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Artificial biomembranes stabilized over spin coated hydrogel scaffolds. Crosslinking agent nature induces wrinkled or flat surfaces on the hydrogel

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ABSTRACT

Hydrogel films possess the ability of retain water and deliver it to a phospholipid bilayer mainly composed by DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine); moisture of the medium favors the stability of an artificial biomembrane when it is subjected to repetitive heating cycles. This hypothesis is valid when the hydrogel film, used as scaffold, present a flat surface morphology and a high ability for water releasing. On the other hand, when the sample presents a wrinkle topography (periodic undulations), free lateral molecular movement of the bilayer becomes lower, disfavoring the occurrence of clear phases/phase transitions according to applied temperature.

Hydrogel films were prepared using HEMA (hydroxyethylmetacrylate), different crosslinking agents and initiators. This reaction mixture was spread over hydrophilic silicon wafers using spin coating technique. Resultant films were then exposed to UV light favoring polymeric chain crosslinking and interactions between hydrogel and substrate; this process is also known to generate tensile stress mismatch between different hydrogel strata, producing out-of-plane net force that generate ordered undulations or collapsed crystals at surface level. DPPC bilayers were then placed over hydrogel using Langmuir–Blodgett technique. Surface morphology was detected in order to clarify the behavior of these films. Obtained data corroborate DPPC membrane stability making possible to detect phases/phase transitions by ellipsometric methods and Atomic Force Microscopy due to their high hydration level. This system is intended to be used as biosensor through the insertion of transmembrane proteins or peptides that detect minimal variations of some analyte in the environment; artificial biomembrane stability and behavior is fundamental for this purpose.

1. Introduction

Tethered bilayer lipid membranes (tBLMs) have attracted large interest from current and modern research in the biotechnology field due to their wide application in technology development for tissue engineering, drug delivery, biosensors and protein channel transduction, among others (Drücker et al., 2014). Supported lipid bilayers (SLBs) is the most common and widely used configuration of tBLMs, corresponding to planar membranes deposited onto hydrophilic solid substrates separated with an ultrathin film of water (1–2 nm) (Rebaud et al., 2014). Thermal studies of these systems elucidate the behavior and properties of cell membranes, and – also – the efficiency of possible devices based on SLBs when are subjected to temperatures near ambient (González et al., 2012). In this way, the determination of bilayer phase/phase transition temperatures becomes in relevant information (Thiam et al., 2013). Some characterization techniques as magnetic resonance, Raman spectroscopy, Atomic Force Microscopy has been utilized for this purpose (Shlomovitz and Schick, 2013; Hain et al., 2013) allowing the detection of minimal structural changes in bilayer conformation such as thickness, smooth/roughness, molecule tilting and

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electric interaction between phospholipids (Jing et al., 2014). However, the complexity of SLBs systems lies in the low structural stability of the bilayer during formation processes and posterior characterization (Andrecka et al., 2013). In order to solve this problem, scaffolds for tBLMs are frequently used; the compound utilized as support must maintain an aqueous (moist) environment that increase their stability during long periods, particularly in unusual conditions (high temperatures, pressure variations, external mechanical stress and pH changes) (Luckey, 2014).

Polymer scaffolds enhance surface-bilayer interactions compared to several others materials commonly used such as aluminium, titanium, iron and silicon oxides (Nellis et al., 2011). Hydrogel is a kind of polymer, which has the capacity to absorb and retain large amounts of solvents into their structural network, being an excellent candidate for membrane support without direct linkage, significantly reducing the frictional coupling between membrane and solid substrate acting as "lubricant cushion" for the interface (Rebaud et al., 2014).

In this study, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was used for bilayer formation. This pulmonary surfactant present three characteristic phases; subgel (L_c), gel ($L\beta$) and liquid crystalline ($L\alpha$) with their respective transitions: laminar gel ($L\beta'$) and ripple gel ($P\beta'$), the superscript of prime means that lipid molecules are oriented tilted from the bilayer plane (Matsuki et al., 2013).

Different types of photo-polymerized hydrogel films, based on polyhydroxyethylmetacrylate (pHEMA), were used as scaffold for DPPC. The technique used to deposit the hydrogel was spin coating, producing a high surface homogeneity; posteriorly, the compounds were exposed to UV light ($\lambda = 365$ nm) in order to complete the polymerization. The systems formed by DPPC/Hydrogel films/Silicon wafer were characterized through different methods. Hydrogel film surface was analyzed using Field Emission gun Scanning Electron Microscopy (FE-SEM) in order to visualize the surface morphology. Atomic Force Microscopy (AFM) was used to detect surface structures dimensions and profiles. Simultaneously, heating cycles were applied during these measurements in order to obtain micrographies at different temperatures, this data was then used to calculate surface roughness for identification of DPPC phases with their respective transitions. Ellipsometric measurements were realized in every deposition step in order to obtain an appropriate thickness control, also, DPPC thickness variations were measured against temperature in order to corroborate phases and phase transitions temperatures detected via AFM.

