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Intracardiac injection of matrigel induces stem cell recruitment and improves cardiac functions in a rat myocardial infarction model

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Abstract

Matrigel promotes angiogenesis in the myocardium from ischemic injury and prevents remodelling of the left ventricle. We assessed the therapeutic efficacy of intracardiac matrigel injection and matrigel-mediated stem cell homing in a rat myocardial infarction (MI) model. Following MI, matrigel (250 μl) or phosphate-buffered solution (PBS) was delivered by intracardiac injection. Compared to the MI control group (MI-PBS), matrigel significantly improved left ventricular function ($n = 11$, $P < 0.05$) assessed by pressure–volume loops after 4 weeks. There is no significant difference in infarct size between MI-matrigel (MI-M; 21.48 ± 1.49%, $n = 10$) and MI-PBS hearts (20.98 ± 1.25%, $n = 10$). The infarct wall thickness of left ventricle is significantly higher ($P < 0.01$) in MI-M (0.72 ± 0.02 mm, $n = 10$) compared with MI-PBS (0.62 ± 0.02 mm, $n = 10$). MI-M hearts exhibited higher capillary density (border 130.8 ± 4.7 *versus* 115.4 ± 6.0, $P < 0.05$; vessels per high-power field [HPF; 400×], $n = 6$) than MI-PBS hearts. c-Kit$^+$ stem cells (38.3 ± 5.3 *versus* 25.7 ± 1.5 c-Kit$^+$ cells per HPF [630×], $n = 5$, $P < 0.05$) and CD34$^+$ cells (13.0 ± 1.51 *versus* 5.6 ± 0.68 CD34$^+$ cells per HPF [630×], $n = 5$, $P < 0.01$) were significantly more numerous in MI-M than in MI-PBS in the infarcted hearts ($n = 5$, $P < 0.05$). Intracardiac matrigel injection restores myocardial functions following MI, which may attribute to the improved recruitment of CD34$^+$ and c-Kit$^+$ stem cells.

Keywords: cardiac regeneration • ischemia • matrigel • stem and progenitor cells

Introduction

The extracellular matrix (ECM) which defines the composition, architecture, signalling and biomechanics of the cellular microenvironment plays a dominant role in regulating the cellular processes, proliferation and differentiation of stem cells, and in maintaining the plasticity of stem cells [1–3]. It is still a challenge that the biological complexity of the native ECM is fully mimicked in the tissue-engineered constructs to which cells can attach, differentiate and function as a living tissue [4].

Matrigel is one of the injectable matrix biopolymers. It is a basement membrane protein mixture secreted by a mouse sarcoma [5]. It contains ECM components such as laminin, collagen IV, entactin and hepran sulphate proteglycan [5], as well as growth factors including basic fibroblast growth factor

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(bFGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), nerve growth factor (NGF), transforming growth factor (TGF-β) and other growth factors which have not been fully identified chemically [6]. The biologically active heterogeneous compositions in matrigel could influence cell gene expression [7–12], regulate complex cellular behaviour including attachment, proliferation and differentiation [13–17], and promote angiogenesis both *in vitro* [18, 19] and *in vivo* [20–22].

Matrigel as engineered ECM is an emerging area of research in cardiac tissue engineering. It showed tremendous promise in the treatment of myocardial infarction (MI). Matrigel can provide the adequate natural ECMs serving as structural support for the cells; it also provides an appropriate environment with above mentioned growth factors for cell survival and function as well. When used in combination with cells such as embryonic stem cells, endothelial cells, cardiomyocytes and bone marrow-derived stem cells, matrigel may serve as cell delivery vehicles by providing a favourable matrix environment that confers cell viability, migration and proliferation [23–28]. This evidence fuelled significant interest in the potential use of matrigel as a cytoprotective agent and a very effective tool for construction of bioactive scaffolds for tissue-engineering applications in the cardiovascular system [24, 26] – intracardiac injection of matrigel can also induce neovascularization after MI [29]. Furthermore, injectable matrigel offers a potentially less invasive and more effective tissue-engineering approach for myocardial reconstruction [30], in comparison with external scaffold patches, which require surgically invasive procedures to be fixed onto the epicardial surface and have thickness restrictions due to the lack of a vascular bed [25]. The ability of injectable matrigel to deform with the dynamically loaded myocardial environment to align their matrix with the injured region may provide better incorporation of implanted cells to the host tissue [23].

In order to fully exploit the potential application of matrigel in the cardiovascular system, it is helpful to understand the mechanisms involved in the matrigel-mediated neovascularization. Therefore, we evaluated the therapeutic efficacy of intracardiac matrigel injection in the infarcted heart. We found that intracardiac injection of matrigel could confer effective cardiac protection and recruit stem cells to the infarcted heart and promote cardiac regeneration after MI.

