Functional evaluation of genetically modified iPSC-derived microglia in a blood-brain barrier assembloid model

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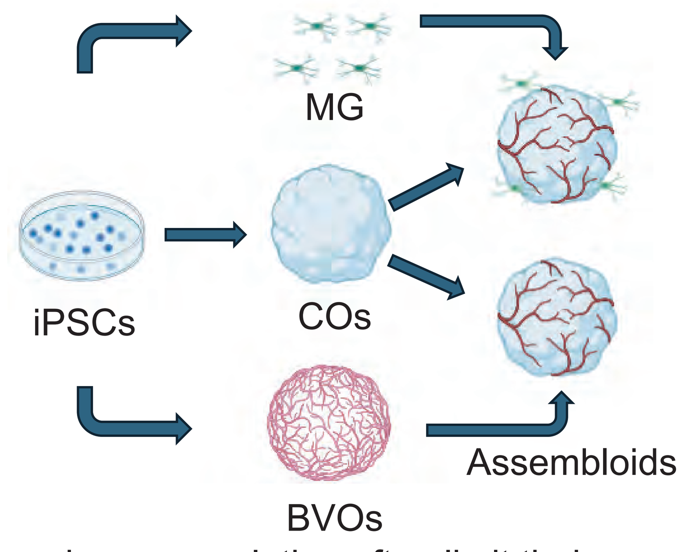
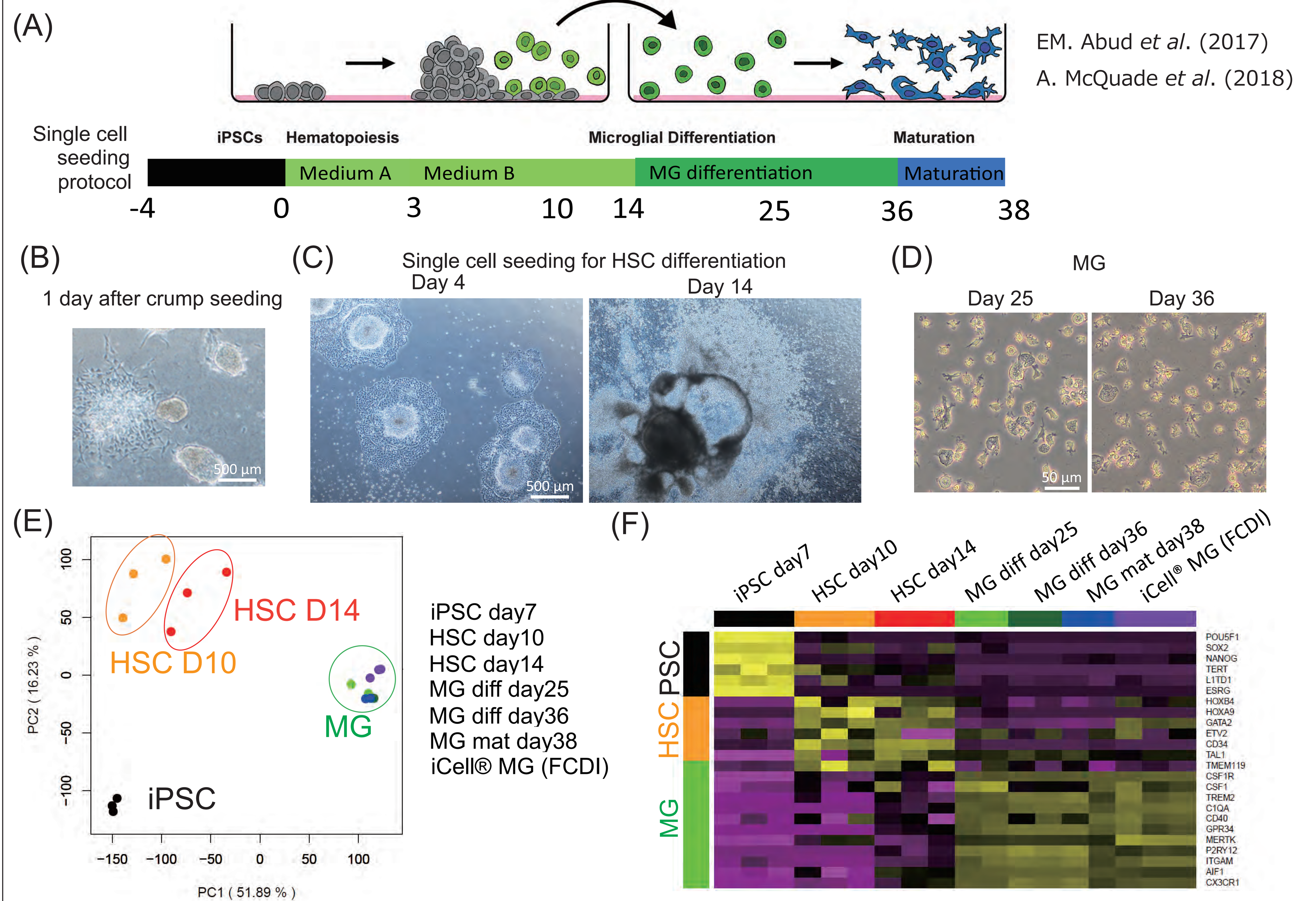
Abstract

