SUPPLEMENTAL METHODS

Characterization of Intact ECM Scaffold

ECM scaffolds were homogenized in 300 microliters of homogenization buffer using a TISSUE TEAROR homogenizer (model 985-370, Biospec Products Inc.; Bartlesville, OK, USA). The homogenization media consisted of 150 mM NaCl, 20 mM Tris buffer (pH 7.5), 0.5% Tween 20 and protease inhibitors (Roche protease inhibitor cocktail tablets, added according to the manufacturer's protocol). Resulting supernatant was run on a 12% sodium dodecyl sulfate polyacrylamide gel. Protein was transferred to a nitrocellulose membrane and blocked with 5% skim milk in TBS-Tween (0.05%) for 1-hour at room temperature. Blots were incubated with anti-FGF-2 primary antibody (1:1000; sc-79; Santa Cruz Bio; Santa Cruz, CA) overnight at 4°C and anti-rabbit secondary antibody conjugated with HRP (Sigma-Aldrich, Missouri, USA) for 1-hour at room temperature. Protein was visualized by chemiluminescence (Thermo Fisher Scientific, Illinois, USA). In addition, in the absence of cells, intact ECM scaffold was incubated in serum-free media (SFM; IMDM; Lonza, Walkersville, MD) for 12-hours at 37°C in 5% CO_2. The resultant conditioned media was analyzed by multiplex assays (Eve Technologies, Calgary, Canada) to quantify FGF-2, VEGF-A, VEGF-C, VEGF-D, EGF and HGF.

Experimental Animals

The experimental protocol was approved by the Institutional Animal Care Committee. All procedures performed were in accordance with the Canadian Council on Animal Care. 170-200g Male rats (Fischer CDF® strain) were obtained from Charles River Canada Inc. (Quebec, Canada).

Animal Sacrifice and Explant

Animals were sacrificed 14-weeks post-treatment by lethal injection of 20mM potassium chloride under anesthesia. The LV of explanted hearts were isolated and divided in long axis through the infarcted anterior myocardial wall. Half of the LV was fixed in 10% NBF (Neutral Buffered Formalin; VWR

International, PA, USA) and embedded in paraffin. The other half was preserved in OCT, flash frozen in liquid nitrogen and stored at -80°C for protein analysis.

Histology and Immunohistochemistry

Histology slides were prepared by Calgary Laboratory Services (Calgary, Canada). In brief, specimens were fixed in 10% Neutral Buffer Formalin (VWR International, Inc., West Chester, PA), embedded in paraffin, and stained with Masson's trichrome. For immunohistochemistry, autofluorescent background was reduced using 50 mM ammonia in 70% ethanol for 180 minutes, followed by 5 minutes in 0.1 % Sudan Black B in 70% ethanol(1). Antigen retrieval was performed by incubating slides in 10mM citric acid in 0.05% tween at a pH of 6.0 for 60 minutes at 95-100°C. Samples were blocked with 5% goat serum 0.1% Triton X-100 in PBS for 1 hour, then stained using primary anti-von Willebrand factor and anti-β-catenin antibodies (Abcam; Toronto, ON, Canada). Nuclei were stained with 4'6-diamino2-phenylindol (DAPI). Stained specimens were examined using a Zeiss Axiovert 40X light microscope (Carl Zeiss Canada, Ontario, Canada) and a Zeiss LSM-5 Laser Confocal Microscope (Carl Zeiss GmBH, Heidelberg, Germany). Vascularity was quantified by measuring the number of alpha-smooth muscle actin positive vascular structures per high power field in the entire infarcted myocardium of 9 animals per group(2). Epicardial progenitor cells were quantified by measuring the percentage of β-catenin positive nuclei in the entire infarcted myocardium of 6 animals per group.

