

Chapter 2

An Alternative Chemical Approach for Development of Polymeric Analytical Platforms

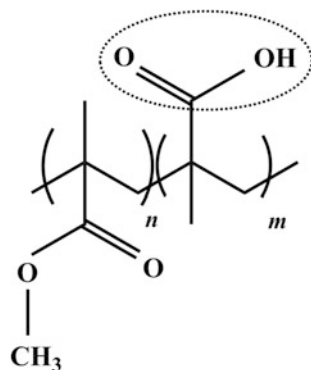
Abstract This chapter introduces a chemical approach as an alternative solution to solve the problems stated in Chap. 1. Chemically synthesized copolymers made from methyl methacrylate (MMA) and methacrylic acid (MAA) monomers in varied molar ratios of the monomers are proposed for fabrication of novel bioreceptor platforms. Our newly developed materials inherit the presence of surface carboxyl groups ($-\text{COOH}$) from the MAA monomers involved in free-radical polymerization reaction. The concentration of surface functional groups can be tuned by variation of the monomers thus a careful control over surface properties can be achieved. This chapter also reviews the principles of applied characterization techniques such as scanning electron microscopy (SEM), atomic force microscopy (AFM), water-in-air contact angle (WCA) measurement and X-ray photoelectron spectroscopy (XPS) that have been used for investigation of the developed platforms. Since developed bioreceptor surfaces are design for their application in virus detection, the chapter covers the major topics such as infectious diseases and neglected tropical diseases (NTDs). Dengue virus (DENV) has been chosen and tested on the developed bioreceptor platforms.

Keywords Carboxyl groups • Surface properties • Free-radical polymerization • Neglected tropical diseases • Dengue virus

2.1 Proposed Chemical Methodology

As a solution to the existing problems discussed in Chap. 1, a copolymeric system is proposed as a substitute material for fabrication of the bioreceptor platforms. Namely methyl methacrylate (MMA, appendix Table 1) and methacrylic acid (MAA, appendix Table 2) have been chosen as monomers for synthesis of the copolymer poly methylmethacrylate-co-methacrylic acid, poly(MMA-co-MAA). Poly methylmethacrylate (PMMA) is one of the widely used materials for mass production of analytical kits. Between the chemical structure of the proposed copolymer and the commercial PMMA, there exists a small alteration that is the

Fig. 2.1 Poly (MMA-co-MAA) chemical structure



presence of the second monomer (MAA) in the polymerization reaction. Such a minor difference can have a great impact on the performance of the substrate in comparison to the PMMA. As the structure suggests (Fig. 2.1), presence of MAA monomer in preparation of the copolymer poly(MMA-co-MAA) introduces one of the desirable functional groups to the polymeric matrix. With this strategy, instead of applying current modification techniques for generation of such functionalities, a copolymeric material can be obtained, which naturally contains the pendent carboxyl groups ($-\text{COOH}$) inside its chemical structure and at the outmost layer of the surface. Poly(MMA-co-MAA) is polymerized in free-radical polymerization reaction while the surface $-\text{COOH}$ groups were derived from MAA monomers of this copolymer [1]. Surface functional groups in the polymeric matrix are part of the chemical structure of the copolymer; hence they would not be deactivated over the time and could not be affected by aging effect or reorientation phenomena [2].

In order to avoid insufficiently or overly functionalized surfaces (Fig. 1.3a, b) and to have a close control over the surface, different molar ratios of the monomers were used in synthesis reaction. As a result different copolymer compositions have been polymerized to have a range of $-\text{COOH}$ concentration in the matrix. The variation in monomers' ratio is the key factor to lead to the optimum concentration of functional groups, which can facilitate effective protein immobilization. Table 2.1 presents percentages of each monomer involved in free-radical polymerization reaction. As a control, pure PMMA has also been synthesized under the exact same reaction condition. To simplify the discussion, further in the text, different copolymer compositions are referred as follows: PMMA, comp.(9:1), comp.(7:3), and comp.(5:5). Expectedly, the concentration of $-\text{COOH}$ groups in copolymer compositions increases as the molar ratios of the MAA segments increases.

