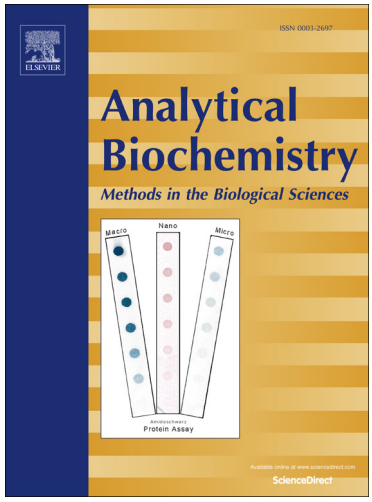

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PII: S0003-2697(14)00514-4
DOI: http://dx.doi.org/10.1016/j.ab.2014.11.001
Reference: YABIO 11901

To appear in: *Analytical Biochemistry*

Received Date: 12 August 2014
Revised Date: 29 October 2014
Accepted Date: 1 November 2014

Please cite this article as: D. Tyagi, J.B. Perez, A. Nand, C. Zhiqiang, P. Wang, J. Na, J. Zhu, Detection of Embryonic Stem cell lysate biomarkers by Surface Plasmon Resonance with reduced non-specific adsorption, *Analytical Biochemistry* (2014), doi: http://dx.doi.org/10.1016/j.ab.2014.11.001

Detection of Embryonic Stem cell lysate biomarkers by Surface Plasmon Resonance with reduced non-specific adsorption

Short Title: ESC lysate study by SPRi

Subject Category: Physical Techniques

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Abstract: Surface Plasmon Resonance imaging (SPRi) emerged as a versatile biosensor to detect wide range of bio-molecular interactions with divergent potential application. Although, the use of this advance level technology for stem cell lysate study are still not much explored. Cell lysates are significant biological analyte utilized for disease diagnostics and proteomic studies, but its complex nature limits its use as an analyte for SPRi biosensor. Here in, we review the problems associated with the use of SPRi for stem cell lysate study and checked the role of surface chemistry, running buffer and blocking solution in order to minimize non-specific adsorption (NSA). We detect the expression of Oct4, Sox2, Nanog, Rex1 and Lin28 biomarkers presents in mouse Embryonic Stem Cell Lysate (mESC), against their corresponding antibodies immobilized on the sensor surface with reduced NSA. The present study shows that the conjunction of the SPRi and microarray can be used as a label-free, high-throughput and rapid technique for detection of biomarkers and their relative abundance in stem cell lysate study.

Keywords: SPR, antibody array, stem cell, non-specific adsorption

Introduction:

Surface Plasmon resonance imaging (SPRi) in combination with microarray technology has been used to detect almost all kinds of biological interactions with wide range of application. It can also prove as a feasible technique in detection and validation of markers in stem cell lysate. Stem cell is an important and hot topic of research due to its biomedical applications as regenerative medicine [1]. Embryonic and induce pluripotent stem cell act as a renewable source, which can form any kind of cells [2]. Therefore, an efficient technique required to evaluate the exact condition of the stem cell lysate during the process of generation. To avoid the problem of immune rejection it is important to develop a convenient method for identification and characterization of biomarkers in stem cell. Rapid and accurate

identification of biomarkers in stem cell is prerequisite before using it for therapeutic application [3-4].

Most of the methods used for stem cell lysate identification are based on the labelling such as reverse transcription polymerase (RT-PCR), Western blot and northern blot. These methods are time consuming and mostly provide semi-quantitative or qualitative data [5-6]. Recent advancements of SPRi makes it a reliable and sensitive biosensor technique with large application range. Nowadays, it also put a step forward in a field of stem cell study [7-9]. SPRi provides label-free, real-time monitoring of bio-molecular interaction with advantage of handling analysis of large data set and requirement of small sample volume. It is a promising approach for biomarker identification and validation [10-11]. The combination of microarray and SPRi acts as a multiplexed assay which covers wide range of biomolecule interactions [12-13]. It provides high-throughput detection and quantitative data even for complex mixes analyte such as serum, blood, cell lysate etc [14-19] and represents an alternative to conventional proteome profiling methods [7, 20].

Non-specific adsorption (NSA) poses a major challenge for surface biosensor and it limited the analysis of cell lysate sample by SPRi [21-24]. In label-free detection NSA signal contributes in the overall signal and reduces the efficacy [25]. In general to get a specific binding response, the signal of target spot is subtracted from the background signal. But sometimes due to high NSA, background signal is higher than the signal on target spot and it gives negative result [26]. Due to this problem it's hard to detect specific low abundant protein in complex mixes [26]. Successful use of SPRi in cell lysate study needs an optimized method which aids in minimizing NSA [27-31]. Smart anti-fouling surface is required to reduce or ideally eliminate the non-specific adsorption [32-36]. Another problems associated with the cell lysate sample are bulk effect and regeneration of the sensor surface for multi-time usage. In literature only limited numbers of articles are found based on the use of SPRi for stem cell lysate study [5, 9, 37].

In the present work we employed SPRi for rapid real time detection of biomarkers in mouse embryonic stem cell (mESC) lysate with the optimization of the factors responsible for non-specific adsorption (NSA). The specific antibodies used for stem cell characterization were immobilized on the sensor surface in array format and mESC lysate were analysed. Biomarkers expressed in mESC lysate sample against the Anti-Oct4, Anti-Sox2, Anti-Nanog, Anti-Rex1 and Anti-Lin28 antibodies were successfully detected. Moreover, this paper focuses on the challenges and problems associated with the use of mESC lysate on SPRi. We limited this study with the most effective factors responsible for NSA and take into consideration signal, background and NSA. We discuss the importance of the parameters affecting the response i.e, surface chemistry, running buffer and blocking solution in terms of minimizing NSA [29, 34-36, 39]. Optimization of these parameters according to experimental requirements helps in getting rid of NSA and makes SPRi a suitable method for detection of biomarkers and their relative abundance in stem cell lysate study.

