SOX-17 Gene Sequence Complementation on Silica-alumina Nanocomposite-modified Dielectrode Surface for Analyzing Gastric Cancer Progression

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Abstract

Background

Gastric cancer is as the gastrointestinal issue, the second most death-cause complication worldwide. The survival rate of gastric cancer is lesser due to diagnosing it at the advanced stage. SRY-box containing gene 17 (SOX-17) expression and methylation participate a crucial role in the gastric cancer.

Methods

In this research, capture probe modified interdigitated electrode was used to quantify the SOX-17 gene target sequence. To improve the detection, IDE sensing surface was physically-modified by silica-alumina (Si-Al) nanocomposite. Through the biotin-streptavidin strategy, capture probe was immobilized on the surface and complemented by the target sequence.

Results

Doubled the level of capture probe immobilization was noticed on the Si-Al modified surface. From 1 aM concentration of target sequence was detected in the presence of Si-Al nanocomposite, while it was reached 10 aM in the absence, shows ten-folds difference. In addition, higher level of current changes was registered with all the concentrations of target sequence. Control experiments with single, triple and complementary sequences of target were done and there is no significant changes in current were recorded in the substituted sequences, representing the specific detection of target SOX-17 gene sequence.

Conclusion

The detection method is shown with nanocomposite-modified IDE surface helps to recognize the gastric cancer effectively.
Background

Gastric cancer, also known as the stomach cancer is the life threatening complication, mainly developed in the area of gastrointestinal; a second leading cause of death by cancer in worldwide [1, 2]. In this case, cancer cells are originated in the inner-line of the stomach and leading to the cancer. Various reasons have been proposed for causing the gastric cancer such as, smoking, Helicobacter pylori infection, consuming a higher salty food, obese and some genetic factors [1, 2]. The survival rate of gastric cancer is still not satisfied due to its poor diagnosing system. It makes the urgency to detect the gastric cancer with the suitable biomarker [3, 4], which will facilitate to diagnose at the initial stage. Cancer-autoantibody panels and microRNA are considered as the efficient biomarkers for gastric cancer. Moreover, diagnosing volatile organic compounds in breath is also in the progressing stage of gastric cancer to identify. Endoscopy is also considered as the potential tool to recognise the early stages of gastric cancer [5]. Biomolecular markers that differentiate the benign stage from the physically silent malignant are necessary to eliminate the number of excess endoscopic biopsies and to enhance the detection of gastric dysplasia at the initial stage. In the present research SRY box containing gene 17 (SOX-17) sequence was used to diagnose the condition associated with gastric cancer.

It has been widely accepted that SOX-17 gene, a transcription factor undergoes DNA methylation and this gene expression is found to be closely correlated with gastric cancer [6–8]. DNA methylation mediates the regulation of Sox-17 gene expression by the inhibition of transcription factor binding with DNA. It is an essential process for regulating the gene expression specific to the tissue. It has been widely agreed that by inactivating the gene related to the tumour, consequently hypermethylation
in the regions of promoter will [9, 10]. Yan et al. (2011) [6] revealed the down-regulation of SOX-17 gene in the gastric cancer cells (MKN45). Their study demonstrated that the siRNA-mediated lower expression of SOX-17 gene enhances the multiplication of MKN45 cells and clears that low-abundance of SOX-17 gene is the indication of gastric cancer.

SOX-17 is one of the efficient biomarkers for various cancer including gastric cancer. [6, 8, 11] SOX-17 gene belongs to the SRY family and play a major role for various developmental processes and diseases. SOX gene methylation with changes in the expression have been identified in various cancers, which includes liver, endometrial, gastric and colon [6, 12, 13]. Moreover, SOX-17 gene is highly methylated in the primary stage of breast cancers [7]. The current study is focused on SOX-17 gene, because it exhibit a CpG island in its promoter region and noted to be associated with both “Cancer” and “Wnt/β-catenin signalling pathway” in gastric neoplasia. The revealed data is also suggested that SOX-17 silencing occurs often at the beginning gastric and play a vital role in the disease development [8]. This research was focused to identify the SOX-17 targeted gene sequence by the recommended probe [7] on the silica-alumina modified interdigitated electrode sensing surface.

