ORIGINAL ARTICLE

DNA APTAMER-IMMOBILIZED BLOCK COPOLYMER SURFACE FOR CIRCULATING TUMOR CELL CAPTURE IN LIQUID BIOPSY

Sıvı Biyopside Sirküle Tümör Hücresi Yakalamak İçin DNA Aptameri-İmmobilize Edilmiş Blok Kopolimer Yüzey

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ABSTRACT

Objective: Detecting circulating liquid biopsy components is essential for early cancer diagnosis and monitoring disease progression. Current biosensors detect circulating tumor cells in later stages, prompting a search for more effective techniques. In this study, we developed a novel surface modified with poly glycidyl methacrylate (PGMA)-b-poly pyridyl disulfide methacrylate (PPDSMA) block copolymer and functionalized with AS1411 aptamer for sensitive detection of circulating tumor cells.

Material and Methods: The PGMA-b-PPDSMA block copolymer was synthesized and characterized by nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC). Glass slides were modified with aminopropyltriethoxysilane (APTES), allowing covalent attachment of the block copolymer via PGMA epoxy groups. The pyridyl disulfide groups of PPDSMA enabled site-specific immobilization of thiol-modified anti-nucleolin DNA aptamer (AS1411). Immobilization efficiency and aptamer specificity were validated using fluorescence microscopy with MCF-7 breast cancer cells.

Results: NMR and GPC results showed that PGMA-b-PPDSMA block copolymer with a molecular weight of 6 kDa, consisting of 4.8 kDa PGMA and 1.2 kDa PPDSMA block, was synthesized and purified. APTES modification on glass surfaces was confirmed by absorbance signals in the range of 1500-1650 cm^{-1} belonging to the NH2 group in the FTIR spectrum. The binding efficiency of thiol-modified AS1411 aptamer to the PPDS group of PGMA-b-PPDSMA block copolymer was followed by absorbance change between 250-400 nm of pyridin-2-thione by-product formed during the reaction with time.

Conclusion: Fluorescence microscopy images showed that MCF-7 cells selectively bound to the AS1411-modified surfaces. These findings indicate that the modified surface has potential for targeted cancer cell binding under in vitro conditions and may hold promise for future application in liquid biopsy following further validation with clinical samples.

Keywords: *Polymers; DNA Aptamers; Biosensors; Liquid biopsy; Breast Cancer*

ÖZET

Amaç: Dolaşımdaki sıvı biyopsi bileşenlerini tespit etmek, erken kanser tanısı ve hastalık ilerlemesinin izlenmesi için önemlidir. Mevcut biyosensörler, dolaşımdaki kanser hücrelerini hastalığın ileri evrelerinde tespit edebilmektedir, bu da daha etkili tekniklerin araştırılmasına yol açmaktadır. Bu çalışmada, dolaşımdaki kanser hücrelerinin hassas tespiti için AS1411 aptameriyle fonksiyonelleştirilmiş ve poliglisidil metakrilat (PGMA)-b- polipiridil disülfit metakrilat (PPDSMA) blok kopolimeriyle modifiye edilmiş yeni bir yüzey geliştirilmiştir.

Gereç ve Yöntemler: PGMA-b-PPDSMA blok kopolimeri, nükleer manyetik rezonans (NMR) spektroskopisi ve jel geçirgenlik kromatografisi (GPC) ile sentezlenmiş ve karakterize edilmiştir. Cam lamlar, aminopropiltrietoksilan (APTES) ile modifiye edilerek blok kopolimerin PGMA epoksi grupları aracılığıyla kovalan bağlanması sağlanmıştır. PPDS'nin piridil disülfid grupları, tiol-modifiye edilmiş anti-nükleolin DNA aptameri (AS1411)'nin bölgesel immobilizasyonunu mümkün kılmıştır. Immobilizasyon verimliliği ve aptamer spesifitesi, MCF-7 meme kanseri hücreleri ile floresan mikroskopi kullanılarak doğrulanmıştır.

