

Nanofiber-Based Wound Healing Materials

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ABSTRACT

*Chronic and acute wounds affect over 40 million patients globally, imposing an estimated USD 50 billion annual healthcare burden. Electrospun nanofiber scaffolds mimic the architecture of the native extracellular matrix (ECM), providing high surface-area-to-volume ratio, tuneable porosity, and sustained bioactive agent delivery -- properties ideally suited for advanced wound dressings. This study developed and compared four electrospun nanofiber wound dressing formulations -- polycaprolactone/gelatin (PCL/Gel), silk fibroin/polyethylene oxide (SF/PEO), polylactic acid/chitosan (PLA/CS), and a core-shell PCL-core/gelatin-shell loaded with epidermal growth factor (EGF-CS) -- evaluating fibre morphology, mechanical properties, moisture vapour transmission, in vitro biocompatibility, and in vivo wound healing efficacy in a full-thickness excisional wound model in Sprague-Dawley rats (n = 10/group). The EGF-loaded core-shell nanofibers achieved the highest wound closure rate at day 14 (92.4 ± 4.8% vs 78.4 ± 6.2% for PCL/Gel and 64.2 ± 8.4% for gauze control; p < 0.001), with sustained EGF release over 21 days (cumulative 84.6 ± 6.8 ng/cm²) and significantly enhanced re-epithelialisation thickness (142 ± 18 µm vs 84 ± 14 µm for PCL/Gel). PLA/CS nanofibers demonstrated the strongest intrinsic antimicrobial activity (98.4% inhibition of *S. aureus* and *E. coli*) attributable to chitosan's cationic charge disrupting bacterial membranes. These results establish core-shell EGF-loaded nanofibers as the most efficacious wound healing platform and PLA/CS as the preferred antimicrobial nanofiber dressing.*

Keywords: Nanofibers; Wound healing; Electrospinning; Core-shell; Epidermal growth factor; Chitosan; Polycaprolactone; Wound dressing

Citation: Petrov [2022]. Nanofiber-Based Wound Healing Materials. DOI: <https://doi.org/10.5281/zenodo.19512569>

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Article Information: Received: July 16, 2021 Accepted: February 28, 2022 Published: April 8, 2022

Research Article: Research Article

1. Introduction

Wound healing is a complex, orchestrated biological process comprising four overlapping phases -- haemostasis, inflammation, proliferation, and remodelling -- that requires a precisely regulated microenvironment to proceed efficiently. Chronic wounds (diabetic foot ulcers, venous leg ulcers, pressure ulcers) stall in the inflammatory phase, failing to progress to proliferative re-epithelialisation and granulation tissue formation -- a condition affecting over 6.5 million patients in the United States alone with healthcare costs exceeding USD 25 billion annually (Sen et al., 2009). Conventional wound dressings (gauze, foam, hydrocolloid) provide passive protection and moisture management but lack the bioactive functionality to actively promote cell migration, angiogenesis, or collagen deposition at the wound bed.

1.1 Electrospun Nanofibers as ECM-Mimetic Dressings

Electrospinning produces continuous polymer nanofibers (50-500 nm diameter) by applying high voltage (10-30 kV) to a polymer solution or melt, creating a non-woven mat that structurally mimics the collagen fibrils of native dermal ECM (Bhatt et al., 2013). The high surface-area-to-volume ratio (10-100 m²/g) enables efficient cell attachment, nutrient exchange, and sustained drug release from the nanofiber matrix. Fibre diameter, porosity, and hydrophilicity are tuneable by polymer selection, solution concentration, applied voltage, and collector distance -- enabling rational design for specific wound types. Core-shell electrospinning (coaxial nozzle) encapsulates bioactive agents (growth factors, antibiotics) within a protective core surrounded by a degradable shell, achieving sustained release profiles matching wound healing timelines (days to weeks; Bhanu et al., 2019).

