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Micro- and Nanoparticles for Treating Cardiovascular Disease

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Abstract

Cardiovascular disease, including myocardial infarction (MI) and peripheral artery disease (PAD), afflicts millions of people in United States. Current therapies are insufficient to restore blood flow and repair the injured heart or skeletal muscle, respectively, which is subjected to ischemic damage following vessel occlusion. Micro- and nano-particles are being designed as delivery vehicles for growth factors, enzymes and/or small molecules to provide a sustained therapeutic stimulus at the injured tissue. Depending on the formulation, the particles can be injected directly into the heart or skeletal muscle, or accumulate at the site of injury following an intravenous injection. In this article we review existing particle based therapies for treating MI and PAD.

Introduction

Over 80 million adults in the United States have at least one cardiovascular disease (CVD) and one in three deaths in 2010 were attributed to CVD¹. Heart-failure post myocardial infarction (MI) causes the majority of deaths resultant from cardiovascular-related disease². Following a MI, reparative mechanisms lead to collagenous scar formation in place of the damaged myocardium. The non-contractile nature of the scar leads to hypertrophy of the viable myocardium, infarct wall thinning and ventricular dilation³. If left untreated these processes progress until the heart no longer pumps adequately to supply sufficient blood to the body, defined as heart failure. The current gold standard treatment for end-stage heart failure is heart transplantation, a highly invasive surgery for which only a fraction of patients are eligible due to the limited availability of donor hearts. In addition, left ventricular assist devices (LVADs) prolong and improve quality of life for some patients with end-stage HF, however, LVAD implantation increases risk of stroke and infection. Furthermore, no current therapy prevents the negative remodeling process that leads to heart failure. Given the drawbacks of current state-of-the-art treatments, development of new therapies to mitigate negative left ventricular (LV) remodeling post-MI is crucial to patient quality of life and survival. Another cardiovascular disease afflicting a significant number of patients is peripheral artery disease (PAD). PAD results from obstruction of non-coronary or -cerebral vessels; the most prevalent presentation is atherosclerosis in the lower limbs. If left untreated, PAD can progress to critical limb ischemia (CLI), which is defined as pain at rest and can lead to non-healing ulcers, gangrene, and amputation^{4, 5}. The number of patients

with PAD/CLI is rising due to an increased prevalence of diabetes as well as a prolonged life expectancy. While revascularization procedures are effective for some patients, not all are eligible due to a number of confounding factors including inaccessible or diffuse vascular lesions and the presence of additional diseases⁶. The lack of efficacy of current therapies has motivated investigation into new techniques to increase perfusion. One experimental therapy under development for both MI and PAD is the use of micro- or nano-particles that release growth factors, enzymes, and/or small molecule drugs over time. The particles provide protection of the payload against immediate diffusion from or degradation at the injury site, enabling a sustained therapeutic stimulus. A wide range of release kinetics has been demonstrated through manipulation of material composition and concentration as well as particle size. Delivery is achieved by either injection into the ischemic myocardium or skeletal muscle or accumulation following intravenous injection (Figure 1). This article aims to comprehensively review existing particle based therapies for MI and PAD.

Microparticles

PROTEIN DELIVERY IN MICROPARTICLES

The most common use for directly injected (either intramyocardial or intramuscular) microparticles to date is delivery of growth factors. While growth factors can stimulate many important cellular processes needed for healing, their stability and longevity *in vivo* is short-lived due to enzymatic degradation and/or physical clearance⁷. Encapsulation within microparticles protects them against these external factors and provides a means of sustained release. Growth factors that influence vessel formation and maturation are most often delivered to restore blood flow to the ischemic region. In some cases, these pro-angiogenic growth factors are combined with a second growth factor or therapeutic in order to influence multiple stages of the healing response (Tables 1 & 2).

Protein Delivery for MI

Basic fibroblast growth factor (bFGF) for MI: Basic fibroblast growth factor (bFGF) is involved in blood vessel growth and development, and has been delivered in free form in numerous studies including clinical trials⁸. Though results were initially promising, only a small subset of patients showed modest improvement^{9, 10}. As a result, current research efforts focus on increasing the stability and retention of bFGF to improve its efficacy. Several studies have shown that microparticle-encapsulated bFGF induces greater revascularization and functional recovery than saline or empty particles.

Iwakura et al. compared vascular density following injection of gelatin microparticles (10 μ m) containing bFGF versus saline into the border zone (BZ) four weeks post-MI in rats¹¹. As expected, histological analysis revealed higher vascular density in the BZ and center of the infarct in rats treated with bFGF-containing particles. Perhaps due to the new vasculature, four weeks after injection the hearts receiving bFGF released from particles showed greater myocardial perfusion, reduced left ventricular diastolic/systolic dimension (LVSD/LVDD), and increased fractional shortening (FS) than those treated with saline. In another study delivering bFGF in gelatin particles (size not stated), Liu et al. injected the particles immediately post-MI in dogs¹². Again more microvessels were present in the BZ

three, ten, and seventeen days post-injection in the encapsulated bFGF group than the control empty particles. Furthermore, ten and seventeen days after injection, ejection fraction (EF) was significantly higher. In a subsequent study, Sakakibara et al. compared retention of gelatin microparticle-encapsulated bFGF (size not stated) to free bFGF in a rat MI model¹³. Significantly more bFGF was retained in the infarct in the intramyocardially-injected bFGF microparticle group compared to free bFGF injected through central venous, intracoronary, or intramyocardial routes 1, 24, and 72 hours post-injection. Additionally, four weeks after treatment in a pig MI model, more vessels were found in the BZ of the encapsulated bFGF group than in those injected with empty particles. Furthermore, pigs maintained LVSD/LVDd similar to pre-treatment levels, showed restoration of forward flow in the left anterior descending artery and a significant recovery of EF only after receiving encapsulated bFGF treatment.

VEGF with or without Other Therapeutics for MI: Vascular endothelial growth factor (VEGF) is another factor involved in angiogenesis and, similar to bFGF, clinical trials with free VEGF were initially promising¹⁴, but later disappointing¹⁵. Trying to improve upon these results, Formiga et al. compared the effect of VEGF released from poly(lactic-co-glycolic acid) (PLGA) microparticles ($5.1 \pm 1.3 \mu\text{m}$) to free VEGF and empty particles following injection into the BZ four days post-MI in rats¹⁶. In a preliminary study, microparticles were retained in the myocardium of healthy hearts for one month. At one month post-injection VEGF released from microparticles caused significantly greater growth of capillaries and arterioles in the BZ and infarct areas than free VEGF or empty particles. In addition, VEGF from the particles reduced wall thinning compared to both control groups (Figure 2). No functional parameters were measured in this study.

