Does Transendocardial Injection of Mesenchymal Stem Cells Improve Myocardial Function Locally or Globally?

An Analysis From the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) Randomized Trial

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Rationale: Transendocardial stem cell injection (TESI) with mesenchymal stem cells improves remodeling in chronic ischemic cardiomyopathy, but the effect of the injection site remains unknown.

Objective: To address whether TESI exerts its effects at the site of injection only or also in remote areas, we hypothesized that segmental myocardial scar and segmental ejection fraction improve to a greater extent in injected than noninjected segments.

Methods and Results: Biplane ventriculographic and endocardial tracings were recorded. TESI was guided to 10 sites in infarct-border zones. Sites were mapped according to the 17-myocardial segment model. As a result, 510 segments were analyzed in 30 patients before and 13 months after TESI. Segmental early enhancement defect (a measure of scar size) was reduced by TESI in both injected (−25.0±4.4%; n=95; P<0.01) and noninjected segments (−25.1±7.8%; n=148; P<0.001; between-group comparison P<0.05). Conversely, segmental ejection fraction (a measure of contractile performance) improved in injected scar segments (19.2±3.3–23.6±3.5%; P=0.003) but not in noninjected scar segments (21.3±2.6–23.5±3.2%; P=0.20; between-group comparison P<0.05). Furthermore, segmental ejection fraction in injected scar segments improved to a greater degree in patients with baseline segmental ejection fraction <20% (12.1±1.2–19.9±2.7%; n=18; P=0.003), versus <20% (31.7±3.4–35.5±3.3%; n=12; P=0.33, between-group comparison P<0.0001).

Conclusions: These findings illustrate a dichotomy in regional responses to TESI. Although scar size reduction was evident in all scar segments, scar size reduction and ventricular functional responses preferentially occurred at the sites of TESI versus non-TESI sites. Furthermore, improvement was greatest when segmental left ventricular dysfunction was severe.

Key Words: cells • magnetic resonance imaging • myocardial infarction • tomography
normal.4 The mechanistic basis underlying the myocardial regenerative effect(s) of MSCs include both direct2,10,11 and paracrine actions.8,12 Whether these actions are exerted locally, at a distance, or globally, the effect of precisely localizing cell injections remains unclear. The POSEIDON trial results indicated improved global ventricular structure and function, but regional effects might be obscured by conventional LV imaging analysis.13

Here, we combined the imaging advantages of multidetector computed tomography (MDCT) and biplane left ventriculography to perform a myocardial segmental analysis of the POSEIDON clinical trial so as to test the hypothesis that sites of cell injection respond more favorably in terms of cardiac repair than sites not receiving cell injections. We investigated whether injected myocardial segments have greater reduction of segmental early enhancement defect (SEED, an indicator of myocardial scar) and improved ventricular performance, measured by segmental ejection fraction (SEF, a measure of regional myocardial contractile performance) in comparison with infarcted noninjected myocardial segments. The findings of this study have important implications for implementing stem cell therapy delivered by transendocardial injection.

Methods

A full description of the study protocol, inclusion and exclusion criteria has been published.4 All patients provided written informed consent for the University of Miami Institutional Review Board–approved protocol; enrollment and exclusions are shown in Online Figure I. In summary, POSEIDON was a phase II/II randomized, open-label clinical trial, designed (1) to explore the safety of allogeneic MSCs and (2) to compare the long-term safety and efficacy of allogeneic MSCs with autologous MSCs in patients with chronic LV dysfunction secondary to myocardial infarction (baseline characteristics shown in Online Table I). Our earlier publication reported clinical safety and efficacy. Here, we used the POSEIDON imaging data and the total population of POSEIDON to explore mechanistic insights related to the site of the injection.

MDCT Analysis

MDCT provides detailed and accurate information that is useful in the preparation of an injection strategy, and in follow-up examination permits quantifying the response to stem cell therapy.14 Cine-angiographic MDCT was used for reconstruction of images in 30 patients at screening and at 13 months after TESI for analysis of scar size, SEF, and wall thickening. Acquisition of images was performed using 128 slice (Siemens AS+; Siemens Medical Solutions) or 320-slice (Aquilion One-Toshiba) CT scanning systems with a spatial resolution of 0.30 mm and 0.35 mm, respectively, and analyzed using iNtuition version 4.4.7.47 (TeraRecon, Inc, Foster City, CA) as described previously.15

We evaluated the regional response to MSCs by measuring myocardial SEED as an indicator of myocardial scar size and SEF as a measure of regional myocardial contractile performance. With regards to scar size, delayed enhancement (DE) obtained with cardiac magnetic resonance (CMR) imaging is often used to measure scar size; however, MDCT-derived DE and EED has also been used in studies to quantify scar size.5,15-17 Schuleri et al15 described cardiac MDCT-DE in a swine model and concluded that it can accurately quantify scar tissue for preclinical and clinical studies for novel myocardial therapies. Mahnken et al17 compared DE-CMR, DE-MDCT, and EED-MDCT in 28 patients within 2 weeks after myocardial infarction and reported strong correlation between DE-CMR and DE-MDCT. DE-CMR and EED-MDCT were tightly correlated; they found that EED-MDCT underestimates the size of myocardial infarction (mean size of myocardial infarction was 31.2 g on DE-CMR, 33 g on DE-MDCT, and 24.5 g on EED-MDCT).17 Nevertheless, MDCT-derived SEED analysis, as described in our earlier publication,2 was preferred as a marker of infarct size because it provided (1) discernible difference in densities between normal and abnormal (infarcted) myocardium, (2) better demarcation of the endocardial border of abnormal myocardium in comparison with DE-MDCT, and (3) better image quality and less susceptibility to beam hardening artifacts secondary to automatic implantable cardioverter-defibrillator leads, which were implanted in all but one of our patients.

