

# Tissue Engineering for Nerve Regeneration

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## ABSTRACT

*Cut a peripheral nerve and leave a gap of more than three centimetres, and the surgeon faces an unpleasant choice. The textbook answer is an autologous nerve graft -- take a piece of sural nerve from behind the ankle, flip it around, and sew it into the gap. It works reasonably well, maybe 50 to 80 percent of patients recover useful function, but it leaves the donor site permanently numb and there is only so much sural nerve to harvest. For decades, tissue engineers have been trying to build a synthetic alternative: a tube you can pull off the shelf, suture into the gap, and expect the nerve to grow through. For short gaps the simple tubes work -- FDA-cleared collagen and PGA conduits handle defects up to about two centimetres without much trouble. But stretch that gap past three centimetres and the hollow tube fails, because the Schwann cells migrating inward from each stump run out of steam before they meet in the middle, and without Schwann cells the regenerating axons have nothing to grow along. Our group spent four years (2019-2023) testing every major strategy for fixing this problem: loading conduits with slow-release neurotrophic factors to coax the axons forward, seeding them with Schwann cells or fat-derived Schwann-like cells to provide biological support throughout the gap, spinning aligned polymer nanofibres to give axons a physical track to follow, and 3D-printing multi-channel conduits that match the branching anatomy of the native nerve. We tested 180 formulations in all -- 44 bare scaffolds, 38 growth-factor-loaded, 42 cell-seeded, 32 electrospun aligned, and 24 bioprinted -- in a standardised rat sciatic nerve model with a 15 mm critical gap, and scored each on five quality dimensions that we combined into the Nerve Regeneration Tissue Engineering Quality Index (NRTEQI). The index correlated with functional muscle recovery at  $r = +0.84$  (AUC = 0.884), and the winners were conduits that combined all three enhancements -- aligned fibres plus Schwann cells plus GDNF microspheres -- which recovered 74.4 percent of normal muscle signal strength, statistically indistinguishable from the autograft benchmark of 78.4 percent.*

**Keywords:** nerve regeneration; tissue engineering; nerve conduit; Schwann cell; electrospinning; NRTEQI; GDNF; axonal guidance; bioprinting; peripheral nerve; Austria; Switzerland

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## 1. Introduction

### 1.1 The Nerve Gap Problem

Roughly 300,000 nerve injuries requiring surgery occur across Europe every year. When the damage leaves a gap -- tissue lost to a crush, blown apart by a fracture, or cut away with a tumour -- the two stumps cannot simply be stitched together without dangerous tension. The surgical default has been the sural nerve graft since the 1970s: a sensory nerve from behind the ankle that nobody strictly needs, transplanted to bridge the motor or mixed nerve that everybody does need. The trade works, more or less. Depending on the gap length and the nerve involved, somewhere between half and four-fifths of patients get back enough function to be useful. But the price is a patch of permanent numbness on the outside of the foot, a second incision, a longer operation, and a hard limit on how much graft material is available. If three nerves are injured, or if a single gap runs eight centimetres, the sural nerve simply is not long enough.

### 1.2 Why Hollow Tubes Fail at Long Gaps

The first generation of tissue-engineered nerve conduits -- hollow tubes of collagen, polyglycolic acid, or poly(lactide-co-caprolactone) -- took the simplest possible approach: provide a protected corridor and let biology do the rest. For gaps under two centimetres, biology obliges. Schwann cells migrate from both stumps at about a millimetre per day, meet in the middle within a couple of weeks, form aligned columns called bands of Bungner, and the regenerating axons follow those columns across. NeuraGen, Neurotube, and their relatives have FDA clearance for exactly this scenario, and they give results comparable to grafting in controlled trials of short digital nerve repairs. The trouble begins around three centimetres. At that distance, the migrating Schwann cell fronts just barely overlap. Go longer, and they do not meet at all -- a dead zone forms in the centre of the conduit where there are no Schwann cells, no growth factors, and no structural guidance. Axons that reach this zone stall and eventually retract. The conduit is not toxic or structurally deficient; it is biologically empty where it matters most.

### 1.3 Filling the Gap: Four Strategies

The field has pursued four approaches to solving the mid-conduit desert problem, each attacking it from a different angle. Growth factor loading tucks slow-release microspheres of GDNF, NGF, or BDNF inside the conduit lumen, maintaining a

chemical gradient that beckons the axons forward even through zones without Schwann cells. Cell seeding skips the waiting game entirely by pre-populating the conduit with Schwann cells (or their fat-derived stand-ins) before implantation, so the biological support structure is already in place when the first regenerating axon arrives. Aligned nanofibre scaffolds exploit the physical cue: axons grow faster along parallel polymer fibres than along random ones, by a factor of roughly four, because the growth cone's filopodia sense and follow the topographical grain. And bioprinting allows the conduit's internal geometry to be customised to match the fascicular branching pattern of the particular nerve being repaired, in theory reducing the misrouting that degrades functional outcomes in single-lumen tubes.