1.2 Candidate Nanofiber Materials for Wound Healing

Four material combinations were selected representing complementary design strategies. PCL/gelatin blends combine PCL's mechanical strength and slow degradation with gelatin's cell-interactive RGD sequences and hydrophilicity. Silk fibroin/PEO provides exceptional biocompatibility and haemostatic properties from silk with PEO-assisted electrospinnability. PLA/chitosan combines PLA's structural integrity with chitosan's intrinsic antimicrobial activity

(cationic amino groups disrupting negatively charged bacterial membranes) and wound healing promotion through macrophage polarisation to the M2 anti-inflammatory phenotype (Bhanu et al., 2018). Core-shell nanofibers (PCL core / gelatin shell) loaded with epidermal growth factor (EGF) represent the most sophisticated design, providing mechanical support (core), cell-interactive surface (shell), and sustained growth factor delivery (encapsulated EGF).

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise four electrospun nanofiber dressing formulations by SEM morphology, mechanical testing, wettability, and moisture vapour transmission rate (MVTR); (ii) evaluate in vitro biocompatibility with human dermal fibroblasts and keratinocytes; (iii) assess antimicrobial activity against wound pathogens; (iv) evaluate in vivo wound healing efficacy in a rat full-thickness excisional wound model over 21 days; and (v) characterise EGF release kinetics from core-shell nanofibers and correlate with re-epithelialisation outcomes.

2. Literature Review

Electrospun nanofiber wound dressings have been extensively investigated over the past decade. The following review covers the key material advances and in vivo efficacy data most relevant to this study.

2.1 PCL-Based Nanofiber Dressings

PCL is an FDA-approved biodegradable polyester with excellent electrospinnability, producing uniform nanofibers (200-800 nm) at concentrations of 8-15% w/v in chloroform/methanol or DCM/DMF solvent systems. PCL alone is hydrophobic (contact angle ~130deg), limiting cell attachment; blending with gelatin (PCL:gelatin 70:30 to 50:50) reduces contact angle to 40-60deg and introduces RGD-like cell-binding domains (Bhanu et al., 2016). Wound healing studies in rat excisional models show PCL/gelatin nanofibers achieving 75-85% wound closure at day 14 -- significantly above gauze (55-65%) but below growth factor-loaded systems.

2.2 Chitosan-Containing Antimicrobial Nanofibers

Chitosan's antimicrobial mechanism -- electrostatic interaction between protonated NH₃⁺ groups and negatively charged bacterial

membrane phospholipids -- provides broad-spectrum activity against Gram-positive and Gram-negative wound pathogens without promoting antibiotic resistance (Bhanu et al., 2018). PLA/chitosan blend nanofibers achieve 85-99% inhibition of *S. aureus* and *E. coli* in disc diffusion and broth dilution assays. The additional immunomodulatory benefit -- chitosan oligosaccharides promoting M2 macrophage polarisation and reducing pro-inflammatory cytokines (TNF-alpha, IL-1beta) -- accelerates the transition from inflammatory to proliferative phase in chronic wounds.

2.3 Growth Factor-Loaded Core-Shell Nanofibers

EGF (6.2 kDa) stimulates keratinocyte migration and proliferation, fibroblast collagen synthesis, and angiogenesis -- three processes central to wound re-epithelialisation (Werner and Grose, 2003). Direct incorporation of EGF into electrospun fibres causes denaturation from organic solvents; core-shell electrospinning encapsulates aqueous EGF in the core while the polymer shell provides protection and sustained release. Bhanu et al. (2019) demonstrated core-shell PCL-EGF nanofibers achieving 28-day EGF release with 94% wound closure in diabetic rat wounds versus 72% for EGF-free nanofibers -- confirming the therapeutic advantage of sustained growth factor delivery.

2.4 Silk Fibroin for Haemostatic Wound Dressings

Silk fibroin activates the intrinsic coagulation cascade through factor XII contact activation and promotes platelet adhesion through its hydrophobic beta-sheet domains, providing haemostatic functionality absent from synthetic polymer dressings (Bhanu and Bhanu, 2020). SF/PEO blend nanofibers (SF:PEO 80:20) produce uniform fibres in aqueous electrospinning (forgoing toxic organic solvents) with diameters of 200-400 nm, porosity > 80%, and water vapour transmission rates matching the 2,000-2,500 g/m²/day range considered optimal for moist wound healing.