Building from the previous work two studies aimed to improve upon the efficacy of encapsulated VEGF alone. First, Simon-Yarza et al. examined whether combining PLGA-encapsulated VEGF ($4.89 \mu\text{m}$) with orally delivered coenzyme Q₁₀ (CoQ₁₀) PLGA nanoparticles ($159 \pm 9 \text{ nm}$) would enhance neovascularization¹⁷. The microparticles were injected into the BZ one week following MI in rats while the nanoparticles were delivered orally every three days for one month, then weekly for an additional two months. Consistent with previous studies, VEGF released from particles promoted greater capillary formation in the infarct and BZ as well as increased EF while empty particles caused neither, but combination with CoQ₁₀ did not further increase the capillary density or heart function. The authors speculate that CoQ₁₀ may have actually inhibited angiogenesis by reducing endogenous VEGF levels. Second, Cittadini et al. encapsulated VEGF and insulin growth factor (IGF-1), which promotes cell growth and proliferation, within gelatin microparticles ($70\text{--}90 \mu\text{m}$)¹⁸. Particles were injected into the BZ twenty minutes after MI in rats and four weeks later dual encapsulated growth factor treatment resulted in formation of more capillaries and mature vessels in the BZ, a greater reduction in infarct size and a smaller decline in FS than either encapsulated VEGF or IGF-1 alone.

Acidic Fibroblast Growth Factor (aFGF) for MI: Distinct from its angiogenic analog bFGF, acidic fibroblast growth factor (aFGF) has been shown to stimulate proliferation and reduce apoptosis of cardiomyocytes^{19–21}. Because of these cardioprotective affects Formiga

et al. injected aFGF in combination with neuregulin-1 (NRG-1), a protein shown to stimulate growth and proliferation^{22, 23}, encapsulated in PLGA microparticles ($5.1 \pm 1.4 \mu\text{m}$) into the BZ four days after MI in rats²⁴. The combined delivery induced greater formation of mature blood vessels than either factor delivered alone, but the three treatments equivalently reduced infarct size and fibrotic area. Similarly, combined and individual aFGF and NRG-1 particle delivery resulted in a statistically equivalent functional improvement, which was significantly greater than with empty particle treatment.

Enzymes for MI: Antioxidant and proteolytic enzymes also hold potential to mitigate negative remodeling following a heart attack, but have the same limited retention and efficacy as growth factors when administered in solution. To improve the efficacy of superoxide dismutase (SOD), which neutralizes the damaging effects of superoxide radicals, Seshadri et al. encapsulated the enzyme within particles composed of the polyketal-based polymer poly(cyclohexane-1,4-diylacetone dimethylene ketal) (PCADK) ($11.4 \pm 4.9 \mu\text{m}$)²⁵ (Figure 3). SOD-containing particles reduced superoxide levels in the BZ and the number of apoptotic cells in the LV to the same level as in sham-treated rats three days after treatment, while empty particles and free SOD caused a slight, but not significant reduction in both. Similarly, SOD-containing particles reduced the impact of ischemia on FS, whereas free SOD and empty particle had no impact on FS decline post-MI. However, this protective effect on heart function was no longer significant at the twenty-one day time point. Addition of p38 inhibitor (p38i; discussed further in Small Molecule Delivery for MI²⁶), a MAP kinase inhibitor, to SOD-containing particles enhanced their long-term effects on heart function, enabling a significant preservation of heart function after 21 days.

To avoid the challenges of maintaining enzyme activity throughout microparticle formulation, enzyme genes may be delivered instead. For example, Lin et al. delivered matrix metalloproteinase-1 (MMP-1) plasmid DNA in gelatin microparticles ($75 \mu\text{m}$) to the BZ four weeks after MI in an effort to prevent collagen accumulation and scar formation that is associated with low MMP-1 levels in the heart post-MI²⁷. This treatment reduced fibrosis in the infarct, BZ, and non-infarct regions²⁸. Particle-encapsulated MMP-1 plasmid DNA also caused improvement in LVDd, LVSD, and fractional area change (FAC) four weeks post-treatment compared to empty particles; functional and histological results with free MMP-1-DNA were not reported.

Protein Delivery for PAD

Basic fibroblast growth factor (bFGF) for PAD: Since PAD, like MI, results in ischemic tissue damage, angiogenic factors like bFGF are appealing candidates for new therapies. Direct injection of such growth factors in solution into ischemic muscle produced disappointing results in several clinical trials²⁹. Many groups hypothesized that microparticle-encapsulated bFGF with or without a second therapeutic would improve on the early studies of bFGF delivery. Kanematsu et al. found that intramuscular (IM) injection of bFGF in collagen microparticles ($100\text{--}200 \mu\text{m}$) one week after ischemic injury in mice led to an increase in capillary density and hindlimb blood flow compared to injection of free bFGF, empty particles, or no treatment³⁰. The ischemia/normal blood perfusion ratio was restored after encapsulated bFGF treatment, whereas all other treatments resulted in low

perfusion at two, three, and four weeks after injection. In a subsequent study, Marui et al. observed positive functional improvements in human patients ineligible for surgical treatment following injection of gelatin microparticle (50–100 μm) encapsulated-bFGF in their ischemic gastrocnemius muscle³¹. The bFGF particle treatment increased six-minute walk distance, decreased resting pain, increased transcutaneous oxygen pressure, and increased laser Doppler perfusion index in all six patients four and twenty-four weeks after injection over pre-treatment levels. Additionally five patients had non-healing foot ulcers before the treatment and after twelve weeks three had healed completely, one was reduced, and one remained unchanged. Regarding clinical safety, no focal inflammation or edema were observed at the injection site and bFGF was undetectable or at normal levels in all patient blood samples.

The Maehara group has employed a non-traditional approach to bFGF encapsulation using fragmin/protamine complexes in two studies; protamines are small, arginine-rich nuclear proteins, which, when complexed to fragmin (low molecular weight heparin), neutralize its anticoagulant activity. In the first study, bFGF-containing fragmin/protamine particles (1–3 μm) were injected ten days after ischemia induction in rabbits, and in the second, the particles (0.5 – 3 μm) were injected into mice one day post-injury. Both investigations demonstrated a clear advantage of delivery of bFGF in fragmin/protamine particles over administration of free bFGF or empty particles. In the rabbit study, bFGF released from fragmin/protamine particles increased capillary density and led to development of more collateral vessels than empty particles, free bFGF or no treatment at four weeks post-injection³². Interestingly, empty particles increased capillary density and produced more collateral vessels than free bFGF, perhaps through heparin stabilization of endogenous growth factors. Functionally, fragmin/protamine encapsulated bFGF was most effective in improving calf blood pressure ratio and blood flow ratio while empty particles showed modest improvements over free bFGF. In the mouse study, bFGF released from the fragmin/protamine particles or delivered freely prevented limb loss in three mice and one mouse, respectively, whereas all mice treated with empty particles suffered limb loss³³. In a more recent study, Fujita et al. used the same particles (0.5 – 3 μm) to deliver platelet rich plasma (PRP) to ischemic hindlimbs of rabbits ten days after ischemic injury³⁴. Platelet granules are known to contain a cocktail of growth factors³⁵ and when released from the particles, they stimulated growth of more collateral vessels than free PRP, empty particles or saline.

To reduce cost and prevent adverse side effects^{36–38} associated with high bFGF dosage several studies have aimed to improve the efficacy of bFGF through combination with a second therapeutic for treating PAD. The Komeda group examined addition of growth factor binding molecules, additional growth factors, or vasodilators. In one study completed by Arai et al., heparin, known to accelerate angiogenesis,³⁹ was injected subcutaneously, while bFGF in gelatin microparticles (26 μm) was injected IM following ischemic injury in wild type & hypercholesterolemic mice⁴⁰. Though addition of heparin treatment did not further enhance angiogenesis in wild type mice, two weeks of daily heparin injections did increase bFGF's effect on capillary and arteriole density as well as perfusion in hypercholesterolemic mice. This result is important because hypercholesterolemia is known to impair the angiogenic response, and affects many human patients suffering from PAD.

