Development of a New Polymeric Material with Controlled Surface Micro-Morphology Aimed for Biosensors Applications

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Abstract—Compositions of different molar ratios of polymethylmethacrylate-co-methacrylic acid (PMMA-co-MAA) were synthesized via free-radical polymerization. Polymer coated surfaces have been produced on silicon wafers. Coated samples were analyzed by atomic force microscopy (AFM). The results have shown that the roughness of the surfaces have increased by increasing the molar ratio of monomer methacrylic acid (MAA). This study reveals that the gradual increase in surface roughness is due to the fact that carboxylic functional groups have been generated by MAA segments. Such surfaces can be desirable platforms for fabrication of the biosensors for detection of the viruses and diseases.

Keywords—Polymethylmethacrylate-co-methacrylic acid (PMMA-co-MAA), Polymeric material, Atomic Force Microscopy, roughness, carboxylic functional groups.

I. INTRODUCTION

SURFACE design of the functional polymeric platforms is of great importance in performance of many diagnostic devices [1]. Immobilization of proteins on the detector surface and measurement of the generated signal through specific coupling between the surface and the proteins are the basic aspects of the bioassay devices [1], [2]. In order to improvement of efficient biosensors, functionalized surfaces with a high degree of control over surface properties (chemistry and morphology) should be produced [1], [2]. It is clear that the polymer surface functional groups play the most important role in protein immobilization. The formation of chemical functionalities such as hydroxyl (-OH), amine (-NH$_2$) or carboxyl (-COOH) groups on the surface is the first step in successful protein immobilization [3]. These functional groups can make an enhancement in surface hydrophilicity. Polymeric materials offer such possibility as they can be processed and designed by the means of synthetic procedures and controlled surface chemistry [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 enzymes, DNA, proteins and metal particles deposition for diagnostic purposes [6]–[8]. The major drawback of the most 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 [9]. For instance, treatment the surface of the PMMA can be conducted in various ways (both chemically and physically) without changes in transparency or in mechanical properties. Plasma processing, wet chemical surface reactions (hydrolysis and aminolysis) or UV treatment are several ways of those treatments. There is an important new insight into the field of surface engineering in the recently reported results [10], but the existing surface treatment techniques present still major concerns. For example, the aging effect is one of the main drawbacks of plasma treated surfaces which some authors are pointing out [11]-[18]. 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 them [12]-[22]. 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 [12]-[22]. It seems that the presentment of a stable material with the high level of control over the surface concentration of functional groups is needed [3]. In a paper by S. Hosseini et al., the synthesis and fabrication of polymethylmethacrylateco-methacrylic acid (PMMA-co-MAA) coatings with a controlled number of surface –COOH groups has been described [3]. The difference in the initial monomer concentration of methyl methacrylate (MMA) and methacrylic acid (MAA) produces plastic material with different MAA segments in the polymer chain; and the –COOH groups generated from MAA polymer segments would be present at the polymer surface [3]. Chemistry and morphology properties of the surface should be analyzed for the development of efficient biosensors. Interaction between biomaterials and biological systems also depends on the surface physical properties [23]. Surface morphology has a significant role in the adsorption of proteins [23]. In the view of the importance of gaining information about the morphology of polymeric surfaces for fabrication of effective

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diagnostic devices, here we show characterization of the PMMA-co-MAA polymer by using atomic force microscopy (AFM).

II. MATERIALS AND METHODS

Methyl methacrylate (MMA), methacrylic acid (MAA) and 1-ethyl-3-(3-dimethylaminopropyl) were purchased from Sigma, US. Polymerization reaction of the monomers MMA and MAA have been done via free-radical polymerization. Distillation method was used for purification of MMA monomer before the free-radical polymerization reaction. Tetrahydrofuran (THF) as the solvent for synthesis the polymer was purchased from Thermo Fisher Scientific, US. The initiator in the free radical polymerization (Azobisisobutyronitrile (AIBN)) was purchased from Friedemann Schmidt Chemical, Germany. The chemical structure of PMMA-co-MAA is shown in Fig. 1. Four compositions of the co-polymer with different concentrations of monomers have been synthesized as follows: MMA/MAA: PMMA-co-MAA (9:1), PMMA-co-MAA (7:3), PMMA-co-MAA (5:5) and pure PMMA as a control. For example, in PMMA-co-MAA (5:5) 5mol of MMA and 5mol of MAA were used in synthesis procedure. The reaction was commenced by adding the solvent (THF) and MAA monomer. The mixture was stirred for 5 minutes. Determined amount of MMA and AIBN were added to the reaction concurrently. The reaction was conducted for 6 hours and a white color polymer was produced after adding distilled water to the mixture. Washing procedure was done several times to eliminate the excess. Then the polymer got dried and stored for the rest of experiments [3].
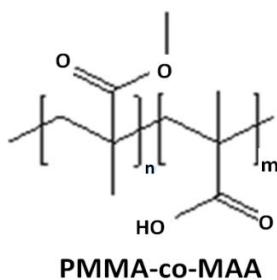

Fig. 1 Chemical structure of PMMA-co-MAA [3]

Coating of the silicon wafers as the substrate was performed by spin-coating procedure. The silicon wafer substrates were cleaned prior to coating. In brief, the substrates were cleaned by (1) immersing in the solution $H_2O:H_2O_2:HCl$ for 5 minutes, (2) dipping into the other solution ($NH_4OH:H_2O:H_2O_2$) as the same time as the previous one, and in the last, they were rinsed with solution of $H_2O:HF$. All the substrates were washed and sonicated in purified water. Then, they were sonicated for the second time. Drying process was carried out under the stream of nitrogen [3]. Coating procedure was performed by using by spin-coater (model WS-650MZ-23NPP, spinning time of 55s at 3000 rpm) with 5% polymer solutions in tetrahydrofuran (THF) on silicon wafers. Coated

surfaces were allowed to dry in oven (60◦C) overnight.

