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Intramyocardial Peptide Nanofiber Injection Improves Postinfarction Ventricular Remodeling and Efficacy of Bone Marrow Cell Therapy in Pigs

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Background—Growing evidence suggests that intramyocardial biomaterial injection improves cardiac functions after myocardial infarction (MI) in rodents. Cell therapy is another promising approach to treat MI, although poor retention of transplanted cells is a major challenge. In this study, we hypothesized that intramyocardial injection of self-assembling peptide nanofibers (NFs) thickens the infarcted myocardium and increases transplanted autologous bone marrow mononuclear cell (MNC) retention to attenuate cardiac remodeling and dysfunction in a pig MI model.

Methods and Results—A total of 40 mature minipigs were divided into 5 groups: sham, MI+normal saline, MI+NFs, MI+MNCs, and MI+MNCs/NFs. MI was induced by coronary occlusion followed by intramyocardial injection of 2 mL normal saline or 1% NFs with or without 1×10^8 isolated autologous MNCs. NF injection significantly improved diastolic function and reduced ventricular remodeling 28 days after treatment. Injection of MNCs alone ameliorated systolic function only, whereas injection of MNCs with NFs significantly improved both systolic and diastolic functions as indicated by +dP/dt and −dP/dt (1214.5 ± 91.9 and -1109.7 ± 91.2 mm Hg/s in MI+NS, 1693.7 ± 84.7 and -1809.6 ± 264.3 mm Hg/s in MI+MNCs/NFs, respectively), increased transplanted cell retention (29.3 ± 4.5 cells/mm^2 in MI+MNCs and 229.4 ± 41.4 cells/mm^2 in MI+MNCs/NFs) and promoted capillary density in the peri-infarct area.

Conclusions—We demonstrated that NF injection alone prevents ventricular remodeling, whereas cell implantation with NFs improves cell retention and cardiac functions after MI in pigs. This unprecedented combined treatment in a large animal model has therapeutic effects, which can be translated to clinical applications in the foreseeable future. (***Circulation***. 2010; 122[suppl 1]:S132–S141.)

Key Words: biomaterials ∎ bone marrow mononuclear cells ∎ cardiac tissue engineering ∎ myocardial infarction

Congestive heart failure is a leading cause of death in the United States and other developed countries. The dominant cause of heart failure is loss of myocardium due to coronary artery disease and the limited regeneration potential of cardiomyocytes. Cardiac tissue engineering is a promising and actively developing area of research aiming to repair, replace, and regenerate the myocardium. Several studies have demonstrated the feasibility of this approach and indicated that direct injection of biomaterials into the infarcted myocardium may be beneficial in preventing deleterious remodeling and reducing cardiac dysfunction.[1–4] Previous studies using intramyocardial injection of self-assembling peptide nanofibers (NFs), a highly biocompatible[5,6] and biodegradable[7] material, have also revealed their therapeutic potentials for angiogenesis, controlled drug/growth factor release, cell delivery, and stem cell recruitment.[5–10] These results indicate that NFs may impact a broad spectrum of applications in myocardial tissue engineering.

Cell therapy is another promising approach to heart disease treatments; however, there are many challenges that call for attention such as the extremely low retention and survival rates of implanted cells.[11] This issue is especially apparent in cardiac therapy due to the forbidding microenvironment of a high blood flow rate in the heart and a high degree of ventricular remodeling after myocardial infarction (MI).[12] Thus, it may be necessary to inject cells contained within

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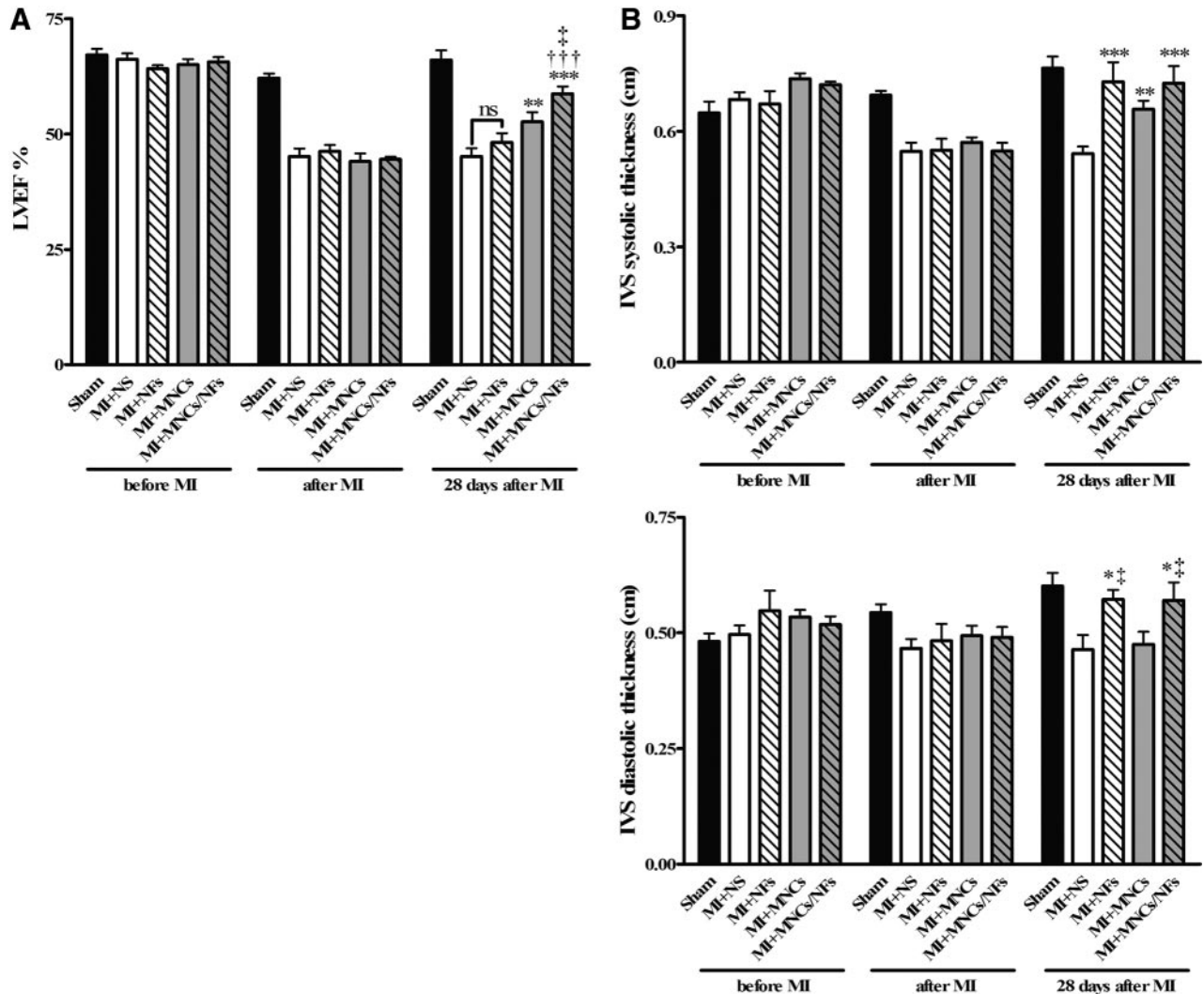
Figure 1. Injection of peptide NFs increases interventricular septum thickness, and injection of bone marrow MNCs improves systolic functions after infarction. A, Histogram of LVEF before, immediately after, and 28 days after MI in sham and experimental groups. B, Statistical analysis of interventricular septum thickness at systole and diastole. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus MI+NS; †††$P<0.001$ versus MI+NFs; ‡$P<0.05$ versus MI+MNCs.

