A dipose-derived stem cells (ASCs) have been extensively investigated for their mesenchymal differentiation, transdifferentiation, paracrine effects, immune modulation, and clinical implications for regenerative medicine.1,2 However, recent studies have indicated that stromal vascular fraction (SVF) also possesses similar potential for regenerative medicine and clinical implications.3–10 Some investigators suggested that SVF may even have an advantage over ASCs because of the presence of endothelial progenitor cells, pericytes, immune cells, and other stromal components along with the ASCs.3 SVF cells are relatively easy and quick to obtain in large quantities without the need of a process of cell culture; therefore, both liposuction and SVF transplantation procedures can be accomplished at same day.

A number of animal studies11–17 and human clinical trials18,19 have demonstrated that intraarterial infusion is a...
safe and effective route for the delivery of bone marrow-derived mesenchymal stromal cells (MSCs) to the target-
ed tissues at a distance for the treatment of stroke,12,14,16,18
myocardial infarction,25,19 renal failure,23 femoral head nec-
crosis,11 and so on. In contrast, several animal studies 20–24
have reported that intraarterial infusion of MSCs in the
settings of xenogeneic or allogeneic transplantations com-
promised blood flow and caused microembolisms and vas-
cular obstruction.

A number of reports have stated that ASCs and SVF
are safe, efficacious, and carry relatively low rates of mor-
bitidy and side effects; however, in most cases, ASCs or
SVF were administrated by in situ injection or topical ap-
lications.1–10 It is unclear whether ASCs or SVF can be
delivered through a systemic route such as intraarterial
infusion. Intraarterial cell delivery could enhance the hom-
ing efficiency to the targeted organs at distance such as
heart and brain. The purpose of this study was to examine
the microcirculatory responses in vivo on local intraarte-
rial infusion of autogenic ASCs or SVF in a vascular pedi-
cle isolated rat cremaster microcirculation model and to
determine whether intraarterial infusion is an appropriate
route for the delivery of autogenic ASCs or SVF.

METHODS

All experimental procedures involving the care of the
animals were approved by our Institutional Animal Care
and Use Committee. Male Sprague–Dawley rats weighing
120–160 g were used. Anesthesia was accomplished using
intraperitoneal sodium pentobarbital (50 mg/kg). Fat
tissue was surgically harvested from rat bilateral flanks,
carefully minced with scissors, and then processed for the
enzymatic isolation of SVF.

Isolation of Stromal Vascular Fraction

The method of SVF isolation has been described in
our previous publication.25–30 Briefly, the fat tissue was
washed with phosphate-buffered saline (PBS) and then
centrifuged at 430g for 10 minutes. After oil removal, the
lipid phase of fat from the top of the conical tube was har-
vested and then diluted with an equal volume of collage-
nase digestion solution (final concentration: 0.3 U/mL,
Collagenase NB 4G proved grade, Serva Electrophoresis,
Heidelberg, Germany). After 30 minutes of incubation, an
equal volume of Dulbecco’s Modified Eagle medium con-
taining 20% fetal bovine serum was added to stop enzy-
matic digestion. The floating layer containing adipocytes
and the pellet containing SVF were separated by centrifu-
gation. The isolated SVF was filtered first through a 100-
µm and then a 20-µm nylon filter. Total number of SVF
cells was counted. The cell size of SVF was measured by a
stage micrometer (Meiji Techno, Japan) under the micro-
scope in each sample before they are used for intraarterial
infusion.

Purification of ASC through SVF Culture

SVF is highly heterogeneous and contains many cell
subsets including ASCs, blood cells, endothelial cell,
pericytes, T cells, B cells, mast cells, and macrophages.

