A Novel Biosensor System on Microfluidic Platform for Early Diagnosis of Breast Cancer

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1. Introduction

Breast cancer still imposes a significant health burden on women worldwide. As is true with most other cancer diseases, early diagnosis and implementation of prompt treatment are crucial in battling this disease.[1] Biological markers from blood, urine, and tissue can signal predisposition, onset, response to drug treatment, and recurrence of breast cancer. Thus discovery and early detection of biomarkers are among the techniques that play significant roles in early diagnosis, effective prognosis, and close monitoring in treatment of breast cancers. Genomic and proteomic-based biomarkers are the most widely studied tumor markers for early detection of cancers; genomic biomarkers are the genetic mutations or alterations in specific genes, while proteomic biomarkers are the changes in protein shape, function, and pattern of expression due to cancerous conditions.

Polymer-based microfluidic devices have attracted significant interest in recent years due to their potential impact on biological analysis, drug development, and medical diagnostics. The promise of these devices in instrument development and assay methodologies is associated with their intrinsic advantages, such as small footprint and easy multiplexing, and with the potential for integration of various components into the system, as well as mass production of the devices at an attractive cost. Since the first fabrication of micro-electrophoresis devices in glass substrates using photolithography and wet chemical etching,[2,3] various microdevices have been constructed and used for bioanalytical applications, such as PCR amplification of oligonucleotides,[4,5] separation of single-stranded[6] and double-stranded DNA,[7] and protein analysis.[8]

Biosensors on the microfluidic device platform are among the applications of these devices. Described here is a methodology development for early diagnosis of breast cancer using simultaneous detection of biomarkers via a microfluidic device platform. This methodology is intended to combine the strengths of microfluidics — high throughput, low sample consumption, and low detection limit — with the highly parallel characteristics of the microarray technologies and the high sensitivity/low limit of detection near-IR fluorescence techniques. By achieving this methodology, it is to make multianalyte (multiple biomarkers originating from the same disease) detection a reality and, reduce false negative and false positive outcomes. In brief, a hybrid biosensor system is to be fabricated on a polymer-based microfluidic device that utilize a unique format — Zipcode universal DNA arrays that will direct each capture probe (genetic and protein capture probes for a given breast cancer biomarker) to a specific locus on the polymer device surface — that decouples the hybridization event from the detection event, and thereby improves the accuracy of the technique.[9] Here, the genetic biomarker (gene mutational sequence) that is named breast cancer type I gene, or *BRCA1,* will be prepared through a polymerase chain reaction (PCR)/ligase detection reaction (LDR) coupled assay using genomic DNA isolated from a breast cancer cell line as the template; the protein biomarker (cell surface over-expressed protein) will be semi-synthesized by cross-linking the anti-protein biomarker antibody with the cZipcode oligonucleotide (oligo) DNA sequence. Signal generation and transduction are achieved through near-IR fluorescence

detection which minimizes background fluorescence interference and therefore increase the sensitivity of detection.

2. Experimental

2.1 Surface modification of the microfluidic substrate: PMMA[10,11]

Poly(methyl methacrylate) sheets, Plexiglass or Lucite, were purchased from Goodfellow and AIN and were machined to various-sized pieces. Before any type of experimental analysis was carried out, PMMA pieces were sonicated in isopropanol (IPA) for 15 min, then rinsed with IPA and subsequently dried with a flow of house nitrogen; scanning force microscopy did not indicate any increases in surface roughness as a result of this cleaning protocol. HPLC-grade isopropanol (IPA) and all other chemicals were obtained from Aldrich and used without any further purification unless noted otherwise. The UV light source used here is a low-pressure mercury lamp possessing an emission spectrum spanning the 240 nm to 425 nm range; the 254 nm band is the strongest with an intensity of 15 $mWcm^{-2}$, while that of all others is less than 1.5 $mWcm^{-2}$ at a 1-cm distance. Characterization and monitoring of the UV-modification process was achieved via a variety of analytical tools.

2.2 Biofunctionalization of the PMMA substrate[12]

Biofunctionalization of the microfluidic device substrate, PMMA, was achieved by utilizing the COOH functional groups that are induced during UV-modification of the PMMA surface. Antibodies or oligonucleotide sequences were covalently immobilized on the UV-modified PMMA surfaces with EDC coupling chemistry, which forms an amide bond between the amino groups on the biomolecules and the COOH groups on the UV-modified PMMA surfaces. Confirmation of the successful immobilization was through fluorescence microscopy and X-ray Photoelectron Spectroscopy (XPS), while the biochemical activity of the immobilized biomolecules was verified to be retained by interactions between the immobilized biomolecuel (probe) with a target molecules, either a secondary antibody or a complementary oligonucleotide sequences.