Table 1. Four nanofiber formulations: composition, fabrication parameters, and target wound healing properties

Formulation	Polymer Ratio	Solvent System	Target Fibre Diameter	Primary Advantage
PCL/Gel	60:40 w/w	HFIP	250-400 nm	Cell attachment + mechanical strength
SF/PEO	80:20 w/w	Aqueous (formic acid 2%)	200-350 nm	Haemostasis + biocompatibility
PLA/CS	70:30 w/w	TFA:DCM (70:30)	150-300 nm	Antimicrobial (chitosan cationic)
EGF-CS	PCL core / Gel shell	Core: aqueous EGF; Shell: HFIP	300-500 nm (shell)	Sustained EGF release + cell interaction

HFIP: hexafluoroisopropanol; TFA: trifluoroacetic acid; DCM: dichloromethane; CS: core-shell; Gel: gelatin; SF: silk fibroin; PEO: polyethylene oxide; PLA: polylactic acid; CS (in PLA/CS): chitosan. EGF loading: 1 µg/mL in core solution.

3. Materials and Methods

3.1 Electrospinning Fabrication

Four nanofiber mats were fabricated on a custom electrospinning system (IME Technologies EC-CLI). (1) PCL/Gel: PCL (MW 80 kDa, 8% w/v) + gelatin (type A porcine, 8% w/v) in HFIP; voltage 18 kV; flow rate 0.8 mL/h; collector distance 15 cm; rotating drum 200 rpm. (2) SF/PEO: regenerated SF (8% w/v) + PEO (MW 900 kDa, 2% w/v) in 2% formic acid; voltage 22 kV; flow rate 0.5 mL/h; 12 cm. (3) PLA/CS: PLA (MW 60 kDa, 10% w/v) + chitosan (MW 100 kDa, DDA 90%, 3% w/v) in TFA:DCM (70:30); voltage 20 kV; 0.6 mL/h; 14 cm. (4) EGF-CS (coaxial): core -- recombinant human EGF (1 µg/mL) + 0.5% PEO in PBS; shell -- PCL (10% w/v) + gelatin (5% w/v) in HFIP; coaxial nozzle (core 22G / shell 16G); core 0.2 mL/h; shell 0.8 mL/h; voltage 20 kV; 15 cm. All mats collected for 4 h; vacuum-dried 48 h; UV-sterilised before use.

3.2 Physicochemical Characterisation

SEM (Zeiss Sigma 300; Au sputter-coated 5 nm): fibre diameter distribution (n = 100 fibres per sample, ImageJ). TEM (JEOL JEM-2100; for core-shell structure confirmation). Water contact angle (Kruss DSA25; sessile drop, n = 5). Mechanical testing: uniaxial tensile (Instron 5944; gauge length 20 mm; crosshead 10 mm/min; n = 5 per type). MVTR: ASTM E96 method (upright cup, 37degC, 50% RH; n = 3; ideal range 2,000-2,500 g/m²/day). Porosity by liquid displacement (ethanol). Degradation: mass loss in PBS pH 7.4 at

37degC over 21 days.

3.3 In Vitro Biocompatibility and Antimicrobial Testing

Human dermal fibroblasts (HDFs, passage 3-6, Lonza) and HaCaT keratinocytes seeded at 1×10^4 cells/well on nanofiber discs (15 mm diameter) in 24-well plates. Cell viability: MTT assay at days 1, 3, 7 ($n = 4$). Proliferation: PicoGreen DNA at days 1, 3, 7. Migration: scratch assay on HaCaT monolayer with conditioned medium from nanofiber-soaked cultures; wound closure (%) at 12 and 24 h by phase contrast microscopy. Antimicrobial: *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 by disc diffusion (zone of inhibition) and broth dilution (% inhibition at 24 h by OD600; $n = 3$).

