Vascular Endoluminal Delivery of Mesenchymal Stem Cells Using Acoustic Radiation Force

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Restoration of functional endothelium is a requirement for preventing late stent thrombosis. We propose a novel method for targeted delivery of stem cells to a site of arterial injury using ultrasound-generated acoustic radiation force. Mesenchymal stem cells (MSCs) were surface-coated electrostatically with cationic gas-filled lipid microbubbles (mb-MSC). mb-MSC was characterized microscopically and by flow cytometry. The effect of ultrasound (5 MHz) on directing mb-MSC movement toward the vessel wall under physiologic flow conditions was tested *in vitro* in a vessel phantom. *In vivo* testing of acoustic radiation force-mediated delivery of mb-MSCs to balloon-injured aorta was performed in rabbits using intravascular ultrasound (1.7 MHz) during intra-aortic infusion of mb-MSCs. Application of ultrasound led to marginalization and adhesion of mb-MSCs to the vessel phantom wall, whereas no effect was observed on mb-MSCs in the absence of ultrasound. The effect was maximal when there were 7 ± 1 microbubbles/cell ($n=6$). In rabbits ($n=6$), adherent MSCs were observed in the ultrasound-treated aortic segment 20 min after the injection (334 ± 137 MSCs/cm²), whereas minimal adhesion was observed in control segments not exposed to ultrasound (2 ± 1 MSCs/cm², $p < 0.05$). At 24 h after mb-MSC injection and ultrasound treatment, the engrafted MSCs persisted and spread out on the luminal surface of the artery. The data demonstrate proof of principle that acoustic radiation force can target delivery of therapeutic cells to a specific endovascular treatment site. This approach may be used for endoluminal cellular paving and could provide a powerful tool for cell-based re-endothelialization of injured arterial segments.

Introduction

DELAYED RE-ENDOTHELIALIZATION, particularly within drug eluting stents,¹ is a major mechanistic contributor to stent thrombosis.² Cell-based therapy presents an attractive approach to restoration of a functional endothelium in this context. For example, endothelial coverage has been accelerated in stents coated with targeting peptides to bind circulating endothelial progenitor cells (EPCs).^{3,4} Another potentially attractive therapeutic cell is the bone marrow-derived mesenchymal stem cell (MSC), which has been shown to facilitate re-endothelialization of prosthetic vascular grafts⁵ and to reduce neointimal formation after vascular injury.^{6,7}

One limitation to the development of cell therapy specifically for vascular healing is the lack of a delivery method that is efficacious in moving cells to, and arresting them at, the vascular wall under the conditions of high shear stress present in arteries. To this end, we hypothesized that acoustic radiation force can be employed to selectively displace therapeutic

cells coated with microbubbles to a specific vascular segment (Fig. 1). Ultrasound radiation force refers to the kinetic energy imparted to a deformable object by absorption of acoustic energy, leading to the object's displacement away from the energy source. Gas-filled microbubble contrast agents are particularly susceptible to the effects of acoustic radiation force exerted by ultrasound.^{8,9} In the current study we demonstrate that ultrasound radiation force can displace MSCs electrostatically coated with cationic microbubbles under physiologic flow conditions *in vitro*. Further, we show that MSCs can be effectively seeded on a denuded arterial segment *in vivo* using ultrasound radiation force.

Methods

Cell culture

MSCs were obtained by plating the bone marrow cells of adult Sprague-Dawley rats or New Zealand rabbits. The cells were expanded using 20% fetal calf serum in α -MEM

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supplemented with antibiotics and 2 mM L-glutamine, and the nonadherent cell fraction was removed after 7 days. The adherent cells were trypsinized at 2 weeks and split every 7–10 days thereafter. MSCs were labeled with 0.5 μ M Cell Tracker Orange (CMRA; Invitrogen), gently trypsinized (0.02% trypsin), and suspended in phosphate-buffered saline (PBS) with microbubbles. The rabbit MSCs (utilized for the *in vivo* studies) were characterized by flow cytometry as CD45 negative, and CD73, 90, and 105 positive (Supplementary Methods; Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/tea).

