Implantation of a double allogeneic human engineered tissue graft on damaged heart: insights from the PERISCOPE phase I clinical trial

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Summary

Background In preclinical studies, the use of double allogeneic grafts has shown promising results in promoting tissue revascularization, reducing infarct size, preventing adverse remodelling and fibrosis, and ultimately enhancing cardiac function. Building upon these findings, the safety of PeriCord, an engineered tissue graft consisting of a decellularised pericardial matrix and umbilical cord Wharton's jelly mesenchymal stromal cells, was evaluated in the PERISCOPE Phase I clinical trial (NCT03798353), marking its first application in human subjects.

Methods This was a double-blind, single-centre trial that enrolled patients with non-acute myocardial infarction eligible for surgical revascularization. Seven patients were implanted with PeriCord while five served as controls.

Findings Patients who received PeriCord showed no adverse effects during post-operative phase and one-year follow-up. No significant changes in secondary outcomes, such as quality of life or cardiac function, were found in patients who received PeriCord. However, PeriCord did modulate the kinetics of circulating monocytes involved in post-infarction myocardial repair towards non-classical inflammation-resolving macrophages, as well as levels of monocyte chemoattractants and the prognostic marker Meteorin-like in plasma following treatment.

Interpretation In summary, the PeriCord graft has exhibited a safe profile and notable immunomodulatory properties. Nevertheless, further research is required to fully unlock its potential as a platform for managing inflammatory-related pathologies.

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Keywords: Cardiac repair; Cardiac regeneration; Engineered tissue; Cardiac tissue engineering; Decellularised tissue; Myocardial infarction

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Research in context

Evidence before this study

The outcomes of strategies employing direct myocardial injection of mesenchymal stromal cells have been variable, often resulting in limited efficacy. The utilization of the PeriCord construct has shown promising regenerative potential in preclinical studies in a swine model.

Added value of this study

This study validated the safety and feasibility of the PeriCord in a first-in-human clinical trial.

Implications of all the available evidence

The unique composition of PeriCord allows it to serve as a versatile platform for delivering active substances without need for patient immunosuppression. Indeed, all components of PeriCord have demonstrated excellent biocompatibility, minimizing the risk of rejection and ensuring favourable tolerability within the body. Furthermore, PeriCord exhibits anti-inflammatory properties that modulated systemic inflammation.

Introduction

The scientific community has been searching for new ways to treat patients with infarcted myocardium, and advanced therapy products have emerged as a potential solution for delivering regenerative cells locally to improve their survival and retention in the target tissue. We developed PeriCord, a double-allogeneic advanced therapy medicinal product (ATMP), which has been approved by the Spanish Agency for Medicines and Healthcare Products (AEMPS) as an Investigational Product (PEI 18–140) with a rigorous manufacturing process for use in clinical trials. This product is composed of an active principle, human allogeneic Wharton's jelly-derived mesenchymal stromal cells (WJ-MSC) and its vehicle, human allogeneic decellularised pericardium sourced from cadavers.[1] WJ-MSC from the connective tissue around the umbilical cord's great vessels were used, given their well-documented immunomodulatory properties, among the various MSCs currently available.[2–5] WJ-MSC have been approved for therapeutic use and shown positive outcomes in various clinical settings (PEI 16–017).[6] Furthermore, preclinical studies have demonstrated that similar double allogenic grafts can promote revascularization of damaged tissue, reduce infarct size, adverse remodelling and fibrosis, and ultimately improve cardiac function.[7,8] In the present study, we report on the safety evaluation of the PeriCord in a first-in-human, phase I, double-blind, single-centre clinical trial (NCT03798353) for the treatment of patients with non-acute myocardial infarction (MI) eligible for surgical revascularization.

Methods

Ethics

The PERISCOPE trial was approved by the Germans Trias i Pujol Ethics Committee with the following reference number: AC-18-106-HGT-CEIM. Las amendment for protocol version #5 was approved by the AEMPS on December, 2019 with number MS03.

Study design

The PERISCOPE trial is a randomized, controlled, double-blind, phase I study (EudraCT N. 2018-001964-49, Clinical Trials Identifier: NCT03798353) that includes a roll-in phase. The trial compares two arms in patients with prior MI who are undergoing coronary artery bypass grafting (CABG) (Fig. 1a).

The study included 12 patients, 2 of whom were open-blinded roll-in patients, and 10 were double-blind randomized patients. To be eligible for the study, patients were required to have a MI with ≥50% transmurality detected by cardiac magnetic resonance imaging (cMRI), be a candidate for revascularization for that or another myocardial territory, be at least 18 years of age, have a Q wave on ECG, and provide informed consent. The study's exclusion criteria were patients with severe valve disease requiring surgical repair, contraindications for MRI (creatinine clearance ≤30 ml/min/1.73 m^2, metal implants, or claustrophobia), extra-cardiac diseases with a prognosis of less than 1 year, neoplastic disease detected in the past 5 years without complete remission, severe kidney or liver failure, abnormal laboratory values that may contraindicate participation, previous cardiac interventions, pregnant or lactating women, heterosexually active women of childbearing age not using effective contraception, participation in another clinical trial or treatment with another investigational product.

