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Peroxidase-mimicking PtNP-coated, 3D-printed multi-well plate for rapid determination of glucose and lactate in clinical samples

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ABSTRACT

Three-dimensional printing (3DP) technologies provide great opportunities for prototyping devices with designed geometric functionality. As a further example of a multifunctional device manufactured using 3DP technologies, this paper describes a multi-well plate—fabricated using a fused deposition modeling-type 3D printer and then treated with a post-printing coating of peroxidase-mimicking platinum nanoparticles (PtNPs)—that (i) catalyzes the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2), (ii) allows determination of substances that can be oxidized by their specific oxidases to produce H_2O_2, and (iii) facilitates measurement of the absorbance of samples after direct loading into a plate reader. After method optimization, the analytical applicability of the PtNP-coated 3D-printed multi-well plate was illustrated in terms of reusability and stability, reaction kinetics, analytical performance, and the respective determination of glucose and lactate concentrations in urine, plasma, serum, and brain microdialysate samples. Such post-printing functionalization schemes should promote 3DP technologies for the future fabrication of multifunctional devices and an expansion of their practical applications.

1. Introduction

Additive manufacturing (three-dimensional printing; 3DP) technologies have accelerated the customization and prototyping of experimental devices/components in general laboratories for a wide range of analytical applications, especially these employing affordable fused deposition modeling (FDM) and stereolithographic technologies [1–10]. Limited by the availability of raw printing materials, 3DP studies have increasingly paid attention to the development of new printing materials, as well as suitable functionalization procedures. For more diverse applications, such fabricated devices can display unique physicochemical properties, enabled through the incorporation of precursors or chemicals into the raw printing materials in advance or applying post-printing functionalization schemes [11–22].

For FDM-type 3DP, the chemical substances added to the thermoplastics must resist the high-temperature extrusion conditions (up to 200 °C) [12,17–19]. There are fewer limitations to the post-printing functionalization of FDM-type printed devices because it is not necessary to consider the thermal stability of the added substances or whether they will be covered by extruded thermoplastics after a printing process. Wang et al. evaluated a 3D-printed acrylonitrile butadiene styrene (ABS) framework, coated with Cu-benzene tricarboxylic acid metal–organic frameworks, as an eco-friendly and reusable adsorbent for the removal of methylene blue [13]. Furthermore, we immobilized glucose oxidase (GOx) and lactate oxidase (LOx) on the surfaces of an ABS-printed flow reactor to allow online oxidation of glucose and lactate, as well as determination of their concentrations in rat brain microdialysates [18]. Post-printing processing is a more appropriate technique when functionalizing 3D-printed devices with fragile or thermally unstable species (e.g., enzymes and other biomolecules).

Nanomaterials (NMs) having intrinsic enzyme-like activities are promising mimics (e.g., peroxidase, oxidase, catalase, superoxide dismutase) for many biochemical applications because they are relatively stable against stringent reaction conditions, have tunable activities, and can be obtained at low cost and in large quantities [23–26]. Nevertheless, technical challenges remain to stabilize these enzyme-like NMs in aqueous media during/after their synthesis, and only a few of them have been designed for reuse [27–29] or fixed on bulk substrates [30,31]. In addition to the introduction of NMs to raw printing materials possibly having a synergistic effect when creating entirely new composites [32], 3D-printed devices coated with these enzyme-mimicking NMs can display the

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attractive features of artificial enzyme mimics while advancing the applicability of 3DP technologies.

In this study we aimed to develop a facile post-printing approach to functionalize multi-well plates manufactured using a low-cost FDM-type 3D printer and ABS filaments. Because peroxidase-mimicking platinum (Pt) NPs readily attach to various applied substrates (e.g., polystyrene microspheres [33], mesoporous silica NPs [34], graphene and graphene oxide nanosheets [35–37], molybdenum trioxide nanosheets [38]) during their synthesis, we optimized a procedure to coat each well of the 3D-printed multi-well plate with peroxidase-mimicking PtNPs that could catalyze the hydrogen peroxide (H_2O_2)–mediated oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB). We then used these PtNPs coated on 3D-printed multi-well plates to determine a variety of chemical substrates by means of coupling their peroxidase activities with oxidation of the oxidase substrate to generate H_2O_2 molecules (e.g., oxidation of glucose by GOx and lactate by LOx). Neither the post-synthesis stabilization of such enzyme mimics nor to remove them before detection was necessary. After optimizing the coating procedure and the reaction conditions, we developed an assay method for glucose and lactate, and its applicability was verified through the respective determination of glucose and lactate concentrations in samples of urine, plasma, serum, and rat brain microdialysate. Accordingly, the PtNP-coated 3D-printed multi-well plates appear to be very useful for routine and rapid analyses of glucose and lactate concentrations in clinical samples, and for the development of suitable functionalization schemes for 3D-printed objects so that they can meet the future requirements for fabricating multifunctional devices capable of a wider range of applications.

