Journal of the mechanical behavior of biomedical materials 68 (2017) 239–246

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Flexible camphor diamond-like carbon coating on polyurethane to prevent *Candida albicans* biofilm growth

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ARTICLE INFO

Keywords:
Diamond-like carbon (DLC)
Candida albicans
Camphor
Raman
Biofilm growth inhibition

ABSTRACT

Camphor was incorporated in diamond-like carbon (DLC) films to prevent the *Candida albicans* yeasts fouling on polyurethane substrates, which is a material commonly used for catheter manufacturing. The camphor:DLC and DLC film for this investigation was produced by plasma enhanced chemical vapor deposition (PECVD), using an apparatus based on the flash evaporation of organic liquid (hexane) containing diluted camphor for camphor:DLC and hexane/methane, mixture for DLC films. The film was deposited at a low temperature of less than 25 °C. We obtained very adherent camphor:DLC and DLC films that accompanied the substrate flexibility without delamination. The adherence of camphor:DLC and DLC films on polyurethane segments were evaluated by scratching test and bending polyurethane segments at 180°. The polyurethane samples, with and without camphor:DLC and DLC films were characterized by Raman spectroscopy, scanning electron microscopy, atomic force microscopy, and optical profilometry. *Candida* albicans biofilm formation on polyurethane, with and without camphor:DLC and DLC, was assessed. The camphor:DLC and DLC films reduced the biofilm growth by 99.0% and 91.0% of *Candida albicans*, respectively, compared to bare polyurethane. These results open the doors to studies of functionalized DLC coatings with biofilm inhibition properties used in the production of catheters or other biomedical applications.

1. Introduction

Diamond-Like Carbon (DLC) is a very attractive material for biological applications because it has physical and chemical properties similar to diamond such as hardness and relatively high modulus of elasticity. DLC is easily deposited on large and 3D areas (Hauert, 2003). Although, there are a significant number of manuscripts about DLC (Choudhury et al., 2016) and DLC with metallic nanoparticles (Widoniak et al., 2005; Marciano et al., 2009), little is known about the use of DLC in polymeric materials (Wang et al., 2004). Some studies mention silver used as a possible inhibitor of microbial growth and catheters coated with a mixture of silver and DLC (Dearnaley and Arps, 2005). On the other hand, only a few studies have used Camphor as a carbon precursor for DLC production, and these prioritized manufacturing process of only carbon bonds, and terpenes radicals were not preserved (Fadzilah et al., 2013; Liu and Kwek, 2008; Zani et al., 2013). No literature was found about camphor:DLC as a material to combat candidiasis proliferation. Candidiasis contamination is related to parenteral nutrition, and it is transmitted through the hands of healthcare workers and especially the use of catheters [6]. Candidiasis contamination has been a big problem for hospitalized patients, especially those in critical condition, and is the fourth leading cause of bloodstream infection. Bloodstream infection increases hospital costs, patient's hospitalization time and mortality rate (Morrison et al., 2006; van Asbeck et al., 2007). Biofilm is responsible for conducting catheter infection, due to a set of micro-organisms that live in association with biofilm (Bazaka et al., 2012; Griffiths and Hall, 2010). Most microorganisms involved in colonization of catheters are not virulent in planktonic form but can cause persistent infection when they are in a group (Co-investigator, 2013).

Camphor is cetonic terpenoid ($C_{10}H_6O$) obtained from the camphor tree that is native to China, and it is part of a class of bioactive compounds synthesized by plants (Frizzo et al., 2000; Gershenzon and Dudareva, 2007; Moreno et al., 2010). Terpenes are several open-

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http://dx.doi.org/10.1016/j.jmbbm.2017.02.013
Received 29 May 2016; Received in revised form 5 February 2017; Accepted 12 February 2017
Available online 16 February 2017

chained or cyclic compounds, usually oxygenates such as aldehydes, alcohols, and ketones. Gershenzon and Dudareva (Gershenzon and Dudareva, 2007) reported previously that plants with terpenes are resistant to diseases, due to their action on fungi and bacteria. Terpenes have detergent properties that are toxic to fungi due to their ability to complex with sterols in fungi membranes, which leads to loss of membrane integrity (Gershenzon and Dudareva, 2007).

The aim of this work was to develop a DLC film that comprises camphor with properties to prevent biofilm of *Candida albicans* for use on catheters and biomedical instruments. For this, we developed a simple apparatus that was coupled in a commercial plasma enhanced chemical vapor deposition (PECVD) reactor for camphor delivery during DLC production. This process allows the deposition of DLC films containing radicals for functionalized surfaces or nanoparticles, covering materials three-dimensionally at low temperature.

2. Material and methods

For this study, we used three samples: bare polyurethane (PU) and polyurethane covered with camphor:DLC (Camphor:DLC/PU) and DLC (DLC/PU) films. The PU sheets used in this work is smooth, flat, transparent, and has 2 mm thickness they are commercial and widely used to produce peripherally inserted central catheters (PICC) - Uni Lumen Biomedical®. Biological tests were performed in three triplicates for each sample. Each triplicate was carried out on different days. Mechanical adhesion tests were also done in triplicate.

