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Electrochemical recycling of gold nanofibrous membrane as an enzyme immobilizing carrier

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HIGHLIGHTS

- Enzymes on nanofibrous membrane showed 226 times higher activity than those on disk.
- Recycle was performed by electrochemical desorption of immobilized enzyme.
- Desorption was confirmed by the loss of enzyme activity and XPS analysis.
- Immobilization and desorption of the enzyme were repeated.

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ABSTRACT

A gold nanofibrous membrane was shown to be an enzyme-immobilizing carrier that can be recycled on-site in a continuous flow-through reactor using a simple electrochemical treatment. The membrane was prepared by electrospinning a polyacrylonitrile/gold salt solution, followed by electroless gold plating. Laccase, used as a model enzyme, was immobilized on the gold membrane surface by a combination of formation of amino-terminated self-assembled monolayers and glutaraldehyde treatment. Enzyme desorption at neutral pH was confirmed by a 98% decrease in enzymatic activity, based on oxidation of a 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) substrate, after electrochemical treatment for desorption, and surface characterization using X-ray photoelectron spectroscopy. The enzymatic activity after re-immobilization on the membrane was restored to 90%, and decreased to 1.0% after redesorption. These results showed that recycling of the gold nanofibrous membrane as an enzyme-immobilizing carrier was possible. The laccase-immobilized membrane was used in a flow-through reactor; the activities of the immobilized and re-immobilized enzymes on the membrane were 62.7% and 58.5% of the respective initial activities after operation for 7 d.

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1. Introduction

Enzyme immobilization has been widely used in biotechnology, and the food and chemical industries, to achieve continuous economic operation and recovery of high-purity products [1]. Traditionally, powdery materials such as macro-sized spheres, microspheres, and nanoparticles of metals, ceramics, carbon, and plastics with large surface area:volume ratios have been used as enzyme-immobilizing carriers to further improve the activity of the immobilized enzyme per unit volume [1–5]. However, apart from macrospheres, powdery materials are only suitable for use in batch reaction systems with slurry reactors, and must be separated after each run [6].

Recently, nanofibrous non-woven membranes consisting of fibers with diameters ranging from less than 100 nm to several micrometers have attracted much attention as enzyme-immobilizing carriers [7–11]. Electrospinning can be used to produce nanofibrous membranes. The nanofibrous membranes obtained using this method have been reported to be potentially good enzyme carriers, because their properties such as high porosities and large surface area:volume ratios result in high enzyme loadings, good mechanical properties, and the possibility of surface modification to give desired functions [11]. In addition, membrane reaction components for continuous flow-through reaction systems can be easily manufactured from nanofibrous membranes.

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Continuous flow-through reactors are often used in industrial reaction processes, because they have high efficiency and catalyst separation from the reaction medium is easier than that from conventional batch reactors with dispersed carriers. In a previous study, Lloret et al. [12] immobilized laccase on polymer beads of diameter several hundreds of nanometers, and used the beads in a continuous flow-through reactor for removal of endocrine-disrupting chemicals. Wang et al. [6] reported urease immobilization on coconut-derived activated carbon and used it for urea degradation in a continuous flow-through reactor. Once the immobilized enzyme has been denatured, the enzyme and carrier must both be removed from the reactor and replaced by fresh ones. The replacement of nanofibrous carriers in a flow-through reactor is sufficiently labor intensive and expensive to limit industrial applications. Recycling of carrier materials would therefore expand the use of nanofibrous membranes as enzyme carriers in flow-through reactors.

The aim of this study was to show that nanofibrous membranes in flow-through reactors can be recycled on-site by in situ enzyme detachment and re-immobilization. Covalent immobilization of enzymes by self-assembled monolayers (SAMs) is a widely used immobilization technique, and it offers a useful way of generating well-ordered monolayers of biological molecules [13–15]. In this study, an enzyme was immobilized by combination with amino-terminated SAMs formed on a gold surface. Gold is a precious metal and can be formed into thin layers to impart electrical conductivity to insulating materials, using electroless plating [16–19]. Various thiol compounds form stable SAMs by strong chemisorption to a gold surface [20] and the SAMs can be removed on demand electrochemically by applying a negative voltage to carriers in neutral pH aqueous solutions [21,22]. It has been reported that proteins immobilized on SAMs of thiolates formed on gold surfaces can be removed on demand by electrochemical treatment [21,22].