Having a clear indication for revascularization of some of the infarcted areas, patients who fulfilled all inclusion and none of the exclusion criteria were offered PeriCord implantation in addition to the planned CABG. After receiving the information about the procedure and given written informed consent, they were planned for the surgical procedure. An open-chest surgery was performed for the CABG under the induction of general anaesthesia with intravenous propofol and subsequent maintenance with sevoflurane with extracorporeal circulation and standard monitoring. The PeriCord was applied and secured with surgical glue (Glubran®2, Cardiolink) at the four edges on the epicardial surface of the transmural MI.

After the feasibility of the study was assessed with two roll-in patients, 10 patients were randomized through a computer system in a 1:1 ratio to either CABG alone (control group, n = 5) or CABG with the implantation of PeriCord (treatment group, n = 5; n = 7 in total)

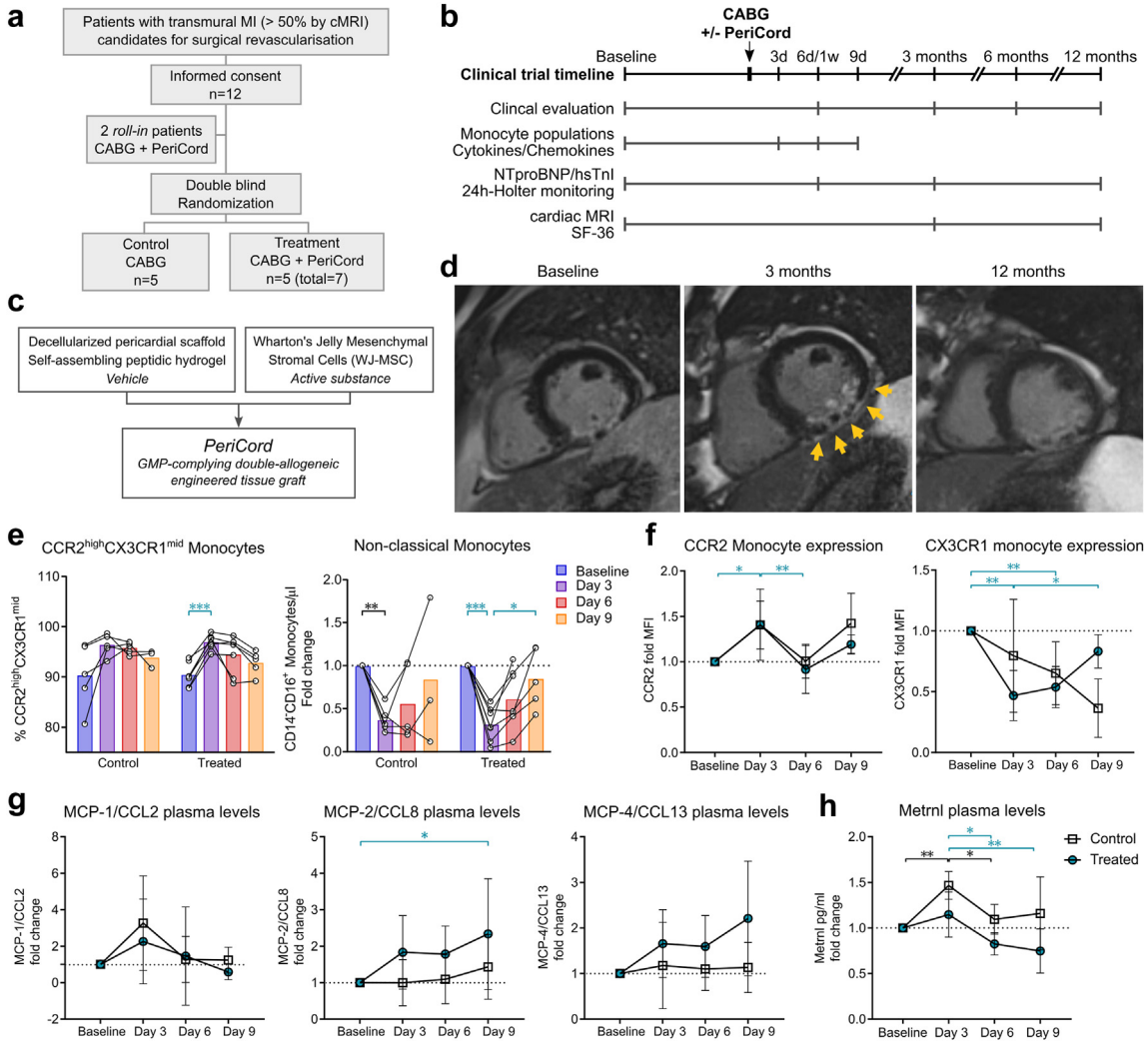

Figure 1: (**a**) Flowchart of the PERISCOPE clinical trial. **(b)** Timeline of patient follow-up. **(c)** Schematic composition of the PeriCord ATMP. **(d)** cMRI analysis of a Treated patient at Baseline (transmural MI), 3 months (the implanted PeriCord is recognizable, yellow arrows) and at 12 months (PeriCord is integrated in the myocardium and hardly visible). **(e)** Percentage of activated $CCR2^{high}CX3CR1^{mid}$ monocytes and fold change of "non-classical" $CD14^{-}/CD16^{+}$ monocyte numbers in peripheral blood of control (n = 5) and treated (n = 7) patients at baseline and 3-, 6- and 9-days post-surgery. **(f)** CCR2 and CX3CR1 surface expression in peripheral blood monocytes. **(g)** Circulating levels of monocyte chemoattractants MCP-1/CCL2, MCP-2/CCL8 and MCP-4/CCL13 and **(h)** Meteorin-like (Metrnl) in plasma of control (n = 5) and treated (n = 7) patients at baseline and 3-, 6- and 9-days post-surgery. Statistical differences are indicated for $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ according to paired Two-way ANOVA. Time-dependent differences are indicated in black and blue for control and treated patients, respectively. MFI, median fluorescence intensity.

based on allocation order. The randomization was blinded to the investigational team, but not to the surgery team or study coordinator. To elaborate, blinded and un-blinded staff had distinct access areas in the case report forms (CRFs). Consequently, the surgical team exclusively handled data related to the procedure, with no access to the remaining eCRF data. Conversely, only the blinded clinical staff had the authority to input data regarding the clinical follow-up, which lasted one year. Fig. 1a shows the flow chart of the clinical trial. Table 1 