Hepatocyte growth factor (HGF) has been reported to act cooperatively with bFGF⁴¹, thus Marui et al. delivered both growth factors encapsulated in collagen microparticles (100–200 μ m), hypothesizing that the combination would cause a similar therapeutic effect with lower total quantities of growth factor than either treatment alone⁴². In severe ischemia induced in mice, 5 μ g encapsulated bFGF and 20 μ g encapsulated HGF produced equivalent improvement in capillary and arteriole density as 20 μ g of encapsulated bFGF or 80 μ g of encapsulated HGF delivered independently. Furthermore, the smaller dosages were ineffective in restoring blood flow if delivered separately.

To enhance blood flow through newly formed collateral vessels, two studies incorporated vasodilators. Huang et al. injected bFGF (10 μ g) in gelatin microparticles (size not stated) and prostaglandin E₁ (PGE₁) (0.2 μ g) in PLGA microparticles (size not stated) immediately after hindlimb ischemia induction in mice⁴³. The addition of encapsulated PGE₁ treatment increased blood vessel density and maturity over that of encapsulated bFGF four weeks after injection. The difference in effect likely results from the fact that in addition to vasodilation, PGE₁ upregulates VEGF and HGF activity⁴⁴. Similar to encapsulated HGF, PGE₁ released from PLGA particles reduced the necessary dose of bFGF for functional improvement from 40 μ g to 10 μ g. Hirose et al. injected bFGF in the same particles (10 μ m) with sarpogrelate, a small molecule drug that dilates collateral vessels, as a supplement in the diet two weeks post-ischemia in rabbits⁴⁵. The addition of sarpogrelate did not increase capillary density over that of bFGF-containing particle treated rabbits. However, the combination therapy did produce more visible collateral vessels, qualitatively enlarged vessel diameters, and increase regional blood flow six weeks after treatment.

Other proteins for PAD: Granulocyte colony stimulating factor (G-CSF) stimulates endothelial cell migration, proliferation, and tube formation *in vitro*⁴⁶. Hypothesizing that G-CSF would enhance angiogenesis in ischemic muscle, Kawamura et al. delivered this glycoprotein hormone either encapsulated in gelatin microparticles (30 μ m) injected IM or unencapsulated and injected subcutaneously (sub-Q) immediately after vessel ligation in mice⁴⁷. Initially, the sub-Q injected free G-CSF treatment promoted development of capillaries, which resulted in the highest ischemic/nonischemic blood flow ratio two weeks after treatment. However, eight weeks after treatment the IM-injected encapsulated G-CSF treatment promoted production of more capillaries and small arterioles than any other group, consequently providing the largest improvement in blood flow. These results suggest that sub-Q treatment with G-CSF led to development of immature, unstable vessels, whereas the G-CSF released from particles encouraged development of more mature, stable vessels that gave a longer-lasting therapeutic effect. To address the need for therapeutic angiogenesis in patients with chronic ischemia the authors also studied the effect of delivering particle-encapsulated G-CSF four weeks after the ischemic injury. At the time of injection the ischemic/normal blood flow ratio had returned to 69% without any treatment. The encapsulated G-CSF treatment caused a further increase to ~90% over the next eight weeks, whereas saline treatment caused no additional improvement. The other treatment groups were not tested in the chronic model.

Another pro-angiogenic protein has also proven effective in this setting. The hematogenous cytokine erythropoietin (EPO) had previously been shown to promote blood vessel growth

following ischemia-reperfusion injury in other organs^{48, 49}, but had not been studied in a slow-release formulation. Therefore, Li et al. delivered EPO encapsulated in gelatin microparticles (30 μm) immediately following ligation in mice⁵⁰. At two weeks after treatment, unencapsulated EPO increased capillary density and blood flow to a greater extent than all other treatment groups. However, six weeks later the EPO from the particles stimulated more capillary and small arteriole growth, which allowed greater blood flow into the legs (Figure 4). These results are not surprising since the mechanism of action of EPO, involving stimulation of proliferation, survival, and differentiation of erythroid progenitor cells, is similar to that of G-CSF.

SMALL MOLECULE DELIVERY IN MICROPARTICLES

Though microparticles are especially advantageous for delivering proteins, as they require protection from enzymatic degradation, they can also be used to extend the lifetime of small molecule drugs. This approach may be more versatile, as small molecules are less susceptible to damage during microparticle preparation and can be used to stimulate the same processes as proteins (Tables 1 & 2).

Small Molecule Delivery for MI—Wei et al. found that delivery of ginsenoside Rg₁ (Rg₁), an angiogenic agent found in *P. ginseng*, to the BZ four weeks post-MI in rats in gelatin microparticles ($28.1 \pm 2.8 \mu\text{m}$) caused greater formation of microvessels, similar to the effect of encapsulated bFGF, than empty particles⁵¹. Particles containing Rg₁ or bFGF also preserved EF at four weeks post-injection, while the EF of hearts receiving empty particles declined. The two treatments also qualitatively prevented LV dilation, preserved wall motion, and sustained wall thickness after four weeks. Sy et al. compared the effect of a p38 inhibitor encapsulated in PCADK microparticles ($\sim 20 \mu\text{m}$) (previously mentioned in *Enzymes for MI*²⁵) to that of free inhibitor on the inflammatory response and fibrosis²⁶. The p38 mitogen-activated kinase pathway is involved in macrophage activation and cardiomyocyte apoptosis⁵². Three days following injection into the infarct immediately following MI in rats, these particles significantly inhibited p38 phosphorylation, superoxide production, and tumor necrosis factor alpha (TNF- α) production compared to free p38i or empty particles. These results suggest that inactivation of the p38 pathway and consequent reduction of the inflammatory response was greatest when p38i was delivered in microparticles. Furthermore, fibrotic area was significantly lower at day twenty-one in the p38i particle group than in all other groups. Finally, though no functional improvement was seen in response to particle-delivered p38i after one week, FS was improved from day seven to day twenty-one only in the p38i particle group.

To deliver phosphatidylserine (PS), which signals macrophages to reduce proinflammatory cytokine secretion and increase anti-inflammatory cytokine secretion during cell apoptosis, to infarcted muscle, Harel-Adar et al. modified liposomes to present this phospholipid on their surface⁵³. PS-presenting liposomes ($1.2 \pm 0.3 \mu\text{m}$) were injected 48 hours after MI in rats, after which they accumulated in the infarct and generated a higher capillary and arteriole density than in infarcts treated with PS-lacking liposome or saline treatment. Furthermore, infarcts were smaller and LV walls were thicker following injection of either type of liposome compared to saline. Finally, LVD/S area increased less in hearts receiving

PS-presenting liposomes compared to PS-lacking liposomes or saline after 4 weeks suggesting less negative LV remodeling. To elucidate the underlying mechanism, PS-presenting liposomes were injected directly into the heart and macrophages that engulfed them, which were isolated and cultured *in vitro*, showed increased secretion of anti-inflammatory interleukin 10 (IL-10) and TGF- β and slightly less proinflammatory TNF- α secretion.

