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Entrapment of Whole Cell Bacteria into Conducting Polymers

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Abstract

In this short review article, we discuss the significant progresses, which have been made in bacterial entrapment techniques over the past decade. Recently, the biocompatibility of conducting polymers as well as the straightforwardness in their preparation has focused more attention on this technique. The key feature of the technique is that bacteria-doped films can be prepared by a single-step electrochemical polymerization, due to the negative surface charges carried by bacteria. We have also included the results of a literature survey on bacterial imprinted polymers, which possess cavities complementary to the imprinted bacteria. Sensory devices using these polymer films will provide a new technology for FIA detectors.

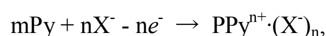
Keywords: bacteria, conducting polymers, polypyrrole, entrapment, molecularly imprinted polymer

1. Introduction

This short review article summarizes some of the recent important progress in attempts on entrapping bulky biological objects, such as bacteria, into conducting polymers (CPs). CPs have been used in a wide range of applications, in which electrical conductivity is required for polymeric materials [1]. Other than the conductivity, CPs have also been providing another valuable property in analytical chemistry; they can incorporate anionic molecules in the textures to compensate for the positive charges introduced on their backbones upon polymerization. This feature has frequently been utilized to immobilize functional biomolecules, such as enzymes and DNA [2].

In spite of this straightforwardness, only a limited number of studies have been available in the literature, concerning the entrapment of much larger objects, such as bacteria and viruses. It was pointed out almost a decade ago that polypyrrole was a biocompatible material on which cell growth could be modulated by changing its redox states [3]. Very recently, we reported that bacilliform bacteria can be quite easily entrapped in a polypyrrole film without loosing their viability [4]. We discuss this technique in this article along with other related studies.

Polypyrrole (PPy) is one of the most commonly used CPs and is prepared by the oxidation of the pyrrole monomer (Py):



$$\mathrm{mPy} + \mathrm{nX^-} - \mathrm{n}e^- \rightarrow \mathrm{PPy^{n+}}\cdot(\mathrm{X^-})_\mathrm{n},$$

where X^- is the dopant, which is taken up into the polymer upon polymerization. The polymeric salt, $\mathrm{PPy^{n+}}\cdot(\mathrm{X^-})_\mathrm{n}$, is denoted as $\mathrm{PPy/X^-}$ hereafter.

The automatic incorporation of a functional substance is a key feature of CPs for its utilization in analytical chemistry in which the immobilization of bulky functional substances, such as bacteria and metal nanoparticles, is expected to occur, provided that these substances carry negative charges [5]. Recently, we have confirmed that the uptake event for bacteria quite easily occurs for several different bacilliform bacteria, and that incorporated bacteria were viable after injection into the polymer texture [4].

2. Entrapping bacteria into conducting polymers

Biomaterials can be immobilized either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these techniques [6, 7]. Entrapment of viable microbes has been studied using matrices, such as calcium alginate, carrageenan, agar, cellulose, polyacrylate, and polyamide. As an application of such immobilization techniques, whole-cell biosensors have been reported [7-9].

Different from previously developed entrapping materials, CPs can automatically entrap bacteria during oxidative polymerization; the entrapment of bacteria is made possible by the outer membranes, which predominantly contain negatively charged lipids, such as lipopolysaccharides [10]. Indeed, the $\mathrm{p}K_\mathrm{a}$ values of *Bacillus subtilis* were reported to be 4.8, 6.9, and 9.4, which likely correspond to the carboxyl, phosphate and hydroxyl sites; the $\mathrm{p}K_\mathrm{a}$ values as well as reported metal sorption evidence indicate that anionic groups dominate the surface in the neutral pH region [11, 12].

Although we cannot find many studies using CPs as

entrapping materials, several interesting studies have appeared in the literature. Whole cell bacterial biosensors were constructed by immobilizing *Gluconobacter oxydans* and *Pseudomonas fluorescens* cells on graphite electrodes modified with CPs based on PPy and polythiophene [13-15]. The cells were spread over the polymer-coated electrodes, followed by covering the surface with a dialysis membrane to conserve bioactivity for the subsequent analytical operations. The sensor response for glucose was studied by monitoring oxygen consumption, which arose from the bacterial metabolic activity, at -0.7 V vs. Ag/AgCl.

Alginate, an anionic polysaccharide, which is found in nature as a structural component of marine brown algae, is a very frequently employed material because of its mild gelling and biocompatible properties [16]. A self-doped PPy was synthesized from a pyrrole-alginate monomer [17]. An electrode coated with the monomer was subjected to electropolymerization after application of a $CaCl_2$ solution [17, 18]. An analytical performance comparison of the amperometric biosensor based on either a regular alginate gel or the synthesized PPy-alginate matrix entrapping algal cells of *Chlorella vulgaris* has been reported [18].