1. Materials & Methods

1.1 **Reagents**

Carboxy-EG_6-undecanethiol (HS-PEG_6-COOH) and Hydroxy-EG_3-undecanethiol (HS-PEG_3-OH) was purchased from Prochimia Surfaces Sp. 2,2-Bipyridyl (Bipy), Copper(II) chloride ($CuCl_2$), 2-Hydroxyethyl methacrylate (HEMA), poly(2-hydroxyethyl methacrylate),

Ascorbic acid (AscA), N,N-Disuccinimidyl carbonate (DSC), 4-(Dimethylamino)pyridine (DMAP), bovine serum albumin (BSA) were purchased from sigma aldrich, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(2-aminoethoxy)ethanol (EG_2-NH_2, 98%), ethanolamine were purchased from Aladdin. □-mercaptoundecyl bromoisobutyrate was provided by Peking University. Superblock, Blocking Buffer in TBS was purchased from Thermo Scientific. PBS, Tris-HCl, HEPES, EDTA were purchased from Solarbio. Phosphate buffer was used for antibody immobilization reactions. Tris-HCl and HEPES buffer supplemented with BSA was used in the SPR analysis as a running buffer according to the lysis buffer. 1M Ethanolamine, BSA, Non-fat Milk, H_2N-PEG_2-OH and superblock were used as blocking solution.

1.2 **Surface Chemistries for antibody array fabrication**

Surface chemistry plays a critical role in achieving accurate results on SPRi [21-22,32-36]. To obtain real binding signals, the difference between the bindings of the analyte to the ligand must be significantly higher in comparison to the binding of the analyte to the surface. Here we investigate NSA on different surface chemistries. PlexArray® gold chips were used to construct the Self Assembled Monolayers (SAMs) of various surface chemistries used in this study. As shown in Figures. 1 (a-c) three different types of surface chemistries were used to check the binding response of mESC lysate with antibody arrays.

1.2.1 Bare Gold

PlexArray® Nanocapture Sensor chips were directly used for antibody immobilization after cleaning it with a plasma cleaner and rinsing it with ethanol. PDC-MG Plasma cleaner from Mingheng Company, China was used to remove the impurities and contaminants from the surface of the all gold chip used (including the chips used to fabricate the PEG and SIP surface chemistry) before any further treatment.

1.2.2 Poly-ethylene-glycol Surface

Self assembled monolayers (SAMs) of Poly-ethylene-glycol (PEG) were prepared using mixture of HS-PEG_6-COOH and HS-PEG_3-OH as shown in Fig 1 (b). Spacer is used to reduce the carboxyl group density which helps in minimizing NSA [4]. The chips were kept overnight in ethanolic solution of 0.1 mM HS-PEG_6-COOH and 0.9 mM HS-PEG_3-OH at 4°C to prepare SAM of PEG with flexible spacer. The slides were washed with ethanol for 30mins under vigorous shaking to remove the unbound particles. The chips were then incubated with a mixture of 0.4M EDC and 0.1M NHS solution for 20mins at room temperature for activation of carboxyl group [38]. Chip was rinsed with water and dried with nitrogen flow before antibody immobilization.

1.2.3. Surface-initiated polymerization (SIP)

SIP as a 3D surface, have higher capacity for capture ligands and has shown promising results for reduction of non-specific protein adsorption [40-41]. A gold chip was modified with SIP chemistry as shown in Fig. 1(C). A mixed SAM solution was prepared by initiators ω-mercaptoundecyl bromoisobutyrate ($BrC(CH_3)_2COO(CH_2)_{11}SH$) and EG_3-thiol in 1: 99 ratio. The chips were immersed in this mix (1mM total concentration) for 16 hours at room temperature, and then thoroughly washed by ethanol and Milli-Q water and dried in a nitrogen stream. Polymerization solution was prepared by 64mg Bipy, 10ml 0.04M $CuCl_2$,

2.6g HEMA, 7.2g OEGMA, 20ml Milli-Q water and 20ml methanol. After 30min deoxygenation, 10ml of AscA (0.04M) were added to the solution and the chips were immersed in this solution for 16 hours at room temperature under an atmosphere of argon. After thoroughly washed with methanol and Milli-Q water, the chip were incubated in a DMF solution containing 0.1M DSC and 0.1M DMAP for 16 hour for acidification. Finally the chip was rinsed with water and dried with nitrogen flow before antibody immobilization

1.3 Antibody array preparation

Several antibodies against specific known biomarker for embryonic stem cell were used to prepare an array by immobilizing on the sensor surface. Anti-mouse Oct4 monoclonal antibody was purchased from Santa cruz, Anti-mouse SOX2 monoclonal antibody, Anti-mouse Nanog antibody and rabbit Anti-Histone were purchased from R&D Systems. Rex1 & Lin28 were provided by Guangzhou Institute of Biomedicine and Health, Guangzhou. Rabbit anti-Histone and BSA were used as a positive control and negative controls respectively. . The PDMS mask was fabricated for printing the chip. After surface activation, PDMS mask was attached on the top of the chip and printing solutions were injected in the corresponding hole according to the planned array format. After all printing solutions were injected the chip was incubated in humid chamber (70% humidity) at 4^0C for 90 minutes. PDMS mask were pulled out and chip was rinsed with water and PBS buffer.The arrays were rinsed with 10x PBS for 15 sec and then wash with 2X PBS for 15 min followed by 1X PBS for another 15 min. All antibodies along with BSA were immobilized in triplet on the corresponding surface with final concentration of 200µg-ml. All printing steps were performed in humid chamber. Un-reacted activated carboxyl groups on the surface were blocked by incubating the slides in corresponding blocking solution followed by washing with phosphate buffer.

1.4 Cell lysate preparation

R1 mouse embryonic stem cells (mESCs) were homogenized with lysate buffer. Embryonic cell lysates were prepared in two lysate buffers separately to check the effect of running buffers, Tris-HCl buffer (50mM Tris-HCl, 150mM NaCl, 0.1% SDS, pH 7.4) and HEPES buffer (20mM HEPES, 150mM NaCl, 0.05% Tween, pH 7.4) containing protease and phosphatase inhibitor cocktail (Roche, 04693124001). The cell lysates were centrifuged at 12,000 rpm for 20min at 4°C, and the supernatants were collected and used for SPRi analysis. Total protein content was determined by Bradford protein assay. Total protein concentration of mESC was 4.55 mg/ml which further diluted in running buffer and the final total protein concentration of 100 µg/ml was used for SPRi experiments. It helps in minimizing bulk effect and NSA.

