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ORIGINAL PRE-CLINICAL SCIENCE

Epicardial infarct repair with bioinductive extracellular matrix promotes vasculogenesis and myocardial recovery

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BACKGROUND: Infarcted myocardium can remodel after successful reperfusion, resulting in left ventricular dilation and heart failure. Epicardial infarct repair (EIR) using a bioinductive extracellular matrix (ECM) biomaterial is a novel surgical approach to promote endogenous myocardial repair and functional recovery after myocardial infarction. Using a pre-clinical porcine model of coronary ischemia-reperfusion, we assessed the effects of EIR on regional functional recovery, safety, and possible mechanisms of benefit.

METHODS: An ECM biomaterial (CorMatrix ECM) was applied to the epicardium after 75 minutes of coronary ischemia in a porcine model. Following ischemia-reperfusion injury, animals were randomly assigned in 2:1 fashion to EIR ($n = 8$) or sham treatment ($n = 4$). Serial cardiac magnetic resonance imaging was performed on normal ($n = 4$) and study animals at baseline (1 week) and 6 weeks after treatment. Myocardial function and tissue characteristics were assessed.

RESULTS: Functional myocardial recovery was significantly increased by EIR compared with sham treatment (change in regional myocardial contraction at 6 weeks, 28.6 ± 14.0% vs 4.2 ± 13.5% wall thickening, $p < 0.05$). Animals receiving EIR had reduced adhesions compared with animals receiving sham treatment (1.44 ± 0.51 vs 3.08 ± 0.89, $p < 0.05$). Myocardial fibrosis was not increased, and EIR did not cause myocardial constriction, as left ventricular compliance by passive pressure distention at matched volumes was similar between groups (13.9 ± 4.0 mm Hg in EIR group vs 16.0 ± 5.2 mm Hg in sham group, $p = 0.61$). Animals receiving EIR showed evidence of vasculogenesis in the region of functional recovery.

CONCLUSIONS: In addition to the beneficial effects of successful reperfusion, EIR using a bioinductive ECM enhances myocardial repair and functional recovery. Clinical translation of EIR early after myocardial infarction as an adjunct to surgical revascularization may be warranted in the future.

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Advances in the management of myocardial infarction (MI) have improved survival after MI. However, the incidence of ischemic heart failure is increasing.[1] Coronary

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artery bypass grafting (CABG) is sometimes performed early after MI, but complete revascularization is not always achieved, and CABG does not directly target the infarcted myocardium. Myocardial remodeling can result in interstitial fibrosis, progressive ventricular dilation, and subsequent heart failure. "Biosurgical" strategies applied at the time of surgical revascularization specifically to target the infarcted myocardium may help promote healing, prevent heart failure, and improve outcomes for patients with preexisting ischemic injury.

The extracellular matrix (ECM) influences cardiac remodeling and function after MI. Healthy ECM provides structural support to tissues and regulates cardiac cell morphology, differentiation, migration, and proliferation,[2] which act in concert to impact tissue function. After tissue injury, ECM is essential for endogenous repair and may mediate the potential for cellular regeneration.[3,4] The application of a biologic ECM construct with bioinductive properties from retained growth factors, cytokines, and matricellular proteins, such as porcine small intestine submucosa extracellular matrix (SIS-ECM), may enhance endogenous tissue repair. SIS-ECM is a decellularized ECM construct that retains its native three-dimensional architecture and cell signaling proteins, providing a homeostatic environment to promote cell function and survival.[5–7] CorMatrix ECM (CorMatrix Cardiovascular, Inc., Roswell, GA) is a commercially available SIS-ECM that is approved by the US Food and Drug Administration and has been used previously in cardiac surgery applications.[8–17]

We established proof-of-concept for epicardial infarct repair (EIR) with SIS-ECM in a rodent model demonstrating that local application of SIS-ECM biomaterial to the epicardial surface of infarcted myocardium limits structural remodeling after MI and improves myocardial function.[8] The epicardium itself is a key player in repair after MI. Following ischemia, endogenous cells within the epicardium become activated, resulting in epicardial thickening.[18] This process mobilizes key progenitor cell niches located within the epicardial space by epithelial mesenchymal transition (EMT).[19–22] Epicardial progenitor cells differentiate into (myo)fibroblasts, vascular smooth muscle cells, or cardiac myocytes.[19,20,22] Restoring local homeostatic queues by application of a healthy biologic ECM construct containing angiogenic growth factors may enhance differentiation toward a vascular phenotype, promoting endogenous healing pathways beneficial in the setting of ischemia.