Currently, the most frequently used CPs are polypyrrole, polythiophene, polyaniline and their derivatives. Although polyaniline is the choice material for studies of chemically modified electrodes, this material has not found favored for the viable cell entrapment due to the difficulty of polymerization in the pH range above 3. However, polyanionic dopants, such as polystyrene sulfonate (PSS), have been reported to accelerate the rate of its polymerization. Using PSS, the aniline monomer and lyophilized bacterial biomass (*Brevibacterium ammoniagenes*), a PSS-polyaniline film entrapping the bacterial cells was deposited at pH 4 between twin Pt-wire electrodes for use in urea determination [19]. The response of this device indicated that the cells could remain viable and retained their enzyme activity within the polymer; the viability was also confirmed by fluorochrome staining (cf. the supplementary content of this paper). Entrapping techniques based on polythiophene have also been reported [15, 20].

The studies discussed above have successfully immobilized microbial cells using CPs; the films functioned either as adsorption platforms, over which microbes were spread, or as inclusion matrices. However, all of these studies did not show any clearly defined structures with respect to immobilized microbes; some microbes were entirely exposed on the surfaces, while others were randomly embedded in the polymer textures.

Very recently, we have successfully developed a technique for the immobilization of bacilliform bacteria into a PPy film in a well-defined manner [4]. Interestingly, all the bacilli studied in that research showed vertical insertion into the PPy films, which was demonstrated by the SEM images and fluorescent microscopic images (Fig. 1). In panel B, *Pseudomonas aeruginosa* was stained by fluorochrome reagents (SYTO® 9 and propidium iodide (PI)); the majority of cells were found as circular radiants due to vertical entrapment. In contrast to previous studies, our technique can arrange bacilliform bacteria in almost perfect order in that they are inserted perpendicular to the polymer surface [4].

The viability of the entrapped cells (80.4%) was almost equal to that for a freshly centrifuged and dispersed specimen (80.2%), thus demonstrating that the entrapment did not damage the cells. It was also found that the PPy layer was important to keep the cells alive.

3. Reversible removal and reentrapment of bacteria in conducting polymers

Molecularly imprinted polymers (MIPs) have been used in separation and sensing applications. The polymers have cavities complementary to template molecules to allow reversible removal and reentrapment of the template. The polymers are usually prepared using functional and cross-linking monomers [21], and there have been many attempts to synthesize microbial imprinted polymers for bacterial cell detection [22].

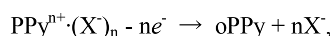
It has been postulated that the restricted diffusion of bulky biological templates, such as bacteria and viruses, from and into the polymer films can neither create a complementary void nor wash them out from the void in an efficient fashion. To circumvent this difficulty, the majority of studies on bacterial imprinted polymers has adopted surface stamping techniques to create the complementary cavities, whereby template cells, which have been immobilized on a flat substrate, is pressed onto a soft polymer layer to create the cavities [23-27]. However, the stamping technique seems to be limited to the applications to cocci (yeast cells) due to difficulties associated with removing the template cells.

Several researchers have also examined the feasibility of CPs as MIP materials targeting these bulky biological objects. The preparation and basic characterization of a PPy-based MIP for label-free detection of bovine leukemia virus glycoprotein gp51 have been reported [28]. A PPy film doped with gp51 was prepared by flow-through electrolysis, followed by treatment with H_2SO_4 to remove the template protein. The prepared film was continuously pulsed between 0 and +0.6 V vs. Ag/AgCl

during the rebinding event in order to observe the dependence of the transient current on the protein concentrations.

A microbial imprinted PPy-polythiophene dual coat electrode was reported for the detection of *Bacillus* endospores [20]. A glassy carbon electrode depositing the spores was subjected to electropolymerization in the presence of pyrrole and a dopant smoother followed by potentiostatic polymerization of 3-methylthiophene. The template spores were removed by soaking the electrode into dimethyl sulfoxide. The susceptance of the film was found to depend on the spore densities in the range between 10^4 and 10^7 CFU mL^{-1}.

We have been preparing CP-based MIP receptors to discriminate amino acid enantiomers, the structural isomers of naphthalene sulfonates, etc [29-33]. A template molecule can be electrostatically ejected from a PPy film by overoxidation, which can remove the intractable washing procedure that the standard MIP protocols require:



$$PPy^{n+}\cdot(X^-)_n - ne^- \rightarrow oPPy + nX^-,$$

where oPPy denotes the overoxidized and electrically neutralized PPy. The important advantage of this technique is that overoxidation cures the polymer texture to preserve the shape complementarity of the cavity in addition to the concomitant electrostatic elimination of a template [34].

