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Polymethacrylate coated electrospun PHB fibers: An exquisite outlook for fabrication of paper-based biosensors

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ABSTRACT

Electrospun polyhydroxybutyrate (PHB) fibers were dip-coated by polymethyl methacrylate-co-methacrylic acid, poly(MMA-co-MAA), which was synthesized in different molar ratios of the monomers via free-radical polymerization. Fabricated platfrom was employed for immobilization of the dengue antibody and subsequent detection of dengue enveloped virus in enzyme-linked immunosorbent assay (ELISA). There is a major advantage for combination of electrospun fibers and copolymers. Fiber structre of electrospun PHB provides large specific surface area available for biomolecular interaction. In addition, polymer coated parts of the platform inherited the premanent presence of surface carboxyl (–COOH) groups from MAA segments of the copolymer which can be effectively used for covalent and physical protein immobilization. By tuning the concentration of MAA monomers in polymerization reaction the concentration of surface –COOH groups can be carefully controlled. Therefore two different techniques have been used for immobilization of the dengue antibody aimed for dengue detection: physical attachment of dengue antibodies to the surface and covalent immobilization of antibodies through carbodiimide chemistry. In that perspective, several different characterization techniques were employed to investigate the new polymeric fiber platform such as scanning electron microscopy (SEM), atomic force microscopy (AFM), water contact angle (WCA) measurement and UV–vis titration. Regardless of the immobilization techniques, substantially higher signal intensity was recorded from developed platform in comparison to the conventional ELISA assay.

1. Introduction

There is a continuous demand for inexpensive and sensitive/selective analytical devices which are reliable, portable, rapid and capable of high-throughput detection in the area of biosensing. To date, many different complex biosensors of the most advanced categories have been developed (Lin et al., 2010; Nie et al., 2014; Xiangjie et al., 2014). When it comes to the actual clinical practice, however, minor percentages of them are playing a vital role in routine diagnostic procedure. This simple and straightforward fact has to lead and encourage researchers to dedicate efforts to the more practical solutions for production of new generations of analytical platforms. Enzyme-linked immunoassay (ELISA) is perhaps the most well-known and widely applied assay for virus detection. Nevertheless, even to date, patients might enter to the acute phase of the illness due to the frequently reported serious drawbacks of this very conventional assay. Some of the major shortages of ELISA assay can be listed as: time consuming and laborious procedure, inconsistency of the detection signal, errors in reproducing the results and large detection range required for relatively more accurate diagnosis (Alcon et al., 2002; Hosseini et al., 2014b). Therefore, the necessity of an additional intermediary that can enhance the performance of ELISA, leading to the timely detection and subsequently better surveillance, is highly desirable.

In the last two decades, polymer fibers and membranes had undergone through significant progress in the field of biomaterials engineering and biotechnology (Chen et al., 2009; Hong et al.,

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2013; Lu et al., 2014; Tang et al., 2014). Having applications in areas such as tissue engineering, controlled drug release, wound dressings, molecular separation, preservation of bioactive agents and biosensors have drawn a great deal of importance in fiber developments (Luo et al., 2010; Zhang et al., 2005). Among existing fabrication techniques, electrospinning remains the most popular and preferred method for fabrication of polymer fibers. Electrospinning has shown major advantages over other techniques as it is a simple and versatile method that can be used for a wide range of polymer solutions (Ma et al., 2006). The laboratory set up can be customized in a relatively low price and the produced fibers can be controlled in diameter range (micro/nanofibers) depending on the size of the needle (Chantasirichot and Ishihara, 2012; Cipitria et al., 2011). Electrospun fibers have proven great potentials in biosensing domain due to the high interconnectivity, porosity, micro/nanointerstitial space and high surface area available for biomolecular interaction (Ma et al., 2006). On the other hand, presence of effective functional groups such as carboxyl (–COOH), amine ($–NH_2$), hydroxyl (–OH) and sulfhydryl (–SH) is essential when covalent immobilization is aimed (Hosseini et al., 2014a,b). Therein, a suitable biosensor material would be credited not only for a large available surface area but also for bearing desirable functionalities. In response to the mentioned factual requirements for a well-designed bioreceptor surface, combination of electrospun fiber and functionalized polymer may offer a protein-friendly platform with high chance of bimolecular interaction associated with binding stability.

