathway on the nerve regeneration process is uncertain and may even be harmful. However, from the perspective of cells, the problem will become relatively simple.

In many studies, the scaffolds are combined with the peripheral nerve regeneration cells or cells that produce certain NGFs, and the nerve repair effect is enhanced (**Table 4**) (38, 40, 41). Berrocal et al. (38) used autologous SCs (200,000 cells/ $\mu$ l) combined with an absorbable collagen catheter, which was applied to the sciatic nerve defect in the rats. The results showed that the combined catheter enhanced the bridge PNIs with the extended segmental defects. Gonzalez-Perez et al. (40) placed MSCs and SCs in the chitosan tubes filled with collagen gel, respectively, which were used to repair a critical size defect of 15 mm in the rat sciatic nerve. And it was found that the SC-aligned scaffolds had the best repair effect. This means that cell technology can be applied to nerve tissue engineering, especially in the long nerve gap, and has a good prospect.

## Schwann Cells

Schwann cells are glial cells in the peripheral nervous system and the most used seed cells in nerve tissue engineering. It can be

made and repaired together with the scaffolds or used to enhance nerve regeneration separately. It is divided into a myelinated cell and a non-myelinated cell according to its structure and function. The former surrounds thicker axons, while the latter surrounds some slender axons. They can support and nourish nerves, have the ability to repair peripheral nerve damage and promote peripheral nerve regeneration. After the PNI, they reversely differentiate into the progenitor-like cells through genetic reprogramming and further transform into a repair phenotype to participate in the peripheral nerve regeneration (18, 64). The complex process involves the downregulation of the myelinating genes and high expression of regeneration genes, such as the upregulation of transcription factors (e.g., c-Jun) and histone deacetylase 1 and 2 (HDAC1/2) are involved (18, 19).

Schwann cells have two main functions for peripheral nerve repair: creating an environment to support the nerve regeneration and guiding the direction of axon growth. After nerve fiber transection, on the one hand, myelin sheath and axon fragments need to be removed timely and effectively. Otherwise, the efficiency and accuracy of reinnervation will be reduced if the time is too long, which is not conducive to the tissue regeneration (65). In the distal stump, the SCs and macrophages can help axons produce a pro-migration environment by removing the axons and myelin fragments.

On the other hand, if nerve stumps atrophy or nerve defect, the gap will be filled with the inflammatory cells and mediators, peripheral nerve cells, fibroblasts, and ECM, which is called the “bridge” of a new composite tissue reconnection. SCs need to cross the new bridge to guide axon regeneration. It cannot migrate directly into the matrix and depend on the macrophages, which secrete vascular endothelial growth factor A (VEGF-A) and other cytokines. Angiogenesis, remyelination, and axon regeneration occur sequentially in the process of nerve regeneration. A VEGF-A is a critical biological factor in angiogenesis, promoting the development and formation of blood vessels and protecting the motor and sensory neurons (66). Neuromodulin 1, endothelin, and Notch signaling molecules are also involved in this process. Neuromodulin 1 regulates the SC migration and proliferation by binding to ErbB2/3 on SCs. Notch signaling accelerates the SC transformation (67). When the SCs form cord-like structures along the new blood vessels and pass through the new bridge, that can guide the axons to grow in the right direction and promote peripheral nerve regeneration (18, 68). In addition, the effects of cytokines secreted by the SCs on the peripheral nerve regeneration cannot be ignored, such as neurotrophic factors (e.g., GDNF and artemin), adhesion molecules (e.g., N-cadherin and N-CAM). The studies have shown that the SCs can synthesize some neurosteroids (such as, PROG and PREG). These synthetic products can interact with the intracellular receptors to activate the synthesis of some myelin proteins (P0 and PMP22) or act on the gene encoding transcription factors Krox-20 (Egr-2) to induce myelination (52).

Many studies have shown that there are many difficulties in using the NGCs to repair the peripheral nerve (3, 32, 57). For example, the effect of repair *in vivo* is poor, and some severe injuries cannot be fully repaired. This may be since nerve conduits can only provide physical support but

**TABLE 4 |** Commonly used cells in neural tissue engineering.

Cell	Repair mechanism	Advantages and disadvantages	Applications	References
Schwann cell	Create an environment that supports nerve regeneration; guide the direction of axon growth	The most commonly used, but lack of some characteristics of ideal transplantable cells for tissue engineering, such as easy harvesting, rapid expansion in culture, and low immunogenicity	NGCs filled with SCs can repair long segment gaps faster and more effectively than using tubes alone.	(3, 32, 57)
Stem cell	Embryonic stem cells	Highly undifferentiated; totipotent; the neural phenotype of embryonic stem cells can differentiate into Schwann cells and perform physiological functions after transplantation.	Easy to obtain, rapid culture expansion, prone to tumorigenesis, moral and ethical issues	Using the peripheral nerve membrane as a natural conduit, implanting embryonic stem cell-derived neural progenitor cells can promote the repair of severely damaged peripheral nerves. (41)
	Neural stem cells	Can differentiate into neurons and Schwann-like cells and secrete various important neurotrophic factors, such as brain-derived neurotrophic factor, fibroblast growth factor, nerve growth factor.	Easy to obtain, rapid culture expansion, prone to tumorigenesis, moral and ethical issues	Intravenous injection of neural stem cells (NSCs) can cause physiological nerve repair, thereby reducing neuropathic pain symptoms. (58, 59)
	Induced pluripotent stem cells	Terminally differentiated cells return to a pluripotent state or form embryonic stem cell lines under specific conditions.	Abundant sources, easy to obtain, rapid expansion, difficult to cultivate, and prone to tumorigenesis, moral and ethical issues	Implantation of polycaprolactone (PCL) scaffolds loaded with activated Schwann cells (ASCs) and neural stem cells derived from induced pluripotent stem cells can promote the recovery of motor function in rats. (60)
	Adipose Stem Cell	Differentiate into various phenotypes, such as osteoblasts, chondrocytes, and muscle cells. Neurophenotypic ASCs have neurotrophic characteristics, can differentiate into Schwann cell-like cells, and secrete a variety of neurotrophic factors (nerve growth factor, brain-derived neurotrophic factor)	The most practical, easy to harvest, and has a strong potential for differentiation, moral and ethical issues	ADSCs can improve neuronal differentiation and nerve repair. (61–63)
Macrophages	M2 type macrophages are activated in a specific microenvironment and participate in anti-inflammatory response and tissue repair; remove axons and myelin fragments in the distal stump to promote axon regeneration.	The function of macrophages can be adjusted by changing the microenvironment, such as up-regulating IL-10 and collagen VI; the use of regulatory factors requires fine control to produce positive therapeutic value.	Electrospinning combined with IL-10 and PCL nanofiber scaffolds can induce the polarization of macrophages to the M2 activation state and participate in nerve repair.	(56)

cannot give endogenous or exogenous regenerative power. Therefore, simple nerve conduits are only suitable for supporting the growth of the nerve cells and transporting nutrients, and cell therapy is an essential basis for repairing the PNI. The first crucial step to introduce the SCs based therapy into the PNI is to add SCs to the guiding catheter. It is proved that the NGCs filled with SCs can repair the long segmental spaces faster and more effectively than the tubes alone (38). In addition, in some studies, SCs were programmed to overexpress neurogenic factors, such as VEGF-A, and then loaded onto the inner wall of hydroxyethyl cellulose/soy protein isolate/polyaniline sponge (HSPS) conduits and transplanted to the damaged site. Three months later, immunofluorescence co-staining showed that MBP + Schwann cells in the HSPS-SC (VEGF) group were far superior to the HSPS-SC group (MBP protein is a biomarker of Schwann cell myelin differentiation). The final results also showed that the NGCs have good

functional and morphological repairs to peripheral nerve injuries (66, 69).

