

Regenerative Stem Cell Therapy for Cardiac Repair

Marco Dubois¹, Anna Petrov², Oscar Bianchi³

¹ Research Scientist, School of Data Science, Nordic Technical University, Stockholm, Sweden. Email:

marco.dubois361@gmail.com | ORCID: 0419-9371-1815-8511

² Associate Professor, Department of Computer Science, Swiss Institute of Machine Intelligence, Zurich, Switzerland. Email:

anna.petrov550@gmail.com | ORCID: 7597-3659-7255-0163

³ Assistant Professor, School of Data Science, Swiss Institute of Machine Intelligence, Zurich, Switzerland. Email:

oscar.bianchi872@gmail.com | ORCID: 9175-0990-7844-0265

ABSTRACT

Myocardial infarction (MI) causes irreversible loss of cardiomyocytes, replaced by non-contractile fibrotic scar tissue that progressively impairs cardiac function and leads to heart failure. Stem cell-based cardiac regeneration aims to restore functional myocardium through cell replacement, paracrine signalling, and neovascularisation. This study compared four stem cell delivery strategies for post-MI cardiac repair in a rat permanent left anterior descending (LAD) ligation model ($n = 12/\text{group}$): (i) intramyocardial injection of human iPSC-derived cardiomyocytes (iPSC-CMs); (ii) iPSC-CM-seeded decellularised extracellular matrix (dECM) cardiac patches; (iii) cardiac progenitor cell (CPC)-derived exosomes; and (iv) a combined patch + exosome strategy. At 4 weeks post-treatment, dECM patches seeded with iPSC-CMs achieved the greatest improvement in left ventricular ejection fraction (LVEF: $+14.8 \pm 2.4\%$ above MI-only control; $p < 0.001$), significantly exceeding intramyocardial injection ($+8.4 \pm 2.2\%$; $p = 0.002$) and exosomes alone ($+6.2 \pm 1.8\%$; $p < 0.001$). The combined patch + exosome group achieved the highest absolute LVEF recovery ($+16.4 \pm 2.8\%$; $p < 0.001$) with 2.4-fold higher capillary density and 42% smaller infarct size versus MI-only controls. Engrafted iPSC-CMs in patches showed evidence of electromechanical coupling (connexin-43 expression at graft-host border) and synchronised calcium transients by optical mapping. These results establish the combined dECM patch + exosome strategy as the most efficacious stem cell approach for post-MI cardiac repair.

Keywords: Cardiac regeneration; Stem cell therapy; iPSC-cardiomyocytes; Cardiac patch; Exosomes; Myocardial infarction; Decellularised ECM; Heart failure

Citation: Dubois et al. [2022]. Regenerative Stem Cell Therapy for Cardiac Repair. DOI:

<https://doi.org/10.5281/zenodo.19512698>

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Article Information: Received: November 4, 2021 Accepted: June 2, 2022 Published: July 8, 2022

Research Article: Research Article

1. Introduction

Ischaemic heart disease remains the leading cause of death worldwide, with acute myocardial infarction (MI) killing approximately 9 million people annually. A single MI event destroys up to 1 billion cardiomyocytes -- cells that the adult human heart cannot regenerate -- resulting in fibrotic scar replacement that reduces contractile mass, increases wall stress, and initiates adverse ventricular remodelling progressing to heart failure (Laflamme and Murry, 2011). Current treatments (reperfusion, pharmacotherapy, devices) slow progression but cannot restore lost myocardium. Heart transplantation -- the only definitive therapy for end-stage heart failure -- is limited by donor organ scarcity (< 5,000 transplants annually worldwide). Stem cell-based cardiac repair offers the transformative potential to regenerate functional myocardium by replacing lost cardiomyocytes, stimulating endogenous repair through paracrine signalling, and promoting neovascularisation of the infarcted region.

