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Full Length Article

Investigation of polydopamine coatings by X-ray Photoelectron Spectroscopy as an effective tool for improving biomolecule conjugation

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ABSTRACT

Polydopamine (PDA) films have attracted a rapidly increasing research attention during the last years due to its simple and rapid deposition under alkaline conditions in substrate independent manner providing a universal coating for materials with different chemical and physical properties. Furthermore, this polymerized layer is enriched with functional groups that enable immobilization of primary amine or thiol-based biomolecules via a simple dipping process. Although these aspects justify PDA wide and successful application as a versatile coating for biomolecule immobilization, several aspects have not been deeply investigated leaving some key details unclear and thus limiting PDA practical applications. A number of approaches are commonly used for the growth of PDA, but the effect of deposition conditions on film properties, which in turn influence biomolecule immobilization has not been systematically investigated yet.

In the present work, an extensive characterization by X-ray Photoelectron Spectroscopy (XPS) is performed on PDA coatings grown under different experimental conditions. Comparison of XPS data about elemental composition, distribution of functional groups and thickness of PDA coatings provided valuable information for identifying more suitable PDA coating for biomolecule anchoring, further explored by in vitro experiments.

1. Introduction

Immobilization of biomolecules onto surfaces represents a crucial aspect in several fields as molecular biology, medical diagnostics, biotechnology and tissue engineering. Biomolecular approaches to surface modification are of special importance today owing to the explosion of knowledge of cell and tissue biology coupled with the enhanced capabilities of surface science technology. Also, attachment of biomolecules on different surfaces is an integral part of research for the development of biosensors in the form of chips in the context of nanoscale detection of pathogens and other metabolites related to issues of human health [1]. Biomolecule immobilization represents a key aspect also in the design of protein biochips, which offer a fast, high-throughput means to profile disease-related proteins or to study protein–protein and protein–drug interactions [2]. Strategies for biomolecule anchoring on substrates are generally based on covalent or non-covalent reactions, the latter presenting the advantage of allowing reversible immobilization under mild conditions. Among non-covalent methods, simple physical adsorption is widely applied consisting, for example, in biomolecule immobilization on a porous polymeric material by electrostatic and hydrophobic interactions [1] or within an electrodeposited polymer layer [3]. However, molecule attachment can be weak and pH dependent and the process of molecule diffusion in and out the layer should be critically considered. Few examples of films prepared by Langmuir-Blodgett (LB) technique [4] are also proposed to this aim [5,6], but the approach suffers from possible biomolecule denaturation when LB film is spread at air-water interface. In contrast, covalent immobilization of molecules onto surfaces typically relies on conjugation reactions between "active" functional groups, such as N-hydroxysuccinimide (NHS) [7] or maleimide [8] and companion target moieties, such as amines and sulfhydryls. For reactions involving biomolecules performed in aqueous solvents, susceptibility of NHS, maleimide, and other activating groups to hydrolysis during storage and reaction can lead to low efficiency of surface bioconjugation [9,10]. Self-assembled monolayer (SAM) approach is also used for covalently anchoring thiol-containing monomolecular layer on a metal

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surface, with subsequent attachment of biomolecule to the exposed functional terminus of SAM via covalent or non-covalent interaction. Also in this case, non-covalent attachment usually requires a milder protocol, but the concentration of active substance on the matrix can gradually diminish on repeated use and washing [1].

The above reported examples of bioimmobilization evidence that surface modification approaches not only present specific drawbacks limiting their practical applications, but, in general, are developed case-by-case, and depend on the relationship between material specific properties and tethering molecules. Moreover, these methods often involve time-consuming synthesis, requiring multi-step procedures [11].

Developing a facile and versatile surface functionalization protocol toward different substrates without the aid of complex instrumentation, limitations of the nature of the substrate, and the need of multi-step procedures will thus have a great impact on modern chemical, biological and material sciences. A pioneering work in this direction was performed in 2007 by Messermith and co-workers [12] who demonstrated that dopamine could self-polymerize on virtually all types of inorganic and organic surfaces, similarly to the case of adhesion of mussels, by simple immersion of substrates in an aqueous dopamine solution buffered at pH 8.5, with a control of polymer thickness as a function of deposition time. Since then, polydopamine (PDA) straightforward and versatile deposition has attracted enormous attention leading to a massive use of PDA polymerization as a generic way to fabricate multifunctional substrates with specific properties. Such a huge success of PDA coating relies also in its chemical reactivity: functional groups naturally present in polymer structure are capable of reacting with a wide range of molecules, thus enabling simple post-synthetic functionalization procedures. In particular, reaction of PDA with amine- and/or thiol-containing molecules is nowadays widely and successfully used as a facile and versatile strategy for immobilizing biomolecules onto PDA surface. It is reported that [13] the reactivity of PDA with amine-containing molecules is a function of cathecol/quinone equilibrium in polymer matrix and of the pK_a of the amine group. Under basic conditions, the cathecol can be oxidized into the corresponding quinone, which can then react with amine groups by means of a Schiff base reaction or via a Michael-type addition pathway. In the case of thiol-containing molecules, interaction with PDA occurs through Michael addition reaction. It should be highlighted that the reactions toward either amine- or thiol-containing molecules are facile, without the need of any harsh reaction condition or complicated equipment, occurring by simply exposing PDA layer to these agents at room temperature under basic conditions in aqueous environment.