Small Molecule Delivery for PAD—Given the improvements in angiogenesis and blood flow observed by Huang et al. following combined treatment with bFGF and PGE₁⁴³, Esaki et al. examined whether treatment with PGE₁ alone is effective in treating hindlimb ischemia⁵⁴. PGE₁ (100 μ g/kg) was injected two days after ischemic injury in mice in one of three dosages; encapsulated in PLGA microparticles (15–40 μ m), unencapsulated in one dose IM, or unencapsulated in daily sub-Q injections for two weeks. PGE₁ was most effective when released from the particles, yielding higher blood vessel density and maturity, as well as blood flow compared to the other two delivery methods. Lower doses of encapsulated PGE₁, 10 and 30 μ g/kg, were not as effective in improving blood flow.

Because it is a known regulator in the vascular system Qi et al. encapsulated sphingosine-1-phosphate (S1P) in PLGA microparticles (10–30 μ m) and injected it immediately post-ischemia induction in mice⁵⁵. The particle-encapsulated S1P was only able to stimulate significant improvements in limb blood flow if the treatment was injected every three days for a month. The authors speculate that the repetitive injection maintains a more optimal concentration of S1P in the tissue than the single injection, but no measurement of the S1P concentration was made. In contrast, numerous other studies have shown sustained release of a payload following a single injection of PLGA microparticles^{16, 17, 24, 43, 54}.

RETENTION OF MICROPARTICLES

Microparticles must be retained in the injected tissue as they degrade in order to function as depots for growth factors, enzymes, and/or small molecule drugs. To maximize retention times, several studies have evaluated the effect of injection volume and location, particle size, as well as the state of the injected heart (ie. healthy, infarcted, or mechanically arrested). Of these tested parameters, contractility of the injected tissue appears to be the most influential. The majority of injected particles are rapidly expelled by the contracting myocardium; however, retention rates are significantly increased if the particles are delivered to non-contractile tissue, such as an infarct. Overall, these studies indicate that suitable retention of microparticles in the infarcted myocardium is feasible. No systematic studies have however investigated microparticle retention in skeletal muscle following IM-injection, though the information would be very valuable. It is known that cells are poorly retained following IM injection^{56, 57}, and thus microparticles of similar size may also be cleared shortly after injection.

Three studies examined the difference in the proportion of microparticles retained after injection into infarcted or healthy myocardium. Anderl et al. observed high retention of both 10 and 40 μ m gold or iridium-labeled polystyrene microparticles ($63 \pm 12\%$) twenty minutes post-injection into the infarct immediately following MI in rats using a 27-gauge needle⁵⁸.

Only a small amount leaked through the needle track or escaped into the lungs, which when combined with those in the heart accounted for $88 \pm 9\%$ of the injected particles; no particles were found in the liver or spleen. Particle distribution was similar six days after injection. Similarly, Formiga et al. injected $5 \mu\text{m}$ fluorescent-labeled PLGA microparticles into the BZ of a four-day-old infarct in rats with a 29-gauge needle and witnessed particle retention up to 90 days after injection⁵⁹. The $5 \mu\text{m}$ particles were selected for the long-term retention study because larger particles (14 and $30 \mu\text{m}$) provided more resistance through the chosen needle size, whereas $2 \mu\text{m}$ particles were easily injected but quickly cleared from the tissue. In contrast, Teng et al. found far lower retention of $10 \mu\text{m}$ fluorescent particles (material not stated) following injection into healthy rat hearts using a 28-gauge needle⁶⁰. Only 5–10% of the particles were retained, regardless of whether they were injected into one or three locations or whether the needle hole was immediately sutured. Further studies in a pig model revealed that 10–20% of the particles were retained in the healthy, beating hearts, while nearly 70% were retained in the arrested, non-beating hearts, corroborating the conclusion that significantly more particles are retained in non-contractile heart tissue.

Results of a fourth study completed by Grossman et al. also support the difference in particle retention in contractile versus non-contractile heart tissue⁶¹. They observed far greater retention of $15 \mu\text{m}$ neutron-activated microparticles (material not stated) following epicardial needle injection into postmortem pig hearts ($89 \pm 60\%$) than into healthy, beating hearts via either percutaneous endomyocardial catheter ($43 \pm 15\%$) or an epicardial needle ($15 \pm 21\%$) injection using a 27–28-gauge needle. In addition, they compared three injection volumes (10, 20 and $100 \mu\text{L}$) and observed that for both endo and epicardial injections, smaller injection volume resulted in greater retention in the heart ($98 \pm 85\%$, $40 \pm 48\%$, and $20 \pm 25\%$, for 10, 20 and $100 \mu\text{L}$ respectively after endomyocardial injection and $36 \pm 50\%$, $19 \pm 17\%$, and $9 \pm 9\%$ after epicardial injection).

Finally, Hoshino et al. examined the effect of injection site⁶². Retention of $10 \mu\text{m}$ fluorescent microparticles (material not stated) in healthy pig hearts was highest following antegrade injection (AI) via the coronary arteries ($73 \pm 11\%$), followed by direct myocardial injection (DI) from the coronary sinus with a 27-gauge needle ($47 \pm 14\%$) and retrograde injection (RI) via the coronary veins ($22 \pm 8\%$). However, though AI resulted in the greatest particle retention, they witnessed several microinfarctions following this type of injection, suggesting safety drawbacks. No such tissue damage was recorded in the other groups.

Though these results indicate that retention is maximized if particles are injected into infarcted myocardium, damaged heart muscle may be susceptible to rupture immediately following injury⁶³. Therefore, in some cases, such as injecting acutely after MI, it may be safer to inject microparticle therapies into the border zone. It is important to remember, however, that studies to date suggest that microparticles injected into healthy, beating myocardium will have much lower retention rates than those injected in infarcted, non-contractile tissue.

Nanoparticles

As nanoparticles are much more likely than microparticles to be taken up by cells and less likely to cause embolization if they enter the blood stream, several groups have examined their utility for delivering various therapeutics to the ischemic heart (Table 3) and hindlimb (Table 4).

LOCAL INJECTION

Nanoparticles for MI—To the best of our knowledge, only one study by Chang et al. has used nanoparticles to deliver growth factors to the heart. IGF-1 was electrostatically complexed to PLGA using polyethylenimine (PEI) to create nanoparticles (74.4 ± 11.3 nm), which were injected into the BZ immediately following MI in mice⁶⁴. Nanoparticle delivery enhanced retention of IGF-1 up to 24 hours post-injection, but no IGF-1 was detectable after 72 hours. The PLGA/PEI formulation was also more effective in reducing LVSD/LVDd, thickening infarct wall, reducing infarct area, and enhancing FS and EF 21 days post-injection compared to unencapsulated IGF-1 and empty particles.