Materials and methods

Experimental design

Surgical and animal care protocols were reviewed and approved by the local animal care committees of Mecklenburg/Vorpommern. Lewis rats (male, 250–280 g, Charles River Laboratories, Wilmington, MA, USA) were randomly assigned to three groups: Sham operation (Sham, $n = 6$), MI followed with matrigel injection (MI-matrigel [MI-M], $n = 11$) and phosphate-buffered solution (PBS) injected MI control group (MI-PBS $n = 11$). The pressure–volume loop analysis was performed at 4 weeks after MI, and then hearts were harvested for histopathological analysis.

MI and local matrigel injection

Rats were anesthetized by intraperitoneal injection with ketamine/xylazine(80/10 mg/kg of body weight; Sigma-Aldrich, St. Louis, MO, USA; intraperitoneal administration), endotracheally incubated and mechanically ventilated. The heart was exposed *via* a left thoracotomy. Anterior MI was created by permanent ligation of the left anterior descending (LAD) artery. Successful infarction was determined by observing a pale discoloration of the left ventricular muscle and an ST-segment elevation on electrocardiograms. Immediately after LAD ligation, matrigel or PBS were injected in a total volume of 250 μl at five injection sites into anterior and lateral aspects of the viable myocardium bordering of the infarction with a 31-gauge needle (BD Biosciences, San Jose, CA, USA). Sham operated rats underwent identical surgical procedures without permanent LAD ligation.

Left ventricular catheterization

Four weeks after surgery, rats (Sham $n = 6$, MI-PBS $n = 11$, MI-M $n = 11$) underwent pressure–volume loops measurements according to the protocol of Cardiodynamics BV (CD Leycom, Zoetermeer, Netherlands). Data were collected with the Millar PV System (Ultra-Miniature Pressure–Volume Catheter (model SPR-838), Millar Pressure Conductance Unit (model MPCU-200) and Millar PowerLab data-acquisition hardware; emka Technologies, Paris, France). Calibration of pressure and volume was performed by equating the minimal and maximal conductances with minimal (0 mmHg) and maximal (100 mmHg) pressures as well as minimal and maximal blood volumes received from venous circulation. After inserting the catheter into carotid artery retrograde access to the left ventricle was achieved. The volume calibration of the conductance system was performed similarly as described in previous reports [31–35]. Briefly, fresh heparinized whole blood of rat was filled into two cylindrical holes with known volume of 95 μl and 300 μl in a block. The volume calibration formula was obtained based on the known absolute cylinder volumes *versus* the raw signals acquired by the conductance catheter conductance. At the end of each experiment, hypertonic saline were injected intravenously, and parallel conductance volume (Vp) was calculated based on the shift of P–V relations parallel conductance volume (Vp) and used for correction for the cardiac mass volume as described previously [34, 35]. Pressure–volume loops of the left ventricle were recorded under normal conditions (baseline). Data were analysed with IOX Version 1.8.3.20 software (emka Technologies).

Infarction size

Four weeks after MI, animals were killed. The hearts were removed, washed with PBS and snap frozen in liquid nitrogen. Frozen sections embedded in optimum cutting temperature medium. From every heart, the tissue sections (8 μm thick) of four levels (15 mm thick) were stained with Sirius red/Fast Green. Sirius Red positive areas (infarction zone) and the infarct wall thickness of the left ventricle (LWT) were analysed using the computerized planimetry (Axio Vision LE Rel. 4.5 software; Zeiss, Jena, Germany).

Table 1 Haemodynamics of the left ventricle 4 weeks after MI

Parameter	Sham ($n = 6$)	MI-PBS ($n = 11$)	MI-M ($n = 11$)	P*
Pmax (mmHg)	142.67 ± 2.60	115.45 ± 3.81	123.63 ± 3.05	0.189
dp/dt max (mmHg)	10,495.83 ± 311.89	4150.45 ± 250.10	5206.81 ± 177.34	0.006
−dp/dt max (mmHg)	9811.83 ± 323.28	3125.72 ± 212.63	3891.36 ± 132.79	0.023
EDV (μl)	211.17 ± 8.51	295.36 ± 13.17	294.00 ± 16.87	0.962
ESV (μl)	70.50 ± 4.92	186.45 ± 12.17	171.72 ± 11.88	0.614
SV (μl)	150.33 ± 4.76	108.72 ± 5.93	124.00 ± 6.27	0.159
EF (%)	66.33 ± 2.29	34.81 ± 2.03	42.72 ± 1.45	0.008
HR	399.50 ± 5.04	348.18 ± 6.66	366.00 ± 10.95	0.298

Values are represented as mean ± S.E.M.
*MI-PBS *versus* MI-M; Pmax: maximum pressure; dp/dt indicates peak rate of maximum pressure rise (dp/dt max) and decline (–dp/dt max); EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume; EF: ejection fraction; HR: heart rate.