summarizes the demographical and clinical data of patients at inclusion, including roll-in patients. Sex was self-reported by study participants. The primary objective was to assess the safety of the PeriCord for the treatment of non-acute MI measured with a combined endpoint of serious clinical events, death or rehospitalisation due to any cause, and serious adverse reactions related to the investigational treatment. Secondary outcome measurements were: rate of death or rehospitalisation due to any cause and/or adverse reactions

	Treatment group (PeriCord) (n = 7)	Control group (n = 5)
Female sex[a]	2 (28.6)	1 (20.0)
Male sex[a]	5 (71.4)	4 (80.0)
Age (years)	63.9 ± 9.4	67.0 ± 4.2
BMI (kg/m^2)	27.1 ± 6.0	29.9 ± 3.5
Diabetes mellitus	4 (57.1)	3 (60.0)
Hypertension	3 (42.9)	3 (60.0)
Hypercholesterolemia	7 (100)	3 (60.0)
PAD	2 (28.6)	0
Heart rate (bpm)	65.0 ± 11.4	70.8 ± 11.4
LVEF (%)	50.7 ± 10.9	51.8 ± 9.6
Haemoglobin (g/dL)	14.4 ± 1.8	14.7 ± 1.9
NT-proBNP (pg/ml), median (IQR)	424 [118–687]	174 [165–180]
Hs-TnI (pg/ml), median (IQR)	7.3 [3.5–20.9]	8.5 [7.9–11.5]
eGFR (ml/min/1.73 m^2)	84.9 ± 6.0	81.4 ± 8.6
Baseline treatment		
Acetylsalicylic acid	6 (85.7)	4 (80.0)
Clopidogrel	0	1 (20.0)
ACEI	4 (57.1)	3 (60.0)
β-blockers	5 (71.4)	2 (40.0)
MRA	1 (14.3)	0 (0)
Furosemide	1 (14.3)	0 (0)
Statins	6 (85.7)	3 (60.0)
Calcium channel blocker	2 (28.6)	2 (40.0)
Nitrate	1 (14.3)	2 (40.0)

Values are mean ± SD, n (%) or median [interquartile range]. BMI, body mass index; PAD, peripheral arterial disease; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-TnI, high-sensitivity Troponin I; eGFR, estimated glomerular filtration rate; ACEI, angiotensin-converting enzyme inhibitors; MRA, mineralocorticoid receptor antagonist. [a]Sex was self-reported by study participants. There were just two reported sex categories: female and male.

Table 1: **Baseline characteristics in the treatment and control groups.**

related to the procedure/product under investigation at follow-up (1 week, 3 and 6 months); death rate or rehospitalisation due to cardiovascular causes at all time-points; rate of relevant arrhythmias in Holter of 24 h, and relevant changes in N-terminal B-type natriuretic peptide (NT-proBNP) and high sensitivity troponin I (hsTnI) levels at all time-points; changes by cMRI at 12 months including infarct size due to gadolinium retention, ejection fraction of the left ventricle, and left and right ventricular geometric remodelling; and change in the score on the quality of life test Short Form 36 Healthy Survey (SF-36) at 3 and 12 months.

Exploratory endpoints included assessing monocyte populations and cytokine/chemokine levels at screening, day 3, day 5, and discharge date/day 9 to evaluate immunomodulation. The timeline of the clinical trial is shown in Fig. 1b.