Microbubble synthesis

Cationic lipid microbubbles were made from a mixture of phosphatidylcholine, the cationic lipid distearoyl-*sn*-glycero-3-ethylphosphocholine and polyethylene glycerol stearate, which was sonicated in saline in the presence of perfluorobutane gas. Control bubbles had distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-biotinyl instead of the charged lipid. The bubble concentration and size were analyzed using a Multisizer 3 Coulter Counter (Beckman), whereas electrical charge was measured using a Zetasizer Nano-Zs90 (Malvern Instruments).

Flow cytometry

Flow cytometry was performed to quantify microbubble coated (mb)-MSC association (FACSCalibur; Beckton Dickinson), based on the observation that microbubble attachment to the MSC surface increases their side scatter (SSC) due to the multiple reflective surfaces of the microbubbles present on the cells. Rat MSCs were labeled with CMRA and mixed with increasing proportions of microbubbles. The number of cells with increased SSC (relative to MSCs alone) and the geometric mean of the SSC arbitrary units in this population were plotted against the proportion of MSCs with microbubbles on their surface and the average number of microbubbles per MSC, respectively, as quantified by microscopy. At least 20 cells were counted for each condition by an operator blinded to treatment assignment.

Ultrasound delivery system

A 5 MHz transducer (Model V326; Olympus NDT) and 1.7 MHz intravascular ultrasound catheter (MicroSonic SV; Ekos Corp) were used for the *in vitro* and *in vivo* studies, respectively. Both transducers were driven with a 5 μ s tone burst, with a 25 μ s pulse interval. The acoustic pressure at the focal point of the 5 MHz transducer was 1.2 mega pascal (MPa). The 1.7 MHz intravascular catheter was calibrated to generate 1 MPa (high radiation force) and 0.5 MPa (low radiation force) at 2 mm from the catheter surface.

MSC viability testing

The effect of microbubble attachment to the MSC surface on cell viability was evaluated. Rat MSCs were incubated in PBS with cationic microbubbles at a 1:50 ratio for 1 h, and then labeled with the Live Dead cell viability kit (Invitrogen) and examined by microscopy after an additional 2 h. Lethal exposure to H₂O₂ was used as a positive control.

In vitro studies in flow phantoms

A vessel phantom was made using a polyvinyl chloride tube (internal diameter 1 mm) placed in a water tank and

under a fluorescence microscope (BX51; Olympus). The 5 MHz ultrasound transducer was immersed perpendicular to the flow direction, with the ultrasound focused at the optical focus. CMRA-labeled MSCs were mixed with cationic microbubbles at different ratios (ratio of MSC to microbubbles 1:12, 1:40, and 1:400). Control experiments were performed with negatively charged microbubbles at 1:400 ratio. mb-MSC complexes (5×10^4 cells) were diluted in PBS and advanced through the vessel phantom at two flow rates resulting in wall shear stress of 0.53 and 4.27 Pa, approximating the coronary wall shear stress during systole and diastole, respectively (the average coronary wall shear stress in humans has been reported at 0.68 Pa¹⁰). The sidewall of the phantom opposite to the ultrasound transducer was continuously microscopically observed for 15 s during simultaneous perfusion with mb-MSCs, with ultrasound being turned on for the middle 5 s of the experimental run. The number of cells adhering or intermittently adherent to the wall (adherent + rolling fraction), as well as the number of cells persistently adherent 5 s after ultrasound was turned off (retained fraction) were counted offline by an operator blinded to experimental conditions.

In vivo studies of rabbit aorta

The animal studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Adult New Zealand rabbits (3–4 kg weight) were sedated with intramuscular xylazine (7 mg/kg) and ketamine (40 mg/kg), intubated, and anesthetized with 1.5%–2.5% inhaled isoflurane. The animals were treated with aspirin (100 mg rectally) and heparin (1000 U iv). The experimental setup is presented in Figure 4A. Vascular access was obtained in the right femoral and the left carotid arteries. To create areas of endothelial injury requiring treatment, an oversized 4.5–5 mm angioplasty balloon was advanced under fluoroscopic guidance via the femoral artery to the thoracic aorta and inflated to nominal pressure in three separate aortic regions spanning T8 to L2 vertebral levels. Each area was treated with three separate 20 s inflations. During each inflation, the balloon was advanced back and forth to ensure effective endothelial denudation, and then removed from the vessel. An over-the-wire occlusion balloon was then advanced antegradely via the carotid artery to the descending thoracic aorta, for use as a flow occluder and cell delivery catheter, whereas the Ekosonic ultrasound catheter was advanced via the femoral access to the denuded section of the aorta.