### Stem Cells

Because of the critical role of endogenous regeneration in the PNI, the SCs are often used as transplantable cells for nerve repair, which has been proved by *in vivo* and *in vitro* experiments. Moreover, due to the limited proliferation ability of SCs, their application is minimal. The self-renewing ability and pluripotency of stem cells are considered the ideal source of seed cells in nerve tissue engineering. Therefore, the scientists hope to repair nerve injury by replacing the cells (35).

Unlike SCs, the stem cells are abundant in variety and source. Nerve tissue engineering needs to be screened from many stem cells. The cells need to include the following characteristics: easy to obtain, rapid expansion in culture, survival in the damaged site, stable transfection and expression of foreign

genes, and binding with NGCs (70). The embryonic stem cells (ESCs), neural stem cells (NSCs), induced pluripotent stem cells (IPSCs), mesenchymal stem cells (MSCs), adipose-derived stem cells (ASCs), neural crest stem cells, dental pulp stem cells, skin, and umbilical cord-derived those can be used to repair the PNI (35, 70).

The ESCs are highly undifferentiated cells with totipotency, differentiating into all the tissues and organs in the adult animals. The ESCs can be induced into a neural phenotype before transplantation and can differentiate into the SCs and play their physiological functions after transplantation, promoting angiogenesis, nerve growth, and myelination. Compared with the SCs, the proliferation of ESCs is more active. Moreover, the SCs differentiated by the ESCs can express relevant markers, glial fibrillary acidic protein, S100, and p75, and induce neuronal myelination (71). In the rat sciatic nerve transection model, using a peripheral nerve adventitia as a natural conduit, implantation of the neural progenitor cells derived from the ESCs can promote the repair of severely injured peripheral nerve (41).

The NSCs are the primitive cells in the nervous system and are also the essential cellular sources of the neurons and glial cells. They can be used as an essential cell source for the nerve regeneration. A study showed that after implantation of the NSCs, the abundance of IL12p80 will increase, which directly stimulates SC differentiation and promotes the peripheral nerve recovery (72). The NSCs combined with the NGCs can differentiate into the neurons and Schwann-like cells and secrete many important neurotrophic factors, such as brain-derived neurotrophic factor, fibroblast growth factor, NGF, insulin-like growth factor, and hepatocyte growth factor, after transplanting to the injured area, to promote nerve regeneration (58).

In addition, intravenous injection of the NSCs can cause physiological nerve repair, thus reducing the neuropathic pain symptoms (59). IPSC has always been a research hotspot, which refers to the recovery of terminally differentiated cells to totipotent state or the formation of ESC lines under the specific conditions. In relevant studies, the implantation of polycaprolactone (PCL) scaffolds loaded with active Schwann cells (ASCs) and IPSC-derived NSCs at the injured site can improve the motor recovery of the rats (60). Bone marrow MSCs are pluripotent adult stem cells obtained by bone marrow aspiration or artificial culture. The rat bone marrow MSCs are used as the supporting cells introduced into the silk fibroin (SF)-based scaffolds. After catheter transplantation, it was found that the gene expression of S100 and several growth factors (brain-derived neurotrophic factor, ciliary neurotrophic factor, and fibroblast growth factor) were upregulated, and many ECM components were secreted, such as collagen, fibronectin, and laminin promote the histological and functional recovery of the damaged sciatic nerve in the rats (73). The chitosan/PLGA nerve scaffolds containing autologous MSCs have been used to repair the long nerve gaps in the large animals (such as bridging the 50-mm-long gap in the sciatic nerve of a dog) (74).

At present, the ASCs are the most valuable source of the transplanted cells (57). The ASCs can be separated from human subcutaneous fat by conventional liposuction under anesthesia and cannot be used directly to repair the nerve

damage. They need to be further separated from the vascular matrix component. Otherwise, it will block the nerve conduit and hinder regeneration (59). They are easy to harvest and have strong differentiation potential. They can differentiate into various phenotypes along the mesoderm lineage, such as osteoblasts, chondrocytes, and muscle cells. The neural phenotypic differentiation of ASCs involves a combination of multiple growth factors. The ECM molecules affect the cell viability, adhesion, and neurotrophic behavior of differentiated ASCs, and coating them with the nerve ducts can increase the regeneration rate. Recent studies have shown that human platelet lysates have neurotrophic properties (such as the release of BDNF). The synergistic effect with laminin can enhance the neurotrophic effect of ASCs on the primary neurons *in vitro* (75). The acquisition site of ASCs (the best subcutaneous and perineal) will also affect the differentiation results (76). The neural phenotype ASCs have neurotrophic characteristics and express SC markers (such as glial fibrillary acidic protein (GFAP), S100, and the low-affinity receptor for NGF p. 75), which can differentiate into the SC-like cells and secrete a variety of neurotrophic factors. Several studies have shown that the ADSCs can improve the neuronal differentiation and neural repair (58, 59). Recent studies have found that the exogenous neurotrophic factors can be used to enhance the neurotrophic capacity of the human adipose derived stem cells *in vitro* (66).

In neural tissue engineering, the use of stem cells requires selecting the appropriate type, optimizing the number and methods of transplantation, and using the exogenous factors to ensure cell survival, reduce tumorigenicity, ensure safety, and maximize therapeutic efficacy (70). The human embryonic stem cells (HESCs) overexpressing the fibroblast growth factor 2 (FGF2) combined with heterogenous fibrin sealant scaffold can support the survival and regeneration of neurons in a mouse model of sciatic nerve injury (42). Beyond that, incorporating the ESCs, NSCs, bone marrow MSCs, adipose-derived cells, skin-derived precursor stem cells, and IPSCs enhances the therapeutic effect of tissue-engineered nerve graft (58).

## Macrophages

The macrophages are mononuclear phagocytes, which show significant plasticity *in vivo* and *in vitro*. As the prominent role of inflammatory cells, there are apparent morphological and functional differences in the different micro-environments. At present, it can be divided into two phenotypes, M1 and M2 macrophages. The M1 macrophages are effectors and induce cells in inflammation and participate in a positive immune response by secreting inflammatory cytokines and chemokines, which activation is related to the lipopolysaccharide (LPS) and interferon- $\gamma$  (IFN- $\gamma$ ) and so on. The M2 macrophages have a weak ability to antigen presentation, secrete anti-inflammatory cytokines, participate in anti-inflammatory response, and are activated in the specific micro-environments to play the role of immune regulation and participate in tissue repair.

After PNI, it will lead to a local inflammatory reaction, a large number of inflammatory factors are released. The macrophages are recruited by chemokines (CCL2) and gather in the area with other inflammatory cells, participate in Wallerian

degeneration of the nerve cells, polarize into anti-inflammatory phenotype (M2) under the effect of IL-4, and participate in axonal regeneration. The results show that the SCs and fibroblasts control and modify the macrophage reactions. And the cytokines secreted by them release into the blood, chemotactic monocytes, and change their behavior (56). In addition, the macrophages can facilitate axon regeneration by removing the axon and myelin fragments in distal stumps (10).