Table 2.1 Different compositions of poly (MMA-co-MAA)

Composition	MMA (%)	MAA (%)
PMMA	100	0
Comp.(9:1)	90	10
Comp.(7:3)	70	30
Comp.(5:5)	50	50

2.2 Application of Developed Polymer Compositions in ELISA

Performance of the synthesized copolymer compositions can be assessed by direct application of the materials in the clinical assay. However, it will be a long journey for each discovery to successfully open its way from the laboratory benches to the industry. Polymer compositions developed in our laboratories have obtained the patent (UMCIC Malaysia/PI 2014700658) and are in the process of commercialization. Nevertheless, there is still no available analytical kits made of poly (MMA-co-MAA) for the careful assessment of the method. For that reason, performance of newly developed platforms has been investigated via intermediary substrates that carry prepared copolymer compositions into the assay. Different materials were used as the supporting substrate and subsequently range of behaviors and performances have correspondingly been obtained due to the different morphologies and surface chemistries. Herein, fabrication and processing of polymethacrylate biochips, by using spin coating technique on the silicon substrate, are reported in a great detail [1]. Developed bioreceptor surfaces, in this study, have been chosen as the mediums to introduce synthesized copolymers into the assay. Proposed platforms were thoroughly characterized by different techniques in order to study the surface property and micro-morphology of the samples. After thorough analysis of the samples, performances of the developed surfaces of different compositions in virus detection have been investigated [1–3].

2.3 Characterization of the Biochips

Every newly developed material has to be carefully analyzed. It is also of a great importance to have a better understanding about the characteristics of the coated biochips made from different compositions. Different characterization techniques were used to study the bulk and surface properties of the biochips. In present book, however, the discussion is limited to the surface characterization techniques, as this study assesses the performances of the developed platforms with the strong emphasis on the influence of surface morphology and chemistry on the detection efficiency. The fundamental principles behind applied characterization techniques are as follows.

2.3.1 Scanning Electron Microscopy (SEM), Morphology Analysis

Frontal view and cross-section images of the biochips were recorded by scanning electron microscopy (SEM, JEOL, JSM7600F) [1, 3, 4]. This characterization

technique operates with the electron beam that can scan the material and provide information in regard to the morphology of the analyzed samples. Accelerated electrons contain significant amount of kinetic energy. When the electron-substrate interaction occurred, electrons become decelerated and it results in dissipated energy that appears in variety of produced signals. Generated signal includes secondary electrons that are responsible for producing SEM images [5–7]. In this technique, samples are routinely placed on the double sided conductive carbon tape and placed inside the chamber. If the samples are from the family of non-conductive materials, imaging can often be troublesome. For that reason and to avoid surface charging, gold or platinum coating is necessary prior to the imaging [3, 8, 9].

2.3.2 Atomic Force Microscopy (AFM), Topography Analysis

Atomic force microscopy (AFM) is the most commonly used technique from the family of scanning probe microscopies (APM). Such techniques collect images by moving a probe over the surface. As the probe moves, it records the height of the surface. AFM offers a nano-scaled three dimensional (3D) profile of the analyzed surface by measuring the force between the probe and the examined substrate. AFM tip gently travels over the surface, scans the forces between the surface and the probe and sends the signals to the feedback method section, where signals can be translated into the images as the final outcomes of the instrument [10–12]. In this study, the surface topography of platforms, were analyzed by AFM (Ambios, Q scope) in a non-contact mode. Important parameters such as mean roughness (Ra), root mean-square roughness (Rq) and total roughness (Rt) were recorded for all of the developed samples [1, 3].

