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CASE REPORT

De novo Biological Coronary Artery Bypass in a Rat Model: Case Report and the Concept of Hybrid Cardiovascular Regeneration

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ABSTRACT

Background: Peripheral blood mononuclear cell-derived progenitor cells (regeneration associated cells: RACs) have been reported to migrate to tissues with inflammation to expedite anti-inflammatory and pro-regeneration actions, but cell sources, administration, timing, dosage, and combined procedures after myocardial ischemia and/or infarction (MI) still remain to be elucidated in preclinical studies.

Materials and Methods: Rats with induced MI were treated with intravenous infusion of 5-day culture-primed autologous RACs or control cell transfusion, followed by weekly echocardiography until 4 weeks after MI, at which time pressure-volume relationship (PVR) analyses and histological studies were performed.

Results: While most rats treated with autologous RACs infusion after MI developed vascular regeneration from surrounding pericardial tissues and significant functional improvements, one rat developed a *de novo* coronary artery bypass ($\phi < 0.3$ mm) mimicking surgical bypass grafting (CABG), echocardiography revealed recovered left ventricular (LV) motion, and PVR analyses showed restored LV function as well as histological changes suggesting revascularization and myocardial regeneration.

Conclusion: Development of isolated bypass-like structure by RAC therapy after MI led us to consider further surgical as well as biological modifications to expedite biological coronary revascularization and myocardial regeneration. A combination with vascular approaches to provide arterial inflow may increase the rate of complete revascularization and regeneration of cardiomyocytes as hybrid procedure, as they complement each other, to realize cardiovascular regeneration.

Keywords: Peripheral blood mononuclear cell, Quality and Quantity control culture, Regeneration-associated cells (RACs), Biological coronary revascularization, Biological myocardial regeneration, Mesenchymal stem cells, Cardiovascular regeneration

Introduction

The history of myocardial revascularization started with the use of the pericardium[1-2], arterial graft onlay placement[3-5], coronary artery bypass grafting (CABG)[6-10] and percutaneous coronary intervention (PCI). While the last two vascular procedures are the current mainstay of coronary revascularization, they do not cover all the patients/lesions with myocardial ischemia, which depend on the balance between the expected benefits and procedural risks involved, including the small target arteries[7], immediacy of ischemic events[8], use of cardiopulmonary bypass[8-9], advanced age[9], repeated operation[9], reduced cardiac function[10], and reperfusion injury[11-12]. These factors increase further risks, rendering the balance to incomplete revascularization[13] despite continued technical advancements.[14] As for cellular cardiomyoplasty, various stem cell therapies have been proposed and clinically tested, but with largely unfavorable results[15-16]. Mesenchymal stem cells are different in that they were reported to possess homing capabilities, tolerance to allogeneic response[17], and *in situ* differentiation[18] to promote angiogenesis and cardiomyogenesis[16-19]. Among them are the peripheral blood mononuclear cell-derived and *in vitro*-expanded progenitor cells[20], i.e., quality and quantity control culture cells[21-24] or regeneration-associated cells (RACs)[25], which may function by migrating to tissues with inflammation irrespective of vascular size, and by expediting their anti-inflammatory as well as pro-regenerative activity[21-25]. In particular, RAC therapy early rather than late after coronary events may enhance its homing efficacy due to accentuated inflammation and intensify vasculogenesis and myocardial regeneration in the target lesions[8-12]. These unique functions may allow intravenous administration to otherwise inaccessible ischemic lesions in order to stabilize inflammation, and revascularize as well as regenerate the myocardium[21-22]. Such functions have been tested via intramyocardial injection[22], topical application[23-24], or intravenous administration[25]. Herein, we report a representative case that substantiated the concept of "biological revascularization" of the ischemic myocardium, from which we obtained clues regarding further enhancement of efficacy in terms of surgical modifications, biological manipulations, and their combination with the current coronary vascular procedures.

