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Nanocomposited coatings produced by laser-assisted process to prevent silicone hydogels from protein fouling and bacterial contamination

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ABSTRACT

Zinc oxide (ZnO) nanoparticles incorporating with polyethylene glycol (PEG) were deposited together on the surface of silicone hydrogel through matrix-assisted pulsed laser evaporation (MAPLE). In this process, frozen nanocomposites (ZnO–PEG) in isopropanol were irradiated under a pulsed Nd:YAG laser at 532 nm for 1 h. Our results indicate that the MAPLE process is able to maintain the chemical backbone of polymer and prevent the nanocomposite coating from contamination. The ZnO–PEG nanocomposited coating reduces over 50% protein absorption on silicone hydrogel. The cytotoxicity study shows that the ZnO–PEG nanocomposites deposited on silicone hydrogels do not impose the toxic effect on mouse NIH/3T3 cells. In addition, MAPLE-deposited ZnO–PEG nanocomposites can inhibit the bacterial growth significantly.

1. Introduction

Silicone hydrogels are polymers consisting of silicon–oxygen bonds (siloxane), which can lead to higher oxygen permeability than other conventional hydrogel [1,2]. Silicone hydrogel has been extensively studied in the fields of contact lenses, tissue engineering, and drug delivery for their good biocompatibility, high oxygen permeability, and proper light transmission. However, silicone hydrogels incur biofouling easily because of hydrophobic surface. Biofouling is the accumulation of proteins, cells and other biological materials on a surface [3,4], including protein fouling, bacterial contamination, etc. Hydrophobic surface can denature the protein structure and prevent the formation of the protein layer (protein fouling) which may act as a "condition film" for the growth of bacteria. It is noted that the bacterial contamination or protein fouling on silicone-based medical devices, such as contact lens, or catheters, may cause severe inflammation to patients [5]. To date, the surface of silicone hydrogels are normally modified with a hydrophilic polymer to enhance their hydrophilicity and protein resistance. Quite recently, a biocompatible coating made of hybrid nanocomposites could be able to enhance the capability of silicone hydrogels to resist protein adhesion, and gain more appropriately chemical and physical properties [6].

Conventional surface modification methods either need direct contact with chemicals or high energy, which will cause contamination or decomposition of materials. Compared to other coating techniques, matrix-assisted pulsed laser evaporation (MAPLE) which derived from pulsed laser deposition (PLD), is able to deposit a wide range of organic materials without solvent and oxygen contamination and maintain the target materials' structure at the same time [7–12]. In this process, the target material within a suitable solvent is frozen by using liquid nitrogen. A laser beam irradiates the frozen target; the energy is mainly absorbed by the chosen solvent, and is converted into thermal energy that allows the solvent to vaporize. Most of the laser energy is absorbed by the solvent of the matrix rather than by target materials, which minimize the photochemical decomposition [7–10]. MAPLE technique is especially suitable for biomaterial surface modification due to its non-solvent and non-oxygen contamination, and maintains the property of hybrid target material at the same time during deposition. However, few studies have been reported on deposit nanocomposites composed of nanostructures and organic materials through this process [11,12].

In this paper, zinc oxide (ZnO) nanoparticles incorporating within polyethylene glycol (PEG) were deposited on silicone hydrogels by MAPLE. PEG is a hydrophilic polymer due to its polyether compound [13]. Zinc-based nanomaterials show excellent

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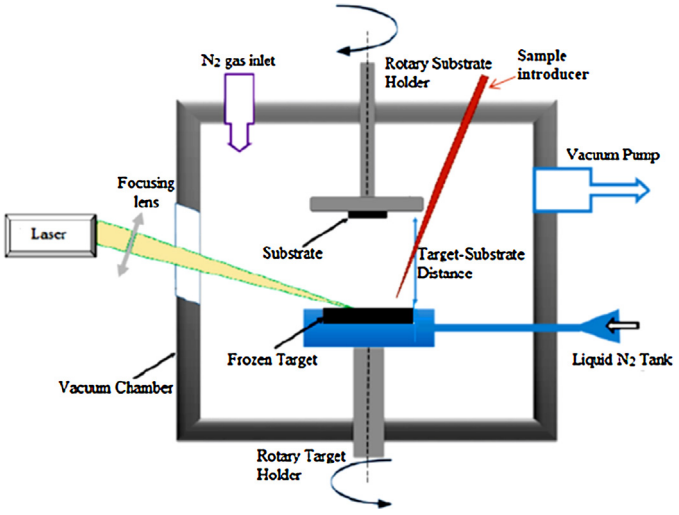

Fig. 1. Illustration of the MAPLE deposition system.

resistance against corrosion and performed good antibacterial activity [14]. The protein absorption and antimicrobial efficiency of the nanocomposite coating on silicone were investigated. Meanwhile, the cytotoxicity of the silicone with nanocomposite coating was evaluated by using the mouse NIH/3T3 cell line.

2. Materials and experimental

All chemicals used in this paper are purchased from Sigma–Aldrich, Canada.