Immunohistochemistry

For immunohistological detection of c-Kit$^+$ and CD34$^+$ stem cells, frozen transverse tissue sections (8 μm) of hearts from MI-PBS and MI-M ($n = 5$ for each time-point and group) were incubated with rabbit anti-c-Kit or goat anti-CD34 polyclonal antibodies. Subsequently, the sections were incubated with donkey anti-rabbit Alexa-Fluor 488 conjugated and donkey anti-goat Alexa-Fluor 488 conjugated secondary antibodies (Invitrogen, Eugene, OR, USA). Nuclei were counterstained with TOPRO3 (Invitrogen). Labelled sections were observed using a Leica SP2 Confocal Microscope (Leica, Hamburg, Germany). The number of c-Kit$^+$ and CD34$^+$ cells were counted in 10 randomly chosen high-power fields (HPFs, 630×) of infarct size. Results were expressed as cells per HPF. All morphometric studies were performed by two examiners who were blinded to the treatment.

Determination of capillary density

The capillary density was assessed at 4 weeks after surgery by counting the number of capillaries of the heart sections immunostained with polyclonal goat anti-CD31 primary antibody followed by donkey anti-goat Alexa-Fluor 568 conjugated secondary antibody. Sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Five sections within the border zone of each animal (Sham $n = 6$, MI-PBS $n = 6$, MI-M $n = 6$) were analysed. Capillaries were counted in 10 border zone randomly chosen fields (400×). Results were expressed as capillaries per HPF.

Statistical analysis

Statistical analysis was performed with ANOVA. Results were expressed as average ± S.E.M. A P-value <0.05 was considered to be statistically significant.

Results

Local matrigel delivery improves cardiac functions

We then evaluated the ventricular function by pressure–volume loop method. Matrigel treatment enhanced systolic and diastolic properties of the infracted left ventricle. Haemodynamic changes are summarized in Table 1. Matrigel treatment produced a 22.7% increase in left ventricular ejection fraction (left ventricular-EF, $P = 0.008$, Fig. 1) relative to MI-PBS (Table 1). Left ventricular peak rate of pressure rise (left ventricular dp/dt max, Fig. 1), a commonly used index of myocardial contractility was significantly enhanced ($P = 0.006$) when compared with MI-PBS. Moreover, we also observed a 24.5% increased in the peak rate of left ventricular pressure decline (left ventricular dp/dt min) compared with MI-PBS (Fig. 1), demonstrating enhanced relaxation in MI-M. Taken together, these results demonstrate that direct administration of matrigel enhanced left ventricular-EF recovery and improved contraction kinetics of left ventricle.

Local matrigel delivery did not reduce infarction size but attenuated the decrease of infarct wall thickness

LAD ligation consistently resulted in transmural MI, exhibiting typical histological changes including thinning of the left ventricular free wall and extensive collagen deposition 4 weeks after MI. The effect of matrigel treatment on myocardial injury 4 weeks after infarction was evaluated by Sirius red/Fast Green staining (Fig. 2A and B). Mean infarct size in the PBS control animals was 21.48 ± 1.49% of the whole heart ($n = 10$, Fig. 2C). Compared with the PBS group, injection of matrigel has no significant reduction the infarct size (20.98 ± 1.25%, $n = 10$, Fig. 2C). However the infarct