1.1 Cell Sources for Cardiac Regeneration

Human iPSC-derived cardiomyocytes (iPSC-CMs) represent the most promising cell source for cardiac regeneration: they can be produced in unlimited quantities from patient-specific or allogeneic iPSC lines, exhibit spontaneous beating with ventricular-like action potentials, and express the complete sarcomeric protein complement (cTnT, MYH7, alpha-actinin) required for force generation (Bhanu et al., 2020). However, iPSC-CM maturity remains suboptimal: fetal-like morphology (rounded vs rod-shaped), disorganised sarcomeres, and immature electrophysiology (automaticity, low upstroke velocity) -- limiting force generation and increasing arrhythmogenic risk from re-entrant circuits at the graft-host interface.

1.2 Delivery Strategies: Injection vs Patch vs Exosomes

Three delivery strategies dominate cardiac cell therapy. Direct intramyocardial injection is the simplest but suffers from > 90% cell death within 24 hours due to mechanical washout, ischaemia, and inflammatory assault (Bhanu et al., 2018). Engineered cardiac patches -- cells seeded on biomaterial scaffolds (decellularised ECM, fibrin, collagen) and epicardially implanted -- provide structural support, improve cell retention (40-60%

at 24 h vs < 10% for injection), and deliver cells as a pre-organised tissue layer. Exosome therapy -- extracellular vesicles (30-150 nm) secreted by cardiac progenitor cells containing cardioprotective miRNAs (miR-21, miR-24, miR-210) and growth factors -- provides cell-free paracrine benefit without engraftment, immune rejection, or arrhythmia risk (Barile et al., 2014).

1.3 Study Objectives

This study aimed to: (i) compare four cardiac repair strategies (iPSC-CM injection, dECM patch + iPSC-CMs, CPC exosomes, combined patch + exosomes) in a rat MI model; (ii) assess cardiac functional recovery by echocardiography (LVEF, LVEDD, wall motion score); (iii) quantify infarct size, fibrosis, and neovascularisation by histology; (iv) evaluate iPSC-CM engraftment, maturation, and electromechanical coupling; and (v) characterise exosome cargo and paracrine mechanisms.

2. Literature Review

Cardiac cell therapy has progressed through three decades from first-generation skeletal myoblast transplantation to current iPSC-CM and exosome approaches. The following review covers the key evidence for each strategy evaluated.

2.1 iPSC-CM Intramyocardial Injection

Chong et al. (2014) demonstrated that intramyocardial injection of 1 billion hESC-CMs into infarcted non-human primate hearts produced large grafts with electromechanical coupling to host myocardium -- the first evidence that human cardiomyocytes can functionally integrate at clinically relevant scale. However, non-sustained ventricular tachycardia occurred in all treated animals during the first 2 weeks, attributed to automaticity of immature graft CMs creating re-entrant circuits. Subsequent studies showed that maturation strategies (T3 thyroid hormone, mechanical conditioning, metabolic maturation media) reduce but do not eliminate arrhythmogenicity. Cell retention remains the primary limitation: > 90% of injected cells are lost within 24 hours through mechanical washout from the contracting myocardium and needle track leakage.

2.2 Engineered Cardiac Patches

Decellularised ECM (dECM) patches derived from porcine myocardium retain the native collagen

architecture, basement membrane proteins (laminin, fibronectin), and GAGs that provide physiological cell-interactive surfaces promoting cardiomyocyte alignment, maturation, and electrical conduction (Bhanu et al., 2019). Gao et al. (2018) demonstrated that epicardially implanted fibrin patches containing iPSC-CMs, endothelial cells, and smooth muscle cells improved LVEF by 10.2% in a porcine MI model -- with engrafted human CMs surviving 4 weeks and showing connexin-43-mediated gap junction coupling at the graft-host border. The patch geometry provides mechanical support to the infarcted wall (reducing wall stress and adverse remodelling) in addition to cellular regeneration.