Analysis of in vivo protein expression by ELISA

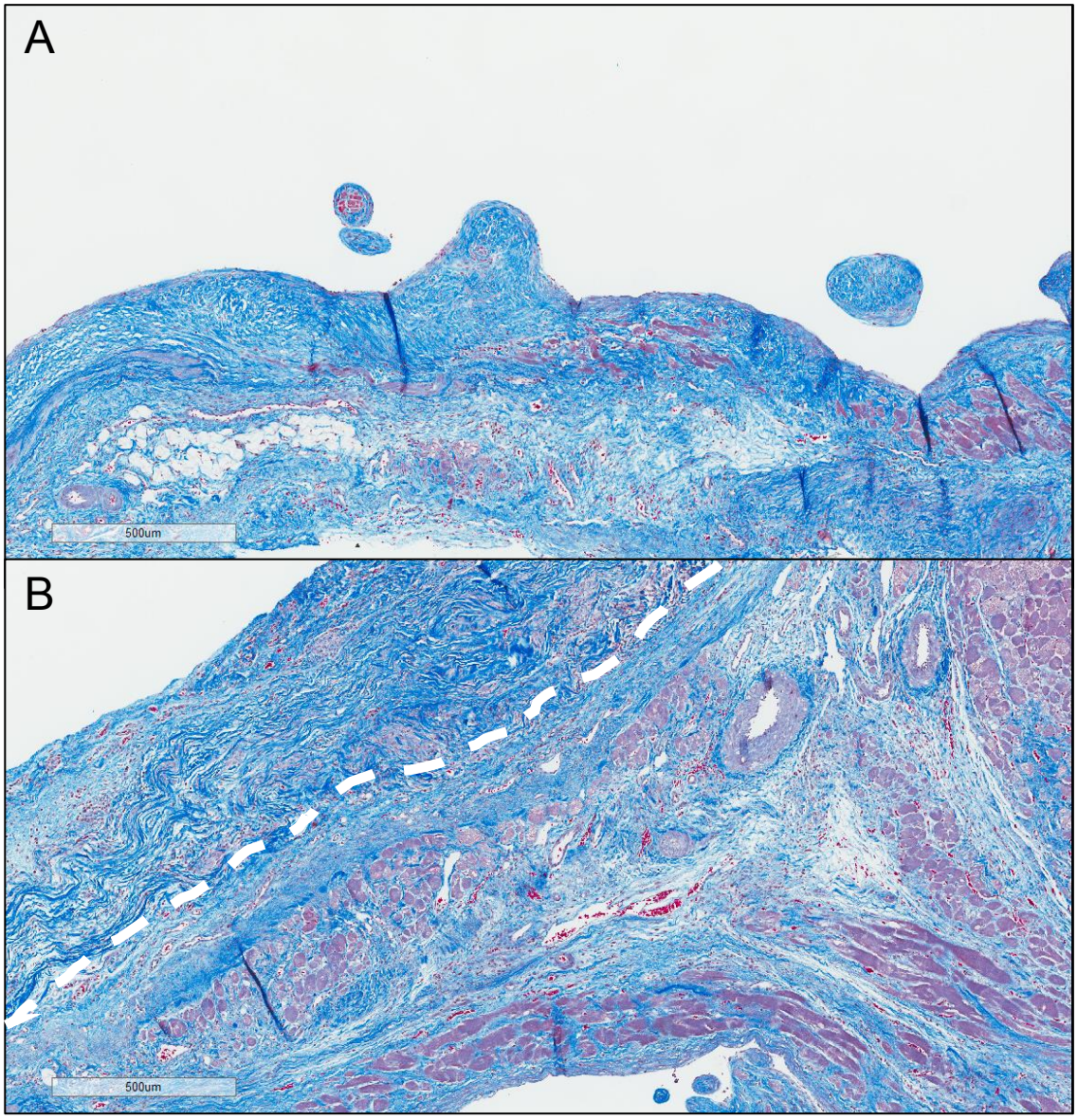
Tissue biopsies of the infarcted myocardium were homogenized for analysis by enzyme-linked immunosorbent assay (ELISA). Transmural tissue sections (20-50 mg) were sliced from the center of each frozen core biopsy and homogenized in 300 microliters of homogenization buffer as previously described. Tissue homogenate was kept on ice, and centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was collected and the protein concentration was determined according to the Bradford method. Samples were diluted to a final protein concentration of 1 mg/ml in homogenization buffer and