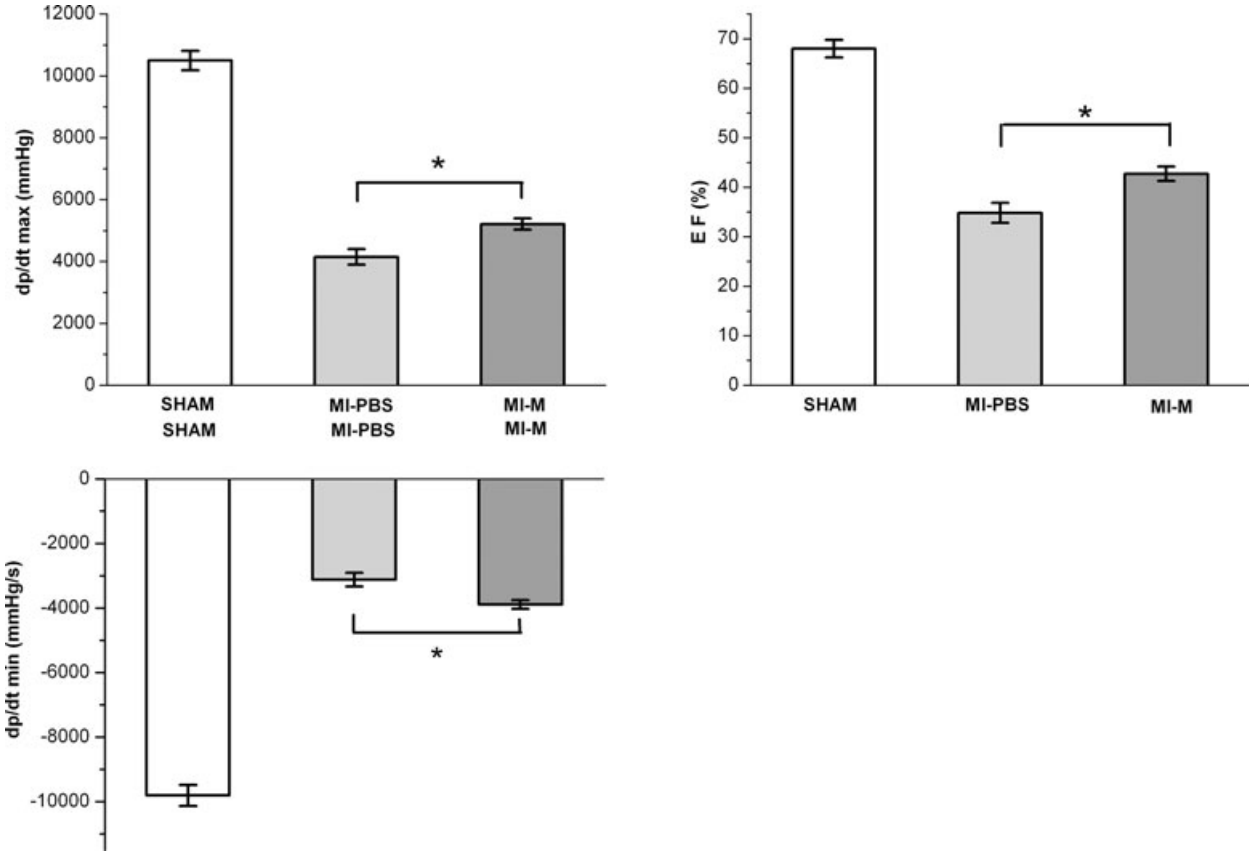

Fig. 1 Local matrigel injection improved cardiac functions 4 weeks after MI assessed by catherization. Left ventricular function (EF, dp/dt max and dp/dt min, $n = 11$) is significantly improved in MI-M compared with MI-PBS. $^*P < 0.05$.

LWT is significantly higher ($P < 0.01$) in MI-M (0.72 $\pm$ 0.02 mm, $n = 10$) compared with MI-PBS (0.62 $\pm$ 0.02 mm, $n = 10$, Fig. 2D).

Local matrigel injection promotes neoangiogenesis

Capillary density was analysed by immune staining with CD31 antibody (Fig. 3A–C) 4 weeks after MI. Compared with PBS-treated group, the capillary density was significantly higher in infarct border zones for rats that received matrigel (MI-PBS *versus* MI-M, 115.4 $\pm$ 6.0 *versus* 130.88 $\pm$ 4.7 vessels per HPF, $n = 6$, $P < 0.05$) (Fig. 3D).

Local matrigel injection augments myocardial stem cell recruitment

To examine whether local matrigel delivery can home c-Kit$^+$ and CD34$^+$ cells to infracted myocardial zone, c-Kit$^+$ and CD34$^+$ cell number in the infarct zone of heart were assessed by immunostaining at 4 weeks after MI (Figs 4 and 5). There was a significant

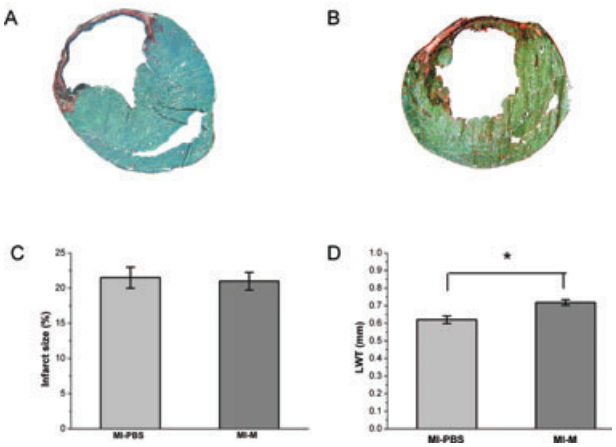

Fig. 2 Effects of local matrigel injection on cardiac remodelling 4 weeks after MI. (**A**), (**B**) Representative heart cross sections stained with Sirius Red (red, fibrosis) and Fast Green FCF (green, myocyte) from rats. (**C**) Ratio of infarction size to entire heart is not significantly decreased in MI-M ($n = 10$) compared with MI-PBS ($n = 10$). (**D**) The infarct LWT is significantly higher in MI-M ($n = 10$) compared with MI-PBS ($n = 10$). $^*P < 0.01$.