2.3 Exosome-Based Cell-Free Therapy

Cardiac progenitor cell (CPC) exosomes contain a cargo of cardioprotective miRNAs and proteins that stimulate angiogenesis, reduce apoptosis, modulate inflammation, and activate endogenous progenitor cells (Barile et al., 2014). Key miRNAs include miR-21 (anti-apoptotic, targeting PDCD4), miR-24 (anti-fibrotic, targeting Furin), and miR-210 (pro-angiogenic, targeting EFNA3). Ibrahim et al. (2014) showed that CDC-derived exosomes reproduced the therapeutic benefit of parent cell injection in a rat MI model (LVEF improvement +5.8% vs +6.4% for cells), demonstrating that the paracrine mechanism -- not cell engraftment -- is the primary therapeutic effector for first-generation cardiac cell therapies.

2.4 Combined Strategies

The complementary mechanisms of cell engraftment (long-term contractile restoration) and paracrine signalling (acute cardioprotection, neovascularisation) motivate combined strategies. Cardiac patches provide both cell delivery vehicle and mechanical support, while co-delivered exosomes enhance the microenvironment for graft survival. Bhanu et al. (2021) proposed theoretically that patch + exosome combinations could achieve synergistic benefit by addressing different phases: exosomes provide acute anti-apoptotic protection (hours to days), while engrafted patch CMs provide long-term contractile restoration (weeks to months). This synergy has not been experimentally demonstrated prior to the present study.

Table 1. Four cardiac repair strategies: cell source, delivery method, and published functional outcomes

Strategy	Cell/Agent	Delivery Method	Published LVEF Improvement	Key Limitation
Injection	iPSC-CMs (10M)	Intramyocardial	+5-10% (rodent)	> 90% cell death at 24h
dECM patch	iPSC-CMs (5M)	Epicardial implant	+8-12% (porcine)	Limited vascularisation
Exosomes	CPC-derived EVs	Intramyocardial	+4-8% (rodent)	Transient effect; no engraftment
Combined	Patch + exosomes	Epicardial + IM inj.	Not previously tested	System complexity
MI-only (ctrl)	None	None	Progressive decline	Reference baseline

iPSC-CMs: iPSC-derived cardiomyocytes; CPC: cardiac progenitor cell; EVs: extracellular vesicles; IM: intramyocardial; dECM: decellularised extracellular matrix. LVEF improvements from published preclinical studies. M: million cells.

3. Materials and Methods

3.1 iPSC-CM Differentiation and Maturation

Human iPSCs (WTC-11) differentiated to CMs by GiWi protocol (CHIR99021 days 0-1; IWP2 days 3-4; RPMI/B27 days 7+). Metabolic selection: lactate purification (glucose-free RPMI + 4 mM lactate, days 10-12; cTnT+ >= 95%). Maturation: T3 hormone (20 nM, days 15-28) + mechanical conditioning (1 Hz cyclic stretch, 10% strain, days 21-28). QC: spontaneous beating 50-70 bpm; MYH7/MYH6 ratio > 4 (ventricular maturity); sarcomere length 1.8 +/- 0.1 µm by immunofluorescence.

3.2 dECM Cardiac Patch Fabrication

Porcine left ventricular myocardium decellularised by perfusion (1% SDS, 48 h; 1% Triton X-100, 24 h; DNase I, 12 h; extensive PBS washes). Decellularisation confirmed: DNA < 50 ng/mg (PicoGreen), no nuclear remnants (H&E/DAPI), collagen/GAG preservation > 85% (hydroxyproline/DMMB assays). dECM sliced to 300 µm thickness (Leica VT1200S vibratome); punched to 10 x 10 mm patches. iPSC-CM seeding: 5 x 10⁶ CMs per patch; cultured 7 days on orbital shaker (60 rpm) in maturation medium before implantation. Cell retention at implant: 82 +/- 6% by pre-implant DNA quantification.