vehicles or biomaterials coupled with nutritional factors to increase cell retention and cell survival rates.[13] Lastly, the lack of angiogenesis in the MI zone is also a critical concern regarding support, survival, and grafting of implanted cells into the host tissue.[14]

Small animal models have been used to attain exciting and promising achievements by different combinations of the mentioned approaches. In contrast, large animal model studies have not been widely performed[15,16] but are crucial to further investigation of clinical applications. Therefore, we carried out experiments to test the hypothesis that intramyocardial injection of NFs thickens the infarcted myocardium and increases cell retention such that attenuation of post-MI cardiac remodeling and dysfunction can be achieved in a pig model of experimental MI.

Methods

A total of 40 sexually mature minipigs (approximately 5 months old, acquired from National Taitung Animal Propagation Station and housed at NCKU Animal Center.) were divided into 5 groups: sham operation, which was performed by opening the chest without coronary artery ligation (sham), MI+normal saline (NS), MI+NFs, MI+autologous bone marrow mononuclear cells (MNCs), and MI+MNCs along with NFs (MNCs/NFs; n=8 in each group). MI was induced by permanent occlusion of the midleft anterior descending coronary artery immediately followed by injection of a total of 2 mL NS or 1% NFs divided among approximately 40 injections into the infarcted area. Freshly isolated 1×10^8 autologous MNCs were mixed in 2 mL NS or NFs for injection. Cardiac functions were assessed by echocardiography before and immediately after MI and together with hemodynamic measurements through catheterization 4 weeks later (Supplemental Figure I, II). For additional information, see online Supplemental Material (available at http://circ.ahajournals.org).

Results

Injection of Peptide NFs Alone Increases Interventricular Septum Thickness and Prevents Ventricular Remodeling After Infarction

The successful induction of MI was confirmed by a comparably decreased left ventricular ejection fraction (LVEF) immediately postinfarction among groups (LVEF: 62.1±1.0% in sham,

Table. Hemodynamic Parameters at 1 Month After MI

Parameter	Sham (n=8)	MI+NS (n=8)	MI+NFs (n=8)	MI+MNCs (n=8)	MI+MNCs/NFs (n=8)
Heart rate, beats/min	102.4±6.3	94.0±4.4	94.0±6.7	95.6±8.1	99.4±6.8
LVESP, mm Hg	89.0±4.1	69.6±2.6	77.5±3.4	77.1±5.7	79.1±4.5
LVEDP, mm Hg	8.3±0.7	12.7±0.9	8.6±1.0†	8.6±0.7†	8.5±0.5†
LVESV, mL	35.6±3.6	103.0±7.0	73.7±3.6‡	58.7±2.4‡§	51.7±4.6‡‖
LVEDV, mL	96.8±6.4	147.5±7.0	120.3±7.0†	115.0±5.7†	113.6±6.1†
SV, mL	64.9±8.6	44.5±5.8	52.0±5.0	56.2±4.8	61.8±5.0
CO, mL/min	6636.0±959.1	4133.3±482.2	5084.6±535.2	5247.0±433.3	5879.9±654.8
SW, mm Hg · mL	4870.5±532.4	2527.0±414.2	3689.1±511.4	4114.5±533.6	4567.0±809.4
AE, mm Hg/mL	1.52±0.12	0.89±0.07	1.26±0.08*	1.12±0.09*	1.29±0.10*
+dP/dt, mm Hg/s	1852.7±82.9	1214.5±91.9	1419.4±71.8	1602.1±135.6*	1693.7±84.7†
−dP/dt, mm Hg/s	−2048.8±293.1	−1109.7±91.2	−1751.0±86.9*	−1401.4±127.6	−1809.6±264.3*
τ (Weiss method), ms	30.8±0.8	42.1±3.4	33.0±1.9*	34.5±0.9*	32.1±1.7*
PRSW, mm Hg	65.1±8.4	42.2±5.6	55.8±6.8	60.5±5.9	65.1±13.8
ESPVR, mm Hg/mL	1.6±.2	0.7±0.1	1.2±0.2	1.3±0.2	1.5±0.2
EDPVR, mm Hg/mL	0.05±0.02	0.06±0.02	0.05±0.02	0.06±0.02	0.05±0.02
E_{max}, mm Hg/mL	3.6±0.4	1.4±0.2	2.9±0.2*	2.5±0.5	3.3±0.4†
+dP/dt-EDV, mm Hg/s/mL	14.3±4.5	6.5±1.6	9.4±3.0	9.5±2.1	12.0±2.9

Values are mean±SEM.
*$P<0.05$ versus MI+NS.
†$P<0.01$ versus MI+NS.
‡$P<0.001$ versus MI+NS.
§$P<0.05$ versus MI+NFs.
‖$P<0.01$ versus MI+NFs.
LVESP indicates left ventricular end systolic pressure; LVEDP, left ventricular end diastolic pressure; LVESV, left ventricular end systolic volume; LVEDV, left ventricular end diastolic volume; SV, stroke volume; CO, cardiac output; SW, stroke work; AE, arterial elastance; τ, time constant of left ventricular pressure decay; PRSW, preload recruitable stroke work; ESPVR, end diastolic pressure–volume relationship; EDPVR, end systolic pressure–volume relationship; E_{max}, maximum chamber elasticity; EDV, end diastolic volume.