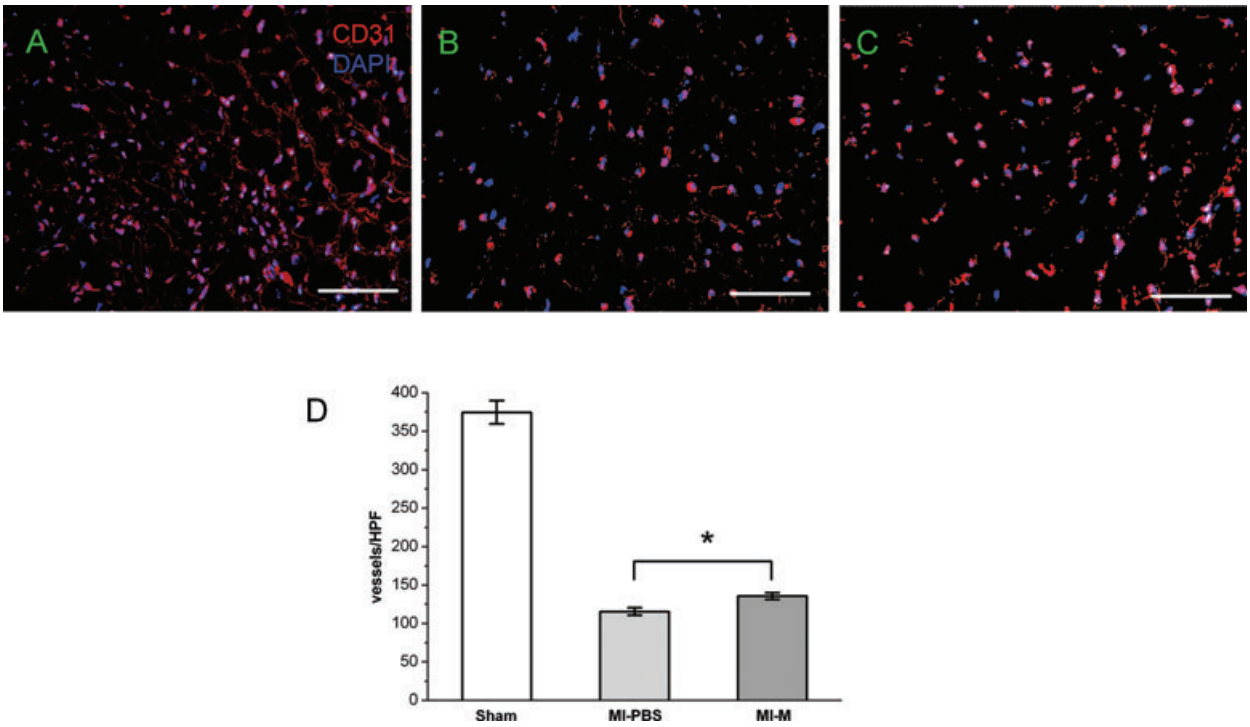

Fig. 3 Local matrigel injection induces neovascularization 4 weeks after MI. Endothelial CD31 were stained in the border of the infarct of hearts. Representative micrographs of the border of the infarct in the three groups of animals [(**A**) Sham, (**B**) MI-PBS, (**C**) MI-M, Blue, DAPI in nuclei, 400×]. (**D**) Morphometric analysis of vessel density in the border of the infarct in the various groups. Data are mean values ± S.E.M. *$P < 0.05$ MI-M *versus* MI-PBS (sham, $n = 6$, MI-PBS, $n = 6$ and MI-M, $n = 6$). HPF: high-power field. Scale bars = 250 μm.

increase in the number of c-Kit$^+$ cells in the MI-M group compared to the MI-PBS group (38.3 ± 5.3 *versus* 25.7 ± 1.5 c-Kit$^+$ cells per HPF [630×], $n = 5$, $P < 0.05$, Fig. 4D). Similarly, the number of CD34$^+$ was also significantly increased compared with MI-PBS (13.0 ± 1.51 *versus* 5.6 ± 0.67 CD34$^+$ cells per HPF [630×], $n = 5$, $P < 0.01$, Fig. 5D). The c-Kit$^+$ cells can seldom be seen in sham-operated rat. These observations therefore revealed that matrigel may enhance stem cells recruitment after MI.

Discussion

The current study presents the first time that intracardiac injection of matrigel after MI significantly improved cardiac function and increased the local numbers of c-Kit$^+$ and CD34$^+$ stem cells. Meanwhile, we also found that local administration of matrigel prompted neovascularization in infract area, which is consistent with previous study [29].