For regional analysis of myocardial function, previous studies,18 using electron beam computed tomography, demonstrated that SEF can detect abnormalities and provide accurate identification of ischemic myocardium in patients with varying degrees of coronary artery occlusion. Similarly, by applying the principle of SEF to regenerative myocardial therapy, we analyzed regional myocardial performance and scarring after TESI in patients with chronic ischemic cardiomyopathy (Online Figure II).

Myocardial Infarct Scar Assessment: SEED

Assessment of myocardial SEED was previously described.4,15,16 Briefly, contiguous 8-mm-thick short-axis reconstructions were made for the end-diastolic, end-systolic, and mid-diastolic (usually 70% of the R–R interval) cardiac phases encompassing the entire LV myocardial volume. Normal reference CT-based myocardial density was measured in Hounsfield units from regions of interest of ≥10 to 15 mm2 in a myocardial segment that demonstrated normal regional function (ie, normal myocardial wall motion and thickening). For optimal evaluation of myocardial SEED, the CT window level was adjusted so that the center of the scale corresponded to 20 Hounsfield units below the normal myocardial density reference value.17,19 This approach was chosen to define a visual threshold of ≥2 SD below the mean of normal myocardium enhancement, allowing optimal delineation of hypoenhancement. A narrow window width was used (100–150 Hounsfield units) to emphasize the threshold between normal and abnormal myocardium and still allow the surrounding anatomical characteristics to be visualized. SEEDs were identified by their crescentic shape, subendocardial or transmural location, and the presence in ≥2 of the examined cardiac phases. Total myocardial SEED mass per patient was calculated by summing all segmental defect areas and multiplying the total by section thickness (0.8 cm) and then by 1.05 g/cm3 (myocardial specific gravity).19 Each SEED identified in each short-axis section was allocated into either 6 (base and mid LV regions) or 4 (apical LV region) myocardial segments according to the American Heart Association (AHA) 17-myocardial segment model.20

EF Analysis: Global EF Analysis and SEF Analysis

Cardiac volumes and global LV EF (LV end-diastolic volume−LV end-systolic volume)/100) were calculated, and 17-segment polar maps were generated. SEF is an expression...
of regional contractile performance, reflecting the volume of blood ejected under a given myocardial segment, contributing to the global LVEF. The left anterior descending artery in the interventricular sulcus between the basal anterior (AHA seg 1) and the basal anteroseptal (AHA seg 2) segments in the short-axis view was used as an anatomic landmark for segmentation. The basal edge of the LV was identified by the mitral valve annulus in 4- and 2-chamber views. Definition of endocardial borders of the LV chamber in end-systolic and end-diastolic phases was manually assessed with contour corrections, when necessary, after the software protocol recommendations.

For SEF calculation, the software assumes that each of the 17 AHA segments is composed of numerous fan-shaped microsegments (Online Figure II). The volume of all microsegments is traced from these images; akinetic segments were marked as infarct motion defects. End-diastolic endocardial contour tracings were function permitted the assessment of myocardial viability and wall motion defects (see Methods). The individual SEF value presented in the polar map (numbers of segments are denoted by red numbers in green color from 1 to 17) was calculated following the 17-segment model for each patient.

Wall Thickening
Segmental wall thickening (systolic wall thickness–diastolic wall thickness) was calculated following the 17-segment model for each patient.

Topographical Analysis
We further evaluated the regional response to MSCs by conducting topographical analyses using MDCT-EED images. To address whether there was a similar response (SEED and SEF) in transmural versus nontransmural scars, all scars were grouped based on baseline transmurality (>50% and <50%).

Strategy of Cell Delivery in the POSEIDON Trial
Catheter left ventriculography guided the sequence of injections and was compared with MDCT for procedural planning and selection of target sites. Biplane left ventriculography was performed in the 60° left anterior oblique and 30° right anterior oblique view. Together these orthogonal projections describe the position and contractility of each myocardial segment. MDCT of the papillary and regional cardiac function permitted the assessment of myocardial viability and wall motion defects. End-diastolic endocardial contour tracings were traced from these images; akinetic segments were marked as infarct zone and the adjacent hypokinetic segments identified as the border zone. During TESI, each site of injection was recorded onto these ventriculographic maps in 2 projections.

Seventeen-Model Segment Reconstruction: Biplane Left Ventriculography-Endocardial Tracings and the Use of the 17-Segment Model for Recognition of Areas Injected
Injection sites were manually translated onto 17-segment polar maps by 2 independent cardiologists (J.P.Z. and K.H.N.), who assessed if a segment received ≥1 injection. Overall agreement between the 2 readers resulted in a κ coefficient of 0.8896 (95% confidence interval, 0.8447–0.9346; P<0.0001). Disagreement was resolved by a third reader (V.K.). All readers were blinded to the SEED and SEF values. Each injection site was allocated to 1 of the 17 segments (Figure 1).