45.1±1.7% in MI+NS, 46.3±3.7% in MI+NFs, 44.0±5.0% in MI+MNCs, 44.5±1.6% in MI+ MNCs/NFs; all $P<0.001$ versus sham; Figure 1A).

At 28 days after MI, 68.5±2.3% NFs were retained in the injected regions as determined by high-performance liquid chromatography (Supplemental Figure III). Injection of NFs alone significantly increased both systolic and diastolic interventricular septum thickness (systole: 0.54±0.02 cm and diastole: 0.46±0.03 cm in the MI+NS group; and systole: 0.73±0.05 cm and diastole: 0.57±0.02 cm in the MI+NFs group; Figure 1B). Furthermore, both left ventricular left ventricular end diastolic volume and left ventricular end systolic volume were improved by NF injection (Table), suggesting that this significantly prevented post-MI left ventricular dilatation. NF injection significantly increased the ratio of scar thickness and decreased the ratio of scar length in the infarct (scar thickness ratio: 66.8±2.6% and scar length ratio: 26.0±1.1% in the MI+NS group, and scar thickness ratio: 85.1±5.4% and scar length ratio: 21.0±1.5% in the MI+NFs group; Figure 2). Together these findings imply that the scar expansion and left ventricular remodeling after infarction were prevented by NF injection.

In addition, the post-MI diastolic function was significantly improved after NF injection (−dP/dt: −1109.7±91.2 mm Hg/s in the MI+NS group, and −1751.0±86.9 mm Hg/s in the MI+NFs group; Table). Consistently, the collagen content at the remote zone (collagen content: 22.5±0.7% in the MI+NS group and 13.0±0.9% in the MI+NFs group; Figure 3) and the global

elasticity (as assessed by arterial elastance and maximum chamber elasticity; Table) both significantly revealed improvement of cardiac compliance by NF injection. However, estimation of LVEF by echocardiography and +dP/dt by catheterization showed no improvement by NF injection (Figure 1A; Table). Combining the finding that there was no significant difference in the ratio of necrotic area between MI+NS and MI+NFs groups (Figure 2C), these results suggest that NF implantation alone may not be sufficient for recovery of cardiac performance and cell implantation may be required to increase the contractile component for the improvement of systolic function.

Injection of Peptide Nanofibers Along With Autologous Bone Marrow MNCs Improves Both Systolic and Diastolic Functions After Infarction

