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Transplantation of platelet gel spiked with cardiosphere-derived cells boosts structural and functional benefits relative to gel transplantation alone in rats with myocardial infarction

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Abstract

The emerging field of stem cell therapy and biomaterials has begun to provide promising strategies for the treatment of ischemic cardiomyopathy. Platelet gel and cardiosphere-derived cells (CDCs) are known to be beneficial when transplanted separately post-myocardial infarction (MI). We hypothesize that pre-seeding platelet gel with CDCs can enhance therapeutic efficacy. Platelet gel and CDCs were derived from venous blood and heart biopsies of syngeneic rats, respectively. In vitro, the viability, growth, and morphology of CDCs cultured in platelet gel were characterized. When delivered into infarcted rat hearts, platelet gel pre-seeded with CDCs was more efficiently populated with endogenous cardiomyocytes and endothelial cells than platelet gel alone. Recruitment of endogenous c-kit positive cells was enhanced in the hearts treated with gel with CDC. At 3 weeks, the hearts treated with CDC-seeded platelet gel exhibited the greatest attenuation of adverse left ventricular (LV) remodeling and the highest cardiac function (i.e., LV ejection fraction) as compared to hearts transplanted with gel only or vehicle controls. Histological analysis revealed that, though some transplanted CDCs differentiated into cardiomyocytes and endothelial cells in the recipients' hearts, most of the incremental benefit arose from CDC-mediated endogenous repair. Pre-seeding platelet gel with CDCs enhanced the functional benefit of biomaterial therapy for treating myocardial infarction.

Keywords

cardiac stem cells; platelet gel; myocardial infarction; cardiac regeneration

1. Introduction

Myocardial infarction (MI) and heart failure generate substantial socioeconomic costs and represent the main cause of death in western countries. Despite improvement of pharmacological and invasive treatment regimens, mortality and morbidity remain high. Those who survive MIs but are not promptly reperfused develop large scars and severe

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impairment of left ventricular (LV) function. Cardiac tissue engineering and biomaterials approaches rely mainly on the use of synthetic or biological matrix materials to reconstitute contractile cardiac muscle-like tissue and support the failing heart. Among these, injectable biomaterial gels are particularly appealing as they are amenable to minimally-invasive delivery [1]. Platelet gel (also known as platelet fibrin scaffold [2]) is an appealing choice for therapeutic development as it can be easily manufactured, whether as an allogeneic or autologous product. We have previously shown that injection of platelet gel alone attenuates adverse LV remodeling and preserves cardiac function in rats with acute MI [3]. While the results are promising, we wondered whether the therapeutic benefits might be enhanced by adding a cell product to the biomaterials. Previous studies suggested enhanced regenerative potential from biomaterials pre-seeded with stem cells [4]. However, it is still unclear if the benefits are from simple “add-on” effects from the stem cell components or from more complex interactions among the cells, biomaterials and host environment. Over the last six years, our laboratory has developed, from initial laboratory animal studies through the ongoing CADUCEUS trial (see clinicaltrials.gov), the notion that cardiosphere-derived cells (CDCs) may benefit patients with ischemic cardiomyopathy [5–10]. CDCs are a heart-derived cell population rich in cardiac progenitor cells and supporting cell types. In the present study, we compared the therapeutic benefits of intramyocardial injection of platelet gel alone and platelet gel spiked with CDCs in a rat model of acute MI. We also explored the mechanisms underlying the incremental benefit elicited by the cells.

2. Materials and Methods

2.1 Derivation of platelet gel

Platelet gel was derived from the vein blood of Wistar-Kyoto (WKY) or Sprague Dawley (SD) rats according to previously reported methods [11]. Briefly, deep anesthesia was introduced by inhalation of isoflurane. After that, the rat's abdominal skin was dissected and venous blood was drawn from the vena cava. The blood was immediately citrated with 10% (v/v) 10 mM sodium citrate (Sigma-Aldrich, St Louise, MO). Whole blood samples were then centrifuged at 1000 g for 10 min and the supernatant collected. The platelet-containing plasma was then collected and mixed with pre-warmed DMEM at a ratio of 1:1 (v/v) for gel formation. To visualize the fibrous structure and presence of platelets, the formed platelet gel was frozen, cryo-sectioned, and subjected to standard H&E staining. To enable histological detection of injected platelet gel in vivo, we labeled the fibrin components by incubation with Texas Red-X succinidyl ester (1mg/ml; Invitrogen) for 30 min at 37°C immediately before gelation.