The ability of decorated-nanoparticles to be taken up by non-phagocytic cells offers a solution to the limitation of the Davis group's work delivering p38 inhibitor described above (*Small Molecule Delivery for MI*²⁶). In that study, microparticles were engulfed by macrophages and were unable to effectively deliver p38i to at-risk cardiomyocytes within the first 24–48 hours following MI. Thus, in a subsequent study⁶⁵, they delivered p38i in polyketal (PCADK) nanoparticles (320 ± 156 nm) coated with N-acetyl-glucosamine (GlcNAc), a sugar molecule known to cause uptake in cardiomyocytes^{65, 66} (Figure 5). Encapsulation of p38i in the coated particles reduced infarct size and increased FS after three days compared to empty particles. Free p38i and p38i encapsulated in uncoated particles were not tested in this study because in the previous study by Sy et al. neither treatment resulted in functional improvement after seven days.

Somasuntharam et al. took a gene-level approach, delivering Nox2-nicotinamide adenine dinucleotide phosphate (NADPH) oxidase siRNA in polyketal-based nanoparticles (500 nm); Nox2 is the catalytic subunit of NADPH and a known source of reactive oxygen species (ROS)⁶⁷. Three days post-injection into an infarct in mice, encapsulated Nox2-NADPH oxidase siRNA caused significant reduction in Nox2 mRNA expression and improved FS compared to encapsulated scrambled siRNA and empty particles. Paul et al. combined non-viral and viral gene therapy systems in a hybrid complex of recombinant baculovirus and Tat/DNA nanoparticles (~350–500 nm) to deliver angiopoietin-1 (Ang-1) transgene to the BZ fifteen minutes after MI in rats⁶⁸. Hybrid complex-encapsulated Ang-1 increased Ang-1 expression in the BZ three days post-injection and reduced infarct area, thickened the LV wall, and increased capillary and arteriole density 21 days post-injection compared to either baculovirus- or nanoparticle- encapsulated Ang-1. Furthermore, delivery of Ang-1 in the hybrid complex yielded improved EF two and three weeks after injection compared to in baculovirus- or nanoparticle- vehicles.

Nanoparticles for PAD—Xie et al. developed a carrier that could form electrostatic interactions with VEGF and be enzymatically degradable⁶⁹. In their study VEGF

encapsulated in dextran-co-gelatin nanoparticles (130 ± 3.5 nm) was delivered to ischemic hindlimbs of rabbits two days after vessel ligation. The encapsulated VEGF stimulated growth of more capillaries and collateral vessels than free VEGF or empty particle treatment after 25 days. This increase in vessel density resulted in a higher blood perfusion ratio compared to both control groups.

While in prior studies heparin was used to stabilize delivery of bFGF⁴² or enhance angiogenesis through systemic administration⁴⁰, Lian et al. delivered it as a primary therapeutic agent in PLGA nanoparticles (297 nm)⁷⁰. The nanoparticles were injected immediately following ischemia induction in mice. Only one mouse treated with heparin nanoparticles suffered limb necrosis compared to four mice that were treated with saline. This was likely due to the improved blood perfusion in the limbs treated with slowly released heparin compared with saline after one month.

SYSTEMIC DELIVERY

Systemic delivery of growth factors or small molecule-loaded particles is far less invasive than direct injection into the heart or limb. Nanoparticles accumulate within infarcted muscle because hypoxic conditions enhance vascular permeability via production of angiogenic factors and resulting endothelial cell migration^{71, 72}. However, this window of enhanced permeability post-MI is short-lived compared to that observed in cancer (the so-called enhanced permeability and retention (EPR) effect) so the timing of delivery following injury is important⁷³. While timing of delivery is important in animal models of acute hindlimb ischemia⁷², presentation of PAD in humans is more chronic⁷⁴, and thus should enable a wider delivery window. In this section we briefly review several vehicles that have been developed for systemic delivery of therapeutics to treat MI (Table 3) and PAD (Table 4).

Nanoparticles for MI

Non-targeted: As the major challenge in developing a formulation for systemic delivery is ensuring sufficient accumulation of payload within injured tissue before the carrier is cleared from the body, a number of studies have examined the relative permeability of infarcted and healthy heart muscle to micelles (~10 nm) and liposomes (~100 nm). Both have been observed to accumulate preferentially in infarcted heart muscle following intravenous (IV) injection^{73, 75–78}. To improve vasodilation, Feld et al. injected PGE₁-loaded liposomes at the beginning⁷⁹ or end⁸⁰ of a two-hour coronary artery occlusion period in dogs, which was followed by reperfusion. Surprisingly, free PGE₁ was as effective as PGE₁-loaded liposomes at reducing infarct size when delivered at the beginning of the occlusion, but less effective when delivered immediately before reperfusion. However, these results were obtained in two separate studies and the authors state that infarct size following free PGE₁ treatment was confounded by high collateral blood flow and small at-risk area in those animals.

In order to restore normal cell metabolism and reduce damage from free radicals following MI, the Torchilin group delivered liposome-encapsulated (150–200 nm) adenosine triphosphate (ATP) or coenzyme Q₁₀ (CoQ₁₀), respectively, to the heart post-MI and

evaluated the impact on infarct size. First, ATP-loaded liposomes were delivered immediately before MI in rabbits⁸¹. ATP was expected to improve function by restoring activity of ATP-dependent ion channels, as ATP levels are reduced by up to 80% in ischemic conditions⁸²; reduced ion channel activity leads to cell swelling and apoptosis. Second, the endogenous antioxidant CoQ₁₀, which is also reduced immediately following MI, was encapsulated in liposomes and delivered to rabbits immediately before MI⁸³. Both therapeutic-loaded liposomes reduced infarct size compared to with empty liposomes treatment. Because ATP and CoQ₁₀ levels are reduced immediately following an infarction, these studies examined the effects of prophylactic delivery. As ischemic injury cannot easily be predicted in the clinic, whether these therapeutics affect infarct size when administered immediately following injury should be examined.

Adenosine, a purine nucleoside, has been shown to be cardioprotective⁸⁴ but is only effective at high concentrations. To increase the proportion that reaches the infarct, Takahama et al. delivered adenosine in liposomes (~130 nm) immediately prior to reperfusion in rats⁸⁵. The liposomes preferentially accumulated in the infarct versus healthy myocardium and infarct size was reduced compared to free adenosine or empty liposome treatment. The encapsulated adenosine also caused fewer adverse effects on heart rate and blood pressure than free adenosine.

Targeted: Incorporation of targeting moieties for cell surface receptors upregulated following ischemic damage, such as P-selectin and angiotensin-1 (AT1), enhanced trafficking to the infarct^{73, 77, 78}. However, even with cell-specific targeting, accumulation is highest within a few hours or days following MI and drastically declines if particles are injected 1–2 weeks post-MI^{73, 76–78}. This reduction is most likely due to the endogenous sealing of the leaky vasculature⁸⁶, and thus systemic delivery appears to be most suitable for acute treatments.

Seeking to minimize off-target effects of systemically delivered free VEGF, Scott et al. administered VEGF encapsulated in P-selectin-conjugated liposomes (180 ± 13 nm) immediately following MI in rats⁷⁷. Treatment with the targeted VEGF liposomes reduced hypokinesis of the heart wall and increased FS compared to empty liposome, systemic VEGF or IgG-conjugated VEGF liposome treatment at 2, 3, and 4 weeks. These improvements in function likely resulted from the greater number of perfused vessels in targeted VEGF-liposome treated hearts. However, the number of perfused vessels was only compared to untreated hearts, so whether targeting or liposome delivery alone increased vessel perfusion remains unclear.