At 28 days after infarction, injection of autologous bone marrow MNCs alone significantly increased LVEF, systolic interventricular septum thickness, and +dP/dt (Figure 1; Table), suggesting that MNC injection improved systolic function after MI. However, neither diastolic interventricular septum thickness nor −dP/dt showed improvement by MNC injection (Figure 1B; Table). Combining the finding that there was no improvement in the maximum chamber elasticity between MI+NS and MI+MNCs groups (Table), these results suggest that MNC implantation alone may not be sufficient for prevention of ventricular remodeling after MI, and NF implantation may be required to increase cardiac compliance and diastolic function.
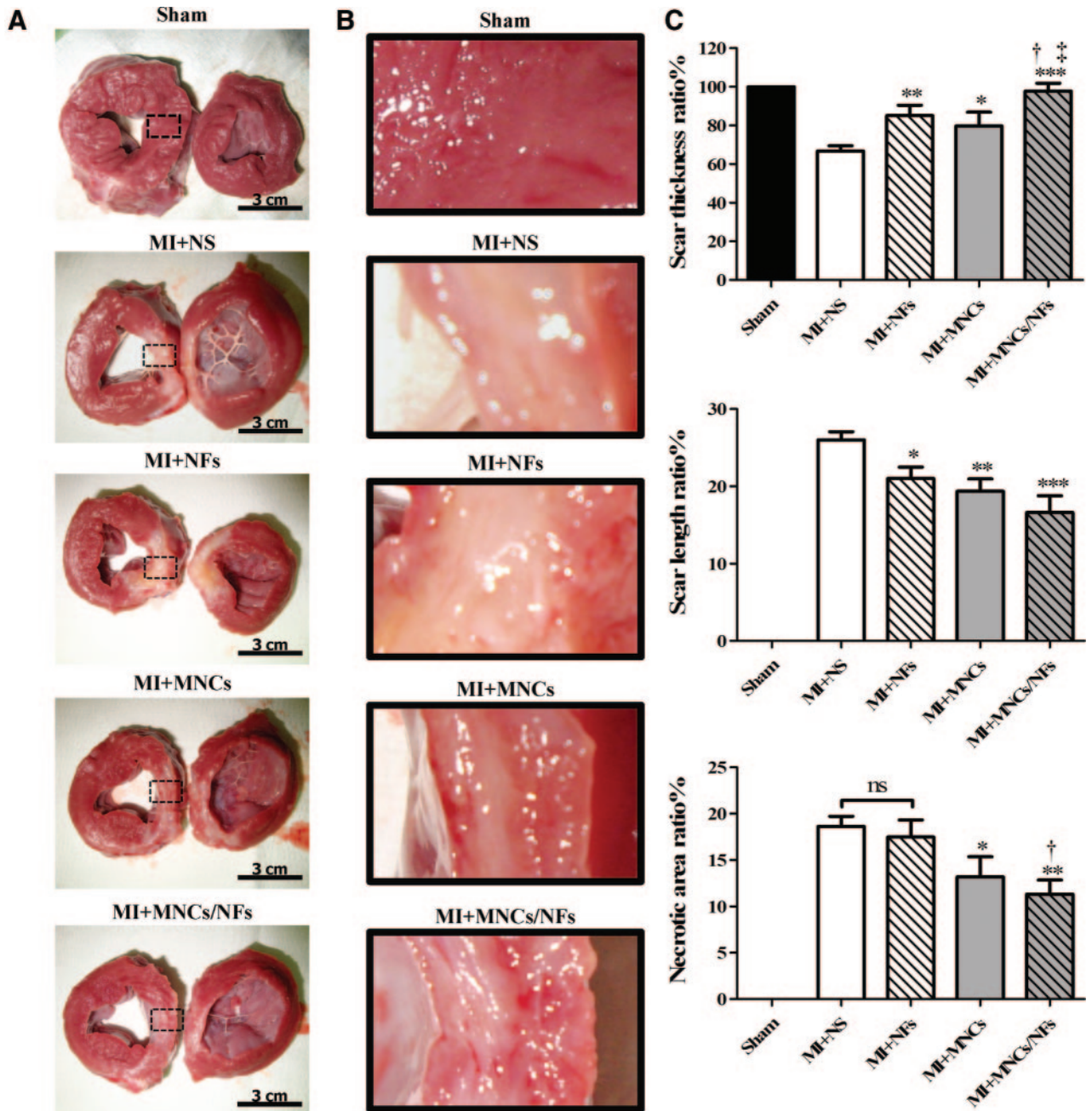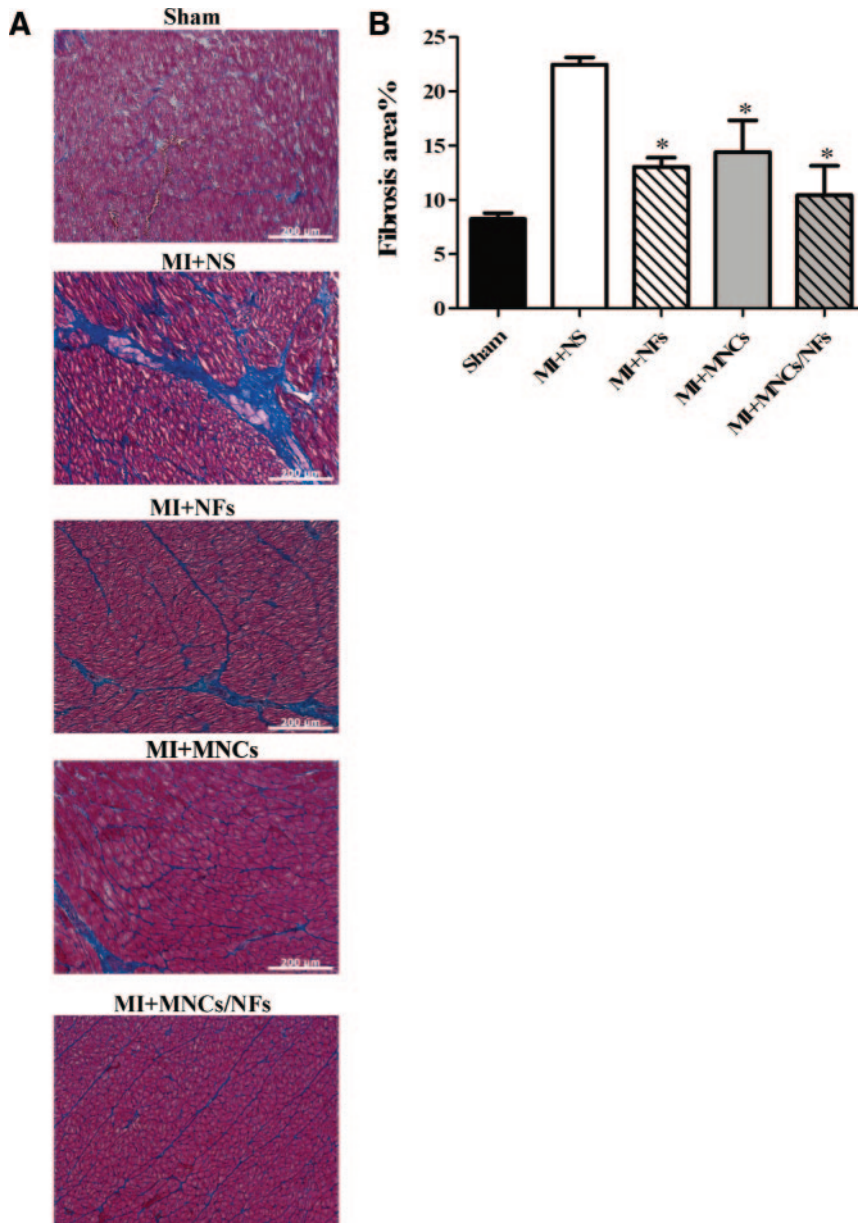
Figure 2. Injection of peptide NFs prevents infarct expansion, and injection of bone marrow MNCs improves tissue viability after infarction. A, Representative cross-section images at the level of papillary muscle insertion of left ventricle from each group. B, Magnified images of nonnecrotic tissue in the peri-infarct zone indicated by the dashed boxes in A. C, Percentages of scar thickness ratio, scar length ratio, and necrotic area ratio determined using computer image analysis. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus MI+NS; †$P<0.05$ versus MI+NFs; ‡$P<0.05$ versus MI+MNCs.

Confirming this hypothesis, we found that the LVEF ($66.0\pm1.0\%$ in the sham group, $45.2\pm1.8\%$ in the MI+NS group, $48.2\pm2.0\%$ in the MI+NFs group, $52.6\pm2.1\%$ in the MI+MNCs group, $58.7\pm1.6\%$ in the MI+MNCs/NFs group; Figure 1A), left ventricular end diastolic pressure, left ventricular end systolic volume, left ventricular end diastolic volume, AE, +dP/dt, −dP/dt, τ, and maximum chamber elasticity were all significantly improved as a result of MNC/NF injection (Table). Although injection of MNCs alone without NFs also improved systolic function after MI, only injection of MNCs combined with NFs improved both systolic and diastolic functions.

Injection of Peptide NFs Along With Autologous Bone Marrow MNCs Decreases Necrotic Tissue and Collagen Content in the Remote Area After Infarction

Consistent with the finding that MNC/NF injection improved both systolic and diastolic functions after MI, the necrotic tissue was significantly reduced after infarction ($18.6\pm1.1\%$

A

Sham

MI+NS

MI+NFs

MI+MNCs

MI+MNCs/NFs

B

Figure 3. Injection of peptide NFs protects the remote zone from fibrosis after infarction. A, Representative images of collagen content at the remote zone from each group. B, Statistical analysis of collagen content from images such as those in A. *$P<0.05$ versus MI+NS.

in the MI+NS group, $17.5\pm1.9\%$ in the MI+NFs group, $13.2\pm2.2\%$ in the MI+MNCs group, and $11.3\pm1.5\%$ in the MI+MNCs/NFs group; Figure 2C). Furthermore, injection of MNCs/NFs significantly attenuated collagen content in the remote area after infarction, but a similar effect was also detected in the other 2 groups of NFs injection alone and MNCs injection alone ($22.5\pm0.7\%$ in the MI+NS group, $13.0\pm0.9\%$ in the MI+NFs group, $14.4\pm3.0\%$ in the MI+MNCs group, and $10.5\pm2.7\%$ in the MI+MNCs/NFs group; Figure 3).