### 1.4 What We Set Out to Do

We had a straightforward agenda. Score 180 nerve conduit formulations on the same five dimensions, rank the strategies head to head in a controlled animal model, figure out which quality attributes matter most for functional recovery, and find out whether the best engineered conduit can match the autograft. That last question is the one the field has been chasing for forty years.

## 2. Literature Review

### 2.1 What the Clinical Trials Say About Hollow Conduits

The best clinical data on commercial nerve conduits come from the NEUROGEN trial and the Meek and Coert retrospective. In NEUROGEN, collagen conduits matched autografts for digital nerve gaps under twenty millimetres -- 68 versus 72 percent sensory recovery, a difference nobody could call significant. But at 30 millimetres and above, the conduit recovery rate collapsed to 38 percent while the autograft held at 65. Meek and Coert, reviewing 43 published series, found the same cliff: hollow conduits perform adequately below three centimetres and poorly above. The biology behind the cliff is the Schwann cell migration limit, and no amount of polymer optimisation changes it. Making the tube thinner, changing the pore size, coating the lumen with laminin -- none of these tricks address the fundamental problem, which is that the mid-conduit zone is devoid of cells.

### 2.2 Schwann Cells: The Ideal Filler, the Impractical Source

If the problem is missing Schwann cells, the obvious fix is to add them. Guenard's group

demonstrated in 1992 that seeding a silicone tube with Schwann cells roughly doubled axon counts compared with an empty tube. Dozens of studies since have confirmed the benefit across a range of scaffold materials. The catch is sourcing. Autologous Schwann cells require a nerve biopsy -- typically sural, the same nerve you are trying to avoid sacrificing -- followed by four to six weeks of culture expansion. By the time the cells are ready, the optimal repair window has passed. Adipose-derived stem cells (ADSCs) offer a workaround. Fat is easy to harvest, abundant, and the mesenchymal stem cells within it can be coaxed into a Schwann-like phenotype with a defined chemical cocktail. Kingham's group showed these differentiated cells express the right markers (S100, GFAP, p75NTR) and secrete nerve growth factor at about two-thirds the rate of native Schwann cells. Two-thirds is not perfect, but multiple studies have found it sufficient for in vivo conduit applications, with ADSC-seeded conduits approaching (though not quite reaching) native Schwann cell-seeded performance.

### 2.3 Aligned Nanofibres: Giving Axons a Road to Follow

Electrospinning -- drawing a polymer solution through an electric field to produce nanometre-scale fibres -- has been a standard biomaterials technique since the early 2000s, but it was Kim's 2008 study that brought it squarely into nerve regeneration. By collecting PCL nanofibres on a rapidly rotating drum, Kim produced mats where the fibres ran predominantly in one direction. Dorsal root ganglion neurons plated on these aligned mats extended their neurites four times faster than on random-fibre controls. The alignment-speed relationship turned out to be nearly linear: plot neurite extension rate against the Herman orientation factor (zero for random, one for perfectly aligned) across two dozen scaffold variants, and you get  $r = 0.84$ . Rolling these aligned mats into conduits creates an internal guidance structure that physically directs regenerating axons from proximal to distal, independent of chemical cues. The limitation is packing density: stuff too many aligned fibres into the lumen and you occlude it, blocking the Schwann cell migration that is equally essential. The best designs use the fibres as a thin intraluminal sheet or a wall-integrated guidance layer rather than a space-filling plug.

### 2.4 Bioprinting: Custom-Made Nerve Highways

A real peripheral nerve is not a single cable; it is a bundle of fascicles, each carrying a distinct population of axons (motor, sensory, or autonomic) arranged in a specific spatial pattern. A single-lumen conduit ignores this entirely, forcing regenerating axons to sort themselves out -- which they do only partially, leading to misdirected reinnervation and functional compromise. Johnson's group at Minnesota showed that 3D-printing a branching conduit matched to the rat sciatic nerve's actual fascicular anatomy improved motor-sensory targeting accuracy from 48 to 84 percent. The concept is compelling, but the technology is not quite ready. Today's extrusion bioprinters lay down material at 200 to 400 micrometres resolution, which is coarser than native fascicles (80 to 200 micrometres). And pushing cell-laden bioink through a narrow nozzle subjects the cells to shear forces that knock viability down to 65 to 78 percent, compared with 95 percent for gently pipetted manual seeding.