Human induced pluripotent stem cell (iPSC)-derived microglia (MG) offer a promising platform for modeling and therapeutic development in neurodegenerative diseases. However, their functional integration within neurovascular environments remains poorly understood. Although animal models of neuro-degenerative diseases, such as Alzheimer's disease model mice, are widely used, species-specific differences in immune responses, cellular interactions, and gene regulation often limit their ability to faithfully recapitulate human MG behavior within the neurovascular niche. Therefore, developing human-relevant *in vitro* systems that enable the study of MG dynamics in a controlled neurovascular context is essential. In particular, blood–brain barrier assembloids generated by integrating cerebral organoids (COs) with blood vessel organoids (BVOs) provide a promising platform to model the neurovascular interface in a human-relevant context. These hybrid 3D systems recapitulate key structural and functional features of the neurovascular unit, including interactions between neural tissue and vascular components that contribute to blood–brain barrier formation and maintenance. Such assembloid models enable the investigation of complex cellular crosstalk within a physiologically relevant environment that more closely reflects human brain architecture. In this study, we aim to utilize a human iPSC-based blood–brain barrier assembloid system incorporating MG to investigate their functional integration and inflammatory behavior under neurodegenerative disease–relevant conditions. By combining stem cell–derived COs with BVOs, this platform seeks to provide deeper insight into the roles of MG in neurovascular regulation and pathology, thereby offering a valuable tool for studying mechanisms underlying neurodegenerative diseases and for evaluating potential therapeutic strategies.

