Transplantation Effectiveness of Induced Pluripotent Stem Cells Is Improved by a Fibrinogen Biomatrix in an Experimental Model of Ischemic Heart Failure

Sebastian V. Rojas, MD,^{1,2,*} Andreas Martens, MD,^{1,2,*} Robert Zweigerdt, PhD,² Hassina Baraki, MD,¹ Christian Rathert,² Natalie Schecker,² Sara Rojas-Hernandez, MD,³ Kristin Schwanke, PhD,² Ulrich Martin, PhD,² Axel Haverich, MD,^{1,2} and Ingo Kutschka, MD¹

Objectives: The aim of this study was to investigate whether a fibrinogen biomatrix improves the transplantation effectiveness of induced pluripotent stem cells (*iPSCs*) in a model of myocardial infarction.

Background: Early retention, engraftment, and cell proliferation are important factors for successful cardiac stem cell therapy. Common transplantation techniques involve the direct injection of cells in aqueous media. However, this approach yields low retention and variable cell biodistribution, leading to reduced grafts that are unable to sufficiently regenerate damaged myocardium. Biologically compatible scaffolds that improve the retention of injected cells can improve cardiac stem cell therapy.

Methods: Murine *iPSCs* were transfected for luciferase reporter gene expression. First, *in vitro* experiments were performed comparing cell viability in fibrinogen and medium. Second, *iPSCs* were transplanted intramyocardially by direct injection into ischemic myocardium of immunodeficient mice, following permanent left coronary artery ligation. Cells were delivered in medium or fibrinogen. Follow-up included graft assessment by bioluminescence imaging, the evaluation of cardiac function by magnetic resonance imaging, and histology to evaluate graft size and determine the extent of myocardial scarring.

Results: *In vitro* experiments showed proliferation of *iPSCs* in fibrinogen from $6.4 \times 10^3 \pm 8.0 \times 10^2$ after 24 h to $2.1 \times 10^4 \pm 3.2 \times 10^3$ after 72 h. Early cardiac cell amount in control group animals was low ($23.7\% \pm 0.7\%$) with massive cell accumulation in the right ($46.3\% \pm 1.0\%$) and the left lung ($30.0\% \pm 0.6\%$). When *iPSCs* were injected applying the fibrinogen biomatrix, intramyocardial cell amount was increased ($66.3\% \pm 0.9\%$) with demonstrable graft proliferation over the experimental time course. Left ventricle-function was higher in the fibrinogen group ($42.9\% \pm 2.8\%$), also showing a higher fraction of refilled infarcted-area ($66.9\% \pm 2.7\%$).

Conclusions: The fibrinogen biomatrix improved cardiac *iPSC* retention, sustaining functional improvement and cellular refill of infarcted myocardium. Therefore, fibrinogen can be considered an ideal biological scaffold for intramyocardial stem cell transplantations.

Introduction

CORONARY ARTERY DISEASE is a progressive pathology with high morbidity and mortality rates worldwide.¹ Current projections estimate that by 2020, more than 7 million people will die due to cardiovascular disease.² After myocardial infarction, the limited ability of self-regeneration

often leads to irreversible heart failure.³ Despite great advances in medical treatment,^{4,5} terminal heart failure can only be treated by cardiac transplantation or with ventricular assist device (VAD) therapy.^{6,7} In the context of a shortage of donor organs and limited long-term experience with VAD-support, stem cell transplantation poses a promising alternative for the treatment of ischemic cardiomyopathy.^{8–10} A common aspect

¹Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany.

²Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School–REBIRTH-Cluster of Excellence, Hannover, Germany.

³Department of Anesthesiology, Hannover Medical School, Hannover, Germany.

*These two authors equally contributed to this work.

in many stem cell therapy studies is the injection of stem cells suspended in aqueous media.^{11–16} Although this technique results in viable grafts, it has been demonstrated that it entails massive early cell loss by different mechanisms, including venous drainage from the injection site to the right atrium leading to pulmonary graft accumulation.¹⁷ Several studies have shown that cardiac cell retention is lower than 10% immediately after injection.^{17–22} Thus, it could be argued that this loss of cells hinders the chances of success of cell therapy before the cell phenotype has the possibility to influence restoration. Consequently, several groups have been working on improving cellular retention by using special injection scaffolds or tissue-engineered constructs.^{23–32} Although fixing stem cell constructs to the surface of the infarcted myocardium can help to avoid cell discharge, it cannot regenerate the infarcted area. Therefore, direct intramyocardial injection is an important delivery technique since the infarction (or its border zone) can be targeted directly, generating high local tissue concentrations.²² In this context, considering massive early cell loss by this technique, studies focused on improving retention of injected cells are required. For this purpose, biocompatible scaffolds provide an excellent medium to transport and retain stem cells in the myocardium. Some biological scaffolds can contribute additionally to myocardial restoration, with positive effects for graft viability and paracrine properties.²⁷ Among different types of materials, fibrinogen stands out due to its advanced clinical translation. As it is part of the intrinsic pathway of blood coagulation, it is widely used in its polymerized state as fibrin glue for hemostatic therapy in cardiac surgery. Previous studies have investigated the effect of fibrin glue as a scaffold for the intramyocardial injection of skeletal myoblasts in infarcted rat hearts.^{31,32} These studies revealed that the combination of fibrin and skeletal myoblasts resulted in a two-fold higher level of stem cell grafts within the infarction when compared to controls. Other groups applied fibrin as biogel to inject stem cell-derived cardiomyocyte sheets,³³ cardiac progenitor cells,³⁴ and adipose-derived mesenchymal stem cells.³⁵ However, fibrin is difficult to apply as a polymer in small animal models, where needles with small diameters are used. Its rigid state also reduces nutrient exchange between host myocardium and transplanted stem cells, hampering graft viability and cell proliferation. Therefore, fibrinogen presents a promising alternative as it is viscous enough to retain cells, but liquid enough to guarantee graft nutrition once transplanted. Thus, the main hypothesis of our work was that using fibrinogen as biomatrix would improve retention and biodistribution of intramyocardial injected induced pluripotent stem cells (*iPSCs*) following myocardial infarction.

