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ANGIOMICS™

Donor-Specific Angiogenic Effects of MSCs in 3D Microvasculature Tissue Constructs

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Introduction

Vascularization is an essential element for tissue homeostasis and function. Oxygen, for example, can diffuse approximately 100 - 200 µm into a tissue from an oxygen source, depending on the tissue. The vasculature is critical in bringing the oxygen source (i.e., blood) in close approximation to the cells of a tissue. Not only does a vessel network transport blood throughout thick tissues, but vascular cells and associated perivascular niche cells play a critical role in overall tissue function. Yet, tissue vascularization remains a key challenge throughout the field of tissue biofabrication and tissue modeling. An important aspect of tissue vascularization is the interplay between the mesenchymal stem/stromal cells (MSCs) of the tissue stroma and the vasculature. It's well known that MSCs condition the tissue environment with a variety of growth factors and cytokines, including angiogenic factors. Additionally, the vascular and perivascular cells of microvessels influence tissue cell behavior important in tissue function.

Increasingly, Angiomics® isolated human adipose-derived microvessel fragments (haMVs) are being used to vascularize 3D tissues and tissue models, including adipose, liver, and bone [1-3]. Angiomics® haMVs, isolated from discarded lipoaspirates, retain their native structure, microvessel phenotype, and cellular complexity [4, 5]. When embedded in a 3D matrix, haMVs undergo sprouting angiogenesis with growing neovessels arising from parent microvessel fragments forming an interconnected neovascular network [1, 5]. When provided a perfusion source, isolated microvessel-derived neovasculatures rapidly inosculate with that source and mature into a persistent microcirculation [6].

RoosterBio's human mesenchymal stem/stromal cells (hMSCs) are widely used in a variety of applications including tissue modeling and advanced therapy manufacturing [7, 8]. In addition to phenotypic plasticity, important in establishing tissue function, MSCs support tissue vascularity [9, 10], in part by promoting angiogenesis [11, 12]. RoosterBio hMSCs secrete a spectrum of pro-vascular growth factors, cytokines, and paracrine factors.

Here, we examined the potential of hMSC lots from different donors to promote angiogenesis in haMV-derived microvasculatures. We focused on specifically the paracrine activity of hMSCs from three different donors, reflecting differences in growth factor secretion profiles, on sprouting angiogenesis from the haMVs. 3D constructs of haMVs were co-cultured with hMSCs suspended above the constructs using cell culture inserts. This allowed the cells and vessels to share the medium compartment, but do not allow for physical interaction. The three hMSC lots promoted angiogenesis, the extent of which depended on the lot, highlighting the benefits of using both technologies in tissue modeling and regenerative applications.

Benefits of Combining Angiomics® haMVs with RoosterBio hMSCs to Vascularize Tissues

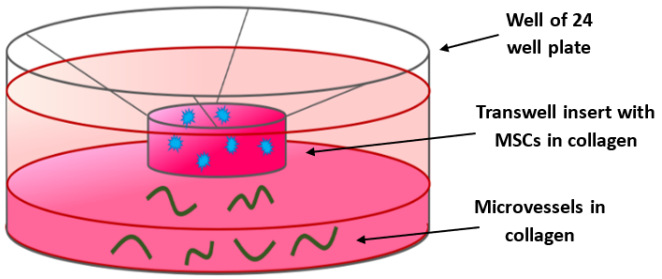
- Accelerated angiogenesis and neovascular growth
- Applicable to a variety of tissue types and systems
- Readily add cellular complexity to tissues
- Higher fidelity to the native, in vivo tissue environment
- Compatible with BioAssembly® automation and high-content imaging analyses

METHODS

Xeno-Free RoosterVial™ bone marrow derived hMSCs from RoosterBio [MSC-031] were cultured using RoosterNourish™-MSC-XF expansion media [part no. KT-016] according to the manufacturer's instructions. Three different lots reflecting 3 different donors were used, designated 257, 277, and 280. hMSCs were suspended in 3 mg/ml collagen type I at a density of 1 million cells per ml and dispensed into Transwell® inserts (200 µl per insert) with three inserts per group. While gelling, ASLS Angiomics® haMVs [ASLS-000123] were thawed, suspended at a density of 100k haMVs/ml in 3 mg/ml cold neutral collagen. In wells of a 24 well plate, 300 µl of cold, neutral acellular collagen was dispensed per well. Before this collagen could gel, 100µl of the haMV/collagen suspension was dispensed into the middle of each well. This creates a central region of haMVs surrounded by a ring of acellular collagen. The inserts containing gelled hMSC/collagen constructs were suspended above wells containing the haMV constructs and

culture medium (RPMI + B27) was added to each well, such that both the hMSC insert and haMVs are covered (**Figure 1**). A control group contained haMVs only without hMSC inserts. After 6 days of culture, haMV constructs were fixed, stained with fluorescent UEA lectin, and imaged using an confocal scanner. Vessel length densities (total vessel length per tissue area) in constructs, a measure of angiogenesis, were determined using BioSegment™ (ASLS).