The underlying mechanism by which intracardiac injection of matrigel improves cardiac function after MI has not been clearly identified, but there are several possible factors to mediate this process: (1) Matrigel contains ECM components such as laminin, collagen IV, entactin, hepran sulphate proteglycan [5], which may prevent negative remodelling of the myocardium by providing three-dimensional support to the infarcted area. (2) Matrigel contains various growth factors like bFGF, EGF, IGF-1, PDGF, TGF-β, which may provide additional nutrients to the ischemic myocardium, promote the proliferation and transmigration of local endothelial cells and mediate the process for angiogenesis. (3) Additionally matrigel provides a suitable natural micro-environment for homing the stem cells. The recruited c-Kit$^+$ stem cells in infarcted area may undergo myogenic differentiation forming synchronously beating cardiomyocytes in the presence of VEGF and bFGF [36] from matrigel, mediate anti-inflammatory process and provide protective effects to the cardiomyocytes directly or indirectly *via* release of paracrine factors in the infarcted heart which limited myocardial damage and scar tissue formation. Hence matrigel could be one of ideal ECMs which are able to provide biochemical signals that influence the migration, proliferation, differentiation and functions of endothelial cells and/or progenitor cells for regenerating the infarcted heart.

The current study provides the first evidence that intracardiac matrigel injection may drive stem cell migration to the injured heart, where the recruited stem cells might support myocardial regeneration. The matrigel-mediated directional migration of stem cells to the infarcted myocardium was particularly important.