This paper presents the fabrication of electrospun polyhydroxybutyrate (PHB) fibers by widely applied electrospinning method. In the second step, different compositions of polymethyl methacrylate-co-methacrylic acid, poly(MMA-co-MAA), were synthesized via free radical polymerization. Dip-coating of electrospun fibers in poly(MMA-co-MAA) solution creates a unique biosensing platform at which the high surface area is originated from the structure of PHB fibers and surface –COOH functional groups are inherited from MAA segments of the copolymer (Hosseini et al., 2014a). In immobilization domain, if one aspect matters more than presence of desirable functionalities, it would be the optimum concentration and proper distribution of them on the surface. An insufficient number of surface functional groups may result in deactivation of the immobilized proteins as biomolecules, in general, could be very sensitive toward solid phases (Hosseini et al., 2014d). On the other hand “too many” surface functionalities might result in an overly functionalized surface which again deactivates proteins due to the steric repulsion (Hosseini et al., 2014c,d). Therefore to assess and optimize versatile surfaces of polymer coated electrospun fibers with variety of –COOH concentrations, different molar ratios of the monomers (MMA/MAA) have been used in polymerization reaction. Designed platforms (uncoated and dip-coated PHB fibers) were employed for detection of the dengue enveloped virus through physical and covalent immobilization in ELISA method. Dengue fever (DF) is a mosquito transmitted viral disease which is mainly widespread in tropical and subtropical regions (Bessoff et al., 2008). However, the risk of this dangerous infection can become worldwide by people who contracted the infection while traveling abroad (Shu et al., 2003). Large reactive surface area that goes hand in hand with stable binding for antibody conjugation, provide a biomolecule-compatible environment that can lead to an adaptive technique for fabrication of new generation of paper-based diagnostic devices with higher performance.

2. Materials and methods

2.1. Chemicals and reagents

Methyl methacrylate (MMA), methacrylic acid (MAA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), polyhydroxybutyrate (PHB), bovine serum albumin (BSA), monosodium phosphate (NaH_2PO_4), chloroform (CHCl3), Tween 20 and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma, US. MMA monomer was purified by distillation technique prior to polymerization reaction. Other materials were used as received. Tetrahydrofuran (THF, solvent in free-radical polymerization and dip-coating), dimethylformamide (DMF) and phosphate buffer saline (PBS) were purchased from Thermo Fisher Scientific, US. Azobisisobutyronitrile (AIBN, polymerization initiator), was purchased from Friedemann Schmidt Chemical, Germany.

2.2. Fabrication of electrospun PHB fibers

PHB fiber membranes were fabricated by electrospinning method. Briefly, 20 ml PHB solution (10 wt%) in chloroform/DMF (9:1) was ejected out of a 20 G needle (inner diameter = 0.9 mm) at a speed of 3 ml/h. 10 kV voltage was loaded between the needle and an aluminum plate (40 cm × 40 cm). The distance between needle and aluminum plate was 18 cm (Scheme 1a). Polymer solution was drawn into fibers and deposited on the aluminum plate to form PHB fiber membrane. The electrospun fiber was then peeled off from the aluminum plate. The white-colored and paper-like PHB membranes with thickness of ~500 μm were cut into the circle shape pieces with dimension of 6 mm (Scheme 1, center) for further experiments.

2.3. Poly(MMA-CO-MAA) synthesis and processing

Four different compositions of poly(MMA-co-MAA) were prepared by free-radical polymerization reaction in THF using AIBN as an initiator. The abbreviations of the copolymers were used to identify the initial molar ratio of the monomers. For instance, Poly (MMA-co-MAA-9:1) corresponds to 90% of MMA and 10% of MAA in reaction mixture. Further copolymer compositions are as follows: poly(MMA-co-MAA-7:3) and poly(MMA-co-MAA-5:5). For the ease of discussion, further in the text, mentioned copolymer compositions are named as follows: comp.(9:1), comp.(7:3) and comp.(5:5). Pure PMMA (when MMA is the only monomer involved in polymerization reaction) was also synthesized under the same reaction conditions and used as control in all experiments. A two-neck round-bottom flask was fitted with a condenser and sealed inlet, used for reactants feed. The set up was charged with pre-calculated amount of MMA in 50 ml of THF under stirring condition for 5 min. A mixture of second monomer (MAA) and initiator (AIBN, 0.164 g) was gradually added to the reaction mixture. Polymerization has been carried out for 8 h at 90 °C and was stopped by adding reaction mixture into 1000 ml of distilled water. Immediate white color precipitation was observed confirming the formation of copolymer compositions. Mentioned precipitation was filtered and thoroughly washed with distilled water and dried in vacuum oven at 40 °C (Hosseini et al., 2014a).

2.4. Coating procedure

Polymer coatings were prepared on fabricated PHB fibers by simple and straightforward method of dip-coating (Scheme 1b). PHB fibers (substrates) were coated by immersing fiber sheets into the polymer solutions (5%) by using THF as solvent. Coated fibers of different copolymer compositions have been taken out after 3 s