Bulgular: NMR ve GPC sonuçları, 4.8 kDa PGMA ve 1.2 kDa PPDSMA bloklarından oluşan 6 kDa moleküler ağırlığa sahip PGMA-b-PPDS blok kopolimerinin sentezlendiğini ve saflaştırıldığını göstermiştir. Cam yüzeylerdeki APTES modifikasyonu, FTIR spektrumundaki 1500-1650 cm^{-1} aralığında NH2 grubuna ait emilim sinyalleriyle doğrulanmıştır. Tiol-modifiye AS1411 aptamerinin PGMA-b-PPDS blok kopolimerinin PPDS grubuna bağlanma verimliliği, reaksiyonla oluşan piridin-2-tiyon yan ürününün 250-400 nm arasında emilim değişimi ile zaman içinde izlenmiştir.

Sonuç: Floresan mikroskobu görüntüleri, MCF-7 hücrelerinin AS1411 ile modifiye edilmiş yüzeylere seçici olarak bağlandığını göstermiştir. Bu bulgular, modifiye yüzeyin in vitro koşullarda hedeflenmiş kanser hücresi bağlanması için potansiyel taşıdığını ortaya koymakta olup, klinik örneklerle yapılacak ileri doğrulama çalışmaları sonrasında sıvı biyopsi uygulamalarında kullanım potansiyeline işaret etmektedir.

Anahtar Kelimeler: *Polimerler, DNA Aptamerleri, Biyoalgılayıcılar, Likit Biyopsi, Meme Kanseri*

INTRODUCTION

Minimally invasive approaches for cancer diagnosis are an important step in the quest to improve cancer survival. Detection of tumor-derived components in the circulation plays an important role in early cancer diagnosis, molecular subtyping, treatment type, and prognosis assessment (1). Circulating tumor cells (CTCs) are often associated with increased metastatic sites and cancer-related morbidity. During metastasis, a cancer cell originating from a primary tumor first invades the surrounding tissue. Then, it translocates to distant tissue microvasculature via the bloodstream. Finally, it initiates secondary tumor development in distant tissues with a suitable environment (2). Clinical studies have shown that the lower the CTC count in patient liquid biopsy material, the longer the survival time. Studies have shown that circulating cancer cells are an important marker of cancer recurrence (3).

One of the main obstacles to creating effective liquid biopsies is the small amount of cancer-derived materials present in a complex mixture of proteins, cells, and nucleic acids (4). In designs aiming to increase selectivity for targeted analytes, polymeric surfaces are used to improve biosensors' analytical performance (5). Polymeric materials, thanks to their tunable physicochemical properties, are widely used in diagnostic tools such as microdevices and biosensors. Their surfaces can be functionalized with groups like hydroxyl, carboxyl, or amine to enable interactions with biomolecules and facilitate surface immobilization (6).

Affinity-based methods based on receptor-ligand interactions are used for circulating tumor cells. These methods are based on the specificity of ligands and analytes for surface receptors. In the design of biological recognition systems, biomolecules such as aptamers, enzymes, and antibodies can be immobilized on polymer surfaces to increase biosensor selectivity. By combining the natural properties of polymers with the specific recognition ability of aptamers, aptamer-conjugated polymeric surfaces can provide an effective and noninvasive approach to early cancer diagnosis (7). Aptamers are short, single-stranded oligonucleotides that bind to molecular targets such as proteins, nucleic acids, and even cells with high affinity and specificity (8). Aptamers offer significant advantages over targeting agents such as antibodies or peptides, such as low cost, low immunogenicity and durability, high tumor penetration, and high affinity. When an aptamer is coupled to a material that serves as a transducer, the system can be defined as an aptasensor (9). Recently, thanks to advances in nanoscience and nanotechnology, nanomaterials with numerous aptasensor applications in healthcare and diagnostics have been produced (10,11). Optical detection methods such as colorimetry, fluorescence, surface-enhanced Raman scattering (SERS), and surface plasmon resonance (SPR) are widely used for signal detection in aptasensors due to their ease of use and high sensitivity (12).