1.5 SPRi experiments and data analysis

SPRi were used as a biosensor technique to detect the interaction of biomarkers in the mESCs cell lysates with biomarker antibody array. All SPRi measurements were performed using the PlexArray® SPR Analyzer. The *PlexArray*® Analyzer is the core of the *PlexArray*® HT surface Plasmon resonance imaging system. It utilizes the Kretschmann configuration for SPR based detection and real-time monitoring of analytes binding to user-defined content arrayed on *PlexArray*® Nanocapture® Sensor Chips. Chips modified with different surface chemistries were loaded separately and initialized by flowing running buffer. Our sample injection cycle consists of a 250 second association phase, and a 500 second dissociation phase with running buffer at 2µL/s flow rate. Results were analyzed by using two software

packages: Plexera SPR Data Analysis Module (DAM) and ORIGIN (OriginLab Corporation). Before analyzing cell lysate, the surface studied were exposed to running buffer (with and without BSA) for two reasons, firstly to rule out non-specific binding and secondly to determine the non-fouling property of the surface. If surface shows any binding signal with the running buffer with BSA, it indicates the non-specific adsorption on the surface. The specific binding of the antibodies spot were determined by subtracting the background signal obtained onto the surface using the Plexera DAM software. All data presented in manuscript is repeated five times to ensure the repeatability of method.

1.6 Western Blot analysis

Western blot (WB) analysis was performed to validate the relative abundance of biomarkers in cell lysate sample. For this purpose 10ug of each cell lysate sample was resolved by 10% SDS-PAGE gel, transferred to a polyvinylidene fluoride (PVDF) membrane, and probed with the diluted primary antibody. After washing with PBST for 3 times, the membrane was incubate with horseradish peroxidase (HRP)-conjugated secondary antibody and target proteins were detected using an enhanced chemi-luminescence system (ECL; Pierce) with optimized exposure times. These pluripotent biomarkers are supposed to be silenced in mouse Fibroblast (MEF) cell lysate sample so it acts as a reference.

2. Results & Discussion

2.1 Effect of Surface chemistry on NSA

Surface chemistry plays a crucial role in obtaining accurate signal with minimal NSA. The choice of surface chemistry depends on the type of ligand (e.g. antibody, protein, DNA) and analyte (e.g. complex mixes, pure analytes). A good surface chemistry has high ligand density [33-36, 42-43], protect the ligand and keep it active after several regenerations. SAMs of PEG is one of effective method with all these advantages and additionally help in getting rid of NSA [32]. Surface modified with the mixed SAM of carboxyl and hydroxyl PEG has been shown to minimize electrostatic nonspecific adsorption [23]. Moreover, some groups consider hydrophilic polymer SIP surface chemistry as an antifouling surface and used for sensitive detection of complex media [22, 41]. Various research groups reported different surface chemistry to get rid of NSA.

We used three different surfaces as shown in Figures 1(a), 1(b) and 1(c) to check the NSA of mESC lysate on antibody array. After the antibody arrays were printed on the corresponding surfaces chemistry the chips were blocked with ethanolamine [1M] at room temperature for 30 minutes. BSA was printed on an array as a negative control and Anti-Histone was considered as positive control. To reduce the bulk effect, mESC lysate was diluted with running buffer [36,38] and used in a final total protein concentration of 50µg/ml. mESC lysate was exposed to the antibody array fabricated on bare gold, PEG and SIP surfaces chemistry for 250 seconds at speed of 2µL/s. Followed by dissociation with running buffer for 500 seconds at the same speed. Figure 2(a-c) shows the sensogram of SPRi signal for bare gold PEG and SIP surfaces respectively. Antibody array fabricated on different surface chemistries showed different SPRi response.

Bare gold surface without any modification was used to fabricate antibody array and rest of the treatment was same as of the other surface modified chips. Figure 2(a) shows SPR sensogram response of antibody array with mESC lysate on bare gold surface. The highest

measured response of anti-Lin28 antibody was 1.83 AU value and the lowest for anti-Sox2 antibody was 1.29 AU. Bare gold surface sensogram shows rapid absorption and desorption phase. Results shows diminished signal with high noise which might be due to NSA. It was assumed that NSA effect of cell lysate masks the signal of interest. Also binding on BSA spot and background were same; this clearly indicates the NSA of cell lysate on surface. Denaturation of antibodies by direct adsorption onto metal surface can also be one of the reasons behind high noise (43). Array prepared on bare gold surface doesn't have repetitive use due to weak interaction between the surface and the immobilized ligand.

Whereas, Figure 2(b) shows the SPR response on SAM of PEG modified surface. The signal was rapid and high in comparison to other surfaces. All antibodies immobilized on to the array surface along with spotted BSA shows binding on PEG modified array surface. The PEG modified surface gave higher response than bare gold. An anti-Lin28 antibody spot shows highest binding response of 6.93 AU, on the same side anti-Sox2 antibody shows lowest binding response of 2.595AU. The recorded AU changes in comparison to bare gold were 5.1 AU and 1.307 AU for anti-Lin28 and anti-Sox2 antibodies respectively. Whereas diminished desorption was observed during washing with the buffer corresponding to less dissociation of binding complex. Carboxyl group's negative charge supposed to have non-specific ionic interactions with target molecules [33]. So spacer of hydroxyl group was used to reduce the density of carboxyl group which suppose to avoid electrostatic non-specific adsorption. Spacer helps in diminishing the effect of NSA which corresponds to high actual binding signal. The PEG surface chemistry with a use of flexible spacer showed highest SPRi response. At last mESC lysate was tested on a SIP surface and SPRi signals of arrayed antibodies were observed as shown in Figure 2(c). Association phase of SIP surface is not as rapid as in PEG and bare gold surface and the dissociation is also slow in comparison. On SIP surface signals are moderate; anti-Lin28 antibody shows highest binding of 3.37 AU. Signal of highest binding was 1.54 AU higher than bare gold and 3.56 AU lower than PEG. The lowest binding signal of anti-Nanog antibody was 0.35 AU higher than bare gold and 0.96 AU lower than PEG. For BSA spots no binding were observed and response was similar to background which indicates the specific binding on antibodies

As shown in Figure 2(d), SIP surface chemistry showed significantly less NSA, than the other two surface chemistries. Whereas background response of other two surfaces (bare gold and PEG) with cell lysate are similar. The higher NSA observed on PEG surface could explain why PEG surface has higher SPRi response than SIP surface. It confirms that in terms of NSA, SIP surface showed better results in comparison to the rest of the tested surfaces.