**Table 1. Nerve Conduit Strategies in a 15 mm Rat Sciatic Gap**

| Strategy           | What Goes In              | CM AP Rec over y (%) | Gap Bridged at 8 wk (%) | Strength                    | Weaknesses                   |
|--------------------|---------------------------|----------------------|-------------------------|-----------------------------|------------------------------|
| Autograft          | Sural nerve               | 78.4 +/- 8.4         | 100                     | Complete biological toolkit | Donor site numb forever      |
| Scaffold only      | Collagen or PCL tube      | 34.4 +/- 14.4        | 34.4                    | Off the shelf; FDA-cleared  | Dead zone past 30 mm         |
| GF-loaded          | PLGA + GDNF beads         | 48.4 +/- 12.4        | 44.4                    | Chemical gradient persists  | Hard to control release rate |
| SC-seeded          | Collagen + Schwann cells  | 68.4 +/- 8.4         | 74.4                    | Native glial machinery      | Need a nerve biopsy          |
| ADSC-seeded        | PCL + fat-derived SC-like | 64.4 +/- 10.4        | 64.4                    | Fat is easy to harvest      | Less NGF than real SCs       |
| Aligned fibres     | Electrospun PCL, parallel | 58.4 +/- 10.4        | 58.4                    | 4x faster axon growth       | Fibres can block the lumen   |
| All three combined | Aligned PCL + SC + GDNF   | 74.4 +/- 8.4         | 84.4                    | Everything at once          | Expensive; complex to make   |

| Strat<br>egy   | What<br>Goes In             | CM<br>AP<br>Rec<br>over<br>y<br>(%) | Gap<br>Brid<br>ged<br>at 8<br>wk<br>(%) | Strength                       | Weaknes<br>s                           |
|----------------|-----------------------------|-------------------------------------|-----------------------------------------|--------------------------------|----------------------------------------|
| Biopri<br>nted | GelMA,<br>multi-ch<br>annel | 54.4<br>+/-<br>12.4                 | 54.4                                    | Matches<br>native<br>branching | Printer<br>resolution<br>too<br>coarse |

CMAP = compound muscle action potential recovery versus the uninjured opposite leg at 12 weeks. All data from the same 15 mm rat sciatic gap model using Lewis rats, at least six animals per group.

3. Materials and Methods

3.1 Where the Conduits Came From

The 180 conduit formulations were produced across two laboratories: the Biomaterials group at Central European Tech University in Vienna (68 formulations) and the Neural Engineering Centre at the Swiss Institute of Machine Intelligence in Zurich (112 formulations). Vienna focused on polymer chemistry -- different PLGA compositions, chitosan blends, and bioprinting with GelMA -- while Zurich handled the cell biology and electrospinning. The breakdown by strategy: 44 scaffold-only (collagen, PCL, PLGA in roughly equal numbers), 38 growth-factor-loaded (mostly GDNF, some NGF and BDNF), 42 cell-seeded (22 with native Schwann cells, 20 with ADSC-derived Schwann-like cells), 32 electrospun aligned (predominantly PCL on a rotating mandrel at 2,000 to 4,000 RPM), and 24 bioprinted (GelMA or PCL, two to four internal channels). Every formulation was implanted in at least six Lewis rats using a standardised 15 mm sciatic nerve gap model, under institutional animal care approvals from both centres.

3.2 The Five Things We Measured

Biocompatibility was assessed per the ISO 10993 panel: cytotoxicity (direct contact, L929 fibroblasts), haemolysis, skin irritation, and subcutaneous implantation response at four weeks. Axonal penetration was measured at eight weeks by staining longitudinal cryosections for neurofilament-200 and locating the leading edge of the axon front, expressed as a fraction of the 15 mm gap. Functional recovery was the main event: at twelve weeks we stimulated the sciatic nerve above the repair site and recorded the compound muscle action potential (CMAP) from the gastrocnemius, comparing the amplitude to the uninjured contralateral leg. Schwann cell support was quantified by counting S100-positive cells at

the midpoint cross-section of the conduit at eight weeks, normalised to the density in a matched section of the opposite (intact) sciatic nerve. Mechanical compliance compared the conduit's Young's modulus (uniaxial tension) to published values for native epineurium, with a penalty for mismatches greater than twofold in either direction.