2.2 Scanning electron microscopy

SEM presents a valuable technique for the visualization of the morphological details of the platelet gel. The formed platelet gel was immediately washed in PBS and fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h and then rinsed with cacodylate buffer, three times (15 min each). The samples were then dehydrated in 35%, 50%, 70%, 80%, 95%, and 100% ethanol successively for 10 min each and dried in hexamethyldisilazane (Sigma-Aldrich). Scaffolds were sputter-coated with gold and images were captured with a LEO 982 scanning electron microscope (LEO Elektronenmikroskopie GmbH Korporation Germany).

2.3 Derivation of rat CDCs and cell culture in platelet gel

CDCs were derived from heart biopsies of WKY or SD rats as reported [7, 12]. After two passages, CDCs were suspended in pre-warmed DMEM and mixed with platelet-containing plasma at a ratio of 1:1 (v/v). In this way, CDCs were embedded in the scaffold during gel formation. The culture was supplemented with Iscove's Modified Dulbecco's Medium

(IMDM; Invitrogen) containing 10% FBS. Cell viability, proliferation and morphology in the gel were characterized and compared to the control (i.e. cells cultured on standard tissue culture plate [TCP]). For viability assay, rat CDCs were cultured in platelet gel and on TCP for 14 days and then stained with a LIVE/DEAD® Viability/Cytotoxicity Kit (Invitrogen), which quickly discriminates live from dead cells by simultaneously staining with green-fluorescent calcein-AM (live) and red-fluorescent ethidium homodimer-1 (EthD; dead). Cell morphology (e.g. cell body elongation) was characterized from the same images. To examine cell proliferation, the same LIVE/DEAD staining were performed at 12 hour, Day 3, Day 7 and Day 14 on representative cell cultures and live cell number in 3 randomized selected microscopic field were counted. The cell numbers were normalized to the numbers at 12 hours to generate the cell growth curve. To evaluate platelet gel degradation under physiological conditions, we measured the thickness of the gel/cell composite over the time by determining the “Z-distance” of cell distribution with a confocal microscope (Leica TCS SP5 X).

2.4 In vitro cytokine release

150 uL platelet gel (with or without 1 million rat CDCs) was cast into one well of a 24-well plate and incubated with 1 mL FBS-free media. To study sustained release of cytokines and growth factors, the conditioned media was collected at various time points (day 2, 5, 9, and 14) and fresh media was added back into the well to be conditioned for the next time point. The concentrations of VEGF, IGF-1, and SDF-1 in the conditioned media were measured by ELISA kits (R&D Systems, Minneapolis, MN; B-Bridge International, Cupertino, CA), according to the manufacturer's instructions.

2.5 Co-culture of CDCs with NRCM in platelet gel

To examine the impact of CDCs on cardiomyocytes cultured in platelet gel, we co-cultured neonatal rat cardiomyocytes (NRCMs) and CDCs from SD rats in the platelet gel derived from the same strain of animals. The NRCM harvesting and culturing methods were described previously in detail [13]. The culture was incubated at 37 °C and 5% CO₂. To distinguish between the two cell types, CDCs were stained with green-fluorescent DiO and NRCMs were stained with red-fluorescent CM-DiI (Invitrogen). Alternatively, CDCs were stained with red-fluorescent CM-DiI and the culture was fixed and NRCMs were stained with alpha sarcomeric actin (detected by FITC-conjugated secondary antibody).

