水溶性二相系を用いた細胞組織生成装置

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本技術の概要

一言で申しますと・・・

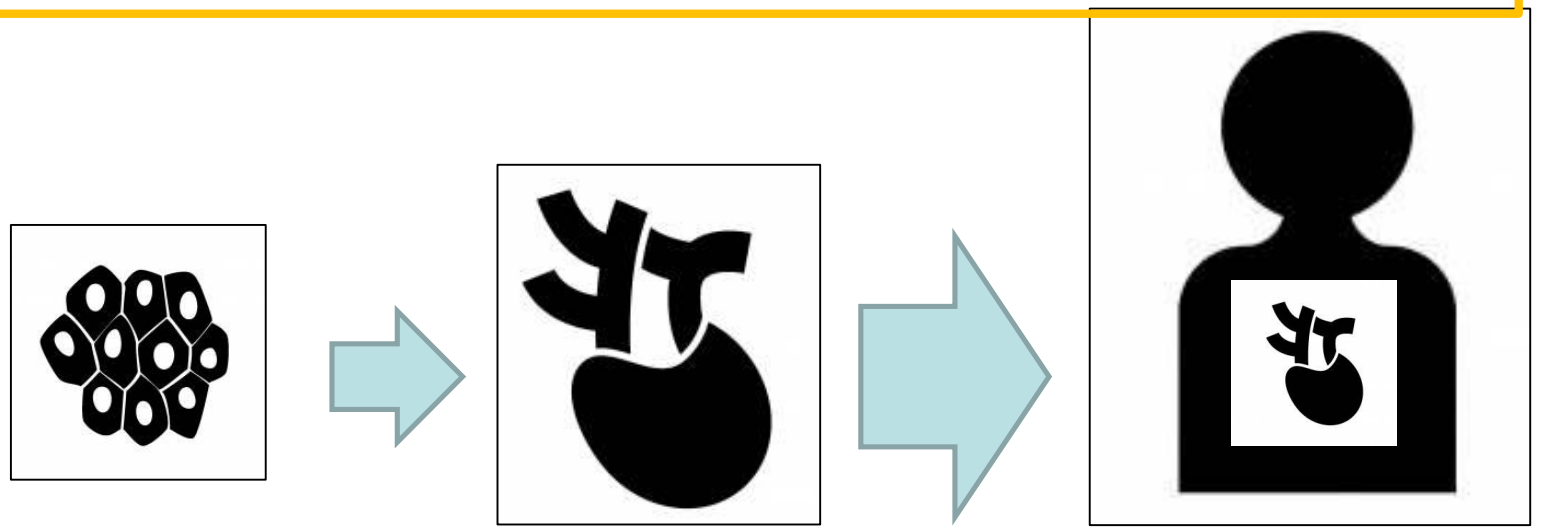
細胞や細菌で
簡単に
お団子（組織？）を作る

技術です.

背景

再生医療：Regenerative Medicine
再生医工学＝組織工学：Tissue Engineering

- 失われた生物学的な機能を修復，置換，増強
- 機能的な**細胞組織**，臓器の代替物を開発
- 多分野融合の領域（理工学，医学，薬学）



細胞組織　**培養臓器**　**再生臓器**

背景

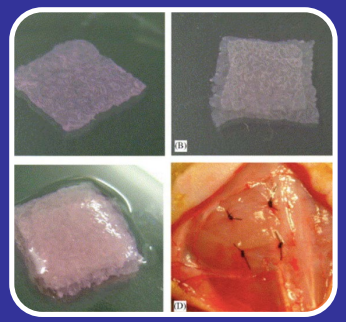
細胞から組織や臓器を作るには？

臓器の特徴を再現する

臓器の組織の特徴

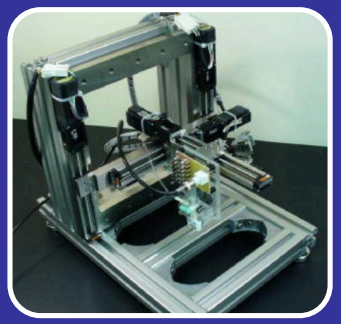
1）**三次元構造**：*大きいサイズ*，*ぶ厚い組織*，*ミクロ構造*
2）毛細血管網が豊富
3）多種細胞で構成

既存の細胞組織構築法の一例と問題点



細胞シート積層技術(1)

- 細胞をシート状に加工し積層
- メリット：厚みのある組織の構築が可能
- デメリット：積層数の限界，内部の栄養，酸素の不足

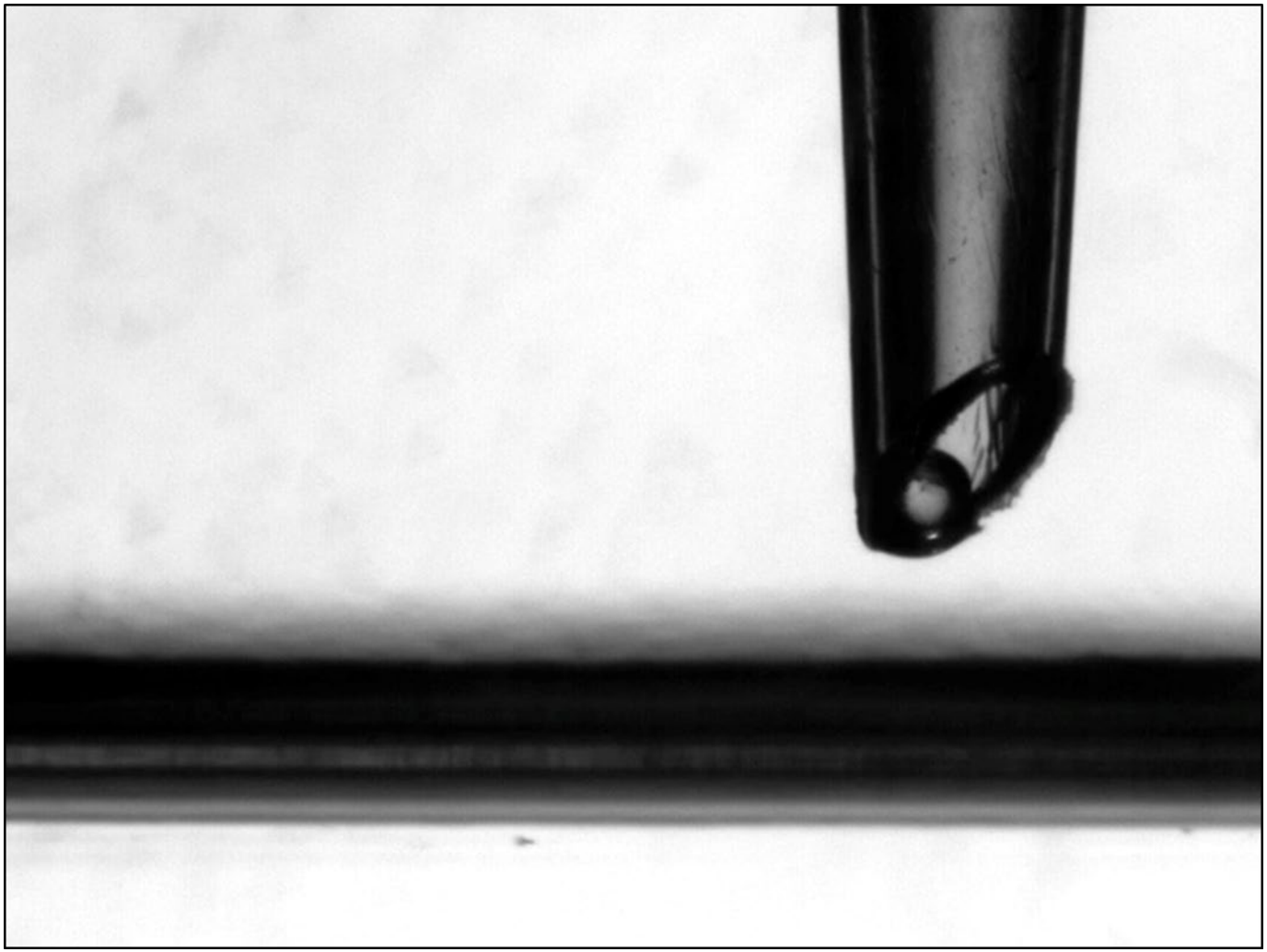


3Dプリント技術(2)