This study aimed to develop a new biosensor surface based on the immobilization strategy of anti-nucleolin aptamer AS1411 on Poly(glycidyl methacrylate)-b-poly(pyridyl disulfide methacrylate) (PGMA-b-PPDSMA) copolymer-coated glass surfaces to capture biomarkers with high specificity and sensitivity. In addition to its multifunctional structure that provides surface binding and bioimmobilization, the PEGylated block copolymer structure has provided superior properties to the surface, thanks to its additional advantages, such as blocking nonspecific binding. Given its demonstrated ability to capture cancer cells, the developed aptasurface represents a promising aptasensor platform for early cancer diagnosis and holds notable potential for detecting various cancer types through the incorporation of appropriate aptamers.

MATERIALS AND METHODS

Glisydyl methacrylate (GMA), methacryloyl chloride, 2-mercaptoethanol, azobisisobutyronitrile (AIBN), and Aldrithiol-2 were obtained from Sigma-Aldrich. All reagents and solvents used in the study were of analytical or high-performance liquid chromatography (HPLC) grade. Phosphate-buffered saline (PBS), fetal bovine serum (FBS), and cell culture medium were obtained from Sigma-Aldrich (St. Louis, MO, USA). The anti-nucleolin aptamer HS-AS1411 (5'-GGT GGT GGT GGT TGT GGT GGT GGT GGT TT-C3-SS-3'; Mw = 9339 Da) was synthesized and purchased from Shanghai Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China). The human breast cancer cell line MCF-7 (ATCC® HTB-22™) was acquired from the American Type Culture Collection (ATCC, Manassas,

VA, USA).

Pyridyl disulfide alcohol (PDS-OH) was synthesized by reacting Aldrithiol-2 (1000 mg, 3 mL methanol) with 65 µL acetic acid, with mercaptoethanol (165 µL in 1 mL methanol) under stirring in an ice bath, followed by 3 h at room temperature. The product was purified by column chromatography. For the synthesis of pyridyl disulfide methacrylate (PDSM), PDS-OH (500 mg) was dissolved in 15 mL anhydrous dichloromethane, and 370 µL triethylamine was added under ice cooling. Methacryloyl chloride (340 µL) was added dropwise, and the reaction proceeded for 16 h at room temperature. The product was purified by column chromatography (13).

Poly(glycidyl methacrylate) (PGMA) was synthesized via Reversible Addition–Fragmentation Chain Transfer (RAFT) polymerization (14). In a typical procedure, 1000 mg of glycidyl methacrylate (GMA), 44 mg of 2-cyano-2-propyl benzodithioate (chain transfer agent (CTA)), and 4.5 mg of azobisisobutyronitrile (AIBN, initiator) were dissolved in 5 mL of acetonitrile. The reaction mixture was transferred into a round-bottom flask, sealed with a septum, and purged with high-purity nitrogen gas for 20 minutes to remove oxygen. Polymerization was then carried out at 70 °C for 16 hours. The resulting PGMA was purified by precipitation into cold diethyl ether.

For the synthesis of PGMA-b-PPDSMA, PGMA was used as a macro chain transfer agent (CTA) in the polymerization of pyridyl disulfide methacrylate (PDSMA). In a typical procedure, 100 mg of glycidyl methacrylate (GMA), 50 mg of PDSMA, and 0.5 mg of AIBN were dissolved in 1.5 mL of acetonitrile. The reaction mixture was transferred into a round-bottom flask, sealed with a septum, and purged with high-purity nitrogen gas for 20 minutes to remove oxygen. Polymerization was then carried out at 70 °C for 24 hours. The resulting PGMA-b-PPDSMA copolymer was purified by precipitation into cold diethyl ether.

The number-average molecular weight (Mn) and polydispersity index (PDI) of the synthesized polymer were determined via Gel Permeation Chromatography (GPC) using a Shimadzu system with tetrahydrofuran (THF) as the mobile phase and poly(methyl methacrylate) (PMMA) as the calibration standard. The structural characteristics of the polymer were further analyzed by 1H Nuclear Magnetic Resonance (1H NMR) spectroscopy. The spectra were recorded on a JEOL JNM-ECZR-500 spectrometer operating at 500 MHz for 1H.