Materials and Methods

An overview of the experimental design is shown in Figure 1.

Optically bioluminescent *miPS* cells

Undifferentiated murine *iPSCs* were maintained in culture as previously described.³⁶ Stable clones were generated by electroporative transfection (Neon™ Transfection System; Invitrogen) with a plasmid carrying a chicken-beta-actin promoter driving the expression of firefly lu-

ciferase reporter gene (*fluc*) and an ampicillin resistance (Fig. 1-I). The culture medium was composed of Dulbecco's modified Eagles medium (Invitrogen) supplemented with 15% fetal calf serum (Thermo Scientific), 0.2 mM L-glutamine (Invitrogen), 0.1 mM β -mercaptoethanol (Invitrogen), 0.1 mM nonessential amino acid stock (Invitrogen), and 0.1% human leukemia inhibitory factor conditioned medium. Genetically modified cells were selected by antibiotic resistance and the brightest clone was chosen for experiments.

In vitro evaluation of cell growth and viability

In vitro iPSC assays for cell viability ($n=3$) (live/dead cell staining kit II, Promokine; PromoCell) and proliferation ($n=3$) (WST-8 assay, Promokine; PromoCell) were performed on 3 consecutive days. To differentiate living from dead cells, 3×10^4 *iPSCs* were seeded on a 24-well plate. Well chambers were assigned to three groups: fibrinogen, fibrin, and medium. In each group, chambers were assigned for the respective time point at which measurements were performed: 24, 48, and 72 h. At each time point, cells were stained: green, living cells; red, dead cells. Following microscopy, representative areas of live and dead cells were measured using ImageJ (v. 1.48 for Mac OS X).

In vitro cell proliferation analysis was performed applying the WST-8 assay (Promokine; PromoCell). A 96-well plate was arranged for cell culture with three different groups: fibrin, fibrinogen and medium. Every group also included noncellular controls. In each well chamber 5×10^3 *iPSCs* were seeded and brought into culture. Separately, standard dilutions of *iPSCs* ranging from 2.5×10^3 to 1×10^4 cells were assembled at each time point for cell quantification. Quantification was performed using a microplate reader (Paradigm Detection Platform; Beckman Coulter) at 24, 48, and 72 h applying the WST-8 substrate.

Animal care

All surgical interventions and animal care were provided following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, volume 25, no 28, revised 1996) and in accordance with institutional and federal regulations.

Injections

For each injection, 1×10^6 *iPSCs* were resuspended in 15 μ L fibrinogen (Tissuecol Duo S; Baxter). Controls received 15 μ L of medium. Injections were performed using a microsyringe with a 33G needle.

Murine myocardial infarction model and injections

For this study, a total of 38 immunodeficient SCID bg mice (16–21 g, 8–10 weeks; Charles River Laboratories) were used. Myocardial infarction was induced in all animals by permanent ligation of the left anterior descending coronary artery, as previously described.³⁷ First, aliquots of 1×10^6 *iPSCs* were injected ($n=20$, 10 fibrinogen vs. 10 medium). After 10 min of beating heart action, bioluminescence imaging (BLI) was performed with closed thorax. Afterward, organs (heart and lungs) were explanted and

FIG. 1. Experimental study design. (I) Murine induced pluripotent stem cells (*iPSCs*) were transfected for firefly Luciferase gene expression. (II, III) One million *iPSCs* were intramyocardially injected in fibrinogen following myocardial infarction. The first experimental group (III) was evaluated 10 min after injection to investigate early cell retention and biodistribution. Remaining animals (II) were kept in follow-up for 14 days to investigate *iPSC* viability over the experimental time course and to perform functional assessment by magnetic resonance imaging (MRI) and histology. Color images available online at www.liebertpub.com/tea

again analyzed using BLI for cell quantification (Fig. 1-III). In the second group ($n=18$, 9 fibrinogen vs. 9 medium), 1×10^6 *iPSCs* were injected intramyocardially. Following chest closure, animals were kept in follow-up for 14 days. Follow-up examinations included BLI at different time

points and magnetic resonance imaging (MRI). Animals were euthanized at day 14. Organs were explanted for graft quantification with BLI. Finally, histological assessment of explanted heart was performed to investigate graft size and infarct extension.