The 1.7 MHz intravascular ultrasound catheter was retrogradely advanced, via the femoral artery, to the level of the denuded section of the aorta. The proximal occlusion balloon was inflated, and 3×10^5 mb-MSCs (rabbit MSCs mixed with cationic microbubbles at 1:40 ratio) were injected anterogradely through the balloon catheter. Simultaneously, ultrasound was delivered by the 1.7 MHz intravascular ultrasound catheter for 3.5 min during gradual retrograde withdrawal of this catheter over the length of the denuded treatment area (10–15 mm). The three injury sites along the descending aorta were randomly assigned to one of three treatment conditions: control (no ultrasound), low radiation force (0.5 MPa), and high radiation force (1 MPa). The occlusion balloon was then deflated, and 20 min after flow restoration, the rabbit was euthanized. The aorta was perfusion fixed and examined en-face by microscopy. The

number of adherent fluorescent MSCs was counted and the area of the aortic segment containing them was measured (ImageJ, National Institutes of Health). For subacute experiments, similarly instrumented rabbits were treated with double the number of mb-MSC complexes and high acoustic power radiation force. The animals were recovered and euthanized at 3 ($n=1$) and 24 h later ($n=2$), and the aortas were prepared as described above.

Statistics

Data are expressed as mean $\pm$ standard error of the mean. Given the relatively small sample sizes, experimental groups were compared using the distribution-independent Kruskal-Wallis test. If significant differences were found ($p < 0.05$), Dunn's test was performed *post hoc* to determine where they resided (Prism 5; GraphPad software).

Results

MSC coating with cationic microbubbles

The electrostatic charge for the cationic microbubbles was $+54.1 \pm 2.1$ mV (diameter 2.5 ± 0.1 μ m), whereas the control microbubbles used in this study had a charge of -4.3 ± 2.3 mV (diameter 2.3 ± 0.1 μ m). The rat MSCs had a surface charge of -23.6 mV. The addition of cationic microbubbles to a concentrated suspension of rat MSCs in PBS led to immediate coating of the MSC surface with bubbles (inset in Fig. 1), whereas control negatively charged microbubbles did not adhere to MSCs. With increasing ratio of the number of cationic bubbles added to the number of MSCs in suspension, the MSC side-scatter on flow cytometry increased (Fig. 2B, C) relative to MSCs alone (Fig. 2A), whereas this was not observed when the MSCs were mixed with control negatively charged microbubbles (Fig. 2D).

The percentage of cells with increased side-scatter (defined based on the upper limit of control MSCs not exposed to bubbles, Fig. 2A) linearly correlated with the number of cells coated with bubbles as identified by microscopy ($n=6$ different batches of MSC and microbubbles, $r^2=0.81$, $p < 0.001$, Fig. 2E). Also, the geometric mean SSC intensity of the mb-

MSCs correlated with the number of microbubbles per cell as quantified by microscopy ($r^2=0.81$, $p < 0.001$, Fig. 2F).

Apoptosis was detected in $13\% \pm 1\%$ of the mb-MSCs, higher than that in control conditions (MSCs alone, $8\% \pm 1\%$, $p < 0.005$), but less than that observed with H_2O_2 ($28\% \pm 3\%$, $n=3$, $p < 0.005$).

mb-MSC displacement in vitro

Time-lapse imaging of the mb-MSC displacement by acoustic radiation force under physiologic flow conditions in the vessel phantom is illustrated in Figure 3A (wall shear stress, WSS 0.53 Pa). There was minimal cell marginalization in the absence of acoustic energy (Fig. 3A, left panel). Immediate rolling and adhesion of the mb-MSCs to the vessel phantom wall was observed with ultrasound exposure (middle 3 panels). MSC rolling ceased and some adherent cells became detached when ultrasound was turned off, but a proportion of MSCs remained attached to the wall (right-most panel). When control microbubbles were used at a 1:400 MSC:microbubble mix ratio, minimal single cell adhesion occurred (Fig. 3B, C).