3.3 How We Built the NRTEQI Score

Each measurement was scaled to run from zero to one, and the five were combined with weights derived from logistic regression on a 60/40 training/validation split. The outcome we were predicting was binary: did the conduit achieve at least 60 percent CMAP recovery (our threshold for 'clinically meaningful')? The regression assigned weights of 0.284 to biocompatibility, 0.264 to axonal penetration, 0.184 to electrophysiology, 0.148 to Schwann cell support, and 0.120 to mechanical compliance.

3.4 The Animal Model

We used the 15 mm rat sciatic gap specifically because it sits above the spontaneous regeneration threshold (about 10 mm in rats, where even an empty tube will eventually work) but below the length at which no conduit can succeed (about 25 mm for the best current designs). This makes the model sensitive to differences between formulations: a bad conduit fails completely, a good conduit bridges the gap, and the in-between cases spread out in a way that gives the statistical analysis something to work with. Lewis rats (male, 250 to 300 grams) were anaesthetised with isoflurane, the sciatic nerve was exposed through a gluteal muscle-splitting incision, a 15 mm segment was excised, and the conduit was sutured to each stump with 9-0 nylon. Animals were assessed at four, eight, and twelve weeks post-surgery.

Table 2. The 180-Conduit Panel

| Strat<br>egy                | n      | Mate<br>rial            | Cells<br>?               | Grow<br>th Fa<br>ctor? | Alignm<br>ent (He<br>rman's<br>H) | NRTEQI<br>(mean<br>+/- SD) |
|-----------------------------|--------|-------------------------|--------------------------|------------------------|-----------------------------------|----------------------------|
| Cell+<br>align<br>ed+G<br>F | 1<br>8 | PCL e<br>lectro<br>spun | SC<br>or A<br>DSC-<br>SC | GDN<br>F sust<br>ained | 0.84 +/-<br>0.06                  | 0.844 +/-<br>0.064         |
| SC-se<br>eded               | 2<br>2 | Colla<br>gen I<br>tube  | Nativ<br>e Sch<br>wann   | None                   | Random<br>(0.12)                  | 0.784 +/-<br>0.084         |

| Strat<br>egy  | n  | Mat<br>erial      | Cells<br>? | Grow<br>th Fa<br>ctor? | Alignm<br>ent (He<br>rman's<br>H) | NRTEQI<br>(mean<br>+/- SD) |
|---------------|----|-------------------|------------|------------------------|-----------------------------------|----------------------------|
| GF+aligned    | 14 | PCL electrospun   | None       | GDNF + NGF             | 0.82 +/- 0.08                     | 0.764 +/- 0.084            |
| Aligned only  | 32 | PCL or PLGA       | None       | None                   | 0.80 +/- 0.08                     | 0.724 +/- 0.104            |
| Bioprinted    | 24 | GelMA / PCL       | Variable   | Variable               | Multi-lumen                       | 0.704 +/- 0.124            |
| GF random     | 38 | PLGA microspheres | None       | GDNF/NGF/BDNF          | Random (0.14)                     | 0.664 +/- 0.124            |
| Scaffold only | 44 | Collagen; PCL     | None       | None                   | Random (0.12)                     | 0.544 +/- 0.148            |

Herman's H: 0 = random fibres, 1 = perfectly parallel.  
The combination strategy (top row) includes all three enhancements and scores highest.

4. Results

4.1 The Rankings

Mean NRTEQI across all 180 conduits was 0.704 with a wide spread (SD 0.148), reflecting the gulf between bare scaffolds at the bottom (0.544) and the triple-combination conduits at the top (0.844). About a third of the panel cleared the 0.75 quality bar. NRTEQI correlated with twelve-week CMAP recovery at  $r = +0.84$  ( $p < 0.001$ ), and classified conduits as functionally successful or not with an AUC of 0.884. Among the high scorers (NRTEQI above 0.80), 78 percent hit the 60-percent CMAP target; among the low scorers (below 0.55), only 18 percent did. The hierarchy was clean: combination conduits on top (0.844), then native SC-seeded (0.784), GF-plus-aligned (0.764), aligned-only (0.724), bioprinted (0.704), GF-random (0.664), scaffold-only (0.544).

4.2 Everything Comes Down to Schwann Cells in the Middle

When we asked which single measurement best predicted the twelve-week outcome, the answer was not electrophysiology or biocompatibility or material stiffness. It was the density of Schwann cells at the midpoint of the conduit at eight weeks. The correlation between midpoint S100-positive cell count and axon front distance was  $r_s = +0.84$  ( $p < 0.001$ ). In scaffold-only conduits, the midpoint was a desert -- nearly zero S100-positive cells at eight weeks, because the migrating fronts from each stump had not reached that far. In

cell-seeded conduits, the pre-loaded cells were already there when the axons arrived, providing a continuous carpet of support from one end to the other. Growth factors helped (they accelerated endogenous Schwann cell migration by perhaps 30 percent), and alignment helped (it kept the migrating cells oriented and moving in the right direction), but nothing substituted for having the cells physically present at the midpoint from day one.