PEG and SIP both surfaces shows enhanced signals when compared with bare gold surface. Even signal to noise ratio is approximately equivalent and higher than bare gold (Figure 3). Signal to noise ratio was calculated by diving the average of maximum intensity with average standard deviation (SD) of base line obtained from spots in triplicate. In all surfaces complete regeneration was achieved using 20mM NaOH solution. For further experiments we used SIP surface chemistry as it shows less NSA than the other surfaces studied.

Biomarkers against Anti-Oct4, Anti-Sox2, Anti-Nanog, Anti-Rex1 and Anti-Lin28 antibodies were successfully detected in mESC lysate sample. Expression of these pluripotent markers has been already reported [54]. We further investigate the performance and efficiency of SIP surface with 5 different total protein concentrations (100, 50, 25, 12.5, 6.25µg/ml) of cell lysate. Figure 4 depicts the SPR response of different concentration of cell lysate over a range of different antibodies immobilized on an array. Moreover, correlation plot of cell lysate

concentration against SPRi response demonstrate sufficient linearity and reproducibility of SIP sensor surface with good pearson correlation factors. This indicates that SIP surface is efficient for the sensitive detection of biomarkers in cell lysate sample. SIP surface showed better performance and well suited for fabricating high density microarrays, and additionally aid in suppression of non-specific protein adsorption.

2.2 Effect of running buffer on NSA

The salt concentration of the running buffer also affects the binding of bio-molecules and therefore there is a need to optimize it in order to minimize NSA. Research suggested that changes in buffer conditions i.e., higher salt concentration, amount of surfactant (Tween 20) or chemicals (EDTA) can helps in reducing NSA [44-46]. Higher salt concentration shield electrostatic charges and aid in reducing NSA. Tween 20 and BSA in running buffer help in reducing both electrostatic and non-electrostatic NSA [23].

Initially we started the experiment with Tris-HCl (50mM Tris-HCl, 150mM NaCl, 0.01% SDS, pH 7.4) buffer as our cell lysate was prepared in the same. But we observed some complicates results i.e, high non-specific adsorption and incomplete regeneration of the surface after sample run even after proper quenching of remaining activated sites. This motivates us to check the effect of running buffer and we prepared our cell lysate in HEPES (20mM HEPES, 200mM NaCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 3mM EDTA, 0.05% Tween 20, pH 7.4) buffer and also used it as running buffer. We compared the results and observed that, signal response in HEPES wasbetter than the Tris-HCl. Both running buffers have lower refractive index than cell lysate samples. To increase the sensitivity and to avoid bulk effect, 1% BSA was added in the running buffers. BSA is known as non-interactive protein and also aid in minimizing NSA [23]. The running buffers were degassed before the usage to avoid bubbles. At the beginning of each experiment, the running buffer was allowed to flow through the array until a stable baseline was achieved.

Figure 5(a-b) shows the results of both buffers Tris-HCl and HEPES. When the Tris-HCl buffer was used, the binding response of the cell lysate was strong and rapid with less dissociation of the binding complex from the surface. Interestingly, it was difficult to regenerate the surface completely when the Tris-HCL based running buffer was used. On the other hand a good regeneration was observed with same regeneration buffer when HEPES was used as running buffer. We assume that NSA was also the reason behind the problem of less dissociation and uncompleted regeneration when Tris-HCL running buffer was used. The cell lysate samples showed less NSA binding to the background when HEPES buffer was used. Figure 5c demonstrates the background signal obtained from the surface on the exposure of cell lysate sample in both running buffers. BSA potentially blocks the non-target sites by saturating the surface. Results suggest that HEPES supplemented with high salt concentration and 1% BSA is a good option while using cell lysates as an analyte and aids in minimizing the NSA.

2.3 Effect of Blocking Solution on NSA

NSA is most likely due to hydrophobic and electrostatic effect of the surface chemistry. After immobilizing the ligands, blocking of remaining activated sites is very important to minimize the NSA [39, 45]. The choice of blocking solution depends on the surface chemistry and ligands used. The blocking solution must not react with the ligand but completely quench the remaining available sites for binding to give accurate results [47-48]. Cell lysate is rich in

lipid which reported to foul the surface [24]. Here, we tested low fouling effect of SIP surface chemistry and for that purpose the effect of 5 different blocking solutions on sensor surface were analyze. We used 2.5% non-fat milk (prepared in PBS buffer), 1M Ethanolamine (pH 8.2), 2% BSA (containing 0.01% Tween 20, pH 7.2), Superblock (in TBS buffer, pH 7.4) and PEG_2-NH_2 (0.1mg/ml) blocking solutions and spotted on the array surface in triplicate [49-52]. An array of blocking solutions was prepared on the SIP surface by PDMS printing method and cell lysate sample were exposed to determine the SPR response on individual blocking solution. Each blocking solution shows different signal intensity with the cell lysate. We presume that the binding response of these blocking solutions with cell lysates directly correlates with their surface blocking efficiency [39]. In case of NSA with cell lysate samples, the background is used as reference to correct the actual binding by subtraction. Hence, the lower signal response on the background indicates reduced NSA and higher efficiency of the blocking solution.

BSA and non-fat milk are commonly used blocking solutions, but they were not effective with cell lysate samples. Amine terminated PEG has become quite popular for reducing NSA due to its anti-fouling property [14]. Blocking solutions studied had different impact in preventing NSA with cell lysate. Figure 6 throws light on the binding response of the cell lysate with different blocking solutions used. Experimental outcomes, suggest that PEG_2-NH_2 and ethanol amine are more effective and better option for blocking while using cell lysate for analysis; it effectively blocks the unreacted NHS ester group [46-48].

