Cellular cardiomyoplasty is a promising approach for the treatment of severe heart failure. However, the question which cell line is the best to use is still a matter of debate. In this study, we aimed to evaluate the efficacy of arterial media–intima cell suspension (AMICS) transplantation in rabbit myocardial infarct model. The study was divided into 2 groups: group A (the cell-treated group, \( n = 9 \)) and group B (the medium injection group, \( n = 8 \)). Group A was further divided into 2 subgroups as branch-1 (treated with unlabeled cells) and branch-2 (treated with iron-labeled cells). The experimental myocardial infarction (MI) was induced by ligation of left anterior descending coronary artery with a combination of cryoinjury. Ten days after the MI, cells obtained from autologous femoral arteries were injected into the injured myocardium of group A, while group B received only DMEM medium. Clinical, echocardiographic, and histopathologic evaluations were done. As compared to the ninth day values, echocardiography showed a significant improvement in systolic functions and left ventricular (LV) dimensions of the cell-treated group on the 30th day. In the heart biopsy sections of branch-1, the immunostained injected cells were observed to exist closely, suggesting an organization. Cells existing separately and lumen-like structure organizations stained positive with both smooth muscle cell (SMC) \( \alpha \)-actin and Prussian Blue were also showed in the histological observation of branch-2. Autologous AMICS transplantation seems to be a feasible and efficacious method for cellular cardiomyoplasty in our rabbit model.
human fetal cells are difficult to obtain and limited to use in routine therapy for ethical, political, practical, and potential rejection reasons. Smooth muscle cells (SMC) were used in a small number of studies. As Pacilli and Pasquinelli [16] mentioned, adult vascular wall has a very dynamic cell population as it contains SMCs, smooth muscle progenitors, and mesenchymal stromal (stem) cells in the media layer, in addition to vascular progenitor cells, end-differentiated endothelial cells, endothelial progenitor cells, and hemangioblasts in the intima layer. The fate of the transplanted cells to the heart is a key question in evaluating the efficaciousness of the injected cell. Whether the injected cells remain in the tissue or migrate is a major question. Therefore, it is important to set up effective cell tracking. Iron labeling is a simple, nontoxic, biodegradable method that allows better cell tracking by magnetic resonance imaging (MRI) and/or simple histological techniques.

To the best of our knowledge, autologous arterial media-intima cell suspension (AMICS) had not been used for the purpose of cellular cardiomyoplasty. The aim of this animal myocardial infarct model was to evaluate the feasibility and effect of autologous AMICS transplantation after experimentally induced myocardial infarction (MI) in rabbits.

Materials and Methods

All animals were studied under guidelines published in Recommendations from the Declaration of Helsinki, and the study was approved by Experimental Medicine Ethics Committee at Selcuk University, Meram Faculty of Medicine. The study was conducted in the Experimental Research Center at Selcuk University, Meram Faculty of Medicine and supported by Turkish Society of Cardiology.

Study design

Study design was presented in Figure 1. The present study consisted of 2 groups: group A (the cell-treated group, n = 9) and group B (the medium injection group, n = 8). Group A was further divided into 2 subgroups as branch-1 (treated with unlabeled cells) and branch-2 (treated with iron-labeled cells). In branch-1, after the first echocardiography, the sample vessel was excised on day 0 for cell culture. Myocardial infarction was induced on the same day. The second echocardiography was taken on day 9, and the third echocardiography was performed on day 30, and the rabbits were sacrificed. All the processes in branch-1 were also applied to branch-2, but additionally, the cells to be injected into branch-2 were labeled with iron on day 8 and MRI was obtained on day 30. In group B, MI was induced on day 0 after the first echocardiography. The second echocardiography was taken on day 9, and medium injection was performed on day 10. The third echocardiography was obtained on day 30 and the rabbits were sacrificed.

Animals

Totally 33 male New Zealand white rabbits (3–3.5 kg) were included in the study. Four rabbits died during the MI procedure, 7 rabbits died in the early postoperative period, and 4 rabbits died due to severe gastroenteritis that started one day after echocardiography. The remaining 18 rabbits were randomized into 2 groups after the induction of MI. While one of the animals died on day 22 in group B, there was no rabbit loss in group A.

Clinical evaluations

Several daily visits were done to evaluate animal behavior and locomotor activity. All cages were observed for about 20 min on each visit. The pulse rates were measured by echocardiography before and after infarct induction. Ventilation rates were also recorded.