Injection of Peptide NFs Along With Bone Marrow MNCs Increases Transplanted Cell Retention and Differentiation as Well as Angiogenesis After Infarction

At the border or infarct zone of treated myocardium, our results showed a higher DiI$^+$ cell density in the MI+MNCs/NFs group than in the MI+MNCs group (29.3 ± 4.5 /mm^2 in

the MI+MNCs group and 229.4 ± 41.4/mm^2 in the MI+MNCs/NFs group, $P<0.001$; Figure 4). This result indicates that NFs also serve as a delivery vector to improve the retention of transplanted cells after intramyocardial injection.

To confirm the differentiation fate of the injected MNCs, we used costaining of primary antibodies with DiI signal to trace these cells. To rule out the cytoplasmic autofluorescence, the DiI signal was checked on multiple wavelengths and was detectable only under red wavelength (Supplemental Figure IVA); serial sections with DiI staining only were also used to confirm that the detected signals were dependent on the primary antibodies to these epitopes (Supplemental Figure IVB–C). Surprisingly, we found that 28 days after injection, the surviving MNCs mainly differentiated into endothelial cells as indicated by von Willebrand factor, VE-cadherin, isolectin, and CD31 staining and few into smooth muscle cells (SM myosin heavy chain, SM22α, and
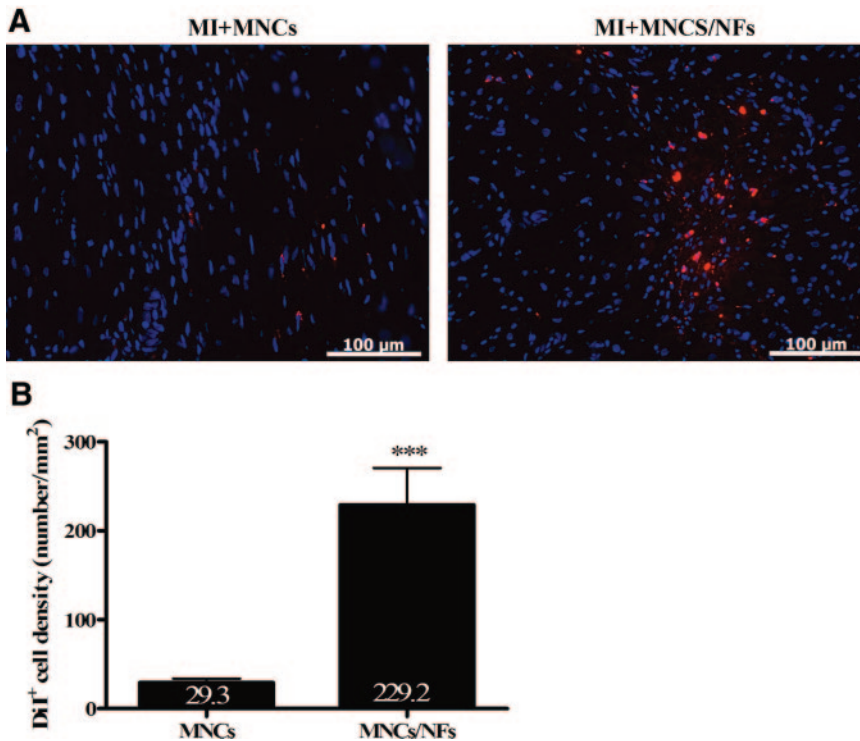
Figure 4. Injection of peptide NFs along with bone marrow MNCs improves transplanted cell retention after infarction. A, Representative pictures of DiI^{+} cells (red) in the area injected with cells alone (left panel) or with cells and NFs (right panel). Nuclei were stained by 4′,6-diamidino-2-phenylindole (blue). B, Cell retention rates as reflected by DiI[+] cell counts. ***$P < 0.001$ versus MI+MNCs.

SMα-actin staining), whereas no evidence of differentiation into cardiomyocytes (stained by cardiac tropomyosin, Nkx2.5, and GATA4; Figure 5; Supplemental Figure V and data not shown). We also detected MNC differentiation into hematopoietic lineage cells (stained by CD45 and an antibody against macrophage; Supplemental Figure VI). Interestingly, when NFs were injected along with MNCs, we found a higher differentiation ratio of MNCs into endothelial cells and smooth muscle cells and still no evidence of cardiomyocytes (MNCs: ratio of endothelial cell differentiation, 56.3±8.8% and ratio of smooth muscle cell differentiation, 3.3±2.1%; MNCs/NFs: ratio of endothelial cell differentiation, 83.0±4.9% and ratio of smooth muscle cell differentiation, 14.5±3.5%; Figure 5). These results indicated that one of the benefits of MNC treatment is angiogenesis and this effect is enhanced by injection of MNCs along with NFs. Supporting the results of MNC differentiation into endothelial cells, there was significant additional increase of capillary density in the peri-infarct zone after treatment with NFs along with MNCs (capillary density: 190.4±20.8 number/mm^2 in the MI+NS group, 352.6±33.5 number/mm^2 in the MI+NFs group, 533.8±53.9 number/mm^2 in the MI+MNCs group, and 644.8±64.6 number/mm^2 in the MI+MNCs/NFs group; Figure 6).

Injection of NFs Reduces C-Reactive Protein Levels After Infarction

Although the biocompatibility of NFs is well established,[5] it has not yet been confirmed in pigs, so we measured C-reactive protein (CRP) levels in the plasma to detect chronic inflammation. There was no CRP level increase at 28 days after NF injection compared with the control. However, CRP is known to rise as a result of MI itself,[17] and our results

confirmed this (sham versus MI only). Surprisingly, all treatment groups showed markedly reduced CRP levels, and the combined NF+MNC group showed the greatest decrease (CRP: 19.5±2.2 μg/mL for sham, 50.0±11.2 μg/mL in the MI+NS group, 26.7±4.0 μg/mL in the MI+NFs group, 29.0±6.4 μg/mL in the MI+MNCs group, and 24.7±4.6 μg/mL in the MI+MNCs/NFs group; Figure 7). Interestingly, we also detected significantly decreased leukocyte infiltration in the NF injection area, suggesting that NF injection may not stimulate inflammatory reaction in the infarcted myocardium, which is consistent with a previous study in rats (Supplemental Figure VII).[5]

Discussion

The outcome of intramyocardial biomaterial injection has been investigated in only a few studies. In contrast, cell therapy is a more commonly explored approach, but cell retention is an issue and is often briefly addressed or ignored. Although both these treatments have potential uses in therapy for cardiac diseases, they have rarely been tested in large animal models, an essential step before proceeding to clinical trials. In this study, we describe a novel approach that combines biomaterial injections with cell therapy and demonstrate that cardiac functions are regained in a large animal model of MI.