Manufacturing and surgical implantation of PeriCord

The PeriCord product was manufactured in compliance with current Good Manufacturing Practice (GMP) at the Blood and Tissue Bank of Barcelona (BST, Spain), as previously described (PEI 18,140)[1] (Fig. 1c and Supplementary Figure S1). Clinical-grade WJ-MSCs were derived from specifically donated umbilical cord tissue following GMP-compliant procedures in the classified areas of the Blood and Tissue Bank of Catalonia (BST; Barcelona, Spain), using a cell banking system containing ready-to-use pure WJ-MSCs. Decellularisation of the human pericardium was also performed in the BST-qualified areas to ensure human tissue quality. PeriCord biofabrication involved multiple steps and in-process controls for batch release for clinical use. The main steps were vehicle immobilisation in the primary packaging, WJ-MSCs thawing, cell conditioning and dose adjustment, vehicle colonisation, and final packaging in a temperature monitored triple-container system with Plasmalyte®148 solution for PeriCord transportation to the hospital before implantation. A total of 4 different WJ-MSC batches were used combined with 11 decellularised pericardial scaffolds from independent donors, obtaining unique combinations for each patient (as specified in Table 2 for each patient).

The final packaged PeriCord product was transported to the surgical room in a temperature-monitored suitcase, containing a triple-container system for safe transportation from the production premises. The product was contained within a secondary sterile packaging along with Plasmalyte® solution and was fixed into the primary packaging. The number of PeriCords administered depended on the extent of the scar to be treated, with patients receiving either 1 or 2 PeriCords (Table 2). Following necessary surgical revascularizations, the PeriCords were applied and secured with surgical glue at their four edges.[1] No immunosuppression was added to patients in the PeriCord arm. Details of surgical procedures and hospital stays of included patients are collected in Supplementary Table S1.

Primary endpoint

Safety of the procedure was evaluated by the presence of major clinical events including hospital admissions and death, and serious adverse reactions related to the investigational product.

Arrhythmias

A 24-h Holter monitoring test was done at each study visit to assess relevant arrhythmic events.

Quality of life

The 36-Item Short Form Survey (SF-36) is an outcome measure instrument used for the objective assessment of the quality of life, and consists of 36 questions that cover eight domains of health: physical functioning (PF), usual role physical activities (RP), body pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH).[9]

PERISCOPE treated patient		1st roll-in	2nd roll-in	1		2		3		4		5	Average[a]	SD
Number of PeriCords implanted		1	1	2		2		2		2		1		
Assay	Specifications	*PER19025*	*PER19077*	*PER20006*	*PER20007*	*PER20054*	*PER20055*	*PER21026*	*PER21027*	*PER21047*	*PER21048*	*PER21113*		
WJ-MSC identity	WJ-MSC batch (PEI 16–017)	*XCU18043*	*XCU18043*	*XCU19041*	*XCU19041*	*XCU18043*	*XCU18043*	*XCU20003*	*XCU20003*	*XCU20009*	*XCU20009*	*XCU20003*	NA	NA
Phenotype	$CD45^-/CD105^+$	95.40%	95.40%	97.70%	97.70%	95.40%	95.40%	97.42%	97.42%	93.80%	93.80%	97.42%	96.08%	1.83%
	$CD31^-/CD73^+$	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	99.65%	99.65%	99.79%	99.79%	99.65%	99.86%	0.17%
	MFI CD73	603.09	603.09	553.24	553.24	603.09	603.09	682.26	682.26	682.44	682.44	682.26	630.26	63.5
	HLA-DR$^-$	99.90%	99.90%	99.90%	99.90%	99.90%	99.90%	99.88%	99.88%	99.89%	99.89%	99.88%	99.89%	0.01%
	$CD90^+$	99.70%	99.70%	99.90%	99.90%	99.70%	99.70%	99.89%	99.89%	99.96%	99.96%	99.89%	99.86%	0.11%
Dose	7–15 × 10^6 viable WJ, MSC	1.25 × 10^7	1.34 × 10^7	8.90 × 10^6	8.20 × 10^6	8.20 × 10^6	7.35 × 10^6	8.16 × 10^6	7.98 × 10^6	1.13 × 10^7	1.17 × 10^7	1.33 × 10^7	1.01 × 107	2.35 × 10^6
Viability	≥70%	85.40%	83.60%	88.70%	92.70%	77.60%	75.50%	70.36%	70.36%	77.90%	79.10%	85.80%	80.64%	7.25%
WJ, MSC Potency	% Proliferation inhibition (PBMC + MSC)	100.0%	100.0%	68.8%	68.8%	100.0%	100.0%	55.1%	55.1%	86.3%	86.3%	55.1%	77.55%	19.67%
Endotoxin	≤4.0 EU/ml	<0.51	<0.501	<1.00	<1.00	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	NA	NA
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	NA	NA
Mycoplasma	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	NA	NA

[a]Average for phenotype and potency data corresponding to the 4 different WJ-MSC batches used.