2. Chemicals and methods

2.1. Chemicals

H_2O_2 (31642), d-(+)-glucose (G7528), l-(+)-lactic acid (L1750), GOx (G7141, from *Aspergillus niger*), LOx (L0638, from *Pediococcus* sp.), TMB (T2885), hexachloroplatinic acid (H_2PtCl_6) solution [8% (w/v) in water; 262587], sodium borohydride (71321), and ammonium acetate (A7330) were purchased from Sigma–Aldrich. Dimethyl sulfoxide (DMSO; 9224) was purchased from J. T. Baker. All chemical solutions were prepared using water purified through a Milli-Q Integral water purification system (Merck Millipore). ABS filaments were obtained from Tiertime Technology (C-23-01; US$36 per kg).

2.2. PtNP-coated 3D-printed multi-well plate

A 48-well plate (well dimensions: 0.6 cm i.d. × 0.9 cm height; well volume: 254 μL) lacking a bottom supporting plate was designed using computer-aided design software (SolidWorks 2013, Dassault Systèmes) and fabricated using a commercial FDM-type 3D printer (UP Plus 2, Delta Micro Factory). The printer was operated with a copper nozzle (0.4 mm) at 260 °C for ABS filaments under the densest filling mode and a *z*-axis (layer) thickness of 0.15 mm. Two fabricated 48-well plates were manually glued (epoxy adhesive) onto a transparent polystyrene microplate and fixed at room temperature for at least 8 h. Accurate alignment of each well with the detector's light path was necessary when loading the fabricated plate into a conventional microplate reader (Infinite M200, Tecan) to directly measure the absorbance of each sample solution. For coating with the PtNPs, the fabricated multi-well plate was cleaned with water and fixed on an orbital mixer (EchoTherm™ SC20); each well was filled with a H_2PtCl_6 solution (196 μL, 0.11%) and then a $NaBH_4$ solution (4 μL, 1%) was added quickly and the reaction allowed to proceed at 35 °C. After 2 h, the solutions were evacuated, and an assay buffer solution (100 mM ammonium acetate, pH 4) was added for a single wash of the residual solution from each well. The fabricated PtNP-coated 3D-printed multi-well plates were preserved at 4 °C until required for experiments. The used plates were gently washed with DMSO and assay buffer solutions for reuse.

2.3. Sample collection and preparation

Urine samples were collected from two volunteers (one healthy, one having type 2 diabetes); a plasma sample was collected from a Sprague–Dawley (SD) rat; a qualified fetal bovine serum was obtained from Thermo Fisher Scientific (FBS; 10437-028; lot number: 1709276). SD rat brain microdialysates were collected from an implanted microdialysis (MD) probe [4-mm-long, 500-μm-diameter polyarylethersulfone (PAES) membrane having a molecular weight cut-off (MWCO) of 20 kDa (8010435; CMA 20, CMA Microdialysis)] to target the hippocampus (2.0 mm anterior-posterior and 2.0 mm laterally from the bregma [39]) in response to switching the perfusion solution from 0.9% NaCl to 1.15% KCl (depolarization model), which was conducted in conformity with the guidelines and approval of the Institutional Animal Care and Use Committee at National Tsing-Hua University (approval number: 10521). Apart from the microdialysates, all of the samples were pre-treated with 3-kDa centrifugal filters (Amicon Ultra-0.5, Merck Millipore) using a microcentrifuge (Z233 M-2, HERMLE Labortechnik GmbH). One part of these filtrates and microdialysates was first diluted with a phosphate buffer solution (10 mM, pH 7.5), and these diluted samples were then treated with addition of an equal volume (two-fold dilution) of a GOx solution (75 U mL^{-1}) for glucose determination; the other part of these filtrates and microdialysates was diluted with a citrate buffer solution (100 mM, pH 6), and these diluted samples were then treated with addition of an equal volume (two-fold dilution) of a LOx solution (1 U mL^{-1}) for lactate determination. Both mixtures were incubated for 30 min at room temperature. Finally, the treated samples (20 μL), TMB solution (20 μL), and assay buffer solution (160 μL) were mixed in the PtNP-coated multi-well plates for 7.5 min at 55 °C. To respectively determine the glucose and lactate concentrations, the absorbance of the mixture in each well was measured directly through the loading of the plate into a microplate reader. Commercial assay kits for glucose (Sigma GAGO20, traceable to the NIST standard) and lactate (K607-100, BioVision) were applied to evaluate the proposed method's accuracy.

3. Results and discussion

3.1. Fabrication of PtNP-coated 3D-printed multi-well plates

We used a FDM-type 3D printer with ABS filaments to fabricate two 48-well plates lacking a bottom supporting plate, and then glued them onto a transparent polystyrene microplate (Fig. 1). Although a multi-well plate with a bottom supporting plate can be fabricated directly within one step by the used FDM-type 3D printer, the rough bottom supporting plate made from stacking layers of thermoplastic threads would lead to high background absorbance due to the scattering when the light source passed through it [40,41]. The printing time of a single 48-well plate was approximately 3.2 h (0.15 mm per layer); its weight was 18.6 g, corresponding to a material cost of US$0.7. Because PtNPs readily attach onto the applied substrate during their synthesis [33–38], we optimized a simple procedure to coat the peroxidase-mimicking PtNPs onto the surfaces of these 3D-printed multi-well plates and then evaluated their catalytic abilities toward the determination of glucose and lactate in clinical samples. Our procedure for coating