Materials Science and Engineering C 58 (2016) 586–594

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Efficient protein immobilization on polyethersolfone electrospun nanofibrous membrane via covalent binding for biosensing applications

Matin Mahmoudifard [a], Sara Soudi [b,c], Masoud Soleimani [d], Simzar Hosseinzadeh [e,f], Elaheh Esmaeili [a,d], Manouchehr Vossoughi [a,g,*]

[a] Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, Iran
[b] Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran
[c] Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[d] Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[e] Nanotechnology and Tissue Engineering Department, Stem Cell Technology Research Center, Tehran, Iran
[f] School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran
[g] Chemical and Petroleum Engineering Department, Sharif University of Technology, Tehran, Iran

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form 26 August 2015
Accepted 3 September 2015
Available online 6 September 2015

Keywords:
Antibody
Immobilization
Electrospinning
Nanofiber
Covalent binding

ABSTRACT

In this paper we introduce novel strategy for antibody immobilization using high surface area electrospun nanofibrous membrane based on ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling chemistry. To present the high performance of proposed biosensors, anti-staphylococcus enterotoxin B (anti-SEB) was used as a model to demonstrate the utility of our proposed system. Polymer solution of polyethersolfone was used to fabricate fine nanofibrous membrane. Moreover, industrial polyvinylidene fluoride membrane and conventional microtiter plate were also used to compare the efficiency of antibody immobilization. Scanning electron microscopy images were taken to study the morphology of the membranes. The surface activation of nanofibrous membrane was done with the help of O_2 plasma. PES nanofibrous membrane with carboxyl functional groups for covalent attachment of antibodies were treated by EDC/NHS coupling agent. The quantity of antibody immobilization was measured by enzyme-linked immuno sorbent assay (ELISA) method. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy was performed to confirm the covalent immobilization of antibody on membrane. Atomic force microscopy, scanning electron microscopy and invert fluorescence microscopy were used to analyze the antibody distribution pattern on solid surfaces. Results show that oxygen plasma treatment effectively increased the amount of antibody immobilization through EDC/NHS coupling chemistry. It was found that the use of nanofibrous membrane causes the improved detection signal of ELISA based biosensors in comparison to the standard assay carried out in the 96-well microtiter plate. This method has the potential to improve the ELISA-based biosensor and we believe that this technique can be used in various biosensing methods.

1. Introduction

The accurate assessment of biomarkers likes proteins, hormones or other biomolecules that amount of them changes with state of disease or level of treatment and gives diagnostic information is a difficult duty. In these samples, pathogens exist in small quantity compared to other background molecules. Therefore, appropriate sampling process for concentrating and separating pathogens from the matrix should be developed in order to provide rapid and accurate pathogen detection test [1].

Electrospinning nowadays attract much more attention due to its facile ability to produce fine fibers which have specific surface area approximately one to two orders of magnitude larger than flat thin films. In this technique, a high voltage is applied to a polymeric droplet emanating from the tip of a nozzle. As soon as voltage passes threshold point, the deformation of a cone-like droplet occurs and continues jet stems from the nozzle tip and accelerates toward the collector to form nanofibrous membrane.

In most of cases, nozzle acts as cathode and collector plays the role of anode [2–3]. Electrospun nanofibrous membranes recently found versatile application in tissue engineering [4], wound dressing [5], drug delivery [6], filtration [7–9], sensor and sampling devices [10] and gas senor [11–12] and so on.

The combination of molecular biomarkers e.g. antibodies, aptamers, peptides, and liposccharides with high surface area electrospun

* Corresponding author at: Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, Iran.
E-mail address: vosoughi@sharif.edu (M. Vossoughi).

http://dx.doi.org/10.1016/j.msec.2015.09.007
0928-4931/

nanofibrous membranes can provide new chance to create both novel sampling and biosensor devices [13]. In most recent studies some researchers have proposed using the perfect capability of high surface area electrospun nanofibrous membranes to improve the efficiency of immunoassays. They studied the capability of surface modified nanofibrous membranes containing primary amine group for covalent immobilization of biological elements likes chymotrypsin and lysozyme [14–16]. They found that nanofibrous membranes could immobilized greater amount of biological molecules compared to that of cast film.

In the most cases, the immunoassay employs the specificity of antibody–antigen interaction for the detection of relevant analytes. Immunoassay is widely used in pharmaceutical research, medical diagnostics and biological analysis. As before reports, wide range of new materials and techniques were proposed to improve the sensitivity and selectivity of traditional immunoassay [17].

ELISA is one of the widely used immunoassay techniques in the biological detection field all over the world and continuous efforts are being done to improve the sensitivity and selectivity of this technique [18–19]. It was accepted that the capacity of solid substrate for adsorbing protein has important effect on sensitivity of this assay [20]. Up to now, various techniques have been proposed to immobilize protein molecules to the different substrates such as magnetic microspheres [21–23], film [24–25], fiber [26–28], and silica microsphere [29]. However, recently electrospun nanofibrous membranes deal with increase interest toward immobilization of proteins on themselves [30–33]. In most of studies the immobilization of antibodies was done through physical adsorption. Physical adsorption on the solid surfaces through hydrophobic interactions is a commonly used antibody immobilization technique for immunoassay platforms [2,34–35]. However, this technique suffers from some lack including non-uniform distribution of antibody, loss of antibody functionality, poor signal to noise ratio, leaching of antibody and protein exchange [36–40]. An ideal antibody immobilization should follow some needs including selectivity and specificity, efficiency and fastness, activity retention and recovery rates [2].