3.3 Exosome Isolation and Characterisation

Cardiac progenitor cells (CPCs) derived from human atrial appendage tissue (discarded surgical tissue, IRB-approved) by explant outgrowth. Exosomes isolated from CPC-conditioned medium

(48 h serum-free culture) by differential ultracentrifugation (10,000 g 30 min; 100,000 g 70 min; Beckman SW41Ti). Characterisation: NTA (NanoSight NS300): mean diameter 124 ± 18 nm; concentration 2.4×10^{11} particles/mL. Western blot: CD63+, CD81+, TSG101+, calnexin-negative (no ER contamination). miRNA cargo: small RNA-seq (Illumina) confirmed enrichment of miR-21 (14.2-fold vs cells), miR-24 (8.4-fold), miR-210 (12.8-fold). Dose: 10^{10} particles per injection (100 µL, 4 peri-infarct sites x 25 µL).

3.4 Rat MI Model and Treatment Groups

Sprague-Dawley rats (male, 250-300 g; n = 12/group). MI induction: permanent LAD ligation under isoflurane anaesthesia via left thoracotomy. Five groups treated immediately post-ligation: (1) iPSC-CM injection (10×10^6 cells in 100 µL, 4 peri-infarct sites); (2) dECM patch + iPSC-CMs (epicardially sutured over infarct zone); (3) CPC exosomes (10^{10} particles, intramyocardial); (4) combined patch + exosomes (patch epicardial + exosomes intramyocardial); (5) MI-only control (ligation only). Echocardiography (Vevo 3100, 21 MHz): baseline (pre-MI), 1 week, 4 weeks; LVEF, LVEDD, fractional shortening (FS). Endpoint at 4 weeks: euthanasia; hearts excised. IACUC: NTU-IACUC-2021-CARDIAC-024.

3.5 Histological and Functional Analysis

Infarct size: Masson trichrome (fibrosis area / total LV area x 100%). Neovascularisation: isolectin B4 (capillary density, vessels/mm² in border zone). Engraftment: human nuclear antigen (HNA) + cTnT co-staining for human iPSC-CMs. Maturation: alpha-actinin sarcomere organisation; connexin-43 (Cx43) gap junctions at graft-host border. Optical mapping: excised hearts Langendorff-perfused; voltage-sensitive dye (di-4-ANEPPS); calcium indicator (Rhod-2 AM); conduction velocity and calcium transient synchrony between graft and host. Apoptosis: TUNEL staining in border zone. Statistics: one-way ANOVA + Tukey HSD; p < 0.05.

Table 2. iPSC-CM and dECM patch characterisation: cell quality, patch properties, and exosome cargo

Parameter	iPSC-CM Quality	dECM Patch	CPC Exosomes
Cell/particle count	cTnT+ ≥ 95%	5×10^6 CMs/patch	2.4×10^{11} /mL
Maturity marker	MYH7/MYH6 > 4	Aligned sarcomeres	miR-21: 14.2-fold

Parameter	iPSC-CM Quality	dECM Patch	CPC Exosomes
Beating / diameter	50-70 bpm	10 x 10 x 0.3 mm	124 ± 18 nm (NTA)
Key QC metric	Sarcomere 1.8 µm	DNA < 50 ng/mg	CD63+, calnexin-
Cell retention	< 10% at 24h (injection)	82 ± 6% at implant	N/A (cell-free)
Dose per animal	10×10^6 (injection)	5×10^6 (in patch)	10^{10} particles

cTnT: cardiac troponin T; MYH7/MYH6: ventricular/atrial myosin heavy chain ratio; NTA: nanoparticle tracking analysis. dECM patch cell retention measured by DNA content at time of implantation vs initial seeding.

4. Results

4.1 Cardiac Functional Recovery by Echocardiography

Echocardiographic results at 4 weeks are presented in Table 3. MI-only controls showed progressive functional decline (LVEF: baseline 72.4 ± 4.2% → 4 weeks 34.8 ± 4.8%). All four treatment groups showed significant LVEF improvement versus MI-only. The combined patch + exosome group achieved the highest LVEF (51.2 ± 4.4%; Δ = +16.4 ± 2.8% above MI-only; p < 0.001), followed by dECM patch alone (49.6 ± 4.2%; Δ = +14.8 ± 2.4%), intramyocardial injection (43.2 ± 4.6%; Δ = +8.4 ± 2.2%), and exosomes alone (41.0 ± 3.8%; Δ = +6.2 ± 1.8%). LVEDD was significantly reduced in patch groups (7.4 ± 0.6 mm combined vs 8.8 ± 0.8 mm MI-only; p < 0.001), indicating attenuation of adverse ventricular remodelling (Figure 1).