The regulation of macrophage function is an effective strategy to improve the peripheral nerve regeneration and repair injury. Currently, it is also included in the nerve tissue engineering to repair the nerve injuries. Jason R Potas et al. used electrospinning combined IL-10 with the PCL nanofibrous scaffolds to successfully induce the macrophages to polarize to the M2 activated state in the scaffolds and adjacent tissues around the nerve, which confirmed that macrophage function could be regulated by manipulating the cell micro-environment, thus affecting the process of nerve regeneration (43). Recent studies have shown that the macrophages polarize into the M2 type in the micro-environment after peripheral nerve injury, which may involve the IL-10 and collagen VI upregulated. Therefore, IL-4 and other factors can be embedded in the nerve guide catheter to promote the transformation of the macrophage M2 phenotype (11). However, the factors that promote the M2 polarization of macrophages may have the property of promoting tumor growth. Therefore, the use of these factors needs fine control to produce positive therapeutic value for the PNI (43). For example, through cell genetic engineering, some cells overexpressing the above factors are loaded on the nerve scaffolds, which can effectively regulate their release. Or changing the physical or chemical properties of scaffolds, and using additive manufacturing technology to construct the biological components of scaffolds layer by layer, so that the factors can be degraded and released at an appropriate time to achieve the purpose of peripheral nerve repair (3).

### 3D Bioprinting in Nerve Injury Repair

A new technique, 3D printing, is also called additive manufacturing, which means adding materials to make things. 3D printing technology was used in the industrial production before, but now bioprinting is common, such as bioprinting of NGCs (77). Micropatterning, injection molding, and unidirectional freezing are the unique manufacturing methods of some NGCs (9). In recent years, 3D printing has become a matured technique, and its most commonly used technologies include extrusion (fuse manufacturing and direct ink writing), powder, and photoinduced polymerization (77). 3D bioprinting is used to make the biomaterials containing cells, that is, to build the complex 3D functional living tissues or artificial organs (49).

Among them, the droplet-based bioprinting (DBB) can print tissue or organs embedded in the cells based on or without a scaffold. It has been reported that in the rat model, the effect of a 3D printed human fibroblast catheter in repairing a 5 mm nerve gap is not significantly different from that in autologous nerve transplantation (33). Because of the personalized manufacturing characteristics of 3D printing, it is possible to customize the size, shape, and structure required. The scaffolds printed

combine with biological factors (such as nanoparticles and cells) to achieve exact control (77). Therefore, the NGCs made of various biomaterials are produced through research. Recently, a catheter is developed, consisting of nanoparticles in hydrogel and hydrogel matrix. The hydrogel provided a physical microenvironment for axonal elongation, and the nanoparticles continuously released drugs to promote the nerve regeneration. The drug is an inhibitor of the Hippo pathway. The previous studies have found that the Hippo pathway affects the peripheral nerve regeneration (44). Therefore, Jie Tao et al. upregulated the downstream gene (yes-associated-protein) by targeting the inhibition of this pathway in the SCs to promote the regeneration of PNI. The experimental results showed that the functional nerve conduit could effectively induce the recovery of sciatic nerve injury (78).

### Muscle Tissue Engineering

The target organs of peripheral innervation include the sensory and motor organs (muscles). After PNI, the sensation will remain for several years, even for a long time to repair, and the function can be restored. In contrast, the denervated muscle progressively loses its ability to become reinnervated (35). In the process of muscle atrophy, the proteasome pathway and the autophagy-lysosomal pathway are activated, leading to different amount of loss of muscle mass and loss of its original function (79).

In neurogenic atrophy, it is proved that the regulation of myogenin is a regulator of muscle development and an inducer of neurogenic atrophy. After denervation, myogenin is upregulated, the expression of E3 ubiquitin ligases is decreased, and the muscle proteolysis and atrophy are promoted (80). A study reported that when the PNI was treated early, the atrophy of the distal muscle would stop, and the function would recover (35).

At present, in addition to exercise, there is no effective treatment for reducing the muscle atrophy. Jin Li et al. demonstrated for the first time that CRISPR-based genome editing can specifically target mir-29b in the mouse denervation model, thus preventing angiotensin-II (Ang-II) and myocyte apoptosis induced by Ang-II (81). Beyond that, intramuscular injection of various growth factors (such as IGF-1) and stem cells (such as muscle satellite cells) can also alleviate the muscle atrophy, but its mechanism is still unknown (35).

Additionally, laser phototherapy has potential therapeutic value. The 780 nm laser phototherapy could temporarily retain the function of denervating muscle and accelerate and enhance the axon growth and regeneration after the PNI or reconstruction surgery (25). However, tissue engineering may be a good choice when needed to reconstruct the muscle, and the regenerated axons cannot be accepted after extensive denervation.

The peripheral nerve regeneration strategy includes the treatment of muscle reinnervation. After the PNI, the innervated muscles will atrophy, and muscle regeneration depends on its endogenous regeneration ability (35). The satellite cells, a group of undifferentiated muscle cells located between the basal layer and the plasma membrane of the muscle fibers, are the main participants in muscle regeneration (79). But when the ability is exhausted, the muscle tissue engineering therapy is essential. However, up to now, muscle tissue engineering has not been able to restore all the functions of the replaced muscle (82). The reason



might be that the mechanical stress stimulation of the human muscle bundle is challenging to simulate fully. Therefore, the bioreactor can be used to stimulate the cells to achieve muscle tissue reconstruction *in vivo*.

However, the above conditions need to be met in nerve tissue engineering because the loose cells cannot regenerate directly, and scaffold support is also required. Perez-Puyana et al. used electrospinning technology to add elastin to the PCL-based scaffolds for skeletal muscle. The scaffolds had more petite size and better hydrophilicity, and the biocompatibility was also improved (83).

## SUMMARY AND FUTURE PROSPECTS

This review describes the pathophysiology of PNI and its repair strategies. The repair strategies involve the intersection of biology, medicine, materials science, and engineering. Neural tissue engineering has proposed many new approaches to manufacture the highly adjustable and functional scaffolds, which have great potential for repairing the PNIs. However, so far, a large number of NGCs developed, whether *in vivo* or *in vitro* experiments, still have a particular gap with autologous nerve transplantation. In the follow-up research, the physical, chemical, and mechanical properties of scaffolds need to be adjusted to optimize the interaction between the cells in the scaffold and the implanted tissue to realize the large-scale promotion and application of NGCs. The biodegradation rate of the scaffold also needs to be controlled to maintain its integrity until the regenerated tissue matures. In addition, the production technology of NGCs is also a hot spot of current research. The 3D bioprinted NGCs support the migration and proliferation of the seed cells. 3D printing based on digital light processing (DLP) technology can develop a more precise internal structure of the scaffold and promote more efficient nerve regeneration.

Given the severity and complexity of the PNI, the current tissue engineering repair strategy still has some problems to be solved. For example, what is the optimal time window for nerve guide catheter implantation? Is it possible to use clinical interventional therapy to reduce the secondary

damage to the injured site by implant surgery? In the above, the delivery of the nerve cells, NGF, and drugs can effectively promote axon regeneration, but the interaction of too many cytokines may cause adverse solid immune reactions. Therefore, the study of single-factor NGCs can become a future research hotspot. Vascularization is an essential factor in the survival and function of nerve grafts. The fascial flaps or vascularized nerve grafts have limited clinical applicability and therapeutic effects. The NGCs carrying angiogenic active substances (such as VEGF) is one of the focuses of current research and may become the development direction of NGCs in the future. In addition, a variety of nerve guiding catheters should be designed, such as multicellular components, pro-angiogenesis, and Büngner-like structures to improve the long-distance growth of axons. In the future, the functionalized NGCs produced by 4D printing will be one of the hot spots in the future research on the peripheral nerve regeneration.