Local Repair by Biomaterial Injection Preserves Cardiac Geometry and Function

Jugdutt suggested that although extracellular matrix expression increases in the entire heart after MI, this increase is only beneficial in the infarct zone at the same time as being detrimental in the noninfarct zone.[18] In this study, we showed that NF treatment increased interventricular septum thickness
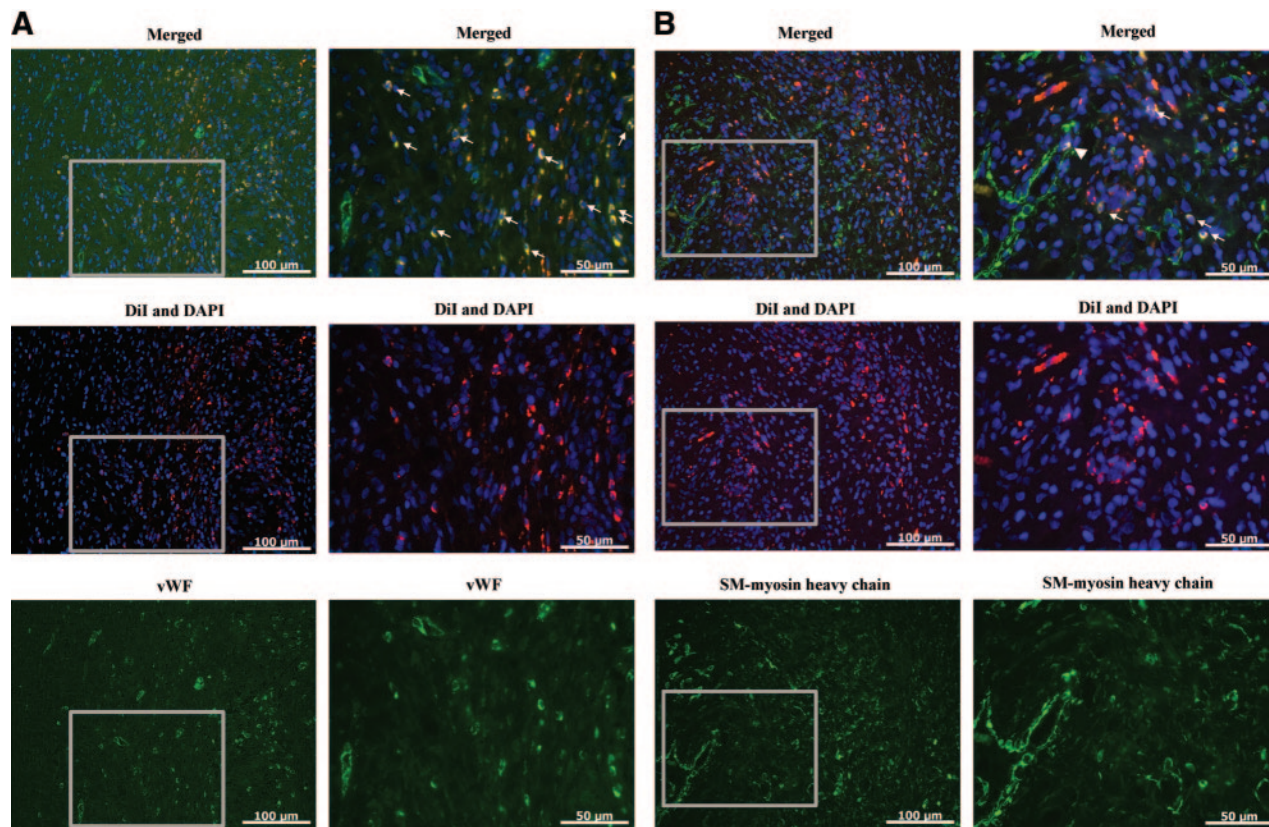
Figure 5. Injection of peptide NFs along with bone marrow MNCs improves transplanted cell differentiation into vascular cells. A–C, Representative images of red DiI fluorescence overlapped with green immunostaining for von Willebrand factor (vWF, A), SM myosin heavy chain (B), and cardiac tropomyosin (C). Nuclei were stained with 4′,6-diamidino-2-phenylindole (blue). The image (right panel) was magnified from left panel indicated in the boxes and costained cells were arrowed. The cell integrated into the vessel was indicated by an arrowhead in B. D, Quantification of ratio of cells stained with endothelial cell (EC), smooth muscle cell (SMC), and cardiomyocyte (CM) markers.

after MI, restrained scar extension, and prevented further harmful fibrosis at the remote zone. Moreover, reduction in global cardiac remodeling and diastolic dysfunction after MI was achieved. Interestingly, these results were very similar to a numeric simulation model proposed by Wall et al, which indicated that intramyocardial noncontractile material injection had all of these effects as well as a reduction in elevated myofiber stresses.[2]

Landa et al reported that intramyocardial biomaterial injection preserves cardiac systolic function 2 months after MI in a rodent model.[3] In contrast, we found that NF injection did not improve systolic function at 28 days after MI in pigs. This inconsistency may be due to the different materials, time points, and animal models chosen for the 2 studies. Because the heart failure indices atrial natriuretic peptide and B-type natriuretic peptide showed no difference between the sham and MI+NS groups (data not shown), the heart of a large animal like the pig may still undergo remodeling beyond 28 days after MI, so long-term studies of the effects of NF injection are needed.

Cell Retention Determines Cardiac Functional Improvement by Cell Therapy

Bone marrow MNC transplantation has been reported to benefit the infarcted myocardium.[19] We demonstrated similar contributions to cardiac functional improvement after MNC implantation in pigs. Beyond direct MNC injection alone, injection of MNCs along with NFs showed even better amelioration of cardiac function. We believe these beneficial results were mainly due to the ability of NFs to increase cell retention. Furthermore, consistent with our previous studies,[6,20] the presence of NFs did not alter the viability of MNCs, which remained approximately 95% viable before injection (Supplemental Methods). NF injection may also increase the cell survival rate due to the increase in capillary density at the border zones 28 days after MI. From this point of view, coinjection of cells with NFs may be highly beneficial for both cell engraftment and angiogenesis effects due to increases in cell retention and survival rates.