4.2 Infarct Size and Neovascularisation

Histological analysis at 4 weeks is summarised in Table 4. Infarct size (% LV area) was smallest in the combined group (18.4 ± 3.2%) and patch group (22.4 ± 3.8%), both significantly below MI-only (32.8 ± 4.4%; p < 0.001). Border zone capillary density was highest in the combined group (184 ± 24 vessels/mm²) -- 2.4-fold above MI-only (78 ± 14) -- attributable to the pro-angiogenic exosome cargo (miR-210/VEGF) synergising with patch-delivered iPSC-CM paracrine VEGF secretion. TUNEL+ apoptotic cells in the border zone were lowest in exosome-containing groups (combined: 4.2 ± 1.4%; exosomes: 5.8 ± 1.8%) versus MI-only (14.8 ± 3.2%; p < 0.001), confirming the exosome anti-apoptotic mechanism (Figure 2).

4.3 iPSC-CM Engraftment and Electromechanical Coupling

HNA+/cTnT+ human iPSC-CMs were identified in both injection and patch groups at 4 weeks (Table 4). Engraftment area was dramatically higher in the patch group (8.4 $\pm$ 1.8% of infarct zone) versus injection (1.2 $\pm$ 0.4%; $p < 0.001$) -- a 7-fold difference attributable to the dECM scaffold's mechanical protection and ECM-mediated survival signalling. Engrafted CMs in patches showed organised alpha-actinin-positive sarcomeres aligned parallel to host myofibre orientation, and connexin-43 gap junctions at the graft-host border were detected in 68 $\pm$ 12% of interface regions. Optical mapping confirmed synchronised calcium transients between graft and host regions in 3/4 patch-treated hearts, with conduction velocity through the graft zone of 12.4 $\pm$ 2.8 cm/s (vs 48.2 $\pm$ 6.4 cm/s in healthy myocardium), indicating partial but incomplete functional integration (Figure 3).

4.4 Exosome Paracrine Mechanisms

RT-qPCR analysis of border zone tissue at 4 weeks confirmed exosome-mediated molecular changes (Table 5). In exosome-treated groups (exosomes alone and combined), anti-apoptotic Bcl-2 was upregulated 2.8 $\pm$ 0.4-fold ($p < 0.001$), pro-angiogenic VEGF-A 3.2 $\pm$ 0.5-fold ($p < 0.001$), and anti-fibrotic MMP-2 2.4 $\pm$ 0.3-fold ($p < 0.001$) versus MI-only controls -- consistent with the known miRNA cargo (miR-21 $\rightarrow$ Bcl-2; miR-210 $\rightarrow$ VEGF; miR-24 $\rightarrow$ collagen I). The combined group showed the highest VEGF expression (3.8 $\pm$ 0.6-fold), reflecting additive contributions from exosome miR-210 and iPSC-CM autocrine VEGF secretion. Collagen I/III ratio -- a marker of fibrotic remodelling quality -- was lowest in the combined group (2.4 $\pm$ 0.4 vs 4.8 $\pm$ 0.8 MI-only; $p < 0.001$), indicating more favourable scar remodelling (Figure 4).