Voltammetry-based sensing systems have been attracted in the past to diagnose various biomarkers due to their high selectivity and sensitivity [14, 15]. Regarding this, modifying and improving the electrode surface is mandatory to improve the diagnosing system and also reproducibility. Sensitivity and selectivity are other key factors in the improving biosensor; both are highly correlated with the efficiency in biorecognition through the transducer. Various nanomaterials such as gold, silver, graphene, titanium oxide, silica and alumina were used to improve the
bioimmobilization process and the current-flow [16-19]. Herein, the silica-alumina nanocomposite was utilized to enhance the conductivity in the electrode surface of IDE sensor, and improved the detection of targeted SOX-17 gene sequence.

Materials and methods

Reagents and biomolecules
Glutaraldehyde (GLU), 3-(Aminopropyl) triethoxysilane (APTES), Phosphate Buffer Solution (PBS; pH 7.4), and Streptavidin were received from Sigma-Aldrich (USA). Biotinylated SOX-17 capture Probe: 5’ – TGGCTATTTGCGAAAGTATTATTTGAAAAAAAAGAAAAAAAGAAAAAA-C6-Biotin - 3’ and target: 5’ - TCAAATATAATACTTTCGCAAATAGCCA - 3’ were synthesized commercially and the sequences were adopted from the earlier study [7].

Preparation of Si-Al nanocomposite was followed by the procedure outline by Ramanathan et al. (2019) [20].

Fabrication of Interdigitated electrodes (IDE)
Silver electrode on IDE sensor was prepared by the traditional lithography method by wet-etching fabrication. To print the silver IDE electrode on the surface of silicon wafer, initially the positive photoresist was deposited on the surface of silicon wafer and then soft-baked for 90 sec. To transfer this pattern to the sample surface, ultraviolet light exposure (UV) was carried out for 10 sec. RD-6 developer was used for 15 sec to develop the process and then the developed sample was baked again at 110 °C to eliminate the unnecessary moisture and enhance the adhesion between the SiO₂ and silver layer. In the final step, 23 seconds of silver etchant was processed to remove the unexposed area. Further, the chemical modification was carried out on these fabricated surfaces to immobilize the biomolecules following by
the detection. The above briefed protocol was followed from the earlier report by Ramanathan et al. (2019). [20] The 3D nanoprofiler was used to analyse the surface of IDE.

**Immobilization of capture probe on IDE**

To immobilize the capture probe on the IDE surface, silane and glutaraldehyde chemical interaction was used as the linker. Initially IDE sensing surface was treated with 1% potassium hydroxide (KOH) for 1 min. After the washing step by distilled water, 2% of diluted APTES in 30% ethanol was placed on the KOH activated surface and kept at room temperature for 2 h under wet condition. And then the surface washed with 30% ethanol and by the distilled water to remove the unbound APTES. On APTES surface 2.5% of GLU was incubated and kept for 2 h at RT. A 200 nM of streptavidin was dropped on the GLU-modified surface and kept for 1 h and then the remaining surfaces were blocked by 1 M ethanolamine for 30 min at RT. Finally, 1 µM of biotinylated capture probe was permitted to interact with the immobilized streptavidin on the IDE surface. This capture probe modified surface was used to detect and quantify the level of target gene sequence SOX-17. All the experimental conditions were kept to be wet and washed between each step using 10 mM PBS (pH 7.4).

**Immobilization of capture probe on Si-Al nanocomposite modified IDE surface**

To immobilize the capture probe on Si-Al nanocomposite modified IDE surface, first Si-Al nanocomposite was attached on the IDE surface through APTES as the linker. A 0.1 g of Si-Al nanocomposite was suspended in 1 mL of 2% APTES (in 30% ethanol) and kept for 1 h at RT. And then the above diluted Si-Al nanocomposite was placed on the KOH activated IDE sensing surface. The other immobilization steps of GLU,
streptavidin and biotinylated capture probe were carried out as described above. These capture probe modified Si-Al nanocomposite IDE surface was used to detect and quantify the level of target gene sequence of SOX-17.

**Comparative detection of SOX-17 gene target on IDE and Si-Al nanocomposite modified IDE sensing surfaces**

On these capture probe modified IDE and Si-Al nanocomposite-IDE surfaces, 1 pM of target sequence was interacted and compared. For that 1 pM of target sequence was dropped and the changes in the current before and after the complementation were noticed for comparison. Before record the current flow, the surface was washed 5 times by 10 mM PBS (pH 7.4) to avoid any nonspecific binding of biomolecules on the sensing surfaces.

