In situ constructive myocardial remodeling of extracellular matrix patch enhanced with controlled growth factor release

Akiko Tanaka, MD, PhD,[a] Keigo Kawaji, PhD,[b] Amit R. Patel, MD,[b] Yasuhiko Tabata, PhD, DMedSci, DPharm,[c] Martin C. Burke, DO,[b] Mahesh P. Gupta, PhD,[a] and Takeyoshi Ota, MD, PhD[a]

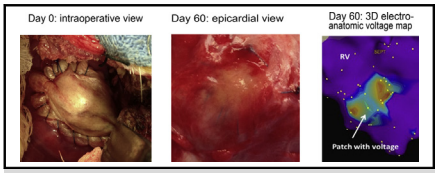
ABSTRACT

Objective: In an effort to expand treatment for advanced heart failure, we sought to develop a tissue-engineered cardiac patch for constructive and functional in situ myocardial regeneration.

Methods: An extracellular matrix patch derived from porcine small intestine submucosa was incorporated with a controlled release of basic fibroblast growth factor. The patch was surgically implanted into the porcine right ventricle (group B, n = 5). Untreated extracellular matrix (group U) and Dacron (group D) patches served as control (n = 5/group). Cardiovascular magnetic resonance was performed in all 3 groups 60 days postsurgery to evaluate regional contractility with peak longitudinal strain, perfusion with relative maximum upslope, and extent of fibrosis/edema with extracellular volume fraction. Electrophysiologic-anatomic mapping was performed in group B. Histology and quantitative reverse transcription-polymerase chain reaction were performed for further tissue characterization.

Results: Cardiovascular magnetic resonance–derived parameters were significantly better in group B compared with groups U and D (strain: group B = −16.6% ± 1.8%, group U = −14.7% ± 1.2%, group D = −9.0% ± 1.5%, $P < .001$; upslope: group B = 13.7% ± 1.1%, group U = 10.8% ± 1.3%, group D = 6.4% ± 1.8%, $P < .001$; extracellular volume: group B = 45% ± 7%, group U = 54% ± 10%, group D = 70% ± 10%, $P = .003$). Histology in group B showed a homogenous distribution of host cells, including tropomyosin and α-sarcomeric actinin–positive maturing cardiomyocytes. Group B demonstrated the greatest degree of vasculogenesis as determined by capillary density analysis (group B = 19.5 ± 6.2/mm^3, group U = 12.7 ± 2.5/mm^3, group D = 6.9 ± 3.7/mm^3, $P < .001$). Quantitative reverse transcription-polymerase chain reaction supported the histologic findings. Electrophysiologic-anatomic mapping in group B indicated positive electrical conductivity in the patch area.

Conclusions: The extracellular matrix patch enhanced with controlled release of fibroblast growth factor facilitated in situ constructive repopulation of the host cells, including cardiomyocyte and functional regeneration, increased regional contractility and tissue perfusion, and positive electrical activity in a porcine preparation. (J Thorac Cardiovasc Surg 2015;150:1280-90)



The ECM patch enhanced with controlled release of growth factor.

Central Message

The ECM patch enhanced with controlled release of growth factor may expedite in situ constructive myocardial regeneration.

Perspective

The ECM patch enhanced with controlled growth factor release has great potential to facilitate constructive myocardial regeneration. Further evaluation is warranted.

See Editorial Commentary page 1290.

See Editorial page 1035.

ET/BS

From the Departments of [a]Surgery and [b]Medicine, The University of Chicago, Chicago, Ill; and [c]Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan.

This study was supported by the American Heart Association Scientist Development Grant (Principal Investigator TO, 11SDG5120010), the National Center for Advancing Translational Sciences of the National Institutes of Health (Principal Investigator TO UL1 TR000430), and Institute of Translational Medicine Pilot Award (Principal Investigator KK CTSA UL1 TR000430).

Received for publication June 3, 2015; revisions received July 20, 2015; accepted for publication July 22, 2015; available ahead of print Sept 3, 2015.

Address for reprints: Takeyoshi Ota, MD, PhD, Department of Surgery, Center for Aortic Diseases, Section of Cardiac and Thoracic Surgery, The University of Chicago, 5841 S. Maryland Ave, MC5040, Chicago, IL 60637 (E-mail: tota@surgery.bsd.uchicago.edu).

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2015.07.073

Abbreviations and Acronyms

CMR = cardiovascular magnetic resonance
ECM = extracellular matrix
ECV = extracellular volume fraction
FGF-2 = basic fibroblast growth factor
LGE = late gadolinium enhancement
MOLLI = modified Look-Locker inversion recovery
ROI = region-of-interest
SENC = strain encoding
SIS-ECM = extracellular matrix derived from porcine small intestine submucosa
SVR = surgical ventricular restoration
vWF = von Willebrand factor

Supplemental material is available online.