stored at -80C until analysis. ELISAs were performed on all tissue homogenates in accordance with the manufacturer's instructions for detection of fibroblast growth factor-2 (FGF-2).

Human Cardiac Fibroblast Isolation and Expansion

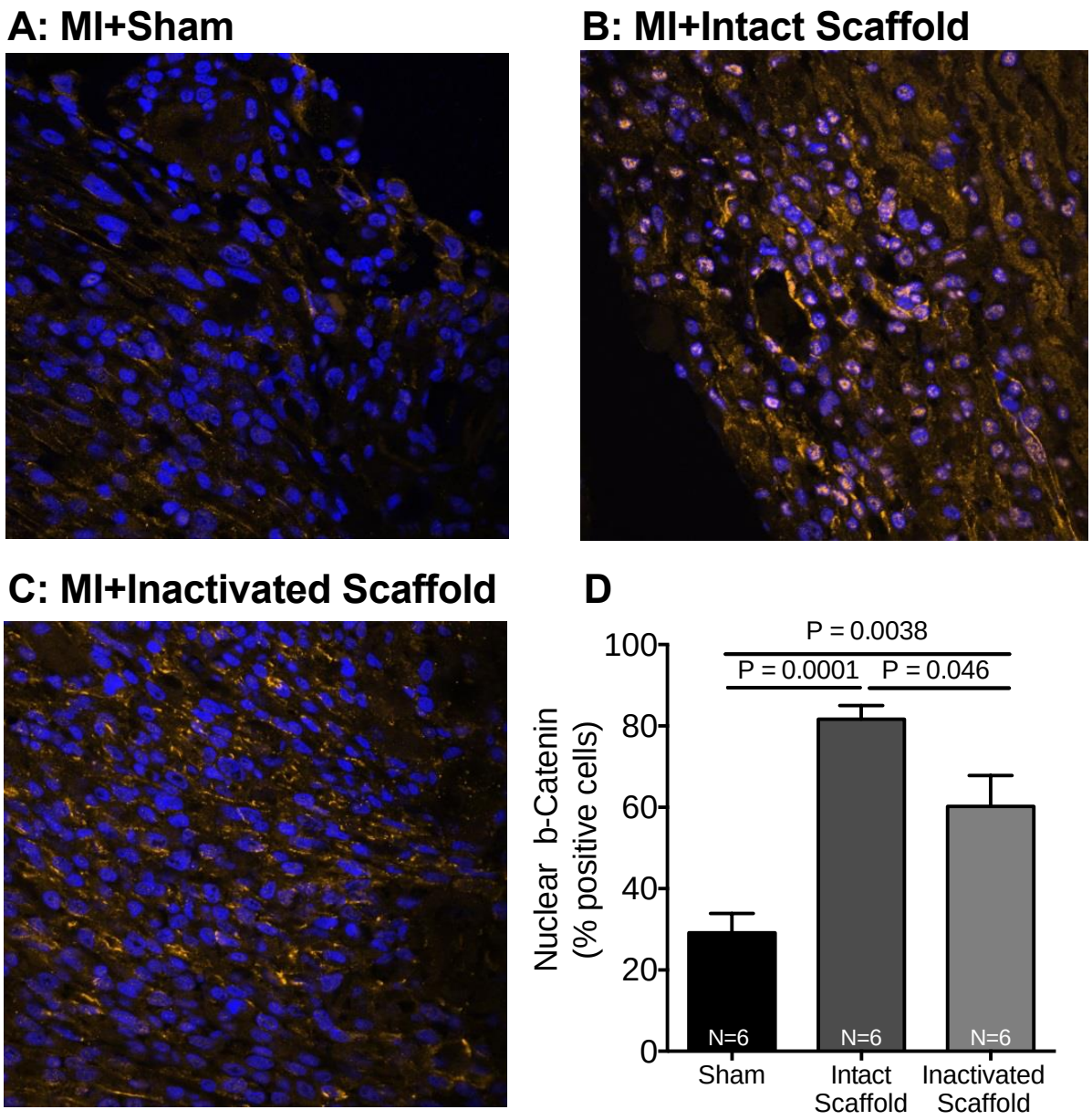
Human cardiac fibroblasts were isolated from right atrial appendage taken from consenting patients undergoing cardiac surgery at Foothills Medical Center (Calgary, Alberta) as previously described(3). All experiments involving human tissue were approved by the Conjoint Health Research Ethics board at the University of Calgary and conform to the Declaration of Helsinki. Informed consent was required. In brief, samples were minced into 0.5-1mm fragments and suspended in Iscove's Modified Dulbecco's Medium (IMDM; Lonza, Walkersville, MD) supplemented with 10% fetal bovine serum (Gibco by Life Technologies, Burlington, Canada) and 50,000 units of penicillin-streptomycin (Life Technologies, Burlington, Canada). Tissue suspensions were plated and cultured at 37°C in 5% CO_2. Passages 1-4 were used for experiments. All cells were serum starved for 24-hours prior to experimental use.

