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Sandwiching multiple dehydrogenases and shared cofactor between double polyelectrolytes for enhanced communication of cofactor and enzymes

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ABSTRACT

Regeneration and reutilization of cofactors remain as the biggest challenge facing the industrial application of cofactor-dependent redox, while efficient communication and synergism between enzymes and cofactors are the major requirements for implementation of such processed. Inspired by swinging arms facilitating multistep catalytic transformations in nature, polyelectrolytes-based artificial swinging arms were introduced to a multienzyme system by coaxial electrospinning technology. The poly(allylamine hydrochloride) (PAH), pre-dissolved in the core-phase solution, would penetrate across the shell of hollow nanofibers, thus act as artificial swinging arms and enable effective tethering and retention of the enzymes and cofactors on the outer surface of nanofibers *via* the ion-exchange interactions. With further intercalary insertion of another negatively charged polyelectrolyte poly(styrene sulfonate) (PSS), a sandwich-like structure was formed on the outer surface of the hollow nanofiber. It was found that PSS layer not only effectively prevented the leaking of enzymes and cofactors, the double-polyelectrolytes also imposed crowding and confinement effects on the sandwiched multienzymes, thus the communication of the dehydrogenases and the shared cofactors were further facilitated. The methanol yield obtained with the sandwiched multienzymes system reached to 98.27%, which was 2.72-fold higher than that of the free system, and even 1.52-fold higher than that without PSS layer.

1. Introduction

The exploration and application of multistep biocatalysis, especially those involving cofactor-dependent enzymes, composes an indispensable part of today's endeavors in green chemical manufacturing [1–4]. While efficient regeneration and reutilization of the expensive cofactors which are consumed at stoichiometric ratios is critical to the economic viability of industrial scale biotransformation using oxidoreductases [1,5,6]. Nowadays, though different approaches based on photochemical and electrochemical principles have been widely explored for cofactor regeneration, the enzymatic methods by coupling two or more oxidoreductases performing redox reactions is particularly preferred for industrial processes due to its high selectivity and efficiency [1,5,7]. Inspired by multienzyme system in cell, NAD(P)H and enzymes have been co-immobilized on solid matrixes allowing their solid-phase recycling and reusability for several operational cycles [1,8]. These heterogeneous systems, however, have shown several disadvantages: the preparation of these systems is complicated and hardly scalable [9], the reusability of these systems is limited [10,11], the enzymatic activity with the immobilized cofactors is low [10], and the turnover numbers of the immobilized cofactors are poor [12]. In that case, efficient shuttling of the shared cofactor between active sites of coupled oxidoreductases is the pre-requisite to success in construction and application of multi-enzymatic system invloving cofactor regeneration [13–15]. Nevertheless, how to create an *in vitro* artificial interface suitable for cofactor-enzyme interactions and the consequent hydride transferring between two coupled dehydrogenases is quite challenging, especially when the catalysts are tethered to solid state supports for heterogeneous catalysis.

In naturally occurring multienzyme complexes, swinging arm plays an important role for the multistep catalytic transformations [13]. *In vitro*, DNA has been explored as nano-scaffolds to create a multi-enzyme complex, whose activity could be manipulated by