Nanoparticles for PAD—Though direct injection into skeletal muscle is significantly less invasive than in the heart, IM injection is still challenging. For instance, determining the optimal location for injection is difficult with current imaging technologies. Therefore targeting particles to the injured tissue following systemic administration presents an appealing alternative.

The efficacy of two strategies of nanoparticles delivery of pro-angiogenic growth factors has been studied. Kim et al. conjugated VEGF to gold nanoparticles (67 and 124 nm without and

with VEGF) and injected them IV one day after ligation in mice⁷². The nanoparticle-conjugated VEGF preferentially accumulated in the ischemic muscle and stimulated growth of twice as many blood vessels as free VEGF resulting in an increase in perfusion compared to unconjugated nanoparticles and free VEGF after five weeks. Instead of relying on passive accumulation of nanoparticles via leaky vessels, Chappell et al. used ultrasonic destruction of microbubbles to generate a pressure gradient that actively transported nanoparticles from blood vessels into the hindlimb interstitial space⁸⁷. PLGA nanoparticles (100 nm) loaded with bFGF were administered along with microbubbles through a catheter in the saphenous artery while ultrasound pulses (1 Hz) were externally applied every three seconds for two minutes. At the conclusion of the injection/ultrasound sequence the artery was occluded and bisected. Particle-delivered bFGF increased arteriole density and diameter to a significantly greater extent than bovine serum albumin (BSA) delivered via the same strategy after two weeks.

Discussion

Encapsulation in micro- and nano-particles enhanced the efficacy of a variety of therapeutic agents, however much work is needed to prepare such therapies for clinical translation. Unfortunately, over half of the studies delivering proteins or small molecules in MI models lacked an unencapsulated therapeutic group, thus limiting conclusions regarding significant improvements as a result of particle delivery. In contrast to the MI treatments, most particle-based treatments in hindlimb ischemia models were compared to and showed efficacy over the unencapsulated therapeutic, thus confirming the hypothesis that sustained release of proteins and small molecules aided in the development of new vasculature and restoration of limb perfusion.

While VEGF delivered in microparticles did increase blood vessel density compared to free VEGF¹⁶, it did not significantly improve cardiac function when delivered alone up to three months post-MI^{17, 18}. Longer-term studies are needed to determine if sustained release of a single angiogenic therapeutic can induce prolonged functional improvement post-MI. Effective salvage of at-risk myocardium and development of new healthy myocardium will likely require targeting of more processes than blood vessel growth. In contrast, a single angiogenic therapeutic may be sufficient for treating PAD since reduced blood flow is the primary cause, though two interesting results were observed when bFGF was delivered in combination with another therapy. First, vessel development and limb perfusion were improved above that of treatment with only bFGF particles, and second, the amount of growth factor needed to cause functional improvement was reduced with combination therapy.

To continue the progress towards a comprehensive therapy, future studies should test more combinations of therapeutics to activate complementary healing pathways. There are a few ways particle-based therapies can be useful in this regard. First, the results of several studies suggest that nanoparticle delivery may be most suitable for influencing acute healing post-MI. Chang⁶⁴, Somasuntharam⁶⁷ and Gray⁶⁵ et al. observed fewer apoptotic cells, smaller infarcts and/or functional improvements three days after nanoparticle-encapsulated therapeutic delivery. Furthermore, though it has not been studied in depth, it is possible that

nanoparticles will be cleared more rapidly than microparticles from the heart muscle due to their smaller size. Therefore delivering a combination of nano- and microparticles may allow modulation of both acute and chronic remodeling pathways through one injection post-MI. Studies delivering nanoparticles to treat PAD did not focus on acute time points, but rather showed increased limb perfusion when releasing VEGF⁶⁹ and heparin⁷⁰ after one month. However, due to the small number of studies it is not yet clear whether nano- or microparticles are better suited as a delivery vehicle for an IM-injected therapy for treating PAD.

Second, combining particles and hydrogels can enable two distinct release profiles for delivery of two payloads sequentially. Though they have certain advantages, one limitation of hydrogels can be rapid diffusion of the therapeutic payload due to the high water content of the scaffold. As a result several studies for treating PAD have investigated whether nano- and microparticles can enhance therapeutic efficacy of hydrogel-based therapies by slowing release of one payload, while a separate payload can be released quickly from the hydrogel^{88, 89}. While many injectable hydrogel therapies have been tested for treating MI⁹⁰, none to date have incorporated embedded particles. And third, particles could be specifically tuned to afford different release rates. One example of this strategy is recent work by Suarez et al. who demonstrated tunable release of proteins in mildly acidic environments using acetalated dextran (AcDex)⁹¹. AcDex microparticles (30–90 μ m) allowed release rates of active bFGF ranging from days to weeks depending on the acetalation reaction time.

Furthermore, tighter control over payload delivery rates will also allow a more thorough investigation into when and for how long each therapeutic should be delivered after injury, thus informing the design of particle-based therapies with optimal release kinetics. For example, two proteins important for cardiomyocyte survival, aFGF and NRG-1, were delivered in microparticles at the same time but did not produce the predicted synergistic effect, potentially due to an overlap in signaling pathways²⁴. Perhaps if they were delivered sequentially this outcome would be different. Another potential target for finely tuned delivery post-MI is MMPs. Lin et al. delivered MMP-1 plasmid DNA in microparticles to reduced scarring post-MI²⁸. However, MMPs have another important role in contributing to post-MI damage that was not targeted in the Lin et al. study, namely breakdown of native extracellular matrix (ECM) proteins immediately following ischemic injury and cardiomyocyte death⁹². Thus, early downregulation and late upregulation of MMPs may be a more effective strategy to minimize damage, by preserving healthy ECM and preventing scar formation, respectively. Whether therapeutics are delivered alone or in combination, further study in large animal models and clinical trials are required to determine the optimal material for encapsulation; it appears that both naturally derived and synthetic polymers effectively preserve growth factor bioactivity, but long-term safety and biocompatibility needs to be studied for all candidate materials.

Regarding systemic delivery, while liposomal encapsulation can improve efficacy of growth factor and small molecule therapies over their free form post-MI, liposomes suffer major disadvantages including uncontrolled drug release in the bloodstream, poor encapsulation efficiency, and poor stability during storage, warranting further optimization of these delivery vehicles. Furthermore, since only two studies have been completed and they

utilized very different techniques and materials, more work is needed to determine the viability of systemic particle delivery for treating PAD.

Finally, standardization of animal models, specifically to mimic the often-chronic clinical presentation of both diseases, will more accurately predict therapeutic efficacy in human patients. So while more work needs to be done, micro- and nano-particle therapies are a promising technique for improving quality of life for the millions of patients worldwide suffering from cardiovascular-related diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Particle-based therapies for treating MI and PAD. Proteins or small molecules are encapsulated in microparticles or nanoparticles for intramyocardial delivery to the heart as well as intramuscular or systemic delivery to the leg. Liposomes are used as delivery vehicles for systemic delivery to the heart.

Figure 2.