- インクジェット技術を用いて細胞組織を3D構造に作製
- メリット：機械による正確な作業
- デメリット：材料の生体適合性

(1)Joseph Yanga et al. Cell sheet engineering: Recreating tissues without biodegradable scaffolds. Biomaterials 26 (2005) 6415–6422
(2) Yuichi Nishiyama et al. Development of a Three-Dimensional Bioprinter: Construction of Cell Supporting Structures Using Hydrogel and State-Of-The-Art Inkjet Technology. Journal of Biomechanical Engineering MARCH 2009, Vol. 131

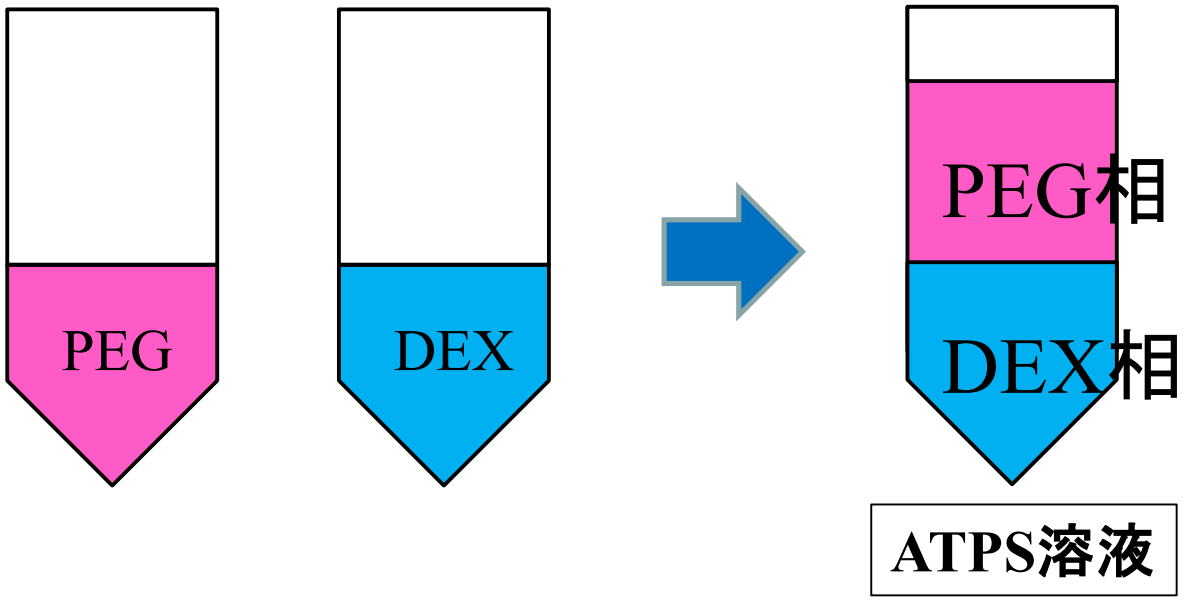
水溶性二相系
Aqueous Two Phase System（ATPS）



水溶性二相系
Aqueous Two Phase System（ATPS）

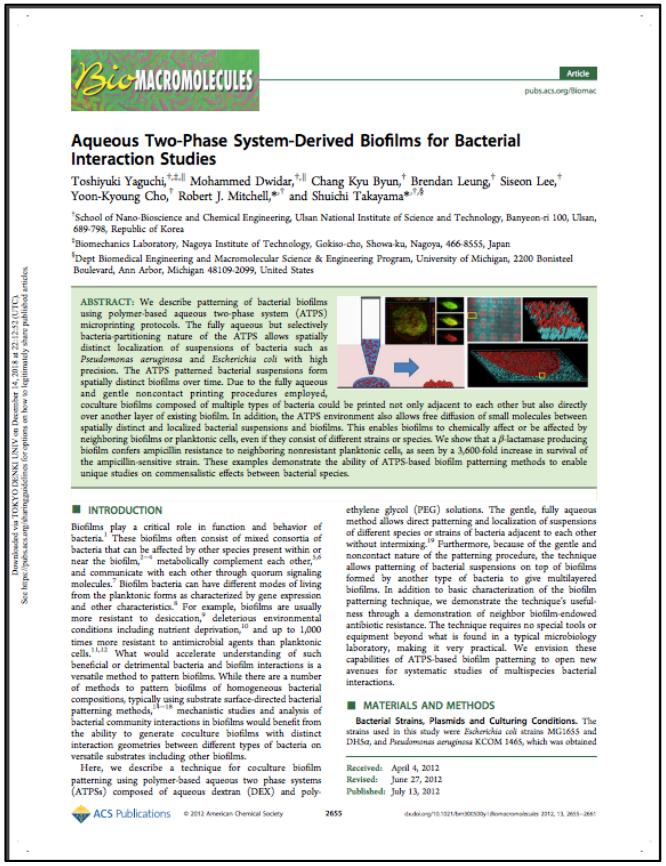
二つの化学的に異なる水溶性ポリマーを組み合わせることよって生成される**二相に分離した溶液**.

例：ポリエチレングリコール（Polyethylene glycol，PEG）
　　デキストラン（Dextran，DEX）



ATPS 法を用いている論文

(1)



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Aqueous Two-Phase System-Derived Biofilms for Bacterial Interaction Studies

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ABSTRACT: We describe patterning of bacterial biofilms using polymer-based aqueous two-phase system (ATPS) microprinting protocols. The fully aqueous but selectively bacteria-partitioning nature of the ATPS allows spatially distinct localization of suspensions of bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* with high precision. The ATPS patterned bacterial suspensions form spatially distinct biofilms over time. Due to the fully aqueous and gentle noncontact printing procedures employed, coculture biofilms composed of multiple types of bacteria could be printed not only adjacent to each other but also directly over another layer of existing biofilm. In addition, the ATPS environment also allows free diffusion of small molecules between spatially distinct and localized bacterial suspensions and biofilms. This enables biofilms to chemically affect or be affected by neighboring biofilms or planktonic cells, even if they consist of different strains or species. We show that a β-lactamase producing biofilm confers ampicillin resistance to neighboring nonresistant planktonic cells, as seen by a 3,600-fold increase in survival of the ampicillin-sensitive strain. These examples demonstrate the ability of ATPS-based biofilm patterning methods to enable unique studies on commensalistic effects between bacterial species.

INTRODUCTION

Biofilms play a critical role in function and behavior of bacteria.[1] These biofilms often consist of mixed consortia of bacteria that can be affected by other species present within or near the biofilm,[2−4] metabolically complement each other,[5,6] and communicate with each other through quorum signaling molecules.[7] Biofilm bacteria can have different modes of living from the planktonic forms as characterized by gene expression and other characteristics.[8] For example, biofilms are usually more resistant to desiccation,[9] deleterious environmental conditions including nutrient deprivation,[10] and up to 1,000 times more resistant to antimicrobial agents than planktonic cells.[11,12] What would accelerate understanding of such beneficial or detrimental bacteria and biofilm interactions is a versatile method to pattern biofilms. While there are a number of methods to pattern biofilms of homogeneous bacterial compositions, typically using substrate surface-directed bacterial patterning methods,[13−18] mechanistic studies and analysis of bacterial community interactions in biofilms would benefit from the ability to generate coculture biofilms with distinct interaction geometries between different types of bacteria on versatile substrates including other biofilms.