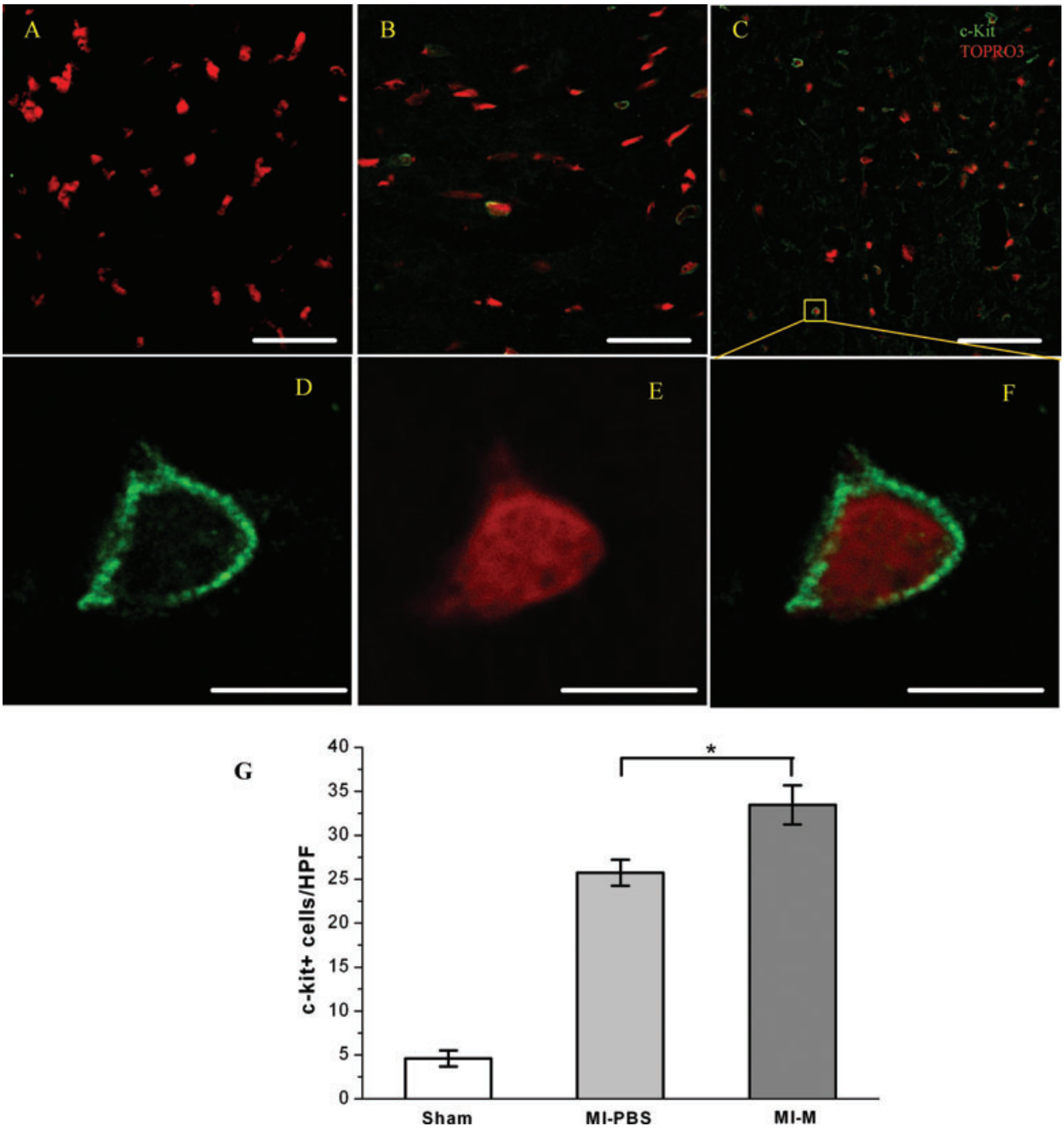

Fig. 4 Local matrigel injection increases the myocardial homing of c-Kit$^+$ cells. Representative immunostaining for c-Kit in the three groups of animals. c-Kit$^+$ cells (green) were identified in infarct border zone at 4 weeks after treatment. Red, TOPRO3 in nuclei. Yellow is shown after green [(**A**), sham, (**B**), MI-PBS, (**C**–**F**), MI-M]. (**D**) The number of c-Kit$^+$ cells per HPF in MI-M hearts ($n = 5$) was significantly higher than in MI hearts ($n = 5$), and c-Kit$^+$ cells can seldom be seen in sham animals ($n = 5$). $^*P < 0.01$. (**A**–**C**) scale bars = 250 μm; (**D**–**F**) scale bars = 25 μm.

Stem cell microenvironment which is defined by various growth factors and ECM is a determined factor for stem cell local activity, such as adhesion, differentiation and proliferation [34, 37]. The functional molecules in matrigel and their interaction with stem cells could be crucial to regulate the survival, self renewal and maintaining of stemness, and control the differentiation of stem cells. In this study, we demonstrated that matrigel can increase myocardial homing of c-Kit$^+$ and CD34$^+$ cells. Kelly *et al.* [38]

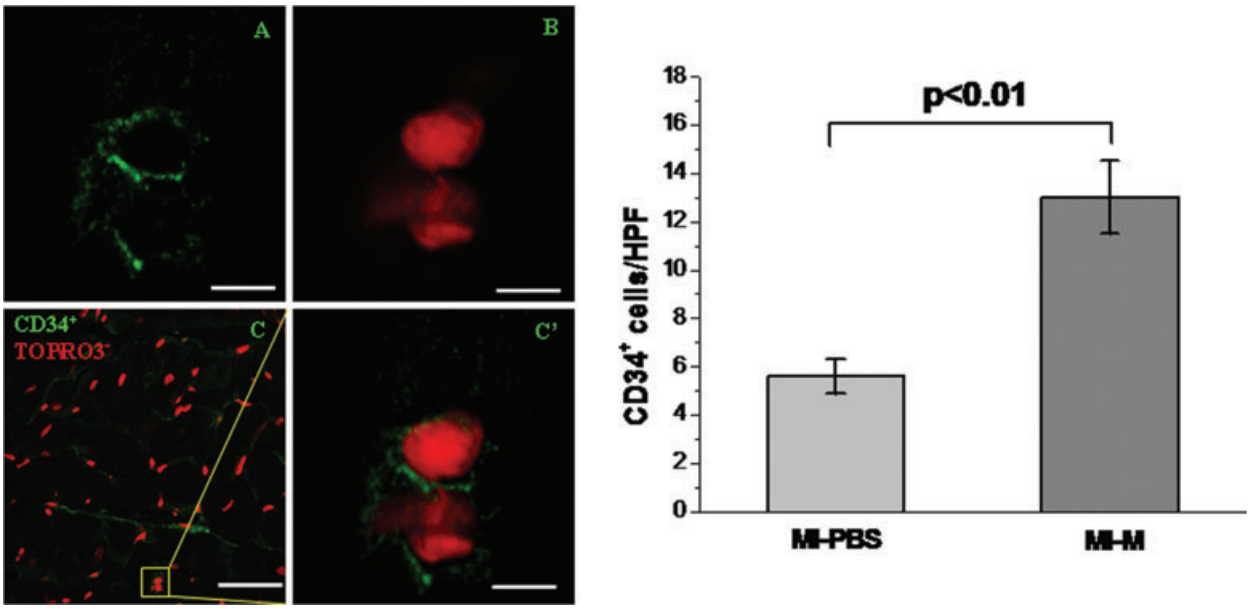

Fig. 5 Local matrigel injection increases the myocardial homing of $CD34^+$ cells. (**A**)–(**C**) and (**C**)' Representative images for $CD34^+$ (green) cells (square) in at 4 weeks after local matrigel injection. Red, TOPRO3 in nuclei. Yellow is shown after green. (**D**) The number of $CD34^+$ cells per HPF in MI-M ($n = 5$) was significantly higher than in MI-PBS ($n = 5$) hearts. $^*P < 0.01$. (**A**), (**B**) and (**C**'): scale bars = 25 μm; (**C**) scale bars = 250 μm.