Recently, we have extended this technique to bacterial recognition; quartz-crystal-microbalance experiments revealed that targeted bacilliform bacteria were discriminated from other bacteria using a overoxidized film, which had been prepared from a PPy film doped with bacteria (cf. Fig. 1). Indeed, the quantitative ejection of the bacteria was confirmed by SEM images of the oPPy film [35]. By utilizing the overoxidation technique, we could efficiently prepare PPy-based bacilli imprinted polymer receptors for the first time.

4. Conclusions

In this short review article, we have discussed the current status of research on CPs that are able to entrap bacteria. Although related studies are presently few in number, the biocompatibility of the polymers as well as straightforwardness in preparing films are focusing more attention on this technique. We have also included the results of a literature survey on bacterial imprinted polymers, which possess voids complementary to imprinted bacteria.

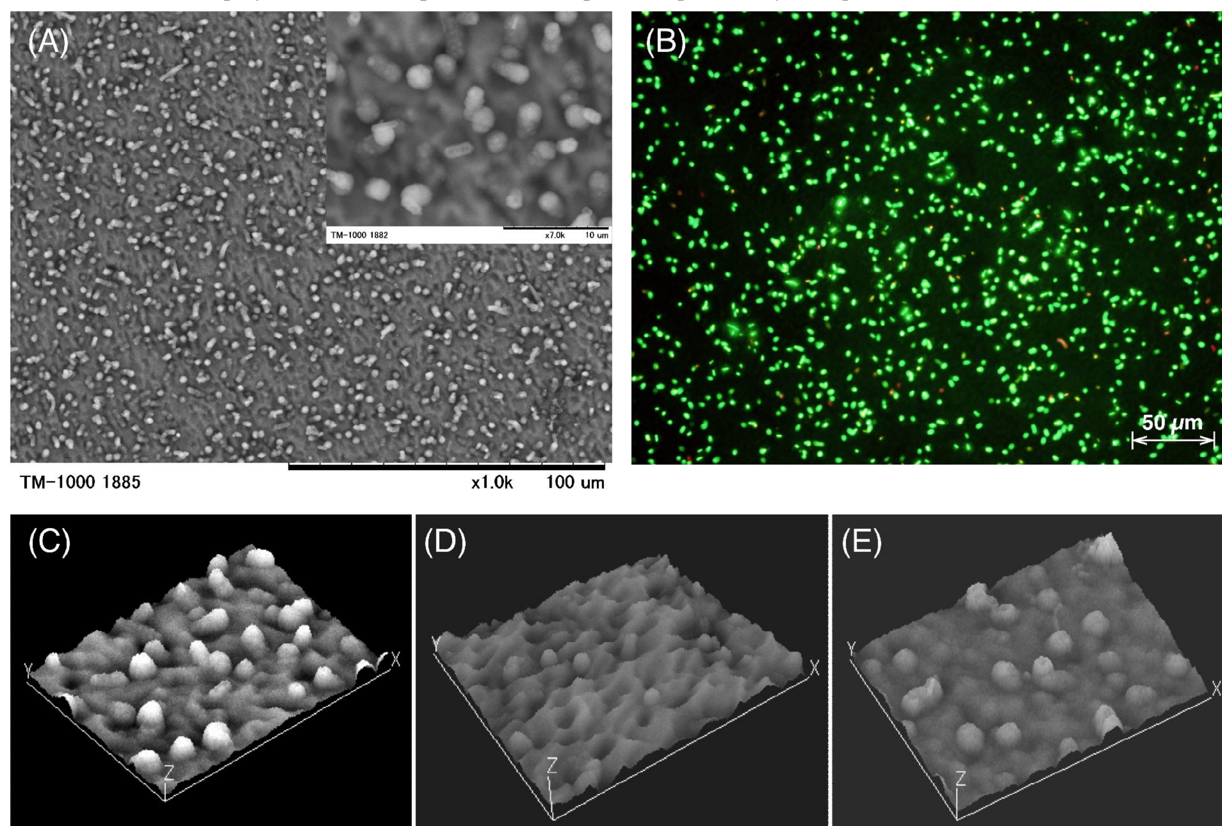


Fig. 1 SEM and fluorescence-microscope images of (A, B and C) PPy/*Pseudomonas aeruginosa*, (D) PPy/*Bacillus subtilis*, (E) PPy/*Escherichia coli*. Pseudo-3D images, (C) – (E), were generated by iSolution software from SEM images with 40 μm x 30 μm dimensions; the image (C) was created from the inset of (A). The films were prepared by polymerizing 0.10 M pyrrole for 300 s at (A, C, D, and E) pH 2.5 and at (B) pH 5.3. *P. aeruginosa* shown in (B) was stained with SYTO 9 and PI prior to the polymerization. Reprinted with permission from Ref. [4]; ©*2012 The Japan Society for Analytical Chemistry.*

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