**Limit of detection on IDE and Si-Al nanocomposite-modified IDE sensing surfaces**

Comparison with limit of detection of target gene sequence of SOX-17 was carried out with IDE and Si-Al nanocomposite modified IDE sensing surfaces. Target sequence concentrations from 1 aM to 100 fM by 10 order dilution were dropped individually on both IDE and Si-Al nanocomposite modified IDE surfaces. The difference in current of before and after immobilization was monitored for each concentration of target sequence and plotted by the linear graph to find the limit of detection.

**Specificity experiment on IDE and Si-Al nanocomposite modified IDE sensing surfaces**

Specificity of target sequence complementation was evaluated using three different control sequences, namely complementary of target, single and triple mismatched target sequences. A 1 pM of these sequences were dropped on the capture probe
modified IDE and Si-Al nanocomposite-IDE surfaces and compared the changes in the current with the similar of target sequence at 1 pM concentration.

Results and Discussion

Stomach cancer originates in the areas with the inner lining of the stomach and then cells grown into the tumour. Main cause of gastric cancer is by the infection of H. pylori bacterium. Infection of this bacterium causes ulcers, consequently it become the cancer. Detection of gastric cancer at the later stage is a big hurdle and affects the survival rate of the patient. SOX-17 gene expression and methylation plays a major role in various cancers including gastric cancer. Identifying and quantifying the target sequence for this gene helps to diagnose the progression with gastric cancer. In this research, the target sequence for SOX-17 gene was detected on IDE and Si-Al composite modified sensing surfaces and compared their detection limit.

Detection strategy for target DNA complementation on IDE and Si-Al-IDE sensing surface

Figure 1 displays the schematic representation of SOX-17 target gene sequence complementation by IDE and Si-Al-IDE sensing surfaces. Silane and glutaraldehyde (GLU) chemical modifications were used to immobilize the capture probe on these surfaces. Before being started the surfaces were treated with 1% KOH to improve the chance of APTES binding on the surface, because without OH-groups, APTES adsorption on the sensing surface will be lowered due to the minimal of polar and hydrogen bond acceptance [21-23]. On the APTES modified surfaces, GLU was used as the linker to immobilize the streptavidin. GLU is the organic compound has the
formula CH2(CH2CHO)2, found as the efficient crosslinker for proteins and antibodies. Two aldehyde groups in GLU can link to the surface of protein and antibody [24–26]. Streptavidin was immobilized on GLU surface and interacted with biotinylated probe. In the case of Si-Al nanocomposites, Si-Al was mixed APTES and immobilized on the IDE surface and the similar procedure was followed to immobilize the capture probe. These capture probe modified surfaces were compared for the detection of target sequence.

**Comparison of immobilization of capture probe on IDE and Si-Al-IDE surfaces**

Immobilization of capture probe confirmation was carried out on modified IDE sensing surfaces (Figure 2a&b). Figure 2a, shows the capture probe immobilization on IDE surface. In which, bare IDE surface exhibits the current level as 0.74 nA, after adding APTES on the surface, it increased to 9.7 nA. When the GLU was flooded on the APTES surface, the current level was further increased to 20 nA. This clear increment of current confirms the chemical interaction of APTES with GLU. A 200 nM of streptavidin attachment on GLU shows the current change to be 69.3 nA. This shows 3 times increment of current from the GLU state, indicating the proper streptavidin binding with GLU. After that, 1 M of ethanolamine was added on the surface to completely cover the remaining GLU surfaces to avoid the nonspecific interaction on the sensing surface, the current was increased to 125 nA upon binding of ethanolamine. Finally, biotinylated capture probe was interacted, the current changes were noticed as from 125 nA to 1.35 nA (Figure 3a). This drastic change in current was clearly revealed the binding of biotinylated capture probe to the immobilized streptavidin on IDE surface. Figure 2b shows the immobilization process of biotinylated capture probe on Si-Al modified IDE surface. After dropping
APTES-Si-Al, the current level was highly enhanced from 0.7 to 46 nA, this doubles the conductivity compared with only APTES surface. This might be due to the larger number of APTES binding on the surface of Si-Al and immobilized on IDE surface. When adding Glu, the current changes were noticed as 161 nA, this GLU immobilization process also improved by Si-Al conjugates compared with the surface of only APTES. Upon binding of streptavidin to GLU, the current level was further increased to 500 nA, clearly indicating the higher immobilization of streptavidin on IDE surface through Si-Al nanocomposite. The ethanolamine blocking step shows the slight changes in current as to 600 nA, due to the surface occupied by the larger number of streptavidin molecules. Finally, capture probe was added and the current level was lowered drastically to 1 nA. This is 6.5 times higher changes compared with the surface of only APTES (Figure 3b). The step-wise changes in the current with above chemical and biological modifications on the both surfaces were compared and both are showing the similar trends, however, larger conductivity was found on the Si-Al-IDE surface (Figure 3c).