also found that matrigel can promote angiogenesis *via* recruitment of precursors from the surrounding tissues. C-kit is a receptor tyrosine kinase [39], and expressed on cardiac progenitor cells [40, 41], endothelial progenitor cells [42] and bone marrow derived cells, including haematopoietic stem/progenitor cells and mesenchymal stem cells [43]. Furthermore, it has been shown that c-Kit-enriched haematopoietic stem cells have the potential to alleviate post-ischemic injury in the myocardium [44]. Fazel *et al.* [45] show coronary ligation leads to a robust increase in the number of peripheral blood $VEGFR2^+$ $c\text{-}Kit^+$ $Sca\text{-}1^+$ cells. Others utilized $c\text{-}Kit^+$ cells for myocardial regeneration and inhibition of damage, and demonstrated the multifaceted potential of this cell population for facilitating reparative processes in the myocardium [46, 47]. The SDF-1 and chemokine (C-X-C motif) receptor (CXCR)4 ligand/receptor pair play a pivotal role in mediating the mobilization of $CXCR4^+$ hematopoietic stem cells (also positive for CD34 or c-Kit) into peripheral blood and guiding the migration of stem cells follow the stromal cell-derived factor (SDF)-1 gradient [48–51] for angiogenesis and cardiac functional restoration [52–54]. The mobilization of $c\text{-}Kit^+$ and $CD34^+$ stem/progenitor cells into the peripheral blood and the significant recruitment of $c\text{-}Kit^+$ and $CD34^+$ stem cells in the infarcted heart are associated with the short time temporally spatially distributed up-regulation of SDF-1 up-regulation [35]. Consequently the accumulation of $c\text{-}Kit^+$ and $CD34^+$ cells in the infarcted heart could be directly related with early homing of the stem cells. Matrigel could reinforce the recruited stem cells by supplying various growth factors to against the apoptosis which could be induced by the arduous microenvironment incurred from ischemia, inflammatory response and pro-apoptotic factors [34]. In addition, the matrigel may mediate the biochemical signals such as SDF-1 and CXCR4 that directly influence the migration of stem cells; or matrigel treatment may induce longer time inflammatory which could indirectly recruit more stem cells to the infarcted heart. All these possibilities for the accumulation of $c\text{-}Kit^+$ and $CD34^+$ cells in the infarcted heart need more investigations.

Despite these encouraging findings, the exact effects of matrigel on the endogenous cardiac stem cells are still not clear. Matrigel has been shown to regulate the proliferation and migration of human circulating endothelial progenitor cells in non-obese diabetic/severe combined immunodeficiency mice [55], and control the differentiation of muscle satellite cells into myocytes lineage [56]. Hence, it can be speculated that matrigel delivered by intracardiac injection may also mediate cardiac stem cell proliferation and differentiation to regenerate the infarcted myocardium. Further studies need to be conducted in order to assess this hypothesis.

Zimmermann *et al.* [27] reported that as one of mechanisms implanted stem cells or resident stem cells could prevent the engineered heart tissue (EHT) induced immunosuppression. It is interesting to note that matrigel could recruit $c\text{-}Kit^+$ and $CD34^+$ cells as demonstrated in our experimental results. Hence matrigel contained in EHT could also mediate stem cell recruitment to the EHT, consequently contributing the prevention of immunosuppression induced by EHT.

In this study, we demonstrated that local matrigel injection improved the left ventricular function. However, the infarct size was

not significant decreased. Huang *et al.* [29] found that the intracardiac injection of matrigel promoted neovascularization in infarct area, but the effect of matrigel injection on the infarct size was not reported. Although martrigel treatment did not reduce the infarct size, the matrigel treatment could attenuate the decrease of the infarct wall thickness which may improve cardiac function. It should be noted that intracardiac injection of fibrin glue also could prevent the infarct wall thinning and attenuated the decrease in ejection fraction after MI [57], which is consistent with the results from the matrigel treatment in our study. Further study is needed to investigate whether the matrigel or fibrin glue functions as the internal space fillers to support the cardiac functional improvement.

In summary, intracardiac matrigel administration improved the cardiac function by mediating c-Kit$^+$ stem cell recruitment to the ischemic myocardium, and demonstrated the beneficial effects of matrigel on cardiac repair. The beneficial effects of matrigel might be closely associated with the targeted migration of stem/progenitor cells. Further studies on the molecular mechanisms associated with matrigel-mediated cardiac protection will reveal whether these encouraging animal data can be translated into clinical applications.

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Conflict of interest

The authors confirm that there are no conflicts of interest associated with this publication.

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