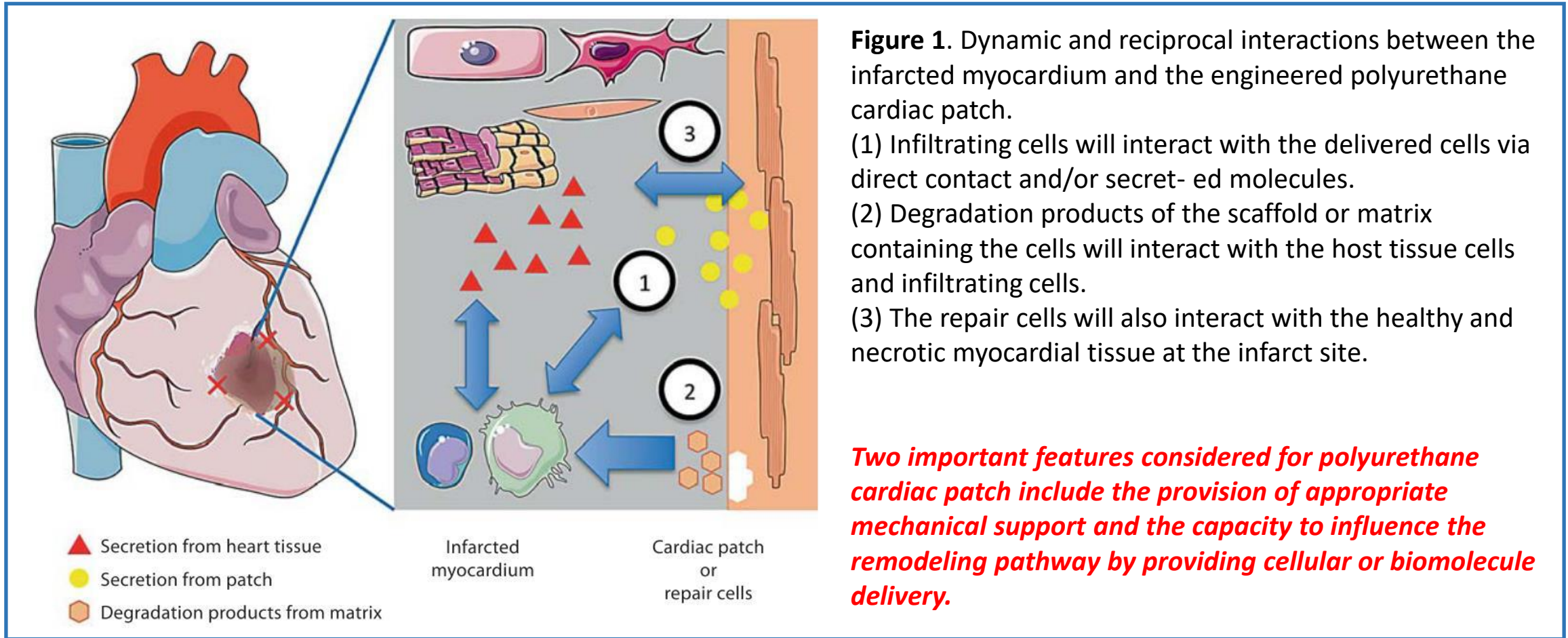**Bio 분야에서의 Polyurethane의 응용**

6장. 폴리우레탄의 재생의학에서의 응용

• Polyurethanes in Regenerative Medicine-heart patch for myocardial infraction

: 재생의학에서 폴리우레탄 biomaterials는 주로 지지체로서 손상된 조직의 물리적 기능을 보조하며, 효능물질을 전달하고, cell transplantation을 보조하는 역할로서 사용된다.
본 보고서에서는 생분해성 폴리우레탄을 이용한 *myocardial infraction heart patch* **에 관한 최신 연구를 소개 하고자 한다.**

Figure 1. Dynamic and reciprocal interactions between the infarcted myocardium and the engineered polyurethane cardiac patch.
(1) Infiltrating cells will interact with the delivered cells via direct contact and/or secret- ed molecules.
(2) Degradation products of the scaffold or matrix containing the cells will interact with the host tissue cells and infiltrating cells.
(3) The repair cells will also interact with the healthy and necrotic myocardial tissue at the infarct site.

Two important features considered for polyurethane cardiac patch include the provision of appropriate mechanical support and the capacity to influence the remodeling pathway by providing cellular or biomolecule delivery.

▲ Secretion from heart tissue
● Secretion from patch
⬡ Degradation products from matrix

Infarcted myocardium

Cardiac patch or repair cells

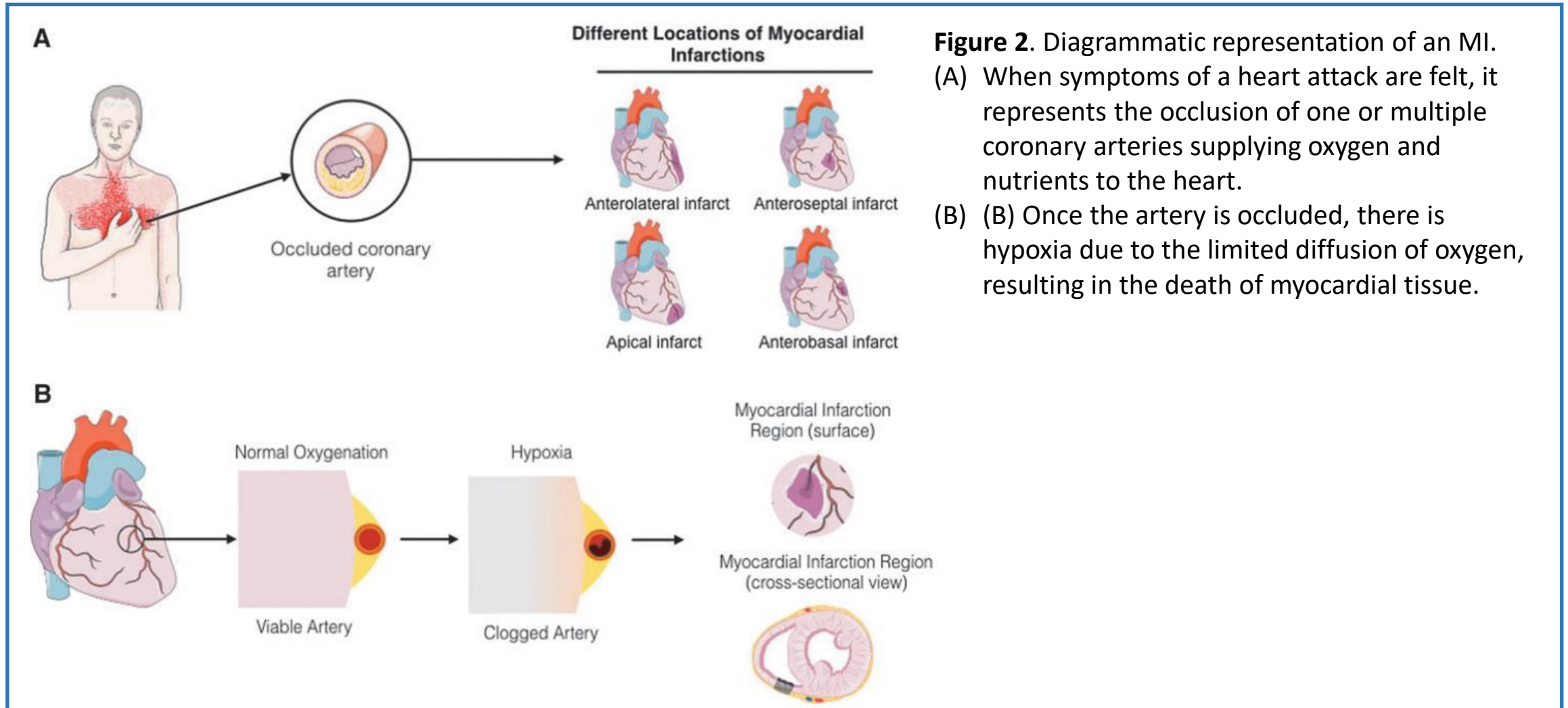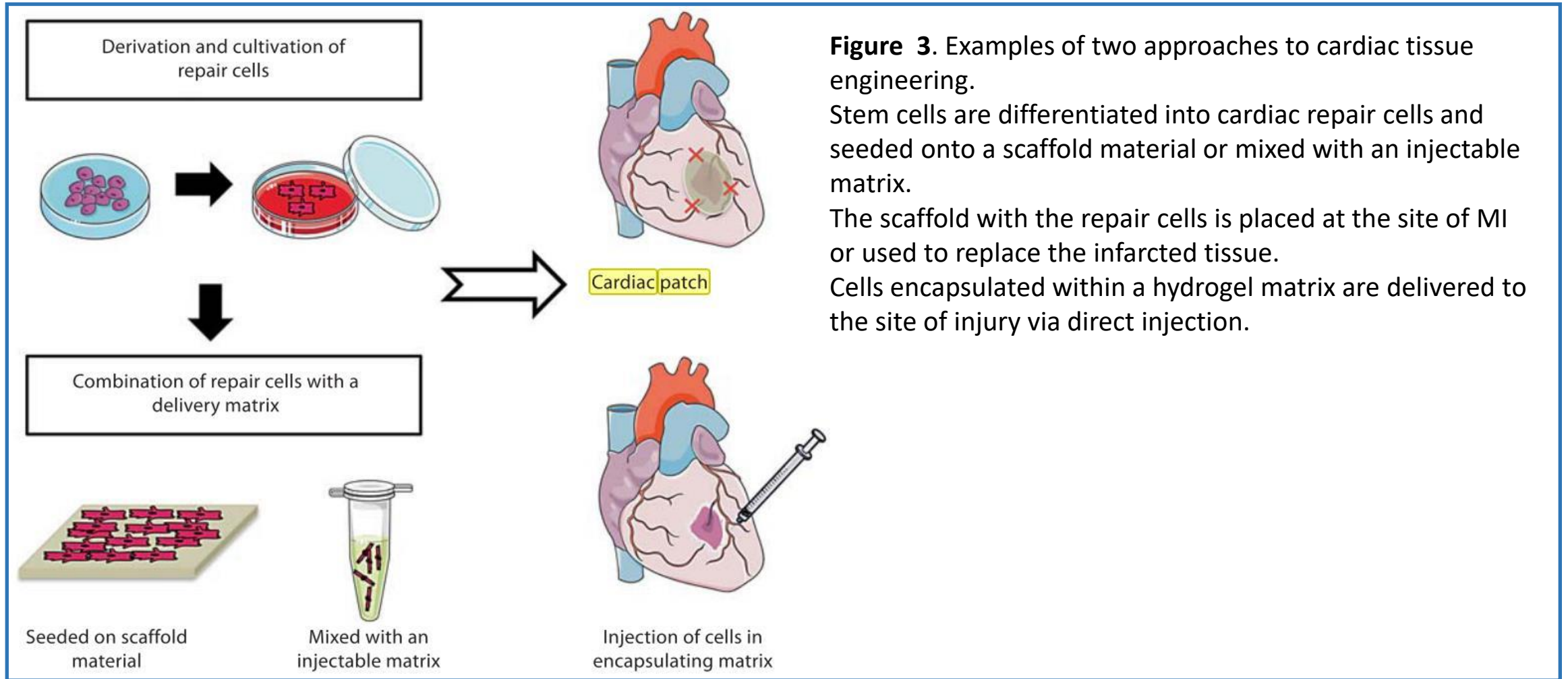- **Myocardial infarction (MI)**