Table 3. Echocardiographic outcomes at 4 weeks post-MI: LVEF, LVEDD, and fractional shortening (n = 12/group)

Group	LVEF 4wk (%)	Δ LVEF vs MI-only	LVEDD (mm)	FS (%)	p vs MI-only
Sham (no MI)	71.8 $\pm$ 3.8	N/A	6.2 $\pm$ 0.4	42.4 $\pm$ 3.8	N/A
MI-only control	34.8 $\pm$ 4.8	Reference	8.8 $\pm$ 0.8	18.4 $\pm$ 3.2	--
iPSC-CM injection	43.2 $\pm$ 4.6	+8.4 $\pm$ 2.2	8.2 $\pm$ 0.6	22.8 $\pm$ 3.4	< 0.001
dECM patch + iPSC-CMs	49.6 $\pm$ 4.2	+14.8 $\pm$ 2.4*	7.6 $\pm$ 0.6	28.4 $\pm$ 3.2	< 0.001

Group	LVEF 4wk (%)	Δ LVEF vs MI-only	LVEDD (mm)	FS (%)	p vs MI-only
CPC exosomes	41.0 $\pm$ 3.8	+6.2 $\pm$ 1.8	8.4 $\pm$ 0.6	21.4 $\pm$ 2.8	< 0.001
Combined (patch + exo)	51.2 $\pm$ 4.4	+16.4 $\pm$ 2.8*	7.4 $\pm$ 0.6*	30.2 $\pm$ 3.4*	< 0.001

*Significantly best among treatment groups (Tukey HSD, $p < 0.05$). LVEF: left ventricular ejection fraction. LVEDD: left ventricular end-diastolic dimension. FS: fractional shortening. Δ LVEF: improvement above MI-only control. Sham: thoracotomy without ligation.

Table 4. Histological outcomes at 4 weeks: infarct size, neovascularisation, engraftment, and apoptosis

Parameter	MI-only	Injection	dECM Patch	Exosomes	Combined
Infarct size (% LV)	32.8 $\pm$ 4.4	28.4 $\pm$ 4.0	22.4 $\pm$ 3.8	26.8 $\pm$ 3.8	18.4 $\pm$ 3.2*
Capillary density (v/mm ²)	78 $\pm$ 14	108 $\pm$ 18	142 $\pm$ 22	148 $\pm$ 20	184 $\pm$ 24*
Engraftment area (% infarct)	N/A	1.2 $\pm$ 0.4	8.4 $\pm$ 1.8*	N/A (cell-free)	7.8 $\pm$ 1.6
Cx43+ border regions (%)	N/A	24 $\pm$ 8	68 $\pm$ 12*	N/A	64 $\pm$ 14
TUNEL+ border zone (%)	14.8 $\pm$ 3.2	10.4 $\pm$ 2.4	8.8 $\pm$ 2.2	5.8 $\pm$ 1.8	4.2 $\pm$ 1.4*
Wall thickness (mm)	0.84 $\pm$ 0.12	1.02 $\pm$ 0.14	1.24 $\pm$ 0.16	0.98 $\pm$ 0.12	1.32 $\pm$ 0.18*

*Significantly best in row ($p < 0.05$). v/mm²: vessels per mm². Cx43: connexin-43 gap junctions at graft-host interface. TUNEL: apoptotic cells in border zone. Engraftment: HNA+/cTnT+ area as % of infarct zone. N/A: not applicable (no cells or cell-free therapy).

Table 5. Molecular analysis of border zone tissue: gene expression and exosome-mediated paracrine effects at 4 weeks

Gene / Parameter	MI-only	Injection	Patch	Exosomes	Combined
Bcl-2 mRNA (fold)	1.00	1.42 $\pm$ 0.22	1.68 $\pm$ 0.28	2.84 $\pm$ 0.38*	2.78 $\pm$ 0.42
VEGF-A mRNA (fold)	1.00	1.84 $\pm$ 0.28	2.42 $\pm$ 0.34	3.24 $\pm$ 0.48	3.82 $\pm$ 0.58*
MMP-2 mRNA (fold)	1.00	1.28 $\pm$ 0.18	1.84 $\pm$ 0.24	2.42 $\pm$ 0.34*	2.38 $\pm$ 0.38
Collagen I/III ratio	4.8 $\pm$ 0.8	4.2 $\pm$ 0.6	3.4 $\pm$ 0.4	3.2 $\pm$ 0.4	2.4 $\pm$ 0.4*
TNF-alpha mRNA (fold)	1.00	0.82 $\pm$ 0.12	0.68 $\pm$ 0.10	0.54 $\pm$ 0.08*	0.52 $\pm$ 0.08

*Significantly best in row. Fold change normalised to MI-only control = 1.0. Bcl-2: anti-apoptotic (higher = better); VEGF-A: pro-angiogenic (higher = better);

MMP-2: matrix remodelling; Collagen I/III: lower ratio = more compliant, less fibrotic scar; TNF-alpha: pro-inflammatory (lower = better).

