In Vitro Vascularization of Human Microtissues

VEGF profiling and angiogenesis in human connective micro tissues

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Abstract: Owing to its dual impact on tissue engineering (neovascularization of tissue implants) and cancer treatment (prevention of tumor-induced vascularization), management and elucidation of vascularization phenomena remain clinical priorities. Using a variety of primary human cells and (neoplastic) cell lines assembled in microtissues (MTs) by gravity-enforced self-aggregation in hanging drops we (i) studied VEGF production of MTs in comparison to isogenic monolayer cultures, (ii) characterized the self-organization and VEGF-production potential of mixed-cell spheroids and (iii) analyzed VEGF-dependent capillary formation of HUVEC (human umbilical vein endothelial cells) cells coated onto human primary cell spheroids.

Key words: Tissue Engineering, Vascularization, Angiogenesis, Regenerative Medicine, Self-organization, Endostatin, VEGF, Drug Screening, Cell-based assay system, Blood vessel formation

1. INTRODUCTION

An artificial tissue with more than a few cubic millimeters cannot survive by simple diffusion and requires formation of new capillaries to supply essential nutrients/oxygen and enable connection to the host vascular system following implantation. A variety of strategies for therapeutic angiogenesis have been designed including (i) delivery of recombinant angiogenic molecules through controlled-release devices and (ii) functionalized matrices or (iii) transfection/transduction of (engineered) angiogenesis-modulating cDNAs. However, the use of growth factors such as the vascular endothelial growth factors (VEGF) bears some risks. The biological effects of VEGF are extremely dose dependent. Loss of even a single allele results in fatal

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vascular defects in the embryo and insufficient levels of VEGF lead to post-natal angiogenesis and ischemic heart disease, whereas uncontrolled VEGF expression may lead to angioma-genesis.

Based on our previous observations that VEGF production in myocardial microtissue is strictly correlated to cell number and microtissue size we have established an entirely human cell-based microtissue format to provide new insight into VEGF production, angiogenesis and blood vessel formation.

2. RESULTS AND DISCUSSION

2.1 VEGF production of human microtissue cultures

ELISA-based technology was used to quantify vascular endothelial growth factor (VEGF) production by human cell lines and primary cells grown assembled as microtissues. Based on previous observations suggesting cell type-specific cell number – microtissue size correlations we have used tailored cell concentrations to obtain microtissues of 350 µm in diameter following a 3-day cultivation period.

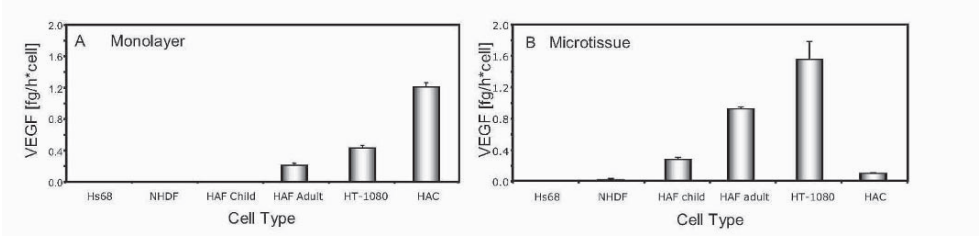

Figure 1. Vascular endothelial growth factor (VEGF) production profiling in monolayer and microtissue cultures (350 µm in diameter). HAC: human aortic chondrocytes; HAF child: human aortic fibroblasts from a newborn donor; HAF adult: human aortic fibroblasts from an adult donor; Hs68: embryonic dermal fibroblast cell line; HT-1080: fibrosarcoma cell line; NHDF: normal human dermal fibroblast.

2.2 Self-organization potential of different cell phenotypes in a microtissue format

In order to investigate self-organization forces underlying cell migration during angiogenesis we assembled HAF:HUVEC and HepG2:HUVEC suspension cocultures to mixed microtissues in hanging drops. According to the differential adhesion hypothesis HAF/HepG2:HUVEC populations resulted in concentric HAF/HepG2-inside:HUVEC-outside structures, reminiscent of blood vessel cross-sections.