Figure 2. Diagrammatic representation of an MI.
(A) When symptoms of a heart attack are felt, it represents the occlusion of one or multiple coronary arteries supplying oxygen and nutrients to the heart.
(B) (B) Once the artery is occluded, there is hypoxia due to the limited diffusion of oxygen, resulting in the death of myocardial tissue.

• <u>Cardiac patch</u> & injectable gel

: The most clinically promising approach to regeneration of cardiac muscle currently under investigation is that of injecting cardiogenic repair cells or implanting a preformed tissue-engineered patch.

Figure 3. Examples of two approaches to cardiac tissue engineering.
Stem cells are differentiated into cardiac repair cells and seeded onto a scaffold material or mixed with an injectable matrix.
The scaffold with the repair cells is placed at the site of MI or used to replace the infarcted tissue.
Cells encapsulated within a hydrogel matrix are delivered to the site of injury via direct injection.

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

: The objective of this report was to focus on these two features by first ***evaluating the incorporation of a cardiac extracellular matrix (ECM) component***, and second by ***evaluating the impact of patch anisotropy on the pathological remodeling process initiated by myocardial infarction***.

The functional outcomes of microfibrous, elastomeric, biodegradable polyurethane cardiac patches have been evaluated in a rat chronic infarction model. Ten weeks after infarction and 8 weeks after patch epicardial placement, echocardiographic function, tissue-level structural remodeling (e.g., biaxial mechanical response and microstructural analysis), and cellular level remodeling were assessed. The results showed that the incorporation of a cardiac ECM altered the progression of several keys aspects of maladaptive remodeling following myocardial infarction. This included decreasing left ventricle (LV) global mechanical compliance, inhibiting echocardiographically-measured functional deterioration, mitigating scar formation and LV wall thinning, and promoting angiogenesis. In evaluating the impact of patch anisotropy, no effects from the altered patch mechanics were detected after 8 weeks, possibly due to patch fibrous encapsulation.

Overall, this study demonstrates the benefit of a cardiac patch design that combines both ventricle mechanical support, through a biodegradable, fibrillary elastomeric component, and the incorporation of ECM-based hydrogel components.

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**
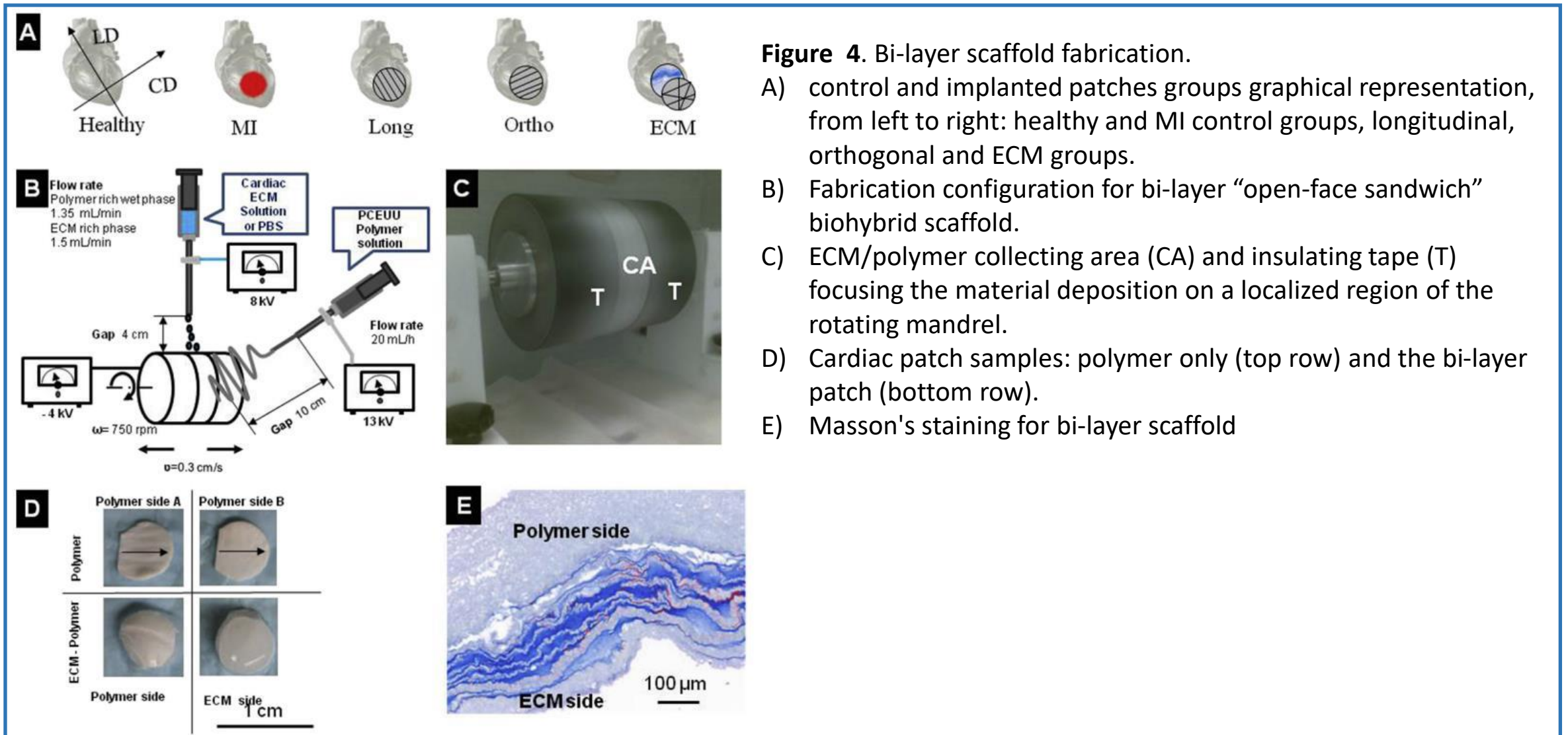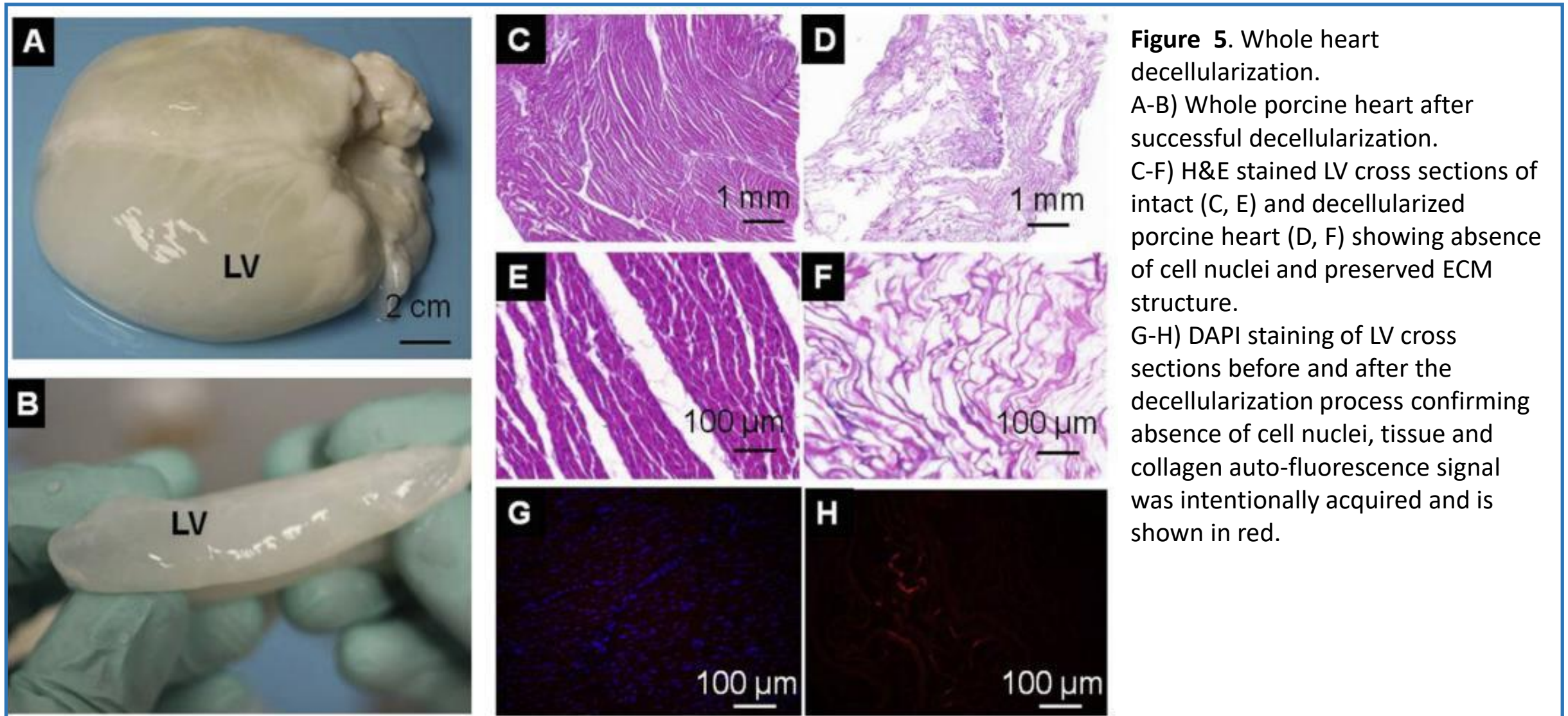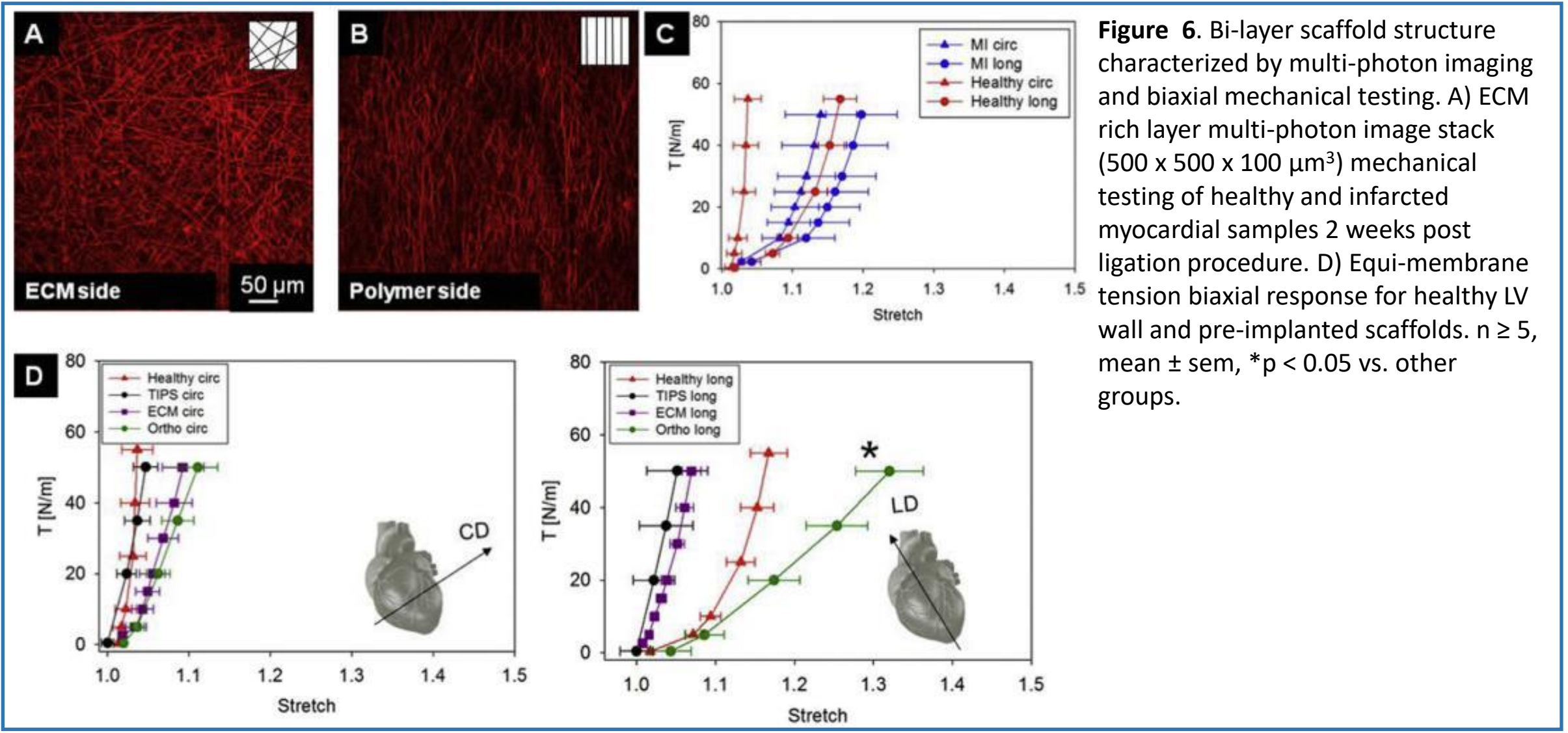