2.3 Preparation of the biomarker targeting materials

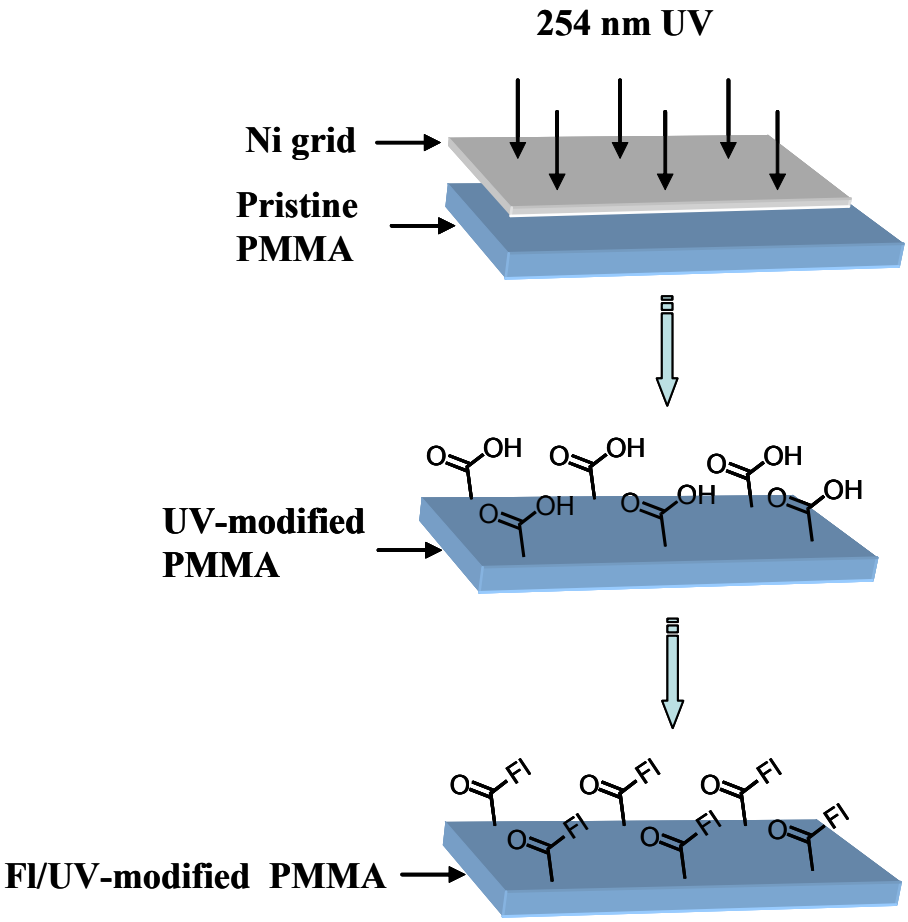
The genetic target material, *BRCA1* gene mutated sequence, was prepared through polymerase chain reaction (PCR)/ligase detection reaction (LDR) combined assay. Genomic DNA isolated from a primary breast cancer cell line was used as the DNA template in the PCR reaction, while the PCR product was used as the DNA template in the LDR reaction. Negative control samples of the LDR reactions were also prepared, one using genomic DNA isolated from a wild-type cell line, the other using a non-specific primer for the LDR reaction. PCR product was characterized on agarose gel electrophoresis while LDR product was analyzed on DNA polyacrylamide gel electrophoresis (PAGE).

Preparation of the protein biomarker targeting material was accomplished by a semi-synthetic scheme, in which a monoclonal antibody was covalently linked to a short oligonucleotide sequence through a heterobifunctional linker, sulfo-SMPB. Investigation and monitoring of the cross-linking was done by UV-Vis spectrophotometry and matrix assisted laser desorption/ionization mass spectrometry coupled to time-of-flight mass analyzer.

3. Results and discussion

Scheme 1 demonstrates the process of UV-modification of the microfluidic substrate, PMMA surfaces and confirmation of the COOH functional groups through fluorescence chemical labeling. Scheme 2 and Figure 1 investigates the possible mechanism behind the

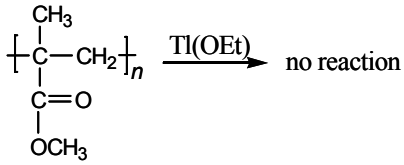
photochemical modification process using thallium labeling of the COOH groups, and subsequently investigated with x-ray photoelectrospectropy deconvolutional method.



