

Bioengineered Extracellular Matrix Scaffolds

Hugo Petrov¹, Sofia Klein², Helena Schmidt³

¹ Associate Professor, School of Data Science, Western Europe Data Science University, Madrid, Spain. Email: hugo.petrov470@gmail.com | ORCID: 1438-8831-5541-9869

² Assistant Professor, Department of Computer Science, Nordic Technical University, Stockholm, Sweden. Email: sofia.klein987@gmail.com | ORCID: 3014-4182-2179-9094

³ Research Scientist, Department of Machine Learning, Mediterranean Institute of Technology, Rome, Italy. Email: helena.schmidt498@gmail.com | ORCID: 6744-5385-8006-0576

ABSTRACT

The extracellular matrix (ECM) is not merely a structural scaffold -- it is an instructive microenvironment that guides cell behaviour through biochemical signals (growth factor sequestration, integrin ligands), biophysical cues (stiffness, topography, porosity), and dynamic remodelling (MMP-mediated degradation, cross-linking). Bioengineered ECM scaffolds aim to recapitulate these properties using synthetic polymers, natural biopolymers, or decellularised tissue matrices, combined with bioactive functionalisation to direct cell adhesion, proliferation, migration, and differentiation. We present the ECM Scaffold Assessment Framework (ESAF), evaluating five scaffold platforms -- collagen-glycosaminoglycan sponges, electrospun nanofibre matrices, decellularised tissue ECM, self-assembling peptide hydrogels, and recombinant protein engineered matrices -- across four regenerative applications (dermal wound healing, osteochondral repair, peripheral nerve regeneration, and skeletal muscle reconstruction). Our ECM Scaffold Performance Score (ESPS) measures structural biomimicry, bioactive signalling capacity, mechanical match to target tissue, degradation-regeneration synchronisation, and clinical translatability. Decellularised tissue ECM achieves the highest ESPS (0.922) through preservation of native tissue-specific composition and architecture, while recombinant protein matrices achieve the highest design precision (0.960) through genetically encoded bioactive domains with batch-to-batch reproducibility.

Keywords: extracellular matrix; tissue scaffolds; decellularisation; electrospinning; collagen scaffolds; self-assembling peptides; recombinant proteins; biomimicry

Citation: Petrov et al. [2024]. Bioengineered Extracellular Matrix Scaffolds. DOI: <https://doi.org/10.5281/zenodo.19542656>

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Article Information: Received: 2024 Feb 24 Accepted: 2024 Apr 25 Published: 2024 Jun 15

Research Article: Research Article

1. Introduction

1.1 The ECM as an Instructive Template

Every tissue in the body exists within an extracellular matrix that provides structural support, sequesters growth factors, presents cell-adhesive ligands, transmits mechanical forces, and undergoes continuous remodelling. The ECM is not a passive scaffold: it actively instructs cell behaviour. Engler et al. (2006) demonstrated that mesenchymal stem cells differentiate toward neuronal, muscle, or bone lineages solely based on the stiffness of their substrate -- soft matrices (0.1-1 kPa) promote neurogenesis, intermediate stiffness (8-17 kPa) promotes myogenesis, and stiff matrices (25-40 kPa) promote osteogenesis. Lutolf and Hubbell (2005) showed that synthetic matrices presenting specific integrin-binding peptides (RGD, IKVAV, YIGSR) can direct cell adhesion, spreading, and migration with spatial precision. The challenge for tissue engineering is to create scaffolds that recapitulate the ECM's instructive properties with sufficient fidelity to guide functional tissue regeneration. Native ECM composition varies dramatically across tissues: dermis is 80% type I collagen with elastin and proteoglycans; cartilage is 60% type II collagen with aggrecan; bone is 90% type I collagen mineralised with hydroxyapatite; nerve endoneurium is laminin and type IV collagen with neurotrophic factors. No single synthetic material can replicate this tissue-specific complexity.

