Modeling human brain angiogenesis with a tissue-engineered brain microvessel

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Statement of Purpose: Brain angiogenesis plays an important role in brain health and diseases. Most of the cellular and molecular process of brain angiogenesis is known from the study of animal embryonic development, in which angiogenic growth factors released from neuron progenitor cells promote the infiltration of endothelial cell followed by stabilization with mural cells [1]. However, the process of angiogenesis in mature brain blood vessels is largely unknown, especially the role of pericytes. Therefore, the overall goal of this study is to model the process of pericyte activities in brain angiogenesis.

Methods: The microfluidic devices were designed to have three channels with a diameter of 150 μm in a collagen hydrogel. The middle channel was used for engineering the co-cultured microvessel. One of the remaining two channels was used as growth factor source and the other one as the sink. Brain microvascular endothelial-like cells (iBMECs) and indued brain microvascular pericytes (iPCs) were differentiated from WTC-iPSC cell lines. To distinguish iBMECs and iPCs, WTC-iPSC cell lines with RFP and GFP labels were used. iBMECs were seeded into the middle channel first and allowed to adhere for a few hours followed by seeding of iPCs (**Fig. 1a**). After the formation of a co-cultured microvessel, angiogenic growth factors were perfused through the source channel. Angiogenesis over the next 2 days was observed with fluorescence imaging and confocal imaging.

Results: A co-cultured blood-brain barrier (BBB) model was successfully created in our microfluidic device using iPSC derived brain vascular cells (**Fig. 1b**). iBMECs formed a confluent monolayer with iPCs at the abluminal side (**Fig. 1c**). After the addition of growth factors, pericyte activation was observed within the first day. Their morphology changed into a typical angiogenic tip cell morphology with extended cell body and several filopodia (**Fig. 1d**). In the next few days, the front pericyte led the growth of angiogenic sprouts. Only one pericyte was observed in each of the angiogenic sprouts (**Fig. 1e**). The growth rate of the angiogenic sprouts with pericyte tip cell was significantly higher than the same one with endothelial tip cell. Our preliminary results show that pericytes could be activated and become the tip cell of an angiogenic sprout leading the sprout.

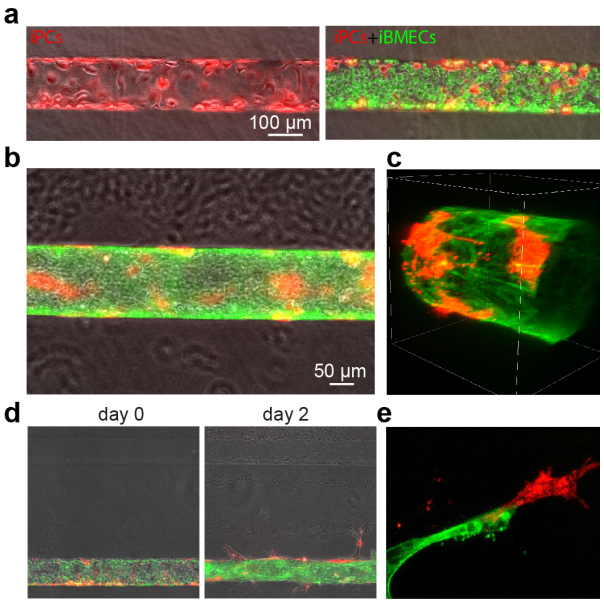

Fig. 1 Engineering of a brain microvessel for modeling brain angiogenesis. (**a**) Sequential seeding of iPCs and iBMECs. (**b**) Formation of a confluent microvessel 24 hours post iBMEC seeding. (**c**) Confocal imaging of a confluent microvessel. (**d**) Angiogenic sprouts of a microvessel over two days. VEGF was perfused via the top channel. (**e**) iPC tip cell of an angiogenic sprout.

References:

[1] Paredes, Isidora, Patricia Himmels, and Carmen Ruiz de Almodóvar. "Neurovascular communication during CNS development." Developmental cell 45.1 (2018): 10-32.