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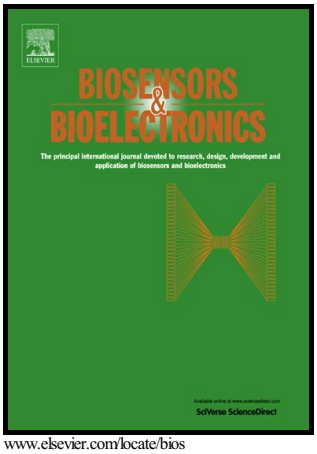

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Label-Free Biosensors in the Field of Stem Cell Biology

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Abstract:

A great body of evidence unveiled the existence of different stem cell types in various tissues with efficient capability for self-renewal and differentiation. Characterization and enumeration of a specific stem cell population seem difficult because these cells consist of a small percentage of cells within different milieu. As such, due to lack of accuracy provided by convenient methods, biosensing techniques are at the center of attention to improve stem cells characterization with high rate of sensitivity and specificity. In line with this statement, bio-sensing, as a sensitive analytical method, opens novel avenues in biological aspects. Physicochemical signals produced by the interaction of analyte-ligands could be applicable for a wide range of scientific fields such as chemical, biochemical, pharmaceutical and immunological purposes. Noninvasive and label free monitoring methods without need to stain, fix, lyse, or fluorescent-tags are desirable in basic stem cell technology and therapeutic applications of cells. We, here, highlighted basic concepts and terms associated with label free biosensor technology for measuring cell entity, evaluating cell surface marker, and peculiarly in the field of stem cell biology. Furthermore, different aspects and methodologies concerning with bio-sensing techniques, including photonic-based detection systems like Surface Plasmon Resonance (SPR) assays, Impedance-based method**,** Quartz Crystal Microbalance (QCM) and paper based detection of lateral flow biosensor (LFB) were discussed.

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Keywords: Label-free biosensors; Cell detection; Stem cell biology; Regenerative medicine; Biomedical field

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Contents:

1. Introduction

Given that stem cells have the ability to transdifferentiate into specific lineages and maintain their self-renewal feature, they are highly regarded by biologists. Even though current analyses in monitoring stem cell function are precise and reliable, but they are destructive and damageable for cell behavior. Some of these techniques include immunocytochemistry (ICC), polymerase chain reaction (PCR), protein immunoblotting and flow cytometry (Araghi et al. 2014; Dalmau et al. 1990; Giulietti et al. 2001; Livak and Schmittgen 2001; Pap et al. 2000; Portmann-Lanz et al. 2006). For instance, PCR needs to extract deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from the cell nucleus. In ICC method, it is essential to permeabilize cell membrane to enhance the entrance and affinity of antibodies. With these comments, both PCR and ICC interrupt the cell membrane integrity (Ginzinger 2002; Toma et al. 2001). As a matter of fact, bio-sensing systems have been developed to characterize physical changes, detect homogeneous and heterogeneous cell populations and study details of cell behavior in real-time monitoring mode. This technique also enables us to follow the dynamic of stem cell surface markers in the time of being multipotential and during differentiation (Chabot et al. 2009; Ebersole et al. 1992; Hu et al. 2013). Bio-sensing facilitates the monitoring of different cell features in real-time, label-free and noninvasive ways and yields profound insight and

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knowledge of physical properties underlying cellular behaviors (Endo et al. 2008; Hide et al. 2002; Quinn et al. 2000).

This review study is an attempt to understand the basic concepts and terms associated with application of biosensor techniques in the field of stem cell biology. There are lots of works about the development of biosensors for immunological and clinical purposes and the most popular use is for cancer cells detection and aptameric-DNA sensors. In the current review, we added the notion of stem cell bio-sensing methods applied for cell detection through the surface markers and biophysical characterization of cells were also studied. We collected data on label-free biosensors such as plasmon resonance (SPR), Impedance-based method, lateral flow biosensor (LFB) and quartz crystal microbalance (QCM) biosensors.

2. Biological characteristics of stem cells

Stem cells have a pivotal role in the homeostasis of various tissues. These cells are commonly quiescent and provide endless cellular reservoir for tissue regeneration. Committed progenitors and daughter cells are originated from stem cells via asymmetric cell divisions, supporting maintenance of tissue functions and replacing exhausted cells (Aponte and Caicedo 2017). Through self-renewal property, stem cells have the potential to regulate their population required during physiological and pathological conditions. Stem cells are also responsible for producing vast array of differentiated lineages after being instructed by neighboring cues (Lane et al. 2014). During different types of insults, several features of stem cells associated with multipotency, self-renewal capacity, ability to preserving undifferentiated/differentiated status and paracrine effect increase the restorative outcomes. The concepts of stemness and multipotentiality were first introduced to define all the unique features correlated with specific genomic profile or epigenetic modifications in the context of tissues (Aponte and Caicedo 2017). Previous works provided evidence of specific cell behavior and capacity after switching on/off distinct genomic pool (Abdyazdani et al. 2017). At present, no single surface molecule or definite markers have been identified related to multipotency and many authorities attempt to determine specific molecules related to stemness feature or multipotentiality (Lv et al. 2014). For instance, the minimum criteria of stemness and multipotency include the mostly-recognized expression of distinct CDs such as CD105, CD90, CD29, CD44, and CD106 in marrow-derived mesenchymal stem cells (Nöth et al. 2008). Notably, the expression of factors Oct-4 and Sox-2 is correlated with more de-differentiated status (Kuroda et al. 2010). Besides expressing and