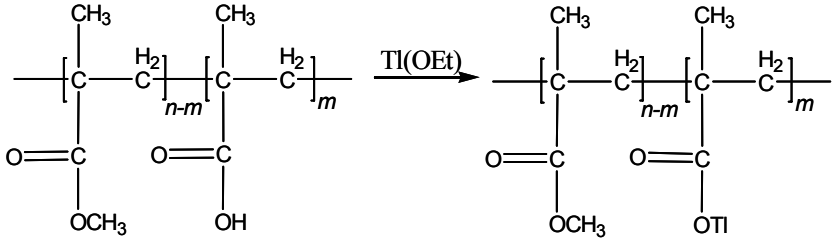
Fl: Fluoresceinyl glycine amide

Scheme 1 Chemical mapping of carboxylic acid functionalities with fluoresceinyl glycine amide. PMMA was first exposed to UV light through a photomask (2000 mesh Ni grid, with square holes of 7.6 μm x 7.6 μm). The groups formed as a result of exposure were derivatized with fluoresceinyl glycine amide in the presence of EDC to produce fluorescence. Fluorescence images were then immediately taken with a Nikon Photoshot FxA fluorescence microscope. The excitation and the emission filters used were 488 nm and 520 nm, respectively.

A. Pristine PMMA



B. UV-modified PMMA



Scheme 2 Tl^{+} labeling of COOH functionalities on pristine and UV-modified PMMA surfaces as was reported by Batich et al.

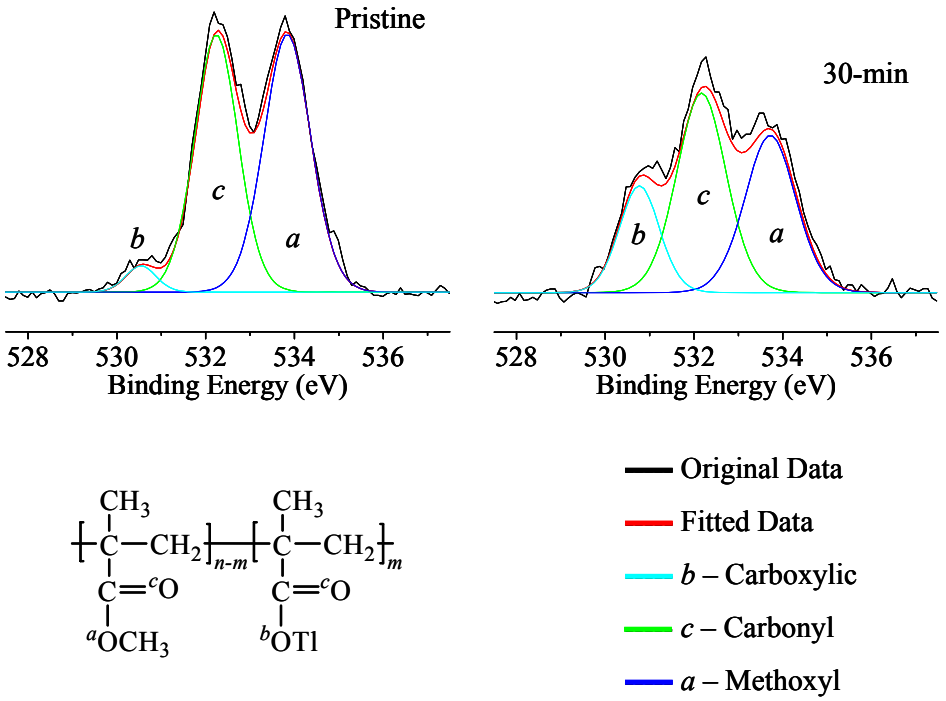
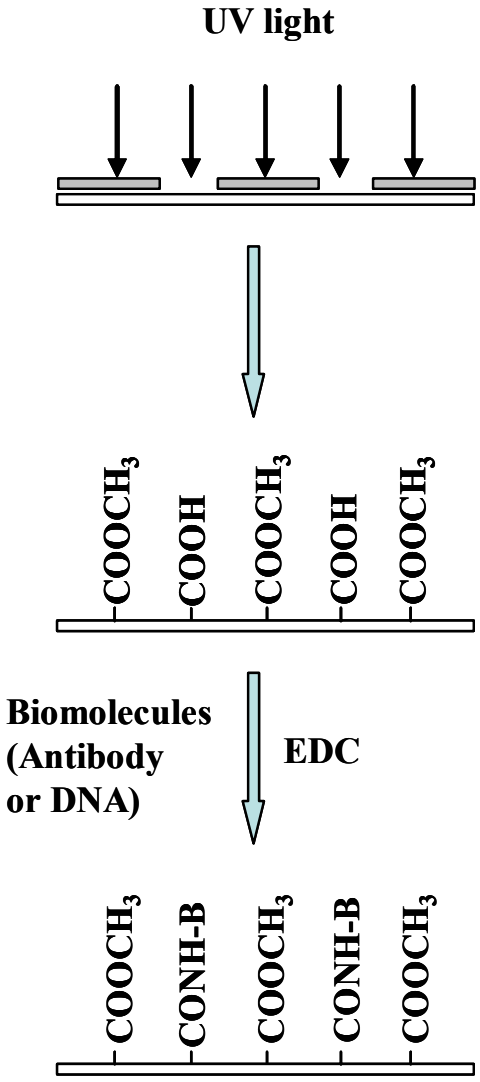