Materials and Methods

Lewis rats were purchased and acclimated for a week before experimentation. These rats were treated according to the animal care requirements of Tokai University. Inbred rats of the same breed were sacrificed to obtain their peripheral blood for culture for the development of RACs[20-22], and other sibling rats underwent ligation of the left anterior descending artery (LAD) or had induced myocardial infarction (MI) via left 4th interspace thoracotomy. Three days later, they received 5-day-cultured RACs at 1 x 10^5 for an aliquot of 0.2 mL via the tail vein to simulate delayed autologous RACs transplantation[25]. The rats were examined by pressure-volume relationship (PVR) analysis[26] as previously reported[27-28] both before and after induced MI, and they were then followed by echocardiography for 4 weeks. They then underwent another PVR analysis (4w) to record left ventricular (LV) responses against "acute afterload increase" by constriction of the descending thoracic aorta and responses against "acute preload increase" by acute intravenous infusion of saline at 1% of body weight as reported previously[27-28]. After these functional studies, the whole heart was excised and submitted to morphological studies by Hematoxylin-Eosin and Trichrome staining. Immunohistochemical studies were performed using monoclonal antibody against myoglobin, CD31 and CD34[21-25].

Results

When the chest was re-entered via the initial thoracotomy 4 weeks after MI, an arterial connection between the sternum and the infarcted myocardium distal to the LAD ligation was observed in a rat treated with intravenous infusion of 5-day culture-primed autologous RACs. The *de novo* coronary artery bypass was associated with collateral vessels radially providing multiple distal connections to the myocardium, similar to a multi-distal CABG but much smaller in diameter ($\phi <$ 0.3mm).

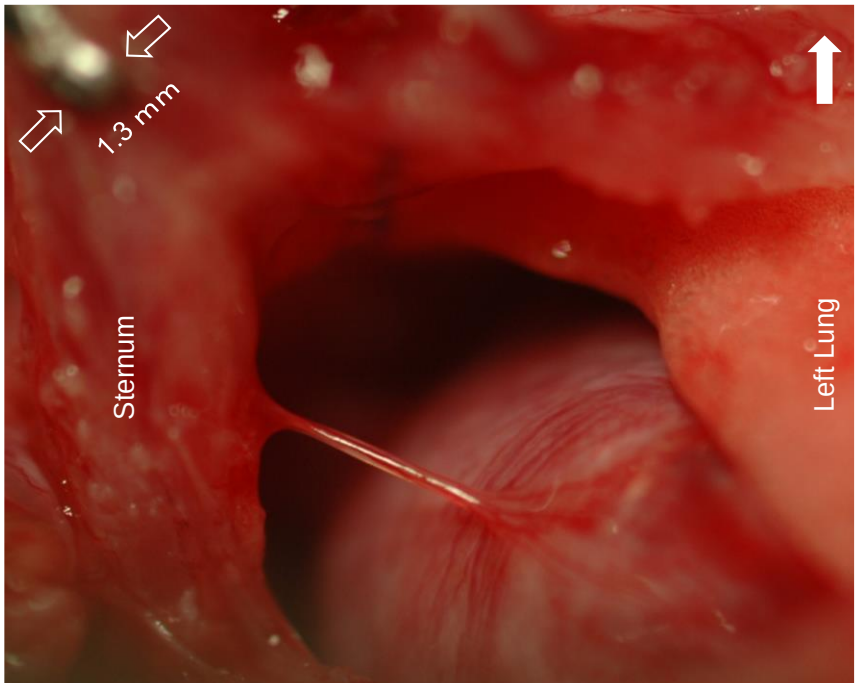

Fig. 1. Biological CABG
A *de novo* vascular connection between the sternum and LV was observed 4 weeks after MI and RAC infusion. It resembles CABG except for its size, less than 0.3 mm in diameter, with arterioles branching from the stem and radially supplying multiple connections over the area of the MI.
Closed arrow indicates the direction to the head. An upper arm of a chest retractor, eye-opener for human use, is 1.3 mm in diameter.

ECHOCARDIOGRAPHY

In this animal, echocardiography revealed that LV dimensions increased for up to 2 weeks and then remained unchanged (Fig. 2A), equivalent to normal control animals[28]. Trans-mitral flows showed similar changes, but they recovered to control levels 4 weeks after MI (Fig. 2B).