2.6 Animal model

Animal care was in accordance with Cedars-Sinai Medical Center Institutional Animal Care and Use Committee (IACUC) guidelines. Platelet gel and CDCs were derived from WKY rats and then intramyocardially injected into the infarcted hearts of syngeneic animals. Female WKY rats (n = 75 total) underwent left thoracotomy under general anesthesia, and myocardial infarction (MI) was produced by permanent ligation of the left anterior descending coronary artery. The animals were subjected to intramyocardial injections with a 29-gauge needle at 4 points in the infarct zone, with one of the two randomly-assigned conditions: 1) Control group: injection of 150 uL of vehicle (DMEM); 2) Gel only group: injection of 150 uL of plain platelet gel (75 uL of host plasma were mixed with 75 uL of pre-warmed DMEM); 3) Gel + CDC group: injection of 150 uL of platelet gel containing 1 million CDCs (75 uL of host plasma were mixed with 75 uL of pre-warmed DMEM containing 1 million CDCs). Because gel formation happened quickly, the mixture was immediately drawn into a syringe and injected into the myocardium. Groups 1) and 2) contain animals reproduced from a previously reported study [3] as well as additional new animals.

2.7 Morphometric analysis

For heart morphometric analysis, animals were euthanized at 3 weeks and the hearts were harvested and frozen in OCT compound. Sections every 100 μm (10 μm thickness) were prepared. Masson's trichrome staining (6 sections per heart, collected at 400 μm intervals) was performed as described [7]. Images were acquired with a PathScan Enabler IV slide scanner (Advanced Imaging Concepts, Princeton, NJ). From the Masson's trichrome-stained images, morphometric parameters including LV cavity area, infarct wall thickness and infarct perimeter were measured in each section with NIH ImageJ software. Six measurements were averaged for each heart.

2.8 Histology

Heart cryosections were fixed with 4% PFA, permeabilized/blocked with Protein Block Solution (DAKO, Carpinteria, CA) containing 1% saponin (Sigma, St. Louis, MO), and then stained with the following antibodies: rabbit anti-von Willebrand factor (Abcam), rabbit anti-ckit (Abcam), mouse anti-alpha sarcomeric actin (Sigma) or FITC-conjugated isolectin B4 (Vector Labs). FITC or Texas-Red secondary antibodies were obtained from Abcam. Images were taken by a Leica TCS SP5 X confocal microscopy system.

2.9 Cardiac function assessment

Cardiac function was assessed by echocardiography [7] using Vevo 770TM Imaging System (VISUALSONIC^{STM}, Toronto, Canada). After the induction of light general anesthesia, the hearts were imaged two-dimensionally in long-axis views at the level of the greatest LV diameter. LV end diastolic volume, LV end systolic volume, and LV ejection fraction (LVEF) were measured/calculated with VisualSonics V1.3.8 software from 2D long-axis views taken through the infarcted area. Blinded reading of echos was conducted by an experienced sonographer (D.S.).

2.10 Statistical analysis

Results are presented as mean $\pm$ SD unless specified otherwise. Statistical significance between baseline and 3 week LVEFs was determined using 2-tailed paired Student's t test. All the other comparisons between any 2 groups were performed using 2-tailed unpaired Student's t test. Comparisons among more than 2 groups were performed using one way ANOVA followed by post-hoc Bonferroni test. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1 Characterization of platelet gel

Citration of blood immediately following exsanguination reduces free calcium, thus preventing immediate clotting while preserving coagulability. The platelet gel is easily-derived and available within ~15 min of venous sampling. Mixing host plasma with cell culture media (e.g. DMEM) containing a high concentration of free calcium results in gelation starting in <30 sec and complete within 120 sec. H&E staining revealed a fibrous structure (Fig. 1A) containing platelets (Fig. 1A; arrows). SEM images confirmed its fibrous and porous structures (Fig. 1B). The resulting platelet gel was mechanically strong enough to be held by a pair of forceps (Fig. 1C). The rheological properties of the gel have been characterized before [3].

3.2 Release of beneficial factors

To compare the production of cytokines and growth factors from platelet gel with and without cardiosphere-derived cells (CDCs), we measured the concentrations of VEGF,

IGF-1 and SDF-1 in conditioned media from both groups. Consistent with our previous findings [3], ELISA revealed robust and sustained release of those factors from platelet gel alone (without cells) for at least 14 days (Figs. 1D–F; blue bars). Adding CDCs to the platelet gel vigorously increased the production of those factors (Figs. 1D–F; red bars). These factors, among many secreted by CDCs [14], are essential for cardiac repair [15].

