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Yi-Dong Lin *et al.*
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Editor's Summary

Nanomaterials Help the Heart to Heal

Normally, the cure for a broken heart is time. After a heart attack, or myocardial infarction (MI), however, time can work against the heart, allowing tissue remodeling, scar formation, and overall heart failure. In an effort to speed up the healing process after MI, Lin and colleagues have created self-assembling peptide nanofibers (NFs) that, when injected into the heart tissue immediately after MI, lead to rapid repair and functional recovery.

The authors first tested the NF with and without varying doses of vascular endothelial growth factor (VEGF) in a rat model. The material –growth factor combination was injected into the heart immediately after MI, and 28 days later had significantly improved cardiac function compared with NF or VEGF alone. The NF/VEGF treatment also prevented tissue remodeling and collagen deposition (which cause heart scarring) and reduced the infarct size. Moving to a large animal that more closely resembles human MI, Lin *et al.* injected the NF/VEGF combination material into heart tissue of pigs immediately after infarction and observed tissue repair and restored function, similar to the rat. The authors found that the NF created the optimal microenvironment for healing by promoting arteriogenesis (increased densities of arteries and arterioles) and by recruiting endogenous myofibroblasts and cardiomyocyte-like cells to the damaged tissue.

Moreover, for translation, the authors showed that their NF material helps to heal the heart in both small and large animal models, without harmful effects to other tissues. Before moving to patients, the material will need to be tested at later time points to mimic the sequence of events after a heart attack. Also, rather than direct myocardial injection, the material will likely need to be delivered via a minimally invasive catheter. With these considerations in mind, this promising NF/VEGF combination is ready to take a shot at healing the human heart.

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BIOMEDICAL ENGINEERING

Instructive Nanofiber Scaffolds with VEGF Create a Microenvironment for Arteriogenesis and Cardiac Repair

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Angiogenic therapy is a promising approach for tissue repair and regeneration. However, recent clinical trials with protein delivery or gene therapy to promote angiogenesis have failed to provide therapeutic effects. A key factor for achieving effective revascularization is the durability of the microvasculature and the formation of new arterial vessels. Accordingly, we carried out experiments to test whether intramyocardial injection of self-assembling peptide nanofibers (NFs) combined with vascular endothelial growth factor (VEGF) could create an intramyocardial microenvironment with prolonged VEGF release to improve post-infarct neovascularization in rats. Our data showed that when injected with NF, VEGF delivery was sustained within the myocardium for up to 14 days, and the side effects of systemic edema and proteinuria were significantly reduced to the same level as that of control. NF/VEGF injection significantly improved angiogenesis, arteriogenesis, and cardiac performance 28 days after myocardial infarction. NF/VEGF injection not only allowed controlled local delivery but also transformed the injected site into a favorable microenvironment that recruited endogenous myofibroblasts and helped achieve effective revascularization. The engineered vascular niche further attracted a new population of cardiomyocyte-like cells to home to the injected sites, suggesting cardiomyocyte regeneration. Follow-up studies in pigs also revealed healing benefits consistent with observations in rats. In summary, this study demonstrates a new strategy for cardiovascular repair with potential for future clinical translation.

INTRODUCTION

Ischemic cardiovascular diseases, such as myocardial infarction (MI), are the leading cause of morbidity and mortality worldwide (*1*). MI is caused by a shortage of the coronary blood supply to the myocardium and results in irreversible damage, including myocardial loss, pathological remodeling, cardiac dysfunction, and heart failure (*2*, *3*). Therefore, therapies that improve neovascularization may prevent pathological deterioration and ameliorate cardiac function after MI (*3*, *4*).

Vascular endothelial growth factor (VEGF) is a key factor for both angiogenesis and vasculogenesis (*5*–*7*). Owing to the efficacy of this factor, therapies with VEGF for the treatment of ischemic diseases have attracted great attention. However, until recently, most clinical trials have not provided convincing evidence for the therapeutic use of VEGF (*4*, *8*, *9*). It has been reported in vitro and in mouse models that the angiogenic efficacy of these therapies is challenged by the ability to maintain the local VEGF concentration at an effective level (*9*–*13*). Sequential vessel regression within 2 weeks after VEGF delivery also restricts functional revascularization because of the lack of additional regulation (*9*, *14*, *15*). To avoid these pitfalls, it is crucial to provide an extracellular matrix (ECM) and recruit mural cells, such as pericytes or vascular smooth muscle cells (SMCs), to envelope and stabilize the nascent fragile endothelial tubes and subsequently achieve capillary stability and durable arteriogenesis (*14*–*17*). Therefore, the creation of an intramyocardial microenvironment with angiogenic incitation and arteriogenic support is likely key to promoting functional neovascularization after MI.

Cumulative studies have revealed that it is possible to use nanotechnology and biomaterials to recapitulate biomimic milieu and manipulate cell activities in vitro (*18*–*20*). Such innovation has opened a new field in regenerative medicine far beyond the original scope of using biomaterials merely for controlled drug release. Accordingly, how to create an engineered niche in vivo that stimulates the tissue regeneration capability is an emerging and imperative question (*21*–*24*). For example, Martino *et al.* recently demonstrated the creation of an engineered microenvironment for bone healing in vivo, which recruits growth factors to the wound and convinces cells to repair the damage (*25*). However, engineered microenvironments with curative efficacy are rarely successful from "bench to bedside," especially for cardiac tissue repair, because of the complexity of the native tissue construction and morphogenesis (*26*–*28*).

Self-assembling peptide nanofibers (NFs) are short oligopeptides that self-assemble into nanofibrous gel when mixed with salt solution at physiological pH (*29*). These unique properties make NFs not only slowly degradable and low in immunogenicity (*30*, *31*) but also therapeutically potent for sustained release of a drug or growth factor via noncovalent coupling or covalent bonding (*31*–*34*). Specifically, recent studies have demonstrated that NF injections give rise to an intramyocardial microenvironment that promotes vascular cell infiltration and maturation (*30*, *35*, *36*). Accordingly, we hypothesized that the injection of VEGF along with NF creates an intramyocardial microenvironment

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suitable for vascular cell recruitment, proliferation, and maturation and would thereby improve neovascularization and cardiac performance after MI in rodents.

RESULTS

Intramyocardial NF/VEGF improves cardiac performance in rats after MI

To verify the functional role of VEGF in vitro, we tested recombinant human $VEGF_{165}$ (165 is the subtype isoform of VEGF) on rat endothelial cells and confirmed that VEGF had a dose-dependent effect on endothelial cell proliferation and reached a saturated functional dose at 10 ng/ml (fig. S1A). This was supported by an increasing level of extracellular signal–regulated kinase (ERK) phosphorylation, which showed a similar dependence on VEGF dose (fig. S1B). For our in vivo studies, a dose of VEGF (100 ng/ml, V100) 10-fold higher than that applied in vitro was used for intramyocardial injection because the weight of the myocardium at the injection site was about 10-fold greater than that of the injected NF (80 mg of NF per 720 mg of myocardium), as described previously (*30*, *36*). In addition, a 10-fold higher dose of VEGF (1000 ng/ml, V1000) was used to test the dose effect of VEGF.

To confirm whether the NF could carry this concentration of VEGF via physical noncovalent interaction, we calculated the maximal binding capacity of NF for VEGF, which was about 3000 to 10,000 ng/ml (fig. S2A). Next, the release of VEGF (100 and 1000 ng/ml) from NF was found to last for at least 14 days in vitro (fig. S2, B and C), which is consistent with the findings reported by Gelain *et al.* in fibers with a similar sequence (*37*). Finally, we investigated the in vivo release kinetics of VEGF injected into rat myocardium immediately after MI, with or without NF (Fig. 1A). We confirmed that both VEGF deliveries (100 and 1000 ng/ml) were retained within the myocardium for 14 days when injected with NF (Fig. 1, B and C).

We used a rat experimental MI model to examine the therapeutic effects of intramyocardial NF/VEGF injection. At 28 days after MI, the intramyocardial NF/VEGF injection significantly improved cardiac systolic function compared with NF or VEGF alone, as indexed by the left ventricular fractional shortening (LVFS) (Fig. 1D). Correspondingly, the NF/VEGF injection also effectively prevented pathological remodeling and ventricular dilation as evidenced by restrained infarct size (Fig. 1E and fig. S3A) and reduced collagen deposition in the non-infarct region (fig. S3, B and C). NF/VEGF treatments also decreased the left ventricle end-systolic and end-diastolic diameters (LVESD and LVEDD, respectively) (Fig. 1, F and G). In contrast, although there was a marginal dose-dependent amelioration of the LVFS, infarct size, collagen deposition of non-infarct region, LVESD, and LVEDD for the VEGF-treated groups at 28 days after MI, there was no significant difference between the phosphate-buffered saline (PBS) and the VEGF-only treatment groups, which indicates that the delivery of VEGF alone was not sufficient to provide cardiac benefits after MI.

NF/VEGF promotes arteriogenesis after MI

Next, the capillary density of the different treatment groups was investigated. A dose-dependent increase was observed after VEGF treatment without NF (fig. S4), suggesting that angiogenesis is achieved by injection of VEGF alone after MI. Moreover, injection of NF alone also significantly improved the level of angiogenesis after MI, indicating that NF provides an angiogenic environment, a finding consistent with previous studies (*30*, *36*). Surprisingly, there was no difference between the groups that were subjected to VEGF (1000 ng/ml), NF/V100, or NF/V1000 treatment after MI. This result is in contrast to the findings reported above where NF/VEGF injection provided a greater cardiac benefit than injection of VEGF alone, suggesting that angiogenesis is unlikely to be the only mechanism involved in the modulation of cardiac function.

