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Emulating human microcapillaries in a multi-organ-chip platform

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ABSTRACT

Current microfluidic chip-based tissue culture systems lack a capillary endothelial vessel system, which would enable perfusion with blood. We utilise spatial cell cultures to populate a perfused multi-organ-chip platform—a microfluidic device recently introduced for substance testing. Complete biological vascularization of such culture systems is vital to properly emulate physiological tissue behaviour. In this study, we incorporated a fibrin scaffold into the two-organ-chip design. Herein, adipose-derived stromal cells (ASCs) directed human umbilical vein endothelial cells (HUVECs) to organise into tube-like structures. The ASCs induced tube formation of HUVECs in static and dynamic conditions. The replacement of full medium enriched with growth factors and foetal calf serum with basal medium resulted in viable cells with similar gene expression profiles. We regard this as a prerequisite for studies with organ constructs that have a need for a different medium formulation. Furthermore, we here address stability issues of the fibrin gel and fibrin composition for optimal microvessel formation.

1. Introduction

Substance testing for human toxicity and efficacy is an ever increasing worldwide issue. Various factors have generated the need for new human *in vitro* solutions: Laboratory animals, for instance, are unsuited due to their phylogenetic distance to humans; *in vitro* systems that are already in place do not usually recapitulate the complex arrangement of different organs and their functions in the human body; and *in silico* models are far from being comprehensive (Esch et al., 2011; Huh et al., 2011; Leist and Hartung, 2013; Moraes et al., 2012). We recently introduced a multi-organ-chip (MOC) platform to model organ–organ interactions (Maschmeyer et al., 2015b; Wagner et al., 2013). The MOC design offers dynamic cultivation of two to four heterogeneous organoid cultures in separate culture compartments connected by microfluidic media transport channels. The integrated micropump and the chip's minute scale provide a dynamic environment and means to interact with an enriched medium across the cultivation compartments. The number and type of organ equivalents in the chip can be altered as required (Ataç et al., 2013; Marx et al., 2012; Maschmeyer et al., 2015a; Materne et al., 2015).

One crucial step in forming three-dimensional (3D) cultures for the chip is the establishment of a vascular network. Without an adequate vascularization, nutrient supply is limited, resulting in

Abbreviations: 2OC, two-organ-chip; 3D, three-dimensional; ACTB, β-actin; ASCs, adipose-derived stromal cells; CD31, cluster of differentiation 31 (also PECAM-1); COX-2, cyclooxygenase-2; eNOS, endothelial nitric oxide synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP, green fluorescent protein; HDMECs, human dermal microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; KDR, kinase insert domain receptor (also: VEGFR2); LDH, lactate dehydrogenase; MOC, multi-organ-chip; PDMS, polydimethylsiloxane; PECAM-1, platelet-endothelial cell adhesion molecule 1; qPCR, quantitative real-time polymerase chain reaction; SEM, standard error of the mean; TEK, tyrosine kinase, endothelial; TGF-β1, transforming growth factor β1; Tie2, tyrosine kinase with immunoglobulin and EGF factor homology domains 2 (also TEK); VE-cadherin, vascular endothelial cadherin; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2 (also: KDR); vWF, von Willebrand factor.

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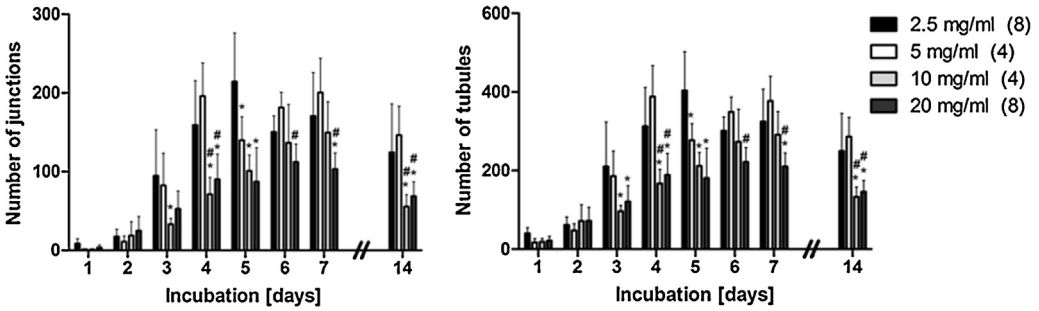

Fig. 1. Quantification of time course experiments with GFP-HUVEC/ASC (ratio 1:1). Fibrin gels contained different amounts of fibrinogen and are incubated under static conditions in full growth media. Number of junctions and vascular tubes increased until day 4 in every condition. Specifically, the number of junctions and tubules increased with incubation time peaking at day 4 (5 mg/ml), day 5 (2.5 mg/ml), day 6 (20 mg /ml), and day 7 (10 mg/ml). After two weeks of incubation, vessel regression occurred. Numbers in brackets indicate number of images per time point analysed. Values are Mean + SD; $*p < 0.05$ *vs.* 2.5 mg/ml; $\#p < 0.05$ *vs.* 5 mg/ml.

impaired tissue function as well as limited size (Nahmias et al., 2006; Vollert et al., 2014). The introduction of a dynamic environment in the MOC improves the supply of the organoid cultures. However, it cannot compensate for vessel formation inside the corresponding organoids. In addition to its task of nourishing tissues, the vasculature is also needed for proper orientation of adjacent heterogenic cells, for example, within liver organoids (Hoehme et al., 2010). Moreover, it is required as a barrier for any biological derivative and for an integral role in the immune system (Li et al., 2005).