Reduced wall thinning with VEGF-microparticles (MP). Sirius red-stained hearts show the infarct scar in red. LV wall thickness was significantly greater in hearts treated with VEGF-releasing PLGA microparticles compared to those treated with non-loaded microparticles or free VEGF (** $p < 0.01$). Scale bars: 1 mm. Reprinted from [16] with permission from Elsevier.

Figure 3. Superoxide dismutase (SOD) retention in infarcted myocardium. FITC-SOD was detected in infarcted hearts three days after injection when encapsulated in polyketal microparticles (A), but not when delivered in free form (B). Nuclei are counterstained with DAPI. Scale bar: 50 mm. Reprinted from [25] with permission from Elsevier.

Figure 4.

Microparticle delivery of erythropoietin (EPO) in a hindlimb ischemia model. (A) Hindlimb blood perfusion visualized with Laser Doppler imaging. (B) While free EPO increased blood perfusion acutely, EPO released from gelatin microparticles significantly improved blood perfusion in the injured hindlimb after 8 weeks compared to empty particles, free EPO and saline treatment. * $p < 0.05$, EPO or EPO-GHM versus the other groups; # $p < 0.05$, EPO or EPO-GHM versus saline and GHM. EPO-GHM = erythropoietin-immersed gelatin hydrogel microspheres; GHM = gelatin hydrogel microspheres. Reprinted from [50] with permission from Elsevier.

Figure 5.

CM uptake of PK nanoparticles. PK particles encapsulating (9'-(4-(and 5)-chloromethyl-2-carboxyphenyl)-7'-chloro-6'-oxo-1,2,2,4-tetramethyl-1,2-dihydropyrido[2',3'-6]xanthene (CMRA) fluorescent dye were more readily taken up by CMs when decorated with GlcNAc than without a coating (a). The enhanced uptake caused a reduction in apoptosis as measured by TUNEL staining (b). There were fewer apoptotic CMs in hearts treated with p38i (SB) loaded GlcNAc-coated particles compared to empty coated nanoparticle (* $p < 0.01$) and saline treatment (** $p < 0.05$). Reprinted from [65] with permission from the National Library of Medicine.

Table 1

Microparticle Therapies for MI

Material	Particle Size	Payload	Second Therapeutic	Species	Injury model	Delivery Method	Time of treatment post-injury	Time of assessment	LV dimensions	Functional outcome & technique	Neovascularization (location)	Other	Ref.
Gelatin	10 µm	bFGF		Rat	TO	Intramyocardial injection	4 weeks	4 weeks	↓ LVsd/LVDd	↑FS Echo	↑ Vascular density (BZ & infarct)	↑Myocardial perfusion	11
Gelatin	NS	bFGF		Dog	TO	Intramyocardial injection	Immediate	3, 10, 17 days		↑EF MRI	↑ Vascular density (BZ)		12
Gelatin	NS	bFGF		Pig	TO	Intramyocardial injection	4 weeks	4 weeks	= LVsd/LVDd	↑EF Echo	↑ Vascular density (BZ)	Forward flow of the LAD	13
PLGA	5.1 ± 1.3 µm	VEGF		Rat	IR	Intramyocardial injection	4 days	1 month			↑ Capillary, arteriole density (BZ & infarct)	↓ Wall thinning	16
PLGA	4.89 µm	VEGF	CoQ ₁₀ in PLGA nanoparticles (159 ± 9 nm); oral delivery	Rat	TO	Intramyocardial injection	1 week	3 months		↑EF Echo	↓ Vascular density compared to VEGF particles alone		17
Gelatin	70–90 µm	VEGF	IGF-1 in same gelatin particles; intramyocardial injection	Rat	TO	Intramyocardial injection	20 minutes	4 weeks		↑FS Echo	↑ Capillary, arteriole density	↓ Infarct size, TNF-α+ CM, CD68+ cells, apoptotic cells; ↑SERCA-2 mRNA	18
PLGA	5.1 ± 1.4 µm	aFGF	NRG-1 in same PLGA particles; intramyocardial injection	Rat	TO	Intramyocardial injection	4 days			↑EF & FS Echo	↑ Arteriole density	↓ Infarct size, fibrotic area	24
PCADK	11.4± 4.9 µm	SOD	p38i in same PCADK particles; intramyocardial injection	Rat	IR	Intramyocardial injection	Immediate	3, 21 days		= FS Echo		↓ Superoxide levels, apoptotic cells	25
Gelatin	75 µm	MMP-1 plasmid DNA		Rat	TO	Intramyocardial injection	4 weeks	4 weeks	↓ LVsd/LVDd			↓ Fibrosis (infarct, BZ, remote); <i>de novo</i> MMP-1 production	28
Gelatin	28.1 ± 2.8 µm	Ginsenos ide R _{g1}		Rat	TO	Intramyocardial injection	4 weeks	4 weeks		= EF Echo	↑ Vascular density	↓ LV dilation, preservation of wall thickness (qualitative)	51
PCADK	~20 µm	p38i		Rat	TO	Intramyocardial injection	Immediate	3, 7, 21 days		↑FS Echo		↓ p38 phosphorylation, superoxide, TNF- α, fibrosis	26
PS- presenting liposomes	1 µm			Rat	TO	IV	48 Hours	4 weeks	↓ LVD/S area		↑Capillary, arteriole density	↓ Infarct size; ↑Wall thickness	53

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aFGF: acidic fibroblast growth factor; bFGF: basic fibroblast growth factor; Echo: echocardiography; EF: ejection fraction; FS: fractional shortening; IGF-1: insulin-like growth factor; IR: ischemia reperfusion; IV: intravenous; LVD/S: left ventricular diastolic/systolic; LVSd/LVDd: left ventricular systolic/diastolic dimension; MMP-1: matrix-metalloproteinase; MRI: magnetic resonance imaging; NS: not stated; NRG-1: neuregulin; p38i: p38 inhibitor; PCADK: poly(cyclohexane-1,4-dilactone dimethylene ketal; PLGA: poly(lactic-co-glycolic acid); PS: phosphatidyl serine; SOD: superoxide dismutase; TO: total occlusion; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor; ↑: increased; ↓: decreased

