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# Paclitaxel/hydroxyapatite composite coatings on titanium alloy for biomedical applications

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ABSTRACT

In order to reduce the side effects of chemotherapy, target therapies have been spotlighted. In this study, paclitaxel, the drug for cancer treatment, is electrochemically deposited on Ti alloy as vascular stents for the tumor localized therapy by sustaining drug releasing to achieve the cancer cells apoptosis or the prevention of cancer metastasis. In the experiment, cathodic polarization tests coupled with electrochemical reactions were analyzed to speculate the deposition mechanism, and the field emission scanning electron microscope (FESEM), focused ion beam (FIB) system and Fourier transform infrared spectroscopy (FTIR) to observe the surface morphology and analyze constituent elements. A spectrophotometer (UV visible spectrometer) was used to measure drug loading and release. Finally, MTT Assay was carried out to analyze the cell viability for drug efficacy. It is concluded that paclitaxel can be successfully deposited on the titanium alloy by electrochemical method. Besides, the post-hydroxyapatite coated specimen with high porosity can enhance the drug loading from 395 ± 95 μg/cm$^2$ to 572 ± 99 μg/cm$^2$, a lower burst release in the first day, a higher sustaining release rate in a month, and the more complete drug release. All results indicate that the paclitaxel/hydroxyapatite composite coating by the electrochemical deposition method is much more effective and promising.



## 1. Introduction

With the increase of human life span and the variation of lifestyle, cancer morbidity continues to rise all over the world [1]. Cancer cells, which can invade any part of the body by spreading to other organs through the blood or lymph system, are deadly [2].

Traditionally, cancer therapies include the initial chemotherapy to reduce tumor cells, the surgery to remove them, and finally by the chemotherapy or radiation to exterminate them [3]. In fact, chemotherapy affects people systemically, which kills not only tumor cells but also normal cells like immune cells. In many situations, people can't help but stop chemotherapy treatment before cancer cells eradicated due to severe side effects including mouth sores, hypersensitivity reactions, nausea, vomiting, diarrhea, low blood counts and hair loss [4].

The target drug delivery system is a direct way for transmitting drug to the certain body area by increasing the local drug concentration. The major advantage of the target drug delivery system is dramatically reducing the therapy doses needed and the side effects of drugs [5]. However, the local drug delivery is not necessary to replace the traditional chemotherapy. It can also be used as adjunctive therapeutic way [6]. The ultimate goal of target drug delivery system is to extend the drug releasing time, succeed in locating the target with neuroprotection, and finally lead to greater efficiency and higher cure rate of the treatment [7].

Paclitaxel (PTX) serves as a mitotic inhibitor for cancer treatment. It plays an important role in the inner microtubules polymerization and stabilization, firmly fixing tumor cells in the mitotic phase without microtubules separation, blocking cells in G2 and M phase of the cell cycle, and resulting in cancer cell death by terminating the cell replication [8]. Unfortunately, a surfactant vehicle in commercial injection is necessary due to the low solubility of PTX. Based on a lot of studies, Cremophor EL® (CEL), which is composed of both poly-oxy-ethylated castor oil vehicle and ethanol (1: 1, V/V), is determined as solvent of PTX injection. Cremophor EL® is a carrier for the preparation of various hydrophobic drugs. The worst side effect of its use has been associated with severe anaphylactoid hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy [9–11]. Therefore, how to reduce or even avoid the side effects of PTX treatment is critical clinically.

Recently, some drug loading methods have been developed, such as liposomes and micelles. However, some drawbacks of large unilamellar liposomes are uncovered, including immediate uptake into the reticuloendothelial system, and the low stability in vitro [12,13]. Though micelles have revealed better stability, they are limited by some shapes and mechanical properties [14].

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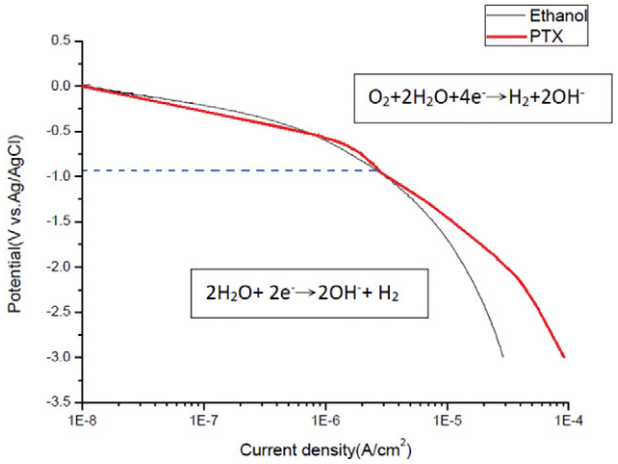


**Fig. 1.** Cathodic polarization curves of Ti alloy specimens in 95% ethanol and PTX alcoholic solutions.

As to the medical applications, there is a kind of PTX-eluting stent for coronary lesions to reduce restenosis. The most methods of drug loading are immersion and spraying. The stent contains PTX 100 μg/cm$^2$ [15,16]. If the dose of the stent can be further enhanced, it could be implanted in blood vessels near tumors to inhibit the cancer cell proliferation. This can not only achieve the target treatment but also avoid the side effects of polyoxyethylated castor oil.

Hydroxyapatite (HAp) is the major component of the human bone structure with chemical formula $Ca_{10}(PO_4)_6(OH)_2$. It has been applied in many references for covering or transferring PTX [17–20].