Symptomatic heart failure is a major health issue with poor prognosis and limited quality of life.[1] Heart transplantation and mechanical circulatory support are the gold standard strategies to treat refractory end-stage heart failure. However, not all patients with advanced heart failure benefit from these treatment options because of donor shortages or strict criteria for surgical indications, such as age. In an effort to develop an alternative therapy for patients with end-stage heart failure, we have sought to develop a tissue-engineered cardiac patch that constructively remodels with in situ functional myocardial regeneration.

Decellularized extracellular matrix (ECM) materials have served as a mechanical framework to repair and replace human tissues.[2] They cause minimal immunologic response and allow site-specific host cell repopulation, possibly by auto-seeding of host progenitor cells from the bloodstream.[3] ECM derived from porcine small intestine submucosa (SIS) has been clinically adapted to repair pericardial or atrial septal defect with excellent results,[4] and its application recently has been extended to heart valve reconstructions.[5] Histologic studies have demonstrated that an explanted SIS-ECM used for pericardium reconstruction was remodeled with connective tissue similar to the native pericardium[4] and that SIS-ECM implanted as a cardiac valve was covered with neointima in 17 days.[6] Toeg and colleagues[7] reported that the injectable form of SIS-ECM improved left ventricular ejection fraction in a mouse myocardial infarction model. Myocardial injection of SIS-ECM with or without circulating angiogenic cells resulted in better preservation and improvement of cardiac function and partially restored myocardial viability.[7] These findings suggest that SIS-ECM could be a promising material for site-specific functional remodeling, including myocardium.

A sufficient supply of nutrients and oxygen is indispensable for survival of cells that regenerate in the ECM scaffold and for these cells to maintain their biological functions.[8] Basic fibroblast growth factor (FGF-2) promotes angiogenesis/vasculogenesis and host cell repopulation,[8] prevents maladaptive fibrosis,[9] and is an antiapoptotic factor for endothelial and smooth muscle cells.[8] Ota and colleagues[10] reported an application of a unique protein combining a growth factor and a collagen-binding domain for in situ myocardial regeneration. The unique protein provided an anchoring effect to the growth factor in an ECM scaffold and allowed the prolonged presence of the growth factor to expedite myocardial regeneration in the ECM scaffold.[10] Tabata and Ikada[11] developed a controlled drug-delivery mechanism using gelatin hydrogel. The gelatin hydrogel binds to FGF-2, preserves its biological properties, and releases it in a sustained manner for up to 5 weeks.

We have developed a novel SIS-ECM cardiac patch enhanced with controlled release of FGF-2. We hypothesized that our tissue-engineered cardiac patch would promote in situ constructive myocardial remodeling and facilitate functional regeneration. We evaluated an implanted SIS-ECM cardiac patch with a comprehensive approach using multiple imaging modalities, including cardiovascular magnetic resonance (CMR), electrophysiologic-anatomic mapping, and histology.

MATERIALS AND METHODS

Preparation of Gelatin Hydrogel Sheet

Gelatin was isolated using an alkaline process from bovine bone with an isoelectric point of 4.9 and a molecular mass of 99,000 kDa (Nitta Gelatin Co, Osaka, Japan).[11] The gelatin sheets were prepared by chemical cross-linking of a 5% bovine bone gelatin solution with glutaraldehyde. The sheets were immersed in 50 mmol/L glycine aqueous solution at 37°C for 1 hour to block the residual aldehyde groups of glutaraldehyde. The gelatin sheets were then freeze-dried and sterilized by ethylene oxide gas.

Cardiac Patch Preparation

SIS-ECM is a collagen construct derived from porcine small intestine (CorMatrix Cardiovascular Inc, Roswell, Ga). A SIS-ECM patch was soaked in natural saline for 30 minutes at room temperature before implantation. For group B, an FGF-2–incorporated gelatin hydrogel sheet was prepared with aqueous FGF-2 (Invitrogen Co, Carlsbad, Calif) with the concentration of 250 μg/mL by impregnation for 30 minutes at room temperature. Then the hydrogel was embedded in the SIS-ECM sheet. For group D, a sterilized Dacron patch was used (Edwards Lifesciences, Irvine, Calif).

Myocardial Repair in a Porcine Model

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Chicago. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (1996).

A female pig, mixed breed of Yorkshire and Landrace (20-30 kg; n = 15), was anesthetized, and the heart was exposed via a right anterolateral thoracotomy. A tangential clamp was placed on the right ventricle free wall. The wall was incised in full thickness and substituted with a 30-mm patch with a continuous 5-0 polypropylene suture. SIS-ECM patches with

ET/BS