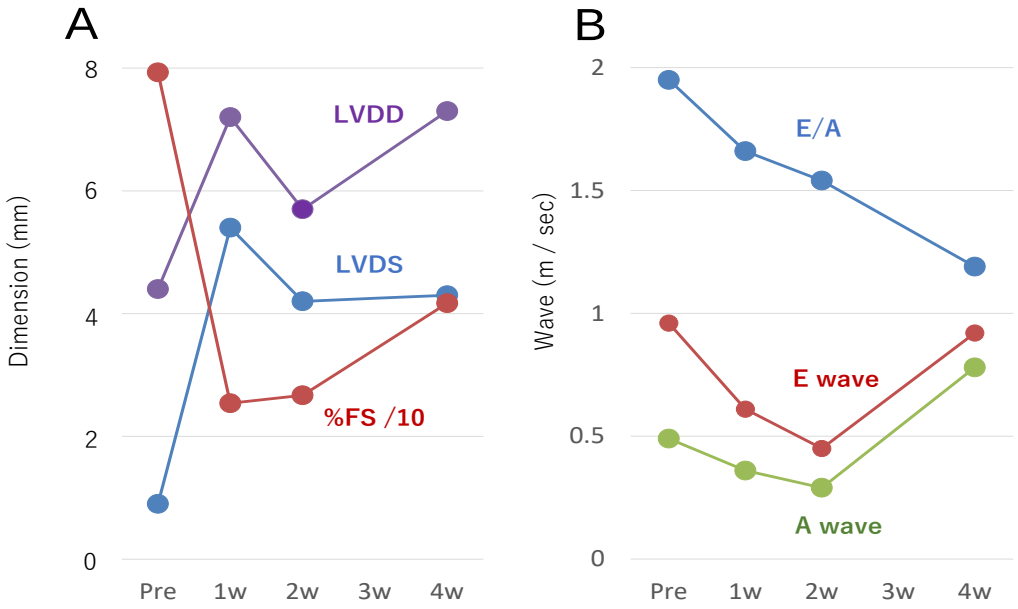

Fig. 2. Echocardiography
Echocardiographic follow-up (A) revealed that LV dimen-sions increased for up to 2 weeks after MI and then remained unchanged, resulting in fractional shortening (%FS) being reduced from the preoperative value to a nadir in 2 weeks, after which they recovered to halfway 4 weeks after MI. Trans-mitral flows (B) showed similar changes, but they recovered almost completely by 4 weeks after MI.

PRESSURE-VOLUME RELATIONSHIP ANALYSES

PVR after MI on day 0 (Fig. 3, top) showed flattened end-systolic PVR (ESPVR, blue line) in response to acute pressure load and steepened end-diastolic PVR (EDPVR, red curve) in response to acute volume load, resulting in reduced stroke volume (SV, 77 µL), increased heart rate (HR, 328 bpm) and elevated EDP (12.4 mmHg) to compensate for reduced cardiac output (CO, 25.4 mL/min). These responses had improved 4 weeks after MI, with steepened ESPVR and flattened EDPVR (Fig. 3, bottom), resulting in increased SV (84 µL) at a lower end-diastolic pressure (6.9 mmHg) and reduced HR (297 bpm) for constant CO (25.0 mL/min). The contractile and relaxation indices such as max positive dP/dt improved from +3676 to +6422 mmHg/sec, and max negative dP/dt alleviated from -3036 to -6179 mmHg/sec as did Tau (τ) from 16.1 to 11.6 msec 4 weeks after MI (bottom panels) to the pre-MI level, as did the recovery in trans-mitral flow in echocardiography. While clamping the bypass structure with a neurosurgical miniature clip (Supplement 2) slowly decreased SV, there was no reversal of the change following release of the clamp for reperfusion.

