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A nanostructured cell-free photosynthetic biocomposite via molecularly controlled layer-by-layer assembly

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ABSTRACT

Thylakoids, on which complete sets of photosynthetic membrane proteins are contained, behave as soft nanoparticles of a few tens nm sizes. Thus, we separated the thylakoid extracts from spinach chloroplasts and densely organized them in a film with either insulating polyethyleneimine (PEI) or conducting polyaniline (PANI) by molecularly controlled layer-by-layer (LBL) assembly. The resulting nanostructured composite films demonstrated photoelectrochemical activities with thylakoids whose stabilities were significantly improved on the charge balanced LBL multilayers compared to one on a randomly deposited film or in solution. The thylakoid LBL films continuously generated photochemical electrons for longer than 130 h with the 2,6-dichlorophenolindophenol (DCPIP) mediated photosynthetic energy conversion cycle while photoactivities of thylakoid moiety slowly decreased in the open circuit potential measurements throughout which the photosynthetic redox cycles were incomplete. As the photofunctional groups of the thylakoid films originated from a biotic system and the photofuctional thylakoids were exceptionally well preserved on the charge-balanced artificial structures, the developed thylakoid LBL film have shown inherent biocompatibility upon PC12 neural cell attachment and differentiation tests, which are essential qualities for emerging human-friendly electronic applications such as disposable electronics, artificial retina, and implantable neural interfacing meditronics as well as cell-free photosynthetic production.

1. Introduction

Photosynthesis is an energy conversion process whereby all living organisms derive their chemical and biological energy either directly or indirectly from sunlight [1]. The quantum efficiencies of water (H_2O) splitting from biological photosynthesis system are surprisingly high – near unity – in comparison with man-made photovoltaic systems, although the overall energy conversion efficiencies of plants, bacteria, and algae become quite poor due to the cellular metabolic constraints of living organisms [2]. Biological photosynthesis is achieved through a series of catalyzed reactions by membrane protein complexes, as in photosystems—photosystem I (PS I) and photosystem II (PS II). Their crystalline structures and complex architecture have recently been revealed so that the super-efficient photo-energy conversion mechanism of the oxygenic photosynthesis including light harvesting, electron-excitation, -conversion, -hopping, and-transfer reactions, are now unraveled and well documented [1,3–5]. Notably, this biological photosynthetic system is naturally biodegradable and biocompatible in terms of its involved materials, reactions, and even energy levels [6,7]. Thus, these biotic photosynthesis complexes can be employed to manufacture recently emerging functional materials and devices such as implantable meditronics for stimulating neurons [8], organic bioelectronics [9,10], biodegradable electronics [11,12], edible electroceuticals [13,14], environmental monitoring biosensors [15,16], and beyond [17]. However, biomaterials are inherently fragile and degradable, obstructing efficient processing for practical uses. Hence, a breakthrough is necessary to prepare biocomposites encapsulating biomolecules into a robust synthetic matrix [7].

In order to utilize biological photosynthetic groups in an artificial system, immobilization of biomaterials and biotic–abiotic hybridization techniques are desirable. Fabricating artificial devices directly from biomaterials requires seamless molecular

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design for bridging large material property gaps between biotic and abiotic systems. Biomaterials are hierarchically organized through complex intermolecular interactions with resultant superior functionality or multifunctionality from ordinary proteins, and work collectively and consecutively from multicomponent, which are in stark contrast with non-living systems. Photosynthetic complexes indeed possess all those unusual qualities. As artificial biotic photosynthesis hybrid systems, PS I [18–22], PS II [23–25], light-harvesting antenna complexes [26,27], and thylakoid membranes [25,28] extracted from cyanobacteria [29], algae, or green plants (i.e. bean leaves [28] and spinach [23]) have proven capable of being porous electrodes [30], organic photovoltaic cells [31], biosensors for microchips [16,32], photobioreactors [7], cell-free hydrogen production [33], and even self-regenerating machines [34]. These photosynthetic groups for hybridization are not limited to protein complexes, but even include whole cells such as in cyanobacteria [35,36].

Biological photosynthesis features a series of light conversions and electron transporting steps that begin with the initial photoreaction in PS II, multi-subunit chlorophyll complexes, followed by excited electrons hopping to plastoquinone, to cytochrome b_6f, to plastocyanin, to ferredoxin, and to PS I, which collaborate sequentially to provide efficient electron collecting pathways [3,14,23,37–39]. (Fig. S1a) Therefore, compared to utilizing one extracted photosynthetic apparatus such as PS I or PS II, using whole cells that retain a selective electron path holds advantages. In most applications, however, whole cell processes are severely restricted since cell dimensions are often too large to be assembled in a controlled manner and the photosynthetic functions are limited to the life span of the cells [31]. Furthermore, two thirds of photoconversion energies must be diverted to cellular breathing and metabolism [40]. Therefore, the use of thylakoids, equipped with all the advantageous electron harvesting paths but free of unnecessary cellular growth consumables, is desirable for the photosynthetic functional unit that can be organized into an artificial cell-free photosynthetic film.