All antibodies on an array are recognized as pluripotent markers and known to express in embryonic stem cells. Western blot were performed to check the relative abundance of the biomarkers in mESC lysate. Fribroblast cell (MEF) lysate were used as a reference to show the specificity of the binding signal. Figure 7 shows the expression of antibodies in mESC and MEF lysate by western blotting. Result shows that Anti-Oct4, Anti-Sox2, Anti-Nanog, Anti-Rex1, Anti-Lin 28 and Anti-Histone are expressed in mESC lysate, whereas these pluripotent markers were silenced and only Anti-Histone expressed in MEF lysate. This clearly reveals the specificity of these biomarkers in mESC lysate. Western blot is based on labeling whereas SPRi is label free and real-time analysis based on surface sensitivity (4). SPR can also be used for quantitative and biomarkers profiling study in stem cell lysate samples if calibration curve of each antibodies are available. In this study we optimized assay conditions for minimizing NSA of mESC cell lysates on antibody array using SPRi. Background noise signal masked the signal of interest, so there is a need to optimize some parameters to minimize false positive results and to achieve actual binding signals. For this purpose, the influence of different surface chemistries, running buffers and blocking solution were examined. Our studies revealed that the optimization of these factors is crucial to achieve minimal NSA. These factors affect the NSA in different ways and interfere with the results of biosensor. Analysis of biological samples like cell lysate, serum, plasma or other complex mixes need more consideration and additional optimization in terms of NSA [53]. Conditions vary according to the ligands and analyte use for analysis. SPRi require less sample volume and provide rapid and accurate detection of biomarkers in stem cell lysate.

3. Conclusion

We have successfully studied the detection of Oct4, Sox2, Nanog, Lin28 and Rex1 biomarkers in mESC lysate with reduced NSA. The results pave the way toward further application of SPRi and antibody array for real-time detection of biomarkers present in different stem cell lysate. It is difficult to completely eliminate the NSA in label-free analysis.

But right surface chemistry (according to the ligand) with a combination of good blocking solution (according to array and surface) and adequate running buffer supplemented with detergent can greatly helps in minimizing NSA. Results suggest that using a SIP surface chemistry with PEG_2-NH_2 blocking of the un-reacted surface, HEPES supplemented with EDTA and BSA as running buffer significantly reduced NSA.

Antibody array and SPRi combination is a big leap forward, converting it into a multiplexed assay which can meet a wide range of research needs. This combination provides qualitative and quantitative results of proteins in complex samples like cell lysates. Our system provided a platform to perform comparative studies of stem cell content. Extension of array with different kind of ligands can be used to efficiently monitor the quality of stem cell lysate. Further development of arrays will help in rapid identification and characterization of proteins in stem cell lysate. Additionally, this application can further effectively applied to investigate the discovery of new potential biomarkers or regulators of pluripotency.

Acknowledgement

This work was partly supported by the National Natural Science Foundation of China Grant 31171381 and the National Basic Research Program of China, 973 Program, 2012CB966701 to (J.N), and the core facility of the Tsinghua-Peking Center for Life Sciences.

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Figure Legends

Figure 1. Different surfaces used to fabricate the antibody array (a) bare gold surface, (b) SAM of 0.1mM HS-PEG6-COOH and 0.9mM HS-PEG3-OH (c) Surface-initiated polymerization (SIP).

Figure 2. SPRi response of the arrayed antibodies after exposure of mESC lysate on the surface chemistries used. (a) Bare gold, (b) PEG (c) SIP surface chemistry (d) SPR signal obtained on the background (spots selected on surface with no ligand attached) of corresponding surfaces (a-c) upon injection of cell lysate sample.

Figure 3. Signal to noise ratio (SNR) on different surfaces. Signal to noise ratio was calculated by diving the average of maximum intensity with average standard deviation (SD) of base line. Results are expressed as mean value ± standart deviations obtained from spots in triplicate.

Figure 4. Graph shows the correlation of average maximal response (AU) achieved versus various total protein concentration (100, 50, 25, 12.5, 6.25µg/ml) of mESC lysate against antibodies (a) anti-Oct4 (b) anti-Lin28 (c) anti-Sox2 (d) anti-Nanog (e) Rex1 (f) anti-Histone. Maximum intensity obtained on the exposure of particular concentration of mESC lysate on each antibody spots were used to calculate the mean ± SD obtained from spots in triplicate and these values were further used to plot the correlation graph. Plexera SPR Data Analysis Module (DAM) was used to analyze the data and to obtain normalized antibody binding curve.

Figure 5. SPR sensogram showing a binding pattern of mESCs cell lysate exposed at 100µg/ml (1:500 dilution) in running buffer onto the SIP surface. (a) exposure of cell lysate in Tris-HCl running buffer (b) exposure of cell lysate in HEPES running buffer (c) SPR signal response on the surface taken as background on exposure of mESC lysate in both running buffer (Tris-HCl and HEPES).

Figure 6. SPR signals response of mESCs cell lysates on array prepared with different blocking solutions (printed in triplicate) on SIP surface. Maximum intensity achieved by the exposure of cell lysate sample on each individual blocking solution was observed to calculate the average maximum intensity and standard deviation. Results are expressed as mean value ± standart deviations obtained from spots in triplicate.

Figure 7. Western blot analysis of anti-Oct4, anti-Sox2, anti-Nanog, anti-Rex1, anti-Lin28 and anti-Histone expression in MEF and mESC lysate.