Definition of Subgroups of Myocardial Segments for Analysis
For segmental analysis, myocardial segments were categorized as follows; scar-injected included all myocardial segments with SEED treated with TESI. Scar-noninjected included all myocardial segments with SEED not treated with TESI. Non–scar-injected included all myocardial segments without SEED treated with TESI. And non–scar-noninjected included all myocardial segments without SEED not treated with TESI. In addition, for radius of activity analysis purposes, the segments around and in the opposite side of the scar-injected were categorized as adjacent and remote myocardial segments, respectively. Within each patient, we compared the accumulated mass value of all SEED treated and not treated with TESI before and after TESI. Finally, to elucidate the changes in each subgroup of segments, we correlated the polar maps obtained from MDCT with those marking injection sites to perform a myocardial segmental analysis of the POSEIDON clinical trial.

Statistical Analysis
For analysis purposes, all 30 patients who received either autologous or allogeneic MSCs were assessed. All data are presented as mean±SEM. GraphPad Prism (version 4.03; La Jolla, CA) was used to analyze all data points. To compare the change in SEF and SEED at 13-month follow-up, paired t tests were applied. For between-group comparisons, 1- and 2-way ANOVA were applied with a Bonferroni/Dunn multiple comparison test when applicable. A P value of <0.05 was considered statistically significant.

Results
Baseline Scar Characteristics
The baseline scar characteristics in the scar-injected and scar-noninjected groups were not significantly different with respect to scar size or thickness of segment with scar; 83.3% (n=25) and 96.7% (n=29) of myocardial segments were transmural in the scar-injected and scar-noninjected, respectively (Online Table II).

Greater Scar Size Reduction at Sites of Stem Cell Injection
Similar scar size reduction was observed in the scar-injected segments with autologous (from 10.2±1.8 to 5.5±0.8 g; n=15; P=0.001) or allogeneic MSCs (from 9.6±1.7 to 5.3±1.1 g; n=15; P=0.001). The scar-noninjected segments had similar reduction in both groups (from 13.2±2.5 to 9.5±1.7 g; P<0.01 for the autologous and from 10.1±1.5 to 7.3±1.2 g; Figure 1).
When considering the autologous and allogeneic groups combined, the scar size was reduced by $-43.7\pm4.4\%$ (from $9.8\pm1.2$ to $5.4\pm0.7$ g; n=30; $P<0.01$) in the scar-injected versus by $-25.1\pm7.8\%$ in the scar-noninjected segments (from $11.7\pm1.4$ to $8.6\pm1.0$ g; n=30; $P<0.001$; between-group comparison scar-injected versus scar-noninjected; $P<0.05$; Figures 2 and 3; Online Movie I). When considering cell type and cell dose, the greatest reduction was found in the injected segments that received 20 million autologous MSCs (from $7.1\pm1.4$ to $3.7\pm0.8$ g; $P=0.02$; between-group comparison $P<0.01$; Online Figure III).

Segmental EF Improves in Injected Segments But Is Unchanged in Noninjected Segments

A total of 510 myocardial segments were analyzed in a total of 30 patients. Improvement in myocardial function was observed in scar-injected segments in both the autologous (from $19.0\pm3.0\%$ to $25.25\pm3.2\%$; n=15; $P=0.03$) and allogeneic groups (from $20.9\pm3.7\%$ to $27.22\pm3.9\%$; n=15; $P=0.02$; between-group comparison $P=NS$). When considering the autologous and allogeneic groups combined, 95 myocardial scar-injected segments were evaluated in a total of 30 patients; SEF improved by $+44.3\pm11.2\%$ (from $19.9\pm3.3\%$ at screening to $26.3\pm3.5\%$) at 13 months after TESI ($P=0.003$) in all scar-injected segments. To analyze the radius of activity, segments adjacent to and remote from scar-injected segments were compared. We observed that SEF improved in adjacent segments in both the autologous (from $18.9\pm1.7\%$ to $26.0\pm2.8\%$; n=15; $P=0.005$) and allogeneic groups (from $29.4\pm4.3\%$ to $34.0\pm4.0\%$; n=15; $P=0.02$; between-group comparison $P>0.05$). However, the remote segments did not demonstrate changes in contractile performance when considering cell type and cell dose or when combining autologous and allogeneic groups (% change in SEF from baseline was $10.1\pm8.9\%$ from $25.8\pm3.2\%$ to $27.0\pm3.6\%$; n=30; $P=0.4$). Importantly, SEF in scar-injected and adjacent segments improved more than in remote segments ($r=0.57$ (95% confidence interval, 0.23–0.78; $P=0.002$; Figure 4). There was no difference between groups when considering cell type and cell dose.

Radius of Activity as Measured by SEF

From our earlier analysis, SEF improved by $+44.3\pm11.2\%$ (from $19.9\pm3.3\%$ at screening to $26.3\pm3.5\%$) at 13 months after TESI ($P=0.003$) in all scar-injected segments. To analyze the radius of activity, segments adjacent to and remote from scar-injected segments were compared. We observed that SEF improved in adjacent segments in both the autologous (from $19.9\pm3.3\%$ to $26.3\pm3.5\%$) at 13 months after TESI ($P=0.003$) in all scar-injected segments. Conversely, SEF did not significantly improve when 148 scar-noninjected segments were evaluated alone or combined. The non–scar-injected (n=63) and non–scar-noninjected (n=204) myocardial segments did not demonstrate changes in SEF at 13 months (Figure 4 and Online Movie II). There was no difference between groups when considering cell type and cell dose. Wall thickening significantly improved in the scar-injected segments ($1.6\pm0.34$ mm at baseline to $2.2\pm0.38$ mm at 13 months; n=30; $P=0.02$) but not in the noninjected segments ($1.5\pm0.24$ mm at baseline to $2.0\pm0.37$ mm at 13 months; n=30; $P=0.2$), between groups $P>0.05$. In addition, the Pearson correlation coefficient between the number of scar-injected segments and the improvement of myocardial SEF was $r=0.57$ (95% confidence interval, 0.23–0.78; $P=0.002$; Figure 4).