Various techniques can be utilised to incorporate a vascular network in microfluidic devices, as reviewed recently (Hasan et al., 2014; Muehleder et al., 2014). Vascular patterns can be designed using microelectromechanical systems (Hsu et al., 2013; Liu et al., 2011; Rosano et al., 2009; Sakaguchi et al., 2013) or 3D printing (Miller et al., 2012). Alternatively, co-culture techniques exploiting the self-mediated arrangement of the cells to tubular networks have also been reported as *in vitro* strategies for tissue vascularization (Akahori et al., 2010; Holnthoner et al., 2012; Kim et al., 2013; Moya et al., 2013). Fibrin was utilised in manifold studies as an easily obtainable and processable scaffold material. In contrast to other naturally occurring hydrogels, such as collagen or Matrigel®, fibrin is structurally and mechanically close to its *in vivo* ideal (Janmey et al., 2009). Thus, the matrix is common to many cell types involved in wound-healing, including endothelial cells. The latter, for example, can alter, metabolise and reorganise fibrin gels to their needs, eventually enabling 3D orientation of the cells (Ghajar et al., 2006; Tian and George, 2011). The formation of vessels, however, only occurs spontaneously by extracellular stimulation. Co-cultivated stromal cells promote paracrine factors that trigger migration and the formation of microcapillary structures (Kachgal and Putnam, 2011; Rohringer et al., 2014a).

We envisage a combined approach of technically created vessels and neo-vascularization in our MOC platform. Firstly, endothelial cells were successfully seeded into the chip's microfluidic channels and exhibited phenotypical behaviour under dynamic conditions (Schimek et al., 2013). Here, we describe the incorporation of tubular networks into the cultivation compartments by using a co-culture of human umbilical vein endothelial cells (HUVECs) and adipose-derived stromal cells (ASCs) encapsulated in a fibrin hydrogel. The set-up was incorporated into a dynamically cultivated two-organ-chip (2OC), demonstrating the feasibility of this approach. Additionally, we focused on the determination of a cell-favouring degree of fibrin density through optimal concentrations of fibrinogen, as well as the cells' ability to self-maintain their phenotype in medium containing no admixed growth factors, cytokines or serum.

2. Methods

2.1. Cell culture

Human umbilical vein endothelial cells stably expressing the green fluorescent protein (GFP) were purchased (Olaf Pharmaceuticals). Adipose-derived stromal cells from one donor were isolated and selected by means of distinct characteristics described elsewhere (Rohringer et al., 2014a; Wolbank et al., 2007). If not otherwise stated, both cell types were cultivated in full Endothelial Growth Medium MV2 (Promocell), including 50 μg/ml gentamicin (Roth) and 0.5 μg/ml amphotericin B (PAA). The cells were used up to passage 11 for HUVECs and passage 7 for ASCs. All cell culture experiments were performed at 37 °C and 5% carbon dioxide in a humid atmosphere.

2.2. Multi-organ-chip fabrication

The microfluidic 2OC platform has been described previously (Schimek et al., 2013). Briefly, the 2OC contained two independent circuits, each with two interconnected cavities capable of embodying organoid constructs (Fig. 2B). Pulsatile perfusion of the system was created by a peristaltic on-chip micropump operated by periodic lowering and lifting of three successively arranged membranes. The volumetric flow rate in the chip can be controlled by the frequency of actuation, pressure and vacuum, which are externally applied to the membranes. The total volume of the chip was 600 μl, superseding external reservoirs that would dilute the conditioned medium.

The assembly of the 2OCs was performed with slight modification of a method described previously (Wagner et al., 2013). In brief, a master mould was created either through photolithography or aluminium milling. From this, a PDMS wafer (Sylgard 184, Dow Corning) was cast containing the microfluidic structure. Areas for later cultivation were kept free with the help of Teflon spacers. The silicon was covalently bonded to a glass microscope slide using physical plasma that activated both surfaces, creating a fluid tight microcirculation.

Immediately following plasma treatment and bonding, the chip was sterilised with 80% ethanol following thorough rinsing with phosphate buffered saline. Subsequently, the 2OC was filled with medium and incubated at 37 °C for three days before further application.

2.3. Time course and quantification of vascular networks in fibrin gels

Both GFP-HUVECs and ASCs were embedded into fibrin matrices as described earlier (Rohringer et al., 2014a). Briefly, fibrinogen