In this study, we examined the influence of EIR using SIS-ECM on regional myocardial recovery as an adjunct to successful reperfusion after MI. We assessed procedural safety, efficacy on regional functional recovery, and possible mechanisms of post-MI repair for EIR.

Methods

Experimental animals

All animal experiments were performed in accordance with the Canadian Council on Animal Care Guide for the Care and Use of Experimental Animals and the National Society for Medical Research Guide for the Care and Use of Laboratory Animals and approved by the University of Calgary Animal Care Committee. Male Landrace pigs weighing 25 kg were obtained from Neufeld Farms (Alberta, Canada).

Ischemia-reperfusion model and EIR procedure

The ischemia-reperfusion model was adapted from the Gorman Cardiovascular Research Group sheep model.[23] Animals were intubated and mechanically ventilated with medical-grade oxygen and 2% to 3% isoflurane and administered continuous infusions of lactated Ringer's solution (0.04 ml/kg/min) and lidocaine (0.04 mg/kg/min). After median sternotomy, diagonal branches of the left anterior descending coronary artery were ligated for 75 minutes and then reperfused. Animals were then randomly assigned 2:1 to receive EIR or a sham procedure. Animals receiving EIR received SIS-ECM (CorMatrix-ECM) secured to the epicardial surface of the heart overlying the infarct territory using a running 5-0 polypropylene (Prolene) suture. Animals receiving sham treatment received a running 5-0 Prolene suture encompassing the infarct border without securing SIS-ECM.

Cardiac magnetic resonance image acquisition

Serial cardiac magnetic resonance (CMR) imaging was performed at baseline (1 week) and 6 weeks after treatment. Animals were mechanically ventilated, and anesthesia consisting of inhaled isoflurane ($\leq$1.0%) and nitrous oxide ($\leq$1.0%) and a continuous intravenous infusion of ketamine (0.3 mg/ml), fentanyl (0.04 mg/ml), and midazolam (0.025 mg/ml) at a rate of 30 to 100 ml/hour was maintained to achieve a mean arterial pressure $>$60 mm Hg. CMR imaging was performed using a 1.5-tesla magnetic resonance imaging scanner (Avanto; Siemens Healthcare GmbH; Erlangen, Germany) at the Stephenson Cardiac MR Center (Calgary, Alberta, Canada). Images were acquired using cine imaging, late gadolinium enhancement (LGE), and T1-mapping by saturation recovery single-shot acquisition[24] protocols.

CMR image analysis

CMR images were analyzed by readers blinded to treatment group using cvi 42 software (Circle Cardiovascular Imaging, Inc., Calgary, Alberta, Canada). The left ventricle was divided into a 3 $\times$ 24-segment model, and infarcted myocardium was defined as all segments with $>$50% LGE at a threshold of $>$5 SD above the mean. The peri-infarct zone was defined as all segments immediately adjacent to any infarcted segment. All remaining segments were defined as remote to the infarct territory.

Regional myocardial function was measured as an average of the percent wall thickening of all segments within a defined territory. Myocardial fibrosis was quantified by measuring the mean extracellular volume (ECV) within a defined territory calculated from regional pre-contrast and post-contrast T1 values. Mean peak systolic strains stratified by territory were measured using a custom-built multi-axial adaptation of the algorithm described by Satriano et al.[26] A clinical cardiologist (J.A.W.) with expertise in CMR imaging blinded to treatment group reviewed all analyses.

Post-mortem assessment

After final CMR imaging, animals were euthanized with intravenous saturated potassium chloride (20 ml) under full anesthesia.