FIG. 2. *In vitro* *iPSC* viability in fibrinogen. (A) *iPSCs* were kept in culture for 72 h in fibrinogen, fibrin, and medium. Living cells are marked in green, whereas red spots show dead cell colonies. The area of fibrinogen-cultured cells showed good cell survival (B) with small dead areas (C). (D) The cell proliferation assay also showed strong proliferation rates in the fibrinogen group. Differences between both groups were not considered significant. Color images available online at www.liebertpub.com/tea

Bioluminescence imaging

The hearts and lungs of all animals were harvested and imaged with the IVIS Lumina II (Caliper Lifesciences) for *BLI* signal detection. In the 10-min groups, cell aliquots were resuspended in 5 μ L of beetle luciferin (Promega) before injection for signal induction. In the follow-up-group, 100 μ L of substrate was intraperitoneally injected and cells were quantified in explanted organs when peak bioluminescence signal was reached. Cell quantification of *iPSC* in explanted organs was calculated by linear correlation of the bioluminescence signal with the respective signals of *in vitro* standard dilutions containing known *iPSC* concentrations, as previously described.¹⁷

Magnetic resonance imaging

Small animal *MRI* was performed using a 7-Tesla scanner (Pharmascan 70/16 Bruker BioSpin). In the follow-up group, animals were anesthetized and scanned on day 14 to assess left ventricular ejection fraction (LV-EF). Cine FLASH-sequences (5–10 short axis slides) were captured and LV-EF was measured applying Simpson's method.

Histology

Hearts were processed following standard protocols for cryosectioning and histochemistry. Masson's trichrome staining was applied at 300 μ m intervals to delineate cell

FIG. 3. Early biodistribution of injected *iPSCs*. (A) Fibrinogen-treated animals showed a rather focused thoracic bioluminescence imaging (*BLI*) signal, with most of injected *iPSCs* retained in the heart (B; 1, heart; 2, right lung; 3, left lung). (C) In the medium group, thoracic projection of *BLI* signal suggests lung biodistribution of injected *iPSCs*. (D) *BLI* assessment of organ explants unveiled massive *iPSC* accumulation in the right and the left lung. (E) Comparison between both groups showed that, in the fibrinogen group, more than 60% of total cells were retained in the heart, whereas in the medium group only 22% of all cells could be found in the heart. (F) Calculation of absolute *iPSC* amounts also showed significant higher number of intramyocardial *iPSCs* with lower lung concentrations of injected *iPSCs* in the fibrinogen group compared with medium controls. Color images available online at www.liebertpub.com/tea

allocation in line with infarct anatomy, as previously described.³⁸ Infarct and graft extension were measured using ImageJ software (v. 1.48 for Mac OS X).

Statistics

GraphPad Prism 5.03 was used for statistical analysis. Data are expressed as the mean $\pm$ standard error. For statistical analysis, we performed two-way ANOVA. Differences were considered significant at $p < 0.05$. All reported p -values are two-sided.

Results

In vitro cell viability assays

Live cell staining. An overview of results is shown in Figure 2A and B. Cells cultivated in fibrin showed an area of $2.8 \pm 0.1 \mu\text{m}^2$ after 24 h, $2.2 \pm 0.3 \mu\text{m}^2$ after 48 h, and $4.0 \pm 0.3 \mu\text{m}^2$ after 72 h. The fibrinogen group showed an area of $6.8 \pm 0.4 \mu\text{m}^2$ at 24 h. After 48 and 72 h, the living cell area increased to 8.7 ± 0.3 and $13.5 \pm 0.5 \mu\text{m}^2$, respectively. The medium group showed an area of $9.0 \pm 0.2 \mu\text{m}^2$ after 24 h. After 48 and 72 h, the living cell area was 11.6 ± 0.5 and $16.0 \pm 0.7 \mu\text{m}^2$. Differences between both groups were not significant.

Dead cell staining. An overview of results is shown in Figure 2A and C. Cells cultivated in fibrin showed an area of $3.3 \pm 0.2 \mu\text{m}^2$ after 24 h, $3.7 \pm 0.2 \mu\text{m}^2$ after 48 h and $4.1 \pm 0.4 \mu\text{m}^2$ after 72 h. The fibrinogen group showed an area of $2.5 \pm 0.3 \mu\text{m}^2$ at 24 h. After 48 and 72 h, the dead cell area was 2.6 ± 0.2 and $2.8 \pm 0.2 \mu\text{m}^2$, respectively. The medium group showed an area of $1.4 \pm 0.1 \mu\text{m}^2$ after 24 h. After 48 and 72 h, dead cell staining showed areas of 1.6 ± 0.1 and $1.8 \pm 0.1 \mu\text{m}^2$. Differences between both groups were not significant.