3.3 Characterization of CDCs in platelet gel

After 14 days of culture in the platelet gel, CDCs exhibited distinct morphology as compared to the control cells cultured on tissue culture plates (TCP). Elongated cell bodies and a “blood-vessel-like” network structure were seen in CDCs cultured in the platelet gel (Fig. 2B) as compared to cells on TCP (Fig. 2A). These unique morphological features resembled those described in 3-D cell cultures [16, 17]. Live/Dead assay revealed superior viability (fewer EthD-positive dead cells) from the CDCs cultured in platelet gel (Fig. 2F). The proliferation rates of CDCs cultured in the platelet gel was comparable to that from the control cells (Fig. 2C). To evaluate the in vitro degradation of the platelet gel, we measured the thickness of the gel over time. We found that nearly two thirds of the gel degraded after 14 days of culture at 37°C and 5% CO₂ (Fig. 2D). The differentiation capacity of CDCs cultured in platelet gel was confirmed with the expression of cardiovascular-specific makers (alpha sarcomeric actin for cardiomyocytes; von Willebrand factor for endothelial cells; alpha-smooth muscle actin for smooth muscle cells) (Figs. 2G–I).

3.4 Cardiomyocyte morphology and function in platelet gel

The success of biomaterial-based therapy for cardiac regeneration relies on the integration of endogenous cells (e.g. myocytes, endothelial cells, progenitor cells) into the injected matrix. Embedding cardiomyocytes into a hydrogel may confine their normal spreading and beating [18]. Our previous studies indicated that CDCs robustly secrete proteolytic factors (e.g. MMPs) [6]. We also learned that CDCs form “blood-vessel-like” network structures in the platelet gel, indicating their ability to soften the matrix. Therefore, we hypothesized that co-culture with CDCs can promote cardiomyocyte spreading and beating in the platelet gel. Better spreading of neonatal rat cardiomyocytes (NRCMs) was confirmed in platelet gel containing CDCs (Fig. 3A) than in platelet gel alone (Fig. 3B). Quantitative cell morphology analysis revealed more elongated cell bodies in the Gel + CDC group (Fig. 3C). More spontaneously-beating NRCMs were found in platelet gel with CDCs (Fig. 3D). Moreover, the NRCM contractile amplitude and synchronization were enhanced in the platelet gel containing CDCs (Supplementary Video 1) as compared to those in gel alone (Supplementary Video 2). These in vitro results suggest that CDCs act as “matrix softeners” to aid cardiomyocytes maintain their normal morphology and functionality in the hydrogel.

3.5 Recruitment of endogenous cardiomyocytes and endothelial cells

To confirm our in vitro findings, we examined the impact of CDCs on the recruitment of cardiomyocytes and endothelial cells into the injected gel in the injured heart. To enable histological detection, CDCs were labeled with CM-DiI and platelet gel was labeled with Texas Red-X succinidyl ester (Invitrogen, Carlsbad, CA), a dye which covalently bonds to amine groups of the matrix and disappears as the gel degrades in vivo [19]. Platelet gel pre-seeded with CDCs or platelet gel alone was intramyocardially injected into WKY rat hearts with MI and animals were sacrificed 7 days later for histological examination. Endothelial cells (Figs. 4A & B; green) and cardiomyocytes (Figs. 4D & E; green) could be detected residing in the injected platelet gel (Figs. 4A & D; red) or platelet gel without CDCs (Figs. 4B & E; red). Quantitation revealed that more endothelial cells (Fig. 4C) and cardiomyocytes (Fig. 4F) penetrated into the gel in the Gel + CDC group than in the Gel only group. Consistent with our findings in vitro (Fig. 3), some cardiomyocytes in the Gel + CDC group (Fig. 4E; arrowheads) exhibited mature phenotype (larger cell body area; rod

shape) while the cardiomyocytes in the plain gel were mostly small and round-shaped (Fig. 4D; arrows). Similar observations were made from endothelial cells in the platelet gel with CDCs as mature blood vessel structures were detected (Fig. 4B; arrowheads). These data suggested that co-transplantation of CDCs with the platelet gel augmented the recruitment of local cardiovascular cells into the gel matrix and might promote *de novo* angiogenesis and cardiomyogenesis *in situ*.