Here, we describe a technique for coculture biofilm patterning using polymer-based aqueous two phase systems (ATPSs) composed of aqueous dextran (DEX) and polyethylene glycol (PEG) solutions. The gentle, fully aqueous method allows direct patterning and localization of suspensions of different species or strains of bacteria adjacent to each other without intermixing.[19] Furthermore, because of the gentle and noncontact nature of the patterning procedure, the technique allows patterning of bacterial suspensions on top of biofilms formed by another type of bacteria to give multilayered biofilms. In addition to basic characterization of the biofilm patterning technique, we demonstrate the technique's usefulness through a demonstration of neighbor biofilm-endowed antibiotic resistance. The technique requires no special tools or equipment beyond what is found in a typical microbiology laboratory, making it very practical. We envision these capabilities of ATPS-based biofilm patterning to open new avenues for systematic studies of multispecies bacterial interactions.

MATERIALS AND METHODS

Bacterial Strains, Plasmids and Culturing Conditions. The strains used in this study were *Escherichia coli* strains MG1655 and DH5α, and *Pseudomonas aeruginosa* KCOM 1465, which was obtained

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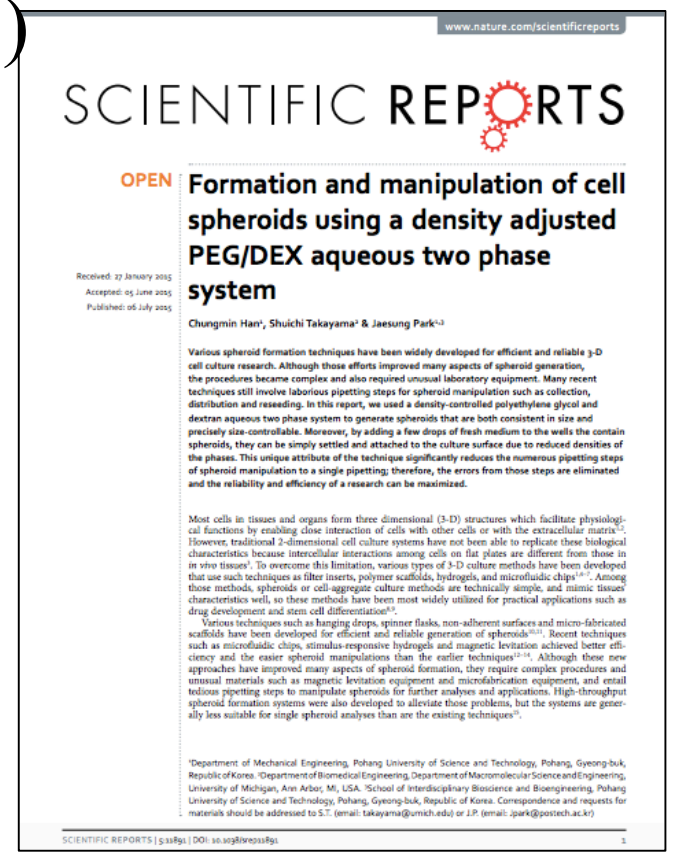

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Formation and manipulation of cell spheroids using a density adjusted PEG/DEX aqueous two phase system

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Various spheroid formation techniques have been widely developed for efficient and reliable 3-D cell culture research. Although those efforts improved many aspects of spheroid generation, the procedures became complex and also required unusual laboratory equipment. Many recent techniques still involve laborious pipetting steps for spheroid manipulation such as collection, distribution and reseeding. In this report, we used a density-controlled polyethylene glycol and dextran aqueous two phase system to generate spheroids that are both consistent in size and precisely size-controllable. Moreover, by adding a few drops of fresh medium to the wells the contain spheroids, they can be simply settled and attached to the culture surface due to reduced densities of the phases. This unique attribute of the technique significantly reduces the numerous pipetting steps of spheroid manipulation to a single pipetting; therefore, the errors from those steps are eliminated and the reliability and efficiency of a research can be maximized.

Most cells in tissues and organs form three dimensional (3-D) structures which facilitate physiological functions by enabling close interaction of cells with other cells or with the extracellular matrix[1,2]. However, traditional 2-dimensional cell culture systems have not been able to replicate these biological characteristics because intercellular interactions among cells on flat plates are different from those in in vivo tissues[3]. To overcome this limitation, various types of 3-D culture methods have been developed that use such techniques as filter inserts, polymer scaffolds, hydrogels, and microfluidic chips[4–7]. Among those methods, spheroids or cell-aggregate culture methods are technically simple, and mimic tissues' characteristics well, so these methods have been most widely utilized for practical applications such as drug development and stem cell differentiation[8,9].

Various techniques such as hanging drops, spinner flasks, non-adherent surfaces and micro-fabricated scaffolds have been developed for efficient and reliable generation of spheroids[10,11]. Recent techniques such as microfluidic chips, stimulus-responsive hydrogels and magnetic levitation achieved better efficiency and the easier spheroid manipulations than the earlier techniques[12–14]. Although these new approaches have improved many aspects of spheroid formation, they require complex procedures and unusual materials such as magnetic levitation equipment and microfabrication equipment, and entail tedious pipetting steps to manipulate spheroids for further analyses and applications. High-throughput spheroid formation systems were also developed to alleviate those problems, but the systems are generally less suitable for single spheroid analyses than are the existing techniques[15].

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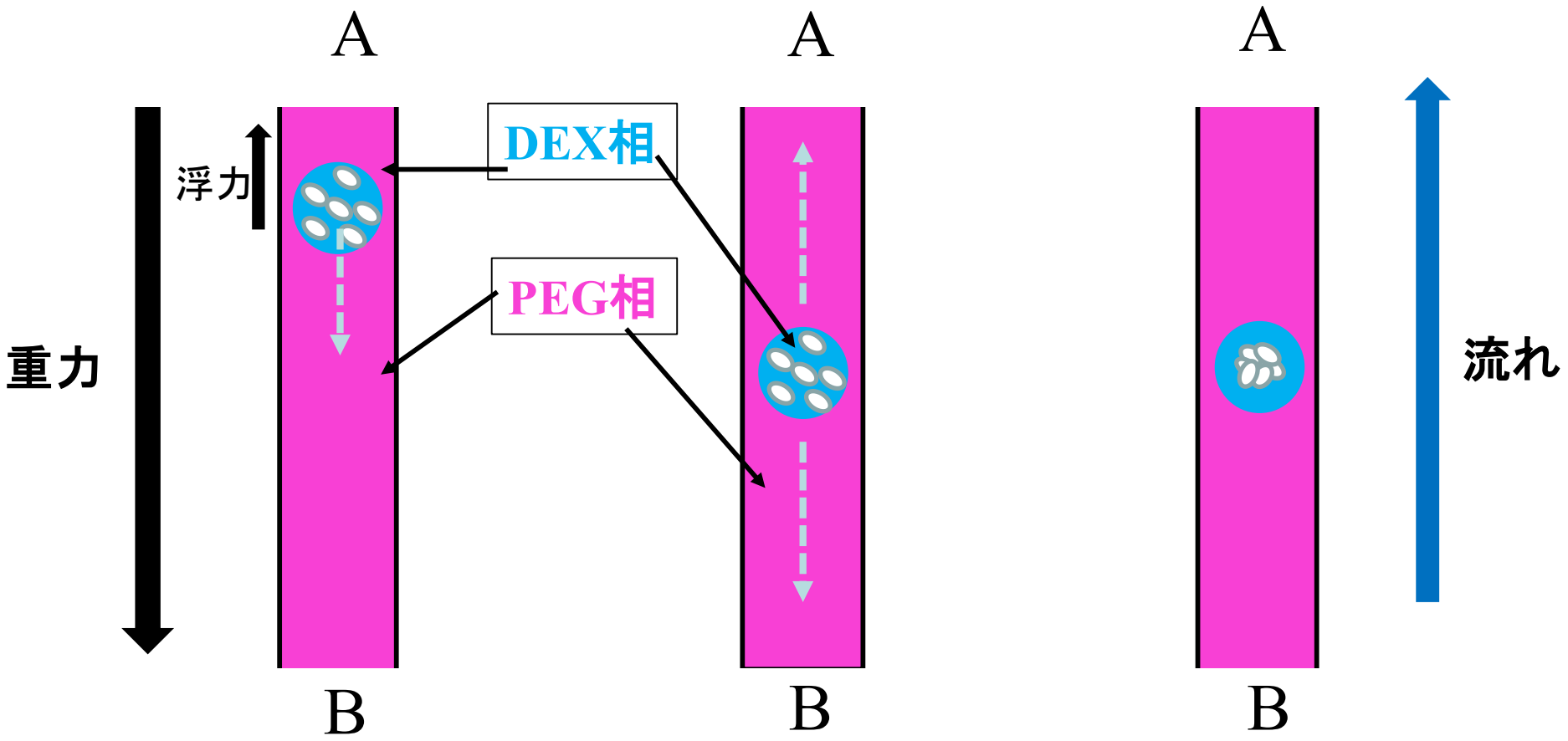
1)**Toshiyuki Yaguchi** et al. :Aqueous Two-Phase System-Derived Biofilms for Bacterial Interaction Studies