## AUTHOR CONTRIBUTIONS

YL: methodology, writing an original draft, and visualization. ZM: conceptualization, methodology, validation, investigation, writing-review and editing, and funding acquisition. YR and DL: writing an original draft and writing-review and editing. WL: writing original draft. TL: investigation and writing-review and editing. JW: conceptualization, validation, and investigation. HM: conceptualization, validation, investigation, writing-review and editing, and supervision. JZ: validation, investigation, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was financially supported by the Shanghai Leading Talents Program in 2020 (No. 110), the National Key R&D Program of China (2020YFC2008700/2018YFA0703000), and the Project of Shanghai Jiading National Health and Family Planning Commission (KYXM 2018-KY-03).

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# Tissue Engineering Strategies for Peripheral Nerve Regeneration

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### Specialty section:

This article was submitted to  
Experimental Therapeutics,  
a section of the journal  
Frontiers in Neurology

Received: 31 August 2021

Accepted: 15 October 2021

Published: 16 November 2021

### Citation:

Li Y, Ma Z, Ren Y, Lu D, Li T, Li W,  
Wang J, Ma H and Zhao J (2021)  
Tissue Engineering Strategies for  
Peripheral Nerve Regeneration.  
Front. Neurol. 12:768267.  
doi: 10.3389/fneur.2021.768267

A peripheral nerve injury (PNI) has severe and profound effects on the life of a patient. The therapeutic approach remains one of the most challenging clinical problems. In recent years, many constructive nerve regeneration schemes are proposed at home and abroad. Nerve tissue engineering plays an important role. It develops an ideal nerve substitute called artificial nerve. Given the complexity of nerve regeneration, this review summarizes the pathophysiology and tissue-engineered repairing strategies of the PNI. Moreover, we discussed the scaffolds and seed cells for neural tissue engineering. Furthermore, we have emphasized the role of 3D printing in tissue engineering.

**Keywords:** peripheral nerve regeneration, nerve tissue engineering, pathophysiology, scaffolds, cells, 3D printing

## INTRODUCTION

A peripheral nerve injury (PNI) is a medical problem mainly caused by external trauma after stretching, tearing, or extrusion of peripheral nerves. (1) It has attracted social attention because of its enormous social and economic pressure. In the United States, the economic loss caused by nerve injury exceeds \$150 billion annually, and the treatment cost exceeds billions of dollars every year. People often underestimate the incidence of PNI, which seriously affects the quality of life of the patients (1–3).

The peripheral nervous system can self-regenerate and repair itself after injury. However, the repair is often slow and incomplete because axon extension depends on the synthesis and transportation of the intracellular substances, and the regeneration speed is similar to that of axon transportation, about 1–3 mm/day (4). In general, the stretch or crush injury results are better than those of transection. The recovery of the distal injury is better than that of the proximal injury, because axon growth only needs a short distance to reach the distal stump.

In the previous studies, substantial functional recovery of mild and moderate nerve injury can be achieved by surgical operation (such as, nerve suture and nerve transplantation) or non-surgical operation (such as, magnetic field, electric field, He-Ne Laser, and traditional Chinese Medicine) (3). The nerve suture and nerve grafts are extensively used for surgical nerve repair in the experiment and the clinic. An end-to-end nerve suture requires that the nerve stumps are aligned to achieve the best repair effect, and only the short nerve gap (<5 mm) can be used (5). When the damage gap is more significant (more than 5 mm), it is not possibly repaired by the meticulous microscopic surgery, and nerve autograft is regarded as the gold standard (3, 5). However, this damages the healthy nerves, and the number of donors is limited (6).

With the rapid development of cell biology and materials science, a new discipline, tissue engineering, is established to construct a different method of peripheral nerve repair. The core



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- Curso de Cirugía de la Mano, Clínica Marly, 2010
- XIV Curso internacional de ortopedia. Octubre de 2010
- III Curso de habilidades y entrenamiento en microcirugía. Febrero 2011
- LXXXIX Curso de actualización y entrenamiento en habilidades de cirugía artroscópica. Agosto 2011
- Congreso Nacional de la Sociedad Colombia de Ortopedia y Traumatología, Mayo 2011
- IV Curso Colombiano de medicina del football y trauma deportivo. Actualización en trauma deportivo pediátrica. Marzo de 2012
- Curso básico de protección radiológica. Agosto 2012
- Congreso de la Asociación Colombia de Cirugía de la Mano, Julio 2013
- Congreso Nacional de la Sociedad Colombia de Ortopedia y Traumatología, Mayo 2013
- XVI Cueso Internacional De ortopedia. Sociedad colombiana de ortopedia y Traumatología regional centro. clínicas Colsanitas. Noviembre 2013
- Curso del capítulo de Cirugía de Columna, Noviembre de 2013
- AO Trauma Básico. Febrero 2014

## PUBLICACIONES

- FABIAN GOMEZ ARDILA, "Aproximación a los enfoques terapéuticos actuales en ontogénesis imperfecta a partir de la biología de la enfermedad " Colombia, Revista de la Sociedad Colombia de Ortopedia y Traumatología , ISSN: 0120-8845 ed: vol 25 2011 Num 1 pag 50-58
- FABIAN GOMEZ ARDILA, " Descripción anatomía de la membrana interósea del antebrazo : estudio en cadáveres" Colombia, Revista de la Sociedad Colombia de Ortopedia y Traumatología , ISSN: 0120-8845 publicado por ELSEVIER España 2013;27(3):140-143
- FABIAN GOMEZ ARDILA, "Asociación de hemimelia de peroné y pie equino varo, reporte de caso " 2104. P, , Revista de la Sociedad Colombia de Ortopedia y Traumatología , ISSN: 0120-8845 publicado por ELSEVIER España 2013;27(4):210-221
- FABIAN GOMEZ ARDILA, POSTER CIENTIFICO, "Osteotomía periacetabular modificada en enfermedad de Perthes y evidencia radiología de extrusión de la cabeza femoral. Descripción de técnica quirúrgica y



experiencia clínica “ 58 congreso Nacional de la Sociedad Colombiana de Ortopedia y Traumatología. Mayo 2013

## **CONFERENCIAS**

- OSTEOTOMIA PERIACETABULAR MODIFICADA EN ENFERMEDAD DE PERTHES , XVI CURSO INTERNACIONAL DE ORTOPIEDIA SOCIEDAD COLOMBIANA DE ORTOPIEDIA Y TRAUMATOLOGIA REGIONAL CENTRO, CLINICAS COLSANITAS NOVIEMBRE DE 2013

---

## **EXPERIENCIA PERSONAL**

---

Experiencia Clínica, Hospital San José.