The glass surfaces (1 cm x 1 cm) were initially cleaned with deionized water, followed by an ethanol rinse. To introduce reactive hydroxyl groups, the surfaces were treated with piranha solution (H_2SO_4/H_2O_2 = 5:1 v/v) for 10 minutes, followed by a 5-minute wash with water. Next, the surfaces were immersed in a 3% (Aminopropyltriethoxysilane) APTES solution in ethanol at ambient temperature for 2 hours. Afterward, the surfaces were washed thoroughly with ethanol. Finally, the modified surfaces were coated with PGMA-block-PPDS (15).

The crosslinking of the glass surfaces and the presence of hydroxyl and amino groups were thoroughly characterized using (Fourier Transform Infrared Spectroscopy) FT-IR (Perkin Elmer, USA). The FT-IR spectra were recorded over the wavenumber range of 4000–450 cm^{-1}. The spectra were collected with a resolution of 4 cm^{-1} and averaged over 32 scans to improve the signal-to-noise ratio. Specific absorption bands corresponding to the characteristic vibrations of the hydroxyl (O-H) and amino (N-H) groups (16).

PGMA-b-PPDSMA block copolymer (4 mg) was dissolved in 2 mL of dichloromethane (DCM). After complete dissolution, 2 mL of the polymer solution in DCM was added to APTES-modified glass slides placed in separate beakers. The slides were incubated for 24 hours in a sealed environment with septa to prevent solvent evaporation. After incubation, the polymer-coated surfaces were washed 3 times with DCM to remove any unbound polymer (17).

Thiol-modified AS1411 aptamer was immobilized onto PGMA-block-PPDSMA-coated glass surfaces via thiol–disulfide exchange by incubating 0.25 mg aptamer in 2 mL 10 mM Tris-HCl buffer (pH 8.0) at room temperature for 2 hours. Aptamer immobilization was monitored by tracking 2-thiopyridone formation spectrophotometrically using a Shimadzu UV-1800 between 250–400 nm at intervals of 5 to 90 minutes, with increasing absorbance confirming time-dependent conjugation (18).

The nucleolin-overexpressing (NUC^+) breast cancer cell line MCF-7 (ATCC® HTB-22™) was employed to

assess the capture efficiency and specificity of AS1411-conjugated surfaces for circulating tumor cells (CTCs). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin (P/S) at 37 °C in a humidified atmosphere containing 5% CO_2.

The cells were trypsinized and removed from the flask, precipitated by centrifugation, and suspended in a PBS solution. The total cell count (5x106 cells/mL) was determined using the automated Cell Counter (TC20 Bio-Rad). The aptamer-conjugated surface was washed with PBS. Cancer cell samples prepared in DMEM (2500 cells/500 µL) were applied to the surfaces. Then, it was kept for 15 minutes for conjugation. After incubation, the surfaces were washed 3 times with PBS. After washing, the cells were incubated with DNA-specific dye (DAPI (4',6-diamidino-2-phenylindole) for another 10 minutes in the dark. Imaging was performed with a fluorescence microscope. The cells captured on the surface were washed three times with PBS (19).

This study used only commercially available cell lines; therefore, no ethical approval was required (2011-KAEK 2-208).

RESULTS

In the first step, PDS-OH was synthesized after the reaction of aldrithiol-2 and 2-mercaptoethanol. Column chromatography (eluting with 10% ethyl acetate:90% hexane solution) was used for purification, affording a yield of 62%. Purified PDS-OH and methacryloyl chloride were reacted as described in Peng and coworkers' procedure (13). After 16 hours of reaction, the crude product was purified by column chromatography using a 10% ethyl acetate / 90% hexane eluent, affording the PDSMA monomer in 65% yield. GMA monomer was preferred to give epoxide functional groups to block polymer, allowing immobilization of PGMA-b-PPDSMA block copolymer via free amine groups of APTES decorated on the glass surface. The RAFT polymerization of GMA was conducted in acetonitrile at 70 °C for 16 h using AIBN as the initiator and CTA agent (2-cyano-2-propyl benzodithioate) (Figure 1A).