Table 2: **Release parameters records of implanted PeriCords.**

Cardiac magnetic resonance imaging

cMRI was performed using a 1.5 T clinical imaging system (Avanto Fit; Siemens Medical Imaging, Erlangen, Germany) with the patient in the supine position and a phased-array coil over the chest. Cine imaging was done using a segmented k-space steady-state free-precession sequence for parallel short-axis and long-axis views. Delayed enhancement images were acquired with a segmented gradient-echo inversion-recovery sequence after gadolinium-DTPA administration.[10,11] The dose of gadobutrol was 0.2 mmol/kg. All images obtained from the cMRI data were analysed using a specialized post-processing software (QMass-MR, v.8.1; Medis Medical Imaging Systems, Leiden, the Netherlands). All cMRI analysis was performed by a CMR expert (A.T.). Collection and interpretation of all imaging data were blinded to the clinical data and outcome.

Left ventricular (LV) volumes and mass were analysed by manually tracing the LV endocardial border (excluding papillary muscles) on all short-axis cine images at the end-diastolic and end-systolic frames. End-diastolic and end-systolic volumes were determined, and LV mass was calculated by subtracting the endocardial volume from the epicardial volume at end diastole and then multiplying by the tissue density of 1.05 g/ml.

The analysis of scar on late gadolinium-enhancement (LGE) images involved manually outlining the endocardial and epicardial contours, and tracing ROIs in the hyperenhanced area and the normal-appearing remote myocardium. Scar volume for each slice was calculated using a full-width at half-maximum (FWHM) algorithm, and scar mass was expressed as total scar volume multiplied by 1.05 g. Scar percentage of myocardium was expressed as a percentage of the total myocardial volume.

Cardiac biomarker monitoring

NT-proBNP and hs-TnI levels were determined by electrochemiluminescence immunoassays using a Cobas E601 platform (Roche Diagnostics, Switzerland) at baseline, 6 days, 3 and 12 months of follow-up.

Peripheral blood monocyte populations and cytokine/chemokine analysis

Whole blood samples were drawn from venepuncture (BD vacutainer citrate tubes) at baseline, 3, 6, and 9 days post-surgery while patients were hospitalized. Due to the COVID-19 pandemic, hospitalization was minimized as much as possible and so some of the planned follow-up of patients was only possible up to day 6 post-surgery. Exploratory analyses were conducted on the 12 included patients.

Whole blood samples were drawn in citrate tubes (BD Biosciences, San Jose, CA, USA) and processed within 3 h for flow cytometry analysis of circulating

monocyte populations at each time-point. To that end, 100 µl were stained for 15 min at room temperature with specific monoclonal antibodies from Miltenyi Biotech: CD86-APC-Vio770 (clone REA968, Cat# 130-116-163, RRID:AB_2727375), CCR2/CD192-PE-Vio770 (clone REA624, Cat# 130-109-597, RRID:AB_2655871), CX3CR1-APC (clone 2A9-1, Cat# 130-096-435, RRID:AB_10828236); and from BD biosciences: CD14-FITC (clone MφP9, Cat# 340682, RRID:AB_400086), CD16-Bv421 (clone 3G8, Cat# 562874, RRID:AB_2716865), CD45-FITC (clone 2D1, Cat# 340664, RRID:AB_400074) and CD73-PE (clone AD2, Cat# 550257, RRID:AB_393561). After incubation, erythrocytes were lysed for 10 min (Lysing Buffer, BD Biosciences), washed at 400×g for 5 min, and resuspended in FACS-Flow (ThermoFisher Scientific). Absolute cell counts were obtained using PerfectCount Microspheres of known concentration (Cytognos SL, Salamanca, Spain). Data was acquired in an LSR Fortessa flow cytometer (BD) using Rainbow calibration particles (6 peaks, 3.0–3.4 µm; BD) before each time-point analysis to ensure median fluorescence intensity (MFI) reproducibility. A minimum of 10,000 events per sample were acquired and analysed using FlowJo software v10.7.1 (BD Biosciences). Doublets and dead cells were excluded based on their FSC-A/FSC-H and FSC-A/SSC-A, respectively, and monocytes gated according to CD86 expression as outlined in Supplementary Figure S2. Fluorescence-minus-one (FMO) controls were used to define positivity for CD16, CD73, CCR2 and CX3CR1. Absolute counts were calculated as: (%subset X) × monocyte count (FSC-A/SSC-A and $CD86^+$).

Cytokine and chemokine levels were analysed with the Olink Target 96 Inflammation panel (Olink Proteomics AB, Uppsala, Sweden) and quantified in terms of arbitrary units (Cobiomic, Córdoba, Spain); or using a 9-plex multiplex ELISA (Millipore) to quantify for human Fractalkine/CX3CL1, G-CSF/CSF-3, GM-CSF, IFN-alpha, IL-1-beta, IL-1RA, IL-12p70, MCP-1/CCL2 and VEGF-D, following manufacturer's instructions. Data was acquired in a Luminex® 100/200 System (Luminex) and analysed using the xPONENT software (version 3.1.971.0).[12–14]

The prognostic marker Meteorin-like protein (Metrnl), a cytokine involved in the attenuation of inflammation was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Meteorin-like/METRNL DuoSet ELISA; R&D Systems, Minneapolis, USA; reference DY7867-05, lot P300680) according to the manufacturers' protocols. Assay range was 15.6–1000 pg/ml.[15–18]

Statistical analysis

For comparison of occurrences of SAEs between groups, person's chi-square or Fisher's exact test was used. For changes within the randomized groups from baseline, we reported the mean or median difference with the respective 95% confidence interval as appropriate. Differences were considered significant if $p < 0.05$. All analyses were performed in STATA V.13.0 (StataCorp, College Station, TX).