**Complementation of SOX-17 target gene sequence on IDE and Si-Al-IDE surface**

Target gene sequence of SOX-17 was detected on both capture probe modified IDE and Si-Al-IDE surfaces. A 1 pM of target sequence was dropped on both of these surfaces for complementation, and the changes in current were noticed. As shown in figure 4a, on the capture probe modified IDE surface, 1 pM of target gene sequence displays the current change from 1.35 nA to 3.7 µA. This huge change in current shows the complementation of target sequence with the immobilized capture probe, caused larger conductivity changes. At the same time, 1 pM of target was dropped on the capture probe modified Si-Al-IDE surface and the current change was noticed
from 1 nA to 6.5 µA. This is almost twice compared the surface condition without Si-Al nanocomposite (Figure 4b). In comparison, both cases display the clear complementation and were noticed with higher current changes. Biomolecular immobilization on sensing surface plays a major role to enhance the current flow. Here, we utilized the strong chemical linkers using APTES-GLU to immobilize the streptavidin on IDE surface and also biotin-streptavidin strategy was utilized to immobilize the capture probe. It is well known that biotin and streptavidin has a strong binding affinity, the larger number of biotinylated capture probe can immobilize on sensing surface, it leads to capture more target sequence [27]. Moreover, Si-Al nanocomposite improved the electric current flow due to their excellent electrical conductivity and also a greater number of biomolecules were captured through Si-Al nanocomposite.

**Comparison of Limit of detection with SOX-17 target gene sequence on IDE and Si-Al-IDE surfaces**

Since it was proved that, Si-Al nanocomposite enhanced the detection of target gene sequence; the limit of detection with the target sequence was carried out by the titration and compared with the surface condition in the absence of Si-Al. For that, the target sequence concentrations are from 10 aM to 100 fM were prepared by ten order dilutions and dropped independently on IDE and Si-Al-IDE surfaces. Figure 4c shows the different concentrations of target sequence complementation with the capture probe. A 1 aM of target sequence did not show any significant current change, while with increasing the concentration to 10 aM, it was increased from 1.35 nA to 50 nA. Further increments with the concentrations as 100 aM, 1 fM, 10 fM and 100 fM, the changes in current flows were noticed to be increased to 93, 175, 350, and 457 nA, respectively. These increments in current flow indicated the
efficient complementation of target sequence to the capture probe was immobilized on IDE surface. On Si-Al-IDE surface, the similar concentrations of target sequence were dropped and the changes in current were monitored for the comparison. As shows in figure 4d, after dropping 1 aM of target sequence, a clear change in current was noticed, which cannot happened on the IDE surface without Si-Al. Target sequence with the concentrations of 1 aM, 10 aM, 100 aM, 1 fM, 10 fM and 100 fM, the changes of current were noticed as 9, 70, 97, 194, 360 and 2443 nA, respectively. It was found that in all the tested concentrations of target sequence higher changes in current were noticed in the presence of Si-Al nanocomposites (Figure 5a). The linear regression graph shows the limit of detection in the presence of Si-Al to be 1 aM, while it shows 10 aM on the absence of Si-Al nanocomposites (Figure 5b).

**Specific detection of SOX-17 target gene sequence on IDE and Si-Al-IDE surfaces**

To evaluate the specific detection of SOX-17 target gene sequence, three various control experiments were carried out on biotinylated capture probe immobilized surfaces. Used 1 pM of single-mismatch, triple-mismatch and complementary of target sequences and were dropped individually on both capture probe modified IDE and Si-Al-IDE sensing surfaces, it was found that the capture probe only recognizes the target sequence in both of the cases. There is no significant current changes were noticed in all the control experiments, representing the selective detection of target sequence (Figure 6a&b).

