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Polymethyl methacrylate-co-methacrylic acid coatings with controllable concentration of surface carboxyl groups: A novel approach in fabrication of polymeric platforms for potential bio-diagnostic devices

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ABSTRACT

The generally accepted strategy in development of bio-diagnostic devices is to immobilize proteins on polymeric surfaces as a part of detection process for diseases and viruses through antibody/antigen coupling. In that perspective, polymer surface properties such as concentration of functional groups must be closely controlled in order to preserve the protein activity. In order to improve the surface characteristics of transparent polymethacrylate plastics that are used for diagnostic devices, we have developed an effective fabrication procedure of polymethylmetacrylate-co-metacrylic acid (PMMA-co-MAA) coatings with controlled number of surface carboxyl groups. The polymers were processed effectively with the spin-coating technique and the detailed control over surface properties is here by demonstrated through the variation of a single synthesis reaction parameter. The chemical structure of synthesized and processed co-polymers has been investigated with nuclear magnetic resonance spectroscopy (NMR) and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-ToF-MS). The surface morphology of polymer coatings have been analyzed with atomic force microscopy (AFM) and scanning electron microscopy (SEM). We demonstrate that the surface morphology and the concentration of surface –COOH groups (determined with UV–vis surface titration) on the processed PMMA-co-MAA coatings can be precisely controlled by variation of initial molar ratio of reactants in the free-radical polymerization reaction. The wettability of developed polymer surfaces also varies with macromolecular structure.

1. Introduction

The development of biosensors has drawn a vital role of research interest due to the high sensitivity and selectivity in detection of diseases and viruses. Of particular interests are polymeric materials used for surface protein immobilization. Those immobilized surface proteins are further engaged in detection of coupling proteins (antibody/antigen) present in diseased blood. The most commonly applied diagnostic device, based on heterogeneous antibody/antigen interaction, the enzyme-linked immunosorbent assay (ELISA), still presents a "golden standard" in clinical diagnostic practice [1,2]. However, conventional ELISA has its own limitations such as: tedious and labor-intensive protocol, long incubation times between each step and inconsistency of the results [3]. In order to overcome those serious limitations, there is a strong need for development of advanced polymer coatings with controllable surface properties such as surface chemistry and morphology. In that perspective, a generation of functionalities such as hydroxyl (–OH), amine ($–NH_2$) and carboxyl (–COOH) groups at the surface of polymer coatings presents the crucial step for further immobilization of proteins and subsequent effective detection of diseases and viruses. Furthermore, the surface concentration of those functional groups must be closely controlled in order to avoid protein de-activation caused by either steric repulsion (over-functionalization) or protein denaturation in close proximity of the polymer surface (low surface concentration of functional groups) [4].

In recent years, polymethyl methacrylate (PMMA) has shown a great potential due to the particular properties and a wide range of applications [5]. PMMA is a low cost polymer with chemical inertness, low specific weight, high impact resistance and flexibility. PMMA has been successfully used for the immobilization of

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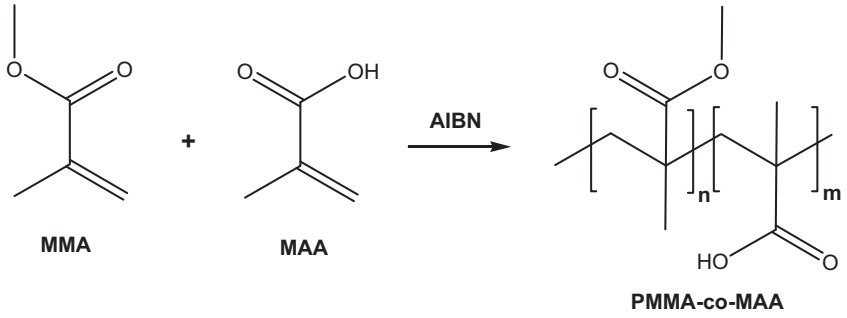

Scheme 1. Free-radical polymerization reaction between MMA and MAA (with AIBN as initiator) and PMMA-co-MAA co-polymer product.