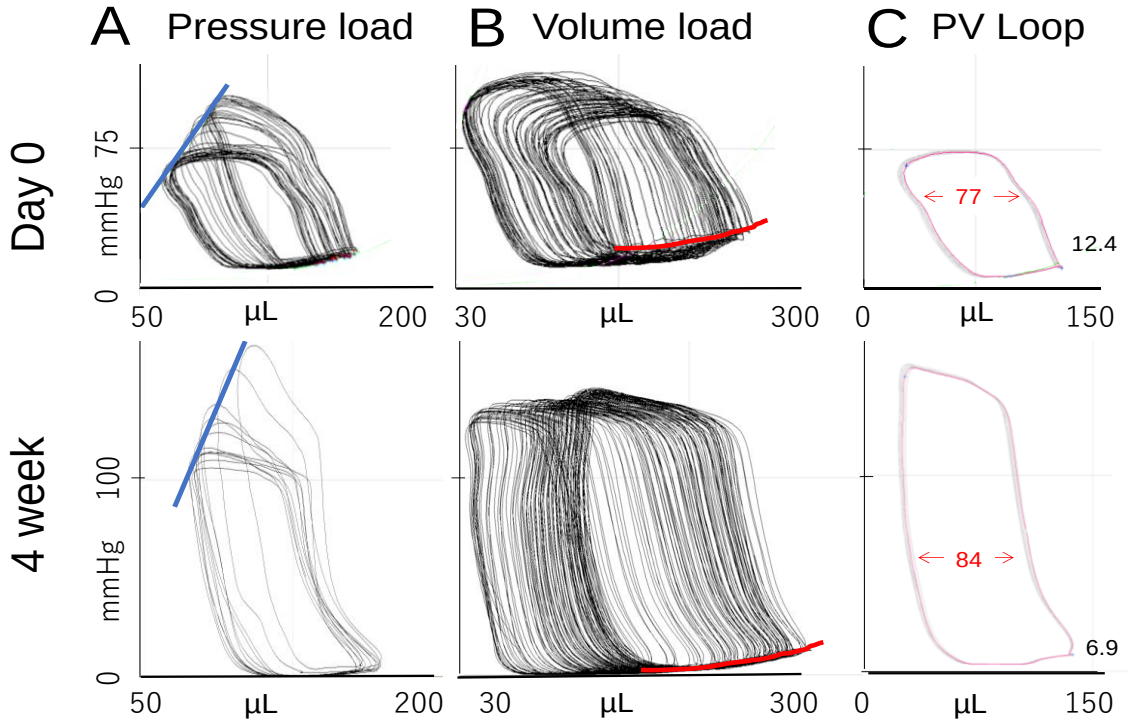

Fig. 3. PVR

PVR showed suppressed ESPVR (blue) and limited EDPVR (red), resulting in reduced SV (77 µL) and increased heart rate (328 bpm) to maintain cardiac output on Day 0 (top panels). These LV functions were improved Pre-MI level 4 weeks after MI (bottom panels).

HISTOLOGY

In Hematoxylin-Eosin staining, there were arterioles, veins, and lymphatics in the bypass structure inserting into the LV wall (Fig. 4A)[4,5], with edema, bleeding, and myocardium of undetermined origin (Fig. 4B), from the recipient or from RACs (Supplement 1)[25]. Trichrome staining (Fig. 4C) showed a thickened myocardium with less infarct (right panel) compared to untreated controls (left panel), comprising an extended area of transmural fibrosis or LV aneurysmal formation.

