（ 続紙 1 ）

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論文題目	Recapitulating Human Development in Spatially and Temporally Controlled Artificial Microenvironments （人工微小環境の時間空間制御とそれを利用したヒト発生の再現）		

（論文内容の要旨）

Understanding human development requires comprehending the intricate interplay between cells and their microenvironment. Human pluripotent stem cells (hPSCs) are vital in this research due to their ability to differentiate into any cell type, thereby mimicking early human developmental stages. Traditional in vitro culture systems often fail to replicate the complexity of the in vivo environment, compromising the accuracy of these models.

hPSCs are highly sensitive to chemical and physical cues in their surroundings. Conventional in vitro systems, typically using plastic substrates, do not replicate the nuanced microenvironment that influences hPSC behavior in vivo. This limitation poses significant challenges in accurately studying cell differentiation and tissue formation. Emerging evidence highlights the crucial role of mechanical and topological factors in hPSC differentiation and maintenance. Therefore, developing culture systems that better mimic the in vivo environment is essential to enhance the relevance and applicability of in vitro research.

Recent advancements have focused on creating sophisticated substrates to support hPSC culture. hPSCs are typically cultured under either 2D or 3D conditions. In 2D cultures, cells grow on rigid surfaces, limiting tissue deformation and constraining differentiation. In contrast, 3D cultures are more flexible for tissue formation but lack stability and reproducibility. Tissues develop under gentle mechanical constraints influenced by chemical and mechanical signals. It's important to mimic these conditions in the lab to induce proper cell fates and create complex tissue structures. Recent research emphasizes the significance of mechanical cues like substrate stiffness and geometry in affecting cell behavior and fate. This study aimed to develop a hydrogel substrate with adjustable stiffness to replicate the natural developmental environment of stem cells.

In Chapter 1, we introduced a photocurable polyethylene glycol–polyvinyl alcohol (PVA-PEG) hydrogel, allowing precise spatial control over surface stiffness and structure at a micrometer scale. This hydrogel can be functionalized with extracellular matrix (ECM) proteins, such as Laminin 511, to promote hPSC growth and differentiation. Cells cultured on these hydrogels showed similar proliferation rates to those cultured on conventional glass-bottom dishes. Immunofluorescence analysis confirmed that the hPSCs maintained their pluripotency, as indicated by the expression of pluripotency markers SOX2, OCT4,

and NANOG, for at least six days. The differentiation potential of hPSCs into the three germ layers—ectoderm, mesoderm, and endoderm—was also evaluated. Neural ectoderm differentiation was induced using a dual SMAD inhibition protocol, with cells on both hydrogels and glass-bottom dishes expressing neural ectoderm markers PAX6 and OTX2. qPCR analysis confirmed similar differentiation efficiency across all substrates, and mesoderm and endoderm differentiation were also achieved. By spatially controlling the stiffness of the patterned gel, we can guide the differentiation of hPSCs into complex, patterned structures, better mimicking the in vivo environment.

Building on the insights from Chapter 1, which emphasizes the importance of the mechanical microenvironment, we focus on enhancing spatial and temporal cues within culture systems to generate 3D structures in Chapter 2. Organoids have emerged as powerful tools for modeling tissue development and diseases, but their application is limited by the inability to replicate the full complexity of developmental processes. This limitation is primarily due to the lack of spatial and temporal cues in traditional culture systems. Chapter 2 addresses this challenge by introducing a photocurable hydrogel with enhanced adhesivity for human embryonic stem cells. This hydrogel allows flexible adjustments in geometry, mechanical properties, and degradability. Using this platform, we have successfully generated notochord and neural tube organoids, enhanced the fidelity of in vitro organoid models, and bridged the gap between simplified culture conditions and the intricate dynamics of in vivo development.

In Chapter 3, we delve into replicating one of the most fundamental processes in early development: gastrulation. Gastrulation is a critical phase in early development, where a single-layered epiblast transforms into a multi-layered structure, forming the basis for all body tissues. Our understanding of this process remains incomplete despite advances in genetic and cellular studies. Utilizing the methodologies from Chapters 1 and 2, we aimed to replicate gastrulation and its morphogenesis in vitro. Using the hydrogel, I generated hPSC epithelia like the epiblast, and upon engineered spatial-temporal stimulation, a simple homogeneous hPSC sheet spontaneously breaks symmetry, generating germ layers and gastrulation-like morphogenesis.

This study aims to propel the fields of developmental biology and regenerative medicine forward by enhancing the precision of in vitro models. By creating advanced hydrogel substrates and implementing precise spatiotemporal controls, we can more accurately replicate the in vivo microenvironment and developmental processes. These innovations offer deeper insights into human development and pave the way for more accurate tissue engineering and medical applications.