2.1. DLC deposition process

The PU substrates were cleaned using multi-enzymatic detergent and sterile distilled water in an ultrasound bath. After this, the samples were dried in an oven at 40°. The samples were placed in a reactor where they were cleaned by argon plasma prior to film deposition.

The Fig. 1 shows a schematic drawing from PECVD reactor assisted by DC pulsed source, containing an apparatus for liquids deliver used to produce the films. The system is composed by an ultrasound bath, a container for liquid precursors, a needle valve and spherical valve to control liquid vapor flux, a vacuum chamber and a turbo pump were also used to control work pressure, as presented in Fig. 1 (1–6), respectively. Inside the vacuum chamber (Fig. 1 (5)), was used a metallic shield to confine the plasma ions. This ionization was controlled by a DC-pulsed power supply allowing the deposition of the Camphor:DLC films keeping some radicals from Camphor, as showed in Raman spectra by the terpene band centered on 1099 cm^{-1} (Fig. 1).

Table 1 shows the parameters used for the camphor:DLC and DLC film deposition. The deposition plasma process started with argon plasma to remove oxides in (PU) surfaces. DLC films were produced using a vapor mixture of hexane and methane and for camphor:DLC was used camphor oil diluted in hexane (10% v.v.) with methane vapor. The glass tube apparatus containing the liquid precursor were sonicated using an ultrasound bath. The flow for each carbon precursor was controlled using flowmeters and pressure valves to maintain the work pressure inside the vacuum chamber at 7 Pa. The power supply was 200 W, and the temperature at substrate surface was kept at 25 °C by cathode refrigeration.

2.2. Biofilm formation and microbiological evaluation

For microbiological evaluation, fungal suspensions were prepared of standard strain ATCC (American Type Culture Collection) of *C. albicans* (10231) in the concentration of 0.5 McFarland scale [106 colony forming units per milliliters (CFU/mL)] in Dextrose Sabouraud broth (Difco™).

The samples (PU, camphor:DLC/PU and DLC/PU) were introduced into different Erlenmeyer flasks containing 15 mL of fungal suspension.They were incubated at 37 °C for 48 h under 110 rpm in constant stirring using an incubator shaker model MA 420 (Marconi). After incubation time, the fungal suspension was transferred to glass tubes with phosphate buffer (PBS pH 7.2 ± 0.1) (de Vasconcelos et al., 2014) and were homogenized in a vortex for 60 s. A 100 µL aliquot of this suspension were spread on Sabouraud agar solidified by "Spread-Plate" technique and incubated at 37 °C for 48 h. This incubation time was used to obtain a mature biofilm (Braga et al., 2008). After this time, the colony-forming units (CFU/mL) were counted and compared between PU, camphor:DLC/PU and DLC/PU samples.

2.3. Camphor:DLC and DLC films evaluation

The modified PU substrates with and without camphor:DLC and DLC films were examined using Raman spectra to verify atomic arrangement. The films were analyzed by confocal Raman microscope Horiba-Model Lab Ram HR Evolution with laser wavelength (λ=514 nm) and calibrated about the diamond peak. The laser diameter spot has submicron lateral resolution and axial confocal performance better than 2 µm. The power on the sample was

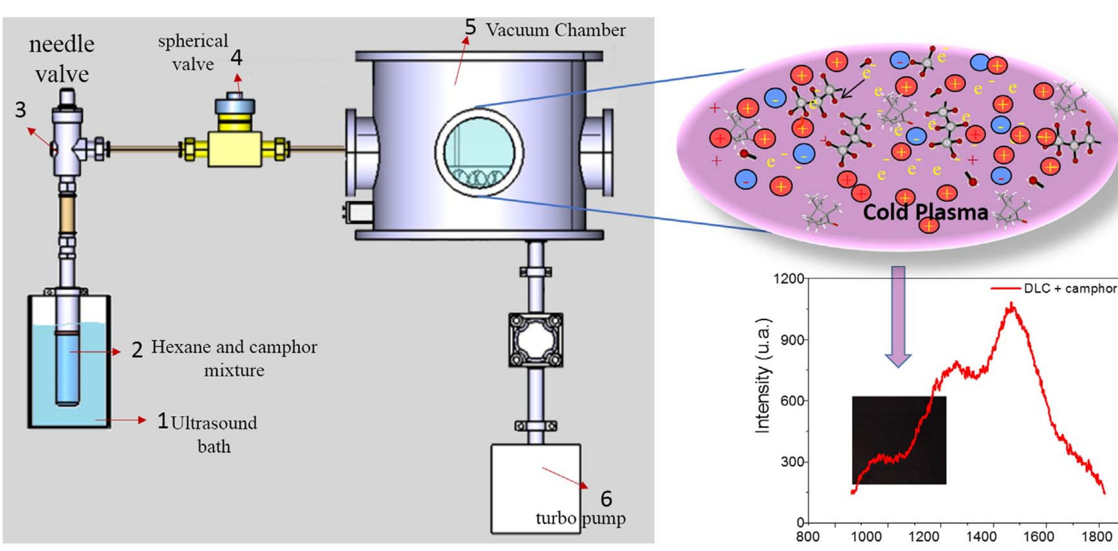

Fig. 1. Schematic drawing of deposition reactor.