The number of MSCs adherent to the vessel wall during ultrasound exposure as well as the number of cells retained on the wall after ultrasound was turned off were quantified (Fig. 3B, C corresponding to low and high shear stress, of 0.53 and 4.2 Pa, respectively). For low shear stress (WSS 0.53 Pa), the number of adherent and retained MSCs varied as a function of cell to microbubble mix ratio (Kruskal Wallis $p < 0.05$), with a significant increase in the number of retained MSCs with increasing cell:microbubble mix ratio from 1:12 to 1:40 ($p < 0.05$). As the mix ratio increased to 1:400, however, cell retention decreased (<0.05 for retained cells, Fig. 3B). Thus, the effect of radiation force on mb-MSC was maximal when the mix ratio of MSC to microbubbles was 1:40, corresponding to $88\% \pm 5\%$ of MSCs being coated by microbubbles and 7 ± 1 microbubble/cell ($n=6$) based on the microscopy data. Increasing the wall shear stress to levels corresponding to diastolic coronary flow decreased cell retention (Fig. 3C, WSS 4.2 Pa, $p < 0.001$ for each mixing ratio).

FIG. 1. Ultrasound radiation force-mediated delivery of stem cells to vascular targets. The cells are coated with microbubbles (inset photomicrograph shows MSCs coated with cationic lipid microbubbles as used in this study). The microbubble cell complexes are injected proximal to the area of interest. As the microbubble-coated cells enter the ultrasound field, they are displaced peripherally by the acoustic radiation force, facilitating cell adhesion to the vessel wall. MSCs, mesenchymal stem cells. Color images available online at www.liebertonline.com/tea

FIG. 2. Quantification of microbubble coating of MSCs using flow cytometry. The flow plots depict the FSC/SSC profile (left column) and the distribution of SSC (right column) of fluorescently labeled MSC alone (**A**), after mixing with cationic microbubbles at MSC to microbubble mix ratio of 1:40 (**B**) and 1:400 (**C**), and after mixing with control negatively charged microbubbles (ratio of 1:400, **D**). In the FSC/SSC plots, the fluorescent events (i.e., MSCs) are in green, whereas the black events represent microbubbles. MSC association with the cationic microbubbles (**B**, **C**) leads to increased SSC of the cells (blue color plots) relative to MSCs alone (**A**) and to control microbubbles (**D**). The increase in side scatter correlates with the proportion of cells coated with microbubbles as identified by microscopy (**E**), whereas the average SSC (for the cells with increased SSC) correlated with the average number of microbubbles per MSC (**F**). SSC, side scatter; FSC, forward scatter; FACS, fluorescence-activated cell sorting. Color images available online at www.liebertonline.com/tea

In vivo endoluminal delivery of mb-MSCs after balloon injury

Cationic microbubbles had a similar affinity for rabbit MSCs. When mixing MSCs with cationic microbubbles at 1:40 ratio, the proportion of cells with increased SSC resulting from microbubble coating was similar between the rat and the rabbit cells (70.3 ± 4.9 , $n=5$, vs. $68.3 \pm 3.8\%$, $n=3$, respectively, $p=NS$). At 20 min after simultaneous ultrasound delivery and mb-MSC infusion, MSCs adhered throughout the balloon injured endoluminal surface of the ultrasound-treated aortic segment, without achieving a confluent layer (Fig. 4,

B2). The spatial distribution of adherent MSCs was not uniform (Fig. 4D, illustrating a composite image of the entire treatment area). Microscopic examination revealed that microbubbles were no longer present on the adherent MSCs (Supplementary Fig. S2). Overall, there was more than a 100- and 150- fold increase in MSC adhesion to aortic segments treated with low ($p < 0.05$) and high acoustic power ($p < 0.01$), respectively, as compared to the control segments (Fig. 4C, $n=6-7$) that were exposed only to mb-MSCs, without ultrasound (Fig. 4B1).