Figure 2. Greater reduction in scar-injected segments. Top left, Three-dimensional reconstruction at baseline and multidetector computed tomography images with 20 Hounsfield units below the normal myocardium density at (A) basal short-axis (B), midventricle short-axis, and (C) long-axis representing early enhancement defect (EED) areas delineated in red. Bottom left, Polar map reconstruction depicting segments with EED delineated in red. Scar mass (EED) at baseline (A–C) has a greater reduction in the injected segments (marked by INJ) located in inferior segments at basal, midventricle, and long-axis, from 5.9 to 1.4 g compared with (D and F) noninjected lateral segments at basal, midventricle, and long-axis images, from 4.6 to 1.7 g 13 months after mesenchymal stem cells.
Improvement in Contractile Performance Is Particularly Evident in the Highly Dysfunctional Segments

In a subgroup analysis of all segments with a SEF<20%, the greatest improvement in contractile performance was observed in the scar injected segments, the mean SEF in scarf-injected myocardial segments was 12.1±1.23% at baseline and improved to 19.9±2.69% (P=0.003) at 13 months after TESI (n=18 patients) compared to the scar-injected segments group with SEF>20%, 31.7±3.4 at baseline and 35.5±3.3% at 13 months after TESI; n=12; P=0.05, between groups P<0.0001 and compared to all other groups P<0.001. The SEF % change from baseline improvement in scar injected segments with SEF<20% and SEF>20% was +64.4±16.04% and +16.5±9.84%, respectively (between groups comparison P=0.03).

In the subgroup analysis of SEF<20%, the scar-noninjected myocardial segments (n=15 patients) showed increasing SEF from 13.3±1.3% to 16.1±2.13% (P=0.05; Figure 6).

Similar Magnitude of Change of the Scar-Injected and Scar-Noninjected With a TransmuralExtent of >50% and <50%, Respectively

Scar-injected and scar-noninjected were grouped based on baseline transmurality of >50% and <50%, respectively. For the scar-injected, there was a similar magnitude of change in SEED and SEF regardless of the transmurality (between-group comparison P>0.05 for all analyses): SEED decreased for the scar-injected segments with a transmural extent of >50% (from 9.8±1.3 to 5.2±0.7 g; n=25; P<0.0001), and transmural extent of <50% (from 10.4±3.3 to 6.7±5.8 g; n=5; P=0.03).

Figure 3. Volume rendered 3-dimensional (3D) reformats of left ventricle with color encoding of scar tissue. A and B, Scar mass (green) of inferior segments treated by transendocardial stem cell injection (TESI) at baseline and 13 months after TESI, respectively. Actual scar mass (grams) is depicted in the lower right corner of each panel. Three-dimensional reconstructions in this figure correspond to segmental early enhancement defect (SEED) measurements of the same patient as in Figure 2 and Online Movie I. E and F, Absolute values and percentage changes of scar mass obtained by segmental imaging analysis approach. When considering the autologous and allogeneic groups combined, there is greater scar size reduction in the scar-injected segments (−43.7±4.4%, from 9.8±1.2 to 5.4±0.7 g; n=30; *P<0.01) when compared with the scar-noninjected segments (−25.1±7.8%, from 11.7±1.4 to 8.6±1.0 g; n=30; **P<0.001; between-group comparison scar-injected vs scar-noninjected P=0.05).

Figure 4. Restoration in contractile performance (segmental ejection fraction [SEF]) and association between number of injected segments vs SEF. A, Greater improvement in SEF was observed in patients with more scar-injected segments (segmental early enhancement defect), when considering the autologous and allogeneic groups combined, scar-injected segments SEF improved from 19.9±2.69% to 26.3±3.5% at 13 months after transendocardial stem cell injection (TESI; P=0.003). See Online Movie II. Conversely, SEF did not significantly improve when scar-noninjected segments were evaluated. The non-scar-injected and non-scar-noninjected segments did not demonstrate changes in SEF at 13 months. B, Association between number of injected segments and the improvement in percent change of SEF, Pearson correlation coefficient r=0.57, 95% confidence interval, 0.23 to 0.78; 2-tailed P value=0.002.
For SEF, the scar-injected segments SEF with a transmural extent of >50% increased from 21.8±3.8% to 28.8±4.0%; n=25; P=0.008) and showed a trend of improvement in segments with a transmural extent of <50% (from 11.0±2.6 to 15.1±2.9; n=5; P=0.09; Figure 7).

Transmural Infarct Size Also Reduces in Scar-Noninjected Segments

For scar-noninjected in patients treated with MSCs, we observed that the scar with a baseline transmurality of >50% had a similar magnitude of change in SEED with the scar-injected

Figure 5. Radius of activity for transendocardial stem cell injections (TESI) with mesenchymal stem cells in segmental ejection fraction (SEF%). A, Improvement in contractile performance was observed in scar-injected (from 19.9±3.3% at screening to 26.3±3.5% at 13 months after TESI (P=0.003), and adjacent segments (from 24.2±2.6% to 30.1±2.6%; n=30; P=0.001). Conversely, the remote segments did not have changes in contractile performance (15.8±12.4% from 25.8±3.3% to 27.1±3.7%; n=30; P=0.4); between-group comparison P>0.05. B, Percentage changes from baseline in SEF showed changes by +44.3±11.2% in scar-injected, by +27.8±6.4% in adjacent and by 10.1±8.9% in the remote segments (1-way ANOVA P=0.003; Dunn post test *P<0.05 in scar-injected vs remote and adjacent vs remote).