The silicone hydrogel was synthesized through a photo-polymerization [6,15]. 3 ml of N,N-dimethylacrylamide, 3-methacryloxypropyltris(trimethylsiloxy)silane, and bis-alpha, omega-(methacryloxypropyl) polydimethylsiloxane were mixed with the volume ratio of 2:4:1, followed by the addition of 15 μl of ethylene glycol dimethacrylate and 0.3 ml ethanol. Nitrogen was purged into the mixture for 15 min before 8 mg of photo-initiator (diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide) was added and stirred for 5 min. After that, the mixture was photo-polymerized under UV irradiation for 50 min. 30% ethanol was applied to wash the hydrogel after photo-polymerization

ZnO nanoparticles (ZnO NPs) were prepared by a sol–gel method where precursor is zinc acetate dehydrate [$Zn(CH_3COOH)_2{\cdot}2H_2O$]. 5.508 g of $Zn(CH_3COOH)_2{\cdot}2H_2O$ and PEG acting as a stabilizer were dissolved in 300 ml of ethanol with a weight ratio of 10:1 (ZnO: PEG). The mixture was stirred at 80 °C for 24 h and then washed three to four times by using methanol. After that, it was calcined at 150 °C for 2 h [16].

Nanocomposited coating made of ZnO NPs and PEG was deposited on the surface of silicone hydrogel by MAPLE deposition as shown in Fig. 1. First ZnO NPs stabilized by PEG were suspended in 10 ml isopropanol with a concentration of 0.5 wt%. The mixture was introduced into the sample holder (Fig. 1) followed by liquid nitrogen freezing [17]. Nd:YAG laser with the wavelength (λ_{em}) at 532 nm was used in the MAPLE deposition. The deposition was conducted for 1 h at a background pressure of 1×10^{-6} Torr. The substrate-to-target distance is 6 cm. The fluence is maintained at 300 mJ/cm^2.

Transmission electron microscopy (TEM, Phillips CM10) and fluorescence spectrometry (PTI QuantaMaster^{TM} 40) were applied to study the microstructures and fluorescence properties of ZnO–PEG NPs. In addition, the MAPLE coated samples were characterized by FTIR (Fourier transform infrared spectroscopy), XRD (X-ray diffraction), SEM (scanning electron microscopy), and fluorescence spectrometry. BioTester 5000 test system (CellScale Biomaterials Testing, ON, Canada) was used to measure the mechanical property of each sample. Triplicate measurements were carried out to obtain the statistic Yong's modulus of the samples.

For protein adsorption test, the samples (1 cm × 1 cm) were immersed in PBS for 24 h, and then soaked in 0.5 mg/ml BSA–PBS solution for 3 h at 37 °C. PBS was used to rinse the samples 3 times to remove the non-absorbed BSA on the surface of hydrogel. After that, the samples were immersed in 1 wt% SDS–PBS solution under sonicate for 20 min to completely detach BSA from hydrogel surface to the solution. The BCA protein assay kit (Micro BCATM Protein Assay Kit, Thermo Scientific, U.S.A.) was used to determine the protein concentration in SDS–PBS solution with a UV–visible plate reader at the wavelength of 562 nm.

The antibacterial efficiency of the nanocomposite coatings was test by drop-test method [16,17]. Gram-negative bacteria, *Escherichia coli* (*E. coli* BL21, ATCC), and Gram-positive bacterium, *staphylococcus aureus* (*S. aureus*) (ATCC) are used in the tests, respectively The samples (1 cm × 1 cm) were placed into sterilized 90-mm Petri dishes. The glass coverslip is used as control sample. Then 100 μl PBS solutions with *bacteria* at a concentration of 10^6 CFU/ml were dropped onto the surface of each sample. The samples were laid at ambient temperature in different time periods (i.e. 1, 2, 4, 8, 12 h). After each time period the bacteria containing drops were washed from the sample surfaces using 5 ml PBS in the sterilized Petri dish. Then 10 μl of each bacteria suspension was spread on the LB Agar plate. The number of surviving bacteria on the Petri dishes was counted after incubation for 24 h at 37 °C. The relative viability of *E. coli* and *S. aureus* was calculated, respectively, which is the counted number of sample plate divided by the counted number of control plate.

Approximate 10,000 3T3 mouse fibroblast cells, determined by cell counting using a haemocytometer, was seeded onto the bottom of 24 well plates and incubated overnight to ensure good cell adhesion onto the plate. Silicone hydrogel samples with nanocomposited coatings were then added into the cultured cells and incubated for 24 h under sterile conditions. The positive control sample was cultured cells without hydrogel samples. After 24 h, the samples were removed and the media aspirated. Then sterilized 40 μl of 0.5% MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide) solution was added to each well containing 500 μl cell media. After 4 h's incubating at 37 °C, the media was aspirated, and plate was rinsed twice with sterile PBS. As per the protocol of Vybrant® MTT Cell Proliferation Assay Kit, cells were labeled by using formazan dissolved in DMSO. Controls and treated cells in microplates were incubated for 10 min followed by the spectrophotometric analysis, and the read absorbance is at 540 nm.

3. Results and discussions

3.1. Characterization of ZnO nanoparticles before and after MAPLE deposition

Fig. 2a is the TEM micrograph of ZnO nanoparticles synthesized by the sol–gel method. The average size of ZnO NPs is estimated at 10 ± 4 nm. Fig. 2b is the TEM micrograph of ZnO NPs deposited on Cu grid directly through MAPLE process. It is estimated that the average size of ZnO NPs deposited in MAPLE process is 9 ± 3 nm. It is noted that different TEM-sample preparation methods may result in different aggregation level of nanoparticles on the Cu grid. In fact, ZnO nanoparticles in term of morphologies and average particle size do not change a lot as shown in the TEM results of the two different process, i.e. "sol–gel process alone", and "sol–gel process followed the MAPLE deposition". It indicates that most energy