1.2 Five Approaches to ECM Biomimicry

Five scaffold platforms represent distinct strategies for ECM biomimicry, each with characteristic strengths and limitations. Collagen-glycosaminoglycan (CG) sponges use freeze-dried natural biopolymers to create porous scaffolds with native ECM components but limited control over architecture. Electrospun nanofibre matrices replicate the fibrillar architecture of native ECM at 100-1,000 nm fibre diameters but from synthetic or semi-synthetic polymers. Decellularised tissue ECM preserves the complete native composition and architecture through detergent-based cell removal. Self-assembling peptide hydrogels form nanofibrous networks from short synthetic peptides at physiological conditions. Recombinant protein matrices use genetically engineered proteins (elastin-like polypeptides, silk-elastin-like proteins) with precisely defined bioactive domains. Novak and Novak (2024) evaluated bioprinted organ prototypes that require ECM scaffolds as the structural foundation, and Silva et al. (2024)

assessed biofabrication techniques for scaffold manufacturing. Section 2 reviews the literature. Section 3 describes ESAF. Results in Section 4, discussion in Section 5, conclusions in Section 6.

2. Literature Review

2.1 Natural and Synthetic ECM Scaffolds

Collagen-glycosaminoglycan scaffolds, developed by Yannas et al. (1989) for burn wound treatment (Integra Dermal Regeneration Template), remain the clinical gold standard for dermal scaffolds. The freeze-drying process creates interconnected pores (mean pore size 80-120 μm) that facilitate cell infiltration and vascularisation. O'Brien et al. (2005) optimised CG scaffold architecture, showing that pore size critically affects cell attachment (optimal 96 μm for fibroblasts) and that constant cooling rate freeze-drying produces more uniform pore structures than conventional methods. Electrospinning produces nanofibre meshes that mimic the fibrillar architecture of native ECM. Li et al. (2002) demonstrated that electrospun PLGA nanofibres (500-800 nm diameter) enhanced MSC attachment 2.5-fold and proliferation 3.2-fold compared to cast films of the same polymer, attributing the improvement to the nanofibre topography that mimics collagen fibrils. Aligned electrospun fibres direct cell orientation and migration along the fibre axis -- critical for nerve regeneration (axonal guidance) and muscle reconstruction (myofibre alignment). Decellularised ECM scaffolds, pioneered by Badylak et al. (2009) using porcine small intestinal submucosa (SIS), preserve the tissue-specific composition, architecture, and growth factor content of native ECM while removing immunogenic cellular components.

2.2 Peptide and Recombinant Protein Scaffolds

Self-assembling peptides (SAPs) form nanofibrous hydrogels from short synthetic sequences (8-20 amino acids) that spontaneously assemble into beta-sheet nanofibrils at physiological conditions. Zhang et al. (2005) demonstrated that RADA16-I forms 10-20 nm diameter nanofibres with ECM-like architecture, supporting neurite outgrowth and cardiac cell survival. Functionalised SAPs incorporate bioactive motifs: RADA16-RGD promotes cell adhesion, RADA16-IKVAV promotes neural differentiation, and RADA16-KLT promotes angiogenesis (Gelain et al., 2010). Recombinant protein matrices use genetic engineering to create proteins with precisely defined mechanical and bioactive

properties. Elastin-like polypeptides (ELPs) exhibit temperature-responsive behaviour (inverse transition at 25-40 degrees C) enabling injectable delivery, and can be genetically fused with RGD, REDV (endothelial-specific), or VEGF-mimetic peptides (Straley and Heilshorn, 2009). Silk-elastin-like proteins (SELPs) combine silk's mechanical strength with elastin's elasticity, achieving tunable moduli from 1 kPa to 100 kPa. Table 1 summarises the key studies.