SUPPLEMENTAL FIGURES



Supplemental Figure 1: Infarcted Myocardium of Sham vs. Intact ECM Scaffold-Treated Animals

Masson's trichrome staining of the infarcted myocardium of sham-treated (A) and intact ECM scaffold-treated animals (B). The infarcted myocardium of intact ECM scaffold-treated animals depicts regions of myocardial cells adjacent to increased vascularity within the completed infarct (dashed white line depicts epicardial surface between intact ECM scaffold and infarcted myocardium).



<u>Supplemental Figure 2</u>: Intact ECM Scaffold Stimulates Epicardial Progenitor Cell Activation

Representative confocal images depicting nuclear re-localization of β-catenin indicative of epicardial progenitor cell activation and epithelial-mesenchymal transition (gold=β-catenin; blue=DAPI nuclear stain; A-C). Nuclear β-catenin measured in the infarcted myocardium of sham (N=6), intact (N=6) and inactivated (N=6) ECM scaffold treated animals 14-weeks post-MI (one-way ANOVA).

<u>Supplemental Table 1:</u> Summary of Patient Demographics

Patient Demographic	N=6
Average Age	60.7
Female	33.3%
Cardiovascular Risk Factors	
Family History of CAD	50.0%
Hypertension	80.0%
Dyslipidemia	50.0%
Diabetes	50.0%
Smoking History	16.7%
Heart Failure	33.3%
Atrial Fibrillation	0.0%
Infectious Endocarditis	0.0%
Pulmonary Disease	0.0%
Cerebral Vascular Disease	16.7%
Renal Insufficiency	33.3%
Cardiac Surgical Procedure	
CABG	2 (33.3%)
CABG + AVR	0 (0.0%)
CABG + MVR	1 (16.7%)
AVR	2 (33.3%)
MVR	0 (0.0%)
LVAD	1 (16.7%)

Patient characteristics for right atrial cardiac tissue biopsies (N=6). CAD = coronary artery disease; CABG = coronary artery bypass graft; AVR = aortic valve replacement; MVR = mitral valve replacement; LVAD = left ventricular assist device.

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3. Svystonyuk DA, Ngu JM, Mewhort HE, et al. Fibroblast growth factor-2 regulates human cardiac myofibroblast-mediated extracellular matrix remodeling. J. Transl. Med. 2015;13:147.