In the group of animals sacrificed at 24 h, surviving MSCs were clearly identified on the endoluminal surface of the

FIG. 3. *In vitro* vessel phantom experiments. **(A)** Selected microscopic images of the vessel phantom depicting acoustic radiation force-induced displacement of mb-MSC complexes during 15 s of flow (wall shear stress 0.53 Pa). The wall of the vessel phantom away from the ultrasound source is at the top of the images. No cell adhesion is observed during the first 5 s without ultrasound (left frame). Application of ultrasound leads to immediate cell rolling and adhesion (middle 3 frames). Some MSCs remain adherent even after the ultrasound is turned off (right frame). The number of cells rolling and adhering (circles) and persistently attached (retained, triangles) under low **(B)** and high flow conditions **(C)** was quantified at different MSC to cationic microbubble mixture ratios. Closed symbols represent the data obtained with control neutral microbubbles, where minimal cell adhesion was obtained **(B, C)**. mb-MSC, microbubble-coated MSC.

treatment area (Fig. 5C). The engrafted cell morphology changed gradually from a rounded appearance at 20 min (Fig. 5A), to an increasingly flattened appearance at 3 h (Fig. 5B), progressively spreading out such that by 24 h, MSC morphology was similar to that observed in cell culture (Fig. 5C). No macroscopic occlusive thrombus formation was detected on direct examination of either the aortic segments containing the adherent MSCs or the control segments.

Discussion

Our study is the first description of targeted delivery of cells using acoustic radiation force. The capacity to impart kinetic energy to gas-filled microbubbles by ultrasound has been previously characterized by Dayton *et al.*,^{8,9} and has been employed to modulate microbubble adhesion to endothelial epitopes for molecular imaging,^{11,12} or to deliver a cytotoxic drug to tumor cells.¹³ The radiation force on a given microbubble is maximal when the incident ultrasound is at the natural resonance frequency of the microbubble. For the lipid-shelled microbubbles used in this study, the average resonance frequency is close to 2.2 MHz.^{9,11} The intravascular ultrasound catheter used in our *in vivo* studies is FDA-approved to facilitate thrombolysis for treatment of stroke. Importantly, no major direct detrimental biological effects to the vessel wall were observed with this type catheter delivering the same amount of energy as in our study.¹⁴ For our application, we drove the transducer with a different pulse sequence than that provided by the commercial catheter, as a longer duty cycle was required (20%), while maintaining a

short pulse length to minimize bubble destruction, to achieve maximal radiation force.

The capacity to displace cells using acoustic radiation force requires that microbubbles attach to the surface of the cells, which was achieved in this study using electrostatic interactions. Cationic microbubble attachment to the MSC surface was confirmed both microscopically and by flow cytometry, which indicated increased side-scatter of the mb-MSC complexes compared to MSCs alone. We exploited this optical property to characterize the association between MSCs and microbubbles, with respect to the number of cells coated with microbubbles, as well as the number of microbubbles per cell. This approach should provide a useful tool to evaluate the efficacy of various methods for coating cells with microbubbles in the effort to maximize the ultimate therapeutic effects of acoustic radiation force for cell delivery in the future.

The number of microbubbles per cell that resulted in the greatest MSC adhesion to the vessel phantom was 7 ± 1 . Interestingly, there was a paradoxical decrease in cell adhesion at higher degrees of microbubble coating, likely because beyond a threshold number of attached microbubbles, the microbubbles themselves physically interfere with MSC apposition against the vessel wall. When MSCs were mixed with negatively charged (control) microbubbles, the cells did not associate with the microbubbles, and there was no significant MSC adhesion to the tube wall during ultrasound exposure. This is an important finding, indicating that the above-described effect was specific for cells coated with microbubbles, and not due to microbubbles transferring kinetic energy to the MSCs simply by colliding with them (a cue-ball effect).