Looking at other possible mechanisms, we found that injecting NF/VEGF significantly increased the vascular densities of both arterioles and arteries at 28 days after MI (Fig. 2, A to C). In contrast, treatment with VEGF alone after MI did not demonstrate a significant effect in terms of arteriogenesis at the peri-infarct area. These results support the idea that arteriogenesis, not angiogenesis, plays a crucial role in improving cardiac performance after MI. Furthermore, there appears to be an increase in both arteries and veins on the basis of vascular wall thickness after injecting VEGF with or without NF. It is interesting that the percentage of veins within large vessels in the myocardium is higher when receiving VEGF (1000 ng/ml) alone than with either of the NF treatments with VEGF (Fig. 2D), indicating that merely increasing VEGF dose without providing essential support may not be sufficient to form strong large vessels.

Local delivery of NF/VEGF does not cause systemic harmful effects

It was reported that the risk of hypotension, proteinuria, and general edema was raised because of vascular leakage resulting from increased VEGF dose (*5*, *7*, *9*, *38*). To test whether the high-dose VEGF injection would cause any systemic adverse effects, we designed an additional experiment to examine vascular leakage into the vital organs (Fig. 3A). Rats were injected with PBS, VEGF (1000 ng/ml), NF alone, or NF/V1000 within the myocardium, which was followed by intravenous injection of red fluorescent nanoparticles (FluoSpheres). Significantly more extravasated FluoSpheres were detected in the brain, lungs, liver, spleen, and kidneys of rats that had received VEGF only than in those that received NF/VEGF (day 0 group; Fig. 3B and fig. S5). This finding indicates that systemic vascular leakage is induced by the injection of high-dose VEGF. In contrast, the injection of NF/VEGF improved FluoSphere retention in the myocardium 7.8-fold compared with all other treatment groups (day 0 group; Fig. 3B and fig. S5). Consistent with these findings, the detrimental proteinuria was significantly increased in rats treated with VEGF alone, but not in those treated with NF/VEGF, as evidenced by examination of proteins within urine (Fig. 3C).

NFs create an intramyocardial microenvironment for myofibroblast engraftment

We conducted a time-course study to explore the underlying mechanism behind enhanced arteriogenesis after NF/VEGF injection. Rats were given intramyocardial injections of PBS, VEGF (100 ng/ml), NF, or NF/V100 after an experimental MI and were then sacrificed at 3, 7, or 14 days after MI. Our results demonstrated that α-smooth muscle actin–positive (α-SMA^+) myofibroblasts populated the infarcted myocardium of all groups at 3 days after MI (Fig. 4 and fig. S6). The myofibroblasts were preserved within the myocardium at 7 days after injection of the NF with or without VEGF, which implies that the myofibroblasts were mostly acquired through the microenvironmental benefits of the NF rather than by the influence of the prolonged VEGF. This finding is consistent with the increased recruitment of nonvascular integrated

SMCs to the myocardium at 28 days after injection of NF alone (Fig. 2A). NF/VEGF injection also preserved more α-SMA$^+$ cells for a long period (Fig. 4B). Because there was no difference in the level of α-SMA$^+$ cell proliferation between the NF and the NF/VEGF groups at 7 days after MI, we speculated that the long-term α-SMA$^+$ cell preservation was attributed to vessel formation, as evidenced

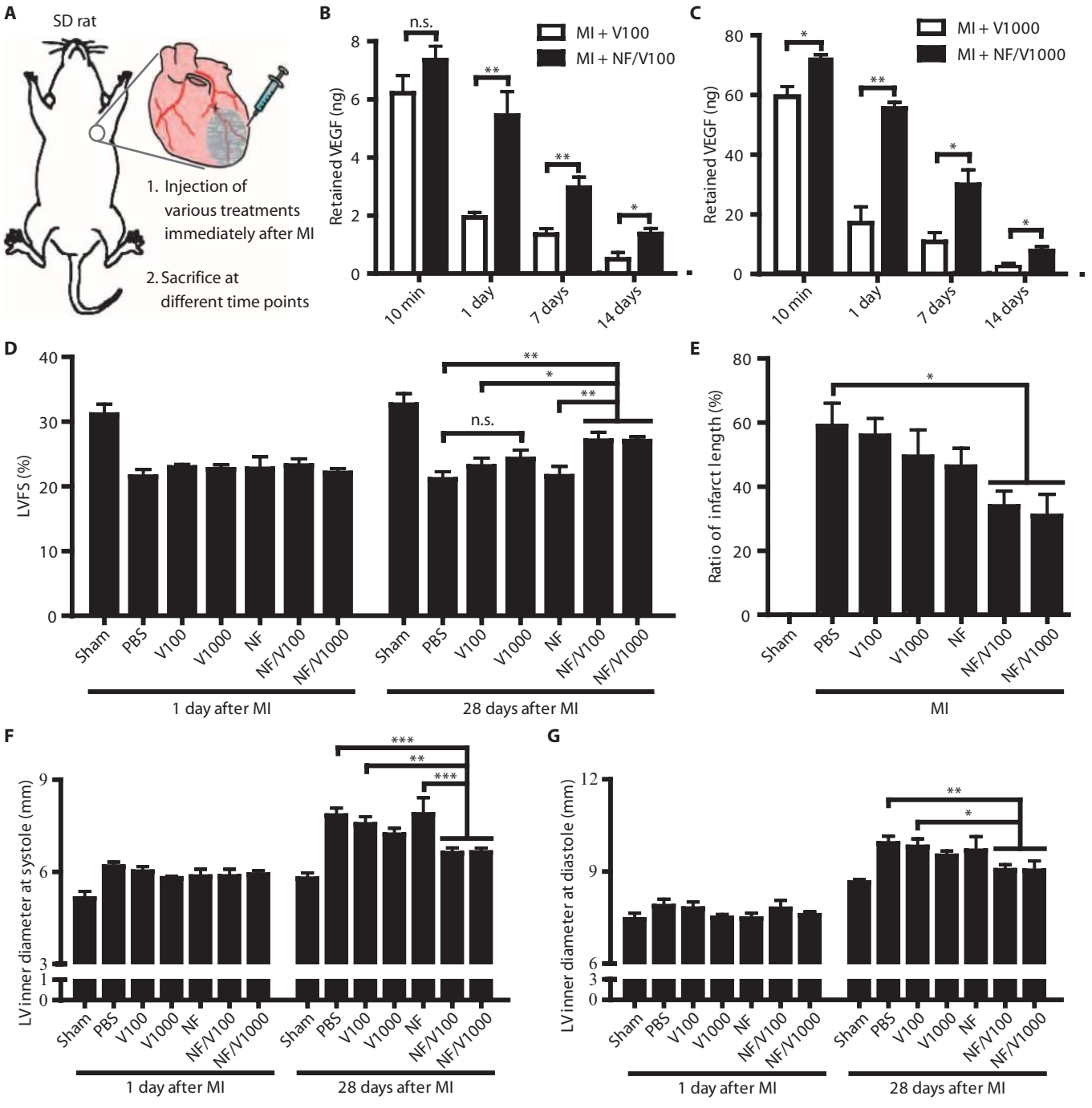

Fig. 1. Intramyocardial NF/VEGF injection achieves a sustained release of up to 14 days after delivery and improves cardiac performance at 28 days after MI. (**A**) The LAD coronary artery was permanently ligated to create MI in Sprague-Dawley (SD) rats, immediately followed by intramyocardial injection of treatments from six directions at the peri-infarct area. (**B** and **C**) In vivo kinetics of VEGF release with or without NF at doses of 100 ng/ml (B) and 1000 ng/ml (C). Data are means ± SEM ($n = 4$ to 5 per group). *$P < 0.05$; **$P < 0.01$; n.s., not significant (two-tailed unpaired t test). (**D**) A histogram showing the percentage of LVFS at 1 and 28 days after MI in the sham and experimental groups. (**E**) Infarct length ratio of the mid-LV at 28 days after MI. Data are means ± SEM ($n = 8$ to 10 per group). *$P < 0.05$, one-way analysis of variance (ANOVA) with Newman-Keuls post hoc test. (**F** and **G**) LV inner dimension at systole (F) and diastole (G). For (D), (F), and (G), data are means ± SEM ($n = 8$ to 10 per group). *$P < 0.05$; **$P < 0.01$; ***$P < 0.01$; n.s., not significant (two-way ANOVA with Bonferroni's post hoc test).

by arteriogenesis at 28 days after MI (Fig. 2, B and C). Together, these results support the concept that functional arteriogenesis requires VEGF-dependent angiogenesis after NF-mediated mural cell acquisition.

A study was then designed to verify the capacity of NFs to capture circulating bone marrow cells (BMCs), which are a source of myofibroblasts and are recruited into the injured area (*39*). Rats received intravenous injections of 1×10^7 allogeneic 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)–labeled BMCs at 7 days after MI, along with intramyocardial injection of PBS, VEGF (100 ng/ml), NF, or NF/V100, and were then sacrificed 8 days after MI (Fig. 5A). The results revealed that significantly more DiI^+ BMCs were retained within the myocardium of rats that received injections of NF compared with rats injected with VEGF or PBS (Fig. 5, B and C). Notably, the level of myocardial DiI^+ BMC infiltration was significantly enhanced by the NF/VEGF injection compared with the injection of NF or VEGF alone. This synergistic effect suggests that a chemotactic or vessel-promoting effect of VEGF couples with an optimally retentive NF microenvironment to retain circulating BMCs.

