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ORIGINAL PRE-CLINICAL SCIENCE

Long-term functional benefits of human embryonic stem cell-derived cardiac progenitors embedded into a fibrin scaffold

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BACKGROUND: Cardiac-committed cells and biomimetic scaffolds independently improve the therapeutic efficacy of stem cells. In this study we tested the long-term effects of their combination.
METHODS: Eighty immune-deficient rats underwent permanent coronary artery ligation. Five to 7 weeks later, those with an echocardiographically measured ejection fraction (EF) $\leq$55% were re-operated on and randomly allocated to receive a cell-free fibrin patch ($n = 25$), a fibrin patch loaded with 700,000 human embryonic stem cells (ESC) pre-treated to promote early cardiac differentiation (SSEA-1^+ progenitors [$n = 30$]), or to serve as sham-operated animals ($n = 25$). Left ventricular function was assessed by echocardiography at baseline and every month thereafter until 4 months. Hearts were then processed for assessment of fibrosis and angiogenesis and a 5-component heart failure score was constructed by integrating the absolute change in left ventricular end-systolic volume (LVESV) between 4 months and baseline, and the quantitative polymerase chain reaction (qPCR)-based expression of natriuretic peptides A and B, myosin heavy chain 7 and periostin. All data were recorded and analyzed in a blinded manner.
RESULTS: The cell-treated group consistently yielded better functional outcomes than the sham-operated group ($p = 0.002$ for EF; $p = 0.01$ for LVESV). Angiogenesis in the border zone was also significantly greater in the cell-fibrin group ($p = 0.006$), which yielded the lowest heart failure score ($p = 0.04$ vs sham). Engrafted progenitors were only detected shortly after transplantation; no grafted cells were identified after 4 months. There was no teratoma identified.

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CONCLUSIONS: A fibrin scaffold loaded with ESC-derived cardiac progenitors resulted in sustained improvement in contractility and attenuation of remodeling without sustained donor cell engraftment. A paracrine effect, possibly on innate reparative responses, is a possible mechanism for this enduring effect.
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At least two important lessons can be drawn from the multiple cardiac stem cell therapies reported so far. The first is that, regardless of whether the transplanted cells directly generate new myocardial tissue or, more likely, act by harnessing endogenous repair pathways to induce host-derived regeneration,[1] the most effective cells seem to be those committed to a cardiac lineage.[2,3] In this setting, along with mesenchymal stem cells exposed to a cocktail of cardiopoietic factors before transplantation[4] and cardiospheres,[5] pluripotent stem cells are attractive because of their intrinsic capacity to be differentiated in any lineage, including the cardiomyogenic one, in response to appropriate cues.[6] The second important lesson is that conventional needle-based intramyocardial injections have several limitations, including random distribution of cells, stress-induced cell damage[7] and, possibly, induction of arrhythmias.[8,9]

Because cardiac surgery provides the unique opportunity for direct control over the heart, several studies have assessed the effects of replacing multiple intramyocardial injections by the epicardial delivery of a cellularized patch. Overall, those results support the superiority of the latter approach with regard to cell survival,[10–13] reduction of fibrosis,[12–14] increased angiogenesis[12–14] and improved function.[10,12–15] Additional advantages of the patch-based approach include strengthening of the infarcted wall, which may contribute to limiting adverse remodeling[16] and provision of a template for cells to proliferate and secrete their own matrix to which they can anchor.[15] Among the diverse materials that can be used, fibrin is attractive due to its biocompatibility, suitability for cell adhesion,[17] potential to act as a reservoir for controlled-release growth factors,[18] and a long-standing safety record when used as an hemostatic sealant in surgery.[19]

The present study was therefore designed to leverage these data by combining the use of human embryonic stem cell (ESC)-derived cardiac progenitors with their incorporation into a fibrin scaffold. We further aimed to assess the outcome of such a composite construct in a rat model of myocardial infarction subjected to a serial and extended (4 months) functional follow-up.

Methods

Animals

Eighty female nude rats (Charles River), weighing an average of 200 to 250 g, were used in this study. All procedures were approved by our institutional ethics committee and complied with European legislation (European Commission Directive 86/609/EEC) on animal care.

Experimental protocol

Myocardial infarction was created as described previously.[14] Five to 7 weeks after myocardial infarction, rats underwent a baseline echocardiographic assessment of left ventricular (LV) function, and all those with an ejection fraction (EF) $\geq 55\%$ were excluded. The remaining rats were re-operated on by a median sternotomy and randomly allocated to receive a cell-free fibrin patch ($n = 25$) or a fibrin patch loaded with 700,000 ESC-derived SSEA-1$^+$ progenitors ($n = 30$). The patch was delivered onto the clearly visible akinetic area and secured to the epicardium by three 6/0 polypropylene sutures. Twenty-five additional rats assigned to a sham group only underwent open-chest placement of similar sutures, without any other procedure. No medical treatment for heart failure was given. Three additional rats receiving a progenitor cell-loaded fibrin patch were used for early post-transplant (1, 2 and 7 days) histologic assessment.

Preparation of SSEA-1$^+$ progenitors

The progenitors were derived from the I6 ESC line (generously provided by J. Itskovitz and M. Amit, Technion Institute, Haifa, Israel), according to a a relatively simple growth factor-based protocol attempting to recapitulate signaling events involved in embryonic cardiogenesis[20–22] (refer to Supplementary Material available online at www.jhltonline.org/). This protocol allowed for reproducibly generat ing a population of SSEA-1$^+$ early cardiac progenitors, primarily expressing *Isl-1*, a transcription factor for both the first and second heart fields,[23] but also additional genes characteristic of different developmental stages of cardiogenesis.[24] The cardiomyocyte-oriented fate of this population has been confirmed in vivo in a non-human primate model of myocardial infarction.[25]

Preparation of the fibrin patch

Seven-hundred thousand SSEA-1$^+$ progenitors were first added to 150 µl of a solution composed of fibrinogen (20 mg/ml) and alpha-minimal essential medium (α-MEM) (Macopharma, Tourcoing, France) in an agarose-coated Petri dish. Four units of thrombin were then added as evenly distributed 25-µl droplets of an equivalent volume of culture medium supplemented with thrombin to induce polymerization of the gel, which occurred in <10 minutes (at 37°C). Both fibrinogen and thrombin were components of the Evicel kit (Ethicon Biosurgery, Omrix Biopharmaceuticals-Ethicon Biosurgery, Rhode Saint Genèse, Belgium). Previous laboratory studies have shown that this fibrinogen-thrombin ratio is appropriate to generate mechanically robust patches with good handling and suturability characteristics and well-preserved cell viability.

Assessment of LV function

LV function was assessed by echocardiography at baseline and every month thereafter until killing at 4 months, as described previously[14] (refer to Supplementary Material online).