For monocytes and chemokines, data is expressed as mean (SD) unless otherwise stated. Statistical significance was considered when $p < 0.05$ according to the appropriate statistical analysis after Normality distribution testing, using Graphpad Prism software (v9.5).

Role of funders

This work was supported in part by grants from MICINN, Instituto de Salud Carlos III and Spanish national research networks. They had no role at all in the study design, data collection, data analyses, interpretation or writing of this report.

Results

The production of the PeriCord product involved the seeding of allogeneic WJ-MSCs, the active principle, at a dose of 7–15 × 10^6 cells per dose, onto a decellularised allogeneic human pericardial scaffold measuring 12–16 cm^2. To facilitate scaffold rehydration and cell colonization, a self-assembling peptide hydrogel (PuraStat®) was utilized (Fig. 1c). All components used in the manufacturing process, as well as the final PeriCord product, adhered to established safety and quality standards, as outlined in PEI 18,140.[1] The release parameters for implanted PeriCords included the identification of the WJ-MSC batch (surface phenotype) and potency (immunomodulatory capacity). Additionally, the final dose and viability of the cells once seeded in the scaffold were assessed, along with safety measurements, as summarized in Table 2. Baseline characteristics of the patients included in the study are shown in Table 1.

The safety of PeriCord implantation is depicted in Table 3. No significant differences between control and experimental group were observed. Among the patients who received a PeriCord implant (2 roll-in and 5 treated) we observed 4 serious adverse effects in the 2 roll-in participants. One patient suffered respiratory failure immediately after the surgery, attributed to multifactorial aetiology, and a surgical wound dehiscence, leading to a slightly prolonged the hospital stay. In the follow-up this patient was also admitted for COVID-19 infection resulting in death. The other roll-in patient presented a femur fracture. One patient in the control group presented death after the surgery due to multifactorial respiratory insufficiency initiated during anaesthetic induction. Importantly, none of these events were associated with PeriCord. Furthermore, the PeriCords successfully integrated into the implanted myocardium as shown by cMRI data (Fig. 1d). PeriCord implantation was not rejected even in absence of immunosuppressive treatment and did not increase the duration of surgery, or the length of hospital stay.

	Roll-in period (n = 2)		Randomized period			
			Treatment group (PeriCord) (n = 5)		Control group (n = 5)	
	Patients	Events	Patients	Events	Patients	Events
Patients with AEs						
Total	2 (100)	7	1 (20.0)	1	3 (60.0)	10
Composite endpoint						
Total	2 (100)	6	0 (0.0)	0	1 (20.0)	2
All-cause death	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
Hospitalization	2 (100)	2	0 (0.0)	0	0 (0.0)	0
Serious adverse events	2 (100)	4	0 (0.0)	0	1 (20.0)	1
Secondary composite endpoint						
Cardiovascular hospitalization	1 (50.0)	1	0 (0.0)	0	0 (0.0)	0
Ventricular arrhythmias	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
Others						
Atrial fibrillation	0 (0.0)	0	0 (0.0)	0	2 (40.0)	2
Pneumothorax	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
Acute renal failure	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
Acute respiratory distress syndrome	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
Anaemia	0 (0.0)	0	1 (20.0)	1	0 (0.0)	0
Rotator cuff syndrome	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1
New onset diabetes mellitus	0 (0.0)	0	0 (0.0)	0	1 (20.0)	1

Table 3: **Safety outcomes: adverse events within the first year in the in the experimental and control groups.**

The study did not show clinical relevant differences in the secondary outcomes, such as NTproBNP and hsTnI levels, SF36 results, or cMRI variables, between the control and treatment groups. For further information, please refer to the Supplementary material (Supplementary Tables S2–S4).