To further explore the cell recruitment and retention mechanisms, we examined the expression profile of adhesion molecules within the infarct myocardium following various treatments at 3 days after MI. Among them, we found that β_2-integrin expression was markedly up-regulated after NF/VEGF injection (Fig. 5D). We thus repeated the BMC injection experiment with cells pretreated with β_2-integrin blockage (Fig. 5A). The effect of cell recruitment and retention disappeared in the NF and NF/VEGF groups, whereas the VEGF group seemed not to be greatly affected (Fig. 5, B and C). A similar finding was observed in the in vitro cell adhesion study, where we cultured freshly isolated BMCs on NF-coated, collagen-coated, and non-coated dishes. Consistently, there were significantly more adhered cells in the NF-coated group than in other groups, whereas this effect was blocked after β_2-integrin neutralization of BMCs (Fig. 5E).

To clarify whether there are other factors affecting the influx of BMCs, we examined the level of sustained myocardial vascular leakage at 7 days after MI (Fig. 3A). Our results revealed that there was only mild vascular leakage within the heart of the NF/VEGF treatment group (day 7 group; Fig. 3B and fig. S7). We also examined the macrophage ($CD68^+$ cells) infiltration at various time points to test whether the inflammatory response affects cell recruitment after MI (fig. S8A). The inflammatory response persisted beyond 7 days after MI in the PBS group, whereas it decreased at 7 days after MI in the NF, VEGF, and NF/VEGF treatment groups (fig. S8B). Together, these results imply that recruitment of reparative cells, such as myofibroblasts or BMCs, may not be directly attributed to vascular leakage or inflammatory response.

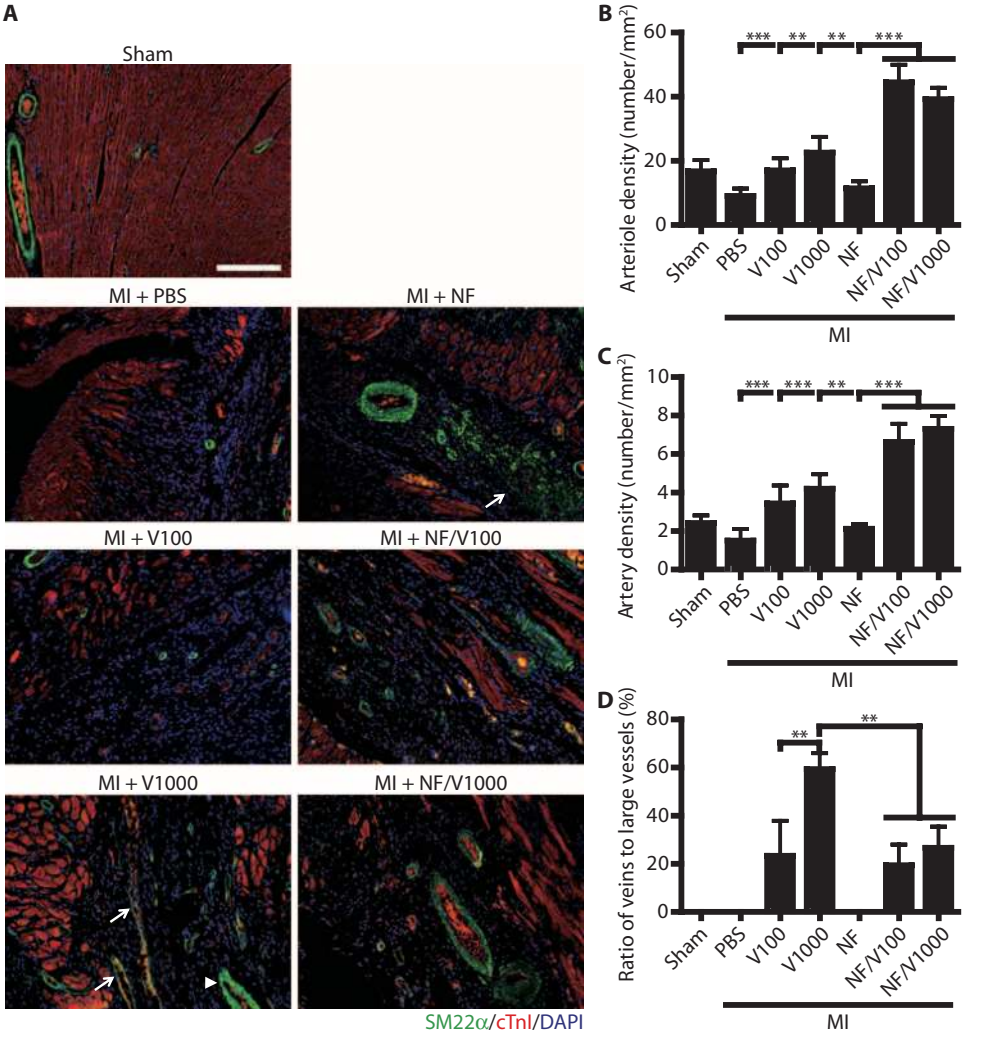

Fig. 2. NF/VEGF injection improves the arteriole and artery densities at the peri-infarct area, and VEGF injection results in increased venogenesis at 28 days after MI. (**A**) Representative immunostained images of SMCs (SM22α; green), cardiomyocytes (cTnI; red), and nuclei (DAPI; blue) at the infarct border zone from each group. The nonvascular integrated SMCs are indicated by arrows in the image from the MI + NF group. Thin-walled veins are indicated by arrows, whereas the thick-walled artery is indicated by an arrowhead in the image from the MI + V1000 group. Scale bar, 200 μm. (**B** and **C**) Quantification of the densities of arterioles (external vascular diameter <75 μm) (B) and arteries (external vascular diameter >75 μm) (C) in the border zone. (**D**) A histogram showing the ratio of veins to large vessels (external vascular diameter >75 μm) at the infarct area. Data are means ± SEM (n = 8 to 10 per group). *P < 0.05, **P < 0.01, one-way ANOVA with Newman-Keuls post hoc test.

NF/VEGF recruits cardiomyocyte-like small cells after MI

Surprisingly, we detected more cardiac troponin I–positive ($cTnI^+$) small cells, which were previously defined as putative myocyte precursors (*35*), within the injected myocardium at 28 days after MI following NF/VEGF injection than in the other groups (fig. S9). To further confirm

that these cTnI$^+$ small cells were not mere debris of remnant cardiomyocytes, we conducted a genetic fate-mapping approach using inducible MerCreMer-ZEG mice as described previously (*40*). In brief, the cardiomyocytes were specifically labeled with green fluorescent protein (GFP) after a short period of tamoxifen pulse, and the GFP$^-$ cardiomyocytes represent newly generated cardiomyocytes derived from endogenous stem/progenitor cells after injury (*40*). Therefore, the GFP$^-$/cTnI$^+$ small cells may indicate stem/progenitor cell–derived cardiomyocyte-like cells in this system. The mice were injected with PBS, VEGF (100 ng/ml), NF, or NF/V100 after experimental MI (Fig. 6A and fig. S10). Injection of NF/VEGF recruited significantly more GFP$^-$/cTnI$^+$ small cells than did the injection of PBS, VEGF alone, or NF alone (Fig. 6, B and C). There were also slightly more GFP-negative mature cardiomyocytes observed in the NF/VEGF treatment group, with no significant differences compared with the control. Nevertheless, the results suggest that the injection of NF/VEGF can provide an intramyocardial microenvironment that is favorable for induction of endogenous cardiomyocyte regeneration.