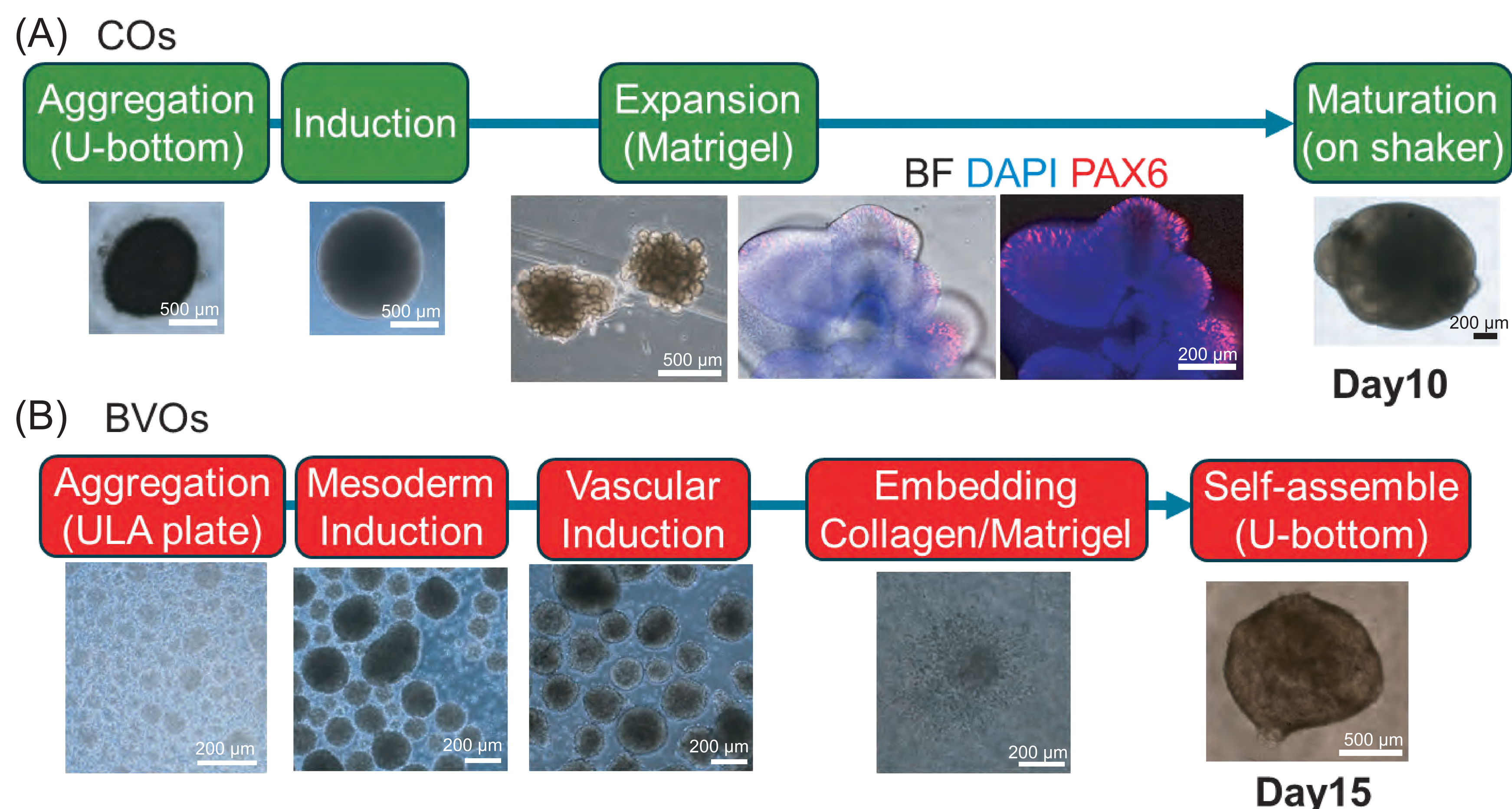


MG differentiation



(A) A schematic drawing of the modified version of the procedure for differentiation MG from iPSCs. (B) A image of iPSCs one day after crump passaging. The shapes and sizes of crumps are variable. (C) Images of hematopoietic stem cells (HSCs) generation. (D) Images of differentiated microglia. (E) PCA of gene expression in iPSCs and their derived HSCs and MG. (F) Heatmap of marker gene expression across individual cells.

