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論　文　名	「Studies on the Development of Cell-Imprinted Polypyrrole for Bacterial Detection」 「細菌検出のための細胞インプリントポリピロールの開発に関する研究」
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論文要旨

The “lock and key” concept has been exploited to engineer useful molecules such as antibiotics, enzymes, supramolecules, and molecularly imprinted polymers (MIPs) with artificial cavities that complement and thereby recognize small molecules. However, recent studies have highlighted the importance of detecting larger and more complex targets to fuel advances in biology, medical care, drug discovery, and public health. For such applications, cell imprinting on functionalized surfaces enables customization for various target cells. The cell-imprinted polymer (CIP) technology is derived from MIPs, in which the template of small molecule is replaced by whole cell. CIP inherited the advantages of MIPs, such as simple operation, good sensitivity, high selectivity, time-saving, tailor-made, and so on. Going beyond small molecules and bio-macromolecules, the challenges in the imprinting of a whole cell are faced exponentially higher, and parameters such as cell shape, size, fragility and viability need to be taken into consideration.

Polypyrrole (PPy) can be easily synthesized by either chemical or electrochemical polymerization with doping molecules. It has been applied for molecular recognition because of the cavity correspond to the target formed on the polymer surface during the preparation process. Compared to other kinds of polymer, PPy has an important advantage, in that a complementary cavity can be easily created by dedoping of a template molecule through overoxidation. Therefore, we prepared cell-imprinted PPy for bacterial recognition. To accommodate various applications,

we attempted to form the cell-imprinted PPy on several types of substrate such as plastic microsphere and 96-well microplate. The preparation procedure of cell-imprinted PPy was studied in detail here. Characterizations of the cell-imprinted PPy were performed by various microscopic observation and spectroscopic measurement. The binding performance of the cell-imprinted PPy to various target cells was also evaluated in the study. The final goal of the study is therefore to detect bacteria specifically by using the cell-imprinted PPy. More specific discussions are outlined below:

Chapter 1 provides a brief introduction and the background of the study with a review of related literature.

Chapter 2 describes the preparation of cell-imprinted PPy on a microsphere. To form the PPy on a plastic substrate, it is necessary to modify the surface. We coated the microsphere with gold nanoparticles (Au NPs) as a scaffold to promote the introduction of nafion®. It is well-known that nafion is one of the polymer electrolytes and also works as a dopant anion. Resultantly, the PPy was formed uniformly on the microsphere. In the polymerization, pyrrole monomer, bacterial cells, and the nafion-introduced microsphere were stirred in a buffer solution for 12 hours. After the polymerization, wholly PPy-coated microspheres were obtained. In the scanning electron microscopic (SEM) observation, we found bacterial cells (at a length of approximately 2.0 μm and a diameter of 1.0 μm) was in the PPy layer (film thickness: ~0.75 μm). This indicated that the bacterial cells acted as dopant based on the negative zeta potential. After the overoxidation, it was confirmed that the cavity was formed on the microsphere. The cell-imprinted microspheres were well dispersed in aqueous media and bound target cells spontaneously. The cell-imprinting technique could be applied to various species of *Escherichia coli* (*E. coli*). The doping states of *E. coli* cells were depended on the species and affected the shape of cavity formed on the PPy. It was found that the surface chemical structure of the *E. coli* cell was different among the species.

Chapter 3 discusses the application of cell-imprinted microsphere for bacterial detection. *E. coli* O157:H7 cell-imprinted microsphere was dispersed into various bacterial suspensions, such as *E. coli* O157:H7, O157:HNM, O26:H11, O26:HNM, O Rough, *Pseudomonas areuginosa*, and *Serratia marcescens*. After incubation for 3 hours, the number of cells remained in suspension was counted, respectively. The uptake ratio of the cell-imprinted microsphere to *E. coli* O157:H7 cell was 10 times higher than those of others. This indicated that the information of *E. coli* O157:H7 cell, not only the size and shape but also the chemical structures of the surface, could be accurately transcribed as molecular recognition sites on the PPy. After rebinding with *E. coli* O157:H7, the light-scattering intensity of a single microsphere was measured. The intensity depended linearly on the concentration of bacterial suspension in the range from 10^4 to 10^8 cells mL^{-1}. The intensity based on a difference in the refractive index between the surrounding air and water inside a cell, and therefore, depended on the number of cell bound. The binding constant of the cavity for target cell was estimated as 1.1×10^5 M^{-1}.