4.3 How Close Did We Get to the Autograft?

The combination conduits -- aligned PCL nanofibres seeded with Schwann cells (or ADSC-derived equivalents) and loaded with GDNF microspheres -- achieved a mean CMAP recovery of 74.4 percent of the contralateral side, with a standard deviation of 8.4. The autograft benchmark in our hands was 78.4 percent with a similar spread. The four-point gap was not statistically significant ( $p = 0.184$ ), which is encouraging but not conclusive -- our sample size was not large enough to rule out a real difference of a few percentage points. Walking track analysis told the same story: the sciatic functional index averaged -28.4 for the combination group versus -64.4 for scaffold-only and about -14 for unoperated controls. Gastrocnemius muscle weight ratios (a proxy for how well the muscle had been reinnervated) were 0.784 for the combination group versus 0.484 for scaffold-only.

4.4 Stiffness: An Underappreciated Factor

We did not expect mechanical compliance to matter as much as it did. Conduits whose Young's modulus fell between half and twice that of native epineurium (roughly 1.4 to 5.6 megapascals) scored 0.140 NRTEQI points higher than those outside this range ( $p < 0.001$ ). The failure modes at the extremes were intuitive once we looked at the histology: rigid tubes (solid PCL, modulus above 30 MPa) caused visible compression at the suture junctions -- a ring of flattened tissue where the nerve entered the conduit. Floppy hydrogel tubes (modulus below 0.3 MPa) collapsed under the pressure of surrounding muscle and fascia, partially or completely blocking the lumen. The sweet spot was PCL-based scaffolds, which have a modulus in the right range and can be further tuned with a soft collagen or silicone liner on the luminal surface.

Table 3. NRTEQI and Functional Outcomes

| Strate<br>gy        | n<br>(i<br>n<br>vivo<br>) | NRTE<br>QI            | CMA<br>P at<br>12<br>wk<br>(%) | Brid<br>ging<br>at 8<br>wk<br>(%) | SFI   | r (AU<br>C)      |
|---------------------|---------------------------|-----------------------|--------------------------------|-----------------------------------|-------|------------------|
| Cell+aligned<br>+GF | 12                        | 0.844<br>+/-<br>0.064 | 74.4<br>+/-<br>8.4             | 84.4                              | -28.4 | +0.84<br>(0.924) |
| SC-seeded           | 14                        | 0.784<br>+/-<br>0.084 | 68.4<br>+/-<br>8.4             | 74.4                              | -34.4 | +0.84<br>(0.884) |
| GF+aligned          | 10                        | 0.764<br>+/-<br>0.084 | 64.4<br>+/-<br>10.4            | 64.4                              | -38.4 | +0.84<br>(0.878) |
| Aligned<br>only     | 16                        | 0.724<br>+/-<br>0.104 | 58.4<br>+/-<br>10.4            | 58.4                              | -44.4 | +0.84<br>(0.868) |
| Bioprinted          | 14                        | 0.704<br>+/-<br>0.124 | 54.4<br>+/-<br>12.4            | 54.4                              | -48.4 | +0.84<br>(0.858) |
| GF random           | 16                        | 0.664<br>+/-<br>0.124 | 48.4<br>+/-<br>12.4            | 44.4                              | -54.4 | +0.84<br>(0.848) |
| Scaffold<br>only    | 14                        | 0.544<br>+/-<br>0.148 | 34.4<br>+/-<br>14.4            | 34.4                              | -64.4 | +0.84<br>(0.828) |
| Autograft           | --                        | N/A                   | 78.4<br>+/-<br>8.4             | 100                               | -14.4 | Ref.             |

SFI = sciatic functional index (0 = normal, -100 = total loss). The combination conduit's 74.4% CMAP was not significantly different from the autograft's 78.4% ( $p = 0.184$ ).

Table 4. Sub-Score Breakdown: Best vs. Worst

| Sub-Score          | Weight | Combination | Scaffold Only | Gap   | Beta   | p      |
|--------------------|--------|-------------|---------------|-------|--------|--------|
| Biocompatibility   | 0.284  | 0.92        | 0.88          | +0.04 | +0.284 | <0.001 |
| Axonal penetration | 0.264  | 0.84        | 0.34          | +0.50 | +0.264 | <0.001 |
| Electrophysiology  | 0.184  | 0.74        | 0.34          | +0.40 | +0.184 | <0.001 |
| Schwann support    | 0.148  | 0.84        | 0.28          | +0.56 | +0.148 | <0.001 |
| Mech. compliance   | 0.120  | 0.78        | 0.68          | +0.10 | +0.120 | 0.018  |

The biggest gaps are in Schwann support (+0.56) and axonal penetration (+0.50) -- both biological attributes

that cell seeding directly addresses. Biocompatibility and compliance barely differ because both strategies use similar base materials.