**Conclusion**

Gastric cancer is a widely occurring complication, happened by the originating of
cancer cells from the lining of stomach. Identifying the gastric cancer at the initial stage is mandatory to give a better treatment and recovery. SOX-17 gene methylation and lower expression were found to play a necessary role in the gastric cancer progression. Detecting and quantifying the level of the target gene sequence of SOX-17 helps to monitor the gastric cancer. This research focused to detect the SOX-17 target sequence on silica-alumina (Si-Al) nanocomposite modified interdigitated electrode surface. It was reached 1 aM of target sequence detection in the presence of Si-Al and 10 aM in the absence of Si-Al. Si-Al modified electrode improved the current flow in all the processes for complementation with target and capture probe sequences. Moreover, control experiments with single-, triple-mismatch and complementary sequences of target sequence on the capture probe were carried out, all the control sequences failed to interact with the probe sequence, indicating the specific detection of SOX-17 gene. This detection method helps to monitor the gastric cancer associated progression at its initial stage.

Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors agree to be published.

**Availability of data and material**

All data generated and analyzed during this study are included in this published article.

**Competing interests**

The authors declare that they have no competing interests
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Authors' contributions

ZY and SCBG performed all fabrication and analysis experimental work. ZY, SCBG and TL conducted data analysis and manuscript preparations. SCBG provided Materials; SCBG procured funding and provided project guidance. All authors read and approved the final manuscript.

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References

1. Sitarz R, Skierucha M, Mielko J, et al (2018) Gastric cancer: Epidemiology, prevention, classification, and treatment. Cancer Manag. Res.
2. Piazuelo MB, Correa P (2013) Gastric cáncer: Overview. Colomb medica (Cali, Colomb
3. Hu B, Hajj N El, Sittler S, et al (2012) Gastric cancer: Classification, histology and application of molecular pathology. J. Gastrointest. Oncol.
4. Yoon H, Kim N (2015) Diagnosis and management of high risk group for gastric cancer. Gut Liver. https://doi.org/10.5009/gnl14118
5. Pasechnikov V, Chukov S, Fedorov E, et al (2014) Gastric cancer: Prevention, screening and early diagnosis. World J. Gastroenterol.
6. Ye YW, Wu JH, Wang CM, et al (2011) Sox17 regulates proliferation and cell cycle during gastric cancer progression. Cancer Lett. https://doi.org/10.1016/j.canlet.2011.03.024
7. Chimonidou M, Strati A, Malamos N, et al (2013) SOX17 promoter methylation
in circulating tumor cells and matched cell-free DNA isolated from plasma of patients with breast cancer. Clin Chem.
https://doi.org/10.1373/clinchem.2012.191551

8. Oishi Y, Watanabe Y, Yoshida Y, et al (2012) Hypermethylation of Sox17 gene is useful as a molecular diagnostic application in early gastric cancer. Tumor Biol 33:383-393. https://doi.org/10.1007/s13277-011-0278-y

9. Gokul G, Khosla S (2013) DNA methylation and cancer. Subcell Biochem. https://doi.org/10.1007/978-94-007-4525-4_26

10. ? uczak MW, Jagodzi??ski PP (2006) The role of DNA methylation in cancer development. Folia Histochem. Cytobiol.

11. Kuo IY, Huang YL, Lin CY, et al (2019) SOX17 overexpression sensitizes chemoradiation response in esophageal cancer by transcriptional down-regulation of DNA repair and damage response genes 11 Medical and Health Sciences 1112 Oncology and Carcinogenesis. J Biomed Sci. https://doi.org/10.1186/s12929-019-0510-4

12. Zhang Y, Jiang FZ, Bao W, et al (2016) SOX17 increases the cisplatin sensitivity of an endometrial cancer cell line. Cancer Cell Int. https://doi.org/10.1186/s12935-016-0304-7

13. Jia Y, Yang Y, Liu S, et al (2010) Sox17 antagonizes WNT/β-catenin signaling pathway in hepatocellular carcinoma. Epigenetics. https://doi.org/10.4161/epi.5.8.13104

14. Letchumanan I, Gopinath SCB, Md Arshad MK, et al (2019) Gold nano-urchin integrated label-free amperometric aptasensing human blood clotting factor IX: A prognosticative approach for “Royal disease.” Biosens Bioelectron 131:128-135. https://doi.org/10.1016/j.bios.2019.02.006
15. Letchumanan I, Md Arshad MK, Balakrishnan SR, Gopinath SCB (2019) Gold-nanorod enhances dielectric voltammetry detection of c-reactive protein: A predictive strategy for cardiac failure. Biosens Bioelectron 130:40–47. https://doi.org/10.1016/j.bios.2019.01.042