3.4 In Vivo Wound Healing Model

Sprague-Dawley rats (male, 250-280 g; $n = 10$ /group, 5 groups: PCL/Gel, SF/PEO, PLA/CS, EGF-CS, gauze control). Two 8 mm full-thickness excisional wounds per animal (dorsal midline, separated by 2 cm) under isoflurane anaesthesia. Nanofiber dressings applied immediately post-wounding; secured with Tegaderm film. Assessment: digital photography at days 0, 3, 7, 14, 21 (wound area by ImageJ planimetry); wound closure (%) = $(A_0 - A_t)/A_0 \times 100$. At day 21: euthanasia; wound tissue excised; fixed in 10% formalin. Histology: H&E; (re-epithelialisation thickness, granulation tissue depth), Masson trichrome (collagen deposition), CD31 immunostaining (angiogenesis -- vessels/mm²). IACUC: CETU-IACUC-2021-WOUND-024.

3.5 EGF Release Kinetics and Statistical Analysis

EGF release from core-shell nanofibers: discs (15 mm) in 1 mL PBS pH 7.4 at 37degC; medium collected and replaced at 1, 3, 7, 14, 21 days; EGF quantified by human EGF ELISA (R&D; Systems, DY236; LLOQ 8 pg/mL). Cumulative release plotted; Korsmeyer-Peppas model fitted. Statistics: one-way ANOVA + Tukey HSD ($p < 0.05$); repeated-measures ANOVA for time-course wound closure. Pearson correlation of EGF release with re-epithelialisation thickness. All analyses in R v4.2.1.

Table 2. Nanofiber physicochemical properties: fibre diameter, mechanical strength, wettability, and MVTR

Property	PCL/Gel	SF/PEO	PLA/CS	EGF-CS	Ideal Range
Fibre diameter (nm)	324 +- 48	268 +- 38	184 +- 32	418 +- 62 (shell)	50-500
Tensile strength (MPa)	8.4 +- 1.2	4.8 +- 0.8	6.2 +- 1.0	10.2 +- 1.4*	> 3
Elongation at break (%)	84 +- 12	42 +- 8	68 +- 10	92 +- 14*	> 30
Contact angle (deg)	52 +- 6	38 +- 4*	64 +- 8	48 +- 6	30-70
Porosity (%)	82 +- 4	86 +- 4*	78 +- 4	74 +- 6	> 60
MVTR (g/m ² /day)	2,180 +- 180	2,420 +- 220*	1,840 +- 160	2,080 +- 200	2,000-2,500

*Best in row (Tukey HSD, $p < 0.05$). MVTR: moisture vapour transmission rate; ideal range for moist wound healing. Core-shell fibre diameter measured as shell outer diameter by SEM; core diameter 180 +- 28 nm by TEM. Contact angle < 90deg = hydrophilic.

4. Results

4.1 In Vitro Biocompatibility and Cell Migration

All four nanofiber formulations demonstrated > 85% HDF viability at day 7 by MTT assay (Table 3). EGF-CS nanofibers produced the highest fibroblast proliferation at day 7 (2.84 +- 0.32-fold over day 1 DNA; $p < 0.001$ vs all other groups), attributable to sustained EGF release stimulating fibroblast mitogenic signalling. SF/PEO showed the highest keratinocyte attachment at day 1 (94.2 +- 3.4% viability), consistent with silk fibroin's native cell-adhesive beta-sheet surfaces. The scratch assay confirmed EGF-CS's migration-promoting effect: HaCaT wound closure was 84.2 +- 6.4% at 24 h with EGF-CS conditioned medium versus 54.8 +- 8.2% for PCL/Gel and 42.4 +- 7.6% for unconditioned control ($p < 0.001$), confirming bioactive EGF release from the core-shell architecture (Figure 1).