Figure 1.



▲**Fig. 1** (*above*) Experimental configuration for paracrine factor testing of hMSCs. hMSCs, embedded in collagen, are suspended above a construct of haMVs in collagen with both compartments sharing culture medium.

RESULTS AND DISCUSSION

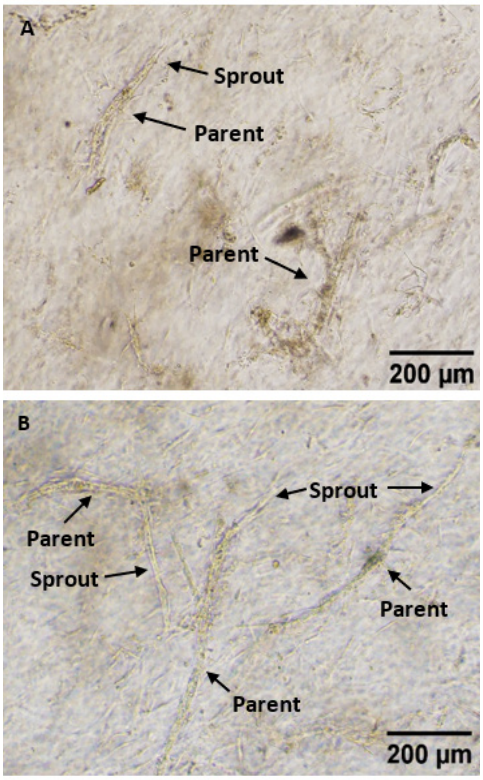
In the present study, we explored the potential of hMSCs to stimulate angiogenesis from pre-existing microvessel segments in 3D in vitro constructs. We have previously observed the pro-angiogenic effects of hMSCs on angiogenesis in a tissue model [1]. Here, we expanded this study to focus specifically on paracrine-mediated angiogenesis by hMSCs from multiple donors. The cell culture insert format of this experiment physically separated the hMSCs and haMV constructs, allowing the hMSCs to condition the culture medium in the absence of direct cell-cell contact (**Figure 1**). Importantly, no exogenous growth factors were added. Thus, any effects on angiogenesis observed reflects the cell activity in the system.

After 6 days, haMV constructs with hMSC inserts exhibited many growing angiogenic sprouts originating from the parent vessels of the haMV isolate as compared to the haMV alone cultures (**Figure 2**).

Differences in vessel length density between control haMV constructs and those co-cultured with the three hMSC lots were measured from confocal images of lectin-stained cultures (**Figures 3-4**). Vessel length density, calculated by summing the length of each individual vessel in the image and dividing by the total area per image, is a common measure of angiogenic growth. Lot 257 hMSCs promoted the most angiogenesis (largest increase in vessel length density), followed by 277 and 280. While lot 257 hMSCs were most strongly angiogenic, all lots promoted angiogenesis.

Because there is no direct hMSC-microvessel contact, the pro-angiogenic effect of the hMSCs is due to paracrine factors and other signaling molecules released by hMSCs

Figure 2.



▲**Fig. 2** (*above*) Angiogenesis in an haMV construct. Parent microvessels were isolated from discarded adipose tissue and embedded in collagen matrix. As compared to the absence of hMSCs (A), angiogenesis, neovessel sprouts arising from parent microvessels, is enhanced in the presence of hMSCs (B).

