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論文要旨

In the general background introduction of this thesis, a new angle and field of study evolved from conventional biological studies is defined as sub-single cellular study. Sub-single cellular studies are the studies that focus on the investigation of subcellular matters (such as proteins, organelles, nucleic acids, vesicles) in a single cell, evolving cell studies from the conventional population level or the single cell level to the sub-single-cell level or even to the single component/vesicle/molecule level. Sub-single cellular studies not only include the investigation of the amount or the chemical/biological characteristics of subcellular matters in a single cell, but also include the investigation of intracellular, intercellular, extracellular behaviors and phenomena regarding subcellular matters of a single cell. Such studies are expected to provide the opportunity to understand and elucidate the detail information of biology in terms of subcellular matters, rather than the whole cells previously. Moreover, sub-single cellular studies hold potential to evolve conventional cellular studies from analyses based on the concept of concentrations of molecules to analyses based on the concept of the actual numbers of molecules, opening a novel angle for the description of cellular studies. Even though, progress in this field is few, because conventional strategies, technologies, methods and tools are difficult to completely match the requirement of sub-single

cellular studies. Nanofluidics which holds the capability in handing nanoscale fluids and objects, is promising in advancing sub-single cellular studies, owing to the match of scales of targets. Nevertheless, despite the advantages and the potentials, the use of nanofluidic devices for sub-single cellular studies is only at a nascent period. Some critical issues towards sub-single cellular studies using nanofluidic devices are urgent to be overcome. Fundamental nanofluidic technologies which can endow or improve the functions of nanofluidic devices, and to promote the applicability and efficiency in use are significant in sub-single cellular studies using nanofluidics, but many of them are still under-developed, resulting in the lacking of functions or methods that are required in sub-single cellular studies using nanofluidics. Hence, the objective of this study in this thesis is to develop fundamental nanofluidic technologies for sub-single cellular studies. Some aspects of fundamental nanofluidic technologies were explored in this study, and the developed fundamental nanofluidic technologies were demonstrated to become methods that satisfied some requirements or conquered some difficulties of sub-single cellular studies, serving as the foundation for further development and application in biological or even other interdisciplinary fields. These contents introduced in this thesis from Chapter 2 to Chapter 6, are summarized as follows.

Chapter 2 introduces the fabrication of nanofluidic devices for sub-single cellular studies, after the general background introduction in Chapter 1. Two types of nanofluidic devices were designed and fabricated. One is a nanofluidic device with gold nanoarray for detecting proteins with single-molecule precision from a sample with a single cell volume. Another is a nanofluidic device with special nano-in-nano structures for single-particle-level analysis of exosomes, which are nanoscale vesicles released from cells. These two types of nanofluidic devices were used in the following explorations in this study.

Chapter 3 introduces a fundamental nanofluidic technology for molecule capture and arraying in nanochannels based on nano-in-nano structures and aptamer technologies. The use of nanochannels with gold nanoarrays enabled the formation of functional nanoarrays that hold capability to capture specific molecules in nanochannels. As a result, the formed aptamer-functionalized nanoarrays exhibited the capability in capturing target proteins in nanochannels. This technology holds the potential in handling subcellular matters from a single cell, which are not only diverse in type but also complicated in composition.

Chapter 4 introduces the development of an application based on aptamer-functionalized nanoarrays in nanochannels. This technology enables to capture single proteins, following a Poisson statistics. Such single molecule capture and detection are expected to enable and evolve single subcellular matter analysis.

Chapter 5 introduces a chemical regeneration technology for nanofluidic devices used in Chapter 3 and Chapter 4, aiming to remove molecule residuals such as biomolecules inside nanochannels and to increase the reusability of nanofluidic devices for sub-single cellular studies. Combining the plasma treatment and the piranha solution treatment in nanochannels, the chemical regeneration method could effectively regenerate nanofluidic devices and made them to be reused in high-sensitive detection. Such chemical regeneration technology is expected to increase the reusability of nanofluidic devices in sub-single cellular studies.

Chapter 6 introduces the exploration of fundamental nanofluidic technologies for exosome studies, aiming to elucidate exosome heterogeneity. Nanofluidic processes for investigating membrane proteins of single exosomes were developed using nanofluidic devices with special nano-in-nano structures. The nanofluidic processes can be used to analyze exosomal membrane proteins on single exosomes. The nanofluidic processes are very useful for elucidating the heterogeneity of single exosomes in terms of membrane proteins.

Chapter 7 present the conclusions and perspectives of the study.

審査結果の要旨

本論文は、サブシングルセル研究のためのナノ流体デバイス基盤技術の開発を系統的に行ったものであり、以下の成果を得ている。

(1) １細胞容量のサンプルからタンパク質を１分子精度で検出するための金ナノアレイを有するナノ流体デバイスと、細胞から放出されるナノスーケルの小胞であるエクソソームを１粒子レベルで分析するためのナノ流体デバイス aifA を設計して開発した。
(2) 金ナノアレイを有するナノ流体デバイスとアプタマー技術を融合して、ナノ流体アプタマーナノアレイを創製した。このナノ流体アプタマーナノアレイはタンパク質を特異的に捕捉できることを明らかにした。これにより、ナノ流路においてタンパク質のナノアレイを形成することを示した。
(3) ナノ流体アプタマーナノアレイを用いて、ポアソン分布に従い、通常濃度の溶液からタンパク質を確率論的に１分子で捕捉できることを実証した。
(4) 酸素プラズマ処理プロセスとピラニア溶液処理プロセスを用いて、サブシングルセル研究のためのナノ流体デバイスの再生法を開発した。この方法で再生したナノ流体デバイスは再利用できることを示した。
(5) ナノ流体デバイス aifA を用いて、単一エクソソームの膜タンパク質を調べるためのナノ流体プロセスを構築した。このナノ流体プロセスを利用して、単一エクソソーム膜タンパク質を単種類レベルでも多種類レベルでも分析できることを示した。これにより、単一粒子レベルでエクソソーム膜タンパク質の不均一性を明らかにした。

以上の諸成果は、サブシングルセル研究のためのナノ流体デバイス基盤技術について、学術的のみならず産業的にも重要な知見を与えるものであり、サブシングルセル領域およびナノ流体デバイス分野において貢献するところ大である。また、申請者が自立して研究活動を行うのに必要な能力と学識を有することを証したものである。