Regarding the exploratory endpoints, recruitment of activated $CCR2^+$ monocytes in peripheral blood increased at day 3 post-surgery in both groups compared to baseline (+6.1 ± 5.7% in Control, *vs* +6.5 ± 1.8% in PeriCord, $p = 0.89$ [Unpaired T-test with Welch's correction] between groups; Fig. 1e, left), concomitant with a peak surface expression of CCR2 chemokine receptor by monocytes at day 3 post-surgery in all patients (Fig. 1f, left) and an increase in MCP-1/CCL2 (Fig. 1g, left). In turn, the non-classical $CD14^-CD16^+$ monocytes decreased regardless of treatment (16.7 ± 11.1% decrease in Control, *vs* 24.0 ± 5, 0% in PeriCord, $p = 0.23$ [Unpaired T-test with Welch's correction]; Fig. 1e, right), with a marked reduction in CX3CR1 in the monocyte population (Fig. 1f, right). Importantly, only PeriCord-treated patients recovered non-classical $CD14^-CD16^+$ monocyte population numbers together with CX3CR1 chemokine receptor expression at day 9 post-treatment (Fig. 1e and f). This was accompanied by increased circulating levels of other monocyte chemoattractants MCP-2/CCL8 and MCP-4/CCL13 in the peripheral blood of treated patients (Fig. 1g, right). Yet, plasma levels of CX3CL1 were undetectable. Also, these effects were independent of the number of PeriCord implanted. On the other hand, PeriCord implantation significantly reduced the peak in MetrnI levels in plasma after surgery (Fig. 1h). Of note, we did not detect differences between patients receiving 1 *vs* 2 PeriCord implants.

Discussion

A first-in-human trial was conducted involving 12 patients with MI to assess the feasibility of manufacturing the PeriCord graft using GMP standards, evaluate its surgical ease of use, and determine its clinical safety. Before proceeding this human trial, we conducted preclinical studies using small-size grafts (1 cm^2), which provided encouraging results in terms of safety and efficacy. However, scaling up to human clinical-sized grafts (12–16 cm^2) presented significant challenges. Close collaboration between the manufacturing facility (BST) and the clinical hospital (HUGTiP) was essential to overcome these challenges and achieve successful scalability. Adhering to GMP guidelines during manufacturing ensured the quality and consistency of the PeriCord grafts.

The PeriCord graft is an innovative 3D cardiac bioimplant that consists of two components: clinical-grade Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) obtained from new-born umbilical cord and a decellularised, lyophilized, and sterilized human pericardium derived from cadaver donors. Detailed information about the manufacturing process of the PeriCord graft has been documented elsewhere.[1]

The application of transendocardial acellular injections of VentriGel, a cardiac extracellular matrix hydrogel has been proven safe and feasible in patients with early and late MI with LV dysfunction.[19] In contrast, our strategy involves modulating the damaged area from the epicardium using the PeriCord construct. We have identified PeriCord as the optimal combination to sustain cell viability and function, providing a conducive niche for the engraftment of pro-regenerative cells, thus facilitating myocardial repair.[7,8] Importantly, this process occurs without disrupting the patient's myocardial structure and coronary flow during the healing phase and circumvents the loss of therapeutic cells after intravenous, intracoronary or transendocardial injection. The risk of coronary embolism is one limitation in intravenous administration of regenerative approaches.[20,21] Another novel technique is cell sheet engineering that has been tested for tissue repair in various tissues, including the heart, cornea, bone, oesophagus, bladder and liver.[22] Cell sheets eliminates the need for traditional tissue engineering procedures such as scaffold-based technologies. However, the number of cell sheets that can be layered without triggering core ischemia or hypoxia is limited.[22] Our approach delivers a structure native to the heart that has been proven to promote an increase in vascularity in preclinical studies with swine model.[8,23,24]

The use of clinical-grade WJ-MSCs in the PeriCord bioimplant offers distinct advantages. These cells possess a unique combination of prenatal and postnatal properties, making them an appealing alternative to other sources of stem cells that face challenges such as immune rejection and teratoma formation. WJ-MSCs secrete various pro-angiogenic factors and have demonstrated functional angiogenic potential in mammalian hearts affected by infarction.[5,25] Furthermore, WJ-MSCs have a natural affinity for cardiac tissue and possess the ability to populate the ventricular myocardium.[2,3] Decellularised cardiac tissues provide a highly compatible microenvironment that closely resembles the native physiological conditions. The decellularisation process preserves the composition and three-dimensional structure of the tissues, with minimal changes in stiffness.[7,26] Comparing decellularised scaffolds from myocardial and pericardial tissues, a study found that the decellularised pericardial scaffold exhibited superior performance.[7] The decellularised pericardial scaffold demonstrated preserved macromechanical and micromechanical properties throughout the decellularisation and recellularisation processes. Moreover, it showed enhanced cell penetration and retention, likely due to its larger pore size.[7] These findings indicate that the decellularised pericardial scaffold is well-suited for tissue engineering applications.[8] Both WJ-MSCs and allogeneic decellularised pericardial matrices can be stored for off-the-shelf clinical use.

The implantation of the PeriCord graft was successfully performed in all patients, with no significant impact on the duration of surgery or length of hospital stay. None of the patients in the PeriCord group experienced any adverse events, including the composite endpoint of all-cause death, hospitalization, or serious adverse events. Ventricular arrhythmias were not observed during the one-year follow-up. In four patients of the PeriCord group, two grafts were required to fully cover the scar. Biomarker analysis showed that both groups exhibited a significant decrease in NTproBNP levels of over 50% at one year. Cardiac structural abnormalities assessed by cardiac MRI did not show significant changes in left ventricular ejection fraction (LVEF) or volumes during the follow-up period. The PeriCord group showed a non-significant reduction in the infarct size (10.0% *vs* 8.0%), while the control group tended to have an increase (9.0% *vs* 10.0%). This study was not specifically powered to assess changes in cardiac function or scar size due to its first-in-human nature and thus, its small size. However, these findings provide valuable insights and serve as a hypothesis-generating basis for larger outcome or imaging trials.