FIG. 4. (A) *In vivo* experimental setup: An occlusion balloon infusion catheter was inflated proximal to sites of angioplasty-induced denudation in the descending thoracic aorta. mb-MSCs were injected through the central lumen of this balloon catheter, whereas ultrasound was delivered to injured region using an intravascular ultrasound catheter. (B) Low magnification *en face* imaging of the endoluminal side of an aortic segment 20 min after delivery of mb-MSCs, showing minimal single fluorescent MSC adhesion in a control proximal segment unexposed to ultrasound (Control, B1), whereas numerous MSCs adhered to the ultrasound-treated areas (Ultrasound, B2). Scale bar 200 μ m. (C) Quantification of the density of retained MSCs for control conditions (black circles), low (0.5 MPa, blue squares) and high (1 MPa, red triangles) acoustic radiation force. * $p < 0.05$, ** $p < 0.01$ versus control, $n = 6-7$. (D) Composite image (images of adjacent segments connected together) of the endoluminal surface of the aorta of the entire treatment area illustrating the distribution of the retained cells 3 h after high ultrasound mediated cell delivery. White lines indicate the borders of the tissue section. Scale bar 1 mm. Color images available online at www.liebertonline.com/tea

FIG. 5. The MSCs adherent to the aortic endoluminal surface after ultrasound mediated delivery survive and undergo morphological changes, between immediate (two examples in A) 3 h (B), and 24 h (C) post delivery. Scale bar 100 μ m. Color images available online at www.liebertonline.com/tea

The rabbit experiments demonstrated up to 150-fold enrichment over control in MSC adherence to the vessel wall when acoustic radiation force was applied. This compares favorably with endovascular seeding with EPCs loaded with supermagnetic iron oxide nanoparticles in arteries of similar caliber, where only up to sixfold enrichment over controls was obtained.^{15,16} As shown in the composite image that lays out a length of treated aorta (Fig. 4D), the MSC adhesion was not homogeneous in spatial distribution around the endoluminal circumference, likely due to the tendency of the relatively soft Microsonic catheter to assume an eccentric position inside the vessel (Fig. 4D). Further, the MSCs were not fully confluent in distribution at the various time point of observation after treatment; this would not necessarily preclude the occurrence of a therapeutic effect via paracrine mechanisms, nor the ultimate development of confluence at later time points. A critical finding was that 24 h after the ultrasound treatment, MSCs had spread out on the aortic wall, consistent with cell survival and engraftment. This observation is critical in that it confirms that the cationic bubbles and the ultrasound were not lethal to the MSCs delivered *in vivo*. In fact, the cationic microbubbles were no longer observed on the adherent MSCs 20 min after delivery and restoration of flow, possibly due to the high hydrodynamic stress and potentially charge neutralization.

It should be emphasized that our study establishes proof of the concept that acoustic radiation force can target the delivery of cells to a vascular target. Clearly, this method can be used for endovascular seeding of other cell types, such as EPCs. The rationale behind using MSCs in our study stems from robust data indicating that seeding of a sufficient number of MSCs on vascular grafts,^{5,17} acellular bioprosthetic valves,¹⁸ or injured vascular segments^{6,7,19} leads to improved endothelialization and decreased neointimal formation, not necessarily by direct MSC endothelial differentiation, but likely via paracrine mechanisms.¹⁹ To this point, MSCs secrete vascular endothelial growth factor, insulin-like growth factor, and hematopoietic growth factor,²⁰ stimulate endogenous angiogenesis, and have a positive effect on the endothelium.²¹ Further, unlike EPCs, MSCs have already been clinically used in an allogeneic fashion, primarily for their indirect regenerative and anti-inflammatory properties,^{22,23} and thus can be readily available to clinicians.

Study limitations

Extended exposure of cationic microbubbles to MSCs *in vitro* led to a 5% excess cell death over control MSCs, likely related to membrane destabilization by cationic lipids.²⁴ For our *in vivo* application, however, MSC exposure to cationic bubbles appears transient, as the microbubbles are no longer observed on the engrafted MSCs, and the 24 h experiments confirmed viability of the engrafted cells. Another limitation is the relative heterogeneity of the distribution of the engrafted cells in the relatively large thoracic aorta of the rabbits. The ultimate therapeutic efficacy of our method and the optimal cell need to be determined beyond the proof of principle established here. Experiments with more extended follow-up periods will be needed to further refine this cell delivery approach, not just in terms of the cell type to deliver, but also the catheter technology involved.