Servicio Social Obligatorio ECOPETROL S.A. ORITO PUTUMAYO. 2006

PRIDE COLOMBIA SERVICES, BOGOTA, 2007

Consulta externa, POLICIA NACIONAL DE COLOMBIA, 2007

Ayudante de quirúrgico, CLINICA LA CAROLINA, 2008-2009

Medico consulta prioritaria ortopedia y traumatología, INSTITUTO DE ORTOPIEDIA Y CIRUGIA PLASTICA, 2008-2010

Ayudante quirúrgico, CECIMIN, 2008-2010

Residente de Ortopedia y Traumatología, FUNDACION UNIVERSITARIA SANITAS, 2010-2014

Medico consulta Prioritaria, INSTITUTO DE ORTOPIEDIA Y CIRUGIA PLASTICA, 2010-2014

ORTOPEDISTA, Consulta prioritaria INSTITUTO DE ORTOPIEDIA Y CIRUGIA PLASTICA, 2014

ORTOPEDISTA, Organizacion SANITAS INTERNACIONAL, EPS SANITAS. Septiembre 2014

ORTOPEDISTA, CAFAM. Septiembre 2014

ORTOPEDISTA Famisanar Plan Complementario. Noviembre 2015

ORTOPEDISTA Allianz Colombia. Noviembre 2015

ORTOPEDISTA Generalli Colombia. Noviembre 2015



ORTOPEDISTA Adscrito Clinica Palermo. Septiembre 2014  
ORTOPEDISTA Adscrito Clinica VIP. Septiembre 2015  
ORTOPEDISTA Adscrito Clinica Los Nogales. Abril 2016  
ORTOPEDISTA Adscrito Instituto de Ortopedia y Cirugia Plastica. Septiembre 2014

---

### **REFERENCIAS PERSONALES**

---

Dr. Cesar Alvarado. Ortopedista Traumatólogo. Expresidente Sociedad Colombiana de Cirugía ortopédica y traumatología  
Instituto de Ortopedia y Cirugía Plástica.  
Tel: 6190311

Dr. Gilberto Sanguino, Ortopedista Traumatólogo, Cirugía de Rodilla, Clínicas Colsanitas. COLTRAUMA  
TEL: 2202727

Dr. Roberto Melendez, Ortopedista Traumatólogo, Cirujano de Mano, Clínicas Colsananitas, Clinica Reina Sofía, CECIMIN  
TEL: 3102543439

**FABIAN GOMEZ ARDILA**



CP- DM – NO. 215

LA CONGREGACION DE LAS HERMANAS DE LA CARIDAD  
DOMINICAS DE LA PRESENTACION DE LA SANTISIMA VIRGEN  
CLINICA PALERMO

CERTIFICA QUE

El doctor, Fabián Gilberto Gómez Ardila, identificado con cédula de ciudadanía número 79948576, es médico especialista en Ortopedia y Traumatología.


El doctor Gómez, es médico adscrito a nuestra Institución desde el 5 de Septiembre de 2014, quien atiende a sus pacientes en forma particular e independiente.

Durante este periodo y hasta la fecha el doctor Fabian Gilberto ha sido una persona responsable y consagrada a su profesión.

Se expide a solicitud del interesado para trámite ante Famisanar Complementario.

Bogotá, D.C., 3 de septiembre de 2015

Cordialmente,

  
DR. CIRO ALFONSO MEDINA TORRES



Copia: archivo

Feney M.

Calle 45C No. 22 - 02 PBX: 572 7777 - 742 0560 Fax: 700 6719  
www.clinicapalermo.com.co  
Bogotá, D.C. Colombia





**Instituto de Ortopedia  
y Cirugía Plástica.**

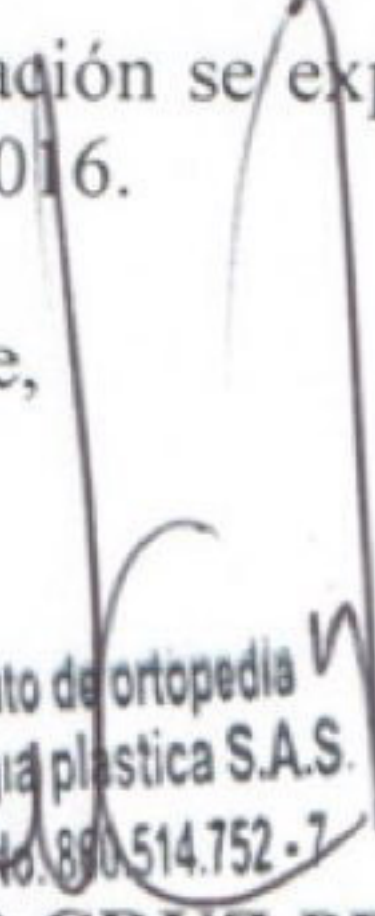

**INSTITUTO DE ORTOPEDIA Y CIRUGIA PLASTICA S.A.S**  
**NIT.860.514.752-7**

**CERTIFICA QUE:**

Yo MAURICIO CRUZ PEREZ identificado con cédula de ciudadanía 19.328.360 de Bogotá, Gerente del Instituto de Ortopedia y Cirugía Plástica S.A.S, certifico que el doctor FABIAN GOMEZ ARDILA identificado con cédula de ciudadanía número 79.948.576, trabaja en la Institución como es médico cirujano, especialista en Ortopedia y Traumatología general, el doctor ha demostrado ser un profesional intachable, de principios, valores dignos de exaltar y cumplidor de sus obligaciones.

Esta certificación se expide a solicitud del interesado a los Once (11) días del mes de Febrero de 2016.

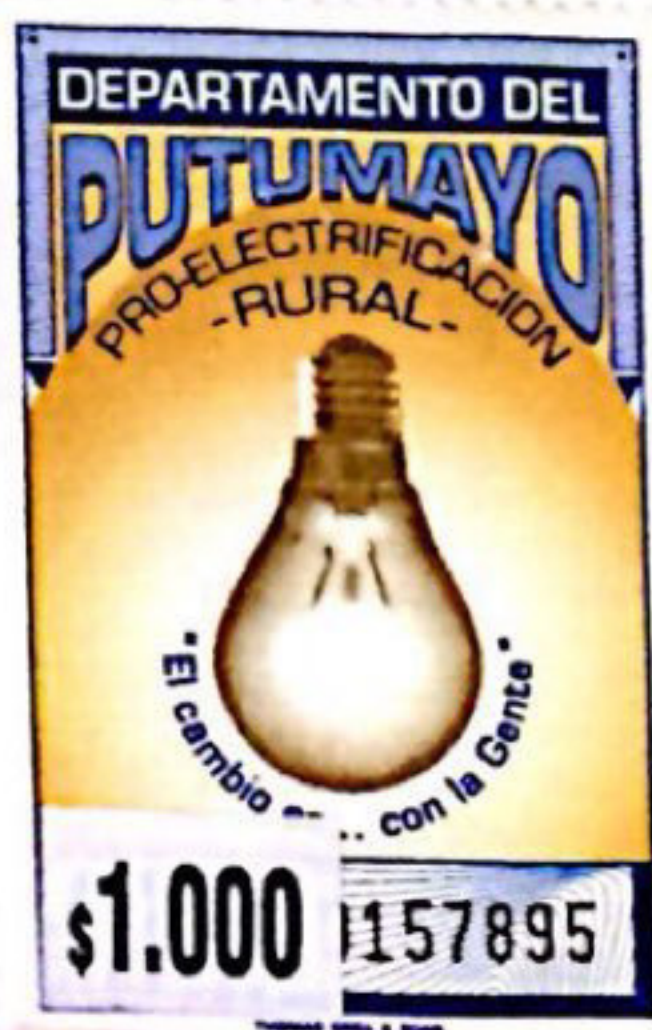
Cordialmente,

  
  