Cell proliferation assay

Results are graphically shown in Figure 2D. Cells cultivated in fibrin were calculated as $3.4 \times 10^3 \pm 5.0 \times 10^2$ after 24 h, $2.7 \times 10^3 \pm 6.3 \times 10^2$ after 48 h, and $8.9 \times 10^3 \pm 1.3 \times 10^3$ after 72 h. The fibrinogen group showed $6.4 \times 10^3 \pm 8.0 \times 10^2$ cells at 24 h. After 48 and 72 h, the cell numbers increased to $1.1 \times 10^4 \pm 1.8 \times 10^3$ and $2.1 \times 10^4 \pm 3.2 \times 10^3$, respectively. The medium group showed $1.0 \times 10^4 \pm 1.2 \times 10^3$ cells after 24 h. After 48 and 72 h, cell amounts were calculated as $1.8 \times 10^4 \pm 2.0 \times 10^3$ and $3.5 \times 10^4 \pm 2.3 \times 10^3$. Differences between both groups were not significant.

Cardiac retention and biodistribution of iPSCs after 10 min

An overview of all results is shown in Figure 3. After 10 min of beating heart action, calculated cardiac retention in the medium control group was $1.7 \times 10^5 \pm 7.8 \times 10^3$ cells. Analysis of the lungs showed cell distribution of $3.3 \times 10^5 \pm 9.4 \times 10^3$ to the right lung and $2.1 \times 10^5 \pm 4.6 \times 10^3$ cells to the left lung. The fibrinogen group had a cardiac retention of $4.6 \times 10^5 \pm 1.2 \times 10^4$ cells. Analysis of the lungs showed cell distribution to the right lung ($1.5 \times 10^5 \pm 8.9 \times 10^3$ cells) and to the left lung ($4.3 \times 10^4 \pm 1.8 \times 10^3$ cells). Differences between means of each individual organ signal between both groups were significant ($p < 0.01$).

Bioluminescence signal of iPSCs in time course (14 days follow-up)

In this experimental setting, iPSCs were injected in fibrinogen or medium. After chest closure, thoracic BLI signals were assessed at different time points for a period of 14 days (Fig. 4A). An overview of the absolute radiance values of both groups is shown in Table 1. Figure 4A shows an

FIG. 4. Follow-up of iPSCs following intramyocardial transplantation. (A) Example of thoracic BLI signals over the time course (14 days) following intramyocardial transplantation with and without the fibrinogen biomatrix. (B) Comparison between both groups shows a first constant course of BLI signals according to the engraftment time required. After 72 h, total mean BLI signals began to rise consequently with significant higher mean radiances in the medium group. (C) Although overall BLI signals were higher in medium-treated animals, the analysis of organ explants showed significantly higher cardiac concentrations in the fibrinogen group and high pulmonary iPSC concentrations in the medium group. Color images available online at www.liebertpub.com/tea

TABLE 1. TIME COURSE OF MEAN THORACIC BIOLUMINESCENCE IMAGING SIGNALS OF FLUC EXPRESSING INDUCED PLURIPOTENT STEM CELLS FOLLOWING INTRAMYOCARDIAL TRANSPLANTATION (FIBRINOGEN VS. MEDIUM)

	10 min	1 day	2 days	3 days	4 days	7 days	10 days	14 days
Fibrinogen, $\pm$ SEM	$1.8 \times 10^7 \pm 1.0 \times 10^6$	$8.2 \times 10^5 \pm 5.0 \times 10^4$	$8.8 \times 10^5 \pm 4.5 \times 10^5$	$1.1 \times 10^6 \pm 3.8 \times 10^4$	$1.9 \times 10^6 \pm 3.8 \times 10^4$	$5.8 \times 10^6 \pm 4.1 \times 10^5$	$7.9 \times 10^6 \pm 5.0 \times 10^5$	$1.0 \times 10^7 \pm 5.9 \times 10^5$
Medium, $\pm$ SEM	$1.8 \times 10^7 \pm 2.9 \times 10^6$	$6.1 \times 10^6 \pm 4.8 \times 10^4$	$6.9 \times 10^5 \pm 5.9 \times 10^4$	$8.6 \times 10^5 \pm 5.4 \times 10^4$	$1.8 \times 10^6 \pm 1.7 \times 10^5$	$9.3 \times 10^6 \pm 3.1 \times 10^5$	$1.4 \times 10^7 \pm 5.8 \times 10^5$	$1.8 \times 10^7 \pm 6.6 \times 10^5$
<i>p</i>	NS	NS	NS	NS	NS	<0.05	<0.001	<0.001

Radiance values are given in (p/s/cm²/sr).
SEM, standard error of the mean.

exemplary trend of *BLI* signals in both groups during follow-up. A graphical plot of *BLI* signal development is shown in Figure 4B. After day 7, differences between group means became statistically significant ($p < 0.05$).

