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A facile synthesis of polydopamine/TiO_2 composite films for cell sheet harvest application

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ABSTRACT

This study presents a convenient and versatile way to prepare functionalized composite polydopamine/titanium dioxide (PDA/TiO_2) film on polystyrene (PS). First, polystyrene substrate was immersed in dopamine chloride solution, and then collosol containing TiO_2 and water was spun on it, to produce uniform, continuous PDA/TiO_2 composite films. The thickness of film was controllable by adjustment of the spin speed. It was found that the films were strongly adhered on the PS substrate, with peel strength and shear strength of 2.78 MPa and 37.78 MPa, respectively. After 20 min of ultraviolet (365 nm) illumination, over 90% of fibroblasts and 77% of osteoblasts detached from the PDA/TiO_2 composite film. Additionally, the detached cells showed good viability, allowing further culture and applications. This preparation method could be widely applied for cell and cell sheet harvesting directly from PS-based culture wares.

1. Introduction

In pastdecades, the use of three-dimensional biodegradable polymer scaffolds with seed cells has become one of the most common methods for tissue engineering, allowing cells to migrate and proliferate to substitute the scaffold when degradation occurs [1,2]. However, there are still some problems limiting widespread applications, including inflammatory response caused by degradation products. To address this issue, several new ideas have been tested for improved tissue engineering [1]. One of these is cell sheet tissue engineering, in which in vitro-obtained cell sheets are used for scaffold-free tissue engineering. Therefore, determination of the best strategies to obtain different kinds of cells/cell sheet has become an important goal of current research [3–5].

For cell sheet harvest to be effective requires the growth and detachment of cell sheets from specific culture surfaces [6]. Because cells are basically cultured on plates made of polystyrene (PS), it would be ideal cell sheets can be directly harvested from the PS plate. Therefore, the appropriate surface modification of polystyrene is required. To date, only a few low-invasive or non-invasive methods have been proposed to harvest cell sheets by promoting cell detachment, including pH change-induced [7,8], electricity-induced [9], temperature-induced [10,11], magnetism -induced [12], and light-induced [13,14], processes. The intensity and direction of light is easy to control as a way to change surface properties, making it a good choice as a switch. Ultraviolet (365 nm) illumination does almost no harm to cells when the illumination time is short, and simple light illumination is an efficient and safe cell sheet harvest method [15,16]. Cell sheets detached from TiO_2 nanodot films showed good cell viability after illumination [13]. Titanium dioxide was one of the most important materials which can be extensively utilized in the fields of biomaterials [17–19], due to its excellent advantages such as stability, nontoxicity, low cost, unique photo-physical and photo-chemical performance [20–22]. In general, the combination of titanium dioxide and polystyrene should be beneficial for cell sheet harvest, providing a more direct and efficient way to obtain cell sheets from the cultured plate via UV illumination.

Recent studies have revealed that the adhesive ability of mussel might be related to the special amino acid composition of proteins [23,24]. Inspired by mussels' property, catecholic compound dopamine (DA), a mussel adhesive protein molecule [25–27] has been used in various applications. DA can attach to all kinds of organic and inorganic surfaces in alkaline condition because of its abundance of bioactive groups, such as OH^-, NH^{2-} and catechol moieties [28–30]. The self-polymerization of DA makes it commonly used for surface modification [27,31], and was selected here for the immobilization of TiO_2 particles onto polystyrene [32–34].

Therefore, in this study, a PDA/TiO_2 composite film was prepared on PS substrate based on dopamine chemistry. The surface properties

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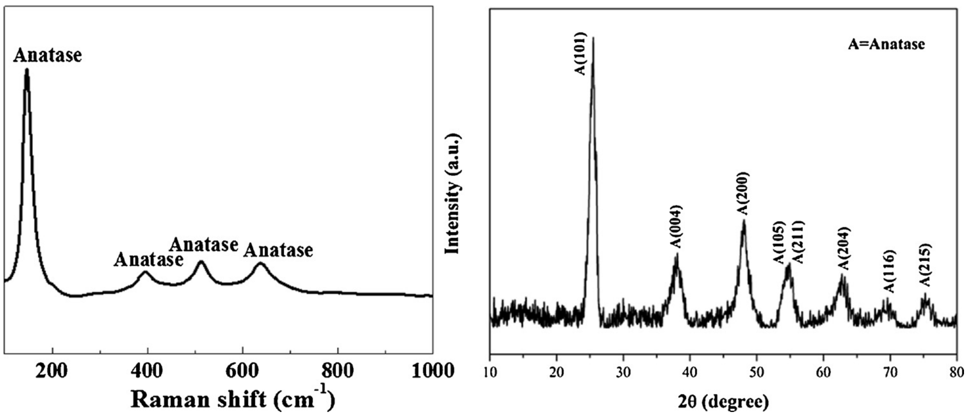

Fig. 1. Typical Raman spectrum and XRD pattern of PDA/TiO_2 composite films.