Electroless gold plating on an electrospun nanofibrous membrane was performed to give electrical conductivity and enable SAM formation on the nanofibrous carrier. Oxidation of ammonium 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate (ABTS) in the presence of laccase was used as a model reaction system. Laccase immobilization on, and electrochemical desorption from, the gold nanofibrous membrane were evaluated by determining the laccase activity, and surface characterization was performed using X-ray photoelectron spectroscopy (XPS). Enzyme immobilization and electrochemical desorption were repeated to investigate the possibility of membrane recycling. The applicability of the system in a flow-through reactor and the stability of the enzymatic activity were determined by using the membranes after enzyme immobilization and re-immobilization.

2. Materials and methods

2.1. Materials

Polyacrylonitrile (PAN, molecular weight: 150,000) was purchased from Polysciences Inc. (PA, USA). $HAuCl_4{\cdot}4H_2O$ was purchased from the Kanto Chemical Corp. (Tokyo, Japan). ABTS, $NaBH_4$, and 2-aminoethanethiol (cysteamine) were purchased from Tokyo Chem. Ind., Ltd. (Tokyo, Japan). $NH_2OH{\cdot}HCl$ and *N*,*N*-dimethylformamide (DMF) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Laccase from *Trametes versicolor* was purchased from JenaBios (Jena, Germany). All chemicals were used without further purification.

2.2. Electrospinning of PAN/gold salt mixed solution

A solution of 14 wt% PAN was prepared by heating a suspension of PAN powder in DMF at 50 °C until the solution turned transparent. $HAuCl_4{\cdot}4H_2O$ powder was dissolved in DMF at 1.0 wt%. $HAuCl_4$/DMF solution (2.0 g) was mixed with the PAN solution (2.3 g). Electrospinning was performed as described elsewhere [23,24]. The mixed solution was electrospun at a tip-to-collector distance of 15 cm, applied voltage of 20 kV, and extrusion rate of 2.0 mL/h from a 5 mL plastic syringe. The obtained membranes were stored in the dark until use.

2.3. Preparation of electroless-gold-plated nanofibrous membrane

Electroless gold plating of the membrane was performed using a procedure similar to those reported in the literature [17,18]. A PAN/gold salt membrane was shaped into a disk of diameter about 4.0 cm and placed in a vacuum filtration apparatus. Aqueous solutions of 1 mM $NaBH_4$ (500 mL) and 0.02 mg/mL $NH_2OH{\cdot}HCl$ (1000 mL), and a yellow aqueous mixture (1000 mL) containing 0.2 mg/mL $HAuCl_4{\cdot}4H_2O$ and 0.02 mg/mL $NH_2OH{\cdot}HCl$, were filtered in turn under reduced pressure until the filtrate was colorless. The thickness of the dried membrane was measured using a micrometer. The sheet resistivity of the membrane was measured with a resistivity meter (RT-70V/RG-5, Napson Corp., Chiba, Japan), using a membrane of average thickness 75 μm. Morphology of the membrane was observed using a scanning electron microscope (SEM, Model S-2250N, Hitachi Ltd., Tokyo, Japan) after drying in vacuo.

2.4. Laccase immobilization

Amino-terminated SAMs were formed by soaking the gold-plated membrane in cysteamine solution. The gold-plated membrane (2.5 mg) or a flat gold disk were incubated in 0.1 M

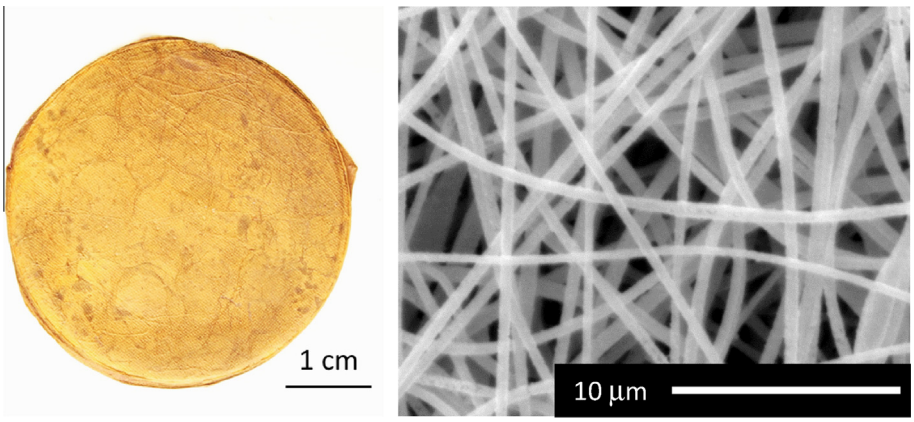

Fig. 1. Photograph (left) and SEM image (right) of gold nanofibrous membrane prepared via electrospinning of PAN/gold salt solution and subsequent electroless gold plating.

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