Figure 4. Bi-layer scaffold fabrication.
A) control and implanted patches groups graphical representation, from left to right: healthy and MI control groups, longitudinal, orthogonal and ECM groups.
B) Fabrication configuration for bi-layer "open-face sandwich" biohybrid scaffold.
C) ECM/polymer collecting area (CA) and insulating tape (T) focusing the material deposition on a localized region of the rotating mandrel.
D) Cardiac patch samples: polymer only (top row) and the bi-layer patch (bottom row).
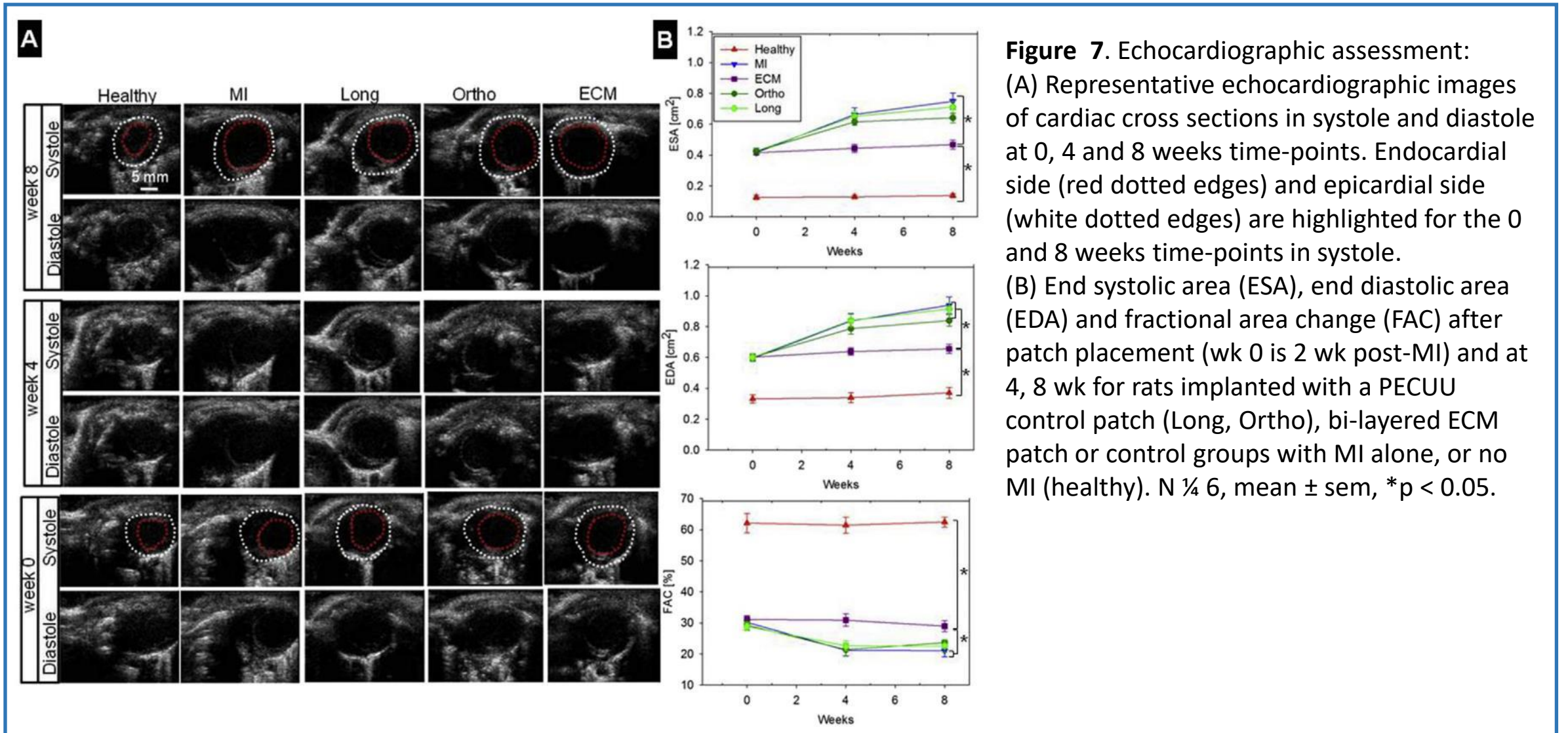E) Masson's staining for bi-layer scaffold

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

Figure 5. Whole heart decellularization.
A-B) Whole porcine heart after successful decellularization.
C-F) H&E stained LV cross sections of intact (C, E) and decellularized porcine heart (D, F) showing absence of cell nuclei and preserved ECM structure.
G-H) DAPI staining of LV cross sections before and after the decellularization process confirming absence of cell nuclei, tissue and collagen auto-fluorescence signal was intentionally acquired and is shown in red.

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

Figure 6. Bi-layer scaffold structure characterized by multi-photon imaging and biaxial mechanical testing. A) ECM rich layer multi-photon image stack (500 x 500 x 100 μm³) mechanical testing of healthy and infarcted myocardial samples 2 weeks post ligation procedure. D) Equi-membrane tension biaxial response for healthy LV wall and pre-implanted scaffolds. n ≥ 5, mean ± sem, *p < 0.05 vs. other groups.

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

Figure 7. Echocardiographic assessment: (A) Representative echocardiographic images of cardiac cross sections in systole and diastole at 0, 4 and 8 weeks time-points. Endocardial side (red dotted edges) and epicardial side (white dotted edges) are highlighted for the 0 and 8 weeks time-points in systole. (B) End systolic area (ESA), end diastolic area (EDA) and fractional area change (FAC) after patch placement (wk 0 is 2 wk post-MI) and at 4, 8 wk for rats implanted with a PECUU control patch (Long, Ortho), bi-layered ECM patch or control groups with MI alone, or no MI (healthy). N ¼ 6, mean ± sem, *p < 0.05.

- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

Figure 8. LV wall thickness at 8 weeks. Control groups (A-B), and patched hearts (C-E) 8 weeks after patch placement (10 weeks post-MI). Mean LV wall thickness (F) was higher when the ECM bi-layer patch was utilized. Pre-Ortho and Pre-ECM represent patch thickness prior to implantation. n ¼ 6, mean þ sem, *p < 0.05, white arrows indicate implantation site.
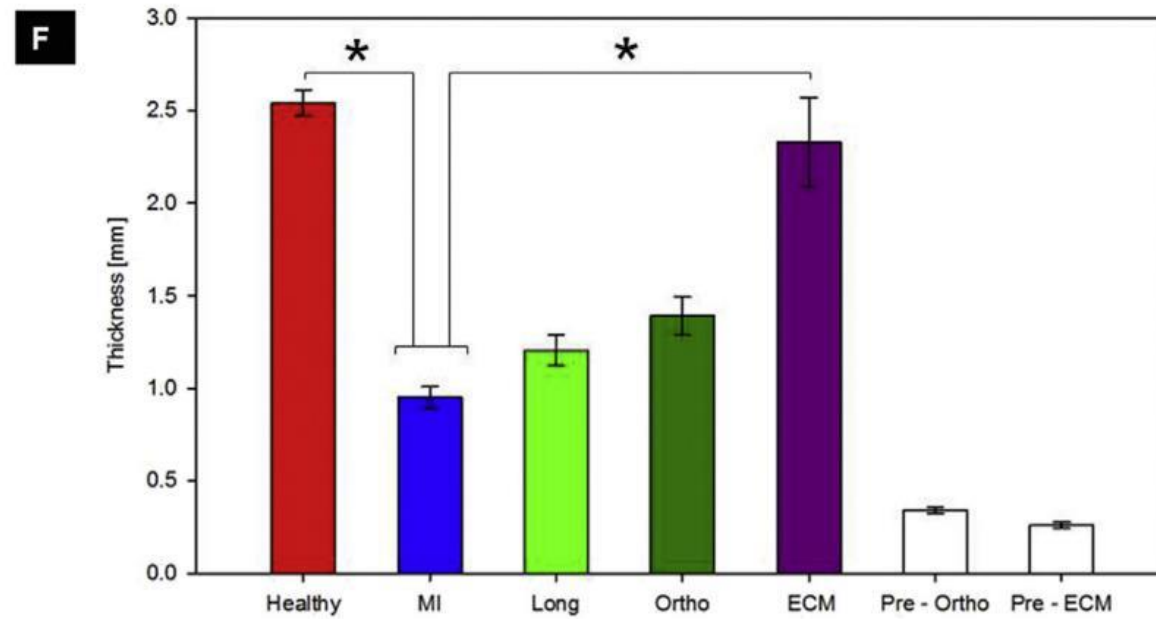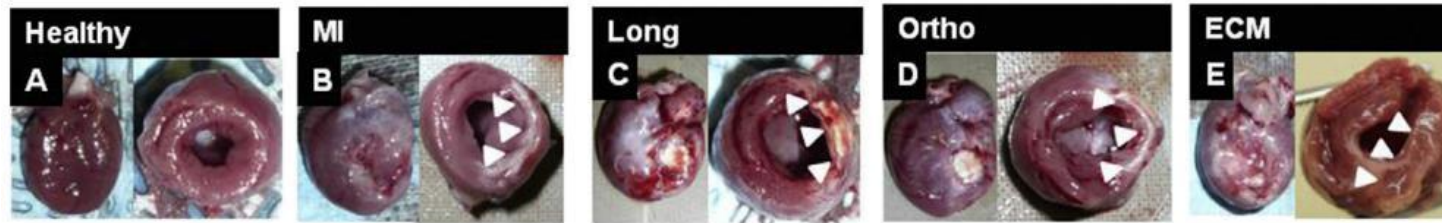
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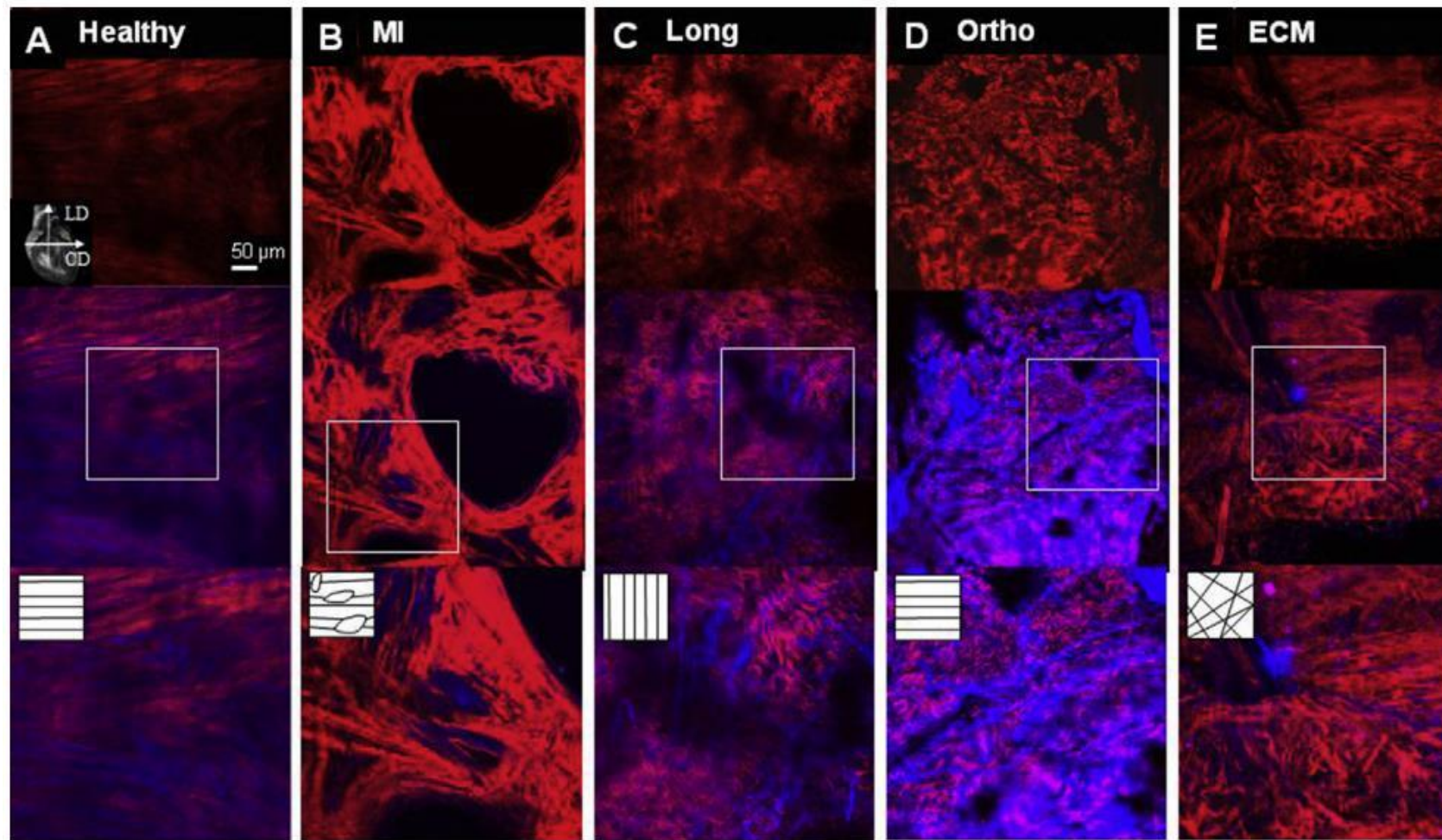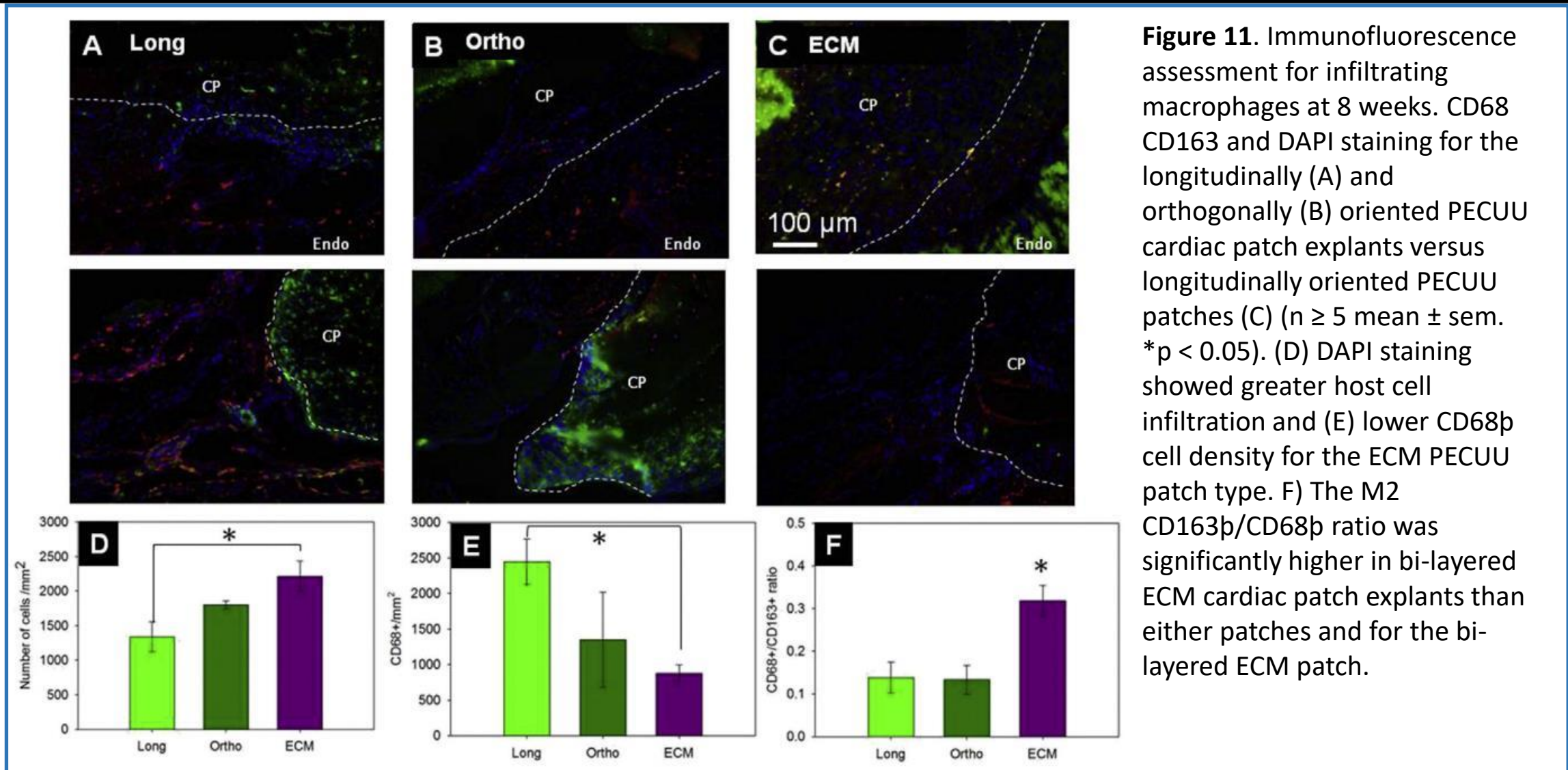