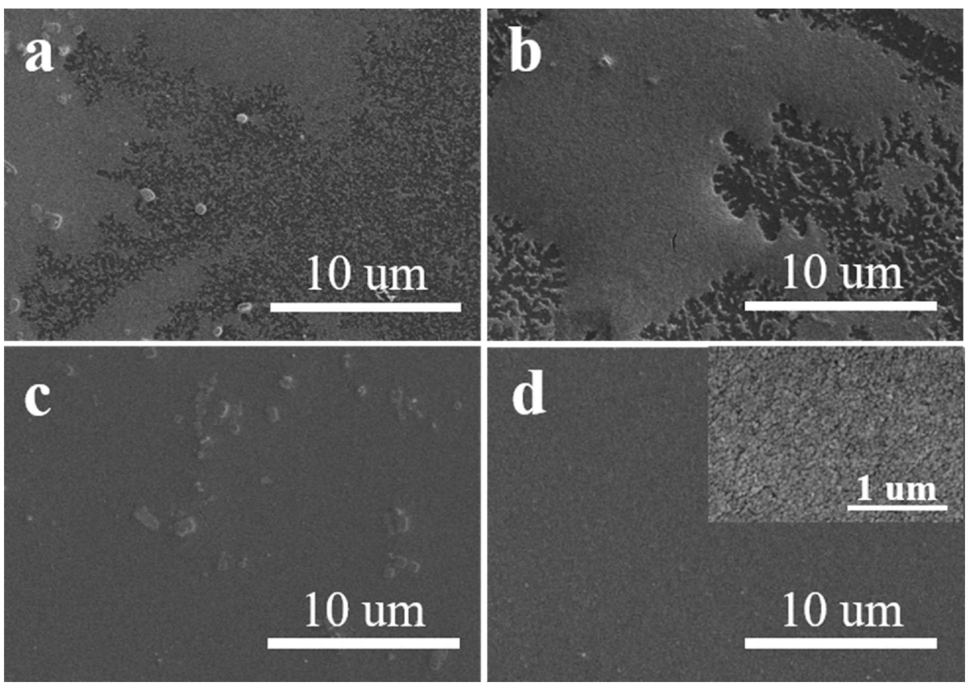

Fig. 2. SEM micrograph and detailed morphology of PDA/TiO_2 composite films prepared at different molar ratios (TiO_2/H_2O), 0.94% (a), 1.41% (b), 2.82% (c), 5.64% (d).

and light-induced cell sheet detachment performance were then characterized and evaluated.

2. Materials and methods

2.1. Preparation and characterization of PDA/TiO_2 film

PDA/TiO_2 film was prepared on polystyrene (PS) substrate using a molecular self-assembly method. Prior to constructing the modification film, the PS substrate was cleaned by ultrasonic treatment and dried. The dopamine solution was prepared by dissolving dopamine hydrochloride (2 mg mL^{-1}, Aladdin) in Tris buffer solution (10 mM, pH 8.5). Then, the PS substrate was immersed in DA-Tris buffer solution for 12 h with stirring. After polymerization, the PS substrate was carefully removed from the beaker, hung for 1 min to remove the extra solution on the surface, and then dried in the oven at 37 °C for 5 min. to preserve a half-wet state. Before that, deionized water and TiO_2 collosol were mixed under different TiO_2/H_2O molar ratios (5.64%, 2.82%, 1.41%, 0.94%), and ultrasonically stirred for 1 h to prepare the coating solution. The TiO_2/H_2O collosol was spin-coated at different speeds onto the PS-PDA substrate. The PDA/TiO_2 composite films were finally obtained after further drying at 60 °C for 1 h.

The surface morphology and the cross section of PDA/TiO_2 composite films at different molar ratios and spin speeds were characterized by scanning electron microscopy (SEM, SU-70, Hitachi). The general distribution of PDA and TiO_2 in the film was examined by energy dispersive X-ray spectroscopy (EDS), and the crystal phase of TiO_2 was analyzed by X-ray diffraction (XRD). The chemical composition of the surface after each step was characterized by X-ray photoelectron spectroscopy (XPS). Considering future use of UV light on PS substrate, the transmittance of ultraviolet light was investigated by UV–vis spectrophotometer (UV–vis).

2.1.1. Wettability

A sessile drop method was utilized to measure the water contact angle (WCA) with a contact angle meter (Dataphysics, OCA20). A droplet (1 μL, water) was dropped on a homogeneous film surface and then the WCA was determined until no change was observed. Before wettability test, PDA/TiO_2 was incubated in the dark overnight and exposed to UV (365 nm) light for 20 min when the WCA was measured.