In transferring photosynthetic thylakoid biomaterials into an artificial device, molecular control in organizing schemes is necessary to preserve complex nanostructures and functionalities [41–43]. Randomly occurring synthesis or mix-and-stirring hybridization techniques are less preferred because lost orientation of biocomplexes causes degraded functionality as well as structural instability [37,41,44]. Thylakoids are valuable molecular units in which highly efficient oxygenic photosynthetic proteins are intensely clustered. This advantage is eclipsed by fast degradation of the photosynthetic proteins that occurs outside of the living cells and even during leaf senescence by structural unfolding [45]. Thus, transferring thylakoids into a biomimetic stabilizing matrix is a prerequisite to achieve durable photosynthetic activities in a biotic–abiotic hybrid device. Here, LBL assembly, a bottom-up molecular multilayering assembly technique [46,47], was adopted to protect the fragile photosynthetic activity of the thylakoid proteins. LBL assembly is used in a wide range of applications due to advantages of being simple, easy to fabricate, robust, and inexpensive to process [48,49]. Charged polyelectrolytes [50,51], biomaterials [52,53], inorganic nanomaterials [54,55], and even uncharged macromolecules [56,57] can be LBL assembled into a permanent and stable nanocomposite film [58,59]. Zhao et al. LBL assembled a photosynthetic reaction center (RC) protein, which was separated from a *Rhodobacter sphearoides* (RS601), with poly(diallyldimethylammonium chloride) (PDDA). The photocurrents of the PDDA/RC LBL films demonstrated a linear increase against the number of adsorbed layers [24]. For thylakoid LBL membranes, Abe et al., isolated thylakoids from the cyanobacterium *Spirulina platensis* and poly(ethyleneimine) (PEI) as LBL components to fabricate herbicide biosensors [25]. A recent work with PS II enriched thylakoid and PEI was also reported for possible application in an herbicide monitoring biochip [15]. The immobilization of thylakoids through LBL assembly is desirable to enhance structural stability and functional durability in addition to producing denser thylakoid packing [60]. Towards this end, we matched a molecular charge distribution of thylakoid with two different types of polymers, insulating PEI and conducting polyaniline (PANI) to increase the thylakoid loading density for LBL assembly. In this study, the resulting thylakoid LBL composite showed durable photoactivity as well as structural integrity up to 130 h in a solution at room temperature. Furthermore, the thylakoid composite showed inherent biocompatibility with animal neural cells, which was proven by PC 12 cell attachment as well as cell differentiation regardless of light shedding on them.

2. Experimental

2.1. Materials

Spinach, *Spinacia oleracea*, was purchased from a food supermarket. Poly(ethyleneimine) solution (PEI solution, ~50% in H_2O) and Polyaniline (PANI, Emeraldine base, average $M_w \sim 65{,}000$) were purchased from Sigma–Aldrich. Ethyl alcohol (assay 99.0%) was supplied by Daejung Chemicals & Metals Company Ltd. Phosphate buffered saline (1x PBS, tablet) and 2,6-dichlorophenolindophenol-sodium salt hydrate (DCPIP, $M_w = 290.08$ g/mol) were purchased from Sigma–Aldrich and used as received.

2.2. Extraction of thylakoid membranes

Thylakoid membranes were extracted from the spinach via a modified centrifugation method [28,31,61,62]. Spinach leaves were physically squeezed in a diluted ethanol aqueous solution. The diluted ethanol treatment not just facilitated extraction of photosynthetic complexes but also increased permeability of thylakoid membranes [63]. The solution was centrifuged multiple times to remove unnecessary cellular components utilizing the Sigma-aldrich chloroplast isolation kits.

2.3. Immobilization of thylakoid membranes

Nanostructured, thin conducting polymer-thylakoid composite films were prepared by a molecular layer-by-layer (LBL) assembly technique [41,47]. A glass substrate was dipped in a positively charged 0.5 wt% PEI or 0.5 wt% conducting polymer, PANI solution. The excessive molecules on the substrate were eliminated by successive washing and drying steps. The substrate was then dipped in a thylakoid solution followed by several washing and drying steps. These processes were repeated for a desired number of times. (Fig. 1)

2.4. Characterizations

The zeta-potential provides the surface charge information of particles by measuring their velocity of movement in solution, which indicates particles' cohesion, interaction, and surface modification in a liquid dispersion. Zeta-potential was measured by the Otsuka electronics Zeta-potential & Particle Size Analyzer (ELSZ-2).

Light absorption spectra were measured by a Varian UV–vis Spectrophotometer (Cary 100). The composite samples were prepared on glass substrates and quartz cuvettes which were cleaned using the piranha solution, a mixture of sulfuric acid and hydrogen peroxide for cleaning organic residues. The quartz cuvette was used to monitor thylakoid activity by measuring DCPIP (0.01 mg/ml) color changes in photosynthesis and the absorbance peak of DCPIP was shown (was observed?) on 600 nm wavelength region by