Clinical implications

In conclusion, we have shown that acoustic radiation force can successfully target stem cells to a treatment site. This approach may ultimately provide a useful tool for accelerated restoration of functional endothelium. We envision this novel strategy as providing the basis for vascular repair in situations in which endothelial injury or dysfunction underlies adverse clinical sequelae, such as late thrombosis after coronary stenting or during atherogenesis.

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Disclosure Statement

No competing financial interests exist.

References

1. Joner, M., Nakazawa, G., Finn, A.V., Quee, S.C., Coleman, L., Acampado, E., Wilson, P.S., Skorjia, K., Cheng, Q., Xu, X., Gold, H.K., Kolodgie, F.D., and Virmani, R. Endothelial cell recovery between comparator polymer-based drug-eluting stents. *J Am Coll Cardiol* **52**, 333, 2008.
2. Finn, A.V., Nakazawa, G., Joner, M., Kolodgie, F.D., Mont, E.K., Gold, H.K., and Virmani, R. Vascular responses to drug eluting stents: importance of delayed healing. *Arterioscler Thromb Vasc Biol* **27**, 1500, 2007.
3. Aoki, J., Serruys, P.W., Van Beusekom, H., Ong, A.T., McFadden, E.P., Sianos, G., van der Giessen, W.J., Regar, E., de Feyter, P.J., Davis, H.R., Rowland, S., and Kutryk, M.J. Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM Registry. *J Am Coll Cardiol* **45**, 1574, 2005.
4. Blindt, R., Vogt, F., Astafieva, I., Fach, C., Hristov, M., Krott, N., Seitz, B., Kapurniotu, A., Kwok, C., Dewor, M., Bosserhoff, A.K., Bernhagen, J., Hanrath, P., Hoffmann, R., and Weber, C. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. *J Am Coll Cardiol* **47**, 1786, 2006.
5. Mirza, A., Hyvelin, J.M., Rochefort, G.Y., Lermusiaux, P., Antier, D., Awede, B., Bonnet, P., Domenech, J., and Eder, V. Undifferentiated mesenchymal stem cells seeded on a vascular prosthesis contribute to the restoration of a physiologic vascular wall. *J Vasc Surg* **47**, 1313, 2008.
6. Yue, W.M., Liu, W., Bi, Y.W., He, X.P., Sun, W.Y., Pang, X.Y., Gu, X.H., and Wang, X.P. Mesenchymal stem cells differentiate into an endothelial phenotype, reduce neointimal formation, and enhance endothelial function in a rat vein grafting model. *Stem Cells Dev* **17**, 785, 2008.
7. Forte, A., Finicelli, M., Mattia, M., Berrino, L., Rossi, L., De Feo, M., Cotrufo, M., Cipollaro, M., Cascino, A., and Galderisi, U. Mesenchymal stem cells effectively reduce surgically induced stenosis in rat carotids. *J Cell Physiol* **217**, 789, 2008.