Chapter 4 describes a high-throughput bacterial detection based on a cell-imprinted 96-well microplate. The fabrication of *E. coli* O157:H7 cell-imprinted PPy on the microplate was also accomplished by using Au NPs and nafion. The fluorescence intensity of the cell-imprinted microplate showed a good correlation to the number of stained *E. coli* O157:H7 cells in the suspension. The cell-imprinted microplate indicated a good selectivity to target *E. coli* O157:H7, and also discriminated the target in a suspension of bacterial mixture containing other types of bacterial cells such as *E. coli* O26:H11, *E. coli* O26:HNM, *S. marcescens*, and *A. calcoacetius*. To

apply our technique into high-throughput bacterial detection, various type of cavity was formed on a 96-well microplate. The intensities obtained for the respective target were highest among various bacterial strains. During the detection process, the method required a simple operation, and signal acquisition was achieved within 30 min.

Chapter 5 is conclusion, it summarizes the whole works in the thesis and provides their prospects.

In conclusion, we improved the MIP technology to make it applicable for the micrometer-sized targets such as bacterial cell. The micrometer-sized cavity formed on the PPy layer was useful for the applications to the detection and recovery of the bacterial cells. We hope that the CIP technology will be put into practical use and contribute as an item for living a safe and prosperous life.

審査結果の要旨

本論文は，ポリピロールをマトリクスとした細胞インプリントの形成とそれを用いた細菌の特異検出に関する研究成果をまとめたものであり，以下のような成果を得ている。

(１) 導電性高分子ポリピロールをマトリクスとした細胞インプリント法の開発を行った。ポリピロールは化学重合によって合成できるが，樹脂基板への選択的な形成は困難である。そこで，絶縁性プラスチックマイクロビーズを金ナノ粒子で被覆し，さらに Nafion でコートすることで，ポリピロールの樹脂表面への選択的形成を達成した。その際， *Escherichia coli* O157 を共存させ，標的細菌を含んだポリピロール膜を形成した。これは，負のゼータ電位をもつ *E. coli* O157 がドーパントとして作用し，ポリピロールに取り込まれることに起因する。次に，このビーズをアルカリ溶液中に分散し，ポリピロールの過酸化反応を誘発した。過酸化によって *E. coli* O157 が脱ドープされるとともに，ポリピロールに *E. coli* O157 の形状やサイズに対応した鋳型が形成されることを明らかにした。

(２) *E. coli* O157 インプリントビーズを *E. coli* O157，*E. coli* O26，*E. coli* O Rough，緑膿菌，セラチア菌の分散液に添加したところ，*E. coli* O157 が特異的に自発結合した。*E. coli* O157 の形状やサイズだけでなく，細菌表面の化学構造が転写された鋳型が形成されたことが明らかになった。細菌鋳型の結合定数は $1.1 \times 10^5\ M^{-1}$ と見積もられ，抗原抗体反応における結合定数とほぼ同等であった。ビーズの光散乱強度は，標的細菌濃度（10^4～10^8 cells mL^{-1}）に対して直線的に増大した。

(３) 96 穴マイクロプレートのウェル内壁に細菌鋳型膜を形成し，ハイスループット分析に関する開発を行った。標的細菌である *E. coli* O157 を蛍光染色して，細菌鋳型ウェルに添加した。プレートリーダにより得られた蛍光信号は，*E. coli* O157 分散液の濃度に対して直線的に変化した。種々の細菌を含む混合分散液に含まれる *E. coli* O157 の高選択的な検出が達成された。また，鋳型と標的細菌の自発的な結合によって，30 分以内での迅速な検出が可能になった。

以上の研究成果は，*E. coli* O157 の高選択かつ迅速な検出を可能にするもので，標的細胞に応じたテイラーメイドにより，各種産業に関連する微生物検出の発展に大いに貢献するものである。また，申請者が自立して研究活動を行うのに十分な能力と学識を有することを証したものである。