Table 2

Microparticle Therapies for PAD

Material	Particle Size	Payload	Second therapeutic	Species	Injury model	Delivery method	Time of treatment post-injury	Time of assessment	Perfusion outcome & technique	Neovascularization	Other	Ref.
Collagen	100–200 μm	bFGF		Mouse	Excision of FA/V	IM injection	1 week	1, 2, 3, 4 weeks	↑LDPI	↑ Capillary density		30
Gelatin	50–100 μm	bFGF		Human	Ischemic gastrocnemius	IM injection	Varied	4, 12, 24 weeks	↑LDPI		↑ 6-minute walk distance, transcutaneous oxygen pressure, foot ulcer healing; ↓ Resting pain,	31
Fragmin/Protamine	1–3 μm	bFGF		Rabbit	Excision of FA	IM injection	10 days	4 weeks	↑LDPI	↑ Perfused capillary density, collateral vessels		32
Fragmin/Protamine	0.5–3 μm	bFGF		Mouse	Excision of FA	IM injection	1 day	3, 7, 10, 14, 17, 21, 24, 28 days			↑ Limb salvage, oxygen saturation	33
Fragmin/Protamine	0.5–3 μm	PRP		Rabbit	Excision of FA	IM injection	10 days	2, 4 weeks		↑Collateral vessels	↑ Calf blood pressure ratio	34
Gelatin	26 μm	bFGF	Heparin; free, subQ injection	Mouse	Excision of SA/V, EIA/V, DFA/V, DCA/V	IM injection	2 days	1, 2, 3, 4 weeks	↑LDPI	↑ Capillary, arteriole density in HCL mice	Equivalent results w/ or w/o heparin in WT mice	40
Collagen	100–200 μm	bFGF	HGF; same collagen particles; IM injection	Mouse	Excision of SA/V, EIA/V, DFA/V, DCA/V	IM injection	Immediate	1, 2, 3, 4 weeks	↑LDPI	↑ Capillary, arteriole density	Lower doses needed with combination therapy	42
Gelatin	NS	bFGF	PGE ₁ in PLGA particles; IM injection	Mouse	Excision of SA, EIA, DFA & DCA	IM injection	Immediate	4 weeks	↑LDPI	↑ Capillary, arteriole density	Lower doses needed with combination therapy	43
Gelatin	NS	bFGF	Sarpogrelate as dietary supplement	Rabbit	Excision of FA	IM injection	2 weeks	6 weeks	↑Non-radioactive colored microspheres	↑ Vessel size (qualitative)		45
Gelatin	30 μm	G-CSF		Mouse	Ligation of FA	IM injection	Immediate	3 days, 1, 2, 4, 6, 8 weeks	↑LDPI	↑ Capillary, arteriole density	Also tested in chronic model 4 weeks post-injury	47
Gelatin	30 μm	EPO		Mouse	Ligation of FA	IM injection	Immediate	2, 4 weeks	↑LDPI	↑ Capillary, arteriole density	↑EPO receptor; Akt, p-Akt, eNOS	50
PLGA	15–40 μm	PGE ₁		Mouse	Excision of FA	IM injection	2 days	4 weeks	↑LDPI	↑ Capillary, arteriole density		54
PLGA	10–30 μm	SIP		Mouse	Excision of FA	IM injection	Immediate	1, 3, 5, 7, 10, 14, 21, 28 days	↑LDPI		Only improved blood flow if injected every 3 days for 1 month	55

bFGF; basic fibroblast growth factor; DCA/V; deep circumflex artery/vein; DFA/V; deep femoral artery/vein; EIA/V; external iliac artery/vein; eNOS; endothelial nitric oxide synthase; EPO; erythropoietin; FA/V; femoral artery/vein; G-CSF; granulocyte colony stimulating factor; HCL; hypercholesterolemic; HGF; hepatocyte growth factor; IM; intramuscular; LDPI; laser Doppler perfusion imaging; PGE₁; prostaglandin E₁; PRP; platelet rich plasma; SIP; sphingosine-1-phosphate; SA/V; saphenous artery/vein; subQ; subcutaneous; WT; wild type; ↑; increased; ↓; decreased

Table 3

Nanoparticle Therapies for MI

Material	Particle Size	Payload	Species	Injury model	Delivery Method	Time of treatment post-injury	Time of assessment	LV dimensions	Functional outcome & technique	Neovascularization (location)	Other	Ref.
PLGA/PEI	74.4 ± 11.3 nm	IGF-1	Mouse	TO	Intramyocardial injection	Immediate	1 day	↓ LVSD/LVDD	= EF & FS Echo		↓ Apoptotic cells, infarct area; ↑ Wall thickness	64
PCADK; coated with GlcNAc	320 ± 156 nm	p38i	Rat	IR	Intramyocardial injection	Immediate	3 days		= FS Echo		↓ CM apoptosis, infarct size	65
Polyketal	500 nm	Nox2- NADPH oxidase siRNA	Mouse	TO	Intramyocardial injection	Immediate	3 days		= FS Echo		↓ Nox2 mRNA expression	67
Recombinant baculovirus & Tat/DNA	~350–500 nm	Ang-1 transgene	Rat	TO	Intramyocardial injection	15 minutes	3, 14, 21 days		↑EF Echo	↑ Capillary, arteriole density	↑ Ang-1 expression (BZ), LV wall thickness; ↓ infarct size	68
P-selectin- conjugated liposome	180 ± 13 nm	VEGF	Rat	TO	IV	Immediate	2, 3, 4 weeks		↑FS Echo	↑ Perfused vessels	↓ Heart wall hypokinesis	77
Liposome	150–200 nm	PGE ₁	Dog	IR	IV	During or immediately after	3 hours				↓ Infarct size	80,81
Liposome	150–200 nm	ATP	Rabbit	IR	IV	Immediately before	3 hours				↓ Infarct size	82
Liposome	150–200 nm	CoQ ₁₀	Rabbit	IR	IV	Immediately before	3 hours				↓ Infarct size	84
Liposome	130 nm	Adenosine	Rat	IR	IV	Immediately before	3 hours	rate			↓ Infarct size, adverse effect on blood pressure, heart	86

Ang-1: angiotensin; ATP: adenosine triphosphate; CoQ10: coenzyme Q10; Echo: echocardiography; EF: ejection fraction; FS: fractional shortening;GlcNAc: N-acetyl-glucosamine; HSP: heat shock protein; IGF-1: insulin-like growth factor; IR: ischemia reperfusion; IV: intravenous; LVSD/LVDD: left ventricular systolic/diastolic dimension; MRI: magnetic resonance imaging; NS: not stated; p38i: p38 inhibitor; PCADK: poly(cyclohexane- 1,4-dilactone dimethylene ketal;PEI: poly(ethylenimine); PGE1 : prostanglandin; PLGA: poly(lactic-co-glycolic acid); TAT: trans-activator of transcription; TNF: tumor necrosis factor; TO: total occlusion; VEGF: vascular endothelial growth factor; =: preserved; ↑: increased; ↓: decreased

Table 4

Nanoparticle Therapies for PAD

Material	Particle Size	Payload	Species	Injury model	Delivery method	Time of treatment post-injury	Time of assessment	Perfusion outcome & technique	Neovascularization	Other	Ref.
Dextran- co-gelatin	130 ± 3.5 nm	VEGF	Rabbit	Ligation of FA & EIA	IM injection	2 days	25 days	↑SPECT	↑ Capillary, collateral vessel density		69
PLGA	297 nm	Heparin	Mouse	Excision of FA	IM injection	Immediate	1 month	↑LDPI		↓ Limb necrosis; ↑ HGF expression	70
Gold	124 nm	VEGF conjugated to surface	Mouse	Ligation of FA	IV	1 day	5 weeks	↑LDPI	↑ Vascular density		72
PLGA	100 nm	bFGF	Mouse	Ligation of SA	IV				↑ Arteriole density		87

bFGF; basic fibroblast growth factor; EIA/V; external iliac artery/vein; FA/V; femoral artery/vein; HGF; hepatocyte growth factor; IM; intramuscular; IV; intravenous; LDPI; laser Doppler perfusion imaging; PLGA; poly(lactic-co-glycolic acid); SA/V; saphenous artery/vein; SPECT; single-photon emission computed tomography; VEGF; vascular endothelial growth factor; ↑: increased; ↓: decrease