One of the unique characteristics of ASC is that they are
adherent to the plastic surface. Therefore, the isolation
of purified ASC can be achieved through SVF culture.
The method for ASC culture has been described in our
previous publication.25–30 In brief, all the isolated SVF
cells were added into a 75-cm² culture flask containing
15 mL of prewarm Nonhematopoietic Expansion
Medium (Miltenyi Biotec, Auburn, Calif.) and 1% of
penicillin–streptomycin. The flask was cultivated at
37°C, 5% CO₂, and 95% humidity. After 24 hours of cul-
ture, the nonadherent cells in the flask were removed
by PBS washing. Trypsin/EDTA 1 mL was added into the
flask and incubated at 37°C for 10 minutes. After com-
plete dissociation, the total number of ASCs was harvest-
ted and counted. The cell size of ASCs was measured by a
stage micrometer under the microscope in each sample
before they are used for intraarterial infusion.

Vascular Pedicle Isolated Rat Cremaster Model

Vascular pedicle isolated rat cremaster model is a mi-
crocirculation model which has been used by the princi-
ple investigator for more than 20 years (Fig. 1).31–36 Briefly,
the vascular pedicle, consisting of the pubic-epigastria ves-
sels, was dissected free up to the iliac vessels. A 2-cm-long
segment of iliac artery distal and proximal to the pedicle
was completely freed from the correspondent veins un-
der the surgical microscope. All other branches arising
from iliac and pedicle arteries were ligated to ensure all
the infused SVF or ASC cells going nowhere but the cre-
master muscle. A tubing (PE-10) presoaked with heparin
and filled with saline was cannulated through femoral ar-
tery and the end of tubing was secured at 0.5 cm distal
to the vascular pedicle of the cremaster muscle (Fig. 1).
After surgical preparation, the cremaster muscle was care-
fully spread on a glass slide and covered with gas-imper-
meable plastic film. This preparation was then placed on
the stage of a trinocular microscope (Olympus, Tokyo,
Japan) equipped with an NEC, NC-8 CCD video camera
(NEC, Tokyo, Japan). The video image was observed in
real time on the monitor, transferred by Pinnacle Video
Transfer, and recorded on a USB 2.0 mass storage device.
Body and muscle temperatures of rats were maintained
at 36–37 and 30–32°C, respectively, using regulated heat
lamps.

Arteriole Diameter and Capillary Density Measurement

The method used to measure arteriole diameter and
capillary density was described in our previous publica-
tions.35–36 Briefly, the images of the vascular tree in the cre-
master muscle were systematically scanned from the major
feeding arteriole (A1: 100–160 µm) down to the middle
arterioles (A2: 40–80 µm) and the terminal arterioles (A3–
A4: 10–30 µm). Baseline diameter measurements were
obtained for every segment of the visible arteriole tree be-
tween each branching point. Inside diameter of each ves-
sel at the narrowest part of each segment was measured.
Two locations in the cremaster muscle were observed to
determine capillary density. At each location, 9 adjacent
fields of view were identified and 18 capillary fields were
counted in each muscle. The same arterioles and capil-
lary fields were reexamined during the infusion of SVF or ASC. Results are expressed as the average number of flowing capillaries/field in each cremaster muscle.

Experimental Protocol
After fat tissue harvesting from rat’s bilateral flanks, wounds were closed by 5-o sutures. Fat tissue was then processed for SVF isolation. During the cell process, the vascular pedicle isolated rat cremaster model was prepared. After the baseline measurement of arteriole diameter and capillary density in the cremaster muscle, the freshly isolated autogenic SVF (1 x 10^5) cells (n = 6) were diluted in 1 mL of normal saline (prewarmed to 37°C) and then infused into the microcirculation of cremaster muscle at a speed from 0.05 mL/min through the cannulated femoral artery. The microcirculatory response in vivo to local intraarteriole infusion of SVF was measured in real time at 5 minutes during SVF infusion.