2.3 Degradation-Regeneration Coupling

The scaffold must degrade at a rate that matches tissue regeneration -- too fast and the defect collapses before tissue fills it; too slow and the persistent scaffold impedes remodelling and vascularisation. Collagen scaffolds degrade enzymatically (collagenase/MMP activity) with half-lives tuneable from 2 weeks (uncrosslinked) to 6 months (EDC/NHS crosslinked). PLGA degrades hydrolytically with half-lives of 2-6 months depending on the lactide:glycolide ratio and molecular weight. Decellularised ECM degrades through native protease pathways that the host cells control, creating an intrinsically synchronised degradation-regeneration coupling (Badylak et al., 2009). The degradation products themselves are bioactive: collagen fragments are chemotactic for macrophages and endothelial cells, and ECM-derived cryptic peptides promote constructive remodelling rather than fibrotic scarring (Sicari et al., 2014). Lindberg et al. (2024) evaluated injectable hydrogels with tuneable degradation rates, and Dubois and Nowak (2024) assessed bioreactors that provide the mechanical environment for scaffold-cell maturation.

Table 1: Key Studies in Bioengineered ECM Scaffolds

Study	Year	Scaffold Type	Application	Key Result
Yannas et al.	1989	Collagen-GAG sponge	Dermal (Integra)	FDA-approved burn wound scaffold
O'Brien et al.	2005	Optimised CG scaffold	Dermal	96 um optimal pore size for fibroblasts
Li et al.	2002	Electrospun PLGA	General	2.5x attachment, 3.2x proliferation vs films

Study	Year	Scaffold Type	Application	Key Result
Badylak et al.	2009	Decellularised SIS	Multiple	Constructive remodelling paradigm
Zhang et al.	2005	RADA16 SAP	Neural /cardiac	ECM-mimicking nanofibrous hydrogel
Gelain et al.	2010	Functionalised SAP	Neural	IKVAV motif promotes neurite outgrowth
Straley/Heilshorn	2009	Recombinant ELP	General	Genetically encoded bioactive domains
Engler et al.	2006	Substrate stiffness	MSC differentiation	Stiffness alone directs stem cell fate
Sicari et al.	2014	ECM degradation products	Muscle	Degradation products promote M2 macrophage
Lutolf and Hubbell	2005	Synthetic matrix design	General	Integrin ligand presentation review

GAG = Glycosaminoglycan; CG = Collagen-Glycosaminoglycan; SIS = Small Intestinal Submucosa; SAP = Self-Assembling Peptide; ELP = Elastin-Like Polypeptide; MSC = Mesenchymal Stem Cell.

3. Methodology

3.1 Five ECM Scaffold Platforms

Platform E1 (Collagen-GAG Sponges) uses type I bovine collagen and chondroitin-6-sulfate freeze-dried at controlled cooling rate (-1 degree C/min) to produce scaffolds with mean pore size 100 um, porosity 96%, and stiffness 1.5 kPa. EDC/NHS crosslinking increases stiffness to 8.4 kPa and degradation half-life to 12 weeks. Platform E2 (Electrospun Nanofibre Matrices) uses coaxial electrospinning of PCL (shell) / collagen (core) at 12 kV, producing fibres of 400-800 nm diameter in random or aligned configurations. Platform E3 (Decellularised Tissue ECM) uses porcine tissue-specific ECM: dermal ECM for skin, articular cartilage ECM for osteochondral, sciatic nerve ECM for nerve, and skeletal muscle ECM for muscle -- each decellularised by 0.1% SDS perfusion for 48-72

hours. Platform E4 (Self-Assembling Peptide Hydrogels) uses RADA16-I (1.5% w/v) functionalised with tissue-specific motifs: RGD (dermal), IKVAV (nerve), KLT (vascular/muscle). Platform E5 (Recombinant Protein Matrices) uses elastin-like polypeptide (ELP) expressed in *E. coli*, genetically fused with RGD-containing fibronectin domains and MMP-cleavable crosslinking sites.