The polymerization of GMA was stopped at a conversion of about 95%, yielding PGMA with Mw = 4800 g mol−1 and PDI = 1.18. The full 1H NMR (CDCl3) spectrum of the PGMA polymer is given in Figure 1B. 1H NMR spectrum showed successful polymerization of GMA monomer, as was evident from the proton resonances at 3.22 (a), 2.83 (b), and 2.62 (b') ppm due to the epoxide group of GMA monomer and the proton resonances at 4.28 and 3.79 ppm epoxide neighboring CH2 protons (c, c'). The absence of characteristic proton resonances belonging to acrylate protons of GMA monomer around 5.0-6.0 ppm showed successful purification.

For the synthesis of PGMA-b-PPDSMA, PGMA was used as a macro-CTA in the polymerization of PDSMA.

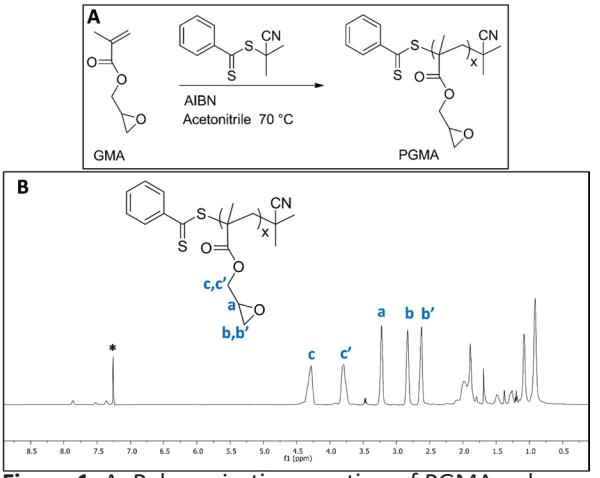

Figure 1. A. Polymerization reaction of PGMA polymer B. 1H NMR spectrum of PGMA polymer (* CDCl3)

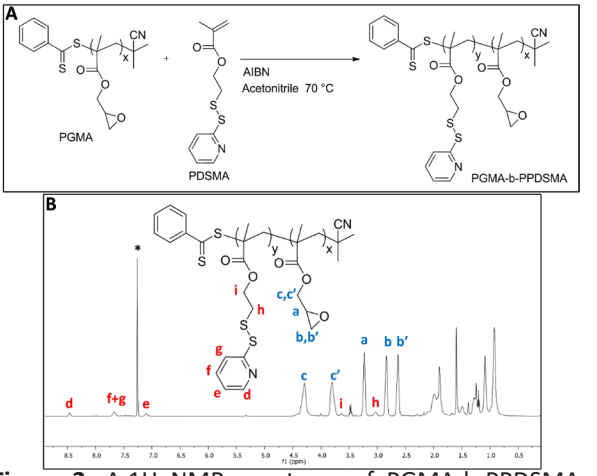

Figure 2. A.1H NMR spectrum of PGMA-b-PPDSMA block copolymer (* CDCl3). Polymerization reaction of PGMA-b-PPDSMA block copolymer. B. 1H NMR spectrum of PGMA-b-PPDSMA block copolymer (* CDCl3)

Bozok Tıp Derg 2025;15(3):345-353
Bozok Med J 2025;15(3):345-353

The RAFT polymerization of PDSMA was conducted in acetonitrile at 70 °C for 24 h using AIBN as the initiator (Fig 2A). After precipitation in cold diethyl ether, PGMA-b-PPDSMA block copolymer was obtained with a conversion of 90%, Mw = 6000 g mol−1, and PDI = 1.19.

The full 1H NMR (CDCl3) spectrum of the PGMA-b-PPDSMA copolymer is given in Figure 2B. The presence of characteristic PGMA proton resonances as well as characteristic aromatic pyridyl proton resonances at 8.46 (d), 7.67 (f+g), and 7.11 (e) ppm and pyridyl disulfide neighboring CH2 proton resonances at 3.63 (i) and 3.03 (h) ppm showed successful polymerization of PGMA-b-PPDSMA copolymer. The absence of characteristic proton resonances belonging to acrylate protons of PDSMA monomer around 5.0-6.0 ppm showed successful purification.