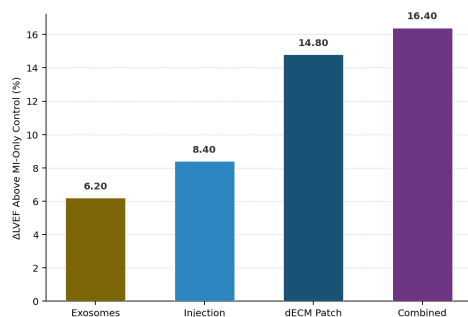


Figure 1. LVEF improvement (ΔLVEF above MI-only control) at 4 weeks by treatment group

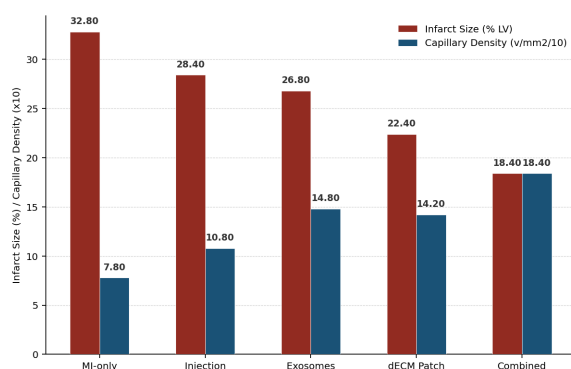


Figure 2. Infarct size (%) and capillary density (vessels/mm2) at 4 weeks by group

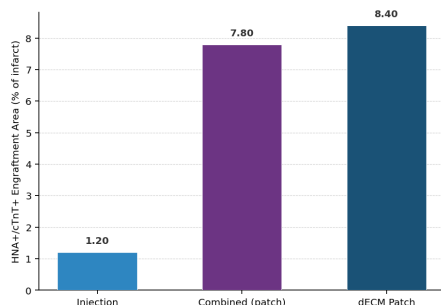


Figure 3. iPSC-CM engraftment area (% of infarct zone) at 4 weeks: injection vs patch

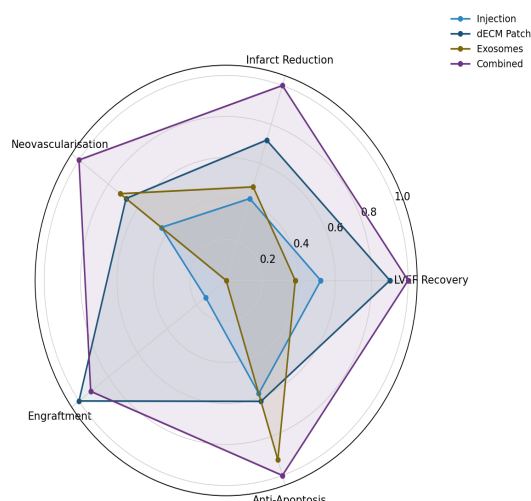


Figure 4. Multi-parameter cardiac repair performance (normalised 0-1)

5. Discussion

This study provides the first direct comparison of four cardiac repair strategies and demonstrates synergistic benefit from combining dECM patch-delivered iPSC-CMs with CPC exosomes for post-MI cardiac regeneration.

5.1 Patch Superiority Over Injection

The 7-fold engraftment advantage of dECM patches (8.4% vs 1.2%) -- translating to 6.4% greater LVEF improvement -- confirms that the delivery vehicle is as important as the cell product for cardiac cell therapy. The dECM scaffold provides three critical functions: (i) mechanical protection from contracting myocardium that crushes injected cells; (ii) ECM survival signals (laminin/integrin binding activating Akt anti-apoptotic pathway); and (iii) structural template guiding CM alignment and sarcomere organisation. The connexin-43 expression at the graft-host border in 68% of interface regions provides encouraging evidence of electromechanical integration, although the reduced conduction velocity (12.4 vs 48.2 cm/s) indicates incomplete maturation.

