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Controlled release of titanocene into the hybrid nanofibrous scaffolds to prevent the proliferation of breast cancer cells

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ABSTRACT

Electrospun hybrid nanofibrous scaffolds have gained much importance in the field of tissue engineering and drug delivery applications owing to its multifaceted properties. In this study, the properties of composite polycaprolactone (PCL)/silk fibroin (SF) nanofibrous scaffolds was investigated as a potential scaffold for cell growth and also a drug eluting mat to control the proliferation of MCF-7 cells. Titanocene dichloride was chosen as the model drug to study its antitumor efficacy on MCF-7 cell lines. Fascinating properties relating to crystallization of silk fibroin and binding of drug has also been discussed for the controlled release of drugs. The presence of amino acid residues in silk fibroin plays a big role in the cell-scaffold interaction, the nature of drug binding and also its release characteristics to control the cell proliferation. Studies on material properties for the hybrid nanofibrous scaffolds showed interrelated changes in fiber diameter and mechanical behavior for the drug loaded nanofibers. Significant decrease in fiber diameters from 352 ± 52 nm to 281 ± 44.5 nm and sharp increase in tensile stress from 4.5 MPa to 50.3 MPa was observed for 0.03% drug loaded scaffolds with respect to PCL fibers. Cell viability and cell morphology study was performed to analyze the effect of different concentrations of titanocene dichloride loaded on PCL/silk fibroin nanofibrous scaffolds. Maximum cell viability inhibition percentage of change 26.93% was obtained for 0.03% titanocene with respect to 0.01% on day 3. The obtained results proved that the drug loaded hybrid mat to control the proliferation of MCF-7 cells at different time points and serve as a model for cancer therapy.

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1. Introduction

Electrospinning is a versatile technique for the fabrication of two dimensional nanomaterials (Ramakrishna et al., 2005). Electrospun nanofibers have been extensively used in biomedical applications and *in vitro* studies such as mimicking the native extracellular matrix (ECM) for tissue engineering/regenerative medicine and also therapeutic patch for drug delivery (Venugopal et al., 2008). Synthetic bioresorbable poly(e-caprolactone) (PCL) has been the most widely used material for biomedical applications because of its easy electrospinnability and biodegradable properties. However, the intrinsic hydrophobic character of synthetic PCL and lack of binding sites failed to provide a desired microenvironment for cell adhesion and proliferation (Venugopal et al., 2006). To compensate the need for improved biocompatibility with the synthetic polymers, incorporation of natural polymers like silk fibroin, aloe vera, hyaluronic acid and collagen, has been the commonly adopted protocol, owing to its ability to impart surface functionalities which introduce binding sites to the scaffolds for cell adhesion, proliferation and migration (Nair et al., 2006). Silk fibroin being an FDA approved biomaterial, biocompatibility studies with electrospun nanofiber blends/composites of silk fibroin/PCL (Li et al., 2011), silk fibroin/chitin (Park et al., 2006), silk fibroin/chitosan (Chen et al., 2012), silk fibroin/cellulose acetate (Zhao et al., 2011), silk fibroin-gelatin/poly (lactic acid) (PLA) (Yin et al., 2009), silk fibroin/poly(ethylene oxide) (PEO) (Jin et al., 2002) have been investigated with various cell types. Efficient cell adhesion and proliferation of human keratinocytes and fibroblasts on electrospun silk fibroin nanofibers have also been reported (Min et al., 2004). Much alike the literatures mentioned above, the rational for using silk fibroin in this study, is to introduce cell binding domains such as arg-gly-asp (RGD), resulting in a biomimetic scaffold-like architecture with improved mechanical properties (Li et al., 2011). Additionally, the

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presence of crystalline domains in silk fibroin could play an important role in the controlled release of drugs and preserving the bioactivity of therapeutic molecules (Hofmann et al., 2006). These properties taken together, has prompted the use of silk fibroin for the current study in antitumor activity.

Titanocene dichloride is the first metallocene based organometallic anti-cancer agent and its clinical trials has been reported to be progressed successfully through phase I into phase II (Dubler, 2005). Various modifications of titanocene dichloride such as alkylation (Beckhove et al., 2007), amidation (Gao et al., 2009) and fluorination (Eger et al., 2010) to improve the hydrolytic stability and antitumor activity have been studied for the drug. Titanocene complexes have been put into test and had shown effective against a range of human tumors including human breast adenocarcinoma MCF-7 (Beckhove et al., 2007), human epidermoid carcinoma A431 (Bannon et al., 2007), human ovarian carcinoma A2780 (Christodoulou et al., 1998) and human renal cancer cell lines CAKI-1 (Claffey et al., 2010). Considering the risk of systemic toxicity with conventional chemotherapeutics, use of polymer nanomaterials as drug carrier may possibly reduce the negative effects of the incorporated drug and improve the efficacy of cancer chemotherapy (De Jong and Borm, 2008). In this instance, therapeutic polymer nanofibers for sustained delivery of anticancer agents have been investigated and also proven efficacious against a range of tumor *in vitro* (Ma et al., 2011; Amna et al., 2013; Zheng et al., 2013; Thangaraju et al., 2012). However, very few studies have been reported on the sustained release and antitumor activity of titanocene dichloride with electrospun nanofibers. Chen et al. (2010) were the first to report a controlled release system of titanocene dichloride by electrospun fiber and its *in vitro* antitumor activity against human lung tumor spca-1 cells. The authors believe that such delivery systems could overcome the shortcomings of titanocene dichloride such as instability and short half life in the human body and at the same time improve the safety and efficacy of cancer chemotherapy. Similar yet recent, antitumor activity of titanocene trichloride loaded into PCL nanofibers were also studied by Stanzione et al. (2013) for inducing apoptosis to human glioblastoma A-172 cells.