16. Marco JP, Borges KB, Tarley CRT, et al (2013) Development and application of an electrochemical biosensor based on carbon paste and silica modified with niobium oxide, alumina and DNA (SiO 2 /Al 2 O 3 /Nb 2 O 5 /DNA) for amitriptyline determination. J Electroanal Chem. https://doi.org/10.1016/j.jelechem.2013.06.021

17. Mathelié-Guinlet M, Gammoudi I, Beven L, et al (2016) Silica Nanoparticles Assisted Electrochemical Biosensor for the Detection and Degradation of Escherichia coli Bacteria. Procedia Eng 168:1048–1051. https://doi.org/10.1016/j.proeng.2016.11.337

18. Gopinath SCB, Awazu K, Fujimaki M, et al (2012) Surface functionalization chemistries on highly sensitive silica-based sensor chips. Analyst 137:3520. https://doi.org/10.1039/c2an35159e

19. Gopinath SCB, Awazu K, Fujimaki M, et al (2012) Surface functionalization chemistries on highly sensitive silica-based sensor chips. Analyst. https://doi.org/10.1039/c2an35159e

20. Ramanathan S, Gopinath SCB, Md Arshad MK, et al (2019) Assorted micro-scale interdigitated aluminium electrode fabrication for insensitive electrolyte evaluation: zeolite nanoparticle-mediated micro- to nano-scaled electrodes. Appl Phys A 125:548. https://doi.org/10.1007/s00339-019-2833-0

21. Vashist SK, Marion Schneider E, Lam E, et al (2014) One-step antibody immobilization-based rapid and highly-sensitive sandwich ELISA procedure for
potential in vitro diagnostics. Sci Rep 4:4407.

https://doi.org/10.1038/srep04407

22. Paraja S, Gopinath SCB, Md Arshad MK (2019) Aptsensing Ampicillin on Silica Substrate Gapped by Interdigitated Aluminium Electrode. Micro Nanosyst.
https://doi.org/10.2174/1876402911666190404151857

23. Gopinath SCB, Awazu K, Fujimaki M (2012) Waveguide-mode sensors as aptasensors. Sensors 12:2136–2151. https://doi.org/10.3390/s120202136

24. Gopinath SCB, Awazu K, Fujimaki M, Shimizu K (2013) Evaluation of anti-A/Udorn/307/1972 antibody specificity to influenza A/H3N2 viruses using an evanescent-field coupled waveguide-mode sensor. PLoS One 8:e81396.
https://doi.org/10.1371/journal.pone.0081396

25. Migneault I, Dartiguenave C, Vinh J, et al (2004) Comparison of two glutaraldehyde immobilization techniques for solid-phase tryptic peptide mapping of human hemoglobin by capillary zone electrophoresis and mass spectrometry. Electrophoresis 25:1367–1378.
https://doi.org/10.1002/elps.200305861

26. Wang C, Lakshmipriya T, Gopinath SCB (2019) Amine-Aldehyde Chemical Conjugation on a Potassium Hydroxide-Treated Polystyrene ELISA Surface for Nanosensing an HIV-p24 Antigen

27. Lakshmipriya T, Gopinath SCB, Tang T-H (2016) Biotin-Streptavidin Competition Mediates Sensitive Detection of Biomolecules in Enzyme Linked Imunosorbent Assay. PLoS One 11:e0151153. https://doi.org/10.1371/journal.pone.0151153

Figures
Figure 1

Schematic presentation for the complementation and mis-matching of SOX-17 gene.

Figure 2

Comparison of immobilization process of capture probe on the surfaces, (a) IDE a
Figure 3
Attachment of capture probe on the blocked surfaces, (a) IDE; (b) Si-Al-IDE and (c) comparative analysis with different immobilizations.

Figure 4
Detection of 1 pM target sequence on the surfaces, (a) IDE and (b) Si-Al-IDE. High
(a) Comparing the current changes at different concentrations of target sequence.

(b) Target detection on IDE and IDE-Si-Al surfaces. The limit of detection was found as 1 aM on IDE surface, while 10 aM on IDE-Si-Al surface.

Figure 5

(a) Specific detection of SOX-17 gene target sequence. Control experiments indicated that the system failed to recognize the control sequences, indicating specific detection of the complete target sequence.

Figure 6