Figure 9. Multi photon explants and control group analysis. A) intact collagen network from the healthy heart, circumferentially oriented, B) damaged collagen fiber network from hearts 8 weeks after MI, C) longitudinally aligned scaffold fibers (blue) and de-novo collagen fiber network (red) for the longitudinally oriented PECUU patch explanted at 8 wk. D) orthogonally/circumferentially aligned scaffold fibers (blue) and de-novo collagen fiber network (red) for the orthogonally oriented PECUU patch explanted at 8 wk. E) ECM bi-layered patched hearts explanted at 8 weeks showed randomly oriented collagen and scaffold fibers.

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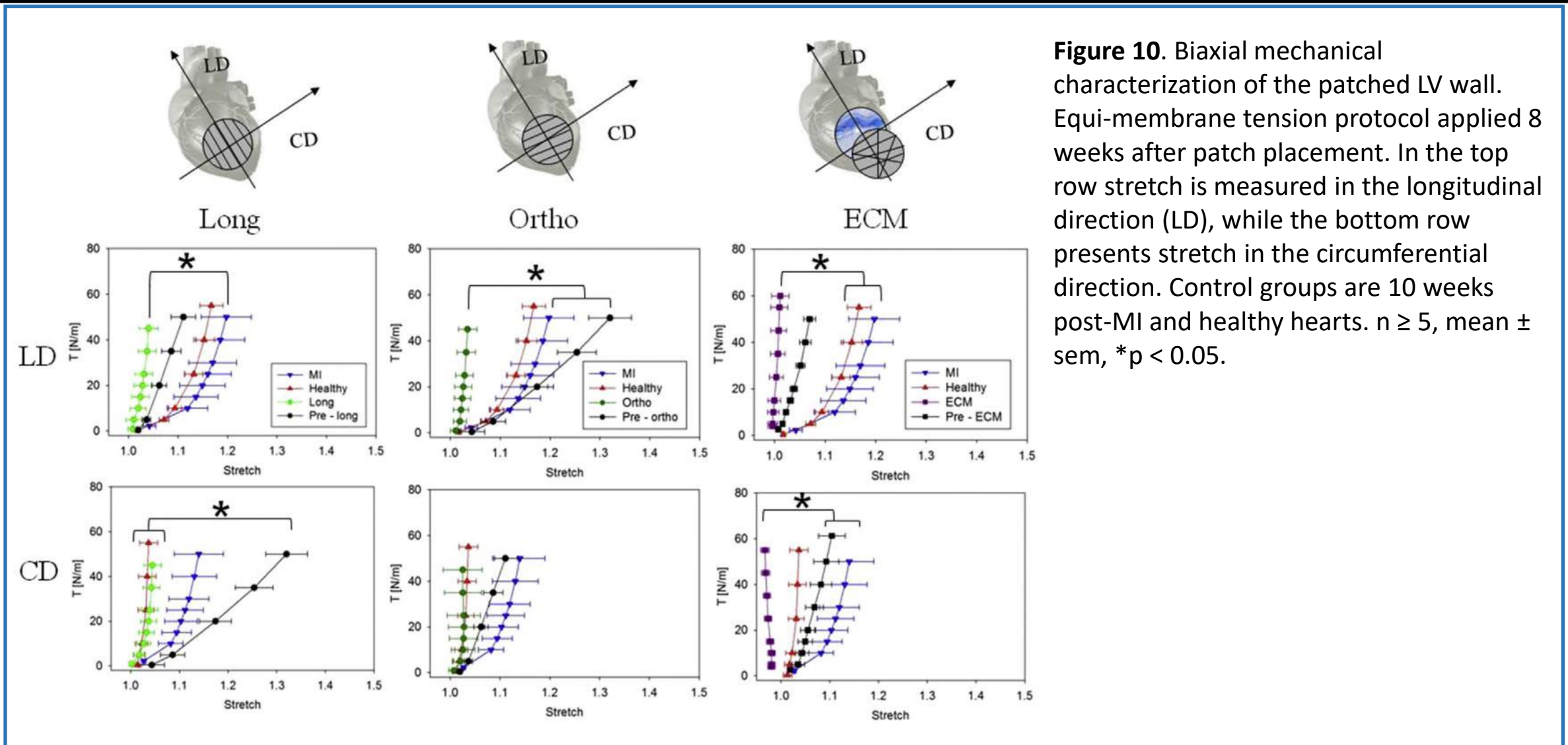

**Figure 10**. Biaxial mechanical characterization of the patched LV wall. Equi-membrane tension protocol applied 8 weeks after patch placement. In the top row stretch is measured in the longitudinal direction (LD), while the bottom row presents stretch in the circumferential direction. Control groups are 10 weeks post-MI and healthy hearts. n ≥ 5, mean ± sem, *p < 0.05.

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Figure 11. Immunofluorescence assessment for infiltrating macrophages at 8 weeks. CD68 CD163 and DAPI staining for the longitudinally (A) and orthogonally (B) oriented PECUU cardiac patch explants versus longitudinally oriented PECUU patches (C) (n ≥ 5 mean ± sem. *p < 0.05). (D) DAPI staining showed greater host cell infiltration and (E) lower CD68þ cell density for the ECM PECUU patch type. F) The M2 CD163þ/CD68þ ratio was significantly higher in bi-layered ECM cardiac patch explants than either patches and for the bi-layered ECM patch.

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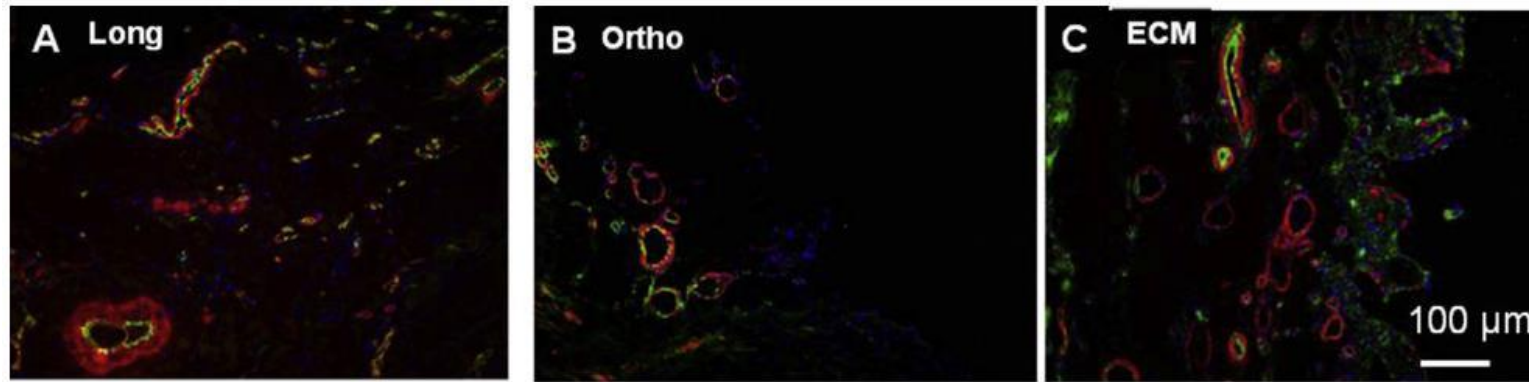
Figure 12. Immunofluorescence assessment for vascularization at 8 wk. CD31 (green), aSMA (red) and DAPI (blue) staining for the longitudinally (A) and orthogonally (B) oriented PECUU patches, and for the bi-layered ECM patch (C) (n ≥ 5 mean ± sem; *p < 0.005 for total vessel number). Vessel were identified by CD31 and αSMA co-localization, vessel typ was determined based on size, intima-media layer thickness ratio, shape, presence/absence of intimal layer.
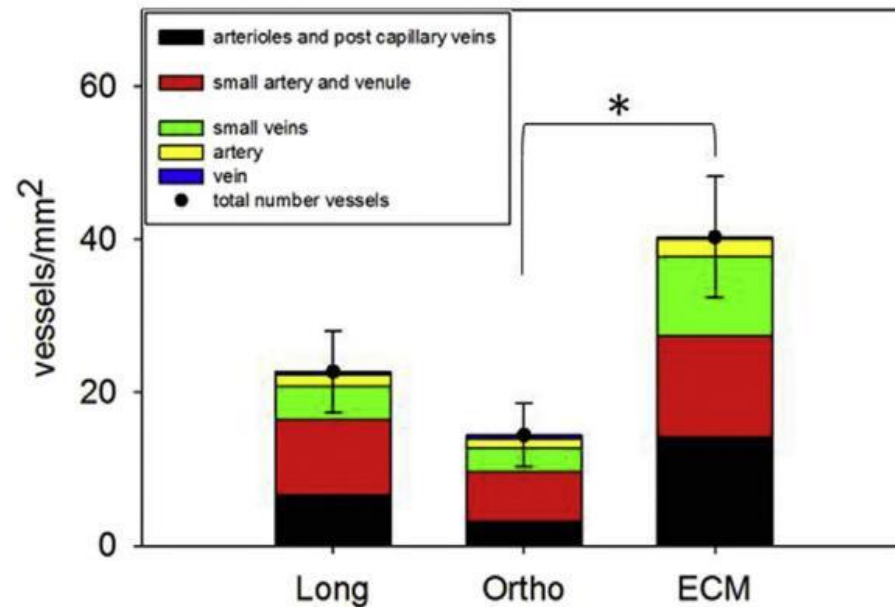
- **Bi-layered polyurethane-Extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model.**