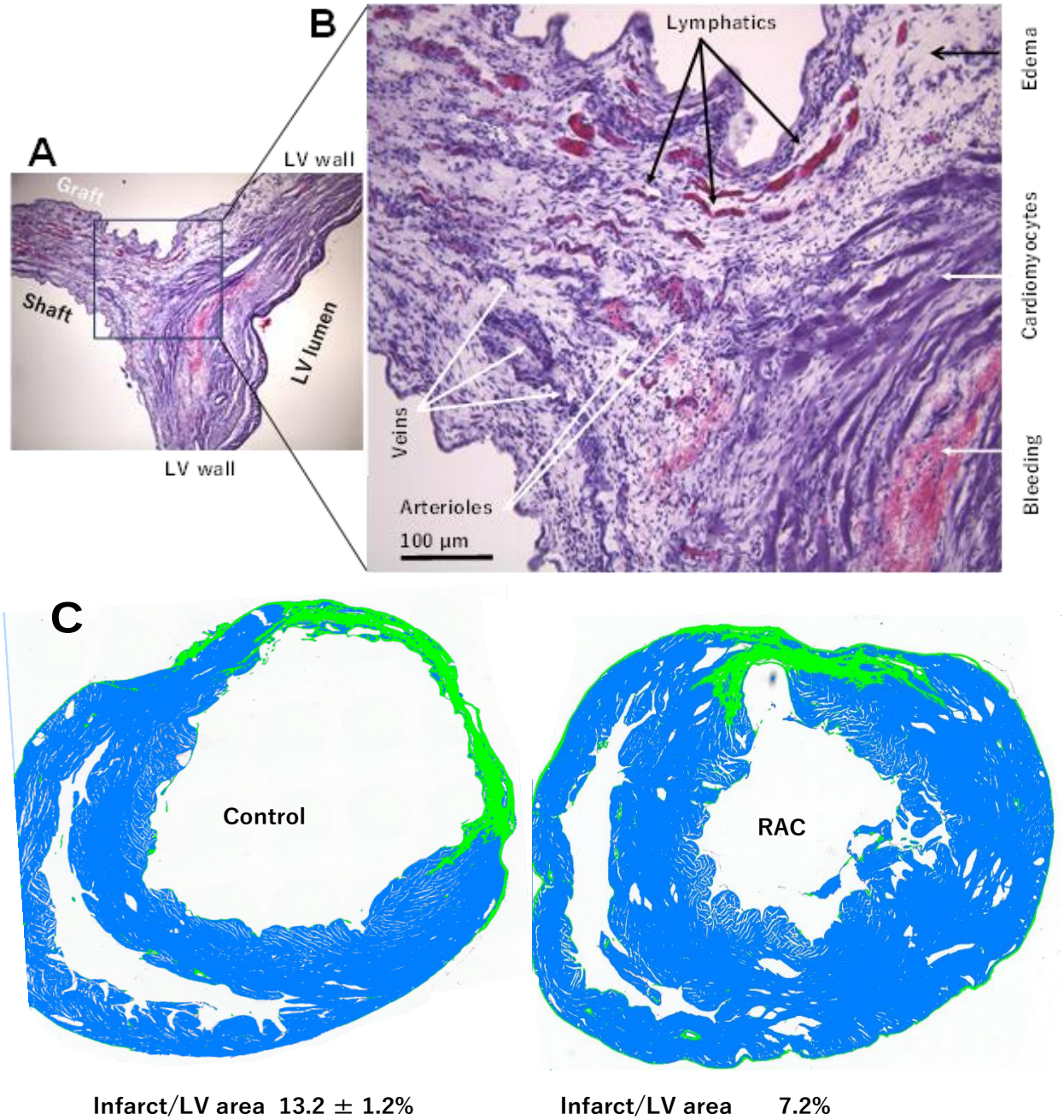

Fig. 4. Histological studies
The vascular structure between the sternum and LV wall was stained with Hematoxylin-Eosin (A), showing arterioles, veins, and lymphatics inserting into the LV wall with edema, bleeding, and cardiomyocytes (B). Trichrome staining (C) showed an increased amount of myocardium (blue) with less infarct (green) in this case (7.2%) than in untreated controls (left) with 13.3 ± 1.2% of infarct[25], with extended area of transmural fibrosis as LV aneurysm.

Discussion

Development of *de novo* biological revascularization as in the current case (Fig. 1) seldom occurred in thusly treated rats, which were usually associated with revascularization from surrounding tissues[25]. It seemed likely that cardiac swelling after MI caused direct contact of the ischemic LV with the sternum and thereby helped develop the bypass structure, blood flow and functional recovery. Nonetheless, clamping and releasing the bypass structure revealed no abrupt changes, suggesting that the bypass flow was insufficient or that regenerated vasculature and cardiomyocytes were no longer dependent on the central bypass 4w after MI. These observations provide hints for further modifications of RAC therapy and remind us of the history of myocardial revascularization; mobilization of the pericardium or arterial graft was to facilitate naturally occurring collaterals[1-5], and the current vascular procedures[6-10] are to supply a viable arterial inflow. Thus, as a surgical modification, it may be advantageous to

provide an arterial inflow by endoscopic closed-chest procedure[14] as an on-lay graft to the center of the infarct (Supplement 2), or a combined Vineberg procedure[3-5]. Such arterial graft in contact with the ischemic myocardium may provide potent arterial support to the target distal arteries and/or regenerated arterioles, which are otherwise inaccessible by the current vascular procedures[6-10]. Since complete revascularization is not always achievable[13], a combination of vascular approaches to provide proximal arterial inflow and mesenchymal progenitor cells[16-19], including RACs infusion[20-25], to regenerate distal vasculature and the myocardium may be attractive for revascularizing ischemic lesions, regenerating cardiomyocytes, and reinstating cardiac function.

Biologically, endothelial progenitor cells were isolated from peripheral blood[20], were greatly expanded more than 200 times by quality and quantity control culture *in vitro*[21-22], and were experimentally tested in skin defects[23], autoimmune myocarditis[24], and induced MI models[25]. Since RACs contain a sizable fraction of type 2 macrophages[29], they migrate to locations of inflammation as homing characteristics. At their destination, RACs have been shown to alleviate hypoxic responses, regulate phagocytosis and apoptosis, and promote vasculogenesis and cardiomyogenesis (Supplement 1)[25]. These characteristics allow remote intervention to reduce procedural risks and therefore offer wider indications than those for CABG or PCI. While these procedures are classified as mechanical/ vascular approaches to supplying proximal inflow, RAC therapy may promote and facilitate biological/cellular revascularization, mutually complementally in advantages as well as in risks, again supporting each other in combination to achieve complete coronary revascularization and myocardial regeneration, or cardiovascular regeneration.

