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New approach for immobilization of 3-aminopropyltrimethoxysilane and TiO_2 nanoparticles into cellulose for BJ1 skin cells proliferation

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ABSTRACT

In the present study, tosylcellulose (TC) was used as a key intermediate for the selective coupling with 3-aminopropyltrimethoxysilane (APTMS) affording amino-propylsilane-grafted tosylcellulose (TC-Si). Solid state ^{13}C NMR and FT-IR analyses confirmed the coupling and self-condensation of APTMS along TC. The changes in the surface morphology of the functionalized cellulose were identified by SEM imaging. The thermal stability of TC-Si was significantly improved as compared to MCC and TC. A new organic/inorganic hybrid cellulosic material was fabricated by embedding TiO_2 nanoparticles into TC-Si network. The new cellulose polymers were investigated for their ability to promote the proliferation of human skin fibroblast (BJ1). The cell cytotoxicity assay showed that both TC and TC-Si possessed moderate toxicity to BJ1 cells by 17% and 23.8%, respectively at 20 μM. Meanwhile, TC-Si/TiO_2 hybrid enhanced the proliferation of BJ1 by 42%. Additionally TC-Si/TiO_2 hybrid demonstrated promising antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

1. Introduction

Among the suggested approaches for tissue engineering is the utilization of hybrid polymeric scaffold as an extracellular matrix which works as solid support that guide the cells to differentiate and proliferate into a desired tissue or organ (Van Vlierberghe, Dubruel, & Schacht, 2011). Ideal scaffold needs to be biocompatible, biodegradable, with characteristic porous structure and appropriate mechanical properties (Pina, Oliveira, & Reis, 2015). Collagen is a natural, biocompatible, and low antigenic biomaterial that have been used in different tissue engineering applications *e.g.* skin, bone, and cartilage (Ferreira, Gentile, Chiono, & Ciardelli, 2012). The poor mechanical properties of collagen remain the main disadvantage because it hinders its processability (Ragothaman, Palanisamy, & Kalirajan, 2014).

Cellulose and its derivatives are promising resources for the fabrication of functionalized hybrid materials OIHs. Hence their sustainability, renewability, recyclability, wide availability, biodegradability and low cost are needed for the innovative applications in different technical and biomedicine areas (Qiu & Hu, 2013). Additionally, several reports revealed the improvement of the thermal stability and its lipophilic characteristic, with increased affinity towards specific substrates of many silica based hybrid cellulosic materials (Angelova, Rangelova, Yuryev, Georgieva, & Müller, 2012; Saini, Belgacem, Salon, & Bras, 2016; Santos & Tavares, 2014; Shao et al., 2017; Shi, Lu, Guo, Zhang, & Cao, 2013).

On the other side, silica based hybrid materials have been the materials of interest in the last four decades due to their durable mechanical properties, light weight, high surface area, ordered porous structures, propitious biological impact, flexibility for further modifications, in addition to their large scale production at a low cost (Rosenzweig, Carelli, Steffen, Jarzem, & Haglund, 2015; Shadjou & Hasanzadeh, 2015; Toskas et al., 2013). These characteristics encouraged the prospective utility of silica based materials in particular those with mesoporous surface morphology in the fabrication of scaffolds for bone tissue regeneration (Arcos & Vallet-Regí, 2010; Muzzarelli, 2011; Shadjou & Hasanzadeh, 2015), or carrier systems for a wide variety of drugs and other bioactive molecules such as nucleic acid, anticancer drugs, and/or imaging agents (Wang, Li, Ma, & Gu, 2013; Yang, Gai, & Lin, 2012; Zhang et al., 2014). 3-aminoalkytrialkoxysilanes were widely used to functionalize the surface of cellulose, cellulose nanocrystals and nanofibers, regenerated cellulose membrane, pullulan (De Souza et al., 2016; Fernandes et al., 2014; Khanjanzadeh

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et al., 2018; Saini et al., 2016; Salimi, Nasseri, Chapesshloo, & Zakerinasab, 2015; Salon, Abdelmouleh, Boufi, Belgacem, & Gandini, 2005; Tee et al., 2013; Wang, Bai, Liang, Bai, & Liu, 2017). Generally, the coupling of 3-aminoalkytrialkoxysilanes onto the surfaces of polysaccharides involves partial or complete hydrolysis of the alkoxy groups in acidic or alkaline aqueous media affording the corresponding 3-amino-alkyltrihydroxysilans, which underwent self-condensation with other silanol molecules generating oligomeric siloxane chains which get adsorbed onto the hydroxyl-rich surface forming siloxane network structure (Salon et al., 2005).