The protocol for ASC intraarterial infusion was slightly different from the protocol of SVF infusion. After the procedure of fat tissue harvesting, rat was moved back to the cage for recovery from anesthesia. Fat tissue was processed for the isolation of SVF. The isolated SVF was then cultured for 24 hours for the purification of ASCs. On day 2, the same rat was anesthetized again for the preparation of the vascular pedicle isolated rat cremaster preparation. The purified autogenic ASC (1 x 10^5) cells (n = 6) were dissolved in 1 mL of normal saline and then infused into the microcirculation of cremaster muscle at a speed from 0.05 mL/min through the cannulated femoral artery. The microcirculatory response in vivo to local intraarteriole infusion of ASCs was measured in real time at 5 minutes during ASC infusion.

Statistical Analysis
All measurements were compared by using analysis of variance followed by t test. A value of P ≤ 0.05 was considered significant.

RESULTS
In each cremaster muscle, 2 of A1 arterioles, 4 of A2 arterioles, 9 of A3/A4 arterioles, and 18 of capillary fields were measured. During the initial baseline period, all vessels and capillaries displayed vigorous blood flow and typical diameters. Before infusion, the cell sizes of SVF and ASC were measured. The average cell size was 17.0 ± 0.7 and 19.8 ± 0.7 µm for SVF and ASC, respectively.

In this study, the first 2 minutes of infusate was actually saline, which was preloaded in the PE-10 tubing. Microcirculation including arteriole diameter and capillary perfusion was observed during saline infusion. No significant microcirculation alteration was found during saline infusion which was corresponded with the findings from our previous studies.31,32,36

There was a significant increase on the diameter of terminal arterioles (A3/A4) and the capillary density during ASC intraarterial infusion. The average diameter (mean ± SEM) during ASC infusion was 95.9% ± 2.4% in A1, 99.8% ± 1.4% in A2, and 113.2% ± 2.8% in A3/A4 (P = 0.049) as compared to the baseline. The average capillary density was 110.2% ± 1.9% (P = 0.02) as compared to the baseline (Fig. 2). Great attention has been paid to the terminal arterioles and capillary perfusion at the time of ASC infusion because the size of ASC is similar to the diameter of the vessels. No ASC trapping-induced microcirculatory alterations were seen. No microembolisms and microcirculation obstruction were found at the time of ASC infu-
sion. After 10 minutes of ASC infusion, a bolus injection was tested. Even when a gentle bolus injection was made with the remaining ASC solution, the microcirculation was quickly returned to normal status within a few seconds.

However, there was a significant change in the blood flow, arteriolar diameter, and capillary density during SVF intra-arterial infusion. As soon as the SVF solution entered the blood vessel, a significant cell aggregation was detected in the arteriole tree. Microembolisms were often flowing within the blood stream or sticking on the arteriole wall with vasoconstriction. (See video, Supplemental Digital Content 1, which displays SVF intra-arterial infusion. Microembolisms were flowing within blood stream or sticking on the arteriole wall (A1) with vasoconstriction. This video is available in the “related videos” section of the full-text article on PRSGlobalOpen.com or available at http://links.lww.com/PRSGO/A272.) Embolisms that are narrowing or blocking the lumen of A3 or A4 arterioles can be found in many places resulting in no flow in the corresponding capillaries. The average diameter was 69.9% ± 3.0% in A1, 60.4% ± 2.6% in A2, and 60.4% ± 1.2% in A3/A4 as compared to the baseline and ASC group (P < 0.01 in all sizes of arterioles). The capillary density was 43.3% ± 4.0% as compared to the baseline and ASC group (P < 0.01). After 10 minutes of SVF infusion, a bolus injection was tested. The bolus injection of SVF solution accelerated embolisms accumulation at the bifurcation of A1 and A2 arterioles resulting in no flow in entire cremaster muscle. (See video, Supplemental Digital Content 2, which displays SVF intra-arterial bolus injection. This video is available in the “related videos” section of the full-text article on PRSGlobalOpen.com or available at http://links.lww.com/PRSGO/A273.)