Figure 6. Improvement in contractile performance is particularly evident in the highly dysfunctional segments. A, Segmental ejection fraction (SEF) <20%, scar-injected segments (baseline) that are encircled with an ellipse in the 17-segment polar map with their corresponding improvement after injection of mesenchymal stem cells. When comparing all subgroups with SEF<20% (B) and >20% (C), the greatest improvement in contractile performance was observed in the scar injected segments with SEF<20% (P<0.001). B, Changes in SEF with highly dysfunctional segments (SEF<20%): The scar-injected SEF was 12.1±1.23% at baseline and improved to 19.9±2.69% (P=0.003) at 13 months after transendocardial stem cell injection (n=18 patients). In this subgroup analysis, the scar-noninjected SEF (n=15 patients) showed a trend of increase from 13.3±1.3% to 16.1±2.13% (P=0.05). C, Changes in segments with SEF>20%.
Discussion

The current study has 3 new major findings: first, the actual site of injection of MSCs plays a fundamental role in the response to cell therapy for chronic ischemic heart disease. Injected sites respond with a reduction in scar tissue and improvement in wall thickening accompanied by functional recovery. Second, although remote segments also exhibit reduction in scar, albeit less compared to injected sites, these segments do not exhibit comparable improvement in myocardial contractile performance. Finally, nonscarred segments do not improve functional regional performance even when cell injections are delivered. These 3 major findings were similar when considering transmural versus nontransmural injected scarred segments. Together these findings offer both mechanistic and practical insights regarding this new and potentially important form of therapy for chronic heart disease.

These findings can best be viewed in the context of mechanistic findings from preclinical models\(^2\),\(^10\),\(^11\),\(^21\) and clinical trials\(^3\),\(^4\),\(^22\)-\(^25\) and are in agreement with both the direct and the indirect mechanisms that underlie these MSC actions.\(^26\) Directly, MSCs are known to engraft and differentiate preferentially in the infarct/border zone\(^10\),\(^11\) producing both reduction of scar size and contractile restoration.\(^21\),\(^31\),\(^32\) In addition, MSCs can release paracrine factors\(^8\),\(^12\),\(^28\) that can have local and remote effects; for example, the release of matrix metalloproteases\(^8\),\(^12\),\(^28\) can contribute to scar size reduction, an effect that could occur remotely. Thus, the improvement in ventricular architecture is driven by a global reduction in scar size, which is accompanied by improved performance that occur preferentially in the injected myocardial segments. These data show that the site of injection matters and that MSCs generate scar size reduction and functional restoration regardless of the transmurality.

The direct and indirect mechanisms underlying these MSC actions have been previously explored in a robust and representative preclinical swine model of ischemic cardiomyopathy.\(^29\),\(^30\) Using this model, our group and others\(^2\),\(^10\),\(^31\) demonstrated that MSCs engraft into chronically scarred myocardium,\(^11\) undergo trilineage differentiation,\(^10\),\(^11\),\(^21\),\(^31\),\(^32\) and enhance the proliferation and differentiation of endogenous cardiac stem cells\(^33\) in the infarct territory,\(^2\),\(^10\),\(^11\) especially at the interface between scar tissue and bordering viable myocardium.\(^2\),\(^10\),\(^11\) As a result, MSCs lead to reduction of scar size and to improvement in LVEF.\(^34\) Moreover, direct hemodynamic assessments of contractility, lusitropy, and myocardial energetics have shown global ventricular improvement, including preload recruitable stroke work, a load independent measure of myocardial contractility.\(^2\),\(^27\)

Another clinical study of intramyocardial MSC therapy that used CMR analysis, also demonstrated a relationship between regional restoration of myocardial contractility, assessed as peak Eulerian circumferential strain\(^8\) and reverse ventricular remodeling. In that study, improved Eulerian circumferential strain in the scar border zone occurred at 3 months after cell injection and correlated with a reduction in ventricular volumes at 12 months after cell therapy. The results of the Cardiopoietic stem Cell Therapy in heart failure (C-CURE) study also showed that MSCs targeted to viable but dysfunctional LV segments resulted in reduction in scar size and increased LVEF in patients with chronic ischemic cardiomyopathy treated with autologous cardiopoietic MSCs.\(^22\)

The imaging approach used in the POSEIDON study provided the means to test the differential effects of local versus remote (paracrine) effects on function and scar size. In the POSEIDON trial, not all scarred myocardial segments were injected, allowing a comparison of TESI-treated and non-treated myocardial segments. Indeed, we found a divergent

**Figure 7.** Dual effects of mesenchymal stem cells, global scar size reduction but restoration of contractile performance only in scar-injected segments, regardless of transmurality. A, Similar magnitude of change in segmental early enhancement defect (SEED) of the scar-injected and scar-noninjected with a transmural extent of >50% (from 9.8±1.3 to 5.2±0.7 g; n=25; P<0.0001) and <50% (from 10.4±3.3 to 6.7±5.8 g; n= 5; P=0.03). B, Similar magnitude of change in segmental ejection fraction (SEF) only in the scar-injected with a transmural extent of >50% (from 21.8±3.8% to 28.8±4.0%; n=25; P=0.008) and <50% (from 11.0±2.6 to 15.1±2.9; n=5; P=0.09).
response between functional restoration and scar reduction. Although myocardial scar segments treated with TESI showed a greater reduction in scar size than those not treated with TESI, the latter also had a significant reduction in scar size. However, only TESI-treated myocardial scar segments exhibited a significant improvement in SEF, indicating that direct cell effects preferentially augmented functional restoration. Furthermore, treated nonscarred myocardial segments did not show changes in contractile performance, implying that the changes occurring in the infarcted myocardium match the mechanisms underlying MSCs therapy. These data also demonstrate that there is a radius of activity for these phenotypic responses: MSCs lead to scar size reduction and restoration in SEF also in adjacent myocardial segments (around scar-injected sites), whereas remote segments only showed reduction in scar size. Collectively, these findings demonstrate a dichotomy in the mechanisms driving the functional (local) and reverse remodeling (local and remote) responses to MSC therapy.

