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Polydopamine coating in organic solvent for material-independent immobilization of water-insoluble molecules and avoidance of substrate hydrolysis

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ABSTRACT

Polydopamine (pDA) coating is the first material-independent surface chemistry utilized in energy storage systems, environmental technology, and biomaterial science. However, the conventional coating method requires use of aqueous solutions, which restrict immobilization of water-insoluble molecules onto surfaces, and the coating cannot be applied to degradable polyester substrates. Herein, methods to form pDA in organic protic solvents (o-pDA) with fast coating kinetics resulting in ~50 nm in thickness just for 4 h. One-step immersion of surfaces in a one-pot mixture of a water-insoluble molecule, dopamine, and piperidine results in o-pDA coating with robust surface immobilization of molecules of interest.

Introduction

Inspired by mussels' adhesive foot proteins containing the repeated chemical moieties of amines and catechols, a synthetic polymer called polydopamine (pDA) was identified as a mussel's adhesive-mimicking polymer which is prepared by oxidative polymerization of dopamine in an aqueous alkaline solution [1,2]. The pDA has two important advantages: First, pDA can be coated onto virtually all types of surfaces of metal oxides, noble metals, semiconductors, polymers, ceramics, and carbon materials. Second, the pDA coating exposes amine and catechol functional groups on the coated surface and supports chemical reactions with various functional groups that are reactive to the amine, thiol, and catechol groups. For example, Sileika et al. demonstrated PEGylation of polymer surfaces through the covalent interaction between thiols of methox-poly(ethylene glycol)-thiol (mPEG-SH) and catechols of the pDA [3]. Feng et al. stably immobilized amine-functionalized multi-walled carbon nanotubes on the pDA through the covalent interaction between amines and catechols to fabricate a membrane for highly efficient solid-phase microextraction [4]. Zheng et al. conjugated folic acid molecules which are utilized for the bio-sensing of folate receptor over-expressed tumor cells to pDA-coated carbon nanotube through EDC/NHS coupling of the carboxylic moieties of folic acids and amine moieties of the pDA [5]. Other than these applications, the pDA coating has also been broadly utilized in areas of science and engineering such as individual encapsulation and functionalization of living cells [6], enhancement of bio-compatibility of surfaces [7,8], cell patterning [9], fabrication of special wettable surfaces [10], removal of heavy metals and organic pollutants [11], detection of biomolecules [12], and synthesis of photo- and chemical-catalysts [13].

Despite the widespread applications, development of the surface functionalization methods has not been fully studied. The original method by Lee et al. utilizes a two-step procedure, *i.e.*, immersion of a substrate in the aqueous alkaline solution of dopamine and subsequent immobilization of molecules of interest (Fig. 1A) [1]. Recently, Kang et al. demonstrated a one-step procedure called one-pot pDA coating. In this case, molecules of interest were co-dissolved with dopamine in the aqueous alkaline

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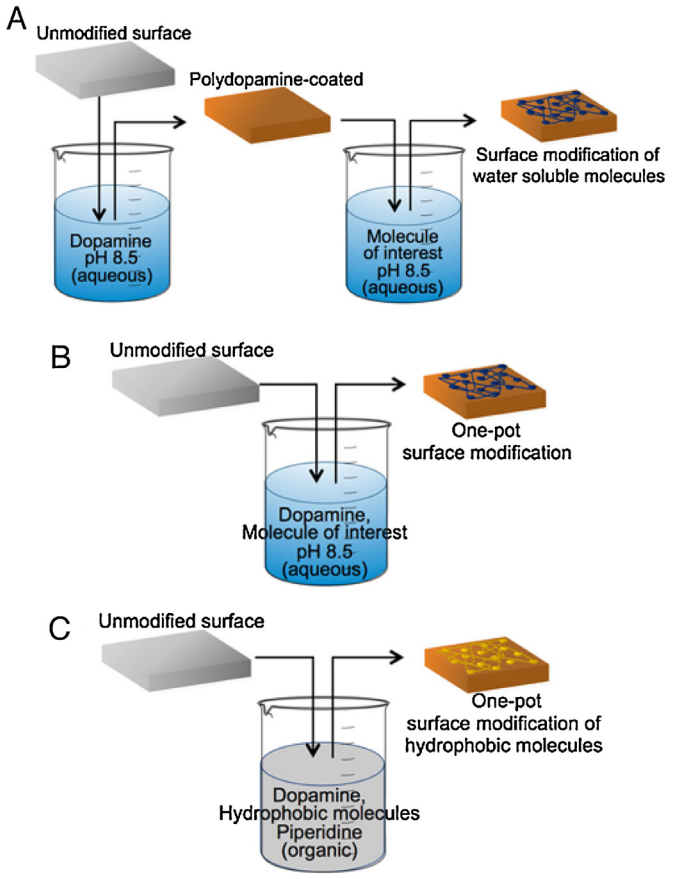