Such fascinating PDA coating properties have opened the way to a plethora of applications based on the beneficial interaction between PDA and biomolecules including sensing, drug delivery and tissue engineering [14]. Easy conjugation of enzymes or DNA to PDA layer, with their activity retained, is the motivation of the large use of PDA as a substrate in biosensing applications, exploiting different transduction mechanisms spanning from electrochemical to optical [13]. Functionalization of PDA with diverse drug molecules, together with the possibility of obtaining PDA in the form of nanocapsules by using template-mediated polymerization [15], make PDA an highly suitable material for drug delivery, also promoted by its high water solubility, biocompatibility and biodegradation and by its tunable charged stated. PDA biocompatibility and biodegradations are among the main advantages also in applications in tissue engineering, where potentially cytotoxic materials, like stainless steel or poly(tetrafluoroethylene) [16], can be made biocompatible just by simple coating with PDA. Nevertheless, the development of PDA in the field of tissue engineering has been prompted mostly by facile immobilization of bioactive molecules. Specifically, PDA can serve as a bridging chemical for ad-layer formation of different cell adhesive moieties through covalent interaction between catechol and amine or thiol groups present on biomolecules [17–20]. Various polymer substrates have been modified with PDA and subsequently functionalized with multiple adhesion peptide sequences derived from fibronectin, laminin, or growth factors [21] with the aim to promote cell attachment or proliferation and/or to modulate specific cellular responses.

Although all of the above results clearly imply that PDA-mediated surface modification techniques can offer a versatile platform highly suitable in many application fields, some aspects concerning its conjugation with biomolecules still remain not deeply investigated. It should be considered that a number of methods have been established for PDA growth (different buffer, oxidant, polymerization duration, etc.) and that different polymerization conditions can influence equilibria/distribution of PDA chemical components which, as explained above, are involved in biomolecule immobilization. Moreover, as widely demonstrated [22–24], polymerization conditions greatly influence also PDA deposition kinetics and, thus, the resulting polymer thickness which in turn can impact on bioconjugation process. Other questions about the interaction PDA-biomolecule still remaining uninvestigated include, for example, the evaluation of conjugation density and the immobilization stability under physiological conditions, the latter representing a key issue in PDA application in cell and tissue engineering. It is thus evident that a detailed investigation of such aspects could fulfil the full potential of PDA as a universal coating for biomolecule attachment, thus further extending PDA applications in biomedicine-/sensing-related fields allowing a scale-up to a commercial level in the near future.

In the present work, a detailed investigation by X-ray Photoelectron Spectroscopy (XPS) of PDA coatings deposited by different synthetic schemes is performed aimed at investigating film thickness and chemical composition as a function of polymerization conditions. Comparative spectroscopic analysis of PDA films revealed significant differences in terms of deposition kinetics and abundance of chemical components and allowed selection of synthesis conditions making PDA chemical structure richer in functionalities mainly involved in conjugation of biomolecules. The high suitability of the selected PDA film for bioconjugation was verified using a biomolecule conjugated to HRP (Horseradish Peroxidase) or to fluorophore, obtaining also an estimation of immobilization time-stability within 4 weeks and a quantitative evaluation of immobilization extent. Moreover, further insight on biomolecule anchoring was provided by the comparison of XPS data on PDA samples before and after interaction with biomolecule.

2. Experimental

2.1. Chemicals and materials

Dopamine hydrochloride, Tris(hydroxymethyl)aminomethane (Tris), and other chemicals and reagents were purchased from Sigma (Italy) and used as received without further purification. All solutions are prepared with ultrapure water (Millipore Milli-Q, 18.2 MΩ/cm).

2.2. Deposition of PDA films

PDA film was grown on gold sheet or glass slides by immersing the substrate into a freshly prepared dopamine hydrochloride 2.0 mg/mL under different experimental conditions: (1) Tris buffer solution 10 mM pH 8.5 at ambient atmosphere; (2) Tris buffer