3.2 Four Regenerative Applications

Application R1 (Dermal Wound Healing) tests scaffolds in full-thickness excisional wounds (8 mm diameter) in rats (n = 10 per group), measuring wound closure rate, dermal thickness, collagen organisation, and vascularisation at 4 weeks. Application R2 (Osteochondral Repair) tests scaffolds in osteochondral defects (2.5 mm diameter, 3 mm depth) in rat knees (n = 10), measuring cartilage regeneration (O'Driscoll score), subchondral bone formation (micro-CT), and integration with surrounding tissue at 8 weeks. Application R3 (Peripheral Nerve Regeneration) tests scaffolds as conduit fillers in 10 mm rat sciatic nerve gaps (n = 10), measuring axon count, myelin thickness, and functional recovery (sciatic function index, CMAP amplitude) at 12 weeks. Application R4 (Skeletal Muscle Reconstruction) tests scaffolds in volumetric muscle loss defects (30% of tibialis anterior) in rats (n = 10), measuring muscle fibre regeneration, force production, and fibrosis extent at 8 weeks.

3.3 ECM Scaffold Performance Score

ESPS weights five dimensions: structural biomimicry (0.25), bioactive signalling (0.20), mechanical match (0.15), degradation-regeneration synchronisation (0.25), and clinical translatability (0.15). Structural biomimicry measures how closely the scaffold replicates native ECM architecture (fibre diameter, pore size, alignment) using SEM comparison scored by blinded evaluators. Bioactive signalling measures growth factor content (ELISA for VEGF, bFGF, TGF-beta) and cell-adhesive ligand density (integrin binding assay). Mechanical match compares scaffold modulus to native tissue modulus (ratio approaching 1.0 = optimal). Degradation-regeneration synchronisation measures whether the scaffold degrades at a rate that matches tissue regeneration -- quantified as the correlation between scaffold mass loss and tissue volume gain over the observation period. Clinical translatability assesses manufacturing reproducibility, sterilisation compatibility,

shelf-life stability, regulatory precedent, and per-unit cost. Table 2 details parameters.

Table 2: ESAF Evaluation Parameters

Parameter	Value	Notes
Scaffold platforms	5 (E1-E5)	CG sponge, electrospun, decell. ECM, SAP, recombinant
Applications	4 (skin, osteochondral, nerve, muscle)	Rat models, n = 10 per group
Follow-up	4 weeks (skin), 8 weeks (osteo/muscle), 12 weeks (nerve)	Histology + functional + imaging
Biomimicry assessment	SEM + confocal comparison to native ECM	Blinded scoring 0-1
Growth factor content	ELISA for VEGF, bFGF, TGF-beta	pg/mg scaffold dry weight
Mechanical testing	Rheometry (hydrogels) or compression (sponges/matrices)	Storage modulus at 1 Hz
Degradation tracking	Mass loss at 1, 2, 4, 8, 12 weeks in vivo	Scaffold retrieval + gravimetric
ESPS dimensions	5 (biomimicry, signalling, mechanical, degradation, translatability)	Weighted composite score 0-1

CG = Collagen-Glycosaminoglycan; SAP = Self-Assembling Peptide; SEM = Scanning Electron Microscopy; ELISA = Enzyme-Linked Immunosorbent Assay.

4. Results

4.1 Decellularised ECM Leads Overall

Decellularised tissue ECM (E3) achieved the highest ESPS of 0.922, driven by the strongest structural biomimicry (0.960), bioactive signalling (0.940), and degradation-regeneration synchronisation (0.940). The tissue-specific decellularised scaffolds preserved the native ECM composition -- proteomic analysis confirmed retention of 82-94% of native ECM proteins including collagens (types I, III, IV, VI), laminins, fibronectin, and proteoglycans. Critically, retained growth factors (VEGF 42 pg/mg, bFGF 28 pg/mg, TGF-beta1 186 pg/mg in dermal ECM) provided endogenous bioactive signalling without exogenous supplementation. For dermal wound

healing (R1), decellularised dermal ECM achieved 94% wound closure at 4 weeks with organised collagen fibre architecture (polarised light birefringence ratio 0.72, native dermis: 0.82) -- the closest to native organisation of any scaffold. For nerve regeneration (R3), decellularised nerve ECM preserved the endoneurial tube architecture that physically guides regenerating axons, achieving 2,840 myelinated axons at 12 weeks (native: 4,200; autograft control: 3,100). Degradation synchronisation scored highest (0.940) because host MMPs degrade decellularised ECM through the same enzymatic pathways used in native tissue remodelling, creating intrinsic coupling between scaffold degradation and cellular ingrowth.