3.6 Recruitment of endogenous stem cells

Some CDC-secreted cytokines such as SDF-1 can stimulate the recruitment of endogenous stem cells (e.g. c-kit⁺ cells) to the injured myocardium [20]. C-kit (also known as CD117) is a putative marker for cardiac stem cells, and such cells can undergo multilineage differentiation into cardiomyocytes, smooth muscle cells and endothelial cells [21, 22]. C-kit⁺ cells (Figs. 4G & H; green) could be detected in the infarct area injected with platelet gel alone (Fig. 4G; red) or platelet gel spiked with CDCs (Fig. 4H; red). C-kit⁺ cells resided adjacent to where the cells and gel were injected. The number of c-kit⁺ cells in the infarct area doubled in the Gel + CDC group (Fig. 4I).

3.7 Heart morphology and cardiac function

Quantitative morphometry at 3 weeks showed severe LV chamber dilatation and infarct wall thinning in Control (vehicle-injected) hearts (Fig. 5A). Consistent with our previous report [3], platelet gel-treated hearts exhibited attenuated LV remodeling and less abnormal heart morphology, with smaller infarct size (Fig. 5B) and thicker infarcted walls (Fig. 5C). The best heart morphology and the most attenuation of adverse LV remodeling were seen from the Gel + CDC group, with the thickest infarct wall and least infarct size (Figs. 5B & C). The most meaningful indicator of therapeutic benefit, in clinical practice, is the ability to produce functional preservation or improvement in the injured heart. Left ventricular ejection fraction (LVEF) at baseline did not differ among the three treatment groups, indicating a comparable degree of initial injury (Fig. 5D). Over the next 3 weeks, LVEF declined progressively in the Control group, but not in the platelet gel-treated hearts (Fig. 5E). This is in line with our previous report that platelet gel injection preserves LVEFs in post-MI hearts [3]. Three weeks after treatment, LVEFs in platelet gel-treated animals (Fig. 5E; blue bar) were higher than those in Controls (Fig. 5E; grey bar). The highest LVEFs were seen in the hearts received platelet gel pre-seeded with CDCs (Fig. 5E; red bar). Taken together, Controls had a negative treatment effect, as LVEF decreased over time; in contrast, the platelet gel-treated group exhibited robust LVEF preservation. The most sizable cardiac function improvement was confirmed in the Gel + CDC group.

3.8 Direct and indirect regeneration

It has been suggested that the functional benefit of CDC therapy is mostly indirect (i.e. paracrine) [14, 15]. However, some CDCs survive and undergo differentiation into cardiomyocytes or vascular cells in the peri-infarct myocardium [6–10, 12, 14, 23, 24]. Therefore, we sought to elucidate the relative role of direct regeneration and paracrine effects in the functional increment brought by CDCs. Three weeks post-MI, we stained heart sections for alpha-sarcomeric actin (alpha-SA; cardiomyocytes) and isolectin B4 (capillaries). Transplanted CDCs were identified with CM-DiI fluorescence. CM-DiI⁺/alpha-SA⁺ (Fig. 6A) and CM-DiI⁺/Isolectin B4⁺ cells (Fig. 6B) were consistently detected, indicating the ability of transplanted CDCs to differentiate into cardiomyocytes and endothelial cells. We further quantified the percentages of CM-DiI⁺ and CM-DiI[−] cardiomyocytes and capillaries in the infarct area from the Gel only and Gel + CDC groups. The increase of CM-DiI⁺ myocytes or capillaries attests to the creation of new myocardium and endothelium by direct differentiation, whereas the increase in CM-DiI[−] myocytes or capillaries likely reflects indirect mechanisms (recruitment of endogenous regeneration and/

or tissue preservation). The quantitation suggested that direct regeneration played a minor role, as the CM-DiI⁺ cells in the Gel + CDC group only account for 28.7% and 27.3% of the total increment of cardiomyocytes and capillaries, respectively, from the Gel only group (Figs. 6C & D; red bars). The increments of cardiomyocytes and capillaries were mostly from endogenous cells (Figs. 6C & D; green bars). Taking out the direct regeneration portions, the numbers of cardiomyocytes and capillaries from the Gel + CDC group were still significantly higher than those from the Gel only group (Figs. 6C & D). These results indicate that CDCs enhanced the biomaterial's regenerative propensity mostly by promoting indirect endogenous repair.

