様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Development of fast-forming 3D cultures of human neurons for modeling Alzheimers disease amyloid and Tau pathology

研究課題名（英文）Development of fast-forming 3D cultures of human neurons for modeling Alzheimers disease amyloid and Tau pathology

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研究成果の概要（和文）：アルツハイマー病(AD)はベータアミロイド(Aβ)ペプチド(Aβプラーク)の細胞外蓄積、細胞内高リン酸化タウ凝集体によるシナプス喪失が原因の神経変性疾患で、認知機能低下と神経細胞死につながります。世界中でテストされた多くの薬にもかかわらず、病気の病理進行を止める薬はありません。その理由の1つは、病態進行を再現できるin vitroモデルがないです。したがって、成功した治療戦略を開発するためには、ADの良いin vitroモデルを開発する必要があります。本研究では、細胞内高リン酸化タウタウとプラークなどの病理が再現できる「ニューロドーム」培養と呼ばれる半3D神経培養について説明しています。

研究成果の学術的意義や社会的意義
The interest for developing adequate in vitro models is huge. The neurodome is easy and fast to culture, and mimics well the main hallmarks of AD. This help with advances in disease understanding, better evaluation of therapeutic interventions, reduced use of animal models, personalized approaches.

研究成果の概要（英文）：Alzheimer’s disease (AD) pathology presents as extracellular accumulation of beta-amyloid (Aβ) peptides (Aβ plaques), intracellular hyperphosphorylated tau aggregates (neurofibrillary tangles), and subsequently loss of synapses leading to progressive cognitive decline and neuronal cell death. Despite extensive effort, and many drugs tested worldwide, there is not successful drug that can stop the progression of the disease. One reason for that is that there are no good disease in vitro or animal models that can reproduce the disease pathology and progression. Thus it is necessary to develop better in vitro models for AD, in order to develop successful treatment strategies.
This research describes a semi-3D neuronal culture named "neurodome" culture, that shows better pathology of AD such as plaques accompanied by tau tangles.

研究分野：Neurobiology

キーワード：Alzheimer's disease Neurodome 3D culture amyloind plaques neurofibrialy tangles tau aggregates tau phosphorylation amyloid beta

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by progressive cognitive decline, memory loss, and impaired daily functioning. It is the most common form of dementia and poses a significant global health challenge. Despite extensive research efforts, the precise mechanisms underlying AD pathogenesis remain elusive, hindering the development of effective treatments. In vitro culture models, which mimic the key aspects of AD pathology, are indispensable tools for investigating disease mechanisms, identifying potential therapeutic targets, and evaluating drug candidates.

Existing in vitro models for AD have contributed significantly to our understanding of the disease. However, they often fail to recapitulate the complexity and dynamic nature of AD pathogenesis, limiting their translational relevance. Traditional models, such as cell lines and primary neuronal cultures, lack the intricate cellular interactions and three-dimensional (3D) architecture found in the brain.

２．研究の目的

The primary objective of this research is to develop an advanced in vitro culture model that more accurately represents the pathological features of Alzheimer's disease. In particularly, the 3D architecture of the model system is critical for adequately mimicking the disease pathology. That is because one of the major hallmarks of AD is extracellular accumulation of amyloid beta plaques. Current 3D culture models have shown that the 3D culture approach has great potential for modelling AD. However culture of 3D models is labourious and takes a long time. Thus the research objective of this project is to develop a semi-3D culture system, which is relatively easy and fast to prepare.

３．研究の方法

The research will involve several key steps in developing the advanced in vitro neurodome culture model for AD:

a. Cell culture and differentiation: Culturing and differentiating human induced pluripotent stem cells (hiPSCs) into neurons.

b. Semi-3D culture system: Testing of several approaches to induce cell crowding and growth into neurodomes.

c. AD pathology induction: I will use application of ApoE4 or Tau pre-formed fibrils into the cultures to induce pathology. Both have been shown before to stimulate the secretion of amyloid-beta (ApoE4), or to cause aggregation of Tau (Tau pre-formed fibrils)

４．研究成果

Using crowding agents in culture medium to create neuronal aggregates resembling neurodomes. Currently, one publication describing synaptic disfunctions caused by pre-formed fibrils is in the final stages of development. Further, I expect to prepare a methods paper describing the neurodome culture.

1. Formation of Neuronal Aggregates:
The addition of crowding agents, such as high molecular weight polyethylene glycol (PEG), to the culture medium successfully induced the formation of neuronal aggregates, called “neurodomes”. These aggregates exhibited a distinctive morphology reminiscent of neurodomes, characterized by a spherical shape with a central core of densely packed neurons surrounded by a more loosely arranged outer layer. These neruodomes formed fast and within 2 weeks.

2. Cellular Organization:
Immunofluorescence staining revealed that the neuronal aggregates formed within the neurodomes exhibited a high degree of cellular organization. The densely packed core consisted predominantly of mature neurons expressing neuronal markers such as NeuN and MAP2 and Tau. These neurons displayed elaborate neuritic processes, forming interconnected networks reminiscent of neural circuitry observed in vivo. The outer layer consisted of a mix of neurons and glial cells, creating a more heterogeneous cellular composition.

3. Disease Modeling:
The neurodomes generated from patient-derived induced pluripotent stem cells (iPSCs) allowed for the establishment of disease models to study neurodegenerative disorders. By differentiating iPSCs from patients with specific neurodegenerative conditions, such as Alzheimer's disease, it was possible to recreate pathological hallmarks within the neuronal aggregates. For instance, the introduction of ApoE4 in Alzheimer's disease models led to the formation of Aβ plaques within the neurodomes, closely resembling the in vivo pathology! Addition of Tau pre-formed fibrils, caused the formation of neurofibrilary tangel of phosphorylated Tau, which were more pronounced when the amyloid-beta plaques were present.

4. Comparative Analysis:
Comparisons were made between the neurodomes generated using crowding agents and other existing in vitro models. The neurodomes exhibited distinct advantages over traditional monolayer cultures and other 3D culture systems. The inclusion of crowding agents provided a more physiologically relevant environment, promoting cell-cell interactions, cellular organization, and the formation of complex neuronal networks. The neurodomes also displayed much easier, cheaper to culture and form much faster compared to existing 3D cultures.

5. Future Directions:
The successful development of neurodomes through the use of crowding agents opens up several avenues for further research. Future studies may focus on refining the culture conditions to enhance the maturity and functionality of the neuronal aggregates. The incorporation of additional cell types, such as microglia or blood-brain barrier components, could further enhance the neurodomes' physiological relevance. Moreover, the neurodomes' potential for high-throughput screening of drug libraries and personalized medicine approaches should be explored.

In conclusion, the utilization of crowding agents in the culture medium resulted in the formation of neuronal aggregates resembling neurodomes, which I newly named “neurodome” cultures. These neurodomes displayed complex cellular organization, fast spontaneous formation, functional neuronal activity, and the ability to model neurodegenerative diseases. The neurodomes hold great promise as an advanced in vitro model for studying neural diseases.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔出願〕　計0件

〔取得〕　計2件

産業財産権の名称 Proteimics-based receptor-ligand matching for optimizing human iPS cells reprogramming	発明者 DD, ZT, MK, TT	権利者 同左
産業財産権の種類、番号 特許、2021-194719	取得年 2023年	国内・外国の別 国内

産業財産権の名称 Proteimics-based receptor-ligand matching for optimizing human iPS cells reprogramming	発明者 DD, ZT, MK, TT	権利者 同左
産業財産権の種類、番号 特許、WO/2023/100949	取得年 2023年	国内・外国の別 外国

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関