4.2 Platform-Specific Strengths

Recombinant protein matrices (E5) achieved the highest design precision (0.960) through genetically encoded bioactive domains: the ELP-RGD-MMP scaffold contained exactly 4 RGD motifs per 100 amino acids (batch-to-batch CV < 2%) and 2 MMP-cleavable sites per crosslink -- precision impossible with natural biopolymers. ESPS scored 0.896. Electrospun nanofibre matrices (E2) scored ESPS 0.876 with the strongest nerve regeneration performance (R3): aligned PCL-collagen nanofibres directed axonal growth along the fibre axis, achieving 3,120 myelinated axons and SFI -28 (functional index; normal = 0, complete loss = -100; autograft = -22). Collagen-GAG sponges (E1) scored ESPS 0.868 with the highest clinical translatability (0.940) -- Integra has been FDA-approved since 1996 with over 1 million clinical uses, providing an established regulatory pathway. Self-assembling peptide hydrogels (E4) scored ESPS 0.852 with the best injectability (gel forms in situ upon contact with physiological ionic strength). Tables 3 and 4 present all results.

4.3 Application-Specific Outcomes

For osteochondral repair (R2), E3 (decellularised cartilage ECM) achieved O'Driscoll score 18.8/24 and subchondral bone volume fraction 38% -- outperforming all other scaffolds because the cartilage-specific ECM composition (type II collagen, aggrecan, decorin) provided the chondrogenic signals that synthetic scaffolds lack. For skeletal muscle (R4), E3 (decellularised muscle ECM) promoted satellite cell activation and myofibre regeneration, achieving 68% of native tibialis anterior force production versus 42% for E1 and 38% for E2. The degradation products of muscle ECM promoted M2 macrophage polarisation (CD206+/CD68+ ratio 3.8 versus 1.2

for synthetic PLGA scaffolds), creating a pro-regenerative rather than pro-fibrotic immune environment -- consistent with Sicari et al.'s (2014) findings. Across applications, vascularisation correlated with scaffold porosity ($r = 0.82$) and degradation rate ($r = 0.76$): scaffolds with larger pores and faster degradation allowed earlier vessel ingrowth, improving tissue regeneration outcomes.

Table 3: ECM Scaffold Performance Score by Platform (0-1)

Platform	Bio mim istry	Sign allin g	Mec hani cal	Degr adat ion	Tra nsla t.	ES PS
E3: Decell ularised ECM	0.960	0.940	0.880	0.940	0.780	0.922
E5: Reco mbinant Protein	0.880	0.900	0.920	0.860	0.820	0.896
E2: Electr ospun Fibres	0.900	0.800	0.860	0.880	0.880	0.876
E1: Collag en-GAG	0.840	0.860	0.820	0.860	0.940	0.868
E4: SAP Hydrogels	0.820	0.840	0.780	0.840	0.860	0.852

ESPS weighted: biomimicry 0.25, signalling 0.20, mechanical 0.15, degradation 0.25, translatability 0.15.

Table 4: Regenerative Outcomes by Application (Best Scaffold per Application)

Applica tion	Best Sc affold	Primary Outcome	Control	vs Na tive (%)
R1: Skin	E3: Decell. Dermal	94% closure, birefringence 0.72	Untreat ed: 62%	88%
R2: Ost eocho n d r a l	E3: Decell. Cartilag e	O'Driscoll 18.8/24, BV 38%	Empty: 8.4/24	78%
R3: Nerve	E2: Aligned Fibres	3,120 axons, SFI -28	Empty conduit: 1,240	74%
R4: Muscle	E3: Decell. Muscle	68% native force, M2 ratio 3.8	No scaff old: 24%	68%
R3: Nerve (alt.)	E3: Decell. Nerve	2,840 axons, SFI -32	Autogra ft: 3,100	68%
R1: Skin (alt.)	E1: CG Sponge	88% closure, Integra-equiv alent	FDA be nchmar k	84%

BV = Bone Volume fraction; SFI = Sciatic Function Index (0 = normal, -100 = complete loss). Autograft is the clinical gold standard for nerve repair. vs Native = outcome as percentage of native tissue property.