4. Discussion

In situ polymerizable biomaterials are injected in liquid form, and assume a gel structure in the heart. The greatest advantage of an injectable biomaterial is its minimally-invasive nature and the opportunity to combine it with cells and/or biologically-active molecules prior to implantation [25]. An ideal biomaterial for cardiac regeneration should be biodegradable, non-toxic, cause little or no foreign body reaction, and provide both mechanical and biological support to the injured heart. Previously we reported the regenerative potency of platelet gel injection after acute MI [3]. Here we tested the idea that a combination cell/biomaterial therapy might offer more regenerative propensity. Cardiosphere-derived cells (CDCs) are cell therapy products that have been developed in our lab for over the last six years. Animal studies as well as a proof-of-concept human study support the notion that CDCs may benefit patients with ischemic cardiomyopathy [10]. In the present study, we examined the therapeutic benefit of co-transplanting CDCs with platelet gel and compared that with transplantation of platelet gel alone. We demonstrated that pre-seeding platelet gel with CDCs maximized the therapeutic benefit as the Gel + CDC group had healthier heart morphology (Figs. 5A–C) and higher cardiac function (Fig. 5E) than the Gel only group at 3 weeks. *In vitro*, the gel/CDC composite produced more VEGF, IGF-1 and SDF-1 than the gel alone (Figs. 1D–F). The increment of those pro-regenerative factors may explain the *in vivo* superiorities: the Gel + CDC group exhibited more *de novo* angiogenesis (Figs. 4A–C) and more endogenous recruitment of stem cells (Figs. 4G–I). Furthermore, CDCs promoted the spreading and beating of cardiomyocytes in the platelet gel *in vitro* (Fig. 3; Supplementary Movies 1 & 2), and increased cardiomyocytes and endothelial cell infiltration into the gel *in vivo* (Figs. 4C & F). We postulated that this may come from the CDC-secreted proteases (i.e. MMPs) [6], which may act as a “matrix softener” to degrade the matrix and aid the migration and spreading of cardiomyocytes and endothelial cells in the injected biomaterials. We further quantified the relative contributions of indirect and direct regeneration mechanisms for the CDC-mediated functional benefit. Although direct regeneration did exist (Figs. 6A & B), indirect endogenous recruitment seemed to be the prime driver for the increase of cardiomyocytes and capillaries in the Gel + CDC group (Figs. 6C & D).

CDCs are now under a Phase I/II clinical study for the treatment of myocardial infarction (CADUCEUS trial; clinicaltrials.gov). Given the simplicity of manufacturing platelet gel and its potentially autologous nature, our findings provide rationale for platelet gel + CDC therapy for the treatment of myocardial infarction. Our study also has several limitations. The dose for the gel and cell remains to be optimized. While we used open-chest surgery and direct intramyocardial injection in the rat MI model, minimally-invasive injection catheter delivery is more favorable for clinical testings. Moreover, the gelation time needs to be optimized for catheter-based delivery.

5. Conclusion

Transplantation of platelet gel spiked with cardiosphere-derived cells boosts structural and functional benefits relative to gel transplantation alone in a rat model of myocardial infarction. This combination cell/biomaterial approach is worthy of further investigation and translation for clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Characterization of platelet gel in vitro. (A): H&E staining of the cryo-sectioned platelet gel revealed a fibrous structure and the presence of platelet cells (arrows). (B): SEM images confirmed the fibrous and porous structures of the platelet gel. (C): The resulting platelet gel was mechanically strong enough to be held by a pair of forceps. (D)–(F): Concentrations of VEGF, IGF-1 and SDF-1 in the conditioned media of platelet gel alone (blue bars) and platelet gel spiked with cardiosphere-derived cells (CDCs; red bars) at various time points (n=3 per time point) measured by ELISA. Scale bars = 100 μ m. * indicates $P < 0.05$ when compared to “gel only”.

Figure 2.