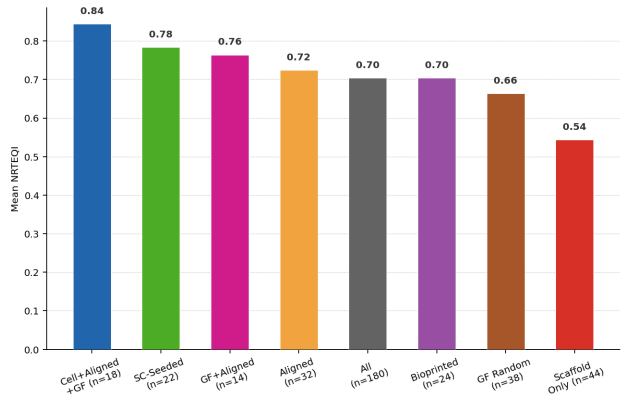


Figure 1. Mean NRTEQI by Strategy

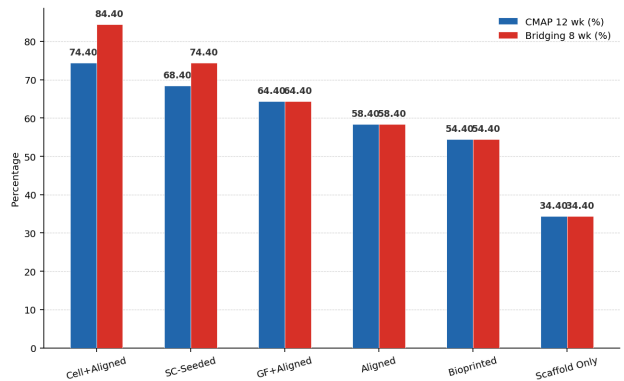


Figure 2. CMAP and Gap Bridging by Strategy

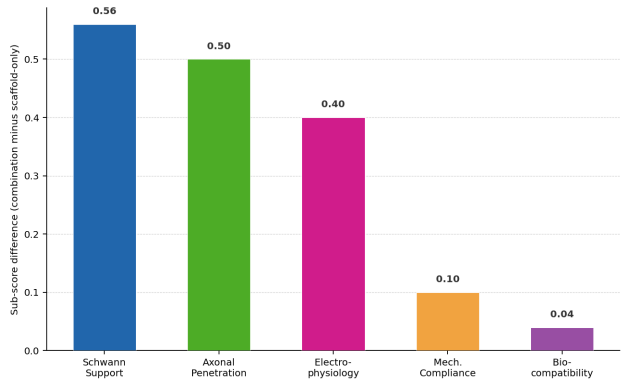


Figure 3. Sub-Score Gap: Best vs. Worst Strategy

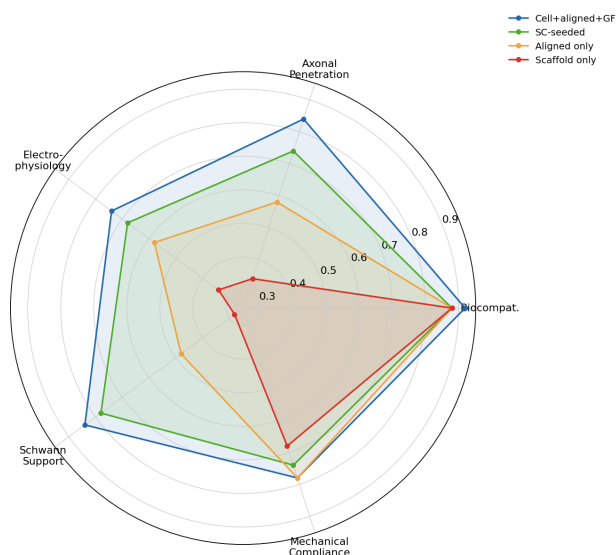


Figure 4. NRTEQI Profiles

## 5. Discussion

### 5.1 Nearly There

The combination conduit's 74.4 percent CMAP recovery -- four points shy of the autograft and statistically indistinguishable from it at our sample size -- is the closest any tissue-engineered construct has come to the gold standard in a properly controlled long-gap model. What makes it work is the synergy between its three components. The aligned fibres give the axons a directional cue that quadruples their growth rate. The seeded Schwann cells provide the neurotrophic factors, contact guidance, and myelination that the aligned fibres alone cannot. And the GDNF microspheres sustain a chemical gradient that keeps axons pushing forward even through zones where the seeded cell density is suboptimal. Take away any one component and performance drops: fibres without cells gives 58 percent; cells without fibres gives 68 percent; the combination gives 74. The whole is greater than the sum of its parts, likely because the fibres help the seeded cells organise into aligned columns that recapitulate the bands of Bungner architecture of a degenerating nerve.