**MAURICIO CRUZ PEREZ**  
Gerente

---

Avenida 19 No. 114-87 - Línea Inmediata: 619 0311 - Fax 214 0358 Bogotá, D.C.  
[servicioalcliente@ortopediayplastica.com](mailto:servicioalcliente@ortopediayplastica.com)





**REPUBLICA DE COLOMBIA  
MINISTERIO DE LA PROTECCION SOCIAL**

**DEPARTAMENTO ADMINISTRATIVO DE SALUD DEL PUTUMAYO**

**RESOLUCION No 86-1715-2006**  
( 22 SEP 2006 )

Por la cual se concede una autorización

**LA DIRECTORA DEL DEPARTAMENTO ADMINISTRATIVO DE SALUD**  
en cumplimiento del Decreto 1875 de agosto 3 de 1994 y,

**CONSIDERANDO**

Que el Doctor FABIAN GILBERTO GOMEZ ARDILA, identificado con cédula de ciudadanía número 79.948.576 expedida en Bogota D.C, ha solicitado el registro de su TITULO de **MEDICO Y CIRUJANO** que le otorgó LA FUNDACIÓN UNIVERSITARIA DE CIENCIAS DE LA SALUD, de la Ciudad Bogota D.C. el día 16 de Diciembre de 2005.

QUE CUMPLIO CON EL SERVICIO SOCIAL OBLIGATORIO CON LA EMPRESA ECOPETROL S.A. EN LA CIUDAD DE ORITO, MUNICIPIO ORITO, DEPARTAMENTO DEL PUTUMAYO, durante el período comprendido entre el 27 de Enero hasta el 27 de Julio de 2.006.

**RESUELVE**

ARTICULO PRIMERO: Autorizar al Doctor FABIAN GILBERTO GOMEZ ARDILA, para ejercer la profesión de MEDICO Y CIRUJANO en todo el Territorio Nacional.

**COMUNIQUESE, NOTIFIQUESE Y CUMPLASE**

Dada en Mocoa, a 22 SEP 2006

**IRMA TULIA CAMACHO TEJADA**  
Directora

Elabora/R.H.D.

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## CERTIFICA

Que el Doctor: **FABIAN GILBERTO GOMEZ ARDILA**

Identificado con la cédula de ciudadanía No. 79.948.576 asistió al **CURSO BASICO DE PROTECCIÓN RADIOLOGICA**, dictado en la ciudad de Bogotá, los días 24 y 25 de Agosto de 2012, con una intensidad de 30 horas.

En constancia firma

  
**HECTOR FABIO CARDENAS C.**  
Física e Ingeniero Nuclear  
Especializado en Seguridad Radiológica  
Licencia Minisud Min. 001893 de 22/05/98









Centro Latinoamericano de Investigación  
y Entrenamiento en Cirugía de  
Mínima Invasión - CLEMI

Certifica que

***Fabián Gómez Ardila MD***  
***Participante***

Asistió al  
***LXXXIX Curso de Actualización y Entrenamiento en habilidades para  
Cirugía Artroscopia***

**Bogotá – Colombia Agosto 22 y 23 de 2011  
Intensidad: 18 Horas**

**Gabriel Oswaldo Alonso MVZ**  
**Coordinador de Formación**  
**CLEMI**

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Centro Latinoamericano de Investigación  
y Entrenamiento en Cirugía de  
Mínima Invasión - CLEMI

Certifica que

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Bogotá – Colombia Agosto 22 y 23 de 2011  
Intensidad: 18 Horas

Gabriel Oswaldo Alonso MVZ  
Coordinador de Formación  
CLEMI





República de Colombia  
Ministerio de Educación Nacional  
y en su nombre la

# Universidad Universitaria de Ciencias de la Salud

Personería Jurídica 10917 del 1° de Diciembre de 1976 del Ministerio de Educación Nacional

## Facultad de Medicina

En atención a que

Fabian Gilberto Gómez Ardila

C.C. 79.948.576 Stafaje de Bogotá

Cumplió satisfactoriamente con todos los requisitos del plan de estudios, le confiere el título de

## Médico y Cirujano

En testimonio de ello se firma y refrenda con los respectivos sellos en Bogotá, D.C., a los 16 de Diciembre de 2005

Presidente Consejo Superior  
Secretario General

Rector  
Decano

Secretario Académico

Número de Registro 0125 Número de Folio 003  
Bogotá D.C. 16 de Dic. de 2005

00698





## Sociedad Colombiana de Cirugía Ortopédica y Traumatología

**JUNTA DIRECTIVA**  
2013 - 2015

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**Suplente**

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**Fiscal Suplente**

Dr. Elkin Augusto Lozano G.

**Revisor Fiscal**

Sr. Fernando Latorre L.

**Gerente General**

Ing. Adrián Hernández A.

**EL DIRECTOR**

**EDITOR DE LA**

### **REVISTA COLOMBIANA DE ORTOPEDIA Y TRAUMATOLOGÍA**

CERTIFICA QUE EL ARTÍCULO

#### **Asociación de hemimelia de peroné y pie equino varo**

Autores

**Dr. Gabriel Ochoa del Portillo**

**Dr. Víctor Vargas**

**Dr. Fabián Gómez Ardila**

Se encuentra en proceso de edición para publicar en la próxima edición Vol. 27 No. 4 Diciembre de 2013 de la Revista Colombiana de Ortopedia y Traumatología.

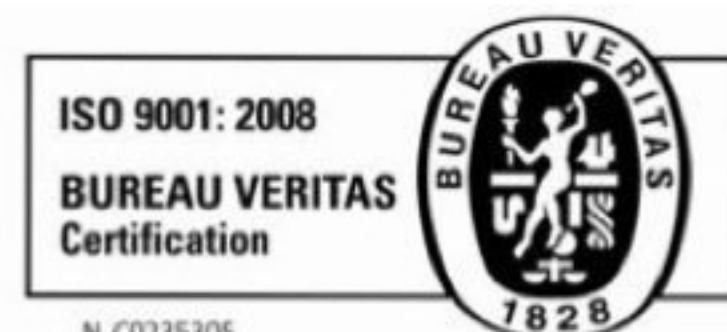
Atentamente,

**Dr. JUAN MANUEL HERRERA ARBELAEZ**

Director Editor

*Está certificación se expide a solicitud de los interesados en la ciudad de Bogotá a los dos días (02) del mes de Julio de 2014.*

Calle 134 No. 7B -83 Oficina: 201  
PBX: (571) 625 7445 – Fax: (571) 625 7417  
E-mail: [secretaria@sccot.org.co](mailto:secretaria@sccot.org.co)  
<http://www.sccot.org.co>  
BOGOTÁ, D.C. – COLOMBIA







FUNDACIÓN  
UNIVERSITARIA SANITAS  
ORGANIZACIÓN VALORES INTERNACIONAL

Personería jurídica reconocida mediante Resolución No. 3015 del 23 de diciembre de 2002

## FACULTAD DE MEDICINA

**ACTA DE GRADO No. EP099**  
(Folio 00099 del libro EP01)

En la ciudad de Bogotá, a los veintiocho (28) días del mes de agosto del año dos mil catorce (2014), la Fundación Universitaria Sanitas, en nombre de la República de Colombia y con autorización del Ministerio de Educación Nacional de la misma, como consta en el registro SNIES No. 54049, llevó a cabo el acto solemne de graduación mediante el cual, previo juramento, otorga el título de

## ESPECIALISTA EN ORTOPEDIA Y TRAUMATOLOGÍA

**A**

**FABIÁN GILBERTO GÓMEZ ARDILA**

Identificado(a) con C.C. No. 79.948.576 de Bogotá, quien cumplió con los requisitos académicos, legales y reglamentarios establecidos para conferir dicho título profesional. Por lo tanto, se le hizo entrega del Diploma No. 14BO57EP000099 que lo(a) acredita como tal.