The surface morphology of the coated PMMA-co-MAA polymer on the silicon substrate was analysed by atomic force microscopy (AFM). The roughness of the surfaces and the surface area of the samples, indeed, have been studied by using AFM (Bruker, Dimension 3000) in contact mode.

III. RESULTS

Surface topography of spin coated samples has been studied by AFM and the results are displayed in Fig. 2. As it can be seen, the surface of the pure PMMA has shown a smooth and uniform coating in comparison to the other analyzed surfaces. The roughness of the coated surfaces was observed to be increased with the increase in the molar ratio of the second monomer (MAA). As it can be seen from Fig. 1, MAA segments of the co-polymer contain –COOH groups. Therefore by introducing this monomer to the polymer reaction it is expected to achieve compositions that prominently have –COOH groups not only on the surface of the materials but also inside the structure. This is also expected to obtain higher surface roughness as the concentration of MAA monomer increases in copolymer system. This is due to the fact that -COOH groups are hydrophilic in their nature and hidrophilicity of the surface is in a direct correlation with the surface roughness [1], [3]. This can be clearly seen from Fig. 2 that mentioned hypotheses have been approved by the behavior of the co-polymer surfaces toward AFM analysis. The surfaces of the PMMA-co-MAA have shown gradual increase in surface roughness as the molar ration of the MAA segments has been increased.
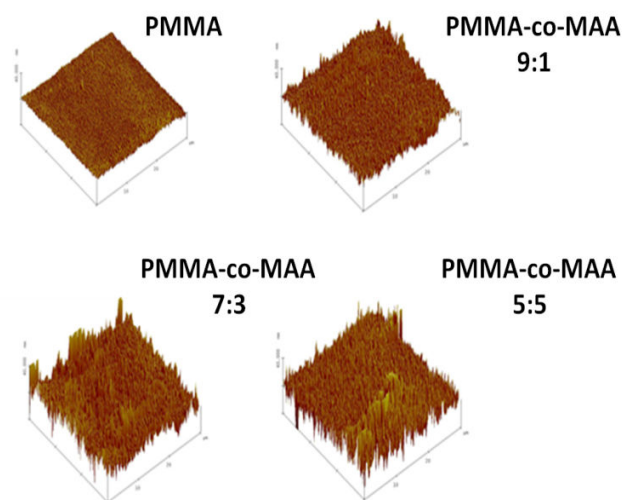

Fig. 2 AFM analysis of PMMA-co-MAA polymer coatings

Table I recorded by AFM presents the results which in the same line confirm our hypothesis about the surfaces of the coated samples. It can be clearly observed from the measurements that the surface area of the coatings was increased by increasing the concentration of surface –COOH groups. The highest surface area is available on the coated samples with composition 5:5. This phenomenon is partially

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the result of highest available surface –COOH groups on this particular composition. In fact, this copolymer (5:5) has formed in the shape of hydrogel material due to the presence of 50% MAA monomer in polymerization reaction. MAA is a very hydrophilic agent that has resulted in synthesis of such gel-like material. This can make the surface potentially more efficient than others as firstly it contains the highest amount of –COOH groups and second the gel structure of material offers more available active sites for protein binding in general. However it should be noted that not too many surface functional groups may always lead to higher efficiency in protein binding as the overly crowded surfaces might cause esteric repulsion.

Preparation of the surfaces with high level of roughness and optimum number of functional groups is desirable for a variety of applications. In the case of protein immobilization such surfaces can be suitable platforms for protein attachment and subsequent virus detection. Current methods of surface modification such as wet-chemical treatment, plasma treatment and UV treatment can also generate such surfaces [4].

TABLE I
SURFACE AREA OF PMMA-CO-MAA COATINGS

Co-polymer compositions	Surface area (μm^2)
PMMA	140.11
PMMA-co-MAA (9:1)	153.88
PMMA-co-MAA (7:3)	169.04
PMMA-co-MAA (5:5)	205.45

Many drawbacks have been reported mentioning that the generated functionalities are not stable by the time. Treated surface tends to rearrange to its original state with lower surface energy [11]. Surface reorientation and aging effect are known as some of the major disadvantages of the mentioned well-known techniques. By using our method there is a possibility for creating the surface with optimal functionalities and controllable surface roughness and surface area available for bioactivation of the platform. Such surfaces are the biomaterial of choice for future fabrication of biosensor devices with high level of sensitivity and selectivity. This is important to note that these potential biosensors can be used for immobilization and detection of any kind of viruses and diseases.

IV. CONCLUSION

In this paper, we present polymer coatings synthesized by using two monomers, methyl methacrylate and methacrylic acid, in the highly controllable manner. Free radical polymerization reaction has been used for synthesis of four different co-polymer compositions with different molar ratios of the monomers. Spin coated chips were produced by using the synthesized copolymers on silicon wafers. These surfaces were produced for development of biosensor devices that can be employed in diagnostic applications. Different compositions of this co-polymer composition were produced in order to have a close control on the availability of the –

COOH functional groups. These pendant acid groups are a function of the methacrylic concentration in the reaction mixture, which are an important feature for protein surface immobilization. The results from AFM analysis show a consistent increase in surface area and surface roughness which can offer a great available active sites for interaction with proteins. It is expected that controllable surfaces would provide better control of protein interaction which is a highly desirable for biosensor devices in general.

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