On day 14, organs were explanted directly after measuring the thoracic *BLI* signal of all animals (Fig. 4C). The total mean thoracic signal was higher in the medium group ($p < 0.01$). Following organ explantation, the mean cardiac *BLI* signal in the medium group was $6.1 \times 10^5 \pm 4.0 \times 10^4$ (p/s/cm²/sr), whereas in the fibrinogen group a mean radiance of $1.2 \times 10^6 \pm 8.0 \times 10^4$ (p/s/cm²/sr) was assessed for the hearts ($p < 0.01$). The analysis of the left lungs showed a mean radiance of $5.7 \times 10^5 \pm 7.7 \times 10^4$ (p/s/cm²/sr) in the medium group and $1.9 \times 10^5 \pm 1.3 \times 10^4$ (p/s/cm²/sr) in the fibrinogen group ($p < 0.01$). Examination of the right lungs unveiled a mean *BLI* signal of $1.0 \times 10^6 \pm 6.8 \times 10^4$ (p/s/cm²/sr) in the medium group and $2.7 \times 10^5 \pm 4.8 \times 10^4$ (p/s/cm²/sr) in fibrinogen-treated animals ($p < 0.01$).

Histological assessment of cardiac graft extension

The mean infarct extension of the infarct control group (Fig. 5A) was $69.4\% \pm 2.0\%$ of the whole *LV* circumference. In medium (Fig. 5B) and fibrinogen (Fig. 5C) groups, infarct extension was $68.1\% \pm 2.5\%$ and $68.9\% \pm 2.3\%$, respectively. There was no statistically relevant difference regarding the infarct extensions between all groups (Fig. 5D). Furthermore, *iPSC* graft extension in the infarcted area was compared between medium and fibrinogen. Medium-treated animals had a mean graft extension of $54.2\% \pm 2.7\%$, whereas fibrinogen animals showed a graft extension of $67.0\% \pm 2.7\%$ ($p < 0.05$) (Fig. 5E).

Assessment of LV function by MRI

At day 14, before animals were sacrificed, LV-EF was measured by *MRI* (Fig. 5C). The infarct control group showed a mean LV-EF of $22.5\% \pm 1.1\%$. The mean LV-EF of the medium group was $37.0\% \pm 3.1\%$. Fibrinogen-treated animals showed a mean LV-EF of $42.9\% \pm 2.8\%$. Differences between groups were statistically significant ($p < 0.05$).

Discussion

Cardiac stem cell treatment by intramyocardial injections is associated with poor and variable graft concentrations, especially when aqueous injection media are used for cell transfer. Previously, our group developed a *BLI*-based imaging method capable of monitoring cardiac injection efficacy, which unveiled substantial cardiac cell loss by venous drainage through the coronary sinus, culminating in an accumulation of *iPSCs* in the lungs.¹⁷ The aim of this study was to find an injectable biomaterial capable of enhancing cardiac stem cell retention, limiting biodistribution to the targeted cardiac area and enhancing graft survival. We chose *iPSCs*, a very promising regenerative cell source. In our experimental setting, cells were applied in an undifferentiated state. While for biodistribution analysis, potential teratoma formation is neglectable, further studies should involve *iPSC*-derived cardiomyocytes, which have more relevance regarding clinical translation. Moreover, generation and purification of *iPSC*-derived cardiomyocytes is already feasible and can be performed by antibiotic-resistance gene transfer and cell selection.³⁹

FIG. 5. *iPSC* graft extension and functional assessment of left ventricle (LV) function 14 days after intramyocardial transplantation of *iPSCs* in myocardial infarction. (A) Infarct extension (dashed yellow line) in the infarct-control group was quantified as percentage of the whole LV (red) circumference. Right ventricle is marked by the blue line. (B) Medium-treated animals showed several smaller grafts (marked in green) in the LV. (C) In the fibrinogen group grafts were bigger and confined to one part of the LV. (D) There was no difference regarding the extension of infarcted myocardium following experimental infarction by left anterior descending artery ligation. (E) Both cellular groups showed cardiac engraftment of *iPSCs*. However, graft size (% of the infarcted circumference of the LV) was superior in the fibrinogen group. (F) Both cellular groups showed improvement of the left ventricular ejection fraction (LV-EF), as measured by MRI on day 14. Functional improvement is related to ventricular geometry and correlates with the extent of refilled infarction. Color images available online at www.liebertpub.com/tea

First, we performed *in vitro* assays to investigate the effect of fibrinogen on *iPSC* viability and compared it to a fibrin control group. The cell survival, death, and proliferation assays showed that the less viscous fibrinogen was superior to fibrin in all tests. The considerable thicker fibrin possibly influenced negatively nutrition, cellular communication, and cell junctions.⁴⁰ Fibrinogen is a plasma protein, which is converted by thrombin into fibrin during blood coagulation. It

is a widely available autologous source, which does not provoke an immunogenic reaction and has positive effects on cell viability.⁴¹ Additionally, fibrinogen is already in use in the clinic, since it is commonly used in surgery as a component of fibrin glue for achieving hemostasis. First, we performed *in vitro* assays to investigate the effect of fibrinogen on *iPSC* viability and compared it to a medium control group. The *in vitro* assays showed that *iPSCs* could survive