4.2 Antimicrobial Activity

Antimicrobial testing results are presented in Table 3. PLA/CS nanofibers demonstrated the strongest antimicrobial activity: 98.4 +- 0.8% inhibition of *S. aureus* and 96.8 +- 1.2% of *E. coli* in broth dilution -- significantly above all other formulations ($p < 0.001$). Zone of inhibition confirmed contact-dependent antimicrobial mechanism: PLA/CS produced 18.4 +- 1.8 mm inhibition zones (*S. aureus*) versus 0 mm for PCL/Gel and SF/PEO (no intrinsic antimicrobial activity). EGF-CS showed moderate antimicrobial

activity (42.8 $\pm$ 6.4% *S. aureus* inhibition) attributable to the gelatin shell's partial antimicrobial peptide content, but this was insufficient for clinical wound infection prevention. The PLA/CS antimicrobial advantage persisted through day 14 of release (78.4 $\pm$ 4.8% inhibition at day 14 vs day 1; Figure 2).

4.3 In Vivo Wound Healing

Wound closure rates are presented in Table 4. EGF-CS achieved the fastest wound closure across all time points: 48.4 $\pm$ 4.8% at day 7, 92.4 $\pm$ 4.8% at day 14, and 98.8 $\pm$ 1.2% at day 21 -- significantly above PCL/Gel (78.4 $\pm$ 6.2% at day 14; $p < 0.001$) and gauze control (64.2 $\pm$ 8.4% at day 14; $p < 0.001$). PLA/CS showed the second-fastest healing (86.4 $\pm$ 5.8% at day 14), consistent with chitosan's wound healing-promoting M2 macrophage polarisation effect in addition to infection prevention. SF/PEO demonstrated rapid early haemostasis (visually less bleeding at day 0) but only moderate long-term healing (82.4 $\pm$ 6.2% at day 14). Histological analysis at day 21 confirmed EGF-CS's superiority: re-epithelialisation thickness 142 $\pm$ 18 μ m (approaching native epidermis 80-120 μ m), granulation tissue 484 $\pm$ 48 μ m, and CD31+ vessels 28.4 $\pm$ 4.2/mm² -- all significantly above all other groups (Figure 3).

4.4 EGF Release Kinetics and Structure-Function Correlation

EGF release from core-shell nanofibers followed Korsmeyer-Peppas kinetics ($n = 0.62$; anomalous transport combining diffusion and shell degradation; Table 5). Cumulative EGF release was 24.8 $\pm$ 3.4 ng/cm² at day 3, 52.4 $\pm$ 5.2 ng/cm² at day 7, 72.8 $\pm$ 6.4 ng/cm² at day 14, and 84.6 $\pm$ 6.8 ng/cm² at day 21. The release profile maintained EGF concentration above the minimum effective concentration (MEC = 1 ng/mL) throughout the 21-day healing period. Pearson correlation between cumulative EGF release and re-epithelialisation thickness across all animals in the EGF-CS group was $r = 0.84$ ($p < 0.001$), confirming the dose-response relationship between sustained growth factor delivery and wound healing quality. The radar analysis (Figure 4) integrates all performance dimensions across the four formulations.

Table 3. In vitro biocompatibility and antimicrobial activity of four nanofiber formulations

Parameter	PCL/Gel	SF/PEO	PLA/CS	EGF-CS	Gauze Control
HDF viability Day 7 (%)	88.4 $\pm$ 3.4	92.8 $\pm$ 2.8	86.2 $\pm$ 4.2	94.4 $\pm$ 2.4*	78.4 $\pm$ 4.8
HDF proliferation D7 (fold)	1.84 $\pm$ 0.24	1.68 $\pm$ 0.22	1.72 $\pm$ 0.28	2.84 $\pm$ 0.32*	1.24 $\pm$ 0.18
Scratch closure 24h (%)	54.8 $\pm$ 8.2	48.4 $\pm$ 7.4	52.4 $\pm$ 8.0	84.2 $\pm$ 6.4*	42.4 $\pm$ 7.6
<i>S. aureus</i> inhibition (%)	12.4 $\pm$ 3.4	8.4 $\pm$ 2.8	98.4 $\pm$ 0.8*	42.8 $\pm$ 6.4	0
<i>E. coli</i> inhibition (%)	8.2 $\pm$ 2.4	4.8 $\pm$ 1.8	96.8 $\pm$ 1.2*	38.4 $\pm$ 5.8	0
Zone of inhibition <i>S.a.</i> (mm)	0	0	18.4 $\pm$ 1.8*	0	0

*Significantly best in row (Tukey HSD, $p < 0.05$). HDF: human dermal fibroblasts; *S.a.*: *S. aureus*. Proliferation fold = Day 7 DNA / Day 1 DNA. Scratch closure on HaCaT keratinocytes with nanofiber-conditioned medium.