The FTIR spectra of unmodified silicon dioxide (SiO_2) and APTES-functionalized SiO_2 (SiO_2–APTES) are presented in Figure 3. In the spectrum of SiO_2–APTES, characteristic absorption bands observed in the region of 1500–1650 cm^{-1} are attributed to the symmetric bending vibrations of N–H bonds, corresponding to the amino (–NH_2) groups introduced by APTES molecules. In contrast, these signals were absent in the spectrum of the unmodified SiO_2 surface, which served as the control. The appearance of these N–H related peaks only after APTES treatment confirms the successful functionalization of the SiO_2 surface with amino groups.

Thiol-modified AS1411 aptamer immobilization onto PGMA-block-PPDSMA coated surfaces was confirmed by UV–Visible spectroscopy (Figure 4). The aptamer was conjugated to the copolymer via a thiol–disulfide exchange reaction, releasing pyridine-2-thione, a yellow byproduct with a characteristic UV–Vis absorption peak. Time-dependent spectra recorded between 5 and 90 minutes showed increasing absorbance of pyridine-2-thione, indicating progressive aptamer binding. These results confirm both the occurrence of the exchange reaction and the covalent immobilization of the aptamer onto the functionalized surface.

The capture of nucleolin-positive MCF-7 cells on AS1411-functionalized aptasurfaces was confirmed by fluorescence microscopy following DAPI staining (Figure 5). Compared to control surfaces without

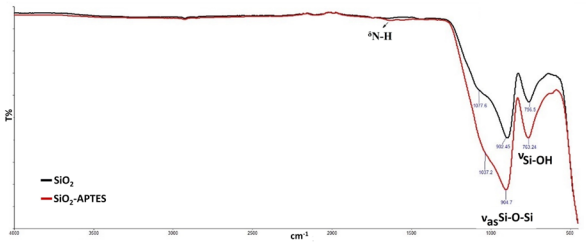

Figure 3. FTIR spectra of SiO_2 and SiO_2-APTES surfaces

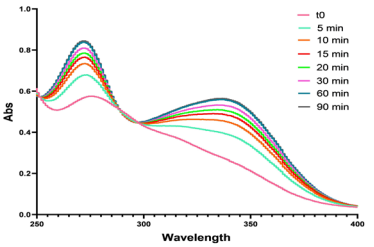

Figure 4. UV–Visible difference spectra recorded during the immobilization of the aptamer onto the polymer surface over a 5–90 min period.

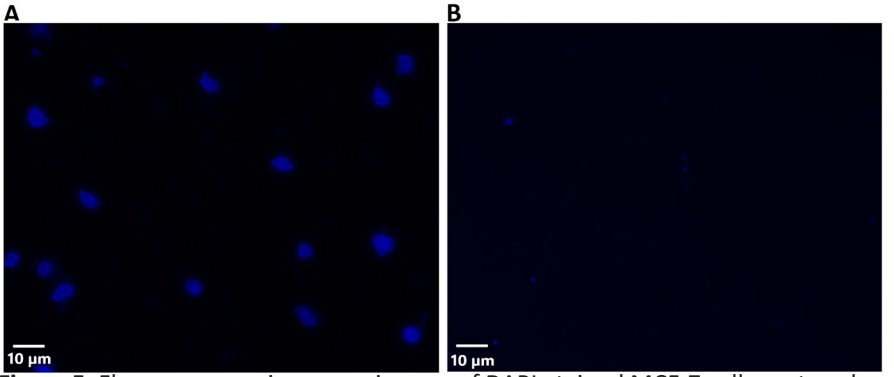

Figure 5. Fluorescence microscopy images of DAPI-stained MCF-7 cells captured on (A) AS1411-conjugated aptamer-coated polymeric surface and (B) non-conjugated control surface. Scale bar = 10 µm.

Bozok Tıp Derg 2025;15(3):345-353
Bozok Med J 2025;15(3):345-353

aptamers, a notably higher number of cells were observed on the functionalized surfaces, indicating effective and selective capture through aptamer–nucleolin recognition.