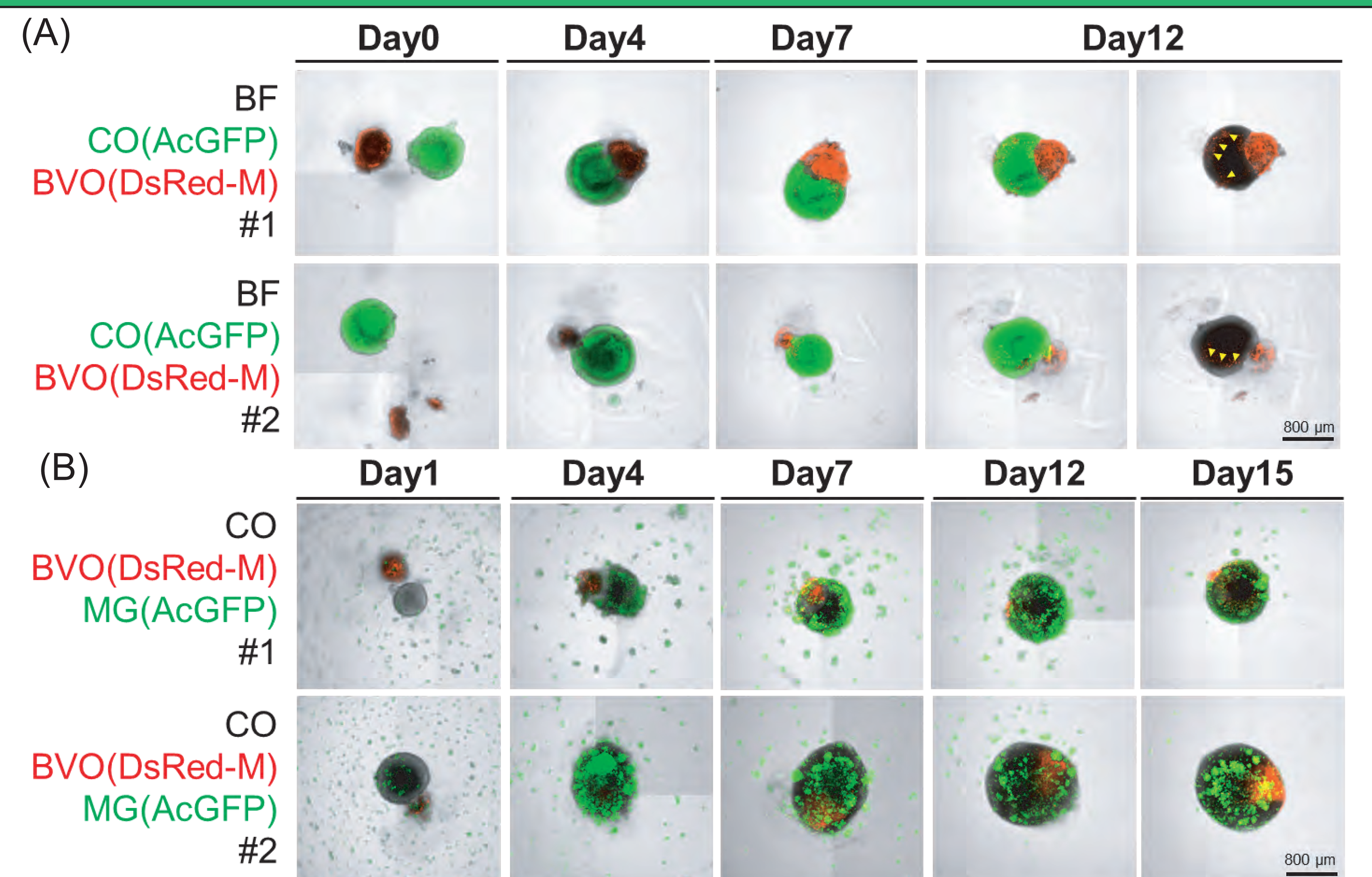
Generation of COs and BVOs



(A) Procedure for COs. Dissociated iPSCs were aggregated in U-bottom wells. Following neural induction, the aggregates were embedded in Matrigel to promote the exansion of neural progenitor cells (PAX6+, red). Each aggregate was then cultured in 24-well plates on shaker to facilitate organoid maturation.

(B) Procedure for BVOs. Partially dissociated iPSCs were seeded onto ultra-low attachment plates to form aggregates. These aggregates were then induced toward a vascular cell fate and embedded into a collagen/Matrigel matrix. Within the collagen/Matrigel matrix, sprouting of vascular networks was observed. The vascular networks were excised and transferred to U-bottom wells, where they self-assembled into BVOs.