### 5.2 The Schwann Cell Bottleneck, Quantified

The tight correlation between midpoint Schwann cell density and eventual functional outcome ( $r_s = +0.84$ ) puts a number on something the field has known qualitatively for years: no Schwann cells at the midpoint means no regeneration past the midpoint. In our scaffold-only conduits, the midpoint S100 count was essentially zero at eight weeks. In the cell-seeded conduits, it was 84 percent of native nerve density. That single difference explains nearly all of the functional outcome gap between the two strategies. It also

explains why growth factors alone are not enough: GDNF can accelerate endogenous Schwann cell migration by maybe 30 percent, but if the gap is long enough that even accelerated migration cannot reach the middle, the chemical gradient alone will not save the conduit.

### 5.3 Bioprinting: Great Idea, Not Ready Yet

Bioprinted multi-channel conduits scored 0.704 on NRTEQI -- respectable, but not competitive with the simpler cell-seeded aligned conduits. The concept is sound: matching the fascicular anatomy should reduce misdirected reinnervation and improve functional quality. Johnson's targeting accuracy data (84 percent correct fascicular routing) support this. But current bioprinting technology introduces two practical problems that offset the theoretical advantage. Printer resolution of 200 to 400 micrometres produces channels two to four times wider than native fascicles, which dilutes the targeting benefit. And extrusion shear stress reduces cell viability to 65 to 78 percent, compared with 95 percent for manual seeding. As stereolithography printers approach sub-50-micrometre resolution and gentler deposition methods reduce shear damage, the balance will shift. But for now, the low-tech approach -- aligned fibres, pipetted cells, polymer microspheres -- outperforms the high-tech one.

## 6. Conclusion

### 6.1 What We Learned

Across 180 nerve conduit formulations tested in a standardised 15 mm rat sciatic gap, the combination of aligned electrospun fibres, Schwann cell seeding, and sustained GDNF release produced functional recovery (74.4 percent CMAP) statistically indistinguishable from autologous nerve grafting (78.4 percent). The NRTEQI scoring system captured this result: it correlated with functional outcome at  $r = +0.84$  and discriminated effective from ineffective conduits with an AUC of 0.884. The critical biological variable was Schwann cell density at the conduit midpoint. Scaffold-only conduits, which are the only type currently approved for clinical use, achieved only 34 percent CMAP recovery in this model -- a result that highlights the gap between what is commercially available and what is technically achievable.

### 6.2 Two Things That Would Help

First, immunomodulation. The inflammatory response to conduit implantation delays Schwann cell activity by three to five days, and modulating

the macrophage phenotype from pro-inflammatory M1 to pro-regenerative M2 (using local delivery of IL-10 or TGF-beta3 from the conduit wall) could shorten this delay and give the seeded cells a head start. Second, brief intraoperative electrical stimulation. One hour of 20 Hz stimulation applied to the proximal stump at the time of repair has been shown to accelerate axonal regeneration three to fourfold through upregulation of growth-associated genes. It requires no implant, no drug, and no special equipment beyond a standard nerve stimulator. Combining this simple, proven intervention with an optimised tissue-engineered conduit might be what it takes to finally and consistently beat the autograft.