The previous studies have shown that the drug/HAp composite coatings by electro-deposition can not only be carried out on implants with complex shape but also reveal sustaining drug release [21,22]. In this study, the PTX was dissolved in ethanol without castor oil and then electrochemically deposited on HAp-coated Ti alloy to optimize the drug loading and releasing, and even avoid the possible side effects.

## 2. Materials and methods

### 2.1. Sample preparation

The ASTM-136 Ti-6Al-4V rod was cut into discs with 14.0 mm diameter and 1.0 mm thickness. All specimens were polished by SiC paper from 600 to 1200 grid. Soon after, all specimens were degreased by detergent, further ultrasonically cleaned by ethanol, and then dried by air.

### 2.2. Cathodic polarization tests and paclitaxel deposition

The Ti-6Al-4V specimen was the working electrode, the platinum electrode the counter electrode, and the saturated Ag/AgCl electrode the reference electrode, respectively. Cathodic polarization test were carried out in 0.01% PTX (AK Scientific) added and 0.01% PTX with 0.01% KCl added in 95% alcohol solutions respectively, the former assigned to PTX and the latter PTX/KCl alcoholic solutions from the open circuit potential to −3.0 V (vs. Ag/AgCl electrode), at a scanning rate of 0.167 mV/s by using EG & G VersaStat™II and Power Suite software 352 (Princeton, USA). The pH values (pH meter; CYBERSCAN-500, USA), $O_2$ concentration ($O_2$ meter) and conductivities (conductivity meter; COSAN CON6, USA) of electrolytes were measured.

The PTX deposition was carried out at −1.8 V (vs. Ag/AgCl electrode) for 1200 s. The hydroxyapatite (HAp) composite deposition was carried out at 1 mA at 65 °C for 600 s in a mixed solution containing 0.042 M $Ca(NO_3)_2 \cdot 4H_2O$ (SHOWA, Japan) and 0.025 M $NH_4H_2PO_4$ (SHOWA, Japan) assigned to CaP solution before PTX deposited [23].

**Table 1**
pH value, $O_2$ concentrations, and conductivities in 95% ethanol, PTX, KCl, and PTX/KCl alcoholic solutions.

| Solutions | pH values | $O_2$ concentrations (ppm) | Conductivities (uS/cm) |
|---|---|---|---|
| 95% ethanol | 7.07 | 8.57 | 2.17 |
| PTX | 6.90 | 8.57 | 7.02 |
| KCl | 7.09 | 8.38 | 60.5 |
| PTX/KCl | 6.91 | 8.47 | 61.5 |

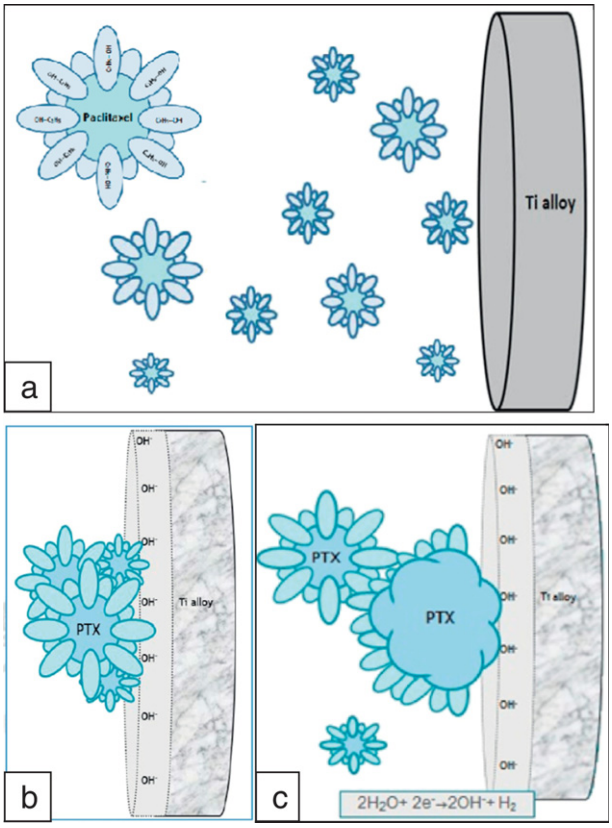


**Fig. 2.** Schematic illustration of PTX deposition (a) ● represents the PTX molecule, ⬬ represents the $C_2H_5OH$ molecule, and each single PTX molecule is surrounded by many $C_2H_5OH$ molecules, (b) they adsorb on Ti alloy after the electrochemical reaction, and (c) finally more PTX molecules are merged into bigger spheres and coated on Ti alloy surface through the electrochemical deposition way.

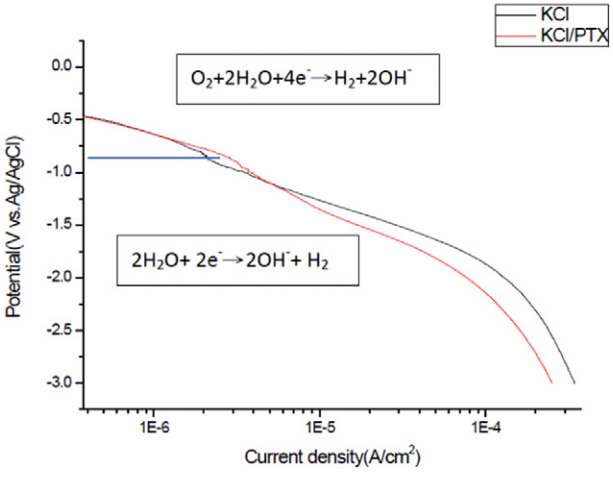


**Fig. 3.** Cathodic polarization curves of specimens in KCl and PTX/KCl alcoholic solutions.