2.3.3 Water-in-Air Contact Angle Measurement, Surface Wettability

Water-in-air contact angle (WCA) measurement is one of the well-known surface analysis methods to explore the wettability of the examined surface. Detailed study on the behavior of the deposited water droplet towards surface provides valuable information that can be referred to the surface properties of the examined surface. The WCA of 90° is ascribed to the wettability of a neutral surface (Fig. 2.2a). Therefore, examined platforms can be categorized in the group of hydrophobic materials if the WCA is greater than 90° (Fig. 2.2b). Conversely, the WCA of less than 90° refers to the relatively hydrophilic materials (Fig. 2.2c).

Super hydrophilic materials are those that have shown very small WCA (only few degrees), while super hydrophobic material are those for which WCA exceeds

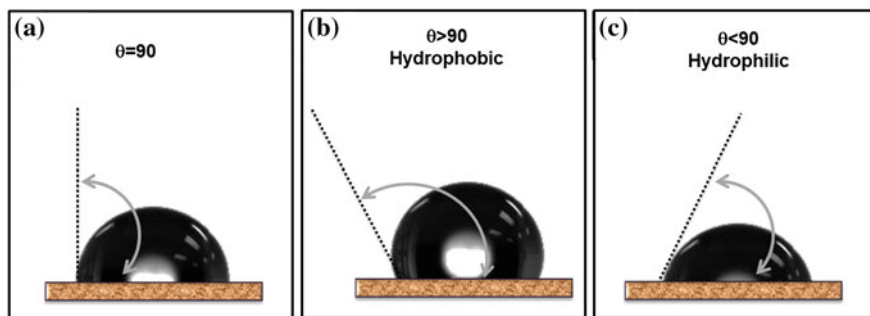


Fig. 2.2 Response of the water droplet deposited on a homogeneous solid surface, which leads to the WCA; **a** neutral; **b** hydrophobic; and **c** hydrophilic

to 150° and above [13, 14]. In this study, WCA measurements were conducted with Dataphysics contact angle system (OCA) by sessile drop method in room temperature by using distilled water (DW). An average contact angle was calculated for five separate measurements with deposited droplets on the center and four corners of the surface. These contact angles were recorded after 1 min of DW deposition while the volume of the droplet was $0.1 \mu\text{l}$. Negligible standard deviations have been obtained ($\pm 2^\circ$) almost for all of the measurements ($n = 15$, 3 samples from each composition).

2.3.4 X-ray Photoelectron Spectroscopy, Surface Chemistry

X-ray photoelectron spectroscopy (XPS) is a sensitive technique from the class of electron spectroscopy (ES) that can analyze the top 10 nm of the surface. In this technique, a soft X-ray beam (low energy) is irradiated to the sample [15]. The beam excites the electrons from the outmost layer of the surface, which have lower binding energy than the radiated X-ray beam. Excited electrons scape from the parent atom in the form of photoelectron and becomes detected by the instrument [16]. In this study, the XPS measurements were performed by using Quantera SXMtm from Ulvac-PHI (Q1) using monochromatic $\text{AlK}\alpha$ -radiation and a take-off angle (θ) of 45° at which the information depth was ~ 7 nm. A spot size of $300 \times 500 \mu\text{m}$ was chosen for the sample analysis. Wide-scan measurements were used to identify the presence of elements on the surface. Precise quantification and detailed identification of chemical states was achieved by using narrow-scans. Standard sensitivity parameters were used to convert peak positions to atomic concentrations. Therefore, the concentrations might be deviated from the real values in the absolute sense (relatively less than 20 %) [2].

2.4 Infectious Diseases

Infectious diseases are illnesses caused by pathogenic microorganisms such as viruses, bacteria, parasites or fungi that might live inside or on the body [17]. Such diseases can be widespread through a direct or an indirect way. For example, some infectious diseases can be passed from one person to another while some others are transmitted by bites from insects or animals. Infectious diseases, in some cases, might also be acquired by consuming contaminated food or water or even being exposed to the microorganisms present in the environment. Signs and symptoms can be different depending on the microorganism that caused the infection. Nevertheless, fever and fatigue can be considered as the common symptoms of the infections. While slight complaints may refer to the home remedies, more life-threatening cases may necessitate hospitalization.