DISCUSSION

In this study, the PGMA-b-PPDSMA block copolymer designed for biomolecule immobilization demonstrated high efficiency in surface functionalization and aptamer attachment. Block copolymers are preferred in such applications due to their unique ability to provide microphase separation, controlled distribution of functional groups, and customizable chemical structures, enabling molecular-level precision in surface architecture (20). The FTIR spectra of unmodified SiO_2 and APTES-functionalized SiO_2 (SiO_2–APTES) showed characteristic absorption bands in the region of 1500–1650 cm^{-1}, attributed to the symmetric bending vibrations of N–H bonds, confirming the successful functionalization of the SiO_2 surface with amino groups (16). These N–H related peaks were absent in the unmodified SiO_2 spectrum, indicating the effectiveness of APTES treatment.

The reversible addition–fragmentation chain transfer (RAFT) polymerization enabled to give of PGMA and PGMA-b-PPDSMA polymers with desired molecular weight and low polydispersity index (PDI) values of 1,18 and 1.19, respectively (14). PGMA block with an average molecular weight of 4800 g mol-1 and PGMA-b-PPDSMA block with an average molecular weight of 6000 g mol-1 were measured using GPC, which means PPDSMA had a molecular weight of an average molecular weight of 1200 g mol-1. 1H NMR spectrum of the polymers showed stability of the functional epoxide and pyridyl disulfide groups after polymerization and purification stages. The ability to control the position and distribution of functional groups with such precision gave an advantage for aptamer immobilization.

The epoxy groups in the PGMA block enabled covalent attachment to the surface, creating a stable and robust coating, while the pyridyl disulfide groups in the PPDSMA block allowed site-specific and controlled immobilization of thiol-modified biomolecules. This strategy helped prevent random and inefficient binding, significantly improving the specificity and sensitivity of the biosensor surface. Moreover, the spatial control over immobilization preserved the three-dimensional conformation and target-binding capacity of the aptamer, directly enhancing biosensor performance (21).

Another key advantage of block copolymers is their ability to combine both hydrophilic and hydrophobic domains, allowing the creation of biocompatible surfaces suited for interaction with biological systems (22). AS1411-functionalized surfaces exhibited high selectivity and binding efficiency for MCF-7 cells. Similarly, Khan et al. utilized ethylene glycol-based block copolymers to regulate selective cell adhesion. Block copolymers like PS-b-PAA effectively immobilize aptamers, enhancing biosensor specificity and sensitivity; for example, a cocaine-specific aptamer covalently bonded to PS-b-PAA preserved its conformation and facilitated target detection in human urine samples (23).

Using aptamers in biosensors offers a significant strategy for the specific detection of target molecules (24). In this study, AS1411 aptamers were immobilized onto glass surfaces using PGMA-b-PPDSMA block copolymers, enabling high specificity and sensitivity in biomolecular recognition. The controlled and localized presentation of functional groups on the block copolymer surface plays a key role in preserving the aptamer's conformation, thereby enhancing its binding capacity to target molecules such as nucleolin (25). Biotin-terminated aptamers can be immobilized on avidin-coated surfaces, while covalent attachment via amino or thiol groups further enhances stability and binding efficiency. Such controlled conjugation directly improves biosensor performance by increasing specificity and sensitivity, underscoring the potential of aptamers for precise and selective biomolecule detection (17).

In this study, the immobilization of the thiol-modified AS1411 aptamer onto the PGMA-b-PPDSMA block copolymer-coated surface was successfully achieved through thiol–disulfide exchange chemistry. The pyridyl disulfide reaction chemistry, known for its efficiency and orthogonality to other conjugation methods, was employed to conjugate the aptamer to the PGMA-b-PPDSMA block copolymer. This chemistry not only ensured stable immobilization but also allowed for the

Bozok Tıp Derg 2025;15(3):345-353
Bozok Med J 2025;15(3):345-353

dynamic and reversible nature of the disulfide bond, providing adaptability in the conjugation process (26).