Beyond cell retention and survival, evidence from our results also showed an increase in MNC differentiation into endothelial cells and smooth muscle cells after injection of MNCs/NFs compared with injection of MNCs alone. The NFs may act as a scaffold that provides a suitable microenvironment for the MNCs to adhere and perform normal cellular functions.[6] Furthermore, differentiation of MNCs into endothelial cells and smooth muscle cells promotes angiogenesis and prevents further apoptosis of cardiomyocytes within the infarct zone, leading to the preservation of cardiac function.[21] These results demonstrate the synergistic effect of NF and MNC injection.
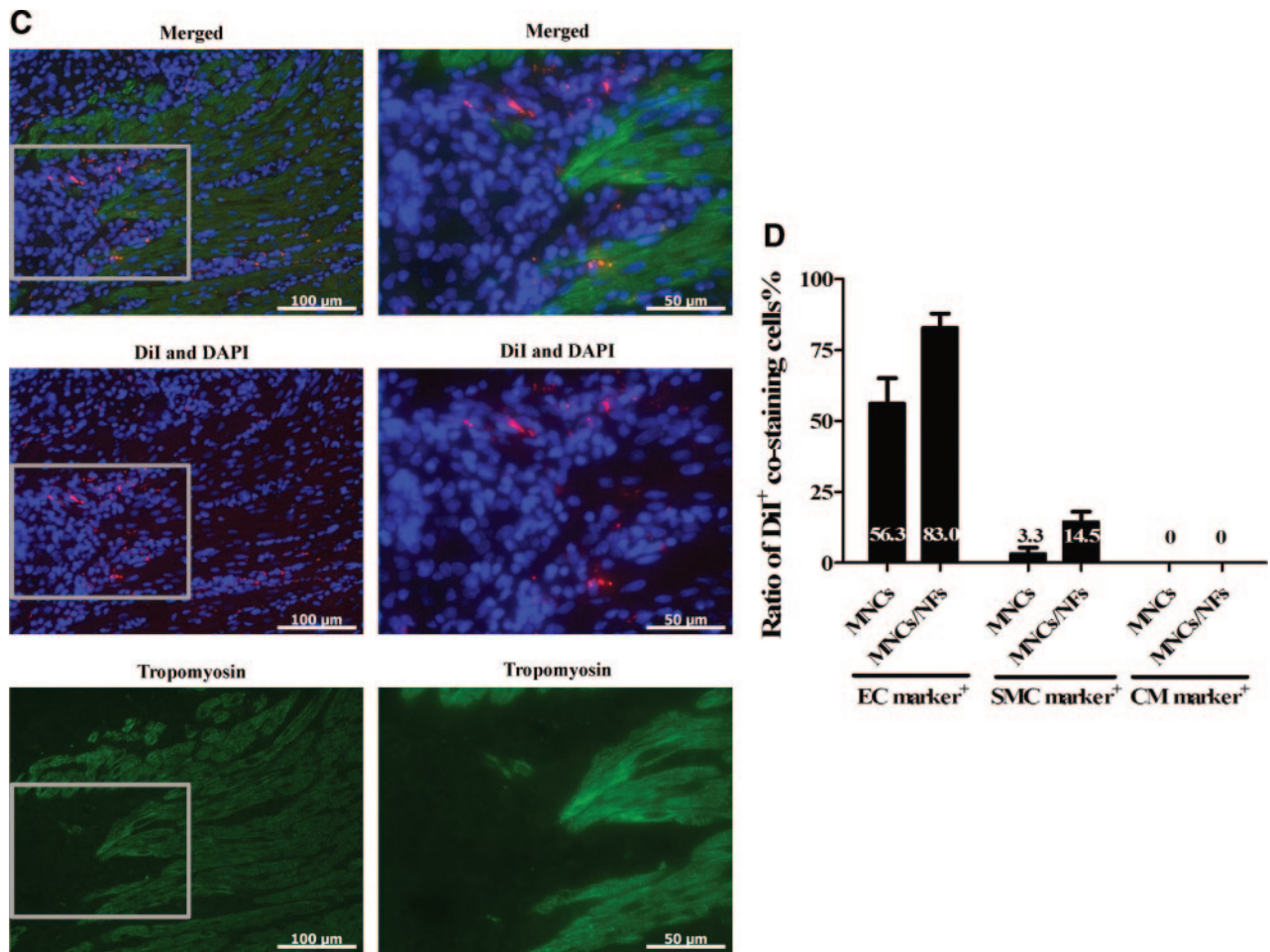
Figure 5 (Continued).

Combined MNC/NF Injection Has Complementary Effects for Clinical Application

MNC injection alone significantly increased systolic but not diastolic function after MI, whereas NF injection alone increased diastolic but not systolic function. Importantly, the combined injection of MNCs and NFs improved both systolic and diastolic function. This combination thus acts in a complementary fashion to synergistically benefit cardiac function and is now shown to be feasible in a large animal model with induced acute MI. Because most cardiac patients have chronic heart disease, whether this treatment is applicable for chronic MI cases as well the appropriate time window requires further investigation.

The different conditions of the treatments in this study such as dosage, timing, and location of injections were tuned to be optimal for 25-kg minipigs. Modification is necessary for clinical trial models. However, one exception is the requirement for spread-out injections to achieve a well dispersed administration of the cells and NFs. In the future, open-chest intramyocardial injection may be replaced by a catheter transendocardial approach or minimally invasive thoracotomy for clinical use to minimize the risk and harm of surgery itself.

From a safety perspective, introduction of foreign materials into the body may be seen as hazardous, possibly leading to mechanical failure or arrhythmia caused by change in conductance of the heart. In our study, 2 of 42 animals died with intractable ventricular fibrillation on the operating table before any treatments were administered. There were no negative side effects due to NF treatments.

Mechanisms of MNC Therapy Remain to Be Determined

The mechanisms of cardiac functional improvement by bone marrow cell therapy are still under investigation.[12,13] Controversies exist concerning myocyte differentiation,[22,23] paracrine effects,[24] and whether stimulation causes endogenous cardiac repair.[25] Another interesting question is if the transplanted bone marrow cells may integrate into the pre-existing vessels or form entirely new vessels. As shown in Figure 5B and Supplemental Figure VIII, we observed not only integration of MNC-derived smooth muscle cells into pre-existing vessels, but also new vessels formed entirely from the injected MNCs. However, to what extent these effects contribute to the increase of functional improvement after MI remains unknown. Therefore, the specific pathways and mechanisms by which bone marrow cells act require further investigation.