In this study, nanofibrous membranes were produced from polyethersulfone (PES) solution using electrospinning technique. PES is a non-biodegradable polymer which is widely used as a membrane material in hemodialysis, filtration and bioreactor technology [41–42]. Oxygen plasma treatment was performed to create functional groups on the surface of produced nanofibrous membrane. Two different strategies were developed to immobilize anti-staphylococcus enterotoxin B (SEB), as an antibody model on the surface of nanofibrous membrane: covalent binding through 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)/N-hydroxysuccinimide (NHS) coupling strategy and the other through hydrophobic interactions. A simple ELISA assay procedure was performed to assess the antibody immobilization efficacy on PES electrospun nanofibrous membrane.

2. Experimental

2.1. Materials

PES was supplied by BASF, Ludwigshafen, Germany. Dimethylformamide, NHS and EDC were obtained from Sigma-Aldrich. Anti-SEB (X) antigen, monoclonal mouse IgG antibody was obtained in our laboratory by hybridoma technique (unpublished data). Secondary antibody (Goat pAb to MS IgG (HRP)) was purchased from Abcam. TMB substrate solution was obtained from Razi Biotech. Bovine serum albumin (BSA) and sulfuric acid (H_2SO_4) were supplied by Merck.

2.2. Nanofibrous membrane preparation and surface treatment

PES nanofibrous membranes were fabricated via electrospinning technique according to a method previously reported in our laboratory [43–44].

In brief, a 24 wt.% solution of PES in dimethylformamide (DMF) was poured into a 10 ml plastic syringe which was connected to a stillness needle with an extension tube. We used electrospinning apparatus (Electroris-NL, Fanavaran Nanomeghyas Co., Iran) to prepare nanofibrous membrane.

The distance between needle and collector was adjusted to 15 cm. The solution was ejected from needle by a syringe pump. High voltage of 18 kV was applied between the nozzle and collector to force the solution droplet from the needle and fabrication of fine fibers with the nanometer diameter on the collector. The flow rate of electrospinning was set to 0.3 ml/h.

In order to modify the surface chemistry of fabricated nanofibrous membrane oxygen plasma treatment was performed by a low frequency plasma generator of 40 kHz with a cylindrical quartz reactor. Pure

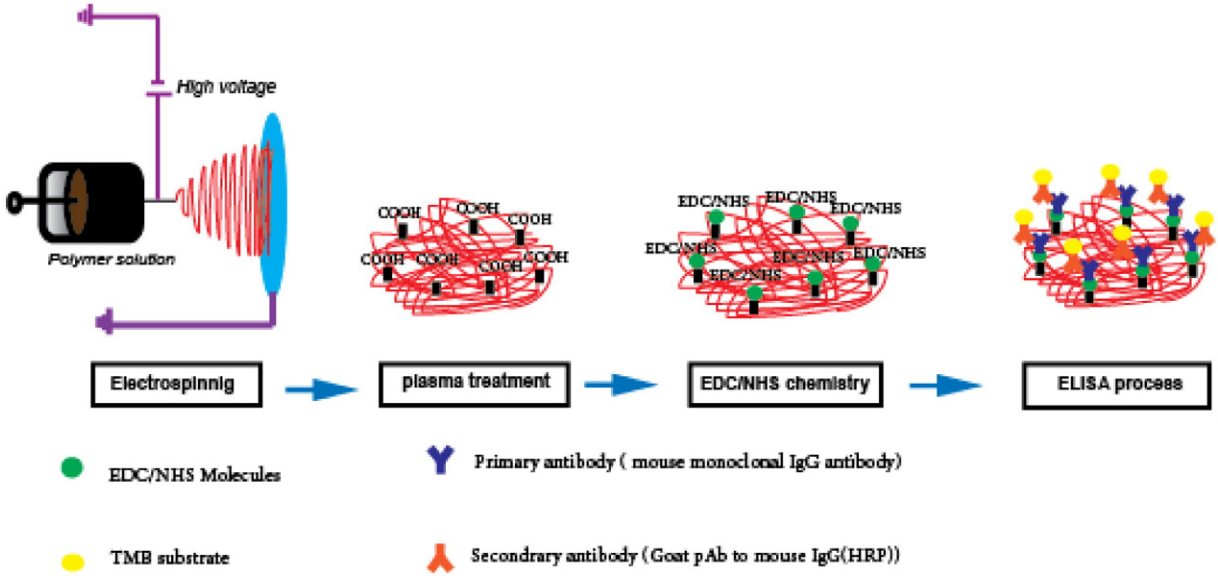

Fig. 1. Schematic of the nanofibrous membrane fabrication through electrospinning, plasma treatment and sandwich ELISA applied on nanofibrous membrane.