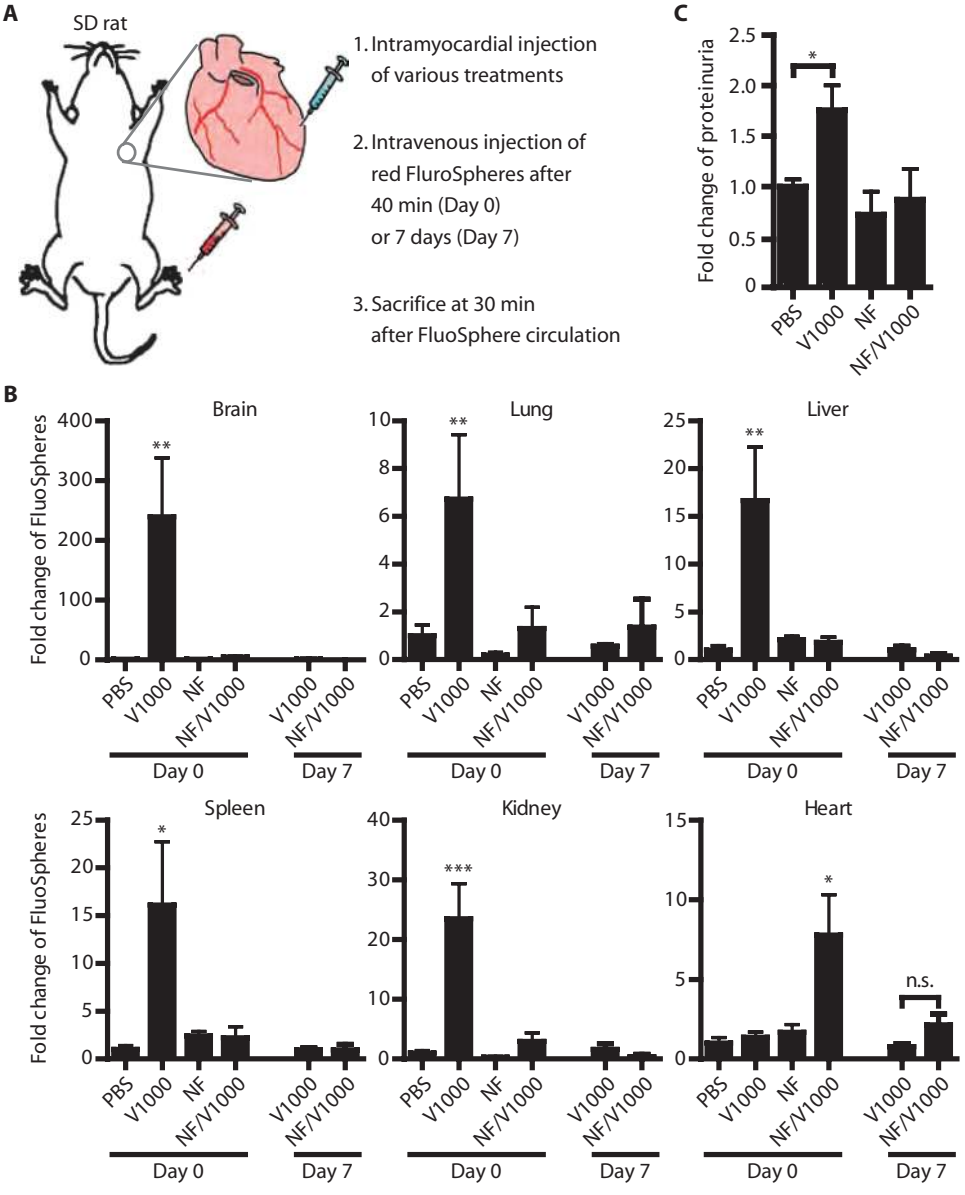

Fig. 3. A high dose of VEGF induces severe general vessel leakage, whereas NF/VEGF exerts myocardium-localized effects. (**A**) Various treatment formulations were injected intramyocardially into the area of the LAD territory. After 40 min or 7 days, red fluorescent FluoSpheres were intravenously injected (day 0 and day 7 groups, respectively). Thirty minutes after FluoSphere injection, animals were sacrificed, and urine and major organs were collected. (**B**) Fold change in extravasated FluoSpheres compared to the PBS-treated group. *P < 0.05, **P < 0.01, ***P < 0.001, compared to all other treatment groups at day 0; n.s., not significant, as indicated by brackets at day 7 (one-way ANOVA with Newman-Keuls post hoc test). (**C**) Fold change in proteinuria compared to the PBS-treated group at day 0. *P < 0.05, one-way ANOVA with Newman-Keuls post hoc test. For (B) and (C), data are means ± SEM (n = 4 to 5 per group).

Intramyocardial NF/VEGF injection increases cardiac performance and arteriogenesis after MI in pigs

We then performed experimental infarction in an established pig model (*30*) to test the therapeutic efficacy and the clinical translational potential (Fig. 7A). PBS, VEGF (100 ng/ml), NF, or NF/V100 was injected immediately after MI. Consistent with the results in rats, VEGF-only injection slightly improved cardiac function with no significant difference compared with control, whereas NF/VEGF injections significantly improved cardiac performance at 28 days after MI (Fig. 7B). The reduced infarct size (Fig. 7C and fig. S11) and hemodynamic parameters, such as +dP/dt, −dP/dt, time constant of LV pressure decay, and maximum chamber elasticity, also showed consistency (table S1).

Moreover, similar to the outcome in rats, delivery of VEGF with and without NF both improved after MI angiogenesis (Fig. 7D and fig. S12), but only the NF/VEGF injection significantly improved arteriogenesis at 28 days after MI (Fig. 7, E and F, and fig. S13). This result suggests that the cardiac benefit relies on arteriogenesis rather than angiogenesis after MI.

DISCUSSION

VEGF therapies have failed to show consistent benefits in recent clinical trials of ischemic cardiovascular disease (*8*, *9*), and intensive efforts have been devoted to determine both the key problems and the potential solutions (*14*, *17*). The results of our study suggest that therapeutic outcome relies more on arteriogenesis than angiogenesis, and that this effect cannot be achieved by a mere increase in VEGF dose. This study demonstrated that NFs

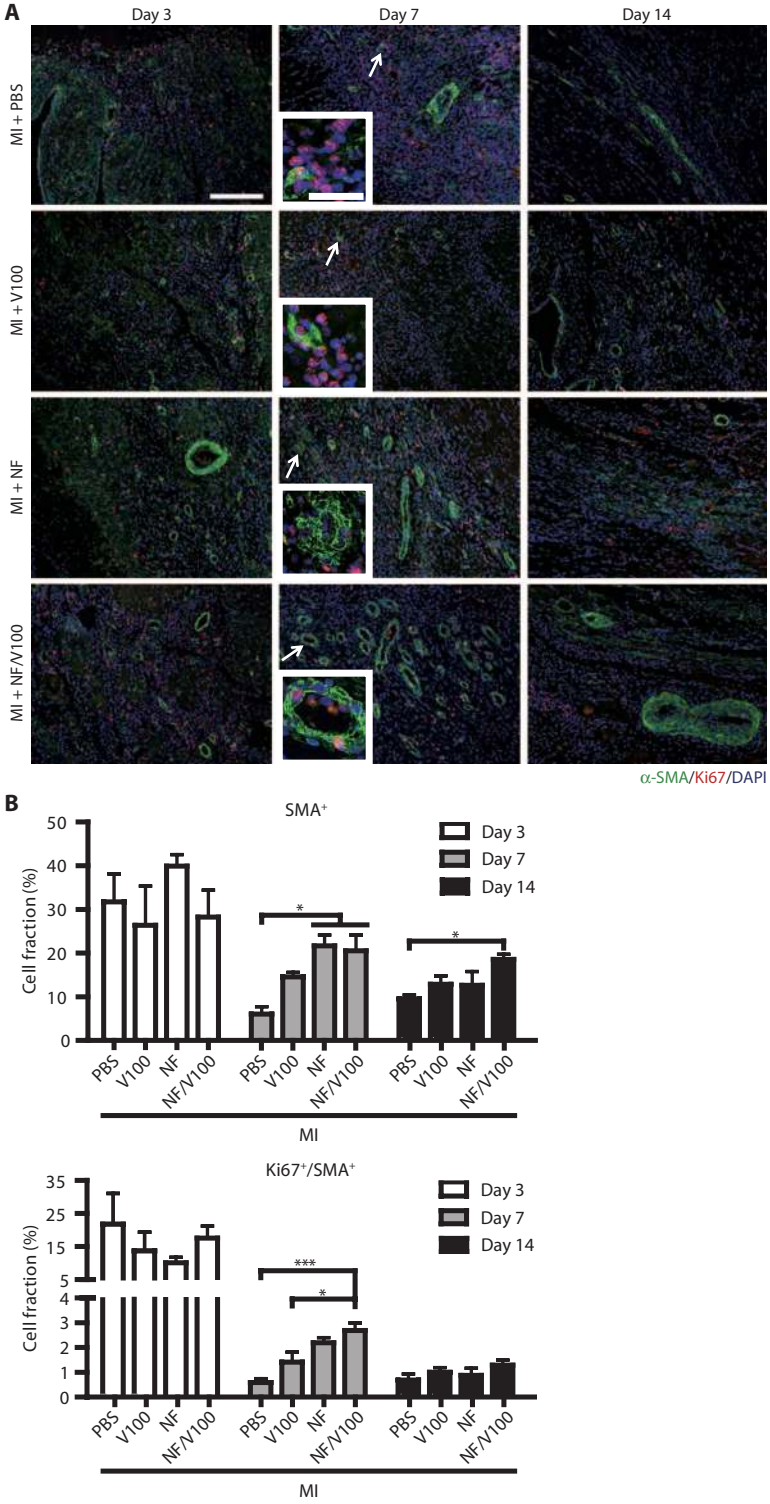

Fig. 4. NF/VEGF promotes replenishment of myofibroblast-derived mural cells. (**A**) Representative immunostaining of myofibroblasts or myofibroblast-derived mural cells (α-SMA; green) and proliferating cells (Ki67; red) at the border zone from each group at 3, 7, or 14 days after MI. Arrows indicate the α-SMA$^+$ and Ki67$^+$ cells at 7 days after infarction and are magnified for clarity. Scale bars, 200 μm for all regular panels and 40 μm for all inset panels. (**B**) Number of α-SMA$^+$ or α-SMA$^+$/Ki67$^+$ cells at the border zone from each group at 3, 7, or 14 days after MI. Data are means ± SEM (n = 4 to 6 per group). *P < 0.05, ***P < 0.001, one-way ANOVA with Newman-Keuls post hoc test.

create an intramyocardial microenvironment that can recruit endogenous myofibroblasts for improved repair after MI. Specifically, the effect of the resultant microenvironment can be further enhanced with sustained VEGF release via NFs and synergistically stimulate arteriogenesis as well as cardiac performance after MI.