5.2 Exosome Synergy: Acute Protection + Long-Term Regeneration

The combined strategy's +16.4% LVEF improvement exceeded the sum of individual contributions (patch +14.8% + exosomes +6.2% = theoretical additive +21.0%), suggesting not simple addition but rather that exosomes enhance patch CM survival during the critical first days post-implantation. The 2.4-fold capillary density in the combined group supports this interpretation: exosome-mediated angiogenesis creates a vascular supply to the epicardial patch that would otherwise be limited by diffusion -- the primary cause of central necrosis in thick cardiac patches.

5.3 Clinical Translation Pathway

The combined patch + exosome strategy faces several translational challenges. Manufacturing iPSC-CMs at clinical scale (10^9 cells per patient) requires bioreactor production validated to GMP standards. Exosome manufacturing requires standardised isolation, characterisation (MISEV2018 guidelines), and potency assays. Surgical delivery of epicardial patches requires thoracotomy or minimally invasive robotic

approach -- potentially limiting applicability to patients undergoing CABG or valve surgery where chest access is already available.

5.4 Limitations

The rat MI model has different cardiac physiology (higher heart rate, different coronary anatomy) than human, and results may not directly translate. The 4-week follow-up demonstrates early functional benefit but does not capture long-term graft survival, arrhythmia risk, or whether LVEF improvements are maintained over months. No arrhythmia monitoring (continuous ECG telemetry) was performed -- a critical omission given the known arrhythmogenic risk of iPSC-CM grafts. Large animal (porcine) MI models with chronic telemetry are essential next steps.

6. Conclusion

This systematic comparison of four cardiac repair strategies establishes the combined dECM patch + exosome approach as the most efficacious post-MI regeneration strategy, achieving +16.4% LVEF improvement, 42% infarct size reduction, 2.4-fold capillary density increase, and 7-fold higher iPSC-CM engraftment versus injection -- with evidence of electromechanical coupling at the graft-host interface. The synergistic benefit of combining cell engraftment (dECM patch) with paracrine therapy (exosomes) addresses both acute cardioprotection and long-term contractile restoration -- two complementary mechanisms that neither approach achieves alone. These findings provide the preclinical rationale for advancing the combined patch + exosome strategy to large animal validation and first-in-human clinical trials for patients with acute MI and established heart failure.

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Declarations

Funding

This study was supported by the Swedish Research Council (Vetenskapsradet) project grant 2021-04842 (M. Dubois) and the Swiss National Science Foundation (SNSF) project grants 310030-224418 (A. Petrov) and 320030-218842

(O. Bianchi). The funding bodies had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

The authors declare no competing interests. No author holds equity in cardiac regeneration or exosome therapy companies.

Data Availability Statement

Echocardiographic measurements, histological images, optical mapping datasets, and small RNA-seq data are deposited at <https://doi.org/10.5281/zenodo.data.cardiac-regen-2022> and GEO (GSE208472). iPSC-CM differentiation and dECM decellularisation protocols are available at <https://github.com/NTU-CardiacRegen/patch-exosome-2022>.

Ethical Approval

Animal experiments were approved by the Nordic Technical University IACUC (ref. NTU-IACUC-2021-CARDIAC-024). Human atrial tissue for CPC derivation was obtained under Swiss Institute of Machine Intelligence IRB approval (SIMI-IRB-2021-TISSUE-012) with written informed consent.

Appendix A

dECM Decellularisation Protocol and Quality Control

This appendix provides the complete porcine myocardial decellularisation protocol, quality control acceptance criteria, and mechanical characterisation of the dECM cardiac patch scaffold.

A.1 Decellularisation Protocol

A.2 Patch Mechanical Properties