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Characterization and multilineage potential of cells derived from isolated microvascular fragments

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ABSTRACT

Background: A number of therapies are being developed that use microvessels isolated from adipose tissue (microvascular fragments [MVFs]) to improve tissue perfusion and implant survival. Because it has been demonstrated that stem cells are associated with microvessels, the purpose of these studies was to gain further insight into the stem cells associated with MVFs to better understand their therapeutic potential.

Materials and methods: Cells derived from MVF explants were compared with adipose-derived stem cells (ASCs) based on the expression of cell surface proteins for mesenchymal stem cells and their capacity for angiogenic, neurogenic, adipogenic, and osteogenic differentiation.

Results: The expression of cell surface proteins for mesenchymal stem cell markers was similar between MVF-derived cells and ASCs; however, the increase in markers consistent with endothelial cells and pericytes was accompanied by an improved ability to form capillary-like networks when cultured on matrigel. MVF-derived cells had increased neuregulin, leptin, and osteopontin expression compared with ASCs when exposed to neurogenic, adipogenic, and osteogenic induction media, respectively.

Conclusions: The stem cell functionality of cells derived from MVFs is retained after their isolation. This helps to explain the ability of MVFs to improve tissue perfusion and has implications for the use of MVFs as a means to deliver stem cells within their niche.

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1. Introduction

Tissue engineering strategies for tissue and organ replacement will lead to significant improvements in surgical outcomes for a large number of diseases. A limitation to the success of tissue-engineered biomaterials is the presence of a vascular supply capable of sustaining perfusion and maintaining implant viability [1,2]. To address this void, a number of prevascularization strategies have been developed that use a combination of cells derived from various tissues (e.g., fibroblasts, mesenchymal stem cells [MSCs], human umbilical vein endothelial cells) for vascular development *in vitro* before their implantation *in vivo* [1,3]. An alternative approach has been the delivery of intact microvessels (arterioles, venules, and capillaries) isolated from adipose tissue, hereafter referred to as microvascular fragments (MVFs), which

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effectively circumvents the need for *in vitro* vessel development [4–7]. MVFs improve perfusion for cardiac and skin tissues and have also been demonstrated to be an effective prevascularization strategy to improve viability of orthopedic and islet implants [4–8]. The observation that freshly isolated MVFs can be used lends to the clinical relevance of their application [6]. Despite the relationship between the therapeutic usefulness of MVFs and the wealth of data ascribing a vascular location to stem cells [9,10], a thorough characterization of the stem cells associated with MVFs is lacking.

The robust angiogenic potential of MVFs even in the absence of supplemental proangiogenic factors (e.g., vascular endothelial growth factor) supports the idea that potent stem cells reside within the vessels that contribute to their vigorous network formation *in vitro* and dynamic remodeling *in vivo* [4–6,11]. Nunes *et al.* [11] suggested that the inherent angiogenic capacity of MVFs may be due, at least in part, to regenerative cells residing within them, a concept supported by a number of studies where MSCs have been shown to reside within the vascular wall [12] or in a perivascular location [9]. The abundant microvasculature within adipose tissue, and accordingly, abundant supply of stem cells that can be derived from adipose tissue are in direct agreement with these concepts. Although adipose-derived stem cells (ASCs) and the freshly isolated stromal vascular fraction have been thoroughly studied, given the differences in the methodologies to procure them, it is not prudent to rely solely on their characterizations to make conclusions regarding the stem cell identity of MVFs.

Given the growing body of literature supporting the idea that the microvasculature is a source of stem cells and the interest in improving vascularization with MVFs, in the present study cells derived from MVFs were characterized using an explant culture method. Comparison with ASCs revealed that the MVF-derived cells (MVF-DC) are heterogeneous and in some regards exhibit evidence of a greater regenerative potential. More importantly, the findings herein support the idea that the isolation of MVFs does not negatively impact resident vascular cells and stem cells. This lends to the possibility that the application of MVFs not only has the advantage of supplying intact microvessels to support tissue perfusion but also supply cellular factors critical for tissue regeneration.

2. Materials and methods

This study has been conducted in compliance with the Animal Welfare Act and the Implementing Animal Welfare Regulations and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals and was conducted in the animal facility at the US Army Institute of Surgical Research. Rats were housed individually in a temperature-controlled environment with a 12-h light–dark cycle.

2.1. MVF-DC and ASC isolation

MVFs were isolated from the epididymal fat pads of wild-type male Lewis rats (350–400 g) as previously described [4]. Briefly, adipose tissue from the epididymal fat pads of rats were subjected to a limited collagenase (Worthington Biochemical Corporation, Lakewood, NJ) digestion (~8 min) at 37° with agitation, washed, and filtered through 500- and 30-μm filters to remove large debris and minimize cell contamination, respectively (Fig. 1). MVFs were plated on tissue culture–-treated plastic dishes similar to that described for the isolation of pericytes from human placental tissue [13] in growth media consisting of Dulbecco Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 100 U/mL each of penicillin and streptomycin (Life Technologies, Grand Island, NY). The cells that emanated from the MVFs, referred to as MVF-derived cells (MVF-DCs), were subjected to characterization as described in the following. For ASC isolation, adipose tissue was digested with collagenase for 45 min at 37° with agitation followed by filtration through 100, 70, and 40 μm mesh filters to remove debris. Remaining filtrate was washed and centrifuged and pelleted cells seeded on tissue culture–treated plastic dishes in growth media [14].

2.2. Cell growth curve

Cell growth curves of MVF-DC and ASC cells were determined by seeding 2.5×10^4 cells per well ($n = 4$ wells per group per time point) in 12-well tissue culture plates. Cells were allowed to attach overnight, and then cells were trypsinized to remove all adherent cells and counted using a hemocytometer for 8 d.

2.3. Multilineage differentiation potential

ASCs (passage 1) and cells derived from MVFs (passage 1) were grown to subconfluency and their ability to differentiate toward various lineages analyzed as described in the following.

2.3.1. Adipogenesis

Adipogenic differentiation of cells was induced by replacing growth media with preinduction adipogenic media consisting of DMEM, 10% FBS, 1% antibiotics, 0.5 mM isobutylmethylxanthine, 200 μM indomethacin, 0.1 μM dexamethasone, and 1 μM insulin (Sigma–Aldrich, St. Louis, MO) for 24 h followed by 2 wk of culture in adipogenic media (same as preinduction media minus the isobutylmethylxanthine). Cells were either harvested for RNA as described in the following or fixed with 4% paraformaldehyde for 20 min followed by staining with Oil Red O for 1 h at room temperature (RT). Excess stain was removed by extensive washes with phosphate-buffered saline (PBS) and cells imaged with an Olympus (Center Valley, PA) IX 71 inverted microscope.

2.3.2. Osteogenesis

Osteogenic differentiation of cells was achieved by replacing growth media with osteogenic media composed of DMEM, 10% FBS, 1% antibiotics, 10 mM β-glycerophosphate, 10 nM dexamethasone, and 150 μM ascorbic acid 2-phosphate (Sigma–Aldrich) for 3 wk. Cells were either harvested for RNA as described in the following or fixed with 4% paraformaldehyde for 20 min followed by staining with Alizarin Red S (40 mM, pH 4.1, 20 min followed by extensive washes with dH_2O) to examine mineralization activity. Images were collected as described previously.