2.5 Neglected Tropical Diseases

Neglected tropical diseases (NTDs) are varied types of diseases that considered being as one of the major issues in tropical and subtropical regions. World health organization (WHO) has listed 18 most common and concerning illnesses in the category of NTDs, which are divided in four categories: (i) protozoan diseases such as chagas disease, human african trypanosomiasis and leishmaniasis; (ii) bacterial diseases such as buruli ulcer, leprosy and trachoma; (iii) helminth diseases such as cysticercosis/taeniasis, dracunculiasis, echinococcosis, foodborne trematodiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis and yaws and; (iv) viral diseases such as dengue, chikungunya and rabies. Such wide-spread diseases affect nearly 1.5 billion people in 149 countries around the world, in particular from the poor populations [17]. NTDs typically grow in underprivileged tropical climates while they have been mostly wiped out in other parts of the world with higher living standards and hygiene [18, 19]. Among different NTDs, dengue fever (DF) has been chosen as the targeted viral infection as the research team which conducted this study is located in one of the tropical countries, Malaysia (Fig. 2.3a).

2.6 Dengue Fever

Along similar infections such as yellow fever, west Nile and Japanese encephalitis, DF can be grouped in the family of *Flaviviridae*. It is a mosquito-borne viral infection, which is mainly transmitted from one person to another by the bite of an aedes mosquito (Fig. 2.3b) [20]. DF cannot be transferred directly from one individual to another.

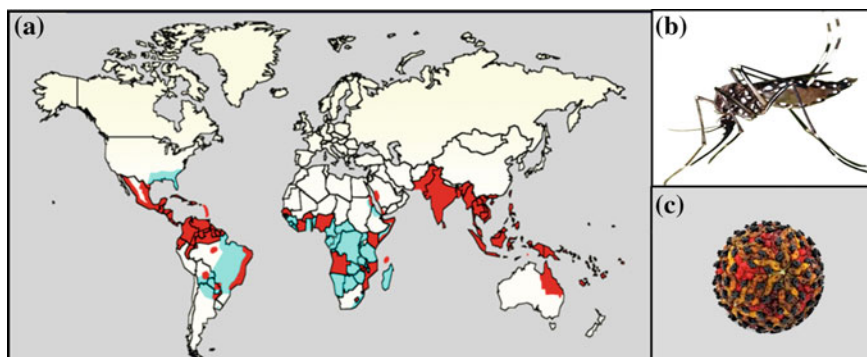


Fig. 2.3 Spreading map of DENV (a); aedes mosquito (b) dengue enveloped virus (c)

According to the WHO's report, the number of the infection with DENV (Fig. 2.3c) exceeds to 50 million cases every year [17] while other sources estimate this number to be up to 100 million infections annually. Only in the first quarter of the year 2014, the outbreak of DF in Malaysia reached to 27,500 cases that consequently resulted in 64 deaths [17]. Although DF is mainly widespread in tropical and subtropical areas, the risk of such infectious illnesses can be worldwide by individuals who contracted the virus while traveling abroad [21]. There are four serotypes for DENV including DENV1, DENV2, DENV3 and DENV4. Infection with any of the serotypes does not immune the person from the infection with another. Although primary infection results in DF, in particular cases, it can develop to the fatal manifestations such as dengue hemorrhagic fever (DHF) and/or dengue shock syndrome (DSS) [21–23]. It is believed that patients who experience DF repeatedly (infection with other serotypes) are more likely to be at risk for syndromes such as DHF and DSS [21–23]. Regular symptoms of DF include fever, nausea, vomiting, headache, skin rashes and pain in the muscles [24–26]. Signs when the patient enters the acute phase of the illness normally are: high fever, restlessness, bleeding under the skin, clammy skin (in the case of DHF) and circulatory collapse (in the case of DSS) [21–23]. With more than half of the world population at risk of this fatal disease, dengue is, indeed, one of the most dangerous mosquito transmitted viral infections [20, 27]. Detection of dengue in the early stages is estimated to reduce the mortality rate from 20 % to below 1 % [24–26].

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