Quevedo et al reported that 14% bone marrow cells differentiate into cardiomyocytes, but our data did not con-
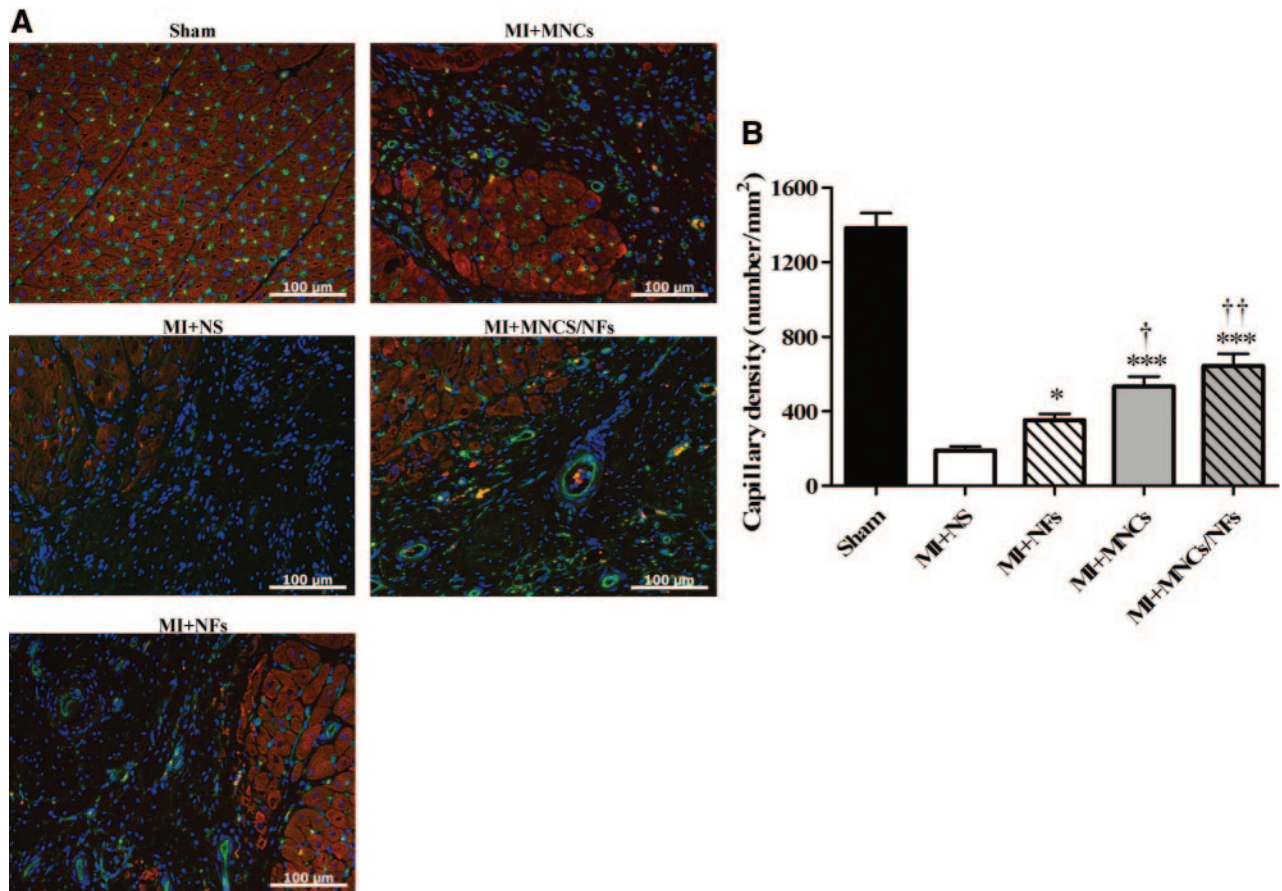
Figure 6. Injection of peptide NFs along with bone marrow MNCs improves capillary density in the peri-infarct area. A, Representative immunostaining of isolectin (green) overlapped with cardiomyocytes stained with cardiac tropomyosin (red) at the border zone from each group. Nuclei were stained by 4′,6-diamidino-2-phenylindole (blue). B, Quantification of capillary density in the peri-infarct zone. *$P<0.05$, ***$P<0.001$ versus MI+NS; †$P<0.05$, ††$P<0.01$ versus MI+NFs.

firm this.[16] The major difference is that the cells they used were allogeneic bone marrow mesenchymal stem cells, which were conditioned by in vitro culture. On the other hand, the cells we used were autologous bone marrow MNCs, which are nonadherent and mainly consist of hematopoietic cells. In
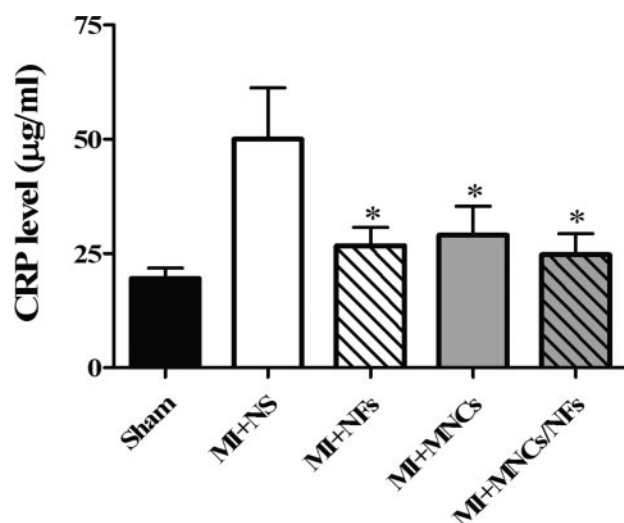
Figure 7. Injection of peptide NFs decreases CRP level after infarction. Statistics of CRP levels. *$P<0.05$ versus MI+NS.

dealing with the controversies surrounding the various results and explanations of bone marrow cell therapy effects, a more stringent approach or confirmation by double experimental methods should be carried out for more convincing results. For example, genetic linage tracking could be considered for tracing the injected cells in future studies.[25] Here, we attempted to simulate the clinical situation of a single procedure, rather than a sequence of steps, so DiI-labeled MNCs were chosen to allow convenient cell isolation and cell labeling concurrently in MI without a complicated and precarious preprocess.

In conclusion, the present study reveals that intramyocardial injection of NFs alone prevents pathological left ventricular remodeling, whereas injection of cells along with NFs helps raise the cell retention rate and improve cardiac performance 28 days after MI in pigs. To our knowledge, this is the first study to use this novel combined treatment approach in a large animal model and demonstrate positive therapeutic effects. We believe these methods and results can be translated to clinical applications in the foreseeable future.

Acknowledgments

Disclosures

None.

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