TiO_2 nanoparticles are an inexpensive photostable inorganic material, which has good optical, photocatalytic, and antimicrobial properties, they have been approved by food and drug administration (FDA) for use in food packaging stuffs, drug formulations, and cosmetics manufacturing (Chawengkijwanich & Hayata, 2008; Snyder, Bo, Moon, Rochet, & Stanciu, 2013). Moreover, several studies emphasized on the impact of addition of TiO_2 nanoparticles to many organic/inorganic hybrid materials which have been investigated as tissues scaffolds and wound dressings due to their antibacterial features and their ability in promoting bone and skin tissues regeneration or wound healing (Behera, Das, Kumar, Bissoyi, & Singh, 2017; Dumitriu et al., 2018; Goncalves, Marques, Pinto, Trindade, & Neto, 2009; Haldorai & Shim, 2014; Khan, Ul-Islam, Khattak, Ullah, & Park, 2015).

In this study, we report a new approach for coupling of APTMS to the microcrystalline cellulose under homogenous conditions through the use of tosylcellulose as a reactive key intermediate. Where APTMS molecules underwent hydrolysis into aminopropyltrihydroxysilane and coupled to TC *via* nucleophilic displacement of the tosyl groups along the cellulose chains affording silanol-functionalized tosylcellulose (TC-Si). Then, a new organic/inorganic hybrid material was fabricated from TC-Si and TiO_2 nanoparticles. The cytotoxicity and the antimicrobial activity of the new cellulosic materials were evaluated to explore the potentiality of the prepared hybrid as antimicrobial skin tissue scaffold.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose (MCC) with average particle size of 20 µm, triethylamine (TEA), anhydrous lithium chloride (LiCl), *p*-toluenesulfonyl chloride (TsCl), and 3-aminopropyltrimethoxysilane (APTMS), Titanium (IV) oxide nanopowder (anatase), with particle size < 25 nm, 99.7% trace metals basis, Ethylenediaminetetraacetic acid (EDTA), Dulbecco's Phosphate Buffered Saline (DPBS) solution, Dulbacco's Modified Eagle's Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Potassium Penicillin, streptomycin sulfate and amphotericin B, L-glutamin were purchased from Sigma–Aldrich. Potassium hydroxide (KOH), Dimethylformamide (DMF), dimethylacetamide (DMA), dimethyl sulfoxide (DMSO), acetone, ethanol, and methanol were of analytical grade and purchased from Thermo Fisher Scientific and used without further purification.

2.2. Methods

2.2.1. Preparation of tosylcellulose (TC)

TC was prepared according to the method described elsewhere (El-Sayed, El-Aziz, Kamel, & Turky, 2018). In brief, 5.0 g of oven-dried MCC (30.8 mmol/AGU) was suspended in 120 mL of DMA, and heated at 160 °C for 2 h under reflux. The suspension was cooled down to 100 °C, this was followed by addition of 10 g of anhydrous LiCl solution (235.9 mmol) dissolved in 25 ml of DMA and the solution temperature was allowed to cool down to room temperature. The stirring was continued till the complete dissolution of cellulose, then, 18 mL of TEA (129.14 mmol) diluted in 10 mL of DMA was added to the stirring cellulose solution and the stirring was continued for additional 30 min at room temperature. After decreasing the solution temperature to 3–8 °C, *p*-toluenesulfonyl chloride (TsCl) solution prepared by dissolving TsCl (40 g, 208.04 mmol) in 25 mL of DMA was added drop wise over 60 min. The stirring of the reaction mixture was continued for another 24 h while its temperature was kept at 3–8 °C. The mixture was poured slowly into 1 L of ice-cold water. The precipitate was filtered off, washed with about 3 L of distilled water, suspended in 250 mL of boiling acetone, re-precipitated in 1 L of distilled water, and filtered off, and finally washed with 500 mL of ethanol. The resulted TC was dried at 50 °C in oven for 48 h. Yield: 85%. Elemental analysis for TC: C = 46.9%, H = 2.15%, N = 0.0385%, S = 8.788%. The degree of tosyl substitution (DS_{Ts}) was calculated based on sulfur content obtained by elemental analysis for TC sample according to the equation as follows (Rahn, Diamantoglou, Klemm, Berghmans, & Heinze, 1996):