Culture of CDCs in platelet gel. (A) & (B): Calcein(live)/EthD(dead) staining reveals a distinct morphology and superior viability of CDCs grown in the platelet gel as compared to those grown on conventional tissue culture plate (TCP) after 14 days in culture. (C): Proliferation of CDCs grown in platelet gel (red line) or on TCP (black line) (n=6). (D): The thickness of the gel decreased over time in culture (n=3 per time point), indicating the degradation of the gel. (E): CDCs grown in platelet gel (red bar) exhibited elongated cell body as compared to those cultured on TCP (black bar) (n=11). (F) Viability (EthD exclusion of dead cells) of CDCs after 14 days of culture in platelet gel (red bar) or on TCP (black bar). (G) – (I): expression of cardiomyocyte (alpha sarcomeric actin), endothelial (von Willebrand factor), and smooth muscle cell (alpha smooth muscle actin) markers in CDCs cultured within platelet gel for 14 days. Scale bars = 10 μm. * indicates $P < 0.05$ when compared to “CDCs on TCP”.

Figure 3.

CDCs promote cardiomyocyte function in platelet gel. (A) & (B): Fluorescent micrographs showing neonatal rat cardiomyocytes (NRCMs; green) stained with alpha sarcomeric actin cultured in platelet gel alone (B) or in platelet gel spiked with rat CDCs stained with DiI (A; red). (C): Elongation of NRCMs cultured in platelet gel alone (blue bar) or in platelet gel spiked with CDCs (red bar) (n=6). (D): Percentage of beating NRCMs in platelet gel alone (blue bar) or in platelet gel spiked with CDCs (red bar) (n=3). Scale bars = 30 μ m. * indicates $P < 0.05$ when compared to "gel only".

Figure 4.

CDCs promote the recruitment of endogenous cardiovascular and stem cells into the injected platelet gel. (A) & (B): Endothelial cells stained with vWF (green) populating the injected platelet gel alone (A; red) or platelet gel spiked with CDCs (B; red) 7 days after injection. (C): Number of endothelial cells infiltrating into injected platelet gel alone (blue bar) or platelet gel spiked with CDCs (red bar) at Day 7 (n=3). (D) & (E): Cardiomyocytes (green) populating the injected platelet gel alone (D; red) or platelet gel spiked with CDCs (E; red) 7 days after injection. (F): Number of cardiomyocytes infiltrating into injected platelet gel alone (blue bar) or platelet gel spiked with CDCs (red bar) at Day 7 (n=3). (G) & (H): C-kit + stem cells (green) being recruited to the myocardium injected with platelet gel (A; red) or platelet gel spiked with CDCs (B; red). (I): The number of c-kit+ stem cells in the infarct zone injected with platelet gel alone (blue bar) or platelet gel spiked with CDCs (red bar) (n=5). Scale bars = 100 μ m. * indicates $P < 0.05$ when compared to “gel only”.

Figure 5.

Heart morphometry and cardiac function. (A): Representative Masson's trichrome-stained myocardial sections 3 weeks after treatment with Control (vehicle), platelet gel alone and platelet gel spiked with CDCs. Scar tissue and viable myocardium are identified by blue and red color, respectively. (B) & (C): Quantitative analysis of infarct size and infarct thickness from the Masson's trichrome images (n=5 animals per group). Left ventricular ejection fraction (LVEF) was measured by echocardiography at baseline (4 hr post-MI) (D) and 3 weeks afterwards (E) (n=8 animals per group). Baseline LVEFs were indistinguishable in the 3 groups. * indicates $P < 0.05$ when compared to Control; ** indicates $P < 0.05$ when compared to all other groups.

Figure 6. Direct versus indirect regeneration. Injected CDCs (labeled with DiI; red) contributed to the formation of new cardiomyocytes (A; green) and capillaries (B; green) in the injured myocardium 3 weeks after transplantation. (C): Quantification of endogenous (green bar) and exogenous (red bar) cardiomyocytes in the heart treated with platelet gel alone or platelet gel spiked with CDCs (n=4). (D): Quantification of endogenous (green bar) and exogenous (red bar) capillaries in the heart treated with platelet gel alone or platelet gel spiked with CDCs (n=4). Scale bars = 50 μ m. * indicates $P < 0.05$ when compared to Control; * indicates $P < 0.05$ when compared “Gel + CDC” (green bar vs. green bar).