Another biological modification may include immediate, without delay allografting of frozen and thawed allogeneic RACs[30-33], or autografting after *in vitro* expansion by quality and quantity control culture[21-25], such as in the current case, or both. Frozen allogeneic RACs may be stored for immediate availability, with preserved viability[30-31] and less immunogenicity[32-33], and they are therefore less likely to provoke allogeneic immunity or related problems. Early RAC therapy is considered to be more effective than late administration after ischemic events due to enhanced inflammation, recruiting more RACs to the lesions. This is contrary to the aforementioned risks associated with vascular approaches, as the immediacy[8] and reperfusion injury[11-12] are known to aggravate the inflammation[11-12] and associated comorbidities, thereby increasing the procedural risks[8-12]. Although autologous RAC *in vitro* culture/expansion may necessarily cause some delays, 3 to 5 days in rodents and 1 week in humans[21-25], they are considered to be effective and long-lasting without immunologic concerns[16-19,25]. Thus, coupled RAC therapy, i.e., immediate allografting and delayed autografting may theoretically be more effective and long-acting for treating acute myocardial ischemia either with or without combined vascular procedures. The application of RAC therapy in terms of timing to the ischemic events, source of cells[16-19], dosage, and combination with the mechanical/vascular procedures needs to be determined experimentally.

Conclusion

While infusion of RACs facilitates and enhances the neovascularization from surrounding tissues[25], the development of the central bypass structure in the current case encourages possible surgical as well as biological modifications. While the current vascular procedures may provide proximal arterial inflow, RAC therapy may promote distal vascular neogenesis and cardiomyogenesis. In addition to the modification of RAC therapy in terms of timing of the ischemic events, source of the cells as well as dosage, a combination with the mechanical/ vascular procedures has yet to be defined in preclinical studies.

Conflict of Interest: None

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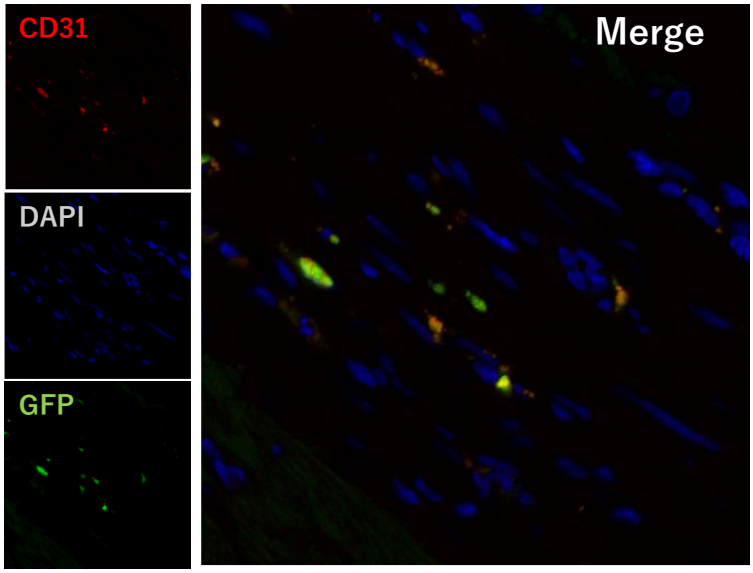
Supplementary Material



Supplement 1

Immunohistochemical studies

Immunohistochemical studies in the infarct of another recipient rat after MI revealed CD31 (red, endothelial cell lineage), DAPI (blue, nuclei) and Green Fluorescent Protein (GFP)-positive cells of RACs prepared from Lew-CAG-eGFP transgenic rats, suggesting their contribution to the biological revascularization and myocardial regeneration.

Supplement 2

Hybrid Revascularization

As a surgical extension, we are currently introducing an arterial inflow on-lay graft, which is placed in direct contact with the infarcted myocardium (dotted area) in line with the distal LAD, as a hybrid technique by the Vineberg procedure[3-5], that requires takedown of an arterial inflow without distal coronary anastomosis. Broken arrows indicate LAD ligation with a 7-0 monofilament suture and a loop guiding an arterial graft along with the distal LAD. The closed arrow indicates the direction to the head. A neurosurgical miniature clip (0.7 mm) is placed for comparison to the surrounding structures.