Echocardiographic evaluation

All echocardiographic evaluations were performed using ATL-5000 (Advance Technology Laboratories, Bothell, WA) under light anesthesia with spontaneous respiration by using intramuscular ketamine and xylazine. Echocardiography was

FIG. 1. Outline of experimental protocol. MI, myocardial infarction.
performed in the slight lateral decubitis position. All echocardiographic measurements were obtained according to the American Society of Echocardiography recommendations [17]. Measurements were obtained from each animal on day 0, day 9, and day 30 (Fig. 1). Left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured using M-mode echocardiography in a parasternal long-axis view (Fig. 2). Ejection fraction (EF) and fractional shortening (FS) were calculated by the formulas: \( LVEF = \frac{(LVEDD - LVESD)}{LVEDD} \times 100 \); \( FS = \frac{(LVED - LVES)}{LVED} \times 100 \) [18]. Myocardial velocities were recorded using a tissue pulse-wave Doppler (TDI) technique as previously described in Mundigler and Zehetgruber [19]. The sample volume was placed at the junction of the LV wall with the mitral annulus of the lateral myocardial segment from the 4-chamber view. Systolic (Sm) and early (Em) and late (Am) diastolic waves were recorded at this region, and the mean values of the measurements obtained during 5 consecutive beats were calculated for day 0, day 9, and day 30 (Fig. 3). Heart rate of each animal was calculated during pulse-wave aortic imaging.

**Vessel preparation**

After premedication with intramuscular ketamine (25 mg/kg) and xylazine (4 mg/kg), the rabbits were prepared for isolation and the harvesting of a femoral artery specimen subsequent to local anesthesia (lidocaine 0.8 mg/kg). A longitudinal incision was made on the medial surface of the right thigh, just distal of ligamentum inguinale. Two sutures were applied to the right femoral artery: the upper suture was just below the branching level and the lower one 1.5 cm distal to the upper. Care was taken to remove the adventitia layer of vessel before excision. The artery biopsy specimen was ~1.4 cm long. The incision was repaired surgically according to tissue layers.

**Cell separation and culture**

Arterial samples were transported to laboratory in Phosphate-Buffered Saline (PBS) (Seromed, Klosterneuberg Austria, L-1815) solution. First, the samples were immersed in DMEM (Sigma, St. Louis, MO; D-5796) containing 0.2% Penicillin–Streptomycin (Biological Industries, Beit HaEmek, Israel, 03-033-1B) and 0.2% Amphotericin B (Biological Industries, 03-029-1B) for 30 min at room temperature. One longitudinal incision was made on each vessel with a fine scissor and then minced to pieces of ~1.0 mm². The pieces were suspended overnight in a medium containing 10 mg/mL collagenase (Sigma; C-6885), 80 IU/mL hyaluronidase (Sigma; H-4272), and Human Serum Albumin 5.0 mg/mL (Vitrolife, Sweden, 10023). The following morning, 1.5 mL FCS (Sigma; F-4135) was added and the mixture was centrifuged 2 times at 400 \( \text{g} \) for 10 min. After adding 2.0 mL DMEM to the pellet, extensive pipetting was made to the final suspension composed of AMICS population. It was recentrifuged and plated to flasks (TPP, Switzerland, 9025) with complete medium prepared as MesenCult medium (StemCell Technologies, Vancouver, Canada, MesenCult 05401) containing 15% FCS (StemCell Technologies, 06472), 10% Mesenchymal stem cell stimulatory supplements (StemCell Technologies, 05402), 0.2% Penicillin–Streptomycin (Biological Industries, 03-033-1B), 0.2% Amphotericin B (Biological Industries, 03-029-1B), and 0.125% L'-Glutamine (StemCell Technologies, 07100). The number of the obtained and plated cells was ~1.2 \( \times 10^6 \) per flask. The culture medium was changed every 2 days.