Figure 13. Immunofluorescence assessment for pericytes and their association with vascular structures at 8 wk. CP (cardiac patch region), vWf(green), NG2 (red) and DAPI (blue) staining for the longitudinally (A) and orthogonally (B) oriented PECUU patches, and for the bi-layered ECM patch (C).
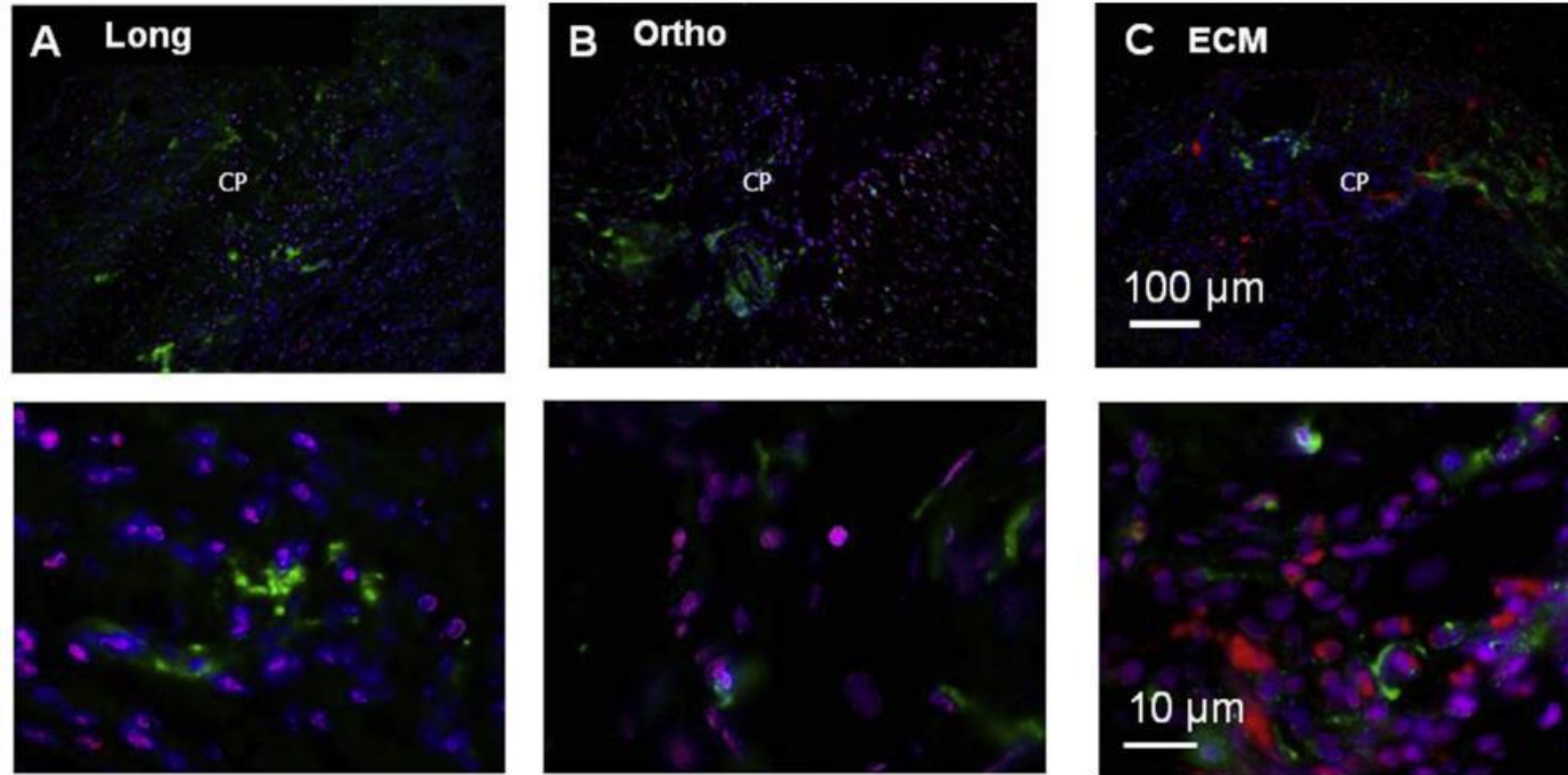
- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

: Although adipose-derived stem cell (ADSCs) have shown promised in cardiac regeneration, stable engraftment is still challenging. Acellular bioengineered cardiac patches have shown promise in positively altering ventricular remodeling in ischemic cardiomyopathy. The author hypothesized that combining an ADSC sheet approach with a bioengineered patch would enhance ADSC engraftment and positively promote cardiac function compared with either therapy alone in a rat ischemic cardiomyopathy model.

Figure 14. Enhancement of functional preservation with adipose-derived stem cell (ADSC) sheet and poly(ester carbonate urethane) urea cardiac patch (*PECUU*).
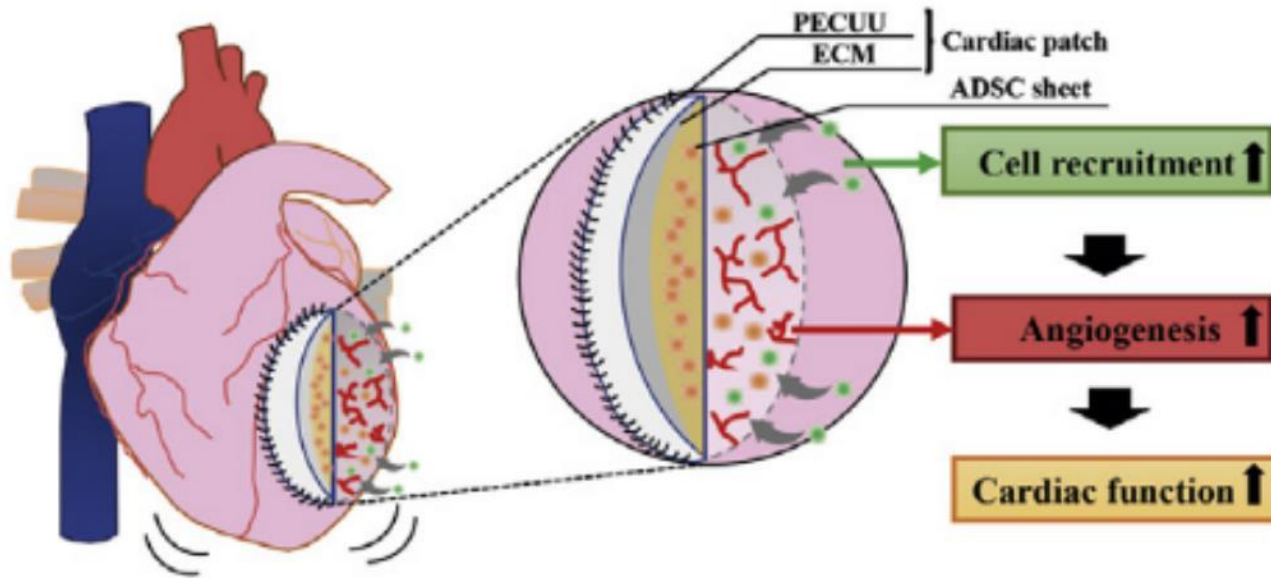

=> As result, *the biodegradable poly(ester carbonate urethane) urea (PECUU) cardiac patch enhanced ADSC engraftment, which was associated with greater cardiac function and neovascularization in the peri-infarct zone following subacute myocardial infraction*.
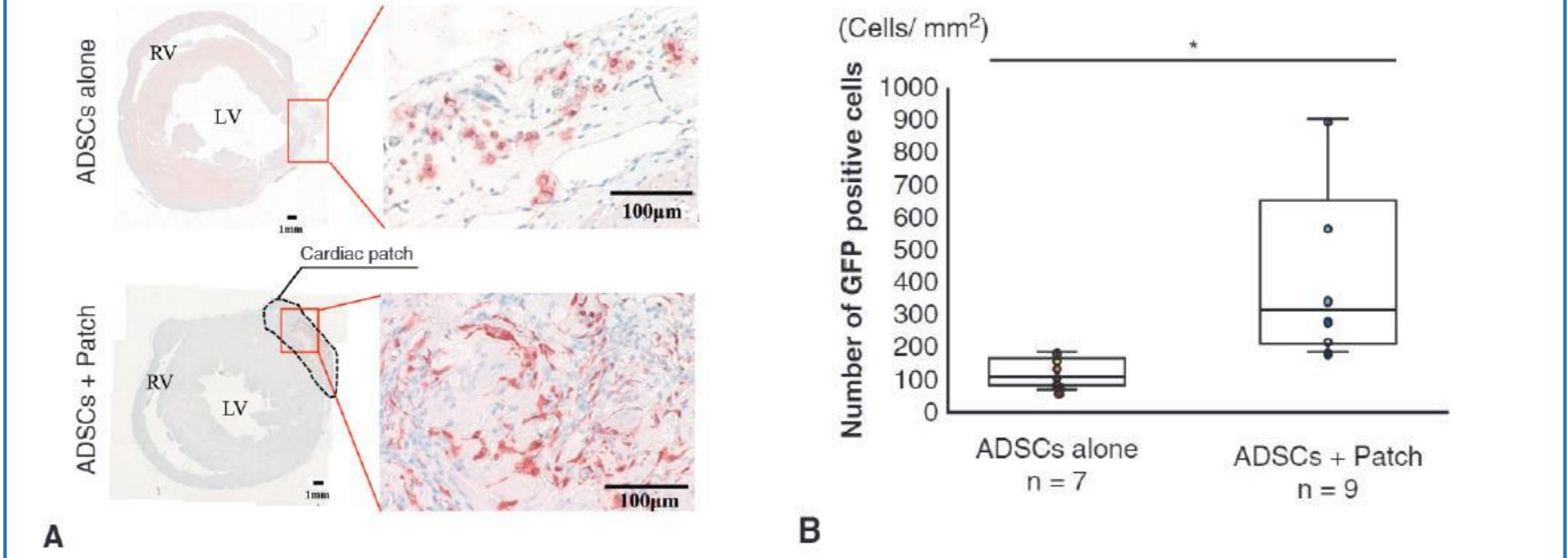
- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