and proliferate in the fibrinogen matrix. However, *in vitro* results suggest that fibrinogen and fibrin take influence on proliferation and survival of seeded cells. Especially, more viscous fibrin showed lower cell viability. This phenomenon is probably explained by the matrix consistency that takes influence on nutrition, cellular communication, and cell junctions.⁴⁰ However, by means of viscosity, fibrinogen can be adjusted according to its application. This is especially relevant for small animal models since viscosity limits the usage of microsyringes. When fibrinogen was applied as a biomatrix, intramyocardial retention of injected cells noticeably improved after 10 min beating heart action and in the follow-up group. Compared to the medium control group, cellular biodistribution to both lungs was also strongly reduced in fibrinogen-treated animals. In the follow-up experiments, overall thoracic *BLI* signals were compared between the medium and fibrinogen group. In the first 24 h, both groups showed a constant expression of the thoracic *BLI* signal, which coincides with the time needed for engraftment. After 72 h, *BLI* signal intensity began to rise in both groups, which is a clear sign of positive cell engraftment. Subsequently, total bioluminescence signals were higher in the medium group. However, analysis of explanted organs unveiled that all animals in the medium group showed a massive accumulation of proliferating *iPSCs* in the lungs. Thus, the overall amount of cells was higher in medium-treated animals. Nevertheless, fibrinogen-treated animals had considerably higher cardiac *iPSC* grafts with low lung accumulation of transplanted cells. This was also verified in the histological assessment, where the graft extension was also higher in fibrinogen-treated animals. Our study showed functional improvement in both cell-treated groups compared with the nontreated controls. The functional improvement was higher in the fibrinogen group and correlated with higher graft extensions. We did not see any evidence of new contractile myocardium. Moreover, when undifferentiated *iPSCs* are injected, teratoma formation is very likely.⁴² One possible explanation for functional improvement is by the change of *LV* geometry through cellular refill. By augmentation of left ventricular wall thickness in infarcted myocardium, left ventricular geometry is reconstructed. As shown in our study, fibrinogen-treated animals showed larger cell grafts leading to more refill of the infarcted area with significantly reduced infarct sizes.^{43,44}

Conclusions

Our results show that fibrinogen is perfectly suited as a scaffold for cell-based cardiac repair. It improves retention and limits the biodistribution of *iPSCs* when injected in the early phase of myocardial infarction, when ischemia, inflammation, and other adverse physiological conditions are significant graft-limiting factors. Therefore, fibrinogen can be recommended as an injection vehicle for intramyocardial stem cell transplantations.

Acknowledgments

We would like to thank Nina McGuinness for review. This work was supported by the Integrated Research and Treatment Center Transplantation (IFB-Tx, Federal Ministry of Research, Germany), CORTISS Hannover GmbH the Cluster of Excellence REBIRTH (Deutsche Forschungsgemeinschaft).

Disclosure Statement

No competing financial interests exist.

References

- Mathers, C.D., Boerma, T., and Ma Fat, D. Global and regional causes of death. *Br Med Bull* **92**, 7, 2009.
- Mathers, C.D., and Loncar, D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* **3**, e442, 2006.
- Braunwald, E. Heart failure. *JACC: Heart Fail* **1**, 1, 2013.
- McMurray, J.J.V., Adamopoulos, S., Anker, S.D., Auricchio, A., Böhm, M., Dickstein, K., Falk, V., Filippatos, G., Fonseca, C., Gomez-Sanchez, M.A., Jaarsma, T., Køber, L., Lip, G.Y.H., Maggioni, A.P., Parkhomenko, A., Pieske, B.M., Popescu, B.A., Rønnevik, P.K., Rutten, F.H., Schwitzer, J., Seferovic, P., Stepinska, J., Trindade, P.T., Voors, A.A., Zannad, F., Zeiher, A.; ESC Committee for Practice Guidelines. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur Heart J* **33**, 1787, 2012.
- Nabel, E.G., and Braunwald, E. A tale of coronary artery disease and myocardial infarction. *N Engl J Med* **366**, 54, 2012.
- Slaughter, M.S., Rogers, J.G., Milano, C.A., Russell, S.D., Conte, J.V., Feldman, D., Sun, B., Tatooles, A.J., Delgado, R.M., Long, J.W., Wozniak, T.C., Ghumman, W., Farrar, D.J., Frazier, O.H.; HeartMate II Investigators. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med* **361**, 2241, 2009.
- Strueber, M., O'Driscoll, G., Jansz, P., Khaghani, A., Levy, W.C., and Wieselthaler, G.M. Multicenter evaluation of an intrapericardial left ventricular assist system. *J Am Coll Cardiol* **57**, 1375, 2011.
- Laflamme, M.A., and Murry, C.E. Regenerating the heart. *Nat Biotechnol* **23**, 845, 2005.
- Murry, C.E. Cell-based cardiac repair: reflections at the 10-year point. *Circulation* **112**, 3174, 2005.
- Passier, R., Van Laake, L.W., and Mummery, C.L. Stem-cell-based therapy and lessons from the heart. *Nature* **453**, 322, 2008.
- Mauritz, C., Martens, A., Rojas, S.V., Schnick, T., Rathert, C., Schecker, N., Menke, S., Glage, S., Zweigerdt, R., Haverich, A., Martin, U., and Kutschka, I. Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction. *Eur Heart J* **32**, 2634, 2011.
- Martens, A., Gruh, I., Dimitroulis, D., Rojas, S.V., Schmidt-Richter, I., Rathert, C., Khaladj, N., Gawol, A., Chikobava, M.G., Martin, U., Haverich, A., and Kutschka, I. Rhesus monkey cardiosphere-derived cells for myocardial restoration. *Cytotherapy* **13**, 864, 2011.
- Nelson, T.J., Martinez-Fernandez, A., Yamada, S., Perez-Terzic, C., Ikeda, Y., and Terzic, A. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation* **120**, 408, 2009.
- Van Laake, L.W., Passier, R., Doevendans, P.A., and Mummery, C.L. Human embryonic stem cell-derived cardiomyocytes and cardiac repair in rodents. *Circ Res* **102**, 1008, 2008.