**Labeling of cells**

Culture medium was removed from flasks, and a new medium was added for cell labeling with iron according to Bulte et al. [20] with the following modifications: (A) MesenCult medium without serum or growth factor supplement that contains only iron nanoparticles (Guerbet, Endorem) at a final concentration of 30 \( \mu \text{g/mL} \) and poly-L-Lysine (Sigma, P-1524) at a concentration of 2.0 \( \mu \text{g/mL} \) was prepared, and (B) cells were treated for 18 h in 5.0% CO\(_2\) incubator. Then flasks were repeatedly washed with DMEM by gentle shaking and discarding until no more decrease of excessive particles or clumps of iron was observed under stereomicroscope. During iron labeling, the confluence of cell culture was around 70%–75%. Cell culture was continued with

![FIG. 2. M-mode echocardiographic evaluations of rabbit myocardial infarct model: (A) before MI, (B) after MI, (C) after cell transplantation.](image-url)
On day 30, intubation was done with a transparent 3F endotracheal tube under light anesthesia, during which time their ventilation rate was recorded in minutes. Finally, the animals were sacrificed.

**Magnetic resonance imaging**

Magnetic resonance imaging studies were performed on day 30 by a 1.5 T clinical MRI scanner (Siemens, Vision Plus, Erlangen, Germany) with a maximum gradient strength of 25 mT/m with a small loop radiofrequency receiver coil. Animals were sacrificed and their chests were opened. In order to obtain a better image, the arterial and venous conduits of the heart were ligated with 3.0 silk sutures to hold intracardiac pressure. Short- and long-axis slices were obtained. Scout transversal and sagittal views ensured correct determination of the short-axis planes. T2-weighted fast spin-echo was applied along both the short and long axis of the heart and gradient-echo sequences were applied only along the short axis by using the following imaging parameters: for the T2-weighted spin-echo sequence, 5000/96 (TR ms/TE ms), 31 × 50-mm field of view, matrix of 126 × 256, and section thickness of 2 mm; for gradient-echo sequence, 800/26, 50 × 100-mm field of view, flip angle of 20°, matrix of 102 × 256, and section thickness of 2 mm.

**Histological staining**

Routine Hematoxylin & Eosin, Masson Trichrome (Sigma) histochemical methods were used to stain 5.0 μm paraffin sections of postmortem infarcted tissue. Smooth muscle cell α-actin (LabVision, 9010) immunostain was used to demonstrate the transplanted arterial cells in the infarcted areas. Iron-labeled cells were stained in one of 2 ways: (a) with only Prussian Blue stain and (b) with Prussian Blue and SMC-specific α-actin, sequentially. As far as we know, there is no precedent staining method for the same slide with both Prussian Blue stain and SMC-specific α-actin immunostain.

**Statistical analyses**

Data were presented as the median values (25th–75th percentiles). We used the Friedman test to evaluate the changes in the echocardiographic parameters among the 3 measurements of the study. If there was a significant difference ($P < 0.05$) among the 3 measurements, we used the Wilcoxon signed rank test with Bonferroni correction method to identify which 2 measurements were significantly different ($P < 0.0167$). In addition, we used the Mann–Whitney $U$-test to compare differences between the 2 groups. All statistical analyses were performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA).

**Results**

**Clinical evaluation**

It was noticed that group A rabbits (the cell-injected group) showed more dynamic locomotor activity than group B after 10–12 days. They were wandering in the cage and were more responsive, while the control group usually stayed motionless.

After MI induction on day 0, the pulse and the ventilation rates of the 2 groups were similar, but, on day 30, they were...
significantly different between group A and group B (pulse rate: 155 ± 17 vs. 182 ± 18 \( P < 0.01 \), respectively; ventilation rate: 42 ± 6 vs. 48 ± 6, \( P = 0.002 \), respectively).

Life-threatening arrhythmia was not detected in the monitored period during echocardiographic evaluation and pre-surgical periods when animals were under light anesthesia. In the follow-up period after the injection time, no animals were lost in group A, but in group B, one animal died.

**Echocardiographic results**

Echocardiographic parameters obtained on day 0 and day 9 showed no significant differences between the 2 groups. However, when day 0 and day 9 parameters were compared, an expansion in LV systolic and diastolic dimensions and a statistically significant decrease in systolic functions were detected in both groups. This showed the efficacy of the MI model applied. The in-group comparison of day 9 and day 30 values showed a decrease in LV dimensions and a statistically significant increase in LVEF, LVFS, and lateral Sm values in group A, indicating an improvement in LV dimensions and functions, while no significant changes were detected in group B. On the other hand, when the day 30 values of the 2 groups were compared, it was observed in all the evaluated echocardiographic parameters that there was a significant improvement in group A, as compared to group B (Table 1).