To date, the largest phase 2 clinical trial to use recombinant $VEGF_{165}$ for the treatment of ischemic cardiovascular disease was performed by Henry *et al.* in 2003 (*8*). This study demonstrated that treatment with low-dose VEGF failed to improve cardiac symptoms and function, whereas treatment with high-dose VEGF reduced angina frequency but did not improve cardiac function when compared with the placebo-treated group. In the current study, we present a similar finding that an increased VEGF dose is slightly more favorable, but not sufficient, to improve cardiac function after MI. It appears that arteriogenesis is more important than angiogenesis for a cardiac benefit, as suggested by an improved LVFS and reduced cardiac dilation and infarct size, as well as the preservation of cardiomyocytes around the matured arteries in both rats and pigs. Nevertheless, our data demonstrate that arteriogenesis cannot be induced by treatment with VEGF alone, even after a 10-fold increase in VEGF dose (up to 1 μg/ml). Indeed, increasing the VEGF dose may not be ideal for clinical applications because this increase is often accompanied by aggravated risks of hypotension, general edema, tumorigenesis, and metastasis (*5*, *9*). Consistently, we also observed augmented proteinuria, increased ratio of venogenesis, and vascular leakage in the brain, lungs, liver, spleen, and kidneys in the group that was subjected to high-dose VEGF. Notably, these harmful complications were not observed when high-dose VEGF was delivered with NF, which demonstrates the safety and efficacy of controlled delivery with NFs, in line with our previous study (*33*).

Consistent with our results, it has been shown that arteriogenesis is therapeutically more critical and beneficial than angiogenesis (*14*, *15*, *17*). Although previous studies have demonstrated that arteriogenesis can be promoted by the delicate dual or triple delivery of growth factors and cells with biomaterials (*15*, *16*, *41*, *42*), it relies on the precise controlled sequential

release or direct serial delivery, which is unfavorable for clinical use. The present study has provided a relatively simple approach and has obtained not only equivalent efficacy of arteriogenesis but also positive cardiac improvements after MI, suggesting that NFs act as more than just a carrier for controlled delivery. We propose that spontaneous and specific regulation of arteriogenesis is activated within the microenvironment of the NF/VEGF injection site, and that this regulation can be attributed to several distinct properties of the NFs. First, the NFs used here are composed of self-assembling peptide fibers with diameters of 10 to 20 nm. This conveniently allowed for a true three-dimensional cell culture environment similar to the natural ECM (10 to 300 nm) (*43*). Conversely, most biomaterials for tissue engineering are fibers ranging from 10 to 100 μm in diameter, which only allow two-dimensional cultures because they are as large as most mammalian cells (*21*, *29*, *44*).

Second, the NFs are degraded slowly, such that about 70% of the material is retained within the injected myocardium for as long as 1 month (*30*). This retention may aid in the provision of a stable milieu and space for cell anchorage as well as vessel formation. By contrast,

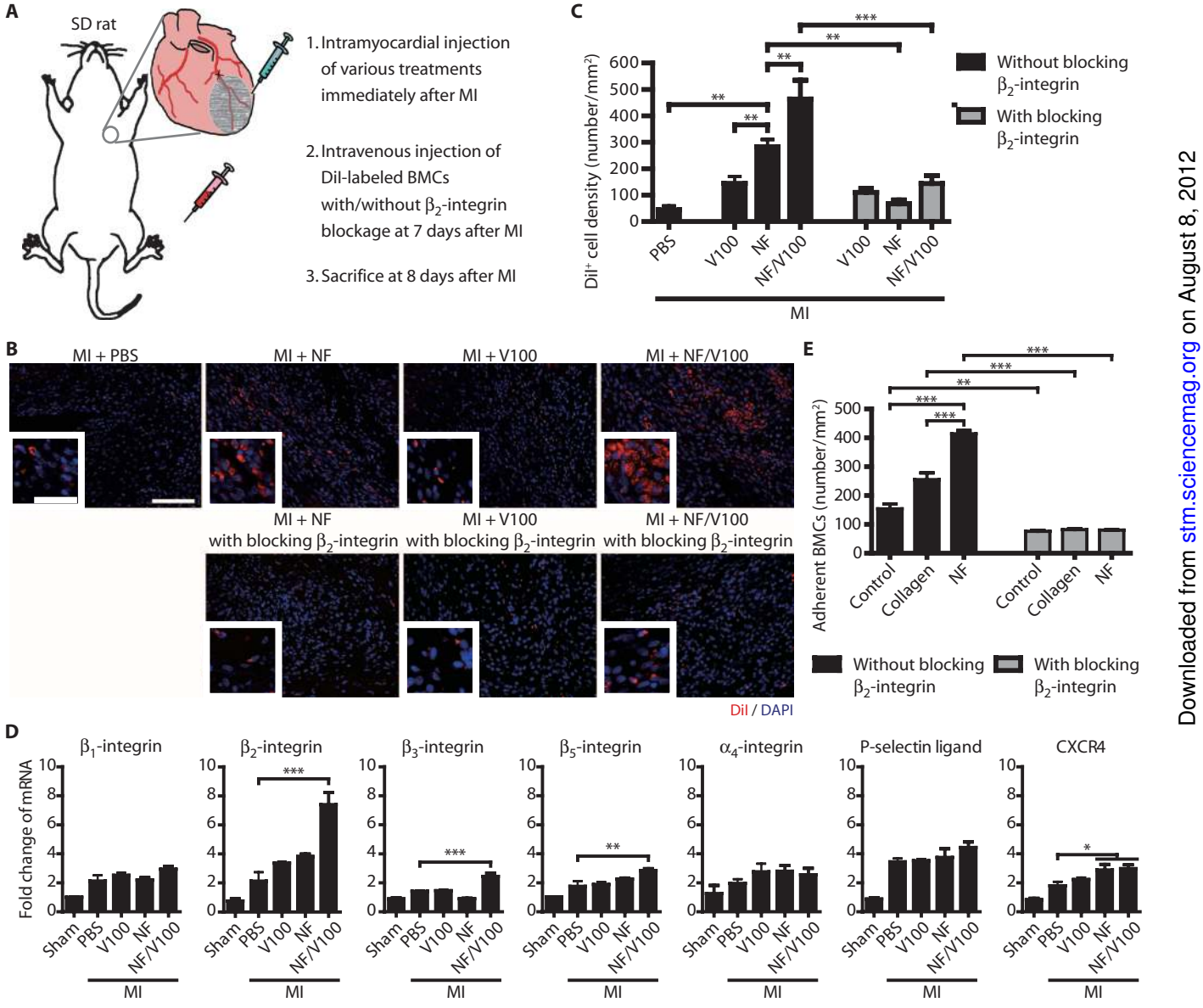

Fig. 5. NF/VEGF promotes recruitment of circulating BMCs at 7 days after MI. (**A**) Various treatment formulations were injected intramyocardially at the border and infarcted areas immediately after MI. DiI-labeled BMCs were intravenously injected at 7 days with or without β_2-integrin blockage after MI. Animals were sacrificed 8 days after MI. (**B**) Representative DiI fluorescence (red) images overlapped with nuclei staining (DAPI; blue) from the border zone of each group. DiI^+ BMCs are magnified in the insets. Scale bars, 100 μm for all regular panels and 20 μm for all inset panels. (**C**) Density of DiI^+ BMCs at the border zone of each group. (**D**) Expression profile of adhesion molecules within the infarct myocardium with various treatments at 3 days after MI. (**E**) Adherent BMCs were counted after 1 hour of culture on various substrates with and without β_2-integrin blockage. For (C) to (E), data are means ± SEM (n = 3 to 6 per group). *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA with Newman-Keuls post hoc test.

most biocompatible hydrogels are either rapidly degraded or nondegradable. Third, the use of NFs also allows for the sustained release of VEGF as well as several other drugs or growth factors via physical embedding or chemical immobilization (*31*–*34*, *45*–*47*). Last, NF implantation creates an intramyocardial microenvironment that promotes the recruitment, retention, and maturation of vascular and cardiac stem/progenitor cells (*30*, *35*), enhancing their therapeutic potential. Together, NFs have all the aforementioned qualities to create an instructive microenvironment for arteriogenesis in vivo.

Myofibroblasts are recruited into the myocardium and undergo apoptosis as part of scar formation and ventricular remodeling after infarction (*39*, *48*, *49*). Our data demonstrated that myofibroblasts were enriched within the myocardium at 3 days after MI and then rapidly decreased in number. The accumulation of nonvascular integrated SM22α^+ cells at 28 days after MI indicated that NF-constructed microenvironments could preserve myofibroblasts within the myocardium for at least 7 days after infarction and could promote further SMC differentiation. The results are consistent with our previous study (*30*), which demonstrated that NF injection could retard harmful collagen deposition (*49*) and diastolic dysfunction due to a reduction in myofibroblast apoptosis. Together, the enhanced replenishment of myofibroblast-derived SMCs and the engraftment of exogenous BMCs by the NFs indicate that the NF/VEGF injection has created a microenvironment that provides a relocation base for circulating BMCs, which can then home to injured sites and differentiate into mural cells for arteriogenesis. Endogenous repair may be facilitated by direct VEGF signaling (such as via a chemoattractant) (*50*), VEGF-induced angiogenic network (improving the arrival of endogenous stem/progenitor cells), or the activated inductive microenvironment termed the vascular niche (*6*, *51*).

Davis *et al.* reported that within the NF-injected region of normal myocardium, there was an infiltration of putative myocyte precursors that expressed α-sarcomeric actin and Nkx2.5 (*35*). Nevertheless, these cardiomyocyte-like cells were not found after MI in the follow-up study (*31*). The present study demonstrated that the NF/VEGF injection gave rise to an infiltration of cardiomyocyte-like small cells within the infarcted myocardium, indicating that endogenous cardiac regeneration after MI may be induced by the recreated vascular niche. Future studies on how to intensify and optimize this effect to obtain mature and functional cardiomyocytes may help reach the ultimate cardiac restoration.