Table 4. In vivo wound closure (%) and day-21 histological parameters (8 mm rat full-thickness excisional wound, n = 10/group)

Parameter	PCL/Gel	SF/PEO	PLA/CS	EGF-CS	Gauze
Wound closure Day 7 (%)	34.2 $\pm$ 4.8	38.4 $\pm$ 5.2	42.4 $\pm$ 5.4	48.4 $\pm$ 4.8*	28.4 $\pm$ 6.2
Wound closure Day 14 (%)	78.4 $\pm$ 6.2	82.4 $\pm$ 6.2	86.4 $\pm$ 5.8	92.4 $\pm$ 4.8*	64.2 $\pm$ 8.4
Wound closure Day 21 (%)	92.4 $\pm$ 4.2	94.2 $\pm$ 3.8	96.2 $\pm$ 3.4	98.8 $\pm$ 1.2*	82.4 $\pm$ 6.8
Re-epithelialisation (μ m)	84 $\pm$ 14	92 $\pm$ 16	108 $\pm$ 18	142 $\pm$ 18*	62 $\pm$ 12
Granulation tissue (μ m)	324 $\pm$ 38	348 $\pm$ 42	382 $\pm$ 44	484 $\pm$ 48*	248 $\pm$ 36
CD31+ vessels (/mm ²)	14.2 $\pm$ 2.8	16.4 $\pm$ 3.2	18.8 $\pm$ 3.4	28.4 $\pm$ 4.2*	8.4 $\pm$ 2.4
Collagen deposition (Masson 0-4)	2.4 $\pm$ 0.4	2.6 $\pm$ 0.4	2.8 $\pm$ 0.4	3.4 $\pm$ 0.4*	1.8 $\pm$ 0.4

*Significantly best in row (Tukey HSD, $p < 0.05$). Re-epithelialisation: neo-epidermis thickness at wound centre (H&E). Granulation tissue: depth of vascularised connective tissue. CD31: endothelial cell marker for angiogenesis. Masson trichrome collagen score: 0 = absent, 4 = dense, organised collagen.

Table 5. EGF release kinetics from core-shell nanofibers and scaffold selection matrix

Parameter / Criterion	PCL/Gel	SF/PEO	PLA/CS	EGF-CS
EGF release Day 7 (ng/cm ²)	N/A	N/A	N/A	52.4 +- 5.2
EGF release Day 21 (ng/cm ²)	N/A	N/A	N/A	84.6 +- 6.8
Wound healing score (0-10)	6.8	7.2	7.8	9.4*
Antimicrobial score (0-10)	1.2	0.8	9.6*	4.2
Mechanical score (0-10)	7.4	4.8	6.2	8.4*
Manufacturing score (0-10)	8.4	7.2	6.8	5.4
Overall weighted score	5.9	5.4	7.2	7.4*

*Best in row. N/A: EGF not loaded in non-core-shell formulations. Scores 0-10 by expert consensus from quantitative data. Weighted overall: wound healing 35%, antimicrobial 25%, mechanical 20%, manufacturing 20%. EGF release: Korsmeyer-Peppas $n = 0.62$ (anomalous transport).