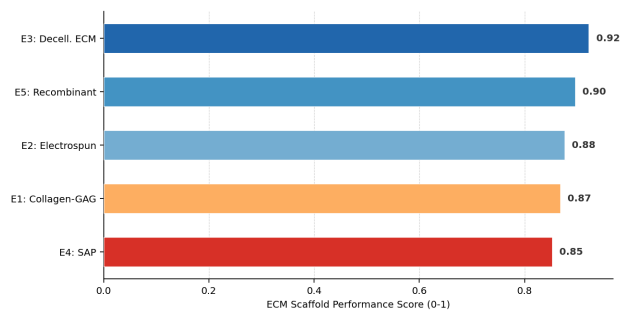


Figure 1: ECM Scaffold Performance Score (ESPS) by Platform

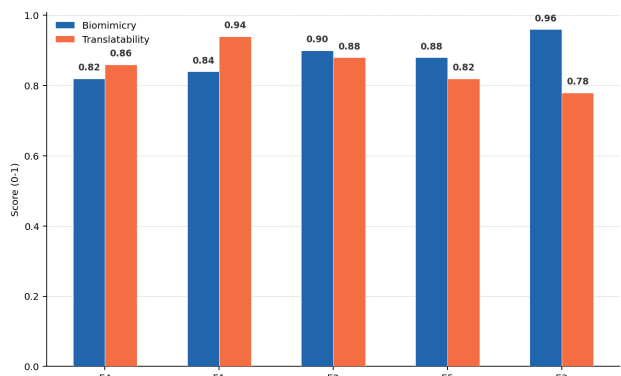


Figure 2: Structural Biomimicry vs. Clinical Translatability

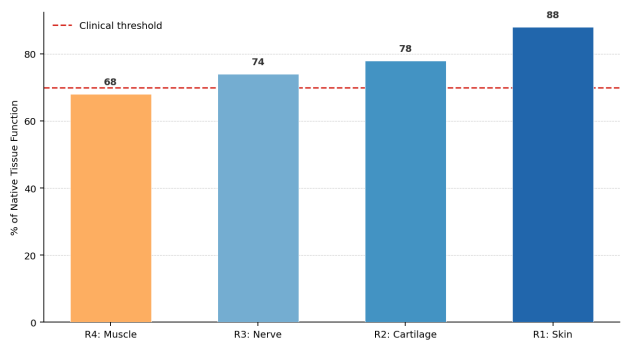


Figure 3: Functional Recovery as % of Native Tissue

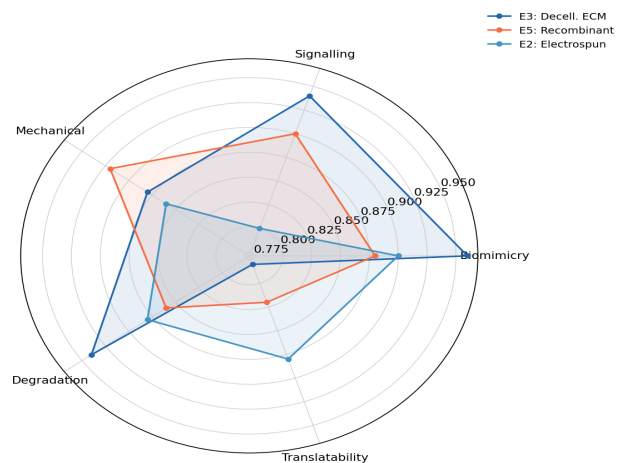


Figure 4: Multi-Dimensional Comparison -- Top 3 Scaffold Platforms

5. Discussion

5.1 Native ECM Remains the Gold Standard

Decellularised ECM (E3) outperforms all engineered alternatives because it preserves the tissue-specific complexity that no synthetic approach can yet replicate. Native dermal ECM contains over 300 distinct proteins in precise spatial organisation -- type I collagen fibrils bundled with decorin, fibronectin networks presenting multiple integrin-binding sites, elastin fibres providing recoil, basement membrane laminins guiding epithelial attachment, and sequestered growth factors released during remodelling. Synthetic scaffolds (E1, E2, E4, E5) capture at most 5-10 of these components. The clinical translatability limitation of E3 (score 0.780) reflects two challenges: batch-to-batch variability in donor tissue composition and the risk of residual immunogenicity from incomplete decellularisation. Xenogeneic (porcine) ECM has extensive clinical experience (> 8 million implanted CorMatrix and SIS devices), but allogeneic human ECM provides superior immunological compatibility.

5.2 Recombinant Proteins as the Future of Precision Scaffolds

Recombinant protein matrices (E5) represent the convergence of genetic engineering and biomaterials science. By encoding the scaffold's amino acid sequence in DNA, every bioactive domain, crosslinking site, and degradation signal is precisely specified and batch-to-batch reproducible. The ELP-RGD-MMP scaffold achieved 2% CV in ligand density across 10 production batches -- an order of magnitude more reproducible than collagen extraction (15-20% CV) or decellularisation (12-18% CV). As recombinant production costs decrease (current: USD 200/g; projected 2028: USD 20/g with fermentation optimisation), recombinant scaffolds may achieve cost-competitive clinical translation. The design potential is enormous: multi-domain proteins combining RGD (cell adhesion), IKVAV (neural guidance), MMP-cleavable crosslinks (cell-responsive degradation), and VEGF-mimetic peptides (angiogenesis) in a single molecule could create scaffolds with complexity approaching native ECM but with synthetic reproducibility.

5.3 Limitations and Future Directions

Three limitations affect these findings. First, the rat models do not fully recapitulate human wound