**Autopsy observations**

Group B heart showed a larger, rougher, and stiffer infarct area caving and pale color in appearance, while group A heart had a perturbing appearance with only small patches of pale color at the infarct area (Fig. 3A and 3B). Peri-infarct heart had a perturbing appearance with only small patches of pale color and had clear demarcation line in group B.

**Histological evaluations**

Eosin Y viability test was done on the suspension before injection. Mean value of viability rate was 89% ± 5%.

Cell injection suspensions contained 83.4% ± 5.6% \( \alpha \)-actin-positive SMCs.

Tissue samples were prepared with routine paraffin processing after neutral-buffered formalin fixation. In the full-thickness infarct area, the scar tissue was observed to be replaced by muscle tissue in patches in group A according to Masson’s Trichrome staining, as opposed to group B, where no signs of muscle appearance were observed in the scar tissue (Fig. 4A). In group B, evaluation of hematoxylin eosin preparations revealed low number of fibroblasts and no signs of inflammation in the scar tissue, while in group A cells resembling muscles were identified. The peripheral regions of scar tissue in both groups contained disorganized muscle tissue with a low number of leukocytes.

Sparsely distributed SMC \( \alpha \)-actin-immunoreactive cells and lumen-like structures with SMC \( \alpha \)-actin-positive wall were observed in branch-1 of group A (Fig. 4B). At some sites, immunostained cells were located closely to each other in the infarct area, resembling an organizational building site suggesting a tubular-like structure that might be an early stage of vessel formation (Fig. 5A). Immunoreactivity was confirmed by the positive muscular vessel stains in a separate healthy area.

The histological observation of branch-2 also showed a lumen-like structure organization stained positive for both SMC \( \alpha \)-actin and Prussian Blue (Fig. 6A–6C). As Prussian Blue stains only the tracked cells, it may be concluded that the injected cells were incorporated into the host tissue. No erythrocytes were detected in the lumen-like structures, so it could not be concluded that these lumens were vessels. However, at some sites, very close organization for angio-genetic bed-like structures was demonstrated (Fig. 5D). At some injected sites, such structures were frequently observed. These findings suggest that our injected cells might be involved in a process reminiscent of an early stage of vessel organization. While some SMC \( \alpha \)-actin- and iron-positive cells were incorporated into lumen-like structures, others were also observed as isolated single cells.

In the slides, iron-loaded cells stained with Prussian Blue were observed 0.4–0.6 cm far from the nearest injected site (Fig. 6A) and organized into lumen-like structures (Fig. 6B).

**Table 1. Echocardiographic Parameters of the Groups**

<table>
<thead>
<tr>
<th></th>
<th>Day 0 (before MI)</th>
<th>Day 9 (after MI)</th>
<th>Day 30 (after interv)</th>
<th>( P ) value</th>
<th>( P1 )</th>
<th>( P2 )</th>
<th>( P3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A, ( n = 9 )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF, %</td>
<td>67.3 (63.7, 69.5)</td>
<td>55.0 (50.5, 58.6)</td>
<td>64.0 (56.1, 67.1)</td>
<td>0.001</td>
<td>0.008</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>FS, %</td>
<td>42.9 (39.7, 44.8)</td>
<td>32.9 (29.7, 35.7)</td>
<td>40.0 (33.8, 43.1)</td>
<td>0.001</td>
<td>0.008</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>15.0 (14.4, 15.8)</td>
<td>16.5 (15.9, 17.0)</td>
<td>15.3 (15.2, 15.4)</td>
<td>0.001</td>
<td>0.008</td>
<td>NS</td>
<td>0.008</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>8.8 (7.9, 9.1)</td>
<td>11.3 (10.1, 11.6)</td>
<td>9.1 (8.8, 10.1)</td>
<td>0.001</td>
<td>0.008</td>
<td>NS</td>
<td>0.008</td>
</tr>
<tr>
<td>Lateral Sm, cm/s</td>
<td>8.8 (7.4, 9.3)</td>
<td>6.4 (6.0, 7.2)</td>
<td>8.6 (7.1, 9.8)</td>
<td>0.003</td>
<td>0.008</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Group B, ( n = 8 )</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF, %</td>
<td>62.9 (61.0, 67.9)</td>
<td>56.2 (47.8, 62.0)</td>
<td>54.7 (49.4, 59.9)*</td>
<td>0.002</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>FS, %</td>
<td>39.1 (37.4, 43.3)</td>
<td>33.8 (27.7, 38.3)</td>
<td>32.7 (28.8, 36.7)*</td>
<td>0.002</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>15.4 (15.0, 15.8)</td>
<td>17.0 (16.3, 17.9)</td>
<td>16.6 (16.0, 16.9)**</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>9.2 (8.8, 9.6)</td>
<td>10.8 (10.5, 12.7)</td>
<td>11.2 (10.5, 11.7)**</td>
<td>0.002</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Lateral Sm, cm/s</td>
<td>8.7 (7.4, 9.6)</td>
<td>6.4 (6.0, 7.9)</td>
<td>6.4 (5.9, 7.8)***</td>
<td>0.009</td>
<td>0.01</td>
<td>0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values were presented as median (25th–75th percentiles).