Different surfaces morphologies were obtained according to the hydrogel type utilized as scaffold (undulated pattern in some cases or flat surface with some crystals structures in others). Patterns morphology and their dimensions are related to the ratio between monomer and crosslinking agent, to the polymerization technique utilized and with the deposition method, these processes generate a stress gradient between film surface and lower strata (Guvendiren et al., 2010). When hydrogel film show tightens wrinkles, DPPC bilayer is found highly packaged affecting their molecular mobility, disfavoring phase (transitions) occurrence. On the other hand, flat topography is ideal for detect thermal behavior of the surfactant and for conserve membrane stability during thermal cycles.

2. Materials and methods

2.1. Materials

For hydrogel synthesis, the following precursors were utilized: 2-hydroxyethyl methacrylate (HEMA, 97%) containing monomethyl ether hydroquinone as inhibitor (≤250 ppm) as main monomer. Four different crosslinking agents were employed: di (ethylene glycol) dimethacrylate (DEGDMA, ≥95%) that includes monomethyl ether hydroquinone as inhibitor (300 ppm). Poly (ethylene glycol) diacrylate (PEGDA), with two different average molecular weights (Mn: 575 and 700 g/mol) and acrylamide (AAm, ≥99%) for molecular biology applications (HPLC purity). 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959, 98%) and 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651, 99%) were utilized as photo-initiators, all the reagents previously mentioned were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Pre-polymer (oligomer) was synthesized using ACS ammonium peroxodisulfate (98%, grade reagent) as thermal initiator. 1-vinyl-2-pyrrolidone (NVP, stabilized with *N*,*N*′-di-*sec*-butyl-1,4-phenylenediamine) was employed for dissolve the photo-initiator; both reagents were acquired from Merck KGaA (Darmstadt, Germany).

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC, ≥99%, powder) was acquired from Sigma–Aldrich Company (St. Louis, Missouri, USA) and used without further purification, while chloroform (99.0–99.4%), hydrogen peroxide (30–32%), sulfuric acid (95–97%) Emparta® ACS and Water for chromatography LiChrosolv® were obtained from Merck KGaA (Darmstadt, Germany). A *p* type <100> ±0.5° orientation silicon wafer was acquired from Siegert Wafer GmbH (Aachen, Germany).

2.2. Equipment and measurements

Hydrogel polymerizations were performed through UV exposure using a lamp with its emission peak centered at $\lambda = 365$ nm (Vilber Lourmat 230 V 50/60 Hz, 9W). A KW-4A spin coater (Chemat Scientific), coupled with an oil free vacuum pump (Rocker Chemker 410), was utilized for deposit hydrogels on hydrophilic silicon wafer. DPPC bilayer deposition, with hydrogel film used as scaffold, was performed with a Langmuir–Blodgett trough model LT-103 (MicroTestMachines).

Micrographies of HEMA–DEGDMA films were obtained with two different high resolution FE-SEM models, JSM 6330F (JEOL Ltd.) and Quanta FEG 650 (FEI Co.), at different magnifications (1000× and 5000×).

The samples that possess a DPPC bilayer on the top, were analyzed using an atomic force microscope (AFM), model NX10 (Park Systems), height profiles of the surfaces were obtained with a scan width of $20 \times 20\ \mu m^2$ and with a super-sharp silicon probe (10 nm radius, 330 kHz resonance frequency and spring constant of 42 Nm^{-1}), this AFM possess a sample heater that permits to increase the temperature between ambient and 60 °C; the equipment is located at Brazilian Nanotechnology National Laboratory (LNNano) in Campinas, Brazil. The topographies of HEMA–$PEGDA_{575}$ and HEMA–$PEGDA_{700}$ were obtained at room temperature using another AFM, model NTEGRA Prima (NT-MDT Co.) in intermittent contact mode using a tips of 75 kHz resonance frequency. Images were treated using the off-line software packages Gwyddion (Klapetek et al., 2011) and WSxM 4.0/8.0 (Horcas et al., 2007) for qualitatively and quantitatively analyze the acquired images.

A Multi-angle laser ellipsometer model SE 400adv (SENTECH Instrument GmbH) was used to perform optical measurements, the equipment possesses a stabilized He–Ne laser ($\lambda = 633$ nm). This instrument was used in conjunction with a home-made copper sample holder coupled with a temperature controller model 325, a Pt100 sensor (model PT-102-2S, useful range 1.4–873 K) and a 25 Watt heater (model HTR-25), all acquired from Lake Shore Cryotronics Inc.; this close-loop system permits the regulation and stabilization of sample temperature between 25 °C and 70 °C.