Abbreviations:

HRP- Horseradish peroxidise
MEF- mouse embryonic fibroblast
mESC – mouse embryonic stem cells

NSA – Non-specific Adsorption
PEG – Polyethylene glycol
PVDF – Polyvinylidene Fluoride
SAM – Self Assembled Monolayer
SD – Standard Deviation
SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPR- Surface Plasmon resonance
SPRi – Surface Plasmon resonance imaging
SIP - Surface-initiated polymerization
WB- Western Blotting

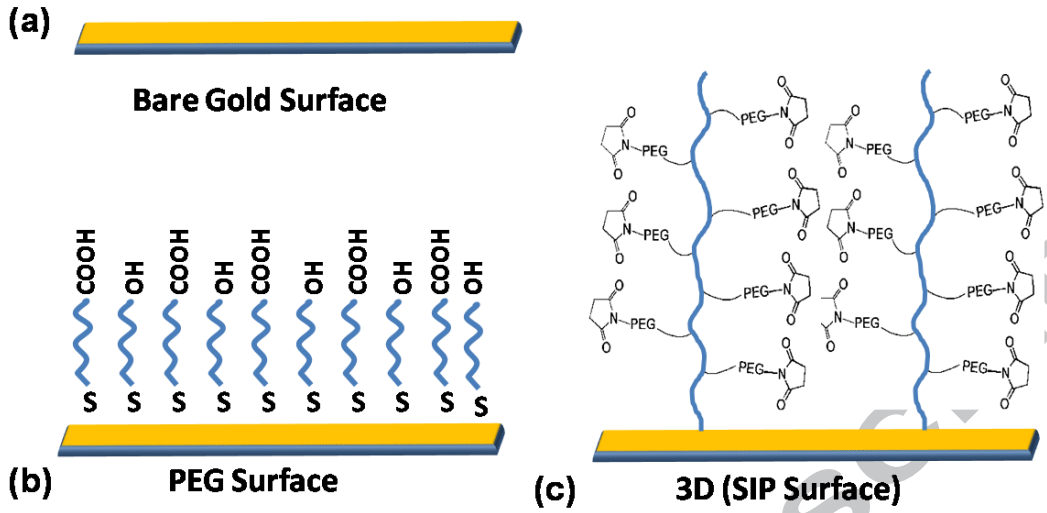


Figure 1

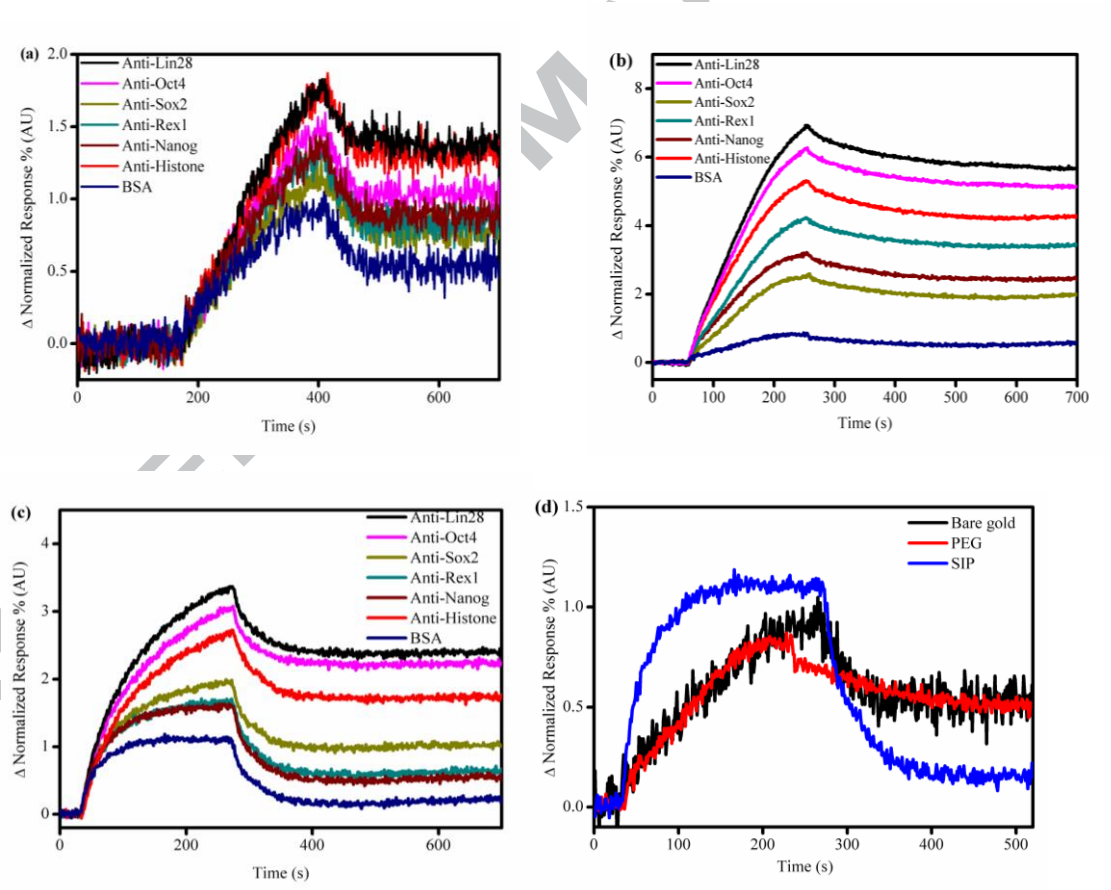
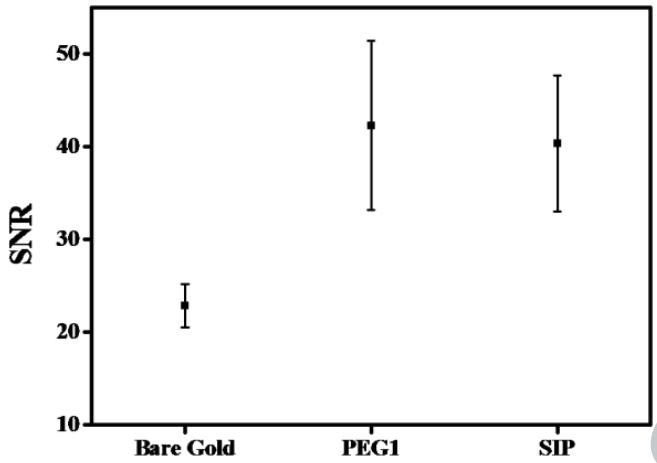