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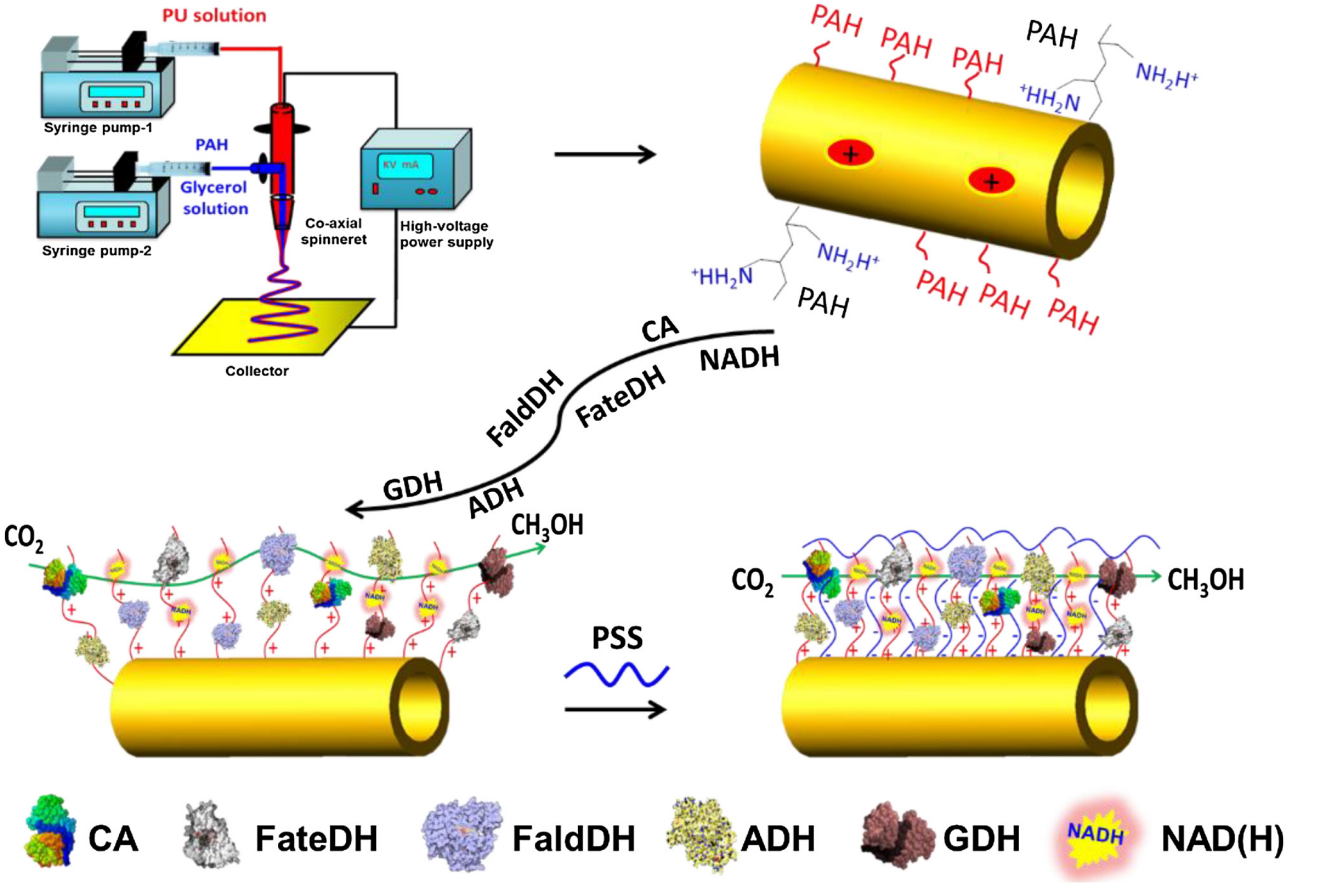
the length and position of the DNA-based artificial swinging arm [14]. Some polymers, such as polyelthene glycol (PEG), have also been used as spacers linking enzyme/cofactor and solid matrix. Improved activities have been reported for the immobilized multienzyme prepared with appropriate spacers of different lengths, with which the molecular interactions of cofactors and enzymes were facilitated [16,17]. Besides, some special proteins also allow the sequential and site-specific assembly of a dehydrogenase-based multienzyme cascade on the yeast surface using the high-affinity interactions. The position-specific and sequential assembly of multienzyme on the displayed scaffold facilitates the efficient substrate channeling, resulting in higher activity than that of the noncomplexed enzymes [18].

Using artificial swinging arms or appropriate spacers have shown effectiveness in facilitating the shuttling of cofactors; while excluded volume effects, which can be reflected either as macromolecular crowding or confinement effects, might provide alternative strategy to improve the intermolecular communication and synergism among enzymes and cofactors [19–22]. *In vivo*, biological cells performing multi-step biochemical reactions catalyzed by group of enzymes in a crowded and confined situation constitute the crucial part of life [23,24]. Despite there are no specific interplays existing among enzymes and other cellular molecules, the mere truth is that the activity and stability of the enzyme is influenced by the space available for its move around [25–30]. Intermolecular communication of enzymes has been found spatially enforced by densely packing enzyme cascade within the capsid architecture of the bacteriophage P22 virus-like particle (VLP) through imposing an *in vitro* "cell-like" crowding and confinement effect on the encapsulated cargos [31–33]. Some other materials-based architurecters, like agarose gels [34], nanofluidic gradient mixer [35], nanoporous materials [20,36,37], and polymer capsules [38], were also applied as artificial nanoreactors mimicking the crowded and confined micro-environment, where normal solution reaction kinetics is no longer valid. Although these methods were proven successfully to some extent, the enzymatic activities with the immobilized cofactors were generally lowered, and the preparation of these systems is complicated and usually hardly scalable.

In one of our previous work, a cationic polyelectrolyte doped polyurethane (PU) hollow nanofibers fabricated by a facile co-axial electrospinning technology have been proven as an ideal scaffold for spatially assembly of multiple enzymes involving NAD(H) [15,39,40]. By simply co-dissolving the poly(allylamine hydrochloride) (PAH) and the four dehydrogenases/NADH in glycerol water mixture solution as the core-phase solution of co-axial electrospinning, *in situ* encapsulation of the multienzyme system inside lumen of the hollow nanofibers were realized [15,39]. The linear polyelectrolyte PAH, which can penetrate across the PU shell, provided positively charged ionizable groups along the shell of the hollow nanofibers and acted as artificial swinging arms to accomplish effective tethering of the negatively charged small molecular NAD(H) to the inner wall of the lumen by ion-exchange interactions between them [15]. Compared with the DNA-based swinging arms, PAH-doped hollow nanofibers were much easier and flexible in preparation and application. What is more important, the shuttling of cofactor between enzymes was efficiently enhanced by the PAH spacers, as evidenced by about 3-fold increase in methanol yield as compared with the solution-based system [15].



Scheme 1. Schematic illustrations of the setup for coaxial electrospinning and sandwiching multiple dehydrogenases and shared cofactor between double polyelectrolytes for enhancing communication of tethered cofactor with multiple dehydrogenases.