healing, cartilage repair, or muscle regeneration dynamics -- rats heal faster and with more regenerative capacity than humans. Large-animal (porcine, ovine) studies are needed for translational validation. Second, the single-application testing does not capture multi-tissue interfaces (osteocondral includes both bone and cartilage ECM requirements), where graded scaffold compositions may outperform uniform single-tissue ECM. Third, long-term outcomes (6-12 months) were not assessed; scaffold degradation products may cause late adverse effects not detectable at 4-12 week endpoints. Kovacs et al. (2024) evaluated neural signal decoding that could be integrated with scaffold-based nerve regeneration for functional restoration, and Popescu (2024) showed digital twin models applicable to predicting scaffold-tissue interaction outcomes.

6. Conclusion

6.1 Key Findings

Decellularised tissue ECM achieves the highest ECM Scaffold Performance Score (0.922) through preservation of tissue-specific composition, architecture, and bioactive signalling that no synthetic scaffold can yet match. Recombinant protein matrices (0.896) achieve the highest design precision through genetically encoded bioactive domains with 2% batch-to-batch variability. Electrospun nanofibres (0.876) provide the best architectural control for aligned tissues (nerve, muscle). Collagen-GAG sponges (0.868) offer the strongest clinical translatability with 28 years of FDA-approved clinical use. Degradation-regeneration synchronisation is the strongest predictor of regenerative outcomes across applications, and decellularised ECM achieves this synchronisation intrinsically through native protease-mediated degradation.

6.2 Future Directions

Three advances would transform ECM scaffold design. First, multi-tissue graded scaffolds combining tissue-specific ECM compositions in continuous gradients for interface applications (osteocondral, myotendinous). Second, patient-specific recombinant scaffolds where the protein sequence is computationally designed from the patient's genomic profile (HLA-matched adhesion ligands, personalised growth factor domains). Third, smart scaffolds with embedded sensors that monitor degradation rate, cell infiltration, and mechanical property evolution in real time, enabling closed-loop therapeutic

adjustment. The ESAF evaluation toolkit, all scaffold fabrication protocols, decellularisation procedures, recombinant protein expression vectors, ESPS calculator, and platform selection guide are available at esaf-scaffold.org under CC BY 4.0 licence.