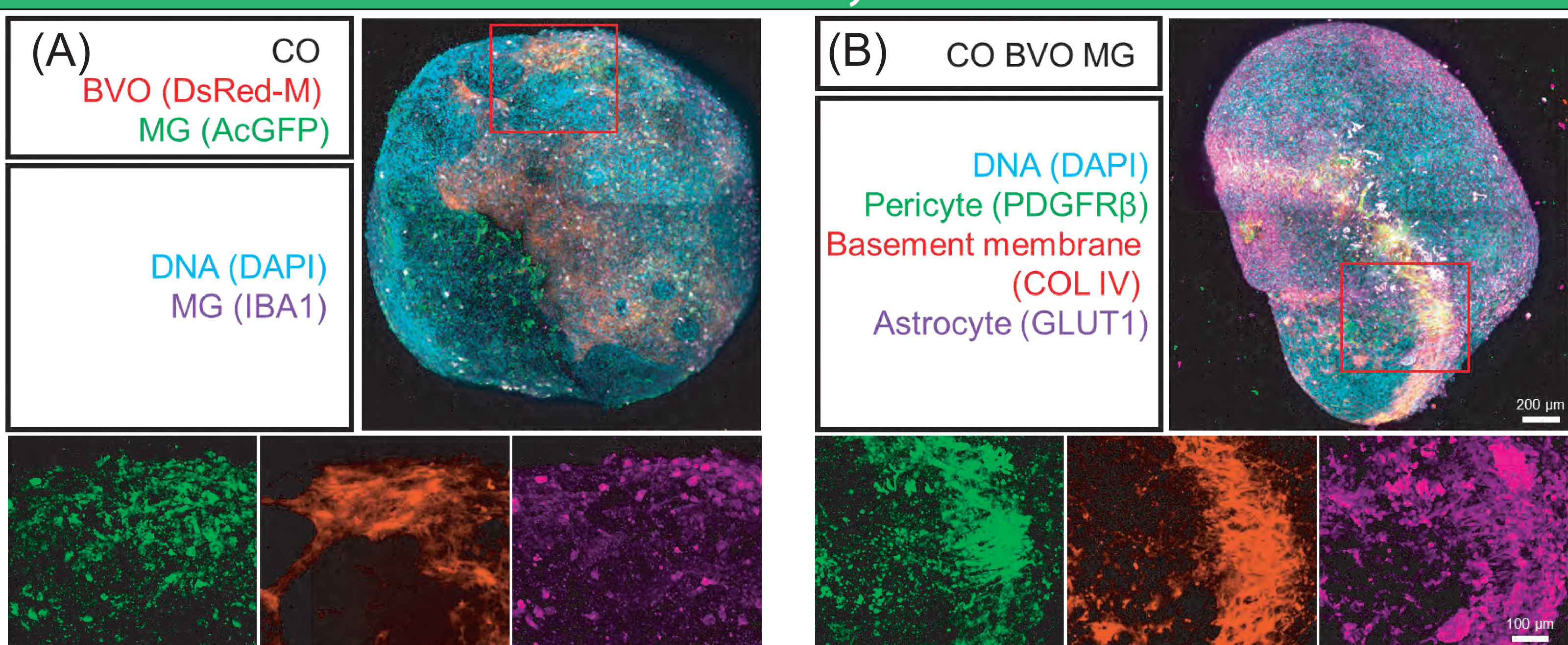
COs, BVOs, and MG assemble



(A) Time course of fusion between COs and BVOs. iPSCs used for COs and BVOs generation were labeled by induction of AcGFP and membrane-tageted DsRed (DsRed-M) expression using PiggyBac vectors, respectively. BVO-derived cells have spread throughout the COs (yellow arrow head).

(B) Time course of fusion among COs,BVOs and MG. iPSCs used for MG and BVOs generation were labeled by AcGFP and DsRed-M, respectively. Each image is a maximum intensity projection (MaxIP) image showing the brightest signal along the z-axis.

Immunohistochemical analysis of BBB assembloids



The assembloids of COs, BVOs, and MG were cultured for approximately 2 months and subjected to immunohistochemical analysis. (A) Non-labeled CO, DsRed-labeled BVO, and AcGFP-labeled MG were assembled. IBA1, a marker for MG, was stained. (B) Non-labeled CO, BVO, and MG were assembled. PDGFRβ (pericyte marker), COL IV (basement membrane), and GLUT1 (astrocyte marker) were stained. Each image is a MaxIP image showing the brightest signal along the z-axis.

Disscussion

- COs and BVOs were successfully generated within 2 weeks following previously reported methods.
- COs, BVOs, and MG self-assembled into a single fused organoid in U-bottom wells without additional Matrigel.
- CO–BVO–MG assembloids contained BBB-like structures composed of pericytes, astrocytes, and endothelial cells.

Limitation of this study

- Variability and limited reproducibility in assembloid formation.
- Uncertainty regarding long-term (more than 2 months) stability and maturation of the assembloid system.

Future plan

- Optimization of observation methods through tissue clearing approaches.
- Elucidation of interactions of genetically modified MG using single-cell RNA sequencing and related approaches.

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COI declaration

The authors declare no conflicts of interest.