In patients treated with PeriCord, we observed a modulation of the kinetics of circulating monocyte populations responding to post-infarction myocardial repair and after an inflammatory insult like CABG surgery. Following myocardial ischemia, the healing myocardium mobilizes two monocyte subsets in a sequential manner with distinct and complementary functions. M1 macrophages dominate during the first three days after the infarction, while M2 macrophages arise afterward and prevail after five days, depending on CCR2 and CX3CR1 recruitment, respectively.[12,13] These monocyte subsets correspond to classical $CD14^{+}CD16^{-}$ and non-classical $CD14^{+}CD16^{+}$ monocytes, respectively, and both are increased in acute MI, peaking on days 3 and 5 after onset, respectively.[14] In patients with MI treated with PeriCord implantation, these monocyte subsets were differentially recruited in response to CCR2 and CX3CR1, and non-classical inflammation-resolving macrophages returned to normal levels by day 9, in contrast to control patients. This indicates a systemic response to PeriCord treatment.

PeriCord implantation was found to regulate the levels of Metrnl in plasma, which is a secreted protein expressed by various tissues including activated macrophages and barrier tissues.[16] Metrnl plays a crucial role in inflammation and innate and acquired immunity. Previous preclinical studies have shown that Metrnl levels can regulate the inflammatory response and aid in post-infarction repair mechanisms such as macrophage polarization to M2 phenotype and angiogenesis.[15,16] Clinical data has indicated that higher levels of Metrnl are associated with worse outcomes for patients suffering from heart failure (HF) and MI with ST-elevation (STEMI).[17,18] Interestingly, PeriCord

treatment managed to reduce Metrnl levels in plasma, which could potentially improve outcomes in patients with HF. These findings warrant further investigation.

Moreover, the systemic anti-inflammatory effects lead by PeriCord treatment may have reduced the incidence of postoperative atrial fibrillation, a serious surgical complication and long-term mortality risk for cardiac surgery patients.[27] This possibility merits long-term assessment in a bigger patient cohort).

This study has several limitations: The COVID-19 pandemic presented challenges in the follow-up of patients, as hospitalizations were minimized as a precautionary measure. It is important to acknowledge that this study was not specifically designed to detect significant changes in cardiac volumes and function as primary outcomes. Furthermore, it is worth considering that patients with established dense myocardial scars, as identified by cardiac MRI late gadolinium enhancement, may exhibit a reduced response to the effects of WJ-MSCs in terms of scar size reduction, fibrosis improvement, and overall cardiac function. In the future, additional research should be conducted to investigate the potential of PeriCord in clinical scenarios characterized by interstitial fibrosis or less dense scars.

In conclusion, the successful development of an advanced ATMP known as PeriCord, which demonstrates both safety and scalability for clinical production, has been accomplished. The unique composition of PeriCord allows it to serve as a versatile platform for delivering active substances without need for patient immunosuppression. Indeed, all components of PeriCord have demonstrated excellent biocompatibility, minimizing the risk of rejection and ensuring favourable tolerability within the body. Furthermore, PeriCord exhibits potent anti-inflammatory properties, capable of modulating systemic inflammation. By shifting the inflammatory response towards an anti-inflammatory state, PeriCord has the potential to mitigate the detrimental effects associated with prolonged inflammation.

Although the present study primarily focused on evaluating PeriCord's safety in the context of myocardial infarction, its anti-inflammatory properties suggest broader applications in various clinical settings where inflammation plays a pivotal role in negative outcomes. This versatility makes PeriCord a promising option for local delivery, providing potential therapeutic benefits in diverse medical scenarios. Continued research and exploration of PeriCord's efficacy and safety in different clinical contexts will help unlock its full potential as a valuable tool for managing inflammation-related pathologies.

Contributors

All authors read and approved the final version of the manuscript. Conceptualisation: ABG, PG, MMT, AV, CMG, SQ; data curation: PG, MMT, CPV, GC, LRG, AT; formal analysis: MMT, CPV, GC; funding acquisition: ABG, SQ; investigation: PG, MMT, MLC, CPV, GC, LRG, AT, ERL, GFC; project administration: PG, CPV, FB, JV, AV, CMG, SQ; resources: SR, CGM, FB, JV; validation: LRG; supervision: ABG, AV, CMG, SQ; writing—original draft: ABG, PG, MMT; and writing—review & editing: ABG, PG, MMT, CPV, SQ.

Data sharing statement

The data used in this study are from patients who consented for use of their coded data for research purposes. All significant results are provided in the Supplementary data files. The anonymized dataset is available upon reasonable request to the corresponding author, subject to the agreement of all co-authors.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105060.

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