In the POSEIDON study, a paradoxical response to the cell dose was found. When comparing 20 versus 100 versus 200 million MSCs, the greatest benefit in total scar size reduction was seen in the group that received 20 million cells. Similar observation was seen in the current results, with a greater reduction of scar size in the scar-injected segments receiving 20 million MSCs. Among all the groups, 20 million autologous MSCs showed a greater reduction of scar size which is consistent. Variables that may affect the response to treatment thus include cell number (total dose), cell concentration, and the number and distribution of injections. The small sample size of our study limits the possibility of making definitive conclusions but provides the basis for better strategies in stem cell therapy. Optimization of the response to TESI is likely to include a number and pattern of injections specific to a given infarction. In this segmental analysis, a linear positive correlation was noted between the number of myocardial segments with scar treated by TESI and the improvement of SEF. A higher number of myocardial segments with scar treated by TESI could promote a greater improvement; nonetheless, other factors also need to be explored. In the Cardiac Stem Cell Infusion in Patients With Ischemic Cardiomyopathy (SCIPIO) clinical trial, patients received cardiac stem cells by intracoronary infusion; both the infusion site and the cell dose were customized according to the infarct location and size. Patients who were followed up with CMR were analyzed for regional EF. The SEF showed improvement by 1.42 units at 4 months and by 17.9 units at 12 months in the infarct-related segments. This improvement was even greater when the dysfunction was severe. Similarly, we observed improvement in contractile performance in the infarcted segments, and that the lower the SEF at baseline the greater the improvement at 13 months after MSC therapy. In light of the above, we can argue that individualized tailoring, including the number of stem cell injection sites and the total cell dose, may offer an optimal strategy when compared with other fixed approaches that do not account for different locations and extent and severity of the infarction.

Together, these findings support the biological activity of MSC therapy to reverse pathological remodeling through a process driven by infarct size reduction. These changes are associated with improvement in myocardial contractile performance that is preferentially found in the scar tissue zones. These data suggest the duality of the functional benefits of MSCs, involving both direct local and also diffusible, potentially paracrine mechanisms. What remains to be determined is whether the local effect of the injected MSCs is specific for structural or functional repair and whether it is more effective or even more enduring than the paracrine effects. Moreover, it is unknown whether the local cellular and paracrine effects occur in synchrony or even in synergy.

Conclusions

The study of segmental myocardial dynamics can expand the current understanding of local changes that guide cardiac remodeling after TESI. This study revealed that significant improvement in SEF occurred in myocardial scar segments treated with TESI but not in myocardial scar segments without TESI, whereas scar size reduction was evident in TESI-treated and nontreated segments. We also demonstrated that myocardial segments with severe regional dysfunction, SEF<20%, showed even more significant improvement in SEF, suggesting that more impaired myocardial segments have better response to TESI. Collectively, these findings suggest that local and paracrine factors differentially influence functional restoration and reverse remodeling. Although this study does not answer the mechanistic issues underlying the functional and structural responses to MSC therapy, it lends support to the transformation of cell therapy into a more customized, targeted therapy. Myocardial regional assessment tools, such as SEF and myocardial SEED, should be considered as potential measures of efficacy for cardiac stem cell therapy.

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Disclosures

Dr Hare reported having a patent for cardiac cell-based therapy, receiving research support from Biocardiia and being a consultant for Kardia. He holds equity in Vestion and maintains a professional relationship with Vestion as a consultant and member of the Board of Directors and Scientific Advisory Board. Vestion Inc did not play a role in the design and conduct of the study. Dr George reported serving on the board of GE-Healthcare, consulting for ICON-Medical-Imaging, and receiving trademark royalties for fluoropertusion-imaging. A.M. Mendizabal is an employee of EMMES Corporation. Dr Altman is employee of Biocardiia-Inc. Dr McNiece reported being a consultant and board member of Proteonomic Inc. Dr Heldman reported having a patent for cardiac...
cell-based therapy, receiving research support from Biocardi, and being a board member, consultant, and having equity interest in Vestion Inc. The other authors report no conflicts.

References


**Novelty and Significance**

**What Is Known?**

- Transendocardial stem cell injection with mesenchymal stem cells improves remodeling, decreases infarct size, and improves function and quality of life in chronic ischemic heart disease.
- Mesenchymal stem cell mechanisms include antifibrotic effects, neovascularization, neomyogenesis, and stimulation of endogenous cardiac cells.
- Whether these actions are exerted locally, at a distance, or globally, the effect of precisely localizing cell injections remains unclear.

**What New Information Does This Article Contribute?**

- The injection site for cardiac cell therapy has an important effect on the phenotypic response, such that cardiac function improved preferentially at injection sites, whereas scar reduction was evident both locally and remotely to sites of cell delivery.
- Cell injections enhanced ventricular performance to a greater extent at sites that had greater baseline dysfunction.
- Cell therapy reduced fibrosis in infarct scars that were both transmural and nontransmural.