Para constancia se expide y firma la presente Acta, válida para todos los efectos correspondientes.

Firmada por: **MARIO ARTURO ISAZA RUGET** - Rector, **JUAN DE FRANCISCO ZAMBRANO** - Decano Facultad de Medicina, y **JOHNS STEVE NAVARRO LARA** - Secretario General.

Es fiel copia tomada del original, expedida en Bogotá, el veintiocho (28) de agosto del año dos mil catorce (2014).

  
**JOHNS STEVE NAVARRO LARA**  
Secretario General





FUNDACIÓN UNIVERSITARIA DE CIENCIAS DE LA SALUD  
Personería Jurídica 10917 del 1º de Diciembre de 1976 del Ministerio de Educación Nacional

## Acta de Grado No. 005

En la ciudad de Bogotá, D.C. dieciséis (16) de diciembre de 2005, en el Auditorio Guillermo Fergusson, del Hospital de San José, de conformidad con el Acuerdo Número 1061 del Consejo Superior de la Fundación Universitaria de Ciencias de la Salud. Sesión Ordinaria No. 182 del (6) de diciembre de 2005 se realizó acto solemne para otorgar el título de :

Médico y Cirujano

A

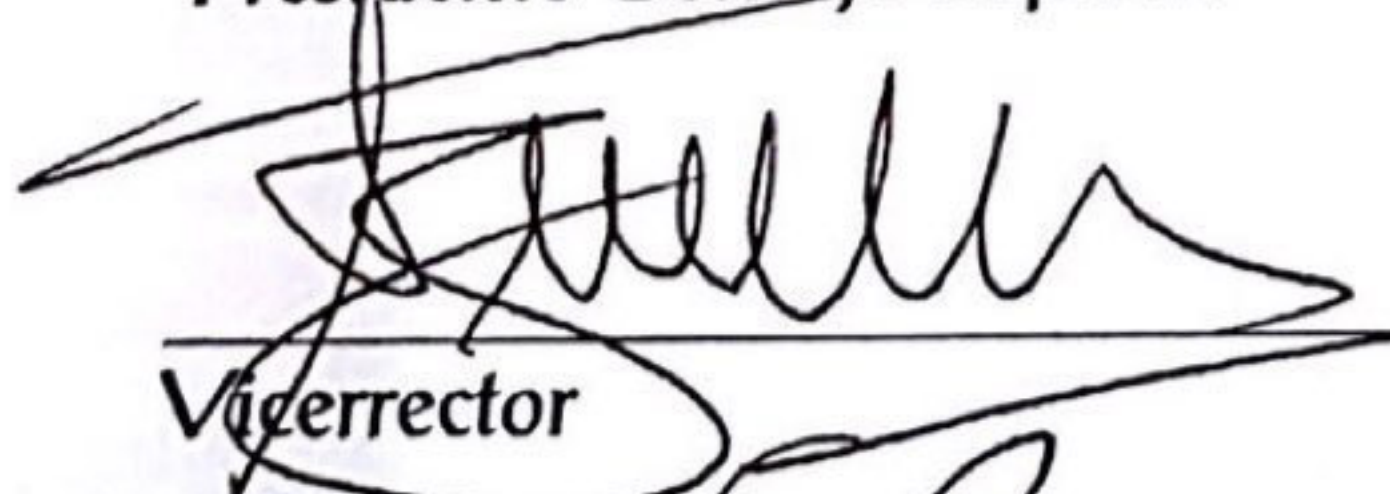
FABIÁN GILBERTO GÓMEZ ARDILA

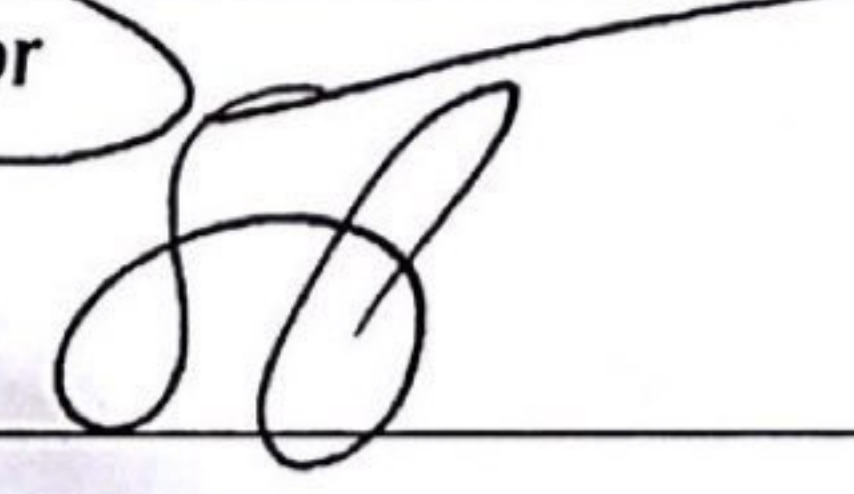
Identificado (a) con C.C. número 79,948,576 expedida en Santafé de Bogotá DC como consta en el acta número 005 del libro de actas de grado de la Facultad de MEDICINA


Se confiere el título en nombre del Ministerio de Educación Nacional en reconocimiento a que el mencionado estudiante cursó y aprobó todas las asignaturas del pénsum reglamentario para el programa de MEDICINA y llenó todos los requisitos exigidos para el efecto por la Fundación Universitaria de Ciencias de la Salud.

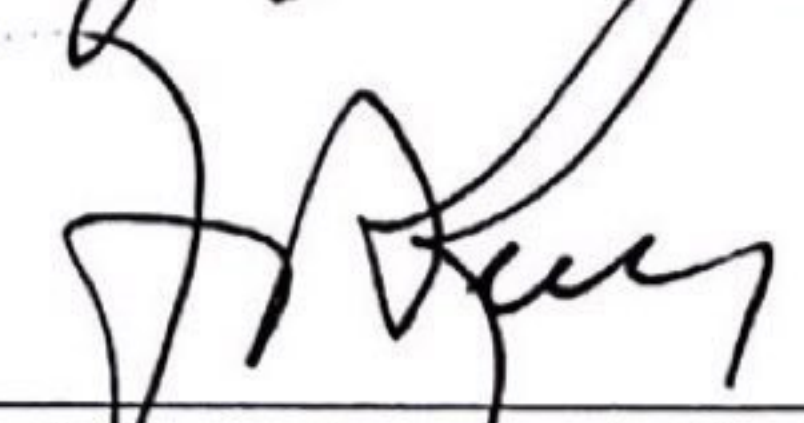
Para constancia de lo anterior se firma la presente acta en Bogotá, D.C. a los (16) días de diciembre de 2005 .