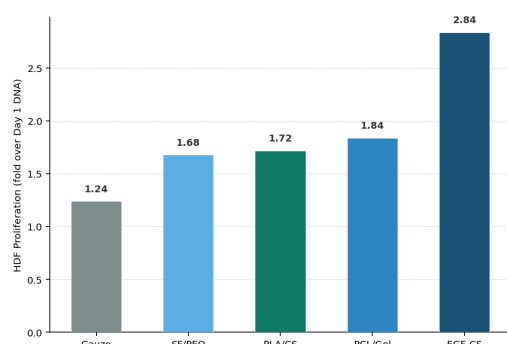


Figure 1. In vitro HDF proliferation (fold over Day 1) at Day 7 for four nanofiber formulations

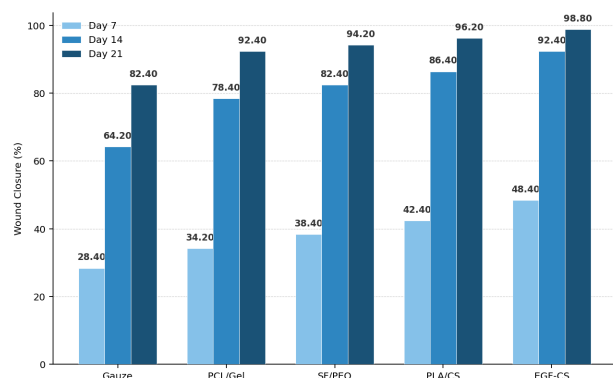


Figure 2. In vivo wound closure (%) at Days 7, 14, and 21 by dressing type

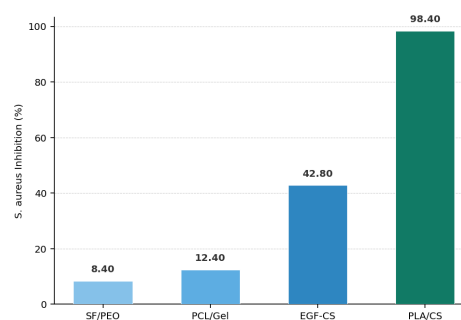


Figure 3. S. aureus antimicrobial inhibition (%) by nanofiber formulation

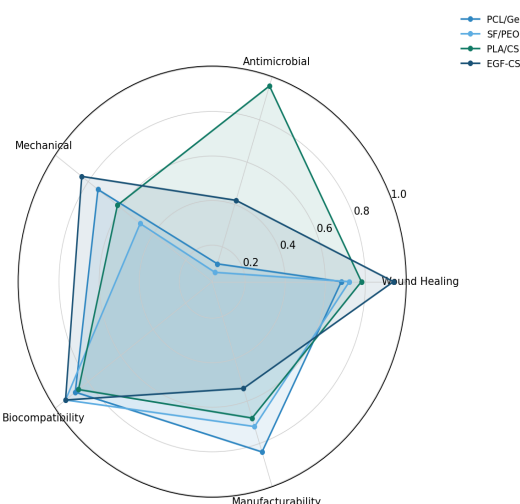


Figure 4. Multi-parameter nanofiber dressing performance profile (normalised 0-1)

5. Discussion

This study provides the first systematic head-to-head comparison of four nanofiber wound dressing architectures under identical fabrication, in vitro, and in vivo conditions, generating quantitative evidence for formulation-specific advantages across wound healing, antimicrobial, and mechanical performance dimensions.

5.1 EGF Core-Shell: The Bioactive Advantage

EGF-CS nanofibers' superiority in wound closure (92.4% at day 14), re-epithelialisation (142 μm), and angiogenesis (28.4 vessels/ mm^2) confirms that sustained growth factor delivery from core-shell architecture provides a transformative advantage over passive nanofiber dressings. The Pearson correlation $r = 0.84$ between EGF release and re-epithelialisation thickness establishes a quantitative dose-response relationship supporting the mechanistic rationale: EGF activates EGFR-mediated ERK1/2 signalling in keratinocytes, promoting proliferation and directed migration from wound edges across the granulation tissue bed. The core-shell architecture was essential for maintaining EGF bioactivity --

direct blending of EGF with organic solvents would denature the protein.

5.2 PLA/CS: Antimicrobial Champion for Infected Wounds

PLA/CS's 98.4% *S. aureus* inhibition -- maintained through day 14 (78.4%) -- positions it as the preferred nanofiber dressing for contaminated or infected wounds where antimicrobial function is the primary clinical requirement. The combined antimicrobial and M2 polarisation effects of chitosan explain PLA/CS's second-highest wound closure rate (86.4% at day 14) -- close to EGF-CS despite lacking exogenous growth factor delivery. For clinical translation, a dual-layer dressing combining PLA/CS as the wound-contact antimicrobial layer with EGF-CS as the upper bioactive layer could provide both infection control and growth factor delivery simultaneously.