We performed a regional analysis after transendocardial stem cell injection to test the hypothesis that sites of cell injection respond more favorably in terms of cardiac repair than sites not receiving cell injections. We report 3 new major findings. First, injected scarred areas respond with a reduction in fibrotic tissue and improvement in contractile performance. Second, although segments remote from the injected area also exhibit reduction in scar, they do not exhibit comparable improvement in performance. Finally, non-scarred segments do not improve performance with transendocardial stem cell injection. These findings elucidate a dichotomy in the phenotypic response to mesenchymal stem cell injections, demonstrating that local and paracrine factors differentially influence functional restoration and scar size reduction. Locally, there is evidence of reduction in scar size associated with significant improvement in performance, whereas remotely only scar size reduction is seen. Collectively, these findings can guide a personalized approach for optimizing the response to cell therapy in humans by tailoring the injection strategy to patient anatomy.
Does Transendocardial Injection of Mesenchymal Stem Cells Improve Myocardial Function Locally or Globally?: An Analysis From the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) Randomized Trial

Vicky Y. Suncion, Eduard Gherin, Joel E. Fishman, Juan Pablo Zambrano, Vasileios Karantalis, Nicole Mandel, Katarina H. Nelson, Gary Gerstenblith, Darcy L. DiFede Velazquez, Elayne Breton, Kranthi Sitammagari, Ivonne H. Schulman, Sabrina N. Taldone, Adam R. Williams, Cristina Sanina, Peter V. Johnston, Jeffrey Brinker, Peter Altman, Muzammil Mushtaq, Barry Trachtenberg, Adam M. Mendizabal, Melissa Tracy, Jose Da Silva, Ian K. McNiece, Alberto C. Lardo, Richard T. George, Joshua M. Hare and Alan W. Heldman

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Supplemental Methods:

MDCT

Acquisition of images was performed with two scanners, a 128 slice MDCT system (Siemens AS+, Siemens Medical Solutions), which has a spatial resolution of 0.30mm, 150ms of temporal resolution, 0.3 of rotation times for sequential scanning, 36mm of maximum z-axis x-ray beam collimation with sub-milimetre slices and a 320-slice Aquillion One (Toshiba) CT scanning system with a 0.35mm spatial resolution, 175ms of temporal resolution, 0.33 of rotation times for sequential scanning and 160mm of maximum z-axis x-ray beam collimation with sub-milimetre slices.

For regional myocardial contractility analysis, previous studies\textsuperscript{20} using electron beam computed tomography (EBCT) demonstrated that SEF can detect abnormalities and provide accurate identification of ischemic myocardium in patients with varying degrees of coronary artery occlusion. Similarly, by applying the principle of myocardial SEF to regenerative myocardial therapy we were able to analyze regional myocardial contractile performance and scar size following TESI in patients with chronic ischemic cardiomyopathy (Online-Figure II).

17-Segment Model Reconstruction: Biplane Left Ventrivulography- Endocardial Tracings and Use of The 17-Segment Model for Recognition of Areas Injected

We used biplane ventriculographic endocardial tracings during TESI for a complete reconstruction of the AHA 17-myocardial segment model and allocation of the sites of injection.

Online Figure I. Study flow diagram. MSCs indicates mesenchymal stem cells; MDCT, multidetector computer tomography; LV, left ventricle.

Online Figure II. Segmental Ejection Fraction (SEF) Calculation. The outer gray circle represents a short axis view of the left ventricle( LV) at the end diastolic phase and the red fan represents the volume of a micro-segment of end diastolic volume. The inner black circle represents a short axis view of the LV at the end-systolic phase and the blue fan represents the same micro-segment of end-systolic volume. The center of the end-systolic phase is used for both cardiac phases (so called fixed axis). The individual SEF value presented in the polar map for all 17 AHA myocardial segments represents the average SEF of all fan shaped micro-segments within the same particular myocardial segment ((Segmental LV End Diastolic Volume – Segmental LV End Systolic Volume) * 100) / Segmental LV End Diastolic Volume).

Online Figure III. Scar mass changes by cell type and dose. (Panel A) Scar size is reduced in the scar-injected myocardial segments treated with autologous MSCs. 20 million autologous MSCs reduce scar size from 7.1±1.4g to 3.7±0.8g \( p=0.02 \), between group comparison with all groups + \( p<0.01 \). A trend of reduction was observed with other autologous doses; 100 million (from 11.3±3.9g to 5.9±1.6g \( **p=0.09 \), 200 million from 12.1±3.4g to 6.5±1.5g \( ***p=0.05 \). (Panel B) Similar changes were observed in the scar-injected segments treated with allogeneic MSCs, 20 million from 9.0±2.5g to 3.5±1.0g \( \dagger p=0.06 \), 100 million 12.7±3.9g to 7.7±3.0g \( p=0.02 \). The scar-non-injected myocardial segments showed a scar size reduction from 12.3±2.3g to 8.9±1.9g \( \dagger p=0.01 \).

Online Table I. Patient baseline characteristics
Online Table II. Scar baseline characteristics

Online Movie I. Volume rendered reformats of left ventricle with color encoding of scar tissue. 3D reconstructions of Multidetector computed tomography (MDCT) images depicting scar mass of myocardial segments treated by transendocardial stem cell injection (TESI) shown in green with numbers from 1-10 representing sites of injection. Scar mass of myocardial segments not treated by TESI is depicted in orange. Actual scar mass in grams is depicted in the lower right corner of each panel. Movies depict change is scar mass after TESI: Baseline (left panel) and 13 months (right panel). 3D-reconstructions in this figure correspond to SEED measurements of the same patient as in Figures 2 and 3.