Figure 2



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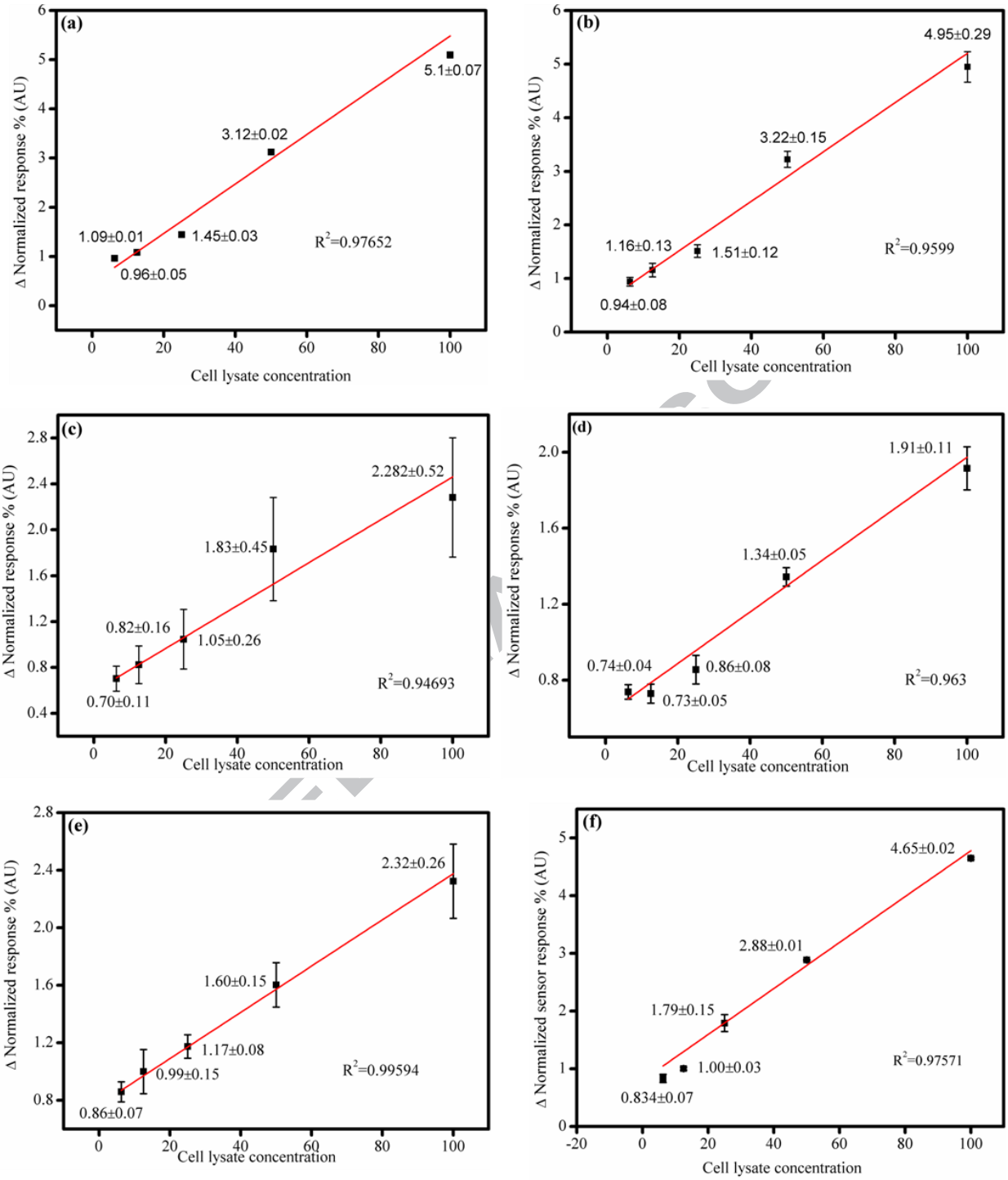