Figure 15. Generation and transplantation of adipose-derived stem cell (ADSC) sheets and biodegradable PECUU cardiac patch with porcine extracellular extracellular matrix (ECM). A, ADSC isolation protocol and the generation of ADSC sheets. B, Macroscopic image (scale bar = 1 mm) of ADSC sheet with hematoxylin and ecsin (H&E) staining (scale bar = 100 μm) and macroscopic image of PECUU patch on top of ADSC sheet and Masson's trichrome (MT) staining (scale bar = 1 mm and 100 μm), and immunohistological staining for green fluorescent protein (GFP) antibody of ADSCs with patch (scale bar = 100 μm). C, Representative flow cytometry data of ADSCs stained with GFP antibodies or isotype control, anti-CD90 antibodies or isotype control, anti-CD 105 antibodies or isotype control, and anti-CD73 antibodies or isotype control, anti-CD45 antibodies or isotype control, and anti-CD34 antibodies or isotype control. D, Transplantation schema for ADSC sheets and PECUU cardiac patch with ECM on the left ventricle surface of rat ischemic model. E, Experimental groups and follow-up examinations after treatments or sham operation. *Tx*, Transplantation of ADSC sheet or PECUU cardiac patch or both; *UCG*, ultrasonic echocardiography.
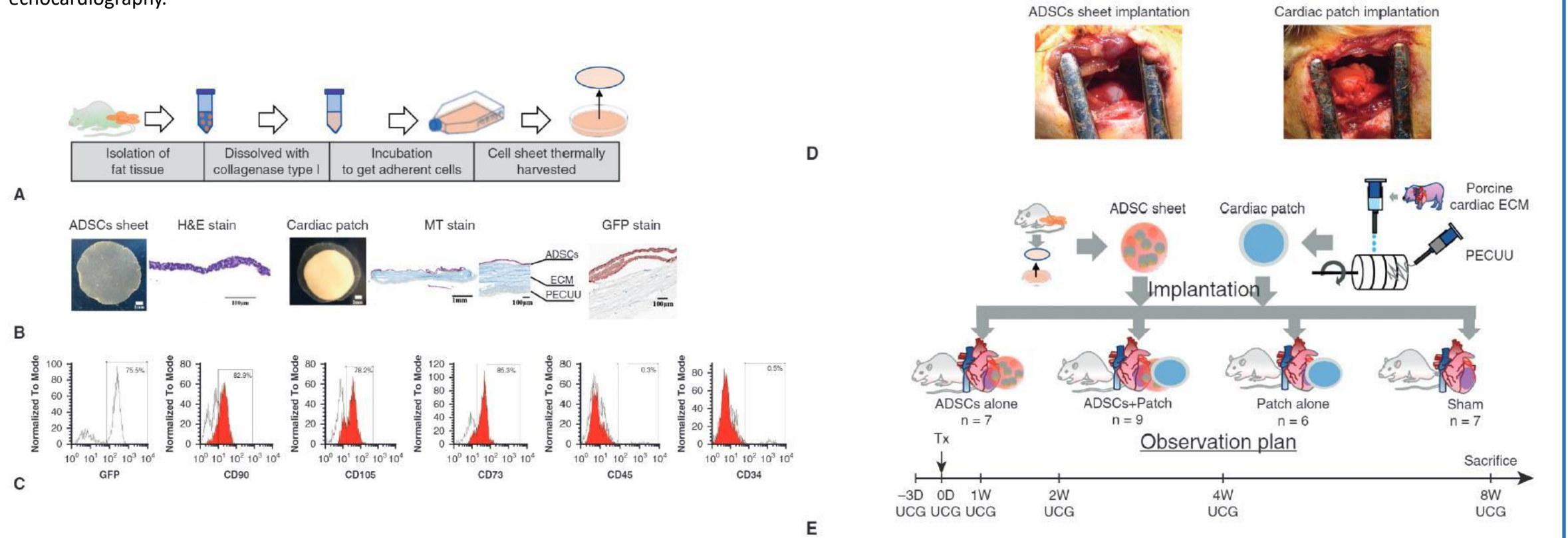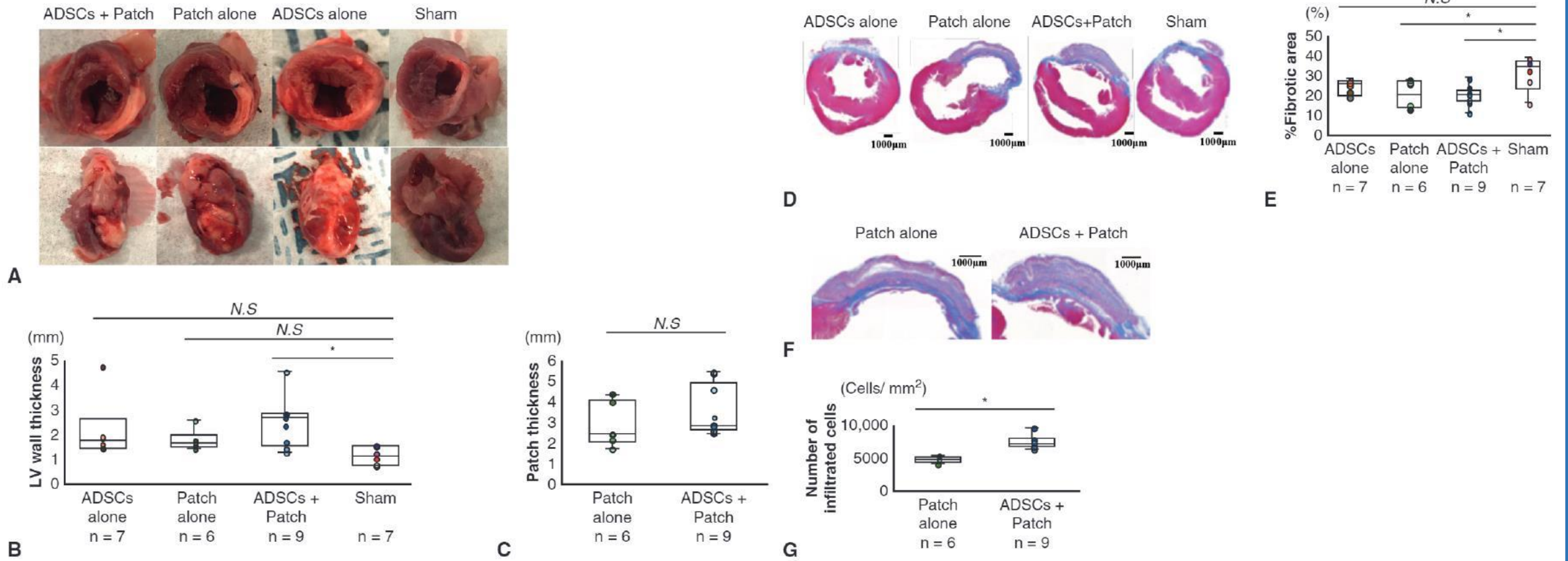
- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

Figure 16. Engraftment of transplanted adipose-derived steam cell (ADSC) sheets around the heart. A, Green fluorescent protein (GFP) staining of the harvested hearts to detect engraftment of transplanted ADSC at 8 weeks after ADSC sheets transplantation. Scale bars = 1 mm and 100 μm. B, The number of engrafted ADSCs, comparing ADSC sheet therapy with and without PECUU cardiac patches. The upper and lower borders of the box represents the median. The upper and lower whiskers represent the maximum and minimum values of nonoutliers. *RV*, Right ventricle; *LV*, left ventricle. *P<0.05.
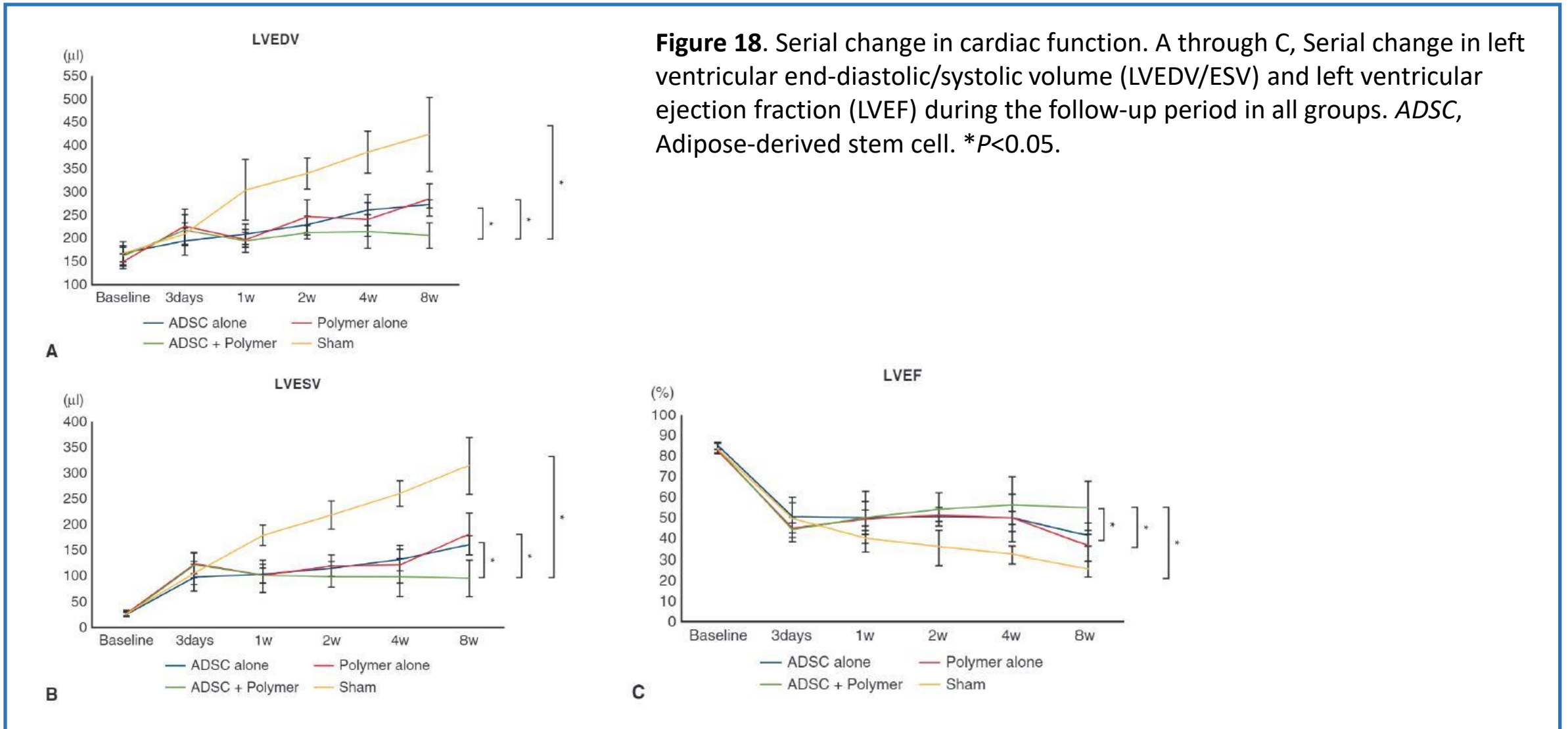
- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