2)Chungmin Han et al. :Formation and manipulation of cell spheroids using a density adjusted PEG/DEX aqueous two phase system

ATPS浮遊培養原理

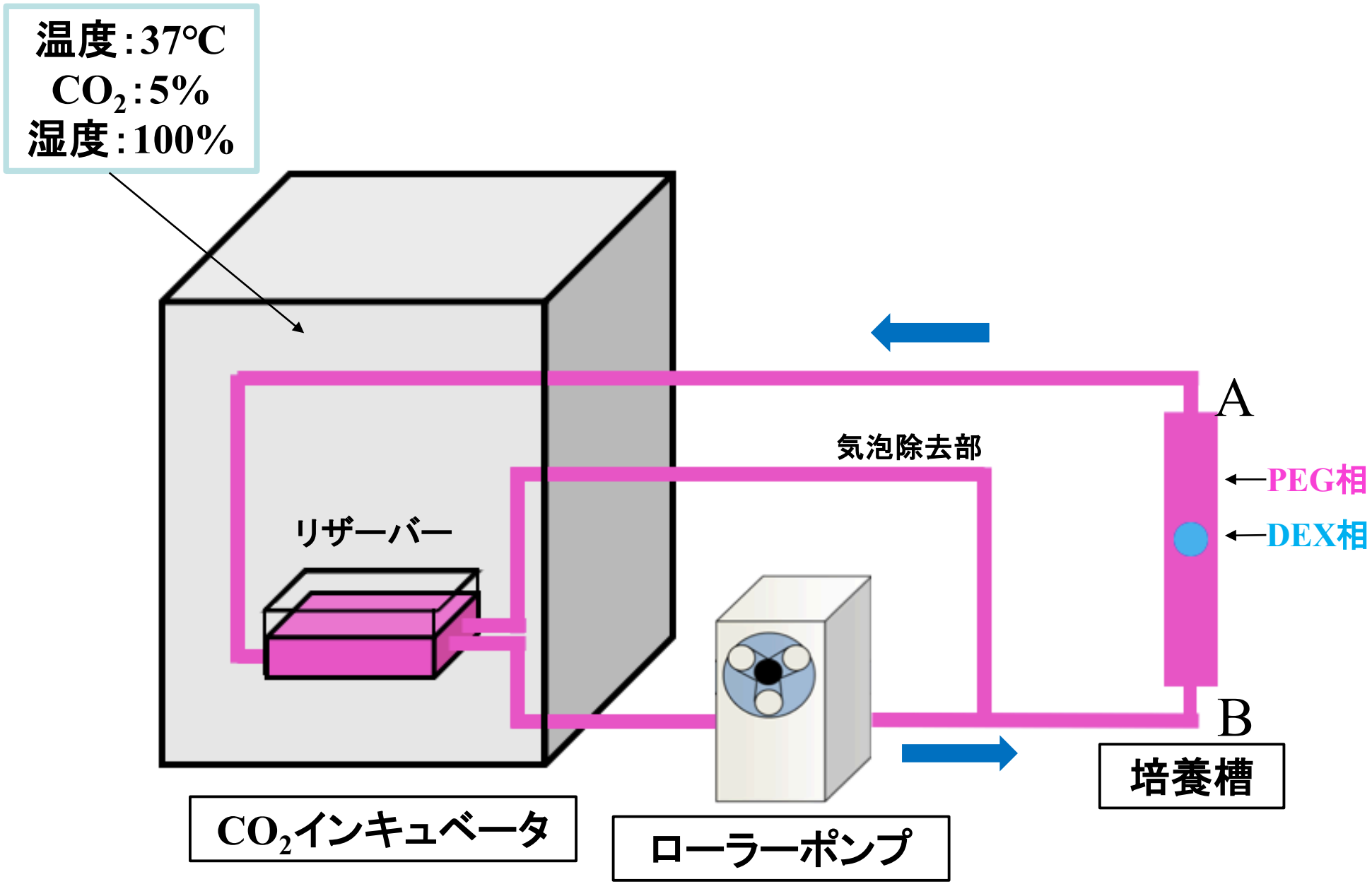


① A端に細胞懸濁DEX液滴を播種
→重力方向に沈降

② PEG相を循環
→AB間で常時浮遊状態を維持

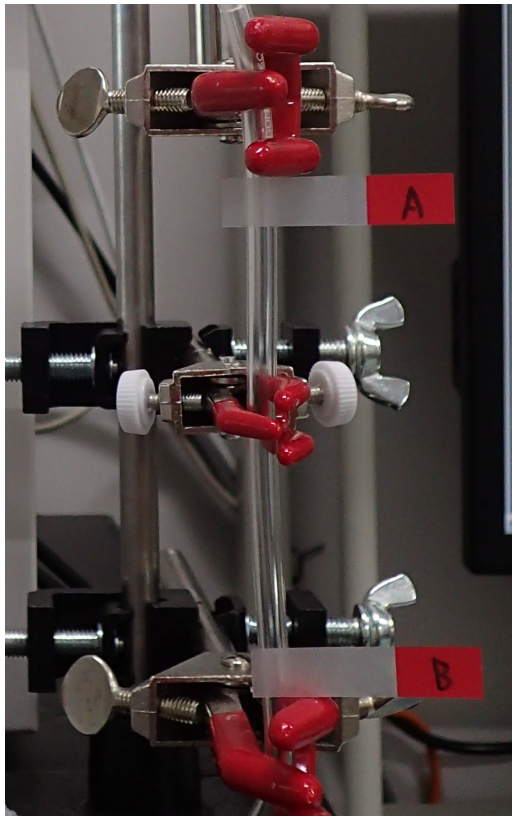
③細胞同士が相互に接着
→**細胞凝集塊**を形成

ATPS浮遊培養システム



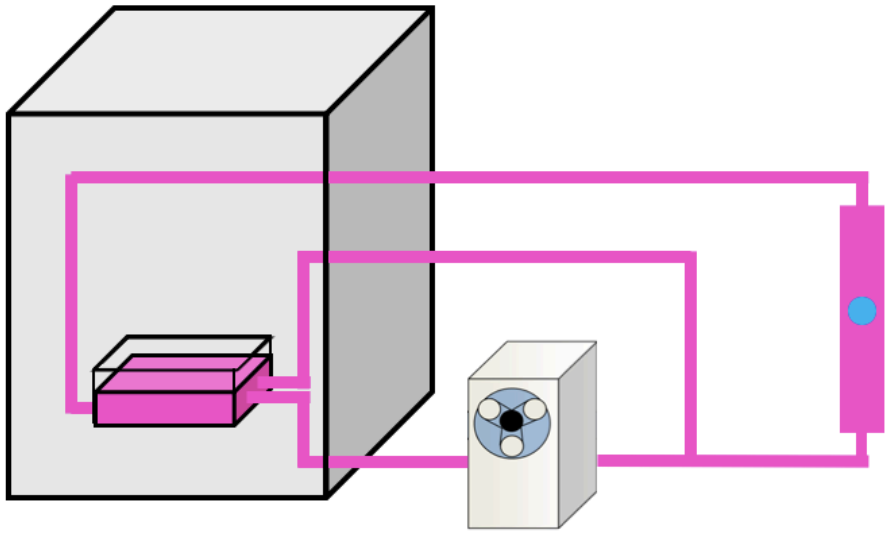
ATPS浮遊培養システム

全体図

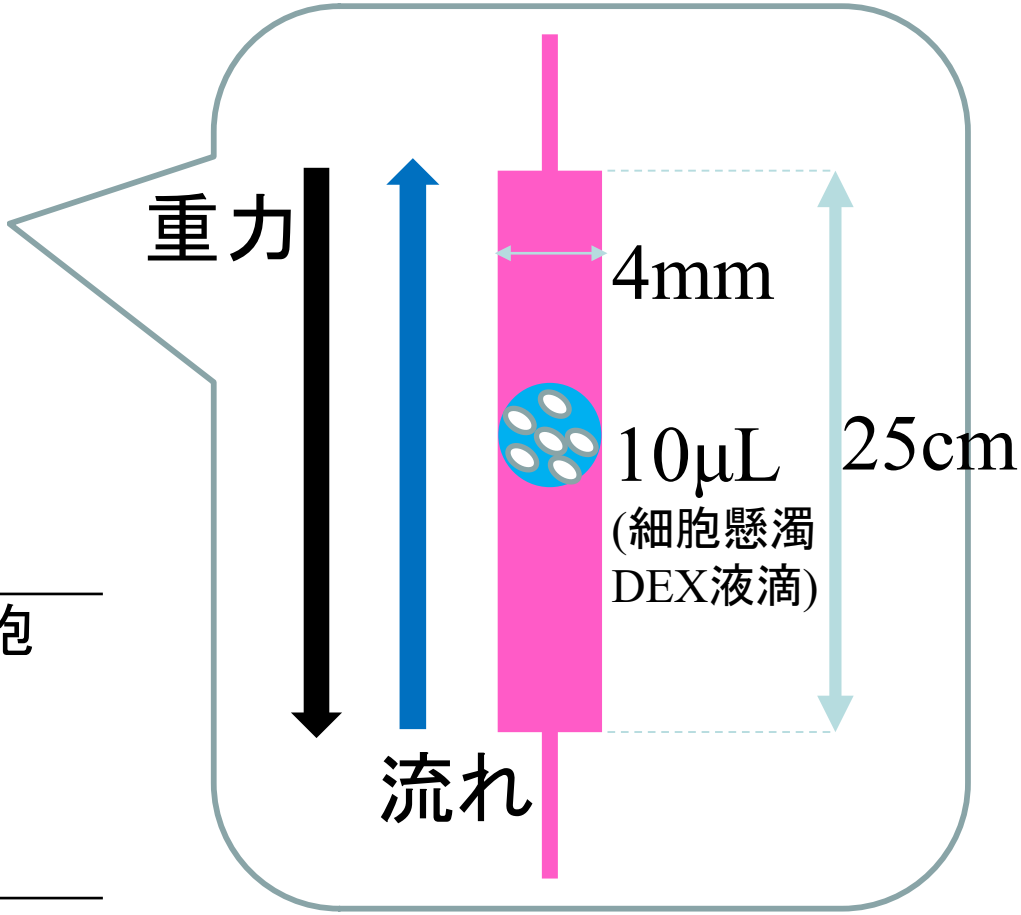


培養槽

ATPS浮遊培養実験



浮遊時間：4hour
流量：8～10[ml/min]



