APPLICATION NOTE

MPS Chip: Boncyte Chip –3-Channel–
Dedicated Rocking Shaker: Boncyte Rocker
Analysis Software: Boncyte Chip Analyzer

Quantitative Evaluation of Sunitinib-Mediated Inhibition of 3D Angiogenesis Using Boncyte Analyzer

Angiogenesis is the formation of new blood vessels from pre-existing vessels, and its dysregulation contributes to various pathological conditions, including retinopathy, chronic inflammation, and ischemic diseases. Conventional two-dimensional tube formation assays do not reproduce lumen formation, three-dimensional sprouting into the extracellular matrix (ECM), or growth factor gradients, while animal models are limited by species differences and low throughput.

In this application note, angiogenesis of human umbilical vein endothelial cells (HUVECs) was reproduced using the Boncyte Chip –3-Channel–, and the effects of the receptor tyrosine kinase inhibitor sunitinib were evaluated. Sunitinib inhibited angiogenesis in a concentration-dependent manner, reducing vessel extension, length, branching, and vascular network area.

This MPS enables quantitative evaluation of angiogenesis-modulating compounds using physiologically relevant three-dimensional vascular structures and may be useful for early-stage drug screening and mechanism-of-action studies.

Highlights

- Reconstitution of angiogenesis in the Boncyte Chip and quantitative evaluation using Boncyte Analyzer
- Concentration-dependent detection of the inhibitory effects of sunitinib

Experimental Overview

A fibrinogen-based gel solution was introduced into the central channel of the Boncyte Chip –3-Channel– and allowed to polymerize. GFP-expressing HUVECs (GFP-HUVECs; Angio-Proteomie; 5×10^6 cells/mL) were introduced into the right channel, and the chip was tilted by 90° to allow the cells to adhere to the gel interface. The right channel was filled with EGM-2 medium (Lonza), while the left channel received EGM-2 containing 50 ng/mL VEGF and bFGF to establish a growth factor gradient. This time point was defined as Day 0. Cultures were maintained at 37°C and 5% CO_2 on the Boncyte Rocker at a tilt angle of 6°, movement speed of 1.2°/s, movement time of 10 s, and pause time of 20 s.

Sunitinib was prepared as a 10 mM stock solution in DMSO and diluted with EGM-2 to final concentrations of 0, 1, 3, 10, 30, and 100 nM, with the final DMSO concentration kept constant. On Day 1, the medium in the right channel was replaced with sunitinib-containing medium.Media in both channels were changed every 48–72 h. Fluorescence and phase-contrast images were acquired at each medium change using a fluorescence microscope (CKX53, Evident).

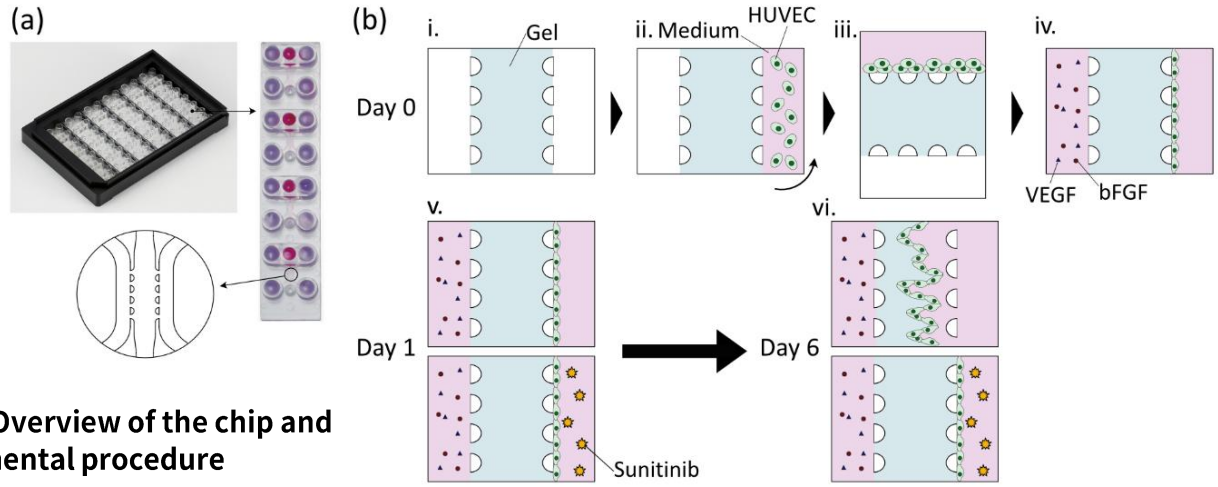

Fig. 1 Overview of the chip and experimental procedure

a. Overview of the Boncyte Chip –3-Channel–.
b. Experimental procedure. A gel solution was introduced into the central channel (i), followed by HUVECs into the right channel (ii). The chip was tilted by 90° to allow HUVEC adhesion to the gel interface (iii). Growth factor-containing medium was introduced into the left channel (iv), and sunitinib was added to the right channel on Day 1 (v). Angiogenesis was evaluated during subsequent culture (vi).

Image Processing and Analysis Using Boncyte Analyzer

Image analysis using Boncyte Analyzer consists of four steps: (1) cropping, (2) binarization, (3) skeletonization, and (4) quantitative analysis. First, the central channel is automatically detected and cropped from the phase-contrast image. For binarization, uneven background fluorescence is corrected using rolling-ball background subtraction, followed by a tubular structure enhancement filter and hysteresis thresholding. Regions incorrectly detected as nonvascular areas due to heterogeneous GFP expression are restored based on residual fluorescence intensity and boundary sharpness. Sheet-like HUVEC regions near the pillars are also detected and filled based on their fluorescence boundaries. Following skeletonization, the distance from the pillar edge to the furthest point of the connected vascular network is defined as the connected sprout reach distance. Total vessel length, vessel area fraction, and branch density are also calculated (Fig. 2).