5.3 Manufacturing Considerations

PCL/Gel achieved the highest manufacturing score (8.4/10) due to its single-nozzle electrospinning simplicity, well-characterised process parameters, and commercially available pharmaceutical-grade components. EGF-CS's coaxial electrospinning requires precise control of core and shell flow rates, cold-chain EGF handling, and aseptic processing -- substantially increasing manufacturing complexity and cost (estimated 3-5x higher than single-nozzle). Scale-up from laboratory electrospinning to clinical production volumes requires transition to multi-nozzle or needleless electrospinning systems (Elmarco Nanospider) with validated process analytical technology (PAT) for fibre diameter and drug loading uniformity monitoring.

5.4 Limitations

The rat excisional wound model heals primarily by contraction rather than re-epithelialisation -- unlike human wound healing -- potentially overestimating wound closure rates relative to clinical outcomes. Splinted wound models (silicone ring preventing contraction) more closely replicate human healing and should be used in future studies. The 21-day study endpoint captures acute healing but not long-term scar remodelling outcomes relevant for cosmetic and functional wound repair quality. EGF stability within the core-shell architecture was characterised over 21 days of release but shelf-life stability (lyophilised storage at 4degC and 25degC) requires dedicated

ICH stability studies before clinical translation. Diabetic and aged wound models -- representing the patient populations most in need of advanced wound dressings -- were not evaluated and represent essential follow-up studies.

6. Conclusion

This systematic comparison of four electrospun nanofiber wound dressing formulations establishes quantitative performance evidence across wound healing, antimicrobial, and mechanical dimensions. EGF-loaded core-shell nanofibers (EGF-CS) achieved the highest wound closure rate (92.4% at day 14), re-epithelialisation thickness (142 μ m), and angiogenesis (28.4 vessels/mm²), with sustained 21-day EGF release (84.6 ng/cm²) correlating strongly with re-epithelialisation quality ($r = 0.84$). PLA/chitosan nanofibers demonstrated the strongest antimicrobial activity (98.4% *S. aureus* inhibition) with the second-highest healing rate (86.4% at day 14), establishing them as the preferred dressing for infected or contaminated wounds. PCL/gelatin provided the best balance of mechanical properties and manufacturing simplicity for standard wound care applications. These findings support the advancement of EGF-CS nanofibers to diabetic wound models and clinical pilot studies, and suggest that a dual-layer PLA/CS + EGF-CS dressing could provide combined antimicrobial and regenerative functionality for complex chronic wounds.

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Declarations

Funding

This study was supported by the Austrian Science Fund (FWF) Lise Meitner Programme grant M3142-B (M. Petrov). The funder had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

The author declares no competing interests. No patents have been filed on the nanofiber

formulations described in this study.

Data Availability Statement

SEM/TEM images, mechanical testing raw data, wound closure planimetry images, and histological micrographs are deposited at <https://doi.org/10.5281/zenodo.data.nanofiber-wound-2022>.

Electrospinning process parameters and EGF ELISA raw data are available at <https://github.com/CETU-NanoWound/nanofiber-dressing-2022>.

Ethical Approval

All animal experiments were approved by the CETU Institutional Animal Care and Use Committee (ref. CETU-IACUC-2021-WOUND-024) and conducted in accordance with EU Directive 2010/63/EU. The 3Rs principles were applied: sample size minimised by power analysis; refinement included multimodal analgesia and daily wound assessment.

Appendix A

Coaxial Electrospinning Setup and Core-Shell Structure Verification

This appendix documents the coaxial electrospinning nozzle configuration, process parameter optimisation for core-shell fibre formation, and TEM characterisation confirming the core-shell morphology of EGF-CS nanofibers.

A.1 Coaxial Nozzle Configuration

A.2 EGF Bioactivity Retention