8. Dayton, P., Klivanov, A., Brandenburger, G., and Ferrara, K. Acoustic radiation force *in vivo*: a mechanism to assist targeting of microbubbles. *Ultrasound Med Biol* **25**, 1195, 1999.
9. Dayton, P.A., Allen, J.S., and Ferrara, K.W. The magnitude of radiation force on ultrasound contrast agents. *J Acoust Soc Am* **112**, 2183, 2002.
10. Doriot, P.A., Dorsaz, P.A., Dorsaz, L., De Benedetti, E., Chatelain, P., and Delafontaine, P. *In-vivo* measurements of wall shear stress in human coronary arteries. *Coron Artery Dis* **11**, 495, 2000.
11. Zhao, S., Borden, M., Bloch, S.H., Kruse, D., Ferrara, K.W., and Dayton, P.A. Radiation-force assisted targeting facilitates ultrasonic molecular imaging. *Mol Imaging* **3**, 135, 2004.
12. Borden, M.A., Sarantos, M.R., Stieger, S.M., Simon, S.I., Ferrara, K.W., and Dayton, P.A. Ultrasound radiation force modulates ligand availability on targeted contrast agents. *Mol Imaging* **5**, 139, 2006.
13. Dayton, P.A., Zhao, S., Bloch, S.H., Schumann, P., Penrose, K., Matsunaga, T.O., Zutshi, R., Doinikov, A., and Ferrara, K.W. Application of ultrasound to selectively localize nanodroplets for targeted imaging and therapy. *Mol Imaging* **5**, 160, 2006.
14. Soltani, A., Singhal, R., Garcia, J.L., and Raju, N.R. Absence of biological damage from prolonged exposure to intravascular ultrasound: a swine model. *Ultrasonics* **46**, 60, 2007.
15. Kyrtatos, P.G., Lehtolainen, P., Junemann-Ramirez, M., Garcia-Prieto, A., Price, A.N., Martin, J.F., Gadian, D.G., Pankhurst, Q.A., and Lythgoe, M.F. Magnetic tagging increases delivery of circulating progenitors in vascular injury. *JACC Cardiovasc Interv* **2**, 794, 2009.
16. Pislaru, S.V., Harbuzariu, A., Gulati, R., Witt, T., Sandhu, N.P., Simari, E.D., and Sandhu, G.S. Magnetically targeted endothelial cell localization in stented vessels. *J Am Coll Cardiol* **48**, 1839, 2006.
17. Hashi, C.K., Zhu, Y., Yang, G.Y., Young, W.L., Hsiao, B.S., Wang, K., Chu, B., and Li, S. Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts. *Proc Natl Acad Sci USA* **104**, 11915, 2007.
18. Vincentelli, A., Wautot, F., Juthier, F., Fouquet, O., Corseaux, D., Marechaux, S., Le Tourneua, T., Fabre, O., Susen, S., Van Belle, E., Mouquet, F., Decoene, C., Prat, A., and Jude, B. *In vivo* autologous recellularization of a tissue-engineered heart valve: are bone marrow mesenchymal stem cells the best candidates? *J Thorac Cardiovasc Surg* **134**, 424, 2007.
19. Takahashi, M., Suzuki, E., Oba, S., Nishimatsu, H., Kimura, K., Nagano, T., Nagai, R., and Hirata, Y. Adipose tissue-derived stem cells inhibit neointimal formation in a paracrine fashion in rat femoral artery. *Am J Physiol Heart Circ Physiol* **298**, H415, 2010.
20. Kinnaird, T., Stabile, E., Burnett, M.S., Lee, C.W., Barr, S., Fuchs, S., and Epstein, S.E. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. *Circ Res* **94**, 678, 2004.
21. Tögel, F., Weiss, K., Yang, Y., Hu, Z., Zhang, P., and Westenfelder, C. Vasculotropic paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol* **292**, F1626, 2007.
22. Le Blanc, K., Frassoni, F., Ball, L., Locatelli, F., Roelofs, H., Lewis, I., Lanino, E., Sundberg, B., Bernardo, M.E., Remberger, M., Dini, G., Egeler, R.M., Bacigalupo, A., Fibbe, W., and Ringden O; Development Committee of the European Group for Blood and Marrow Transplantation. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* **371**, 1579, 2008.
23. Hare, J.M., Traverse, J.H., Henry, T.D., Dib, N., Strumpf, R.K., Schulman, S.P., Gerstenblith, G., DeMaria, A.N., Denktas, A.E., Gammon, R.S., Hermiller, J.B., Jr., Reisman, M.A., Schaer, G.L., and Sherman, W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells after acute myocardial infarction. *J Am Coll Cardiol* **54**, 2277, 2009.
24. Soenen, S.J., Brisson, A.R., and De Cuyper, M. Addressing the problem of cationic lipid-mediated toxicity: the magnetoliposome model. *Biomaterials* **30**, 3691, 2009.

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