Increasing reports have revealed the advantage and importance of biomaterials in cardiac tissue engineering (*20*, *52*, *53*). Despite the enthusiasm, there are relatively few ongoing clinical trials using

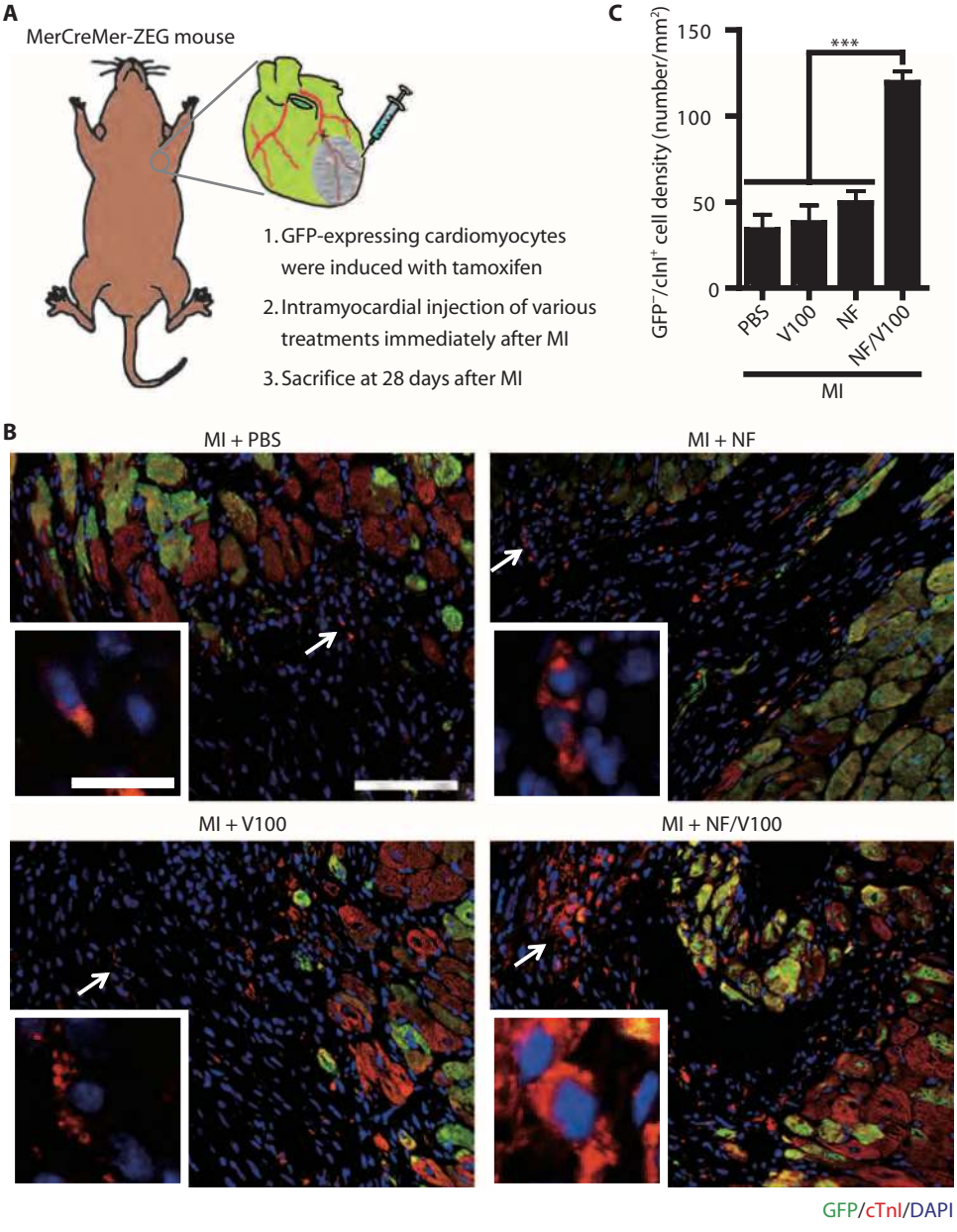

Fig. 6. NF/VEGF increases the cardiomyocyte-like small cell population at 28 days after MI. (**A**) Cardiomyocytes were prelabeled with GFP in MerCreMer-ZEG mice. Immediately after MI, the mice were injected intramyocardially with various treatments. Animals were sacrificed at 28 days after MI. (**B**) Representative immunostained images of cardiomyocytes (cTnI; red), cardiomyocyte-specific Cre recombinase–meditated GFP expression (GFP; green), and nuclei (DAPI; blue) at the border zone from each group. The GFP$^-$/cTnI$^+$ small cells are indicated by arrows and are magnified in the inset images. Scale bars, 100 μm for all regular panels and 20 μm for all inset panels. (**C**) Quantification of GFP$^-$/cTnI$^+$ small cells at the border zone. Data are means ± SEM (n = 4 to 5 per group). ***P < 0.001, compared to all other treatment groups (one-way ANOVA with Newman-Keuls post hoc test).

injected materials for cardiac repair (ClinicalTrials.gov identifiers: NCT00557531, NCT01311791, NCT01226563), maybe due to a lack of evidence in large-animal studies, which are necessary before moving to humans. We performed a large-animal study with a pig model to demonstrate the translational potential. However, because the immediate treatment after MI may not be relevant to clinical situations, whether this approach also works in the chronic case and whether there exists an optimal therapeutic time window require further examination. The optimization of NF/VEGF dose and long-term studies are also needed for clinical translation. In addition, although we showed the major merit of NFs to build beneficial microenvironments, the exact pivotal cues may be attributed to the biometric fiber diameter, tendency to induce cell adhesion, slow degradability, or a combination of all of these. Therefore, the underlying mechanism as well as the criteria for biomaterial design still requires further investigation. In conclusion, here we report that NFs are able to create an in vivo microenvironment for cardiovascular regeneration and also provide positive therapeutic effects after MI in both small and large animals. This strategy holds promise for future research not only in basic tissue engineering but also in translational medicine.

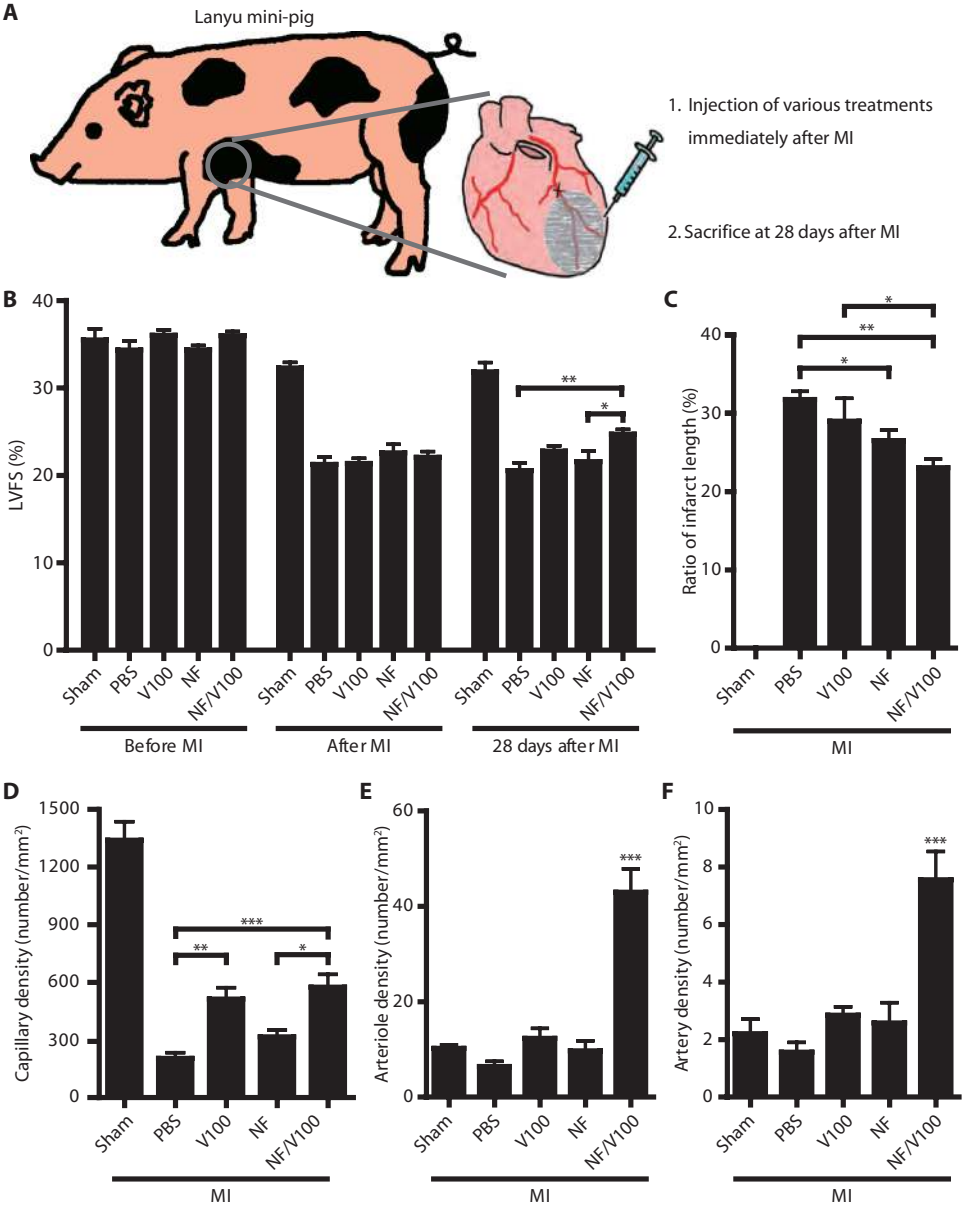

Fig. 7. NF/VEGF increases cardiac performance and arteriogenesis after MI in pigs. (**A**) Immediately after MI, pigs were injected intramyocardially with various treatments. Animals were sacrificed at 28 days after MI. (**B**) LVFS before, immediately after, and 28 days after MI in the sham and experimental groups. Data are means ± SEM (n = 5 to 6 per group). *P < 0.05, **P < 0.01, two-way ANOVA with Bonferroni's post hoc test. (**C**) Infarct length ratio of the mid-LV at 28 days after MI. (**D**) Capillary density 28 days after infarction at the border zone from each treatment group. (**E** and **F**) Densities of arterioles (external vascular diameter <75 μm) (E) and arteries (external vascular diameter >75 μm) (F) in the border zone. For (C) to (E), data are means ± SEM (n = 5 to 6 per group). *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA with Newman-Keuls post hoc test.