\( ^{*}P = 0.034 \); \( ^{**}P < 0.001 \); \( ^{***}P = 0.015 \) for comparison of Day 30 in group A. \( P1 \) refers to comparison of Day 9 and Day 0. \( P2 \) refers to comparison of Day 0 and Day 9. \( P3 \) refers to comparison of Day 30 and Day 9.

Abbreviations: MI, myocardial infarction; EF, ejection fraction; FS, fractional shortening; interv, intervention; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; NS, non-significant; Sm, systolic motion.
A population of iron-loaded cells (around 60%) was not SMC α-actin-positive, which suggested cell differentiation (Fig. 6C). It was also observed that the cells loaded with iron particles had a tendency to accumulate near and inside the adventitial layer of arteries (Fig. 6D).

Group B heart histology showed diffuse collagen tissue with fibroblastic activity and rare vessel structures.

**FIG. 4.** (A) Scar tissue stained with Masson’s Trichrome, large collagen bundles are seen with scattered smaller collagen fibers. Red stained cytoplasm of scar tissue cells are dispersed (bar represents 50 μm). (B) Injected scatteredly located unorganized α-smooth muscle-specific immunostained cells are seen in concordance with our injection site (bar represents 50 μm).

**FIG. 5.** Colored cells are the smooth muscle cell (SMC) α-actin-positive cells. They are also stained with Prussian Blue. The injected cells are in an organization form. (A) Longitudinal section, (B) higher organization, 2 layers of cells are seen, (C) single cells (short arrow), and a higher organization (longer arrow), (D) site where increased angiogenesis is seen (bar represents 50 μm in all figures).

**FIG. 6.** (A) Prussian Blue stained cell 1.1 cm far from the nearest injected site, (B) Prussian Blue stained cells showing a lumen-like structure, (C) This slide was prepared with both staining of smooth muscle cell (SMC) α-actin and Prussian Blue. Here cells are stained with Prussian Blue but did not take SMC α-actin immunoreactivity, (D) Cells stained with Prussian Blue and SMC α-actin immunoreactivity located at media and adventitia layer of a vessel (all figures were viewed under immersion oil field).

**MRI evaluation**

As well as wall thinning at the site of the infarct area, hypointense areas on the surface of the infarcted area due to superparamagnetic iron oxide susceptibility were visible (Fig. 7). Lesions were observed on both fast spin-echo and gradient-echo images. Areas of signal intensity loss were better identified on T2-weighted gradient-echo images than on T2-weighted fast spin-echo images. There were susceptibility artifacts on T2-weighted gradient-echo images, and thus, the gradient-echo images were not as good as fast spin-echo images. However, the areas containing labeled cells were more prominent on T2-weighted images because of superparamagnetic iron oxide susceptibility. The injected cells labeled with iron were detected far from their original injection cites as hypointense signal on T2-weighted gradient-echo images. This suggests that our injected cells survived for at least 3 weeks and migrated in the meantime.

**Discussion**

In this study, autologous AMICS was used for the purpose of cellular cardiomyoplasty. This suspension of cells was able to reverse the dilatation of the left ventricle and improved the LV systolic function at an early stage. Also, it was demonstrated by TDI that the tissue contraction velocity improved to an extent close to its preinfarct values. The injected SMCs in branch-1 were observed to be arranged in rows almost in parallel to each other. As for branch-2, the injected cells survived for 3 weeks after injection, and some of them contributed to the newly formed vessel-like structures. Much to our surprise, while some iron-loaded cells were SMC α-actin immune-positive, most were immune-negative (~40% and 60%, respectively, data not shown). However, the high rate of SMC-negative cells may be accounted for by possible differentiation mechanisms. Cells do not continue to exist as SMCs but differentiate into other lines, instead.
Such other cells as hemangioblasts are not stained by α-actin but labeled with iron, resulting in a higher rate of α-actin immune-negative cells. Pacilli and Pasquinelli (16) emphasized the dynamic heterogeneous cell population of vascular wall. Another possible explanation is that the SMCs in the transplanted cell population might have survived less than other cells.