**DISCUSSION**

A number of animal studies11–17 and human clinical trials18,19 have demonstrated that bone marrow-derived MSCs can be delivered systemically through an intra-arterial infusion. For example, Jin et al11 reported the intra-arterial infusion of autologous MSCs as a feasible and relatively safe method for the treatment of femoral head necrosis in dog model. Silachev et al12 found that the intra-arterial injection of autologous MSCs to the brain through the carotid arteries substantially promoted functional recovery after traumatic
The purpose of this study was to examine the microcirculatory responses in real time in vivo on local intra-arterial infusion of autogenic ASCs or SVF in a vascular pedicle isolated rat cremaster microcirculation model and to determine whether intra-arterial infusion is an appropriate route for the delivery of autogenic ASCs or SVF. Here, the point of emphasis is that this was a local intra-arterial infusion rather than a systemic intra-arterial infusion. In a systemic infusion model, multiple concentrations and infusion speeds can be examined. Although higher concentrations of cell can be administrated to the whole body in systemic infusion model, the actual number of cells entering into the target tissue of interest (such as cremaster) could be very limited and is difficult to be quantitated. However, for this local intra-arterial infusion with our unique preparation, all the vascular branches arising from pedicle artery were ligated to ensure all the cells going nowhere but the cremaster. Therefore, it is possible to determine how many cells were administered into the cremaster in the limited time period. Moreover, the size of the rat cremaster is comparable to the size of human thumb tip and paper thin. If such a small piece of tissue can sustain $1 \times 10^5$ cells infusion without causing microcirculation obstruction, then systemic infusion at any reasonable higher concentrations should be possible.

The cell size, concentration, and infusion speed are not considered responsible for SVF-induced microcirculatory obstruction because no embolisms and no microcirculatory obstruction were found during infusion of ASCs that have the same cell size, concentration, and infusion speed as SVF. A great attention has been paid to observe the A5/A4 terminal arterioles. No significant cell trapping was seen in the terminal arterioles during ASC or SVF infusion. On the contrary, many microembolisms flowing from proximal to distal (from A1 to A4) and then narrowing or blocking the lumen of terminal arterioles during SVF infusion were seen. The normal blood flow in the feeding arteriole (A1) of cremaster is about 220 nL/s. This infusion speed was used to study ischemia and reperfusion injury for more than 20 years. As this is a microcirculation study, no significant change in infusion speed was made because it could alter microcirculatory dynamics and physiology.

Moreover, the bolus injection was used to deliver bone marrow-derived MSCs to animals and human in clinical trials. However, this method is not appropriate for a microcirculation study presented here because it could mechanically alter the microcirculatory dynamics and physiology. Therefore, the bolus injection was not even planned in the experimental protocol and the bolus-injection-induced microcirculatory response was not intended to be measured. For the curiosity, however, after the completion of the planned experiments, a gentle bolus injection was tested by using the remaining ASC or SVF solution. A significant vasodilatation was observed immediately after gentle bolus injection; however, arteriole diameter was quickly returned to normal status within a few seconds.

The mechanism of ASC-induced vasodilation in the terminal arterioles is unclear. However, based on our previous study, it was hypothesized that the ASC-induced vasodilation in terminal arterioles could be due to the growth factors such as vascular endothelial growth factor produced by ASCs. It is unknown why the intra-arterial infusion of autogenic SVF, but not autogenic ASC, caused rapid cell
aggregation with microembolisms formation. Compared to the purified ASC, SVF is a heterogeneous collection of cells that include ASCs, blood cells, endothelial progenitor cell, pericytes, T cells, B cells, mast cells, and macrophages. Dr. Wang has interpreted that some components in the SVF cells isolated from adipose tissue might not be compatible with the blood components that might result in blood coagulation and embolism formation. Nevertheless, a further study is definitely warranted to explore the mechanism for this autogenic SVF-induced embolism formation.

**SUMMARY**

Intra-arterial infusion is an appropriate route for delivery of autogenic ASCs, but not of SVF. SVF-induced microembolisms were the cause that narrowing or blocking the lumen of terminal arterioles resulting in no-flow in the corresponding capillaries.

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