MATERIALS AND METHODS

All animal procedures were approved by the National Cheng Kung University Institutional Animal Care and Use Committee.

Rat experimental MI model

Male Sprague-Dawley rats (~250 g) were anesthetized, and the chest cavity was opened either without coronary artery ligation (sham) or with permanent ligation of the left anterior descending (LAD) coronary artery. Then, a total of 80 μl of each of the various treatment formulations was given by intramyocardial injection divided among six different sites at the border zones and the infarcted area of the heart. Cardiac performance was assessed by echocardiography; animals were then sacrificed and their tissues stained for levels of pathological remodeling, angiogenesis, and arteriogenesis (Supplementary Methods).

Preparation of NF/VEGF treatments and the in vivo release kinetics of VEGF

Self-assembling peptide NFs (peptide sequence AcN-RARADADARARADADA-NH_2; SynBioSci) were gel-formatted with sterile PBS as solvent, as described previously (*30*). Recombinant human $VEGF_{165}$ was thoroughly mixed with either the NF or PBS at a volume ratio of 1:9 to obtain the required concentration for each experiment. Rat hearts with various treatments were harvested at various time points, and the border and infarct myocardium were trimmed and dissolved in 400 μl of nonreducing buffer containing 1% Triton X-100, 50 mM

tris (pH 7.4), 300 mM NaCl, 5 mM EDTA, and 0.02% NaN_3, supplemented with a proteinase inhibitor cocktail (Sigma-Aldrich) at a 1:200 dilution for protein extraction. The soluble protein extracts were then subjected to a human $VEGF_{165}$ enzyme-linked immunosorbent assay (eBiosource).

Exogenous BMC injection study

At 7 days after treatment, rats were intravenously injected with 10^7 rat BMCs, which had been freshly isolated from the tibias and femurs and labeled with DiI (Invitrogen) for tracing. For β_2-integrin blockage, 0.5×10^6 cells were incubated with anti–β_2-integrin monoclonal antibody (BD Pharmingen) at a concentration of 20 μg/ml for 30 min on ice immediately before injection (*54*). The rats were then sacrificed 1 day after the BMC injection, and hearts were harvested for tissue processing as described above. The myocardial sections were stained with 4′,6-diamidino-2-phenylindole (DAPI), and four pictures were taken blindly for each section. DiI signal was detected with a red fluorescence filter set at a constant exposure time. Only cells whose nuclei were surrounded with DiI signal were counted as DiI^+ cells.

Cardiomyocyte-specific fate-mapping study

Double-transgenic MerCreMer-ZEG mice were generated as described previously (*40*). Tamoxifen (Sigma) was dissolved in sunflower oil (Sigma) at a concentration of 5 mg/ml and was injected intraperitoneally at a dose of 40 μg per gram body weight per day for 14 days. The $GFP^-/cTnI^+$ cells were quantified blindly, and the nuclei of the counted cells were surrounded with cTnI, but not GFP, signals.

Pig model of experimental MI

Sexually mature Lanyu mini-pigs (~5 months old) were induced with MI by a permanent occlusion of mid-LAD coronary artery, immediately followed by injection of 2 ml of PBS or 1% NF with or without VEGF (100 ng/ml) into the peri-infarct and infarct areas. Cardiac functions were assessed by echocardiography before and immediately after MI and together with hemodynamic measurements through catheterization 4 weeks later.

Pigs were then sacrificed, and hearts were harvested as previously described (*30*). In brief, the atria and right ventricles were removed, and then the LVs were cut into two parts at the papillary muscle insertion site and placed upright. Necrotic tissue (pale) was quantified by manual tracing and software calculation (ImageJ).

Statistical analysis

Differences were determined by two-tailed unpaired *t* test or one-way repeated-measures ANOVA with Newman-Keuls post hoc test to compare means between multiple groups, or by two-way ANOVA with subsequent Bonferroni's post hoc test to compare means between multiple groups on multiple time points. A *P* value of <0.05 was considered statistically significant.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/4/146/146ra109/DC1

Methods

Fig. S1. Human VEGF acts on rat endothelial cells in a dose-dependent manner.

Fig. S2. Peptide NFs exhibit a high binding capacity and a stable release kinetics profile of VEGF in vitro.

Fig. S3. NF/VEGF retards pathological remodeling at 28 days after MI.

Fig. S4. NF/VEGF and high-dose VEGF improve the peri-infarct capillary density at 28 days after MI.

Fig. S5. FluoSpheres estimate vessel leakage in vital organs at day 0.

Fig. S6. Representative flow cytometry–like scattergram of Ki67- and α-SMA–positive cells under various treatments and time points.

Fig. S7. FluoSpheres estimate vessel leakage in vital organs at day 7.

Fig. S8. Macrophage infiltration is attenuated by NF and/or VEGF injection after infarction.

Fig. S9. Immunostaining of cardiomyocyte-like small cells at the border zone from each treatment group at 28 days after MI in rats.

Fig. S10. Background signals from erythrocytes, but not $GFP^-/cTnI^+$ cardiomyocyte-like cells, were detected in the negative control of staining.

Fig. S11. Cross sections at the level of papillary muscle insertion of left ventricle from each pig group.

Fig. S12. Capillaries at the border zone from each treatment group at 28 days after MI in pigs.

Fig. S13. Arterioles and arteries at the border zone from each treatment group at 28 days after MI in pigs.

Table S1. Hemodynamic parameters in pigs at 28 days after MI.

REFERENCES AND NOTES

1. V. L. Roger, A. S. Go, D. M. Lloyd-Jones, R. J. Adams, J. D. Berry, T. M. Brown, M. R. Carnethon, S. Dai, G. de Simone, E. S. Ford, C. S. Fox, H. J. Fullerton, C. Gillespie, K. J. Greenlund, S. M. Hailpern, J. A. Heit, P. M. Ho, V. J. Howard, B. M. Kissela, S. J. Kittner, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, D. M. Makuc, G. M. Marcus, A. Marelli, D. B. Matchar, M. M. McDermott, J. B. Meigs, C. S. Moy, D. Mozaffarian, M. E. Mussolino, G. Nichol, N. P. Paynter, W. D. Rosamond, P. D. Sorlie, R. S. Stafford, T. N. Turan, M. B. Turner, N. D. Wong, J. Wylie-Rosett, Heart disease and stroke statistics—2011 update: A report from the American Heart Association. *Circulation* **123**, e18–e209 (2011).
2. M. Jessup, S. Brozena, Heart failure. *N. Engl. J. Med.* **348**, 2007–2018 (2003).
3. M. A. Laflamme, S. Zbinden, S. E. Epstein, C. E. Murry, Cell-based therapy for myocardial ischemia and infarction: Pathophysiological mechanisms. *Annu. Rev. Pathol.* **2**, 307–339 (2007).
4. M. Simons, J. A. Ware, Therapeutic angiogenesis in cardiovascular disease. *Nat. Rev. Drug Discov.* **2**, 863–871 (2003).
5. N. Ferrara, Vascular endothelial growth factor: Basic science and clinical progress. *Endocr. Rev.* **25**, 581–611 (2004).
6. L. Coultas, K. Chawengsaksophak, J. Rossant, Endothelial cells and VEGF in vascular development. *Nature* **438**, 937–945 (2005).
7. A. K. Olsson, A. Dimberg, J. Kreuger, L. Claesson-Welsh, VEGF receptor signalling—In control of vascular function. *Nat. Rev. Mol. Cell Biol.* **7**, 359–371 (2006).
8. T. D. Henry, B. H. Annex, G. R. McKendall, M. A. Azrin, J. J. Lopez, F. J. Giordano, P. K. Shah, J. T. Willerson, R. L. Benza, D. S. Berman, C. M. Gibson, A. Bajamonde, A. C. Rundle, J. Fine, E. R. McCluskey; VIVA Investigators, The VIVA trial: Vascular endothelial growth factor in ischemia for vascular angiogenesis. *Circulation* **107**, 1359–1365 (2003).
9. S. Ylä-Herttuala, T. T. Rissanen, I. Vajanto, J. Hartikainen, Vascular endothelial growth factors: Biology and current status of clinical applications in cardiovascular medicine. *J. Am. Coll. Cardiol.* **49**, 1015–1026 (2007).
10. M. L. Springer, A. S. Chen, P. E. Kraft, M. Bednarski, H. M. Blau, VEGF gene delivery to muscle: Potential role for vasculogenesis in adults. *Mol. Cell* **2**, 549–558 (1998).
11. R. J. Lee, M. L. Springer, W. E. Blanco-Bose, R. Shaw, P. C. Ursell, H. M. Blau, VEGF gene delivery to myocardium: Deleterious effects of unregulated expression. *Circulation* **102**, 898–901 (2000).
12. C. R. Ozawa, A. Banfi, N. L. Glazer, G. Thurston, M. L. Springer, P. E. Kraft, D. M. McDonald, H. M. Blau, Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *J. Clin. Invest.* **113**, 516–527 (2004).
13. G. von Degenfeld, A. Banfi, M. L. Springer, R. A. Wagner, J. Jacobi, C. R. Ozawa, M. J. Merchant, J. P. Cooke, H. M. Blau, Microenvironmental VEGF distribution is critical for stable and functional vessel growth in ischemia. *FASEB J.* **20**, 2657–2659 (2006).
14. P. Carmeliet, Angiogenesis in health and disease. *Nat. Med.* **9**, 653–660 (2003).
15. M. J. Frontini, Z. Nong, R. Gros, M. Drangova, C. O'Neil, M. N. Rahman, O. Akawi, H. Yin, C. G. Ellis, J. G. Pickering, Fibroblast growth factor 9 delivery during angiogenesis produces durable, vasoresponsive microvessels wrapped by smooth muscle cells. *Nat. Biotechnol.* **29**, 421–427 (2011).
16. N. Koike, D. Fukumura, O. Gralla, P. Au, J. S. Schechner, R. K. Jain, Tissue engineering: Creation of long-lasting blood vessels. *Nature* **428**, 138–139 (2004).
17. R. K. Jain, Molecular regulation of vessel maturation. *Nat. Med.* **9**, 685–693 (2003).
18. L. G. Griffith, M. A. Swartz, Capturing complex 3D tissue physiology in vitro. *Nat. Rev. Mol. Cell Biol.* **7**, 211–224 (2006).