The aptamer was conjugated with the PGMA-b-PPDSMA block copolymer through thiol groups. Pyridyl disulfide-based conjugation is among the most commonly employed strategies due to its high efficiency and compatibility with other conjugation methods, allowing it to be used under mild conditions in a wide range of applications. The reaction generates pyridine-2-thione as a side product, which can be conveniently monitored in situ via UV/vis spectroscopy, allowing for easy tracking of the process. Additionally, the reaction is compatible with a wide range of organic solvents and aqueous solutions across a broad pH range. The resulting disulfide bond acts as a stable linker, which can be reversibly cleaved through redox reactions, providing a dynamic and adaptable connection between the aptamer and the block copolymer (27).

The immobilization process was confirmed through UV-Visible spectroscopy, which showed a characteristic absorption peak corresponding to pyridine-2-thione, the byproduct of the thiol–disulfide exchange reaction. The reaction was monitored at multiple time points ranging from 5 to 90 minutes, revealing a time-dependent increase in pyridine-2-thione absorbance. This gradual increase in pyridine-2-thione concentration indicated the continuous and covalent immobilization of the aptamer onto the surface (28). Furthermore, the resulting immobilized aptamer exhibited high specificity and sensitivity in biomolecule binding, which is essential for the application of aptamers in biosensor platforms.

Using this conjugation strategy, we ensured that the aptamer maintained its structural integrity and binding capacity to nucleolin, which is overexpressed in various cancer cell types (29). The PGMA-b-PPDSMA block copolymer provided a suitable surface for aptamer immobilization, enhancing both its functionality and stability. In this study, we demonstrated the potential of a polymer-based, label-free detection platform for selectively capturing circulating tumor cells in breast cancer. Integration of the MCF-7 sensor developed by Khoshroo et al., with a detection limit of 7 cells/mL, supports its applicability for real-time monitoring of minimal residual disease and metastatic progression (30). The ability to capture circulating tumor cells efficiently is crucial for cancer diagnostics, and aptamers have proven to be valuable tools in this regard (31).

Aptamer-conjugated magnetic nanoparticles, for instance, enable rapid and specific CTC capture, with detection limits as low as 75 cells/mL in buffer and 200 cells/mL in whole blood, demonstrating strong potential for clinical applications (32).

Furthermore, the development of micro-well-supported multi-aptamer immunomagnetic platforms has greatly improved the purity and capture efficiency of CTCs, facilitating mutation analysis in cancer patients and contributing to the clinical utility of these technologies. These advancements in nanotechnology-based aptamer platforms offer promising avenues for the early detection and molecular characterization of cancer, which could greatly impact clinical decision-making and patient outcomes (33). Polymeric surfaces, through surface modification or functionalization, enable the conjugation of receptor agents specific to defined targets. The integration of single or multiplex sensor systems and surface conjugation via chemical modifications is crucial for ensuring high sensitivity and validation (34).

Although the results obtained from the AS1411-functionalized PGMA-b-PPDSMA surfaces demonstrated high selectivity and sensitivity for target cell capture under in vitro conditions, the current study is limited to laboratory-scale assessments. The clinical applicability of this platform requires further validation under in vivo conditions and in complex biological matrices such as whole blood. Additional preclinical and clinical studies are essential to evaluate the biocompatibility, specificity, and reproducibility of the system in real patient samples. Addressing these translational challenges will be crucial for advancing this biosensor platform from bench to bedside. Future work will aim to optimize the platform for clinical environments and validate its performance in larger cohorts for early cancer diagnostics.

CONCLUSION

In this study, we developed an aptamer-based biointerface by conjugating thiol-functionalized AS1411 aptamers onto polymeric surfaces after attaching pyridyl disulfide groups to glass slides. This biointerface

was demonstrated to specifically capture human breast cancer cells in vitro. Along with the outputs of this project, further clinical studies will enable the analysis of even low-concentration tumor cells in blood samples using aptamer-functionalized polymeric surfaces. In this context, the aptamer-based biosensor kit developed in this study could serve as a laboratory system for early cancer diagnosis, prognosis monitoring, and the earlier evaluation of chemotherapeutic efficacy.

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The authors declare that they have no conflict of interest to disclose

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