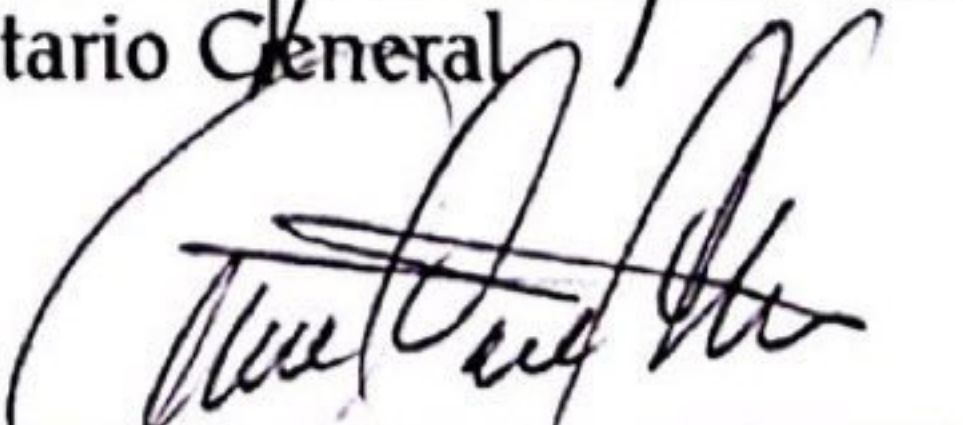
  
Presidente Consejo Superior

  
Vicerrector

  
Decano

  
Rector

  
Secretario General

  
Secretario Académico

00536

THOMAS GREG & SONS.

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Centro Latinoamericano de Investigación  
y Entrenamiento en Cirugía de  
Mínima Invasión - CLEMI

Certifica que  
***Fabián Gómez Ardila MD***  
***Participantes***

Asistió al  
***III Curso de Actualización y Entrenamiento en  
Habilidades para Microcirugía***

Bogotá – Colombia Febrero 18 y 19 de 2011  
Intensidad: 18 Horas

Francisco José Camacho MD  
Director de Investigación, Patentes y  
Desarrollo CLEMI  
Coordinador Curso

Gabriel Oswaldo Alonso MVZ  
Coordinador de Formación  
CLEMI

Jorge Felipe Ramírez MD  
Presidente CLEMI





# Sociedad Colombiana de Cirugía Ortopédica y Traumatología

EL DIRECTOR

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## REVISTA COLOMBIANA DE ORTOPEDIA Y TRAUMATOLOGÍA

CERTIFICA QUE LOS DOCTORES

**FRANCISCO JOSÉ CAMACHO GARCÍA**  
**CAROLINA RAMÍREZ MARTÍNEZ**  
**FABIÁN GÓMEZ ARDILA**  
**GABRIEL OSWALDO ALONSO CUÉLLAR**

Publicaron el artículo "**Descripción anatómica de la membrana interósea del antebrazo: estudio en cadáveres**" en la edición Vol. 27 No. 3 Septiembre de 2013 de la Revista Colombiana de Ortopedia y Traumatología.

Atentamente,

**Dr. JUAN MANUEL HERRERA ARBELAEZ**  
Director Editor

*Está certificación se expide a solicitud de los interesados en la ciudad de Bogotá a los dos días (02) del mes de Julio de 2014.*

### JUNTA DIRECTIVA 2013 - 2015

#### Presidente

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#### Vicepresidente

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#### Presidente Saliente

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#### Fiscal Principal

Dr. Edgar Estrada S.

#### Fiscal Suplente

Dr. Elkin Augusto Lozano G.

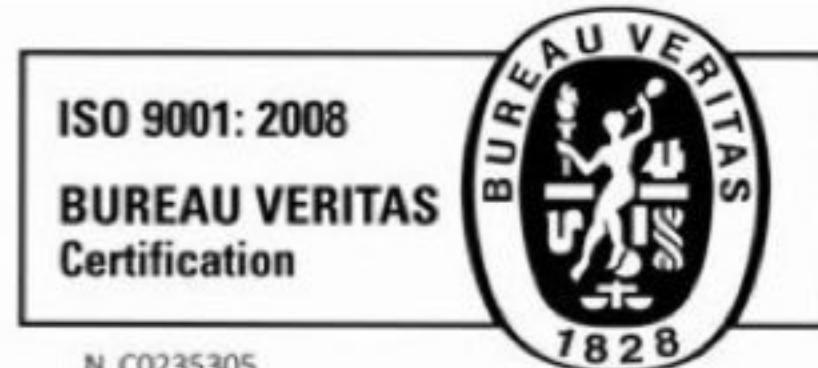
#### Revisor Fiscal

Sr. Fernando Latorre L.

#### Gerente General

Ing. Adrián Hernández A.

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E-mail: [secretaria@sccot.org.co](mailto:secretaria@sccot.org.co)  
<http://www.sccot.org.co>  
BOGOTÁ, D.C. – COLOMBIA







# Sociedad Colombiana de Cirugía Ortopédica y Traumatología

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**EL DIRECTOR**

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## **REVISTA COLOMBIANA DE ORTOPEDIA Y TRAUMATOLOGÍA**

CERTIFICA QUE LOS DOCTORES

**FABIÁN GÓMEZ ARDILA  
MAURICIO LARA GARAVITO  
CÉSAR E. ÁLVAREZ QUINTERO**

Publicaron el artículo "**Aproximación a los enfoques terapéuticos actuales en osteogénesis imperfecta a partir de la biología de la enfermedad**" en la edición Vol. 25 No. 1 Marzo de 2011 de la Revista Colombiana de Ortopedia y Traumatología.

Atentamente,

**Dr. JUAN MANUEL HERRERA ARBELAEZ**  
Director Editor

*Esta certificación se expide a solicitud de los interesados en la ciudad de Bogotá a los dos días (02) del mes de Julio de 2014.*

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E-mail: [secretaria@sccot.org.co](mailto:secretaria@sccot.org.co)  
<http://www.sccot.org.co>  
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## Course Participant Certificate

Certifica que  
**Fabian Gómez**

Ha participado en el  
AO Trauma Curso - Básico de los Principios del Tratamiento  
de las Fracturas

Fecha/Ciudad  
Febrero 19 - 22, 2014      Medellín, Colombia

Director del Curso  
  
**Claudia Medina**

This certificate pertains only to the participant's completion of the educational activity and does not  
in any way attest to the proficiency of the participant's clinical or surgical expertise

  
Francisco Javier Pineda  
Vicepresidente de la  
Comisión Latinoamericana

  
Roberto Torres  
Miembro Honorario  
Comisión Latinoamericana

  
Luis Quiroga  
Presidente AO Foundation





República de Colombia  
**FUNDACIÓN UNIVERSITARIA SANITAS**

Personería Jurídica No. 30115 del 23 de diciembre de 2002

**Considerando que**

**Fabían Gilberto Gómez Ardila**

C.C. 79.948.576 de Bogotá


Cumplió los requisitos académicos exigidos para optar al grado de especialista, le confiere el título de

**Especialista en Ortopedia y Traumatología**

En testimonio de ello se firma y refrenda con los respectivos sellos el presente Diploma en Bogotá D.C. a los veintiocho (28) días del mes de agosto del año dos mil catorce (2014)

  
**Mario Arturo Isaza Ruget**  
Rector

Anotado al Folio No.000999 Libro de registro No. EP01  
14BO57EP000999

  
Francisco Zambrano  
Decano

  
Steven Navarro Lara  
Secretario General