19. D. E. Discher, D. J. Mooney, P. W. Zandstra, Growth factors, matrices, and forces combine and control stem cells. *Science* **324**, 1673–1677 (2009).
20. M. P. Lutolf, P. M. Gilbert, H. M. Blau, Designing materials to direct stem-cell fate. *Nature* **462**, 433–441 (2009).
21. M. P. Lutolf, J. A. Hubbell, Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat. Biotechnol.* **23**, 47–55 (2005).
22. H. J. Kong, D. J. Mooney, Microenvironmental regulation of biomacromolecular therapies. *Nat. Rev. Drug Discov.* **6**, 455–463 (2007).
23. L. Ferreira, J. M. Karp, L. Nobre, R. Langer, New opportunities: The use of nanotechnologies to manipulate and track stem cells. *Cell Stem Cell* **3**, 136–146 (2008).
24. N. Huebsch, D. J. Mooney, Inspiration and application in the evolution of biomaterials. *Nature* **462**, 426–432 (2009).
25. M. M. Martino, F. Tortelli, M. Mochizuki, S. Traub, D. Ben-David, G. A. Kuhn, R. Müller, E. Livne, S. A. Eming, J. A. Hubbell, Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. *Sci. Transl. Med.* **3**, 100ra89 (2011).
26. K. R. Chien, I. J. Domian, K. K. Parker, Cardiogenesis and the complex biology of regenerative cardiovascular medicine. *Science* **322**, 1494–1497 (2008).
27. E. S. Place, N. D. Evans, M. M. Stevens, Complexity in biomaterials for tissue engineering. *Nat. Mater.* **8**, 457–470 (2009).
28. T. Dvir, B. P. Timko, D. S. Kohane, R. Langer, Nanotechnological strategies for engineering complex tissues. *Nat. Nanotechnol.* **6**, 13–22 (2011).
29. X. Zhao, F. Pan, H. Xu, M. Yaseen, H. Shan, C. A. E. Hauser, S. Zhang, J. R. Lu, Molecular self-assembly and applications of designer peptide amphiphiles. *Chem. Soc. Rev.* **39**, 3480–3498 (2010).
30. Y. D. Lin, M. L. Yeh, Y. J. Yang, D. C. Tsai, T. Y. Chu, Y. Y. Shih, M. Y. Chang, Y. W. Liu, A. C. L. Tang, T. Y. Chen, C. Y. Luo, K. C. Chang, J. H. Chen, H. L. Wu, T. K. Hung, P. C. H. Hsieh, Intramyocardial peptide nanofiber injection improves postinfarction ventricular remodeling and efficacy of bone marrow cell therapy in pigs. *Circulation* **122**, S132–S141 (2010).
31. P. C. H. Hsieh, M. E. Davis, J. Gannon, C. MacGillivray, R. T. Lee, Controlled delivery of PDGF-BB for myocardial protection using injectable self-assembling peptide nanofibers. *J. Clin. Invest.* **116**, 237–248 (2006).
32. M. E. Davis, P. C. H. Hsieh, A. J. Grodzinsky, R. T. Lee, Custom design of the cardiac microenvironment with biomaterials. *Circ. Res.* **97**, 8–15 (2005).
33. P. C. H. Hsieh, C. MacGillivray, J. Gannon, F. U. Cruz, R. T. Lee, Local controlled intramyocardial delivery of platelet-derived growth factor improves postinfarction ventricular function without pulmonary toxicity. *Circulation* **114**, 637–644 (2006).
34. F. B. Engel, P. C. H. Hsieh, R. T. Lee, M. T. Keating, FGF1/p38 MAP kinase inhibitor therapy induces cardiomyocyte mitosis, reduces scarring, and rescues function after myocardial infarction. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15546–15551 (2006).
35. M. E. Davis, J. P. M. Motion, D. A. Narmoneva, T. Takahashi, D. Hakuno, R. D. Kamm, S. Zhang, R. T. Lee, Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. *Circulation* **111**, 442–450 (2005).
36. D. A. Narmoneva, O. Oni, A. L. Sieminski, S. Zhang, J. P. Gertler, R. D. Kamm, R. T. Lee, Self-assembling short oligopeptides and the promotion of angiogenesis. *Biomaterials* **26**, 4837–4846 (2005).
37. F. Gelain, L. D. Unsworth, S. Zhang, Slow and sustained release of active cytokines from self-assembling peptide scaffolds. *J. Control. Release* **145**, 231–239 (2010).
38. M. H. Kim, F. E. Curry, S. I. Simon, Dynamics of neutrophil extravasation and vascular permeability are uncoupled during aseptic cutaneous wounding. *Am. J. Physiol. Cell Physiol.* **296**, C848-C856 (2009).
39. S. W. M. van den Borne, J. Diez, W. M. Blankesteijn, J. Verjans, L. Hofstra, J. Narula, Myocardial remodeling after infarction: The role of myofibroblasts. *Nat. Rev. Cardiol.* **7**, 30–37 (2010).
40. P. C. H. Hsieh, V. F. M. Segers, M. E. Davis, C. MacGillivray, J. Gannon, J. D. Molkentin, J. Robbins, R. T. Lee, Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat. Med.* **13**, 970–974 (2007).
41. T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney, Polymeric system for dual growth factor delivery. *Nat. Biotechnol.* **19**, 1029–1034 (2001).
42. S. Levenberg, J. Rouwkema, M. Macdonald, E. S. Garfein, D. S. Kohane, D. C. Darland, R. Marini, C. A. van Blitterswijk, R. C. Mulligan, P. A. D'Amore, R. Langer, Engineering vascularized skeletal muscle tissue. *Nat. Biotechnol.* **23**, 879–884 (2005).
43. M. M. Stevens, J. H. George, Exploring and engineering the cell surface interface. *Science* **310**, 1135–1138 (2005).
44. S. Zhang, Beyond the Petri dish. *Nat. Biotechnol.* **22**, 151–152 (2004).
45. M. E. Davis, P. C. H. Hsieh, T. Takahashi, Q. Song, S. Zhang, R. D. Kamm, A. J. Grodzinsky, P. Anversa, R. T. Lee, Local myocardial insulin-like growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 8155–8160 (2006).
46. M. E. Padin-Iruegas, Y. Misao, M. E. Davis, V. F. M. Segers, G. Esposito, T. Tokunou, K. Urbanek, T. Hosoda, M. Rota, P. Anversa, A. Leri, R. T. Lee, J. Kajstura, Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. *Circulation* **120**, 876–887 (2009).
47. M. J. Webber, J. Tongers, C. J. Newcomb, K. T. Marquardt, J. Bauersachs, D. W. Losordo, S. I. Stupp, Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13438–13443 (2011).
48. J. J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, R. A. Brown, Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3**, 349–363 (2002).
49. B. I. Jugdutt, Ventricular remodeling after infarction and the extracellular collagen matrix: When is enough enough? *Circulation* **108**, 1395–1403 (2003).
50. M. Grunewald, I. Avraham, Y. Dor, E. Bachar-Lustig, A. Itin, S. Yung, S. Chimenti, L. Landsman, R. Abramovitch, E. Keshet, VEGF-induced adult neovascularization: Recruitment, retention, and role of accessory cells. *Cell* **124**, 175–189 (2006).
51. O. Cleaver, D. A. Melton, Endothelial signaling during development. *Nat. Med.* **9**, 661–668 (2003).
52. A. A. Rane, K. L. Christman, Biomaterials for the treatment of myocardial infarction: A 5-year update. *J. Am. Coll. Cardiol.* **58**, 2615–2629 (2011).
53. V. F. M. Segers, R. T. Lee, Biomaterials to enhance stem cell function in the heart. *Circ. Res.* **109**, 910–922 (2011).
54. Y. Wu, J. E. Ip, J. Huang, L. Zhang, K. Matsushita, C. C. Liew, R. E. Pratt, V. J. Dzau, Essential role of ICAM-1/CD18 in mediating EPC recruitment, angiogenesis, and repair to the infarcted myocardium. *Circ. Res.* **99**, 315–322 (2006).

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