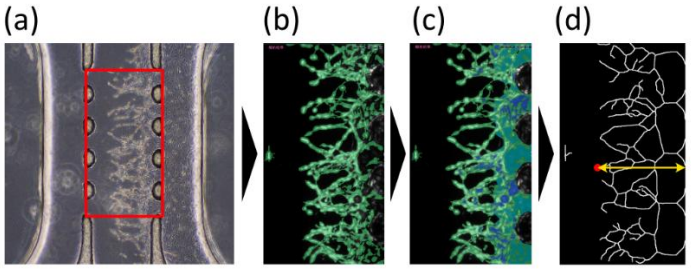

Fig. 2 Image analysis workflow using Boncyte Analyzer

a. Automatic cropping based on the phase-contrast image.
b. Binarization of the vascular region (green).
c. Correction of falsely detected nonvascular regions (blue) and sheet-like regions (cyan).
d. Skeletonization. The yellow line indicates the connected sprout reach distance.

Evaluation of the Inhibitory Effects of Sunitinib

Fluorescence imaging showed suppression of angiogenesis at sunitinib concentrations of 30 nM or higher (Fig. 3a). Quantitative analysis also showed that, on Days 6 and 8, all angiogenesis parameters were significantly lower in the ≥30 nM groups than in the control group (Fig. 3b).The IC_{50} based on connected sprout reach distance was 25 nM on Day 6. Previous studies reported IC_{50} values of 4–12 nM for inhibition of VEGF-induced HUVEC proliferation[1,2] and 43 nM for VEGFR2 kinase activity[3]. Thus, the value obtained in this MPS was within a comparable range. These results demonstrate the potential of this MPS as a quantitative platform for evaluating angiogenesis inhibitors.

References:
1. Nacev BA, Liu JO. PLoS One. 2011;6(9):e24793.
2. Zhang K, et al. Thorac Cancer. 2020;11(6):1636–1647.
3. Bakri SJ, et al. PLoS One. 2024;19:e0304782.

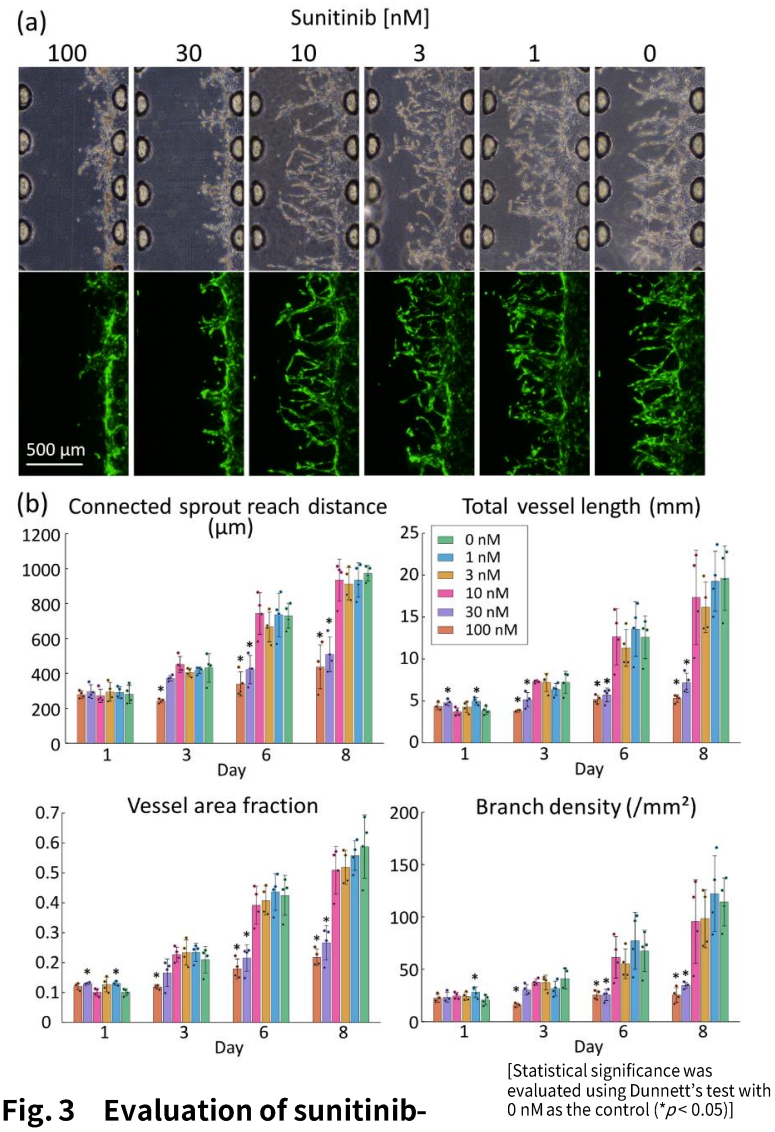

Fig. 3 Evaluation of sunitinib-mediated inhibition of angiogenesis

a. Fluorescence and phase-contrast images on Day 6.
b. Quantitative analysis of angiogenesis: connected sprout reach distance, total vessel length, vessel area fraction, and branch density.