Figure 4

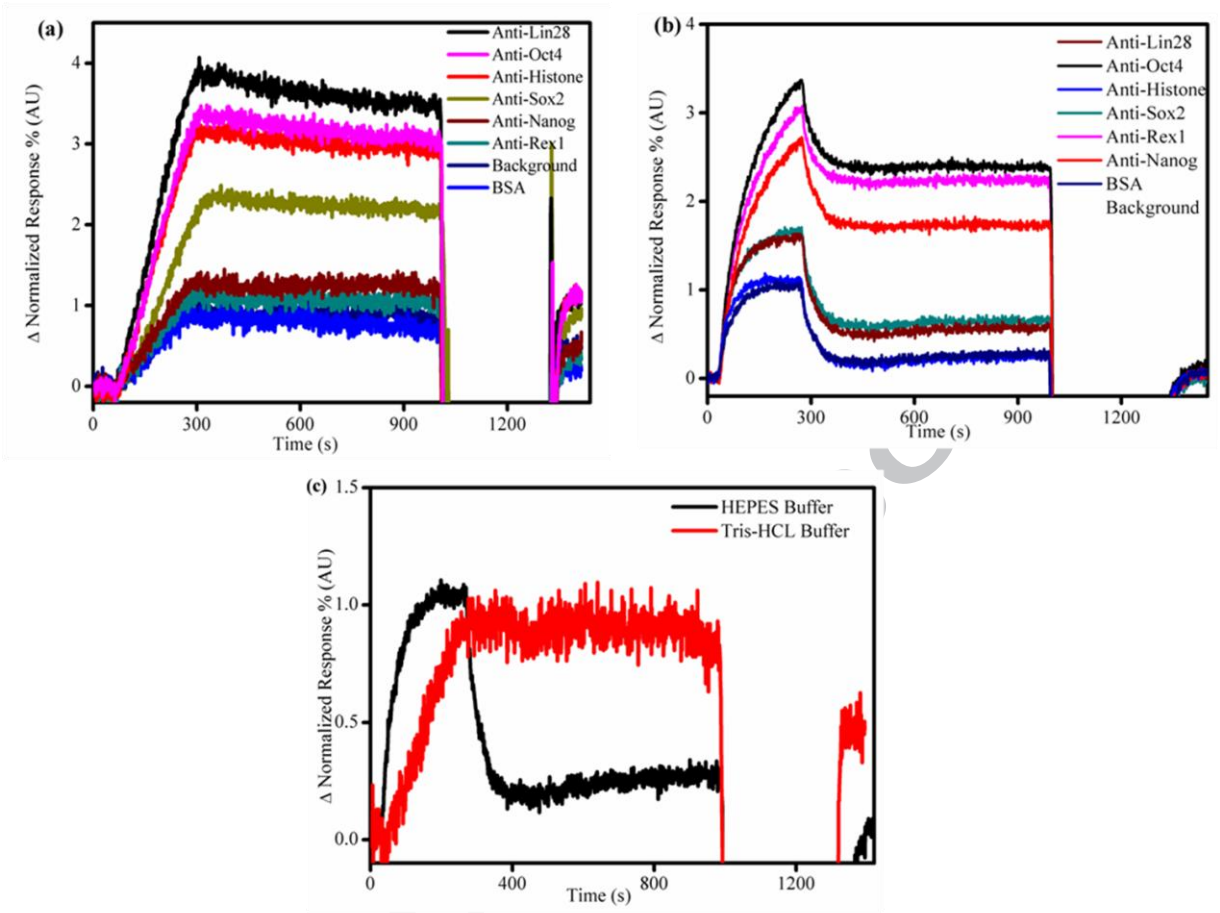

Figure 5

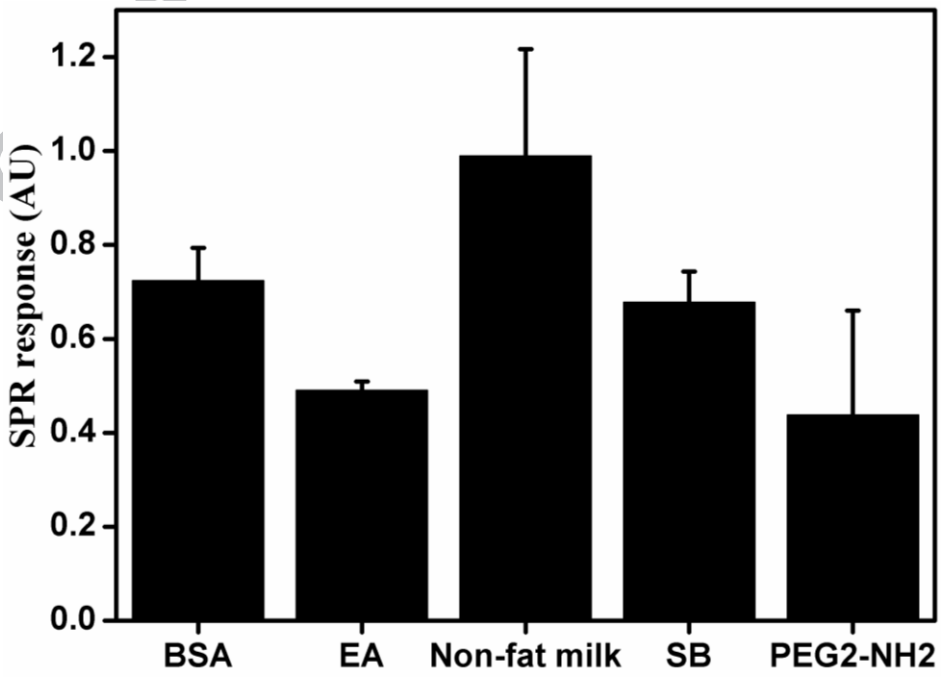

Figure 6

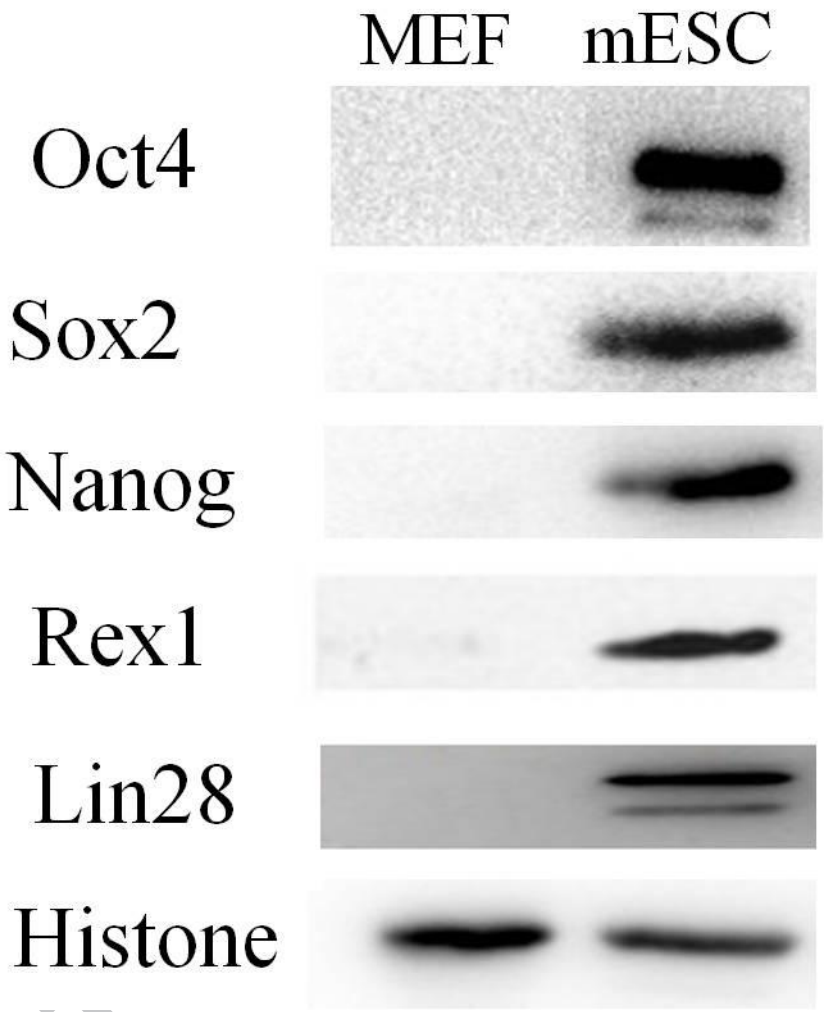

Figure 7