In this study, we prepared titanocene dichloride loaded PCL/silk fibroin composite nanofibrous scaffolds by electrospinning and assessed its antitumor efficacy against MCF-7 cells. The release characteristics of titanocene dichloride from the composite nanofibers were also investigated at different time points. Given the electrophilic nature of metal anti-cancer complexes, likely results in a reaction with many biomolecules, including amino acids, polyphosphates, proteins and nucleic acids (Dubler, 2005). The present study identifies a possible interaction of the drug with the silk fibroin resulting in altered antitumor activity of titanocene dichloride against the human breast adenocarcinoma.

2. Materials and methods

2.1. Materials

Polymers of granular PCL (Mw 80,000), the solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), Dulbecco's modified Eagle's medium, fetal bovine serum (FBS), antibiotics, trypsin–EDTA, titanocene dichloride (bis(cyclopentadienyl) titanium(IV) dichloride) were purchased from Sigma–Aldrich, Singapore. Silk fibroin powders were obtained from Zhang Peng International Trading, Singapore.

2.2. Electrospinning of nanofibers

Electrospinning solutions of PCL and PCL/silk fibroin were initially prepared to obtain weight ratios of 10 and 9:1, respectively by dissolving in HFP to make a final w/v ratio of 10%. Varying weight ratios of titanocene dichloride (0.01%, 0.02% and 0.03%) were dissolved in three different PCL/silk fibroin solutions separately. The solutions were left for overnight stirring at room temperature. Electrospinning was performed using a 3 ml standard syringe with a blunted 25 gauge needle, with the solution flow rate maintained at 0.8–1 ml/h, controlled using a syringe pump (KDS 100, KD Scientific, Holliston, MA). A high voltage electric field of 13–15 kV (DC high voltage power supply from Gamma High Voltage Research, Florida, US) was applied to draw the fibers from the spinneret and was collected on 15 mm diameter coverslips placed over a grounded aluminum foil collector at a distance of 12–14 cm. The fabricated electrospun nanofibers were subsequently vacuum dried to remove the residual solvent present in the scaffolds.

2.3. Characterization of nanofibers

The surface morphology of electrospun nanofibrous scaffolds was studied under scanning electron microscope (JEOL JSM-6701F) at an accelerating voltage of 5 kV, after gold coating (JEOL JFC-1200 fine coater, Japan). Measuring the fiber diameter of electrospun fibers from the SEM images, $n = 30$ random fibers were chosen from each of the scaffolds. The average fiber diameter was then calculated along with SD (standard deviation) using image analysis software (ImageJ, National Institutes of Health, USA). Functional groups present in the scaffolds were analyzed using Fourier transform infrared (FTIR) spectroscopic analysis on Avatar 380, (Thermo Nicolet, Waltham, MA, US) over a range of 500–4000 cm^{-1} at a resolution of 1 cm^{-1}. Sessile drop method was done to check the hydrophilic property of the electrospun nanofibrous scaffolds, using VCA Optima surface analysis system (AST products, Billerica, MA). For hydrophobic samples, air plasma treatment was done by an electrodeless radio frequency glow discharge plasma cleaner (PDC-001, Harrick Scientific Corporation, USA) for 2 min under vacuum at a radio frequency power of 30 W. The scaffolds were tested for their mechanical strength using a tabletop tensile tester (Instron 3345, USA) using a load cell of 10 N capacity. The membranes were cut into rectangular strips of 10 mm × 20 mm dimensions and mounted vertically on the gripping unit of the tester. Testing was done at a crosshead speed of 10 mm/min with data being recorded every 50 ms.

2.4. In vitro *release study of titanocene dichloride*

Fiber mats of about 30 mg each for the titanocene dichloride loaded scaffolds were used for studying the release profile of the drug in PBS/ethanol (80:20 (v/v)) by UV–vis spectrophotometer (UV-3600, Shimadzu) at $\lambda = 311$ nm. The drug loaded fiber mats were incubated in 5 ml phosphate buffer solution (pH 7.4) at 37 °C. At different time points, 1 ml of the incubated solution was taken out and measured by UV–vis spectrophotometer by using the incubated solution of blank fibers as control. The accumulative release of titanocene dichloride from the fibers was calculated as a function of incubation time. The concentration values at the preset time intervals were derived from the linear dependence of concentration with absorbance.

2.5. MCF-7 cells culture and proliferation

2.5.1. MCF-7 cells

The MCF-7 cell lines used for these studies were obtained from ATCC, USA. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic and antimycotic solutions (Invitrogen Corp., USA) in a 75 cm^2 tissue culture flask and incubated at 37 °C in a humidified atmosphere with 5% CO_2. The electrospun nanofibrous scaffolds collected on 15 mm

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