Figure 1 Deconvoluted X-ray photoelectron spectra in the O1s region for pristine and 30-min, UV-modified PMMA. A. pristine PMMA after exposure to Tl(OEt); B. 30-min UV-modified PMMA after exposure to Tl(OEt). There are three components for both spectra, designated as: *c*. carbonyl oxygen; *a*. methoxyl oxygen; *b*. carboxylic oxygen (bound to Tl^{+}).

Scheme 3 depicts how biomolecules like antibodies and oligonucleotides are immobilized on UV-modified PMMA surfaces in a simply patterned way. Figure 2 is the XPS spectra of pristine, UV-modified, and antibody-immobilized PMMA surfaces, which are to confirm the successful immobilization of biomolecules such as antibodies using the simple EDC coupling chemistry. The presence of nitrogen photon emission signal at 399.8 eV only on the antibody (Ab)-PMMA surfaces is the evidence of the successful immobilization.



Scheme 3 Approach for patterning biomolecules on UV-modified PMMA surfaces.

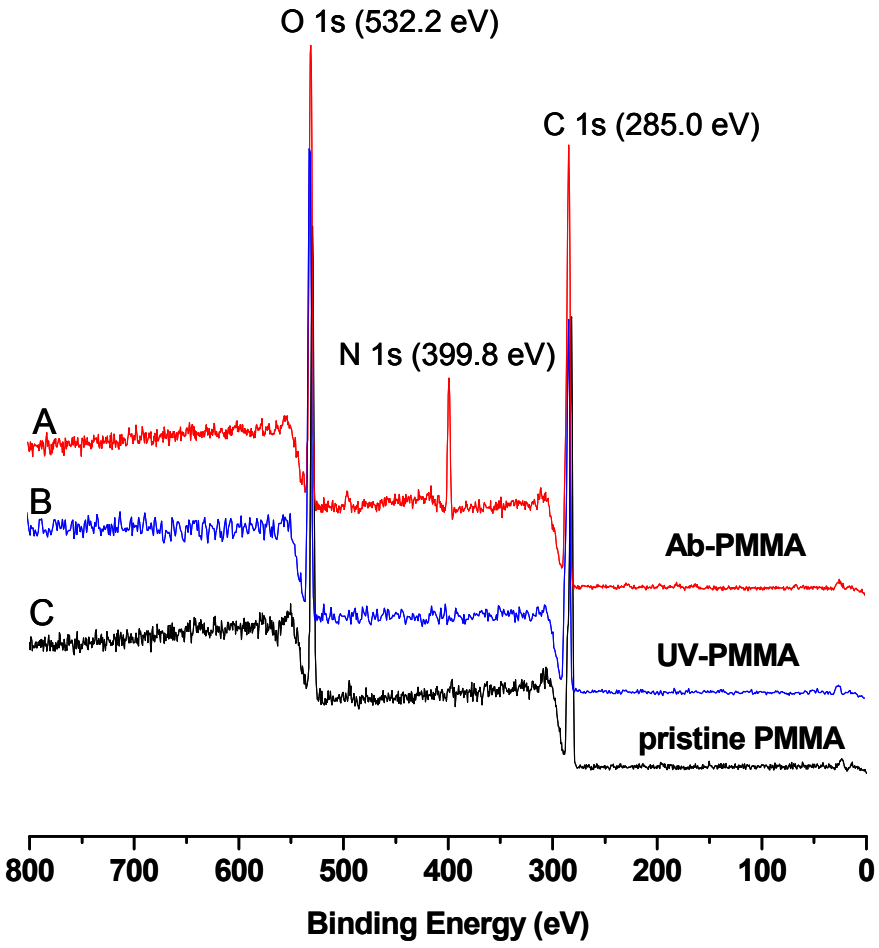
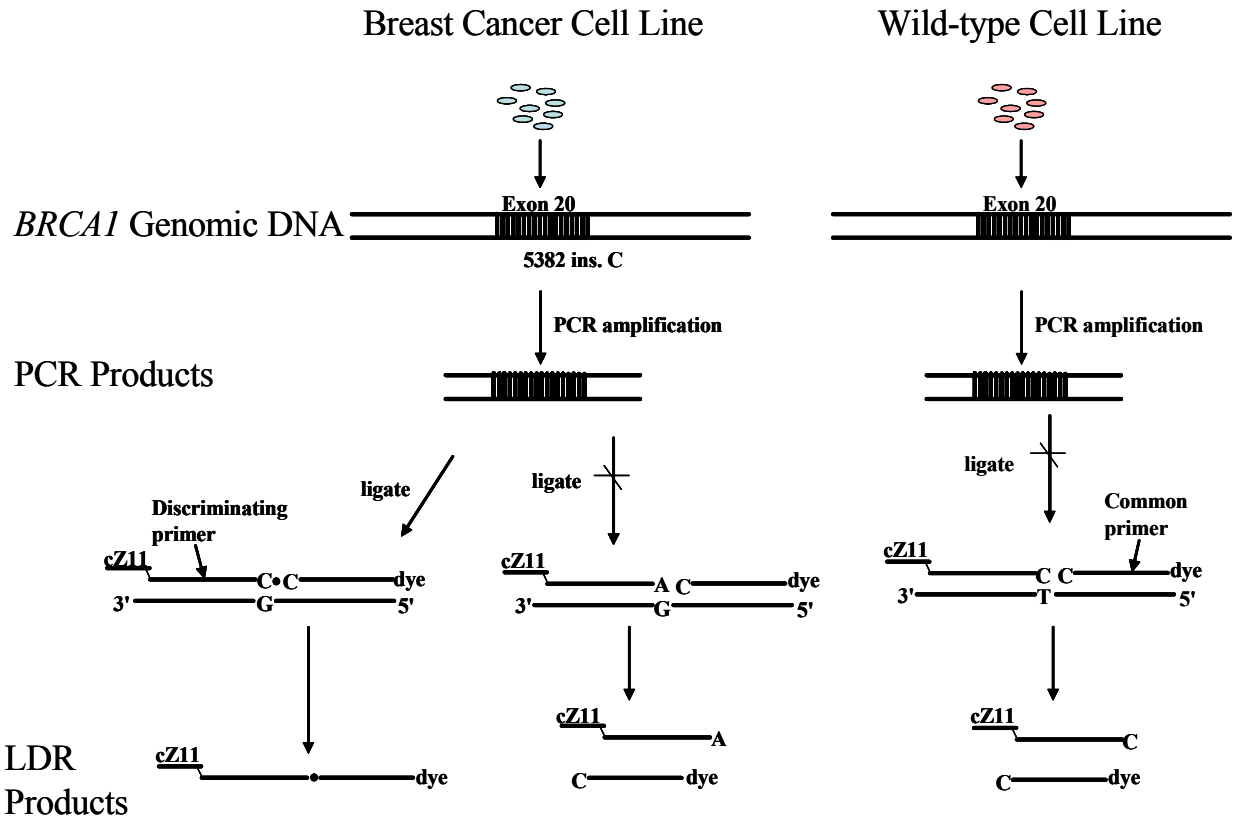


Figure 2 Confirmation of the immobilization of antibody molecules on UV-modified PMMA surfaces by X-ray Photoelectron Spectroscopy. Shown are XPS survey spectra of: A. antibody-immobilized PMMA; B. 30-min, UV-modified PMMA; and C. pristine PMMA. There are only two components in the spectra for both pristine and UV-modified PMMA surfaces, namely carbon and oxygen, while there is an additional nitrogen peak in spectrum C indicating the immobilization of antibody molecules.

Scheme 4 demonstrates the protocol of the PCR/LDR combined assay in preparing the genetic targeting materials for fabrication of the hybrid biosensor system. The scheme comprises preparation steps for both the test sample and controls. Characterization and monitoring of the preparation procedure were achieved by slab gel electrophoresis followed by gel imaging and quantification.



Scheme 4 The adapted PCR/LDR combined assay for detection of single insertion mutation in the *BRCA1* gene.

Conclusions

Surface modification and manipulation of the microfluidic substrate PMMA have been achieved by simple and straightforward photochemical methods. Immobilization of biomolecules, one of the first steps in fabricating biosensors, was achieved via EDC coupling chemistry upon the UV-modification of the PMMA substrate surface. Protocols for preparation of biomarker targeting material was developed and verified.

Acknowledgments

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