Online Movie II. Restoration in contractility only in scar injected myocardial segments. MDCT-CINE short axis videos of a patient with anterolateral and inferior scars and chronic ischemic cardiomyopathy. (Left Panel) Baseline: Inferior myocardial segments before transendocardial stem cell injections (TESI); Segmental ejection fraction (SEF) 31%, segmental early enhancement defect (SEED) 5.9g, end diastolic volume (EDV) 173.0 mL, end systolic volume (ESV) 124.76 mL and sphericity index (SI) 0.419. (Right panel) 13 months after TESI of allogeneic MSCs only into inferior myocardial segments; SEF 43.7%, SEED1.4g, EDV 131.9 mL, ESV 84.68 mL and SI 0.303. Movie corresponds to same patient as in Figures 2 and 3.

Online Figure I.
Online Figure II

**Blue fan**: A segment in end-systolic phase. Volume of this fan is "Segmental End Systolic Volume"

**Red fan**: The same segment in end-diastolic phase. Volume of this fan is "Segmental End Diastolic Volume"

**SEF Formula**:

\[
\text{SEF} = \frac{\text{Segmental End Diastolic Volume} - \text{Segmental End Systolic Volume}}{\text{Segmental End Diastolic Volume}}
\]
# Online Table I.

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Allogeneic MSCs</th>
<th>Autologous MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treated</strong></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>62.8 (10.5)</td>
<td>63.7 (9.3)</td>
</tr>
<tr>
<td><strong>Gender:</strong> Male</td>
<td>13 (86.7%)</td>
<td>13 (86.7%)</td>
</tr>
<tr>
<td><strong>Ethnicity:</strong> Hispanic or Latino</td>
<td>3 (20.0%)</td>
<td>3 (20.0%)</td>
</tr>
<tr>
<td><strong>Race:</strong> White</td>
<td>13 (86.7%)</td>
<td>15 (100.0%)</td>
</tr>
<tr>
<td><strong>Median Years since last MI (range)</strong></td>
<td>9.0 (0.2-27.1)</td>
<td>12.8 (2.4-31.8)</td>
</tr>
<tr>
<td><strong>History of Coronary Interventions</strong></td>
<td>13 (86.7%)</td>
<td>13 (86.7%)</td>
</tr>
<tr>
<td><strong>History of Atrial or Ventricular Arrhythmia</strong></td>
<td>13 (86.7%)</td>
<td>11 (73.3%)</td>
</tr>
<tr>
<td><strong>History of Hypertension</strong></td>
<td>13 (86.7%)</td>
<td>13 (86.7%)</td>
</tr>
<tr>
<td><strong>New York Heart Association Class</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I - No Limitation</td>
<td>2 (13.3%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Class II - Slight Limitation of Physical Activity</td>
<td>9 (60.0%)</td>
<td>9 (60.0%)</td>
</tr>
<tr>
<td>Class III - Marked Limitation of Physical Activity</td>
<td>4 (26.7%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td><strong>History of Smoking</strong></td>
<td>7 (46.7%)</td>
<td>12 (80.0%)</td>
</tr>
<tr>
<td><strong>History of Diabetes</strong></td>
<td>4 (26.7%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td><strong>Treatment Pre-TESI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-I</td>
<td>9 (60.0%)</td>
<td>12 (80.0%)</td>
</tr>
<tr>
<td>Angiotensin 2 Blockers</td>
<td>3 (20.0%)</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Beta Blockers</td>
<td>15 (100.0%)</td>
<td>13 (86.7%)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>10 (66.7%)</td>
<td>10 (66.7%)</td>
</tr>
</tbody>
</table>

Abbreviations: MSCs, mesenchymal stem cells, TESI, transendocardial stem cell injection, ACE-I, angiotensin converting enzyme-Inhibitor. Data are presented as No. (%) unless otherwise specified.
Online Table II.

<table>
<thead>
<tr>
<th>Scar Injected</th>
<th></th>
<th>Scar Non-injected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autologous n=15</td>
<td>Allogeneic n=15</td>
<td>Autologous n=15</td>
</tr>
<tr>
<td>Scar Mass-SEED in grams</td>
<td>10.2 (1.8)</td>
<td>9.6 (1.8)</td>
<td>13.3 (2.4)</td>
</tr>
<tr>
<td>Thickness of segment mm</td>
<td>7.7 (0.5)</td>
<td>7.7 (0.3)</td>
<td>7.5 (0.3)</td>
</tr>
<tr>
<td>Segmental EF %</td>
<td>19.0 (3.0)</td>
<td>20.6 (3.7)</td>
<td>20.0 (2.4)</td>
</tr>
</tbody>
</table>

| | Autologous and Allogeneic combined n=30 | |
| Scar Mass-SEED) in grams | 9.8 (1.2) | 11.7 (1.4) |
| Thickness of segment mm | 7.7 (0.3) | 7.2 (0.3) |
| Transmurality Extent (% of Wall Thickness) | >50% Mean (SEM, N) | 71.4 (2.8, 25) | 65.4 (2.2,29) |
| | <50% Mean (SEM, N) | 46.7 (0.8, 5) | 43.6 (-, 1) |
| Segmental EF % | 19.6 (2.4) | 21.5 (2.0) |

Abbreviations: SEED, segmental early enhancement defect, EF, ejection fraction. Data are presented as: Mean, SEM (standard error of mean).