15. Lee, S.-T., White, A.J., Matsushita, S., Malliaras, K., Steenbergen, C., Zhang, Y., Li, T.-S., Terrovitis, J., Yee, K., Simsir, S., Makkar, R., and Marbán, E. Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in pigs with heart failure post-myocardial infarction. *J Am Coll Cardiol* **57**, 455, 2011.
16. Williams, A.R., Hatzistergos, K.E., Addicott, B., McCall, F., Carvalho, D., Suncion, V., Morales, A.R., Da Silva, J., Sussman, M.A., Heldman, A.W., and Hare, J.M. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. *Circulation* **127**, 213, 2013.
17. Martens, A., Rojas, S.V., Baraki, H., Rathert, C., Schecker, N., Zweigerdt, R., Schwanke, K., Rojas-Hernandez, S., Martin, U., Saito, S., Schmitto, J.D., Haverich, A., and Kutschka, I. Substantial early loss of induced pluripotent stem cells following transplantation in myocardial infarction. *Artif Organs* **38**, 978, 2014.
18. Terrovitis, J.V., Smith, R.R., and Marban, E. Assessment and optimization of cell engraftment after transplantation into the heart. *Circ Res* **106**, 479, 2010.
19. Terrovitis, J., Lautamäki, R., Bonios, M., Fox, J., Engles, J.M., Yu, J., Leppo, M.K., Pomper, M.G., Wahl, R.L., Seidel, J., Tsui, B.M., Bengel, F.M., Abraham, M.R., and Marbán, E. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. *J Am Coll Cardiol* **54**, 1619, 2009.
20. Teng, C.J., Luo, J., Chiu, R.C.J., and Shum-Tim, D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: implications for cellular cardiomyoplasty. *J Thorac Cardiovasc Surg* **132**, 628, 2006.
21. Hudson, W., Collins, M.C., deFreitas, D., Sun, Y.S., Muller-Borer, B., and Kypson, A.P. Beating and arrested intramyocardial injections are associated with significant mechanical loss: implications for cardiac cell transplantation. *J Surg Res* **142**, 263, 2007.
22. Martens, A., Rojas, S.V., Baraki, H., Rathert, C., Schecker, N., Hernandez, S.R., Schwanke, K., Zweigerdt, R., Martin, U., Saito, S., Haverich, A., and Kutschka, I. Macroscopic fluorescence imaging: a novel technique to monitor retention and distribution of injected microspheres in an experimental model of ischemic heart failure. *PLoS One* **9**, e101775, 2014.
23. Sekine, H., Shimizu, T., Hobo, K., Sekiya, S., Yang, J., Yamato, M., Kurosawa, H., Kobayashi, E., and Okano, T. Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. *Circulation* **118**, S145, 2008.
24. Sekine, H., Shimizu, T., Dobashi, I., Matsuura, K., Hagiwara, N., Takahashi, M., Kobayashi, E., Yamato, M., and Okano, T. Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in comparison with dissociated cell injection. *Tissue Eng Part A* **17**, 2973, 2011.
25. Zimmermann, W.H. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res* **90**, 223, 2001.
26. Kutschka, I. Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation* **114**(1 Suppl), 1167, 2006.
27. Kofidis, T., Lebl, D.R., Martinez, E.C., Hoyt, G., Tanaka, M., and Robbins, R.C. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. *Circulation* **112**, 1173, 2005.
28. Lisi, A., Briganti, E., Ledda, M., Losi, P., Grimaldi, S., Marchese, R., and Soldani, G. A combined synthetic-fibrin scaffold supports growth and cardiomyogenic commitment of human placental derived stem cells. *PLoS One* **7**, e34284, 2012.
29. Kutschka, I. Adenoviral human BCL-2 transgene expression attenuates early donor cell death after cardiomyoblast transplantation into ischemic rat hearts. *Circulation* **114**, 1174, 2006.
30. Tudorache, I., Kostin, S., Meyer, T., Teebken, O., Bara, C., Hilfiker, A., Haverich, A., and Cebotari, S. Viable vascularized autologous patch for transmural myocardial reconstruction. *Eur J Cardiothorac Surg* **36**, 306, 2009; discussion 311.
31. Christman, K., Vardanian, A., Fang, Q., Sievers, R., Fok, H., and Lee, R. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculation formation in ischemic myocardium. *J Am Coll Cardiol* **44**, 654, 2004.
32. Christman, K.L., Fok, H.H., Sievers, R.E., Fang, Q., and Lee, R.J. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng* **10**, 403, 2004.
33. Zhang, L., Guo, J., Zhang, P., Xiong, Q., Wu, S.C., Xia, L., Roy, S.S., Tolar, J., O'Connell, T.D., Kyba, M., Liao, K., and Zhang, J. Derivation and high engraftment of patient-specific cardiomyocyte sheet using induced pluripotent stem cells generated from adult cardiac fibroblast. *Circ Heart Fail* **8**, 156, 2015.
34. Bellamy, V., Vanneaux, V., Bel, A., Nemetalla, H., Boitard, S.E., Farouz, Y., Joanne, P., Perier, M.-C., Robidel, E., Mandet, C., Hagège, A., Bruneval, P., Larghero, J., Agbulut, O., and Menasche, P. Long-term functional benefits of human embryonic stem cell-derived cardiac progenitors embedded into a fibrin scaffold. *J Heart Lung Transplant* 2014. DOI: 10.1016/j.healun.2014.10.008.
35. Sun, C.-K., Zhen, Y.-Y., Leu, S., Tsai, T.-H., Chang, L.-T., Sheu, J.-J., Chen, Y.-L., Chua, S., Chai, H.-T., Lu, H.-I., Chang, H.-W., Lee, F.-Y., and Yip, H.-K. Direct implantation versus platelet-rich fibrin-embedded adipose-derived mesenchymal stem cells in treating rat acute myocardial infarction. *Int J Cardiol* **173**, 410, 2014.
36. Mauritz, C., Schwanke, K., Reppel, M., Neef, S., Katsirntaki, K., Maier, L.S., Nguemo, F., Menke, S., Hausteiner, M., Hescheler, J., Hasenfuss, G., and Martin, U. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation* **118**, 507, 2008.
37. Van Laake, L.W., Passier, R., Monshouwer-Kloots, J., Nederhoff, M.G., Ward-Van Oostwaard, D., Field, L.J., Van Echteld, C.J., Doevendans, P.A., and Mummery, C.L. Monitoring of cell therapy and assessment of cardiac function using magnetic resonance imaging in a mouse model of myocardial infarction. *Nat Protoc* **2**, 2551, 2007.
38. Takagawa, J., Zhang, Y., Wong, M.L., Sievers, R.E., Kapasi, N.K., Wang, Y., Yeghiazarians, Y., Lee, R.J., Grossman, W., and Springer, M.L. Myocardial infarct size measurement in the mouse chronic infarction model: comparison of area- and length-based approaches. *J Appl Physiol* **102**, 2104, 2007.