$$DS_{Ts} = M_{AUG}.\ w_S\ (\%)/\ M_S.\ 100\ (\%) - M_{Ts}.\ w_S\ (\%)$$

Where M_{AGU} is the molar mass of the anhydroglucose unit (AGU) = 162.05 g/mol, M_S is the molar mass of sulfur = 32.065 g/mol, M_{Ts} is the molar mass of the tosyl group = 155.02 g/mol, and w_S (%) is the mass fraction of sulfur of TC = 8.788%, and the DS_{Ts} = 0.77

2.2.2. Coupling of APTMS to tosylcellulose

In typical preparation, 2 g (15.8 mmol/AGU) of TC with DS_{Ts} = 0.77 were dissolved in 5 mL of DMF and heated at 80 °C. Then 3 mL of APTMS (17.07 mmol) was diluted in 15 mL of DMF and added drop wise to TC solution with stirring. The mixture was kept stirring at 80 °C for 24 h. The resulted gel was suspended in 100 mL of distilled water and centrifuged at 6000 rpm for 10 min. To ensure the removal of unreacted components or other byproducts, the gel was washed with 200 mL of ethanol. TC-Si gel was oven dried at 50 °C for 48 h for structure characterization or stored in refrigerator at 4 °C in refrigerator for further use. Yield: 55%. Elemental analysis for TC-Si: C = 44.18%, H = 3.09%, N = 2.05%, S = 2.18%; DS_{Ts} = 0.11

2.2.3. Fabrication of TC-Si/TiO_2 hybride

TC-Si gel was prepared as described in Section 2.2.2, 120 min following the addition of APTMS to TC at 80 °C, a suspension of TiO_2 nanoparticles prepared by dispersing 20 mg of TiO_2 nanoparticles (1.0% wt/wt from TC) in 5 mL of DMF and sonication for 10 min using digital ultrasonic disperser (a IKA high-shear mixer, T-T18 ULTRA TURRAX Basic) was added to TC-Si gel and stirring was continued for additional 24 h at room temperature (Haldorai & Shim, 2014). The resulted gel was suspended in 100 mL of distilled water and centrifuged at 6000 rpm for 10 min. The gel was further washed with 200 mL of ethanol to remove unbound APTMS and TiO_2 nanoparticles or other byproducts. The resulted TC-Si/TiO_2 gel hybrid was dried in oven at 50 °C for 48 h for structure characterization or stored in dark vial in refrigerator at 4 °C till its use. Yield: 56%. Elemental analysis for TC-Si/TiO_2: C = 45.51%, H = 3.83%, N = 1.97%, S = 2.36%; DS_{Ts} = 0.13

2.2.4. Fourier transfer infrared spectroscopy (FT-IR)

The FT-IR spectra of MCC, TC, TC-Si, and TC-Si/TiO_2 were recorded with FT-IR spectrometer (Nicolet Impact-400 FT-IR spectrophotometer) in the range of 400–4000 cm^{-1}.

2.2.5. Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

ICP-AES for the samples was performed on a Perkin Elmer Optima 7000DV. Before analyses, silicon was dissolved by alkaline fusion while titanium was dissolved by wet acid digestion.

2.2.6. Nuclear magnetic resonance spectroscopy (NMR)

The solid state ^{13}C and ^{29}Si NMR experiments for MCC, TC, and TC-Si samples were recorded on a Varian VNMRS 300 MHz solid spectrometer using a two-channel probe with 3.2 mm ZrO_2 rotors. The ^{29}Si solid state NMR spectra were recorded using both CP MAS and one pulse (OP) sequences with samples spinning at 6 kHz. CP MAS was used