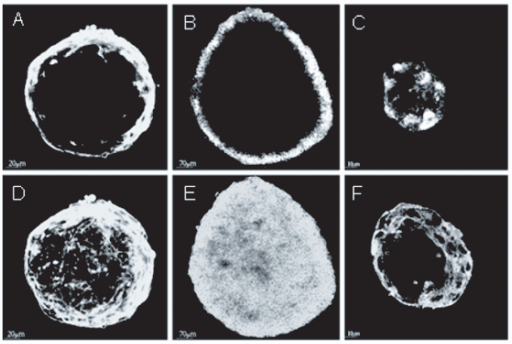

Figure 2. Mixed populations of HUVECs, stained for von Willbrand factor (A-C) and HAFs, stained for F-Actin (D), HepG2, stained for F-Actin (E), as well as human umbilical aortic smooth muscle cells, stained for smooth muscle alpha-actin (F).

2.3 In vitro vascularization

To vascularize it was not sufficient to produce mixed HUVEC microtissue cultures. Therefore we coated pure HAF microtissues of different size (125 µm – 335 µm; produced by 2-day gravity-enforced assembly) by cocultivation with monodispersed HUVECs in hanging drops. Transmission electron microscopy revealed that the migrated endothelial cells are characterized by lumen formation, with endothelial-periendothelial cell contacts. Thus, lower VEGF production was measured in the vascularized microtissues.

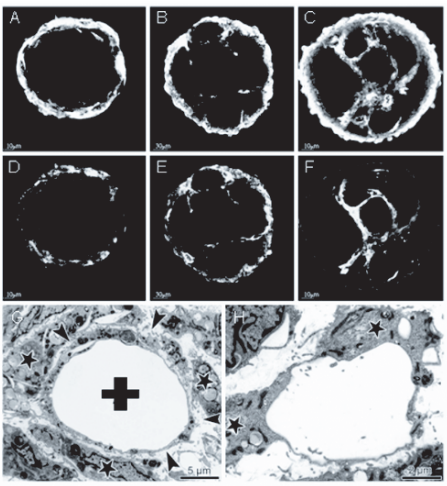

Figure 3. Migration of HUVECs, stained for von Willbrand factor (A-C) and CD31 (D-F) in different sized microtissues, initiated with 500 HAFs coated with 600 HUVEC (A, D); 5,000 HAFs coated with 900 HUVECs (B, E); 10,000 HAFs coated with 1,200 HUVECs (C, F), 6 days post coating. Endothelial cells are typically characterized by intracellular lumen formation (+) and are tightly covered by human aortic fibroblasts (asterisks) (G, H).

The C-terminal cleavage product of collagen XVIII known as endostatin is a key anti-angiogenic factor. We evaluated endostatin action on HAF microtissues coated with HUVECs.

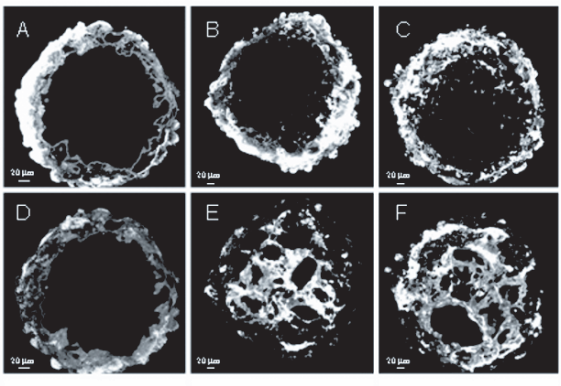

Figure 4. Endostatin induced inhibition of HUVEC-based capillary formation. HUVECs were stained for von Willebrand factor (A-C) and CD31 (D-F). Endostatin was supplemented with HUVECs (A, D), two days postcoating (B, E) and the control culture, grown without addition of Endostatin, is shown in (C, F).