Table 1. Growth factors secreted by RoosterBio hMSCs (pg/10^5 cells/day)

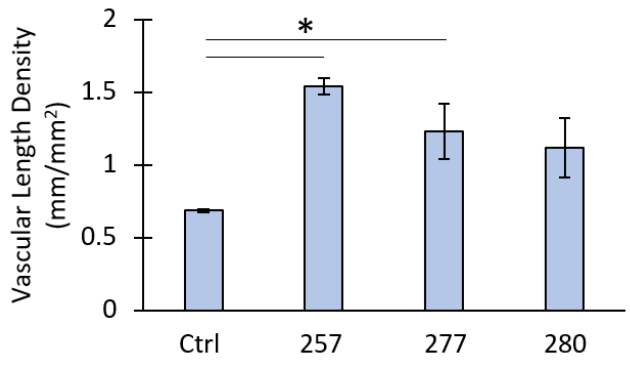
Lot #	bFGF	HGF	IL-8	TIMP-1	TIMP-2	VEGF
257	14.5	882.3	151.3	98592.3	10694.5	687.4
277	27.9	508.4	239.8	107253.8	9009.1	592
280	21.5	671	226.3	185800.8	18188.2	725.3

into the culture medium. Examining the growth factor secretion profiles of the three hMSC lots used in this study indicates the cells are producing a variety of growth factors, many of which are known to stimulate angiogenesis (**Table 1**). Thus, it is not surprising that angiogenesis is stimulated in the hMSC presence.

Interestingly, Lot 257, which stimulated angiogenesis the most, did not produce the highest levels of factors, except for HGF. HGF is also a known pro-angiogenic growth factor [13], and may be contributing to the higher angiogenic potential of this lot. Of course, there are a variety of factors produced by hMSCs that were not measured, including small molecule metabolites and cytokines [14], that could be driving the angiogenesis from haMVs. Additionally, as fragments of native microvasculature, the haMVs are comprised of multiple cell types in addition to endothelial cells, including pericytes and macrophages. These other cell types may be responding to the hMSC-derived factors by releasing their own, angiogenic factors. An important aspect of this assay is that the cellular complexity reflects more the in vivo tissue environment, and all assay readouts reflect the interplay between pro- and anti-angiogenesis generating a "net angiogenic" outcome.

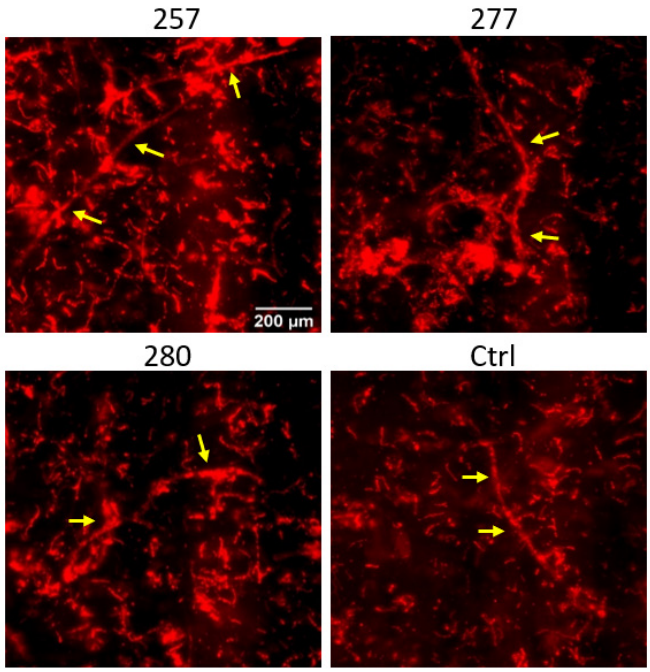
The Angiomics® haMVs have been used to vascularize a variety of tissues and tissue models, including adipose, bone, liver, endocrine, and cardiac [15-19], and we have previously demonstrated their dynamics for in vitro studies or implantation purposes [20, 21]. In all cases, endogenous or included parenchyma and stromal cells contribute to microvessel dynamics in the absence of exogenous factors. Here, we show that hMSCs similarly accelerate angiogenesis and neovessel growth from the haMVs without the need for growth factor supplementation. Thus, combining RoosterBio's hMSCs with haMVs has broad applicability related to tissue construction and vascularization for in vitro 3D tissue modeling and potentially clinical applications as well. In addition, differences in hMSC angiogenic potential enable fine tuning/optimizing tissue vascularization strategies. Furthermore, the study highlights the utility of haMV cultures as an angiogenesis assay in which neovessel arise from parent microvessels, as it happens in the native tissue.

Figure 3.



▲**Fig. 3** (*above*) Vascular length density of haMV cultures without and with hMSC lots (257, 277, or 280). *P<0.05, one way ANOVA with Tukey post-hoc test, n=3.

Figure 4.



▲**Fig. 4** (*above*) Representative confocal maximum projections of microvessel cultures conditioned with different lots of MSCs. During analysis, only intact parent and neovessels were counted towards vessel length density (arrows). Red = lectin stain (endothelial cells).

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