enzymes, DNA, proteins and metal particles deposition for diagnostic purposes [6–9]. For developing a fluorescence based biosensor devices, PMMA has shown many advantageous properties such as transparency, low intrinsic fluorescence and ease of fabrication [10]. The major drawback of most of the polymeric surfaces (including PMMA) is their hydrophobic nature, usually with low surface energy and the absence of above mentioned surface functionalities. In most cases the polymeric surfaces need to be treated in order to obtain the optimum concentration of the surface functional groups [11]. For example, the surface of the PMMA can be treated in various ways (both chemically and physically) without changes in transparency or in mechanical properties. Those treatments include plasma processing, wet chemical surface reactions (hydrolysis and aminolysis) or UV treatment. Although the recently reported results present an important new insight into the field of surface engineering [12], there are still major concerns about existing surface treatment techniques. For example, some authors are pointing out that the main drawback of plasma treated surfaces is aging effect [13]. Functional groups formed on the treated surface are not stable during the time and the surface tends to return to its untreated state as the functional groups reorient themselves [13]. The simplicity and cost effectiveness of wet chemical surface treatments seems to be compromised by non-specific reactions resulting in a range of oxygen-containing surface functional groups. Another serious concern is the surface etching which often results in a non-regular surfaces, difficult to control [4,14]. Similar to other existing surface treatments (such as plasma or UV treatments) the important aspect is the stability of chemically modified surfaces and surface "relaxation" to the previous, non-treated state [15]. Obviously there is a strong need for stable and robust materials with the high level of control over the surface concentration of functional groups.

In the view of the importance of polymeric surfaces for development of effective diagnostic devices, here we report the synthesis and fabrication of polymethyl methacrylate-co-methacrylic acid (PMMA-co-MAA) coatings with controlled number of surface –COOH groups. We have carefully chosen the co-polymer composition for a well-established free-radical polymerization synthesis protocol (Scheme 1) and subsequent surface fabrication by spin-coating technique. The design and control over the surface chemistry of PMMA-co-MAA coatings is based on the hypotheses: (i) the variation in the initial monomer concentration of methyl methacrylate (MMA) and methacrylic acid (MAA) would yield plastic material with varying MAA segments (Scheme 1) in the polymer chain; and (ii) the –COOH groups generated from MAA would be present at the polymer surface. The tuning of the polymer composition has the potential to provide a simple and effective method to control the surface properties of the polymer coatings, important for their future application as diagnostic devices.

2. Experimental procedure

2.1. Materials

Methyl methacrylate (MMA), methacrylic acid (MAA), 2,5-dihydroxy benzoic acid (DHB), sodium iodide (NaI), toluidine blue ((7-amino-8-methyl-phenothiazin-3-ylidene)-dimethylammonium, TB) and ethanol (EtOH) were purchased from Sigma Malaysia. Deuterated dimethyl sulfoxide-d_6 (DMSO-d_6) was purchased from Merk, Germany. Tetrahydrofuran (THF, Thermo Fisher Scientific, US) has been used as solvent in polymer synthesis and processing procedures. The free-radical initiator azobisisobutyronitrile (AIBN) was purchased from Friedemann Schmidt Chemical, Germany. Diced silicon OFET substrates (2 cm × 2 cm) were purchased from Ossila, UK. MMA monomer was purified by distillation prior to the free-radical polymerization synthesis. All other materials have been used as received.

2.2. Synthesis

Four different compositions of the PMMA-co-MAA co-polymers were prepared by free-radical polymerization reaction in THF using AIBN as an initiator. The abbreviations of the co-polymers have been used identifying the initial molar ratio of the monomers. In particular, PMMA-co-MAA (9:1) corresponds to 90% of MMA and 10% of MAA in reaction mixture. Further co-polymer compositions are as follows: PMMA-co-MAA (7:3) and PMMA-co-MAA (5:5). The pure PMMA (polymerization of MMA with AIBN initiator) was synthesized in 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 50 ml of THF and pre-calculated amount of MMA and stirred for 5 min. A mixture of MAA and AIBN (0.328 g) was gradually added to the solution. Reaction was allowed to proceed for 6 hours at 90 °C. The reaction mixture was poured into 1000 ml of distilled water. White color precipitation was filtered and washed thoroughly with water by using centrifuge. Freeze-drying has been used to remove the residual water and the samples were stored in the fridge before dissolution and subsequent chemical analysis.

2.3. Polymer sample preparation by spin-coating technique

The polymer coatings were prepared on silicon wafers (substrate) by spin-coating procedure. Silicon wafer substrates were previously cleaned in three following steps: (1) substrates were first immersed for 5 min in the solution of H_2O:H_2O_2:HCl with the ratio of 6:1:1; (2) secondly, substrates were taken out and immersed for another 5 min in solution of NH_4OH:H_2O:H_2O_2 with the ratio of 1:5:1; (3) the final step has been completed by rinsing the substrates in the solution of H_2O:HF with the ratio of 1:1 for