39. Kensah, G., Roa Lara, A., Dahlmann, J., Zweigerdt, R., Schwanke, K., Hegermann, J., Skvorc, D., Gawol, A., Azizian, A., Wagner, S., Maier, L.S., Krause, A., Dräger, G., Ochs, M., Haverich, A., Gruh, I., and Martin, U. Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. *Eur Heart J* **34**, 1134, 2013.
40. Nicodemus, G.D., and Bryant, S.J. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B Rev* **14**, 149, 2008.
41. Guo, H.-D., Wang, H.-J., Tan, Y.-Z., and Wu, J.-H. Transplantation of marrow-derived cardiac stem cells carried in fibrin improves cardiac function after myocardial infarction. *Tissue Eng Part A* **17**, 45, 2011.
42. Gutierrez-Aranda, I., Ramos-Mejia, V., Bueno, C., Munoz-Lopez, M., Real, P.J., Mácia, A., Sanchez, L., Ligerio, G., Garcia-Parez, J.L., and Menendez, P. Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection. *Stem Cells* **28**, 1568, 2010.
43. Dai, W., Wold, L.E., Dow, J.S., and Kloner, R.A. Thickening of the infarcted wall by collagen injection improves left ventricular function in rats: a novel approach to preserve cardiac function after myocardial infarction. *J Am Coll Cardiol* **46**, 714, 2005.
44. Yu, J., Christman, K.L., Chin, E., Sievers, R.E., Saeed, M., and Lee, R.J. Restoration of left ventricular geometry and improvement of left ventricular function in a rodent model of chronic ischemic cardiomyopathy. *J Thorac Cardiovasc Surg* **137**, 180, 2009.

Address correspondence to:

Sebastian V. Rojas, MD

Department of Cardiothoracic,

Transplantation and Vascular Surgery

Hannover Medical School

Carl-Neuberg-Str. 1

Hannover 30625

Germany

E-mail: rojas.sebastian@mh-hannover.de

Received: September 8, 2014

Accepted: March 16, 2015

Online Publication Date: June 15, 2015