Fabrication of Vascularized 3D-Wound Healing Fibrous Tissues and Application for In Vitro Tumor Invasion Models

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Statement of Purpose: A use of in vitro tissue models for drug assessments have been attracted increasing attention, because previous models such as cell monolayer and animal models have difficulties in the evaluation of tissue response and species differences. Especially, in vitro tumor models composed of cancer cells, blood vessels, and fibrous tissues are desired for tumor invasion and metastasis assay. However, there are no reports on 3D-in vitro tumor invasion models containing blood capillary networks. In this study, we developed novel tumor invasion models with blood-capillary networks constructed by cell-accumulation technique (Figure 1a). We have reported bottom-up approach to develop multilayered tissues by cell surface coating with nanometer-sized ECM films, fibronectin and gelatin (FN-G) films (A. Nishiguchi et al., Adv. Mater. 2011;23:3506–10.). About 7 nm of the FN-G films induce cell-cell interaction in three dimensions. Moreover, endothelial tubular networks were formed in the fibroblast tissues by sandwich culture of endothelial cells. Since the vascularized 3D-fibrous tissues are similar histological morphology to wound healing place, this may be useful for in vitro tumor invasion analysis, especially to evaluate the effect of tumor to blood-capillary networks (Figure 1a). Comparing the results of invasion process and the effect to blood vessels between in vitro and in vivo models, we show the similarity to living tissues and usefulness of this model. The novel in vitro tumor invasion models will be valuable as a cancerous peritonitis model.

Methods: The 5 x 10^6 cells/mL normal human dermal fibroblasts (NHDF) were alternatively incubated with 0.04 mg/mL FN (M_w = 4.6 × 10^5) and G (M_w = 1.0 × 10^5) in 50 mM Tris-HCl (pH = 7.4) for 1 min at 30 rpm. After repeating the nine steps of immersion, the $(FN/G)_4FN$ films with about 7 nm thickness were prepared on the cell surface. The FN-G coated NHDF were seeded into a cell culture insert and cultured for 1 day to construct multilayered tissues. In the same manner, human umbilical vein endothelial cells (GFP-expressing HUVEC) were sandwiched between 10-layered NHDF tissues to form vascularized tissues. After that, two types of 1 x 10^5 cells /well of human cancer cells (RFP-expressing MiaPaCa-2 and BxPC3) were seeded onto blood-capillary models to make invasion models. The invasion process of RFP-MiaPaCa-2 and RFP-BxPC3 and the morphology of GFP-HUVEC networks were observed by confocal laser scanning microscopy (CLSM) for 7 days.

Results: When MiaPaCa-2 were cultured on the surface of the 3D-fibrous tissues, the cancer cells adhered and started invasion within 24 hours and then reached to the bottom of the substrate after 7 days of incubation. The MiaPaCa-2 did not significantly affect the blood-capillary

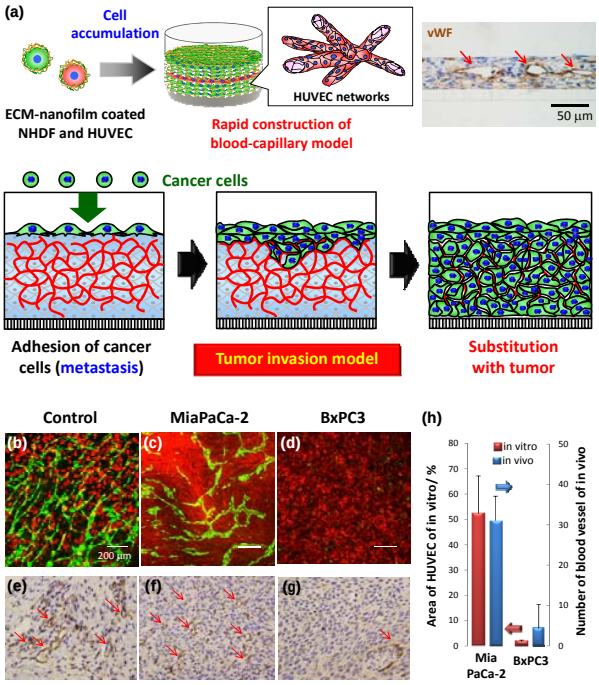

Figure 1. (a) Schematic illustration of reconstruction of vascularized 3D-wound healing fibrous tissues and 3D-tumor invasion models by ECM-nanofilm coating. (b-d) CLSM images of HUVEC networks of in vitro models after 96 hours. (e-g) Immunohistochemistry images of blood vessels in tumors of in vivo models. (h) Quantitative analysis of blood vessels of in vitro and in vivo models.

structures, but the surrounding NHDFs disappeared completely (Figure 1,b c). In contrast, the almost capillary networks disappeared during the culture and invasion of BxPC3, and then hypo-vascularized tumor tissues were obtained (Figure 1d). We confirmed the same trend as invivo nude mouse experiments by transplantation of these cancer cells on the surface of the wound healing place of peritonea (Figure 1e-g). The quantitative comparison of in vitro model versus in vivo revealed high correlation as shown in Figure1h. The effect of blood-capillary networks on anticancer drugs is now in progress using in vitro and in vivo models.

Conclusions: We demonstrated reconstruction of 3D-tumor invasion models in vitro and found the similarity of the structures of blood vessels between in vitro and in vivo mouse models. This 3D-tumor invasion model with blood vessels would be useful for the establishment of cancerous peritonitis treatment.