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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.

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 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.

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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 succimidyl ester (1 mg/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 Electronenmikroskopie 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 h, 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 h 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% CO_2. 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:

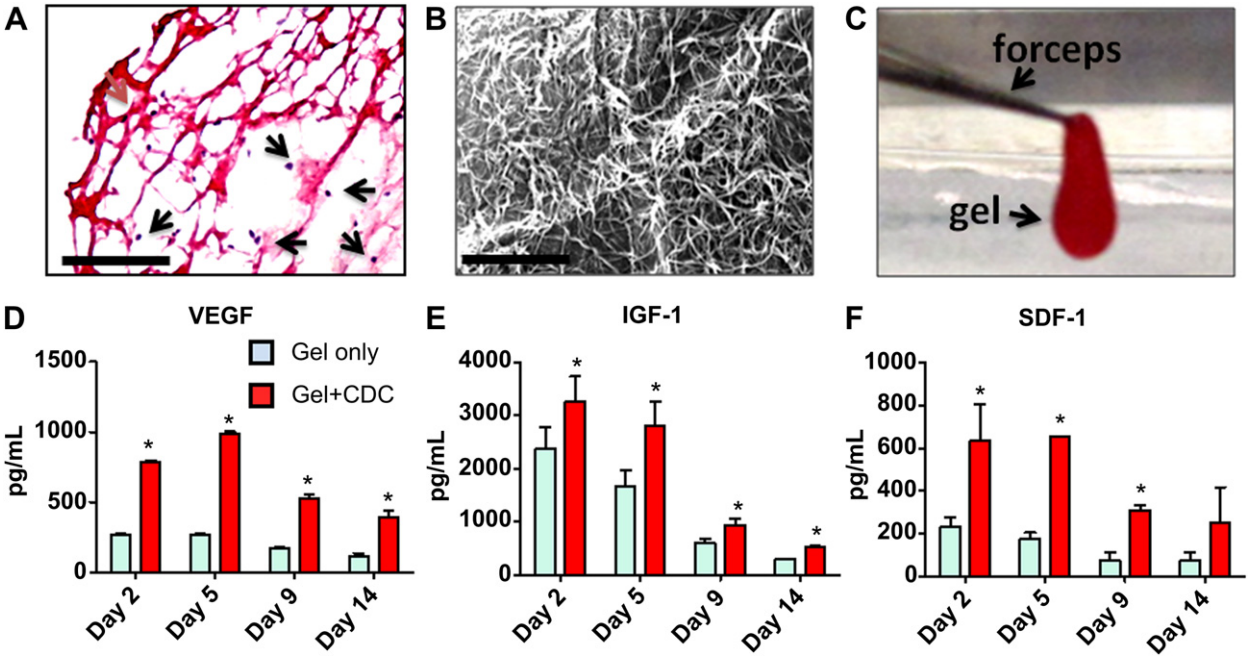

Fig. 1. Characterization of platelet gel in vitro. (A): H & E staining of the cryo-sectioned platelet gel revealed a fibrous structure and the presence of platelet cells (arrows). (B): SEM images confirmed the fibrous and porous structures of the platelet gel. (C): The resulting platelet gel was mechanically 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 μm. *indicates $P < 0.05$ when compared to "Gel only". (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)