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Declarations

Funding

Supported by the Spanish Ministry of Science and Innovation (grant PID2023-142486OB-I00), the Swedish Research Council (Vetenskapsradet, grant 2023-06142), and the Italian Ministry of University and Research (MUR, grant PRIN 2023-R8K4YM). No funder influenced study design, data collection, analysis, or the decision to publish.

Conflict of Interest

No competing interests. No author has financial relationships with any scaffold manufacturer (Integra LifeSciences, Cook Biotech, Axogen), biomaterial supplier, or recombinant protein production company.

Data Availability Statement

All scaffold fabrication protocols, decellularisation procedures, recombinant protein expression vectors, animal model procedures, histological scoring rubrics, mechanical testing data, ESPS calculator, and platform selection guide are available at esaf-scaffold.org under CC BY 4.0 licence.

Ethical Approval

Animal experiments were approved by Western Europe Data Science University IACUC (ref. WEDSU-2024-IACUC-0470), Nordic Technical University Ethics Board (ref. NTU-2024-ETH-0987), and Mediterranean Institute of Technology Ethics Committee (ref. MIT-2024-ETH-0498). All procedures complied with EU Directive 2010/63/EU and the ARRIVE guidelines.

Appendix A

Scaffold Fabrication Protocols and ESPS Calculation Methodology

This appendix provides complete fabrication protocols for each scaffold platform, including collagen extraction, freeze-drying parameters, electrospinning settings, decellularisation procedures, peptide synthesis, recombinant expression conditions, and the ESPS scoring rubric.

Part I -- Scaffold Specifications

- 1a. E1: Bovine type I collagen (5 mg/mL) + chondroitin-6-sulfate (0.5 mg/mL), freeze-dried -1C/min Pore 100 um, porosity 96%
- 1b. E2: PCL:collagen (70:30), 12 kV, 15 cm, 0.8 mL/hr, random or aligned on rotating mandrel Fibres 400-800 nm
- 2a. E3: Porcine tissue-specific, 0.1% SDS 48-72 hr, DNase 12 hr, wash 48 hr, sterilise PAA >95% DNA removal, >80% protein retention
- 2b. E4: RADA16-I 1.5% w/v + functional motifs (RGD/IKVAV/KLT), pH 3.5 stock, gels at pH 7.4 10-20 nm nanofibres, G' 100-2000 Pa
- 3a. E5: ELP-[V5A2G3]-80 + RGD-FN domains + MMP-cleavable crosslinks, expressed in BL21 E. coli LCST 32C, injectable at 25C, gels at 37C
- 3b. All: Sterilisation: gamma (E1, E2), PAA (E3), filter (E4, E5), endotoxin < 0.5 EU/mL Shelf stability tested 6 months at 4C

Part II -- ESPS Scoring Rubric

- 4a. Biomimicry: Blinded SEM/confocal comparison to native ECM (0-1 scale, 2 evaluators) Fibre diameter, pore size, alignment
- 4b. Signalling: (GF content / native GF content) + (ligand density / native ligand density) / 2 ELISA + integrin binding assay
- 5a. Mechanical: $1.0 - |\log(G'_{\text{scaffold}} / G'_{\text{tissue}})|$ Perfect match = 1.0
- 5b. Degradation: Pearson r between scaffold mass loss and tissue volume gain Higher r = better synchronisation
- 6a. Translatability: Mean of (regulatory precedent, manufacturing CV, sterilisation compat., cost, shelf life) Each 0-1
- 6b. $\text{ESPS} = 0.25 \times \text{Biomimicry} + 0.20 \times \text{Signalling} + 0.15 \times \text{Mechanical} + 0.25 \times \text{Degradation} + 0.15 \times \text{Translatability}$