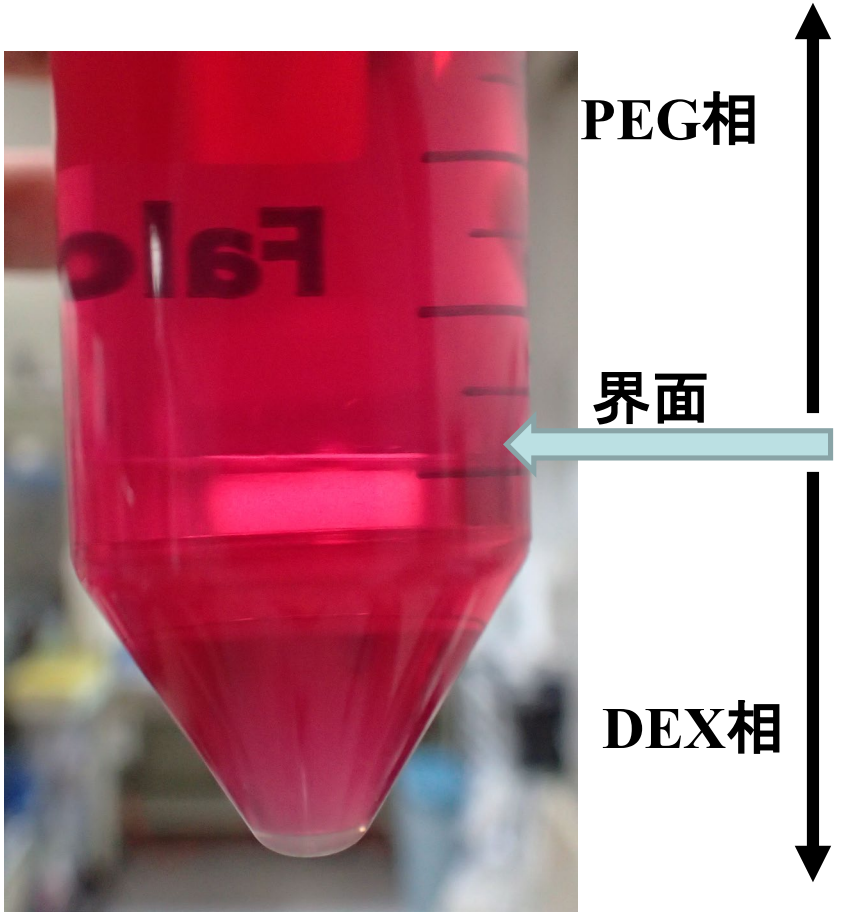
条件

細胞種	マウス線維芽細胞
DEX液滴体積[μl]	10
細胞数[cells]	1.4×10^4

ATPSの調整

ATPSの調整条件

構成	分子量（Mw）	濃度 %(w/w)
PEG	35K	5
DEX	250K	10

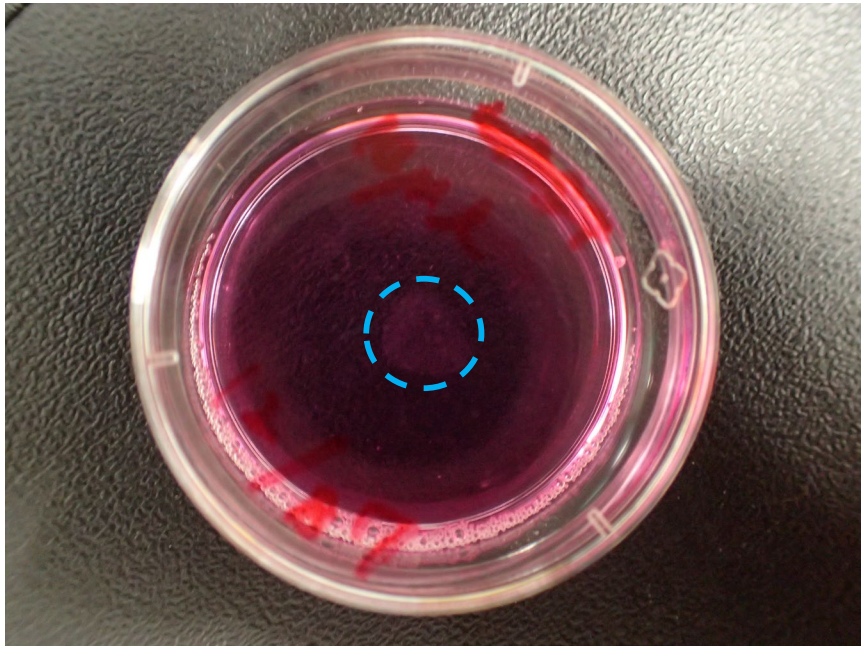


作製したATPS溶液

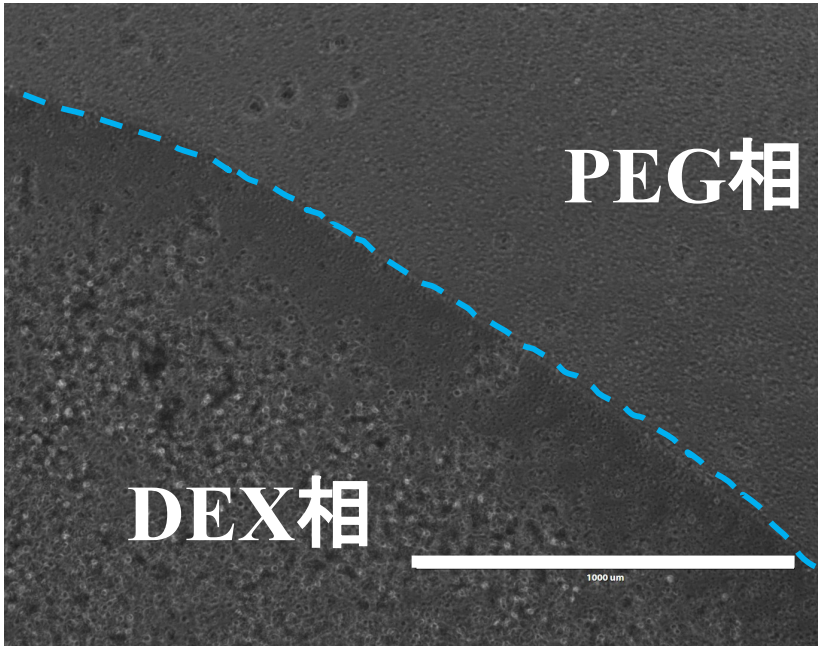
細胞懸濁DEX液滴の調整

条件

細胞種	マウス線維芽細胞
DEX液滴体積 [μl]	10
細胞数[cells]	1.4×10^4

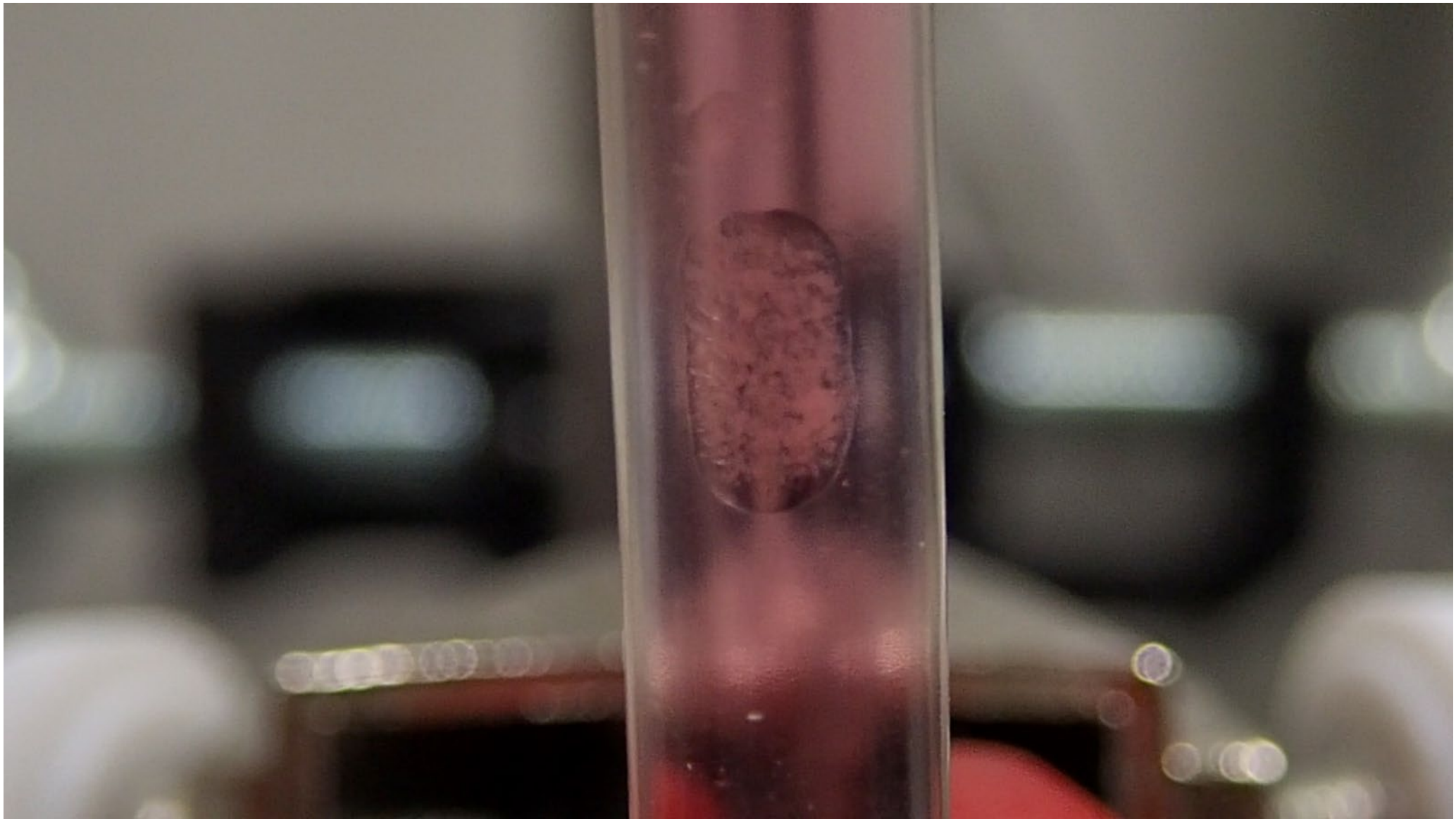


ディッシュに滴下した様子
（液滴：10μL）