Figure 17. Left ventricle (LV) wall thickness, fibrotic areas, and cell recruitment after treatments. A, Macroscopic images of whole and transected hearts at the end of the experiment in all groups. B, LV wall thickness comparing all groups. C, Cardiac patch thickness comparing adipose derived stem cell (ADSC) sheets + PECUU cardiac patch group and cardiac patch alone group. D, Masson's trichrome staining of the harvested hearts. Scale bars = 1000 μm. E, The percentages of fibrotic areas in LV of transected hearts in all groups. F, Masson's trichrome staining of the harvested hearts for infiltrated cells into the cardiac patches comparing PECUU cardiac patches with and without ADSC sheets. The upper and lower borders of the box represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values of nonoutliers. Extra dots represent outliers. N.S., Not significant. *P<0.05.
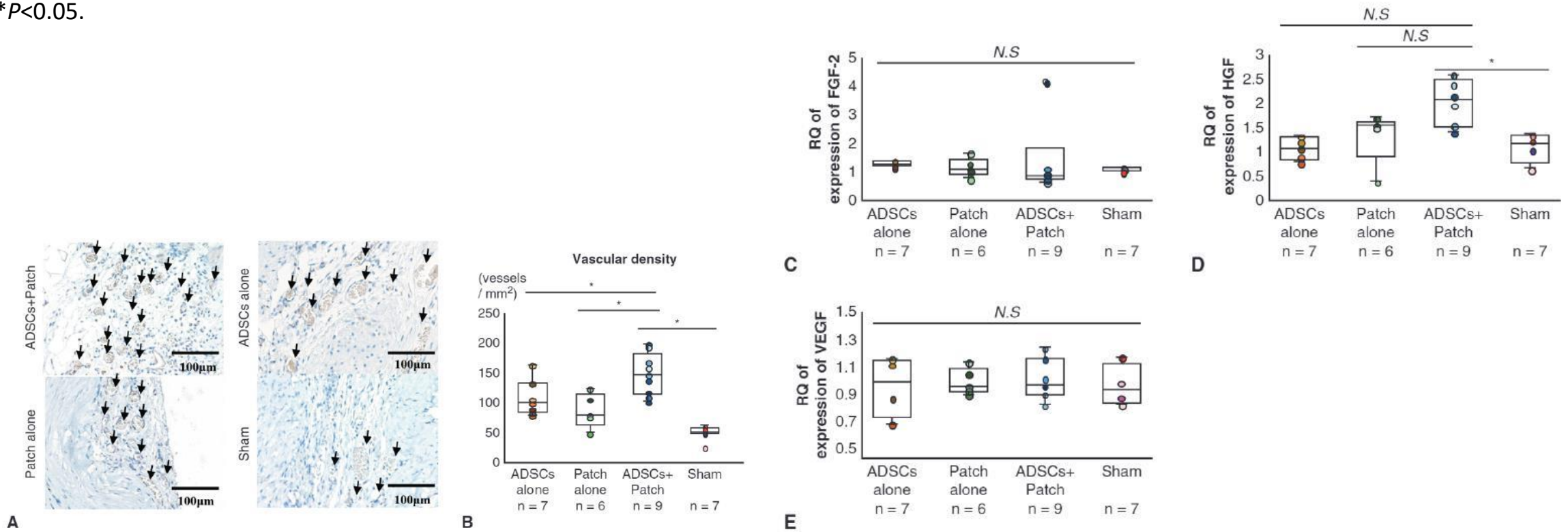
- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

Figure 18. Serial change in cardiac function. A through C, Serial change in left ventricular end-diastolic/systolic volume (LVEDV/ESV) and left ventricular ejection fraction (LVEF) during the follow-up period in all groups. *ADSC*, Adipose-derived stem cell. *P<0.05.

- **Adiopose-derived stem cell sheet under an elastic patch improves cardiac function in rats after myocardial infraction.**

Figure 19. Comparing vascular density and the gene expression of markers related to neovascularization among the all groups. A, CD31 stains of peri-infract area of the heart transected at the middle level of the left ventricle (LV) in all groups. Arrows indicate CD31-positive cells. Scale bars = 100 μm. B, Comparison the vascular density among all group. C through E, Comparing fibroblast growth factor 2 (FGF-2), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) expression in cardiac tissues at 8 weeks after treatments among the all groups. The upper and lower borders of the box represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values of nonoutliers. Extra dots represent outliers. *ADSC*, Adipose-derived stem cell; *RQ*, relative quality; *N.S.*, not significant. *$P<0.05$.

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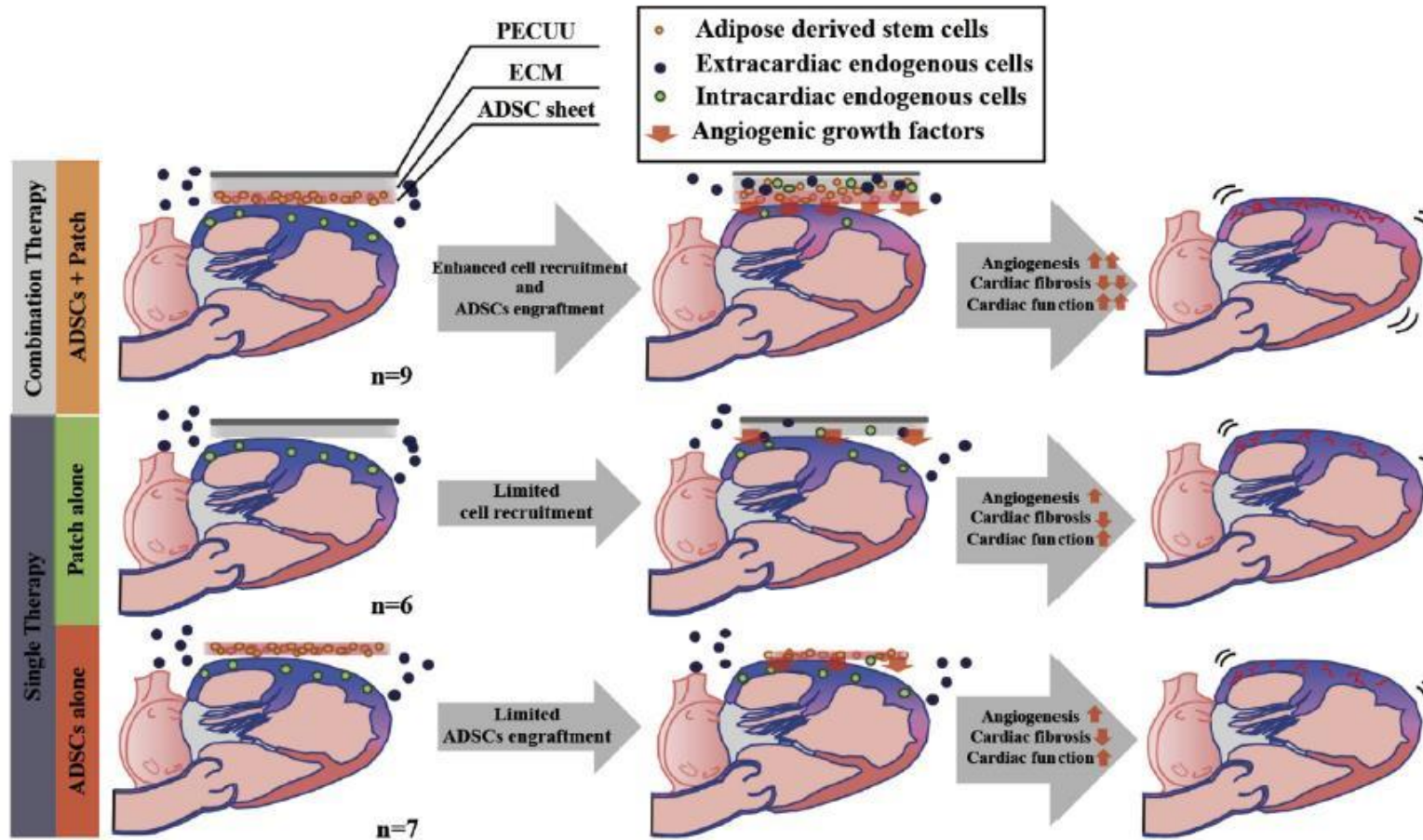

Figure 20. Enhancement of functional preservation with adipose-derived stem cell (ADSC) therapy by PECUU cardiac patch with extracellular matrix (ECM). Combining a biodegradable, elastic cardiac patch, incorporating a cardiac ECM-derived hydrogel, with ADSC sheet therapy enhanced cardiac function over individual therapy, and was associated with greater engraftment of ADSCs and neovascularization with greater cell recruitment following subacute myocardial infraction.