Fig. 1. Schematics of the surface functionalization by pDA coating. (A) The conventional two-step surface functionalization. A substrate is immersed in the aqueous alkaline solution of dopamine and then, immersed in the alkaline solution of molecules of interest. (B) The conventional one-pot surface functionalization. Molecules of interest (water-soluble) are co-dissolved with dopamine in the aqueous alkaline solution and a substrate is immersed in the solution. (C) One-pot surface functionalization in organic solvents. Water-insoluble molecules of interest, dopamine, and piperidine are co-dissolved in the protic organic solvents and a substrate is immersed in the solution.

solution so that the molecules were immobilized onto the immersed substrates during the pDA coating (Fig. 1B) [14]. This one-pot pDA coating turned out to be very effective when it comes to surface modification of proteins such as a metalloenzyme alkaline phosphatase (ALP). For example, Nijhuis et al. demonstrated that larger amount of ALP was immobilized by the one-pot coating strategy than by the original two-step coating method because the one-pot method provides double chances for the target biomolecules to be physically entrapped as well as to be covalently bonded with the pDA [15]. So far, regardless of the two- or one-step procedures, the solvents for the pDA coating has been primarily aqueous solutions. Therefore, most of the pDA studies significantly limits the molecules that are not dissolved in aqueous solutions. Examples include the hydrophobic molecules such as paclitaxel (PTX), sirolimus (SRL), and tamoxifen (TM) or lipid-like molecules such as organic alkanethiols and alkane-phosphates. In addition, the conventional, aqueous-solution-based pDA coating methods could not be applied to the surface modification of water-hydrolysable or water-soluble materials such as poly(lactic-*co*-glycolic acid) (PLGA) and poly(vinyl alcohol) (PVA) due to degradable nature. In fact, Yue et al. reported pDA coating on the surface of iron-oxide nanoparticles in ethanol. However, the study used co-solvent of ethanol and alkaline Tris buffer that triggers the oxidative polymerization of dopamine [16]. The use of co-solvent prevents surface functionalization of the aforementioned degradable substrates as well as the immobilization of hydrophobic molecules if the molecules are precipitated in the co-solvent conditions.

Here, we demonstrate a new pDA coating method that is effective in pure organic solvents named as the organic-pDA coating (o-pDA). The important key factor in this method is the use of piperidine as an organic base which deprotonates/oxidizes the dopamine molecules in the pure organic solvents for rapid formation of the pDA layer, and it is found that this o-pDA coating is effective only in polar protic solvents such as alcohols. We also demonstrated that one-pot o-pDA coating (*i.e.* co-immobilization of target molecules on surfaces) was possible. The immersion of surfaces in one-pot mixtures of water-insoluble target molecules, dopamine, and piperidine resulted in the surface immobilization of the molecules such as anticancer drugs as well as alkainethiols (Fig. 1C). It is believed that the o-pDA coating method can provide a new strategy of surface modification for hydrolysable or degradable substrates as well as immobilization of water-insoluble, functional molecules.

Experimental

Materials

Dopamine hydrochloride, PVA granules (MW: 85–124 kDa, 87–89% Hydrolyzed), methanol (99.8%) ethanol (99.8%) and piperidine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). (Si) substrates and (Ti) substrates were purchased from Nanofab center (Korea). Stainless steel was purchased from Kumjeong (Korea). Human cervical carcinoma (HeLa) and human breast cancer cell (MCF-7) were obtained from American Type Culture Collection. Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), fatal bovine serum (FBS), penicillin-streptomycin were obtained from GIBCO. Live/Dead® cell viability assay kit was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Paclitaxel, 1-hexanethiol, 1-decanethiol, and 1-octadecanethiol were also purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

One-pot Surface functionalization of the o-pDA coating

Dopamine hydrochloride (10 mM) was dissolved in methanol or ethanol solvents. Piperidine (20 mM) was added into the solutions. To immobilize water non-soluble molecules, paclitaxel (200 μM) or *n*-alkanethiol molecules (10 mM) were co-dissolved together with dopamine (10 mM) in methanol or ethanol solvents, and piperidine (20 mM) was added.

Preparation of PVA nanofibers

PVA of the concentration of 10 wt% was dissolved in D.I water through the repeated boiling and cooling using a microwave oven. The resultant PVA solution was electrospun at a voltage of 10 kV controlled using a voltage generator (Korea Switching, Inc., Republic of Korea). A gas-tight glass syringe with a stainless steel needle of 23 gage was used with a syringe pump (KDS-100, KD-Scientific, USA) to maintain a flow rate of 0.5 mL/h. The distance between the tip of the needle and the aluminum plate was 15 cm.

Live/dead assay for cell viability

Cell line Human cervical carcinoma (HeLa) and human breast cancer cell (MCF-7) were used in this study. They were grown as a monolayer culture in Dulbecco's Modified Eagle Medium, supplemented with 10% (v/v) fatal bovine serum, and 1% (w/v)