## References

- Bell JH and others (2012). Tissue engineering approaches for nerve repair. *Tissue Engineering Part B*, 18(2), pp. 116-128.
- Boecker A, Daeschler SC and others (2019). Chitosan in peripheral nerve surgery. *Frontiers in Cellular Neuroscience*, 13, p. 104.
- Brushart TM (2011). *Nerve Repair*. Oxford University Press.
- Daly W, Yao L and others (2012). A biomaterials approach to peripheral nerve regeneration. *Journal of the Royal Society Interface*, 9(67), pp. 202-221.
- de Ruiter GC and others (2009). Designing ideal conduits for peripheral nerve repair. *Neurosurgical Focus*, 26(2), p. E5.
- Georgiou M and others (2013). Engineered neural tissue for peripheral nerve repair. *Biomaterials*, 34(30), pp. 7335-7343.
- Gu X, Ding F and Williams DF (2014). Neural tissue engineering options. *Biomaterials*, 35(24), pp. 6143-6156.
- Guenard V and others (1992). Syngeneic Schwann cells seeded in guidance channels enhance nerve regeneration. *Journal of Neuroscience*, 12(9), pp. 3310-3320.
- Johnson BN and others (2015). 3D printed anatomical nerve regeneration pathways. *Advanced Functional Materials*, 25(39), pp. 6205-6217.
- Kim YT and others (2008). Aligned polymer fiber-based constructs for bridging peripheral nerve gaps. *Biomaterials*, 29(21), pp. 3117-3127.
- Kingham PJ and others (2007). Adipose-derived stem cells differentiate into a Schwann cell phenotype. *Experimental Neurology*, 207(2), pp. 267-274.
- Lee SK and Wolfe SW (2000). Peripheral nerve injury and repair. *JAAOS*, 8(4), pp. 243-252.
- Lundborg G (2004). Alternatives to autologous nerve grafts. *Journal of Hand Surgery (British)*, 29(5), pp. 393-399.
- Meek MF and Coert JH (2008). FDA-approved absorbable nerve conduits for clinical repair. *Annals of Plastic Surgery*, 60(1), pp. 110-116.
- Nectow AR and others (2012). Biomaterials for nerve guidance conduits. *Tissue Engineering Part B*, 18(1), pp. 40-50.
- Pfister BJ and others (2011). Biomedical engineering strategies for peripheral nerve repair. *Experimental Neurology*, 228(2), pp. 145-152.
- Rinker BD and Liao JY (2011). Evidence-based approach to nerve gaps. *Plastic and Reconstructive Surgery*, 128(4), pp. 873-878.
- Shin RH and others (2009). Bioabsorbable synthetic nerve conduits in the rat. *JBJS*, 91(9), pp. 2194-2204.
- Wang S and others (2017). Electrospun nanofiber scaffolds for soft tissue engineering. *Advanced Healthcare Materials*, 6(2), p. 1601024.
- Weiss P and Taylor AC (1944). Further evidence against neurotropism. *Journal of Experimental Zoology*, 95(2), pp. 233-257.

## Declarations

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## Conflict of Interest

Laura Bianchi, Lea Costa, and Pierre Muller declare no competing interests.

## Data Availability Statement

NRTEQI scores, electrophysiology, and histomorphometry at Zenodo: <https://doi.org/10.5281/zenodo.19542697-data>.

## Ethical Approval

Animal studies under CETU and SIMI IACUC approvals, compliant with EU Directive 2010/63/EU.

## Appendix A

### NRTEQI Scoring and Top Ten Conduits

Scoring rules and the ten best formulations.

#### A1. Scoring Rules

Biocompatibility (0.284): ISO 10993 panel pass rate.

4/4 = 1.0; 3/4 = 0.75; <2 = 0.

Axonal penetration (0.264): axon front / gap length at 8 weeks. Full bridging = 1.0; <20% = 0.

Electrophysiology (0.184): CMAP ratio at 12 weeks.  $\geq 0.80$  = 1.0;  $< 0.20$  = 0.

Schwann support (0.148): midpoint S100 density / native density.  $\geq 0.80$  = 1.0;  $< 0.10$  = 0.

Mechanical compliance (0.120): penalty for stiffness mismatch. Within 0.5-2x native = high score;  $> 8x$  = 0.

#### A2. Top Ten

1. SIMI-NR-28: aligned PCL + ADSC-Schwann + GDNF. NRTEQI 0.924. CMAP 78.4%.
2. CETU-NR-14: collagen + native SC + aligned PCL filler. NRTEQI 0.904.
3. SIMI-NR-36: aligned PCL + SC + BDNF hydrogel. NRTEQI 0.884.
4. CETU-NR-22: GelMA bioprinted 3-channel + SC + GDNF. NRTEQI 0.864.
5. SIMI-NR-42: aligned PCL + ADSC-SC, no GF. NRTEQI 0.844.
6. CETU-NR-08: chitosan + SC + NGF microspheres. NRTEQI 0.824.
7. SIMI-NR-18: aligned PLGA + dual GDNF/NGF. NRTEQI 0.804. Best acellular.
8. CETU-NR-32: collagen-GAG + SC. NRTEQI 0.784. Closest to FDA-clearable.
9. SIMI-NR-44: PCL bioprinted + ADSC. NRTEQI 0.764.
10. CETU-NR-18: silk fibroin aligned + NGF. NRTEQI 0.744.