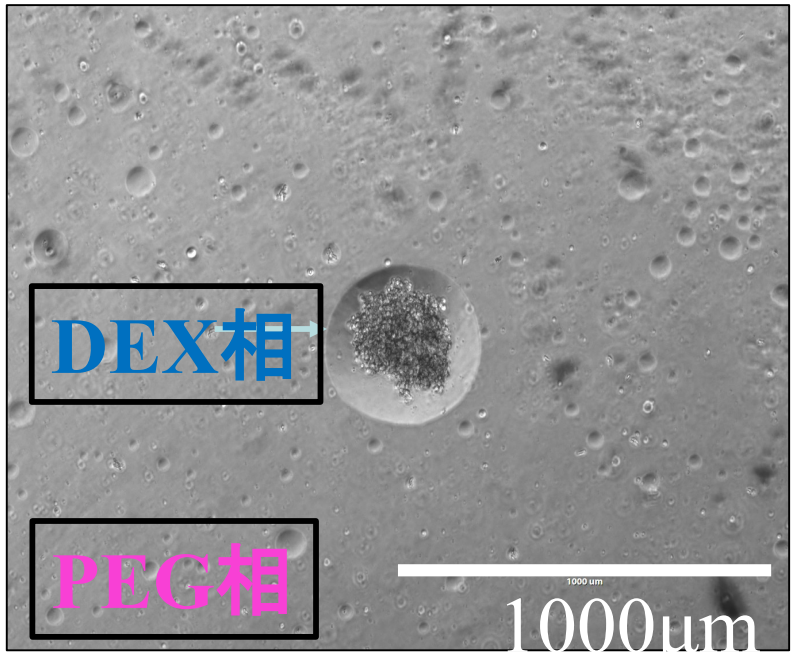


拡大図（スケールバー：1mm）

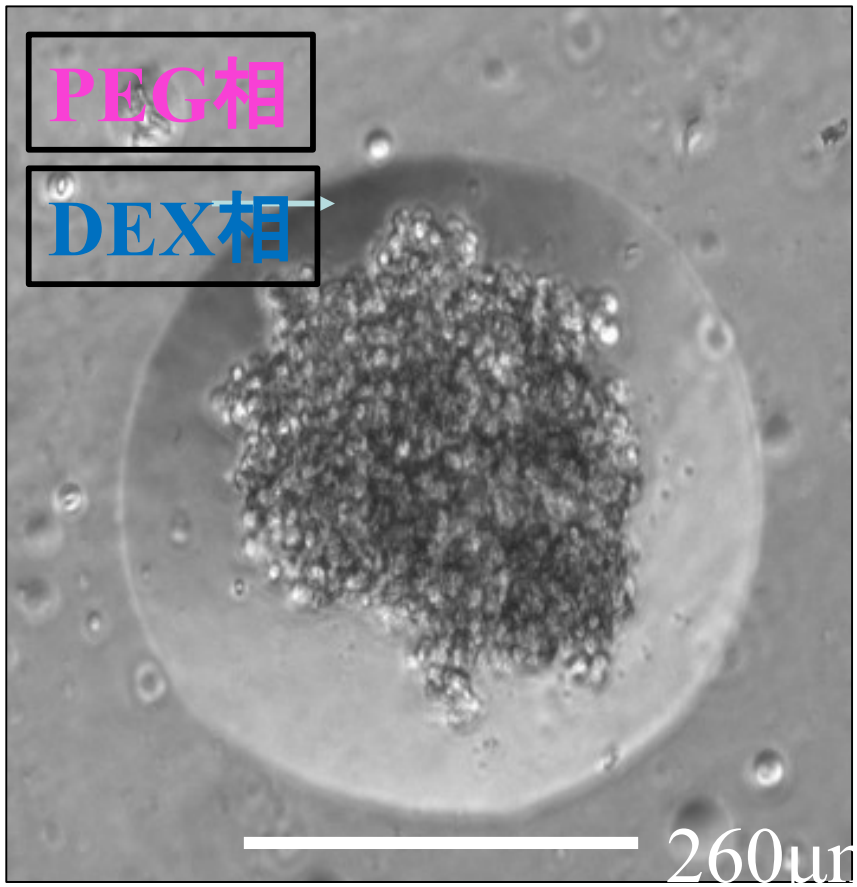
チューブ内で液滴が浮遊している様子



浮遊開始から4時間後の液滴の一部



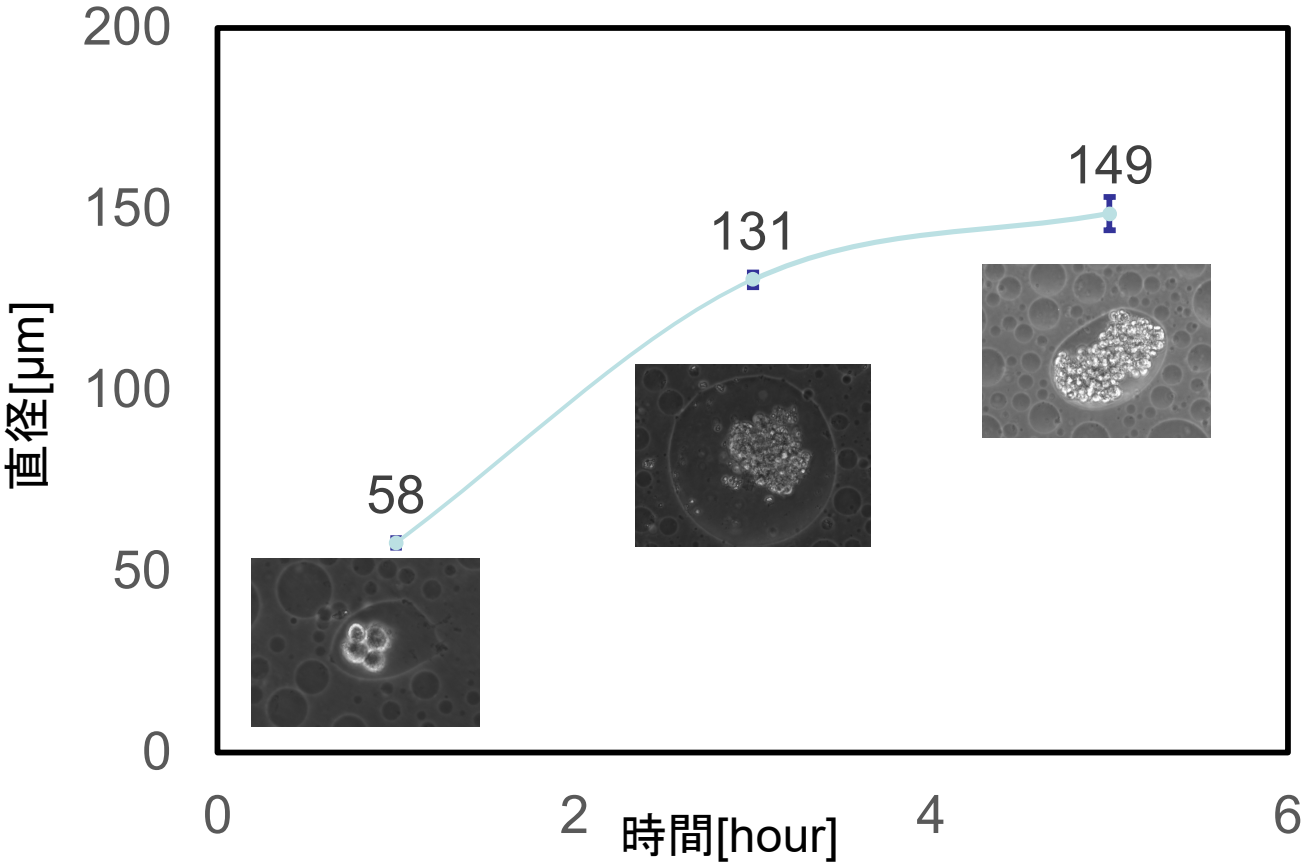
(a)倍率4倍



(b)拡大図

約260μmの細胞凝集塊を回収

培養時間に対する細胞凝集塊サイズ



凝集塊のサイズ変化

浮遊時間 [h]	平均直径値 [μm]
1	58±1.34
3	131±2.02
5	149±4.57

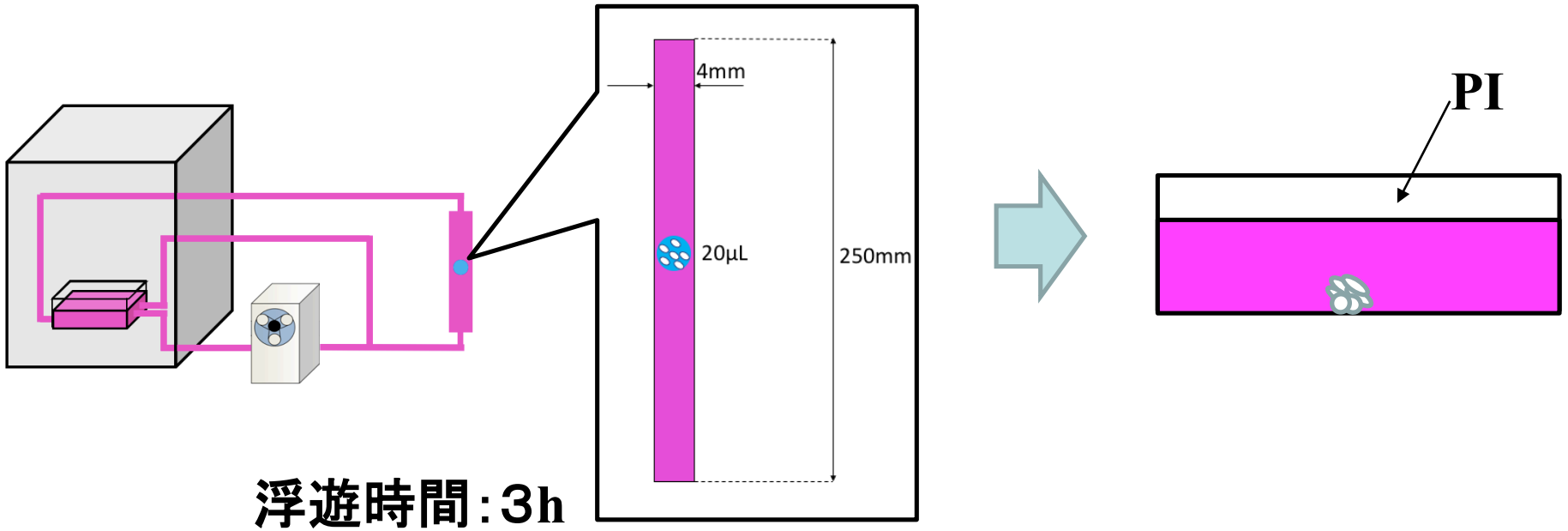
凝集塊サイズの大型化の条件を追求してゆく

新技術の特徴･従来技術との比較

- 従来技術より簡便な装置と手技で細胞凝集塊（スフェロイド様形態）を作成可能