The MR findings showed a wider distribution of iron-labeled cells than we have expected. Our expectation was to observe cells only at the injection sites. The wider distribution showed that the cells had the potential for migration while protecting their viability.

NYHA functional class is an important predictor of clinical outcome in humans, and we aimed to carry this valuable classification to our animal model. For this purpose, we focused on locomotor activity and responses to sound and touch. Our results revealed that the cell-injected animal group had higher clinical functional status and that cell therapy had a favorable effect.

Smooth muscle cells were previously selected from fetal rat stomach by Li et al. [21]. The same authors later worked on syngenic rats [22], obtaining SMC from aorta tissue. They used a patch seeded with SMC and applied it to the scar on the heart by an open chest surgery operation. The same author also used SMC obtained from autologous ductus deference [23].

Using AMICS has some advantages; first of all, obtaining these cells would be practical, especially from the radial arteries (which are used for coronary artery bypass operations). Second, the autologous nature eliminates the need for immunosuppression. As opposed to cell applications of fetal origin, our method does not pose any ethical difficulties. Third, it is easy to obtain a high number of such cells with simple protocols or to identify and make in vitro culture. It is also relatively simple to propagate SMCs, which were reported to proliferate in myocardial scar tissue [21]. Another advantage is that transferring an impulse with coupling is relatively simple to propagate SMCs, which were used for coronary artery bypass operations. AMICS can survive while being in a new microenvironment. On the other hand, this new environment may lead them to apoptosis [27], or they may tend to migrate to other locations, which will reduce their number in the scar area.

In this study, an improvement in systolic functions was shown, which is directly related to contractility. How the injected cells contribute to this contractility is unclear. Arterial cells actively secrete mediators and an extensive number of cytokines that regulate, induce, or accelerate wound-healing phase in the tissue [28,29]. As is known, vascular SMCs secrete elastic fibrils that may add strength to scar tissue and help the mechanical contraction of ventricular muscles. Secretion and migratory properties of vascular SMCs were helping to improve cardiac functions in their new microenvironment. On the other hand, this new environment may lead them to apoptosis [27], or they may tend to migrate to other locations, which will reduce their number in the scar area.

During our study, we faced some limitations that we hope future researchers will consider. First, as we focused only on vascular SMC, we made our design based on its markers, but as the study progressed, the need for a wider spectrum of markers such as vascular endothelial growth factor, connexin, von Willebrand factor, and so on arose. Another advantage is that injecting AMICS can promote scar connective tissue turnover in the myocardial scar. Still another advantage is that SMC can migrate, as we also detected iron-labeled cells about 0.5 cm far in all directions from the nearest injection site (based on MRI and histological observations).

One of the most important advantages is that AMICS work under rhythmic pressure regulations in their natural conditions, which is not the case for cells of bone marrow or skeletal muscle origin. They can survive while being in direct contact with blood flow. Furthermore, AMICS are far from being a source for carcinogenic development as compared to embryonic cell population.

Approximately 1.2 × 10^6 cells were transplanted into each heart in our study, which is a lower number when compared to other studies. However, Suzuki et al. [25] stressed the qualitative value of the cells instead of the quantity. Interestingly, Li et al. did not give a conclusion as to why 10^8 SMCs were helping to improve corrupted heart functions. Instead, they addressed and questioned the possible secrections [26]. When SMCs are transplanted into infarcted heart tissue, they may help improve the cardiac functions in their new microenvironment. On the other hand, this new environment may lead them to apoptosis [27], or they may tend to migrate to other locations, which will reduce their number in the scar area.

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limitation was that, to the best of our knowledge, the clinical status of experimental MI model in animals was for the first time documented by observation, but it may need more detailed quantifications and methods.

In conclusion, AMICS transplantation seems to be feasible and efficacious in rabbit MI model.

**Author Disclosure Statement**

No competing financial interests exist.

**References**


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