- 一般的にはスフェロイドを自発的に形成する細胞種は限られているが，本法では広範な種類の接着性細胞で凝集塊を作成可能
- 細胞だけでなく細菌のバイオフィルム形成も可能と思われる

細胞の生死判別： PIによる死細胞の染色

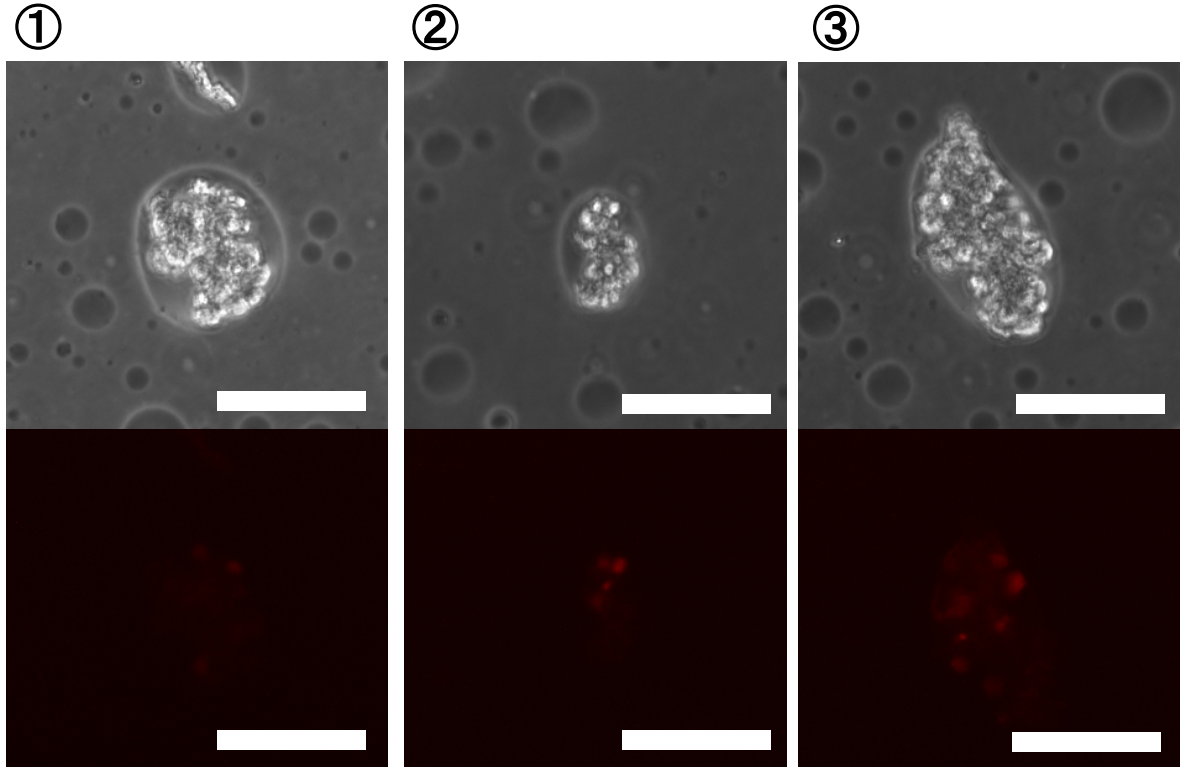
- 浮遊培養を3時間行い凝集塊を形成.
- 凝集塊にPI (1000倍希釈) を滴下.
- 蛍光顕微鏡を用いて観察.



結果：PIによる死細胞の染色

NIH-3T3 凝集塊
明視野像

PIを用いて死細胞
核染色をした蛍光像



スケール：100μm

生細胞で形成されていることを確認

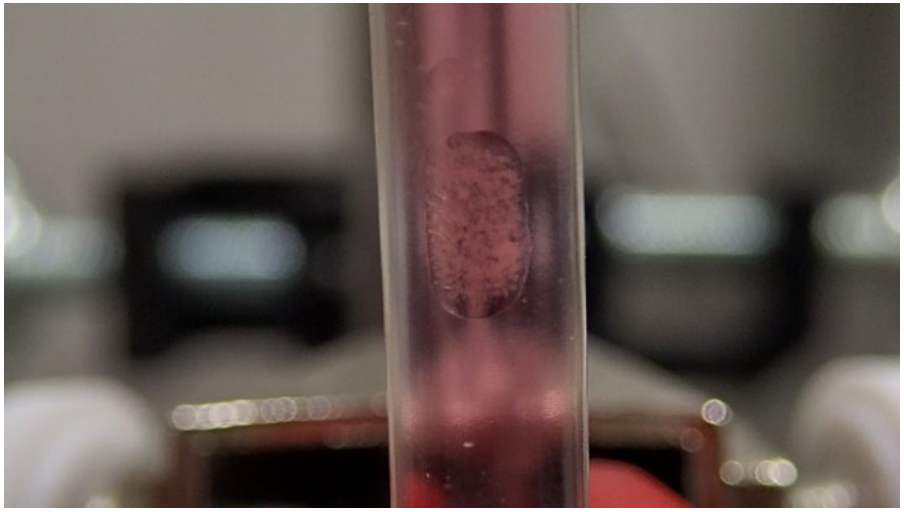
想定される用途

- 本技術は，様々な細胞製品の製造に適用することが可能.
- 一度に多くの細胞凝集塊を作成可能.
- 作成した異なる細胞種の凝集塊を組み合わせて生理的機能を持った「ミニ臓器」作製を目指す.
- 再生医療分野，創薬分野での活用に期待.

実用化に向けた課題

- さらに大きな細胞凝集塊を形成するにはシステムの大型化，もしくは培養時間の延長が必要　→　システムの自動制御

- DEX液滴内の循環により細胞凝集が低下？
  →　流体力学的シミュレーションで最適化



企業への期待

- 再生医療分野、創薬分野の企業と共同研究
  - 細胞凝集塊形成システムの研究開発
  - 臓器作製に向けた研究開発

本技術に関する知的財産権

- 発明の名称 : 浮遊培養装置及び浮遊培養方法
- 出願番号 : 特願2019-191368
- 出願人 : 東京電機大学
- 発明者 : 矢口俊之，井上　聡

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