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cellular distribution of above-mentioned markers in mesenchymal stem cells, the protein encoding activity of genes HLA-DR, CD45, CD11b, CD34, and CD14 must be so trivial (P et al. 2011). Hematopoietic stem cells differ fundamentally from mesenchymal stem cells by the expression of CD90/Thy1^{+}, CD34^{+}, CD59^{+}, CD38$^{low/-}$, c-Kit$^{-/low}$, and Lin^{-} (Taussig et al. 2005). Based on previous experiments, two types of stem cells markers have been proposed; sole and stemness markers. Sole markers bear a number of molecules used for identification and isolation of stem-like cells while stemness markers are used usually for confirmation of stem cell subsets with superior clonogenic and multipotency features. The cell distribution of sole markers is thought to be more than stemness markers (Lv et al. 2014). In contrast to normal niche, increasing data revealed the existence of small cancer subpopulation namely cancer stem cells, exhibiting stem-like appearance, self-renewal, and differentiation capacity. Similar to normal stem cells in various tissues, some factors such as CD133, CXCR-4, and CD44 are commonly expressed by cancer stem cells (Wei et al. 2014). Stem cells coming from different tissues may contain common and tissue-specific markers. For example, neural stem cells could express CD133, nestin, Sox-1, Sox-2, Pax-6 and etc. (Yuan et al. 2011). In addition, the profile and expression pattern of stem cell surface markers are changed during differentiation and normal cell turnover (Hassanpour et al. 2016). Upon exposure to various cytokine and factors, morphological adaptation in line with changes in the expression of specific markers helps stem cells to give rise to final identity (Bonaventura et al. 2015). Commensurate with these comments, markers and molecules proposed for stem cells are required for the detection of developmental stages of stem cells and different methodologies and approaches must be applied to monitor these changes.

3. Concept and current terms in biosensing

A biosensor may be defined as an analytical device that combines a biological component with a physicochemical detector. This technique has the potential to convert any changes in an immediate environment to signals conductive via an immobilized bioreceptor probe that is selective for target analyte molecules. Biosensors have two intriguing characteristics; they could selectivity bind and respond to biological or biologically active analytes in the context of a liquid phase. In Fig.1 the overall schematic picture of biosensor systems that is composed of a biological component (bioreceptor) and a physical detector (transducer) has been shown. For detecting target molecules, it is essential to immobilize bio-receptor in close proximity to

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transducer through physical or chemical interaction (Eggins 2008; Florinel-Gabriel 2012). During the interaction of analyte with receptor on immobilization probe, a transducer converts these changes into measurable signals. For instance, in EIS biosensors any electron transporting and mass changing on the surface of electrode can be easily shown in the form of impedance signals by transducer in SPR based biosensor any mass and refractive index changing on the gold surface chip can be detected by recording angle shift during passing time. The component and types of signals in four sensor systems have been outlined in Table 1.

"Fig.1"

"Table.1"

Due to label-free and real-time detection, the non-invasive systems are more useful than invasive techniques for measuring biological and cellular activities thereby enabling us to monitor cellular response and kinetic data in live cells. Based on the data of these biosensors, not only cellular behavior including cell attachment and spreading, but also information regarding contact area with the gold sensors, cytoskeleton, adhesion strength, and cell–cell adhesion could be analyzed. The methods like western blot, flow cytometry, reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence assays need multi-step processes of labeling of different cell compartments, leading to cell damage and loss of biological properties in living cells. To successfully study the dynamic cellular processes in live cells, non-destructive and non-invasive real-time and label-free monitoring approaches are required.

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4. Surface Plasmon Resonance (SPR) Biosensor

4.1 The application of SPR in cell biology

One of the best ways to investigate an accurate biomolecule-cell interaction is SPR method with fascinating range of applications in various fields of science. The theory of SPR is founded on the optical phenomenon that can occur when plane-polarized light transfers a thin metal film (gold sensor) under total internal reflection conditions (TIR). In this method, one binding partner such as antibody, DNA, different peptides or even cell fraction is attached on the surface of gold chip while the other counterpart could be applied with distinct affinity, such as different antigens, DNA, RNA, drugs or cell surface markers, during fluidic systems (Fig. 2). By virtue of reciprocal interactive properties between ligand and analyte, any substantial changes in

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mass and refractive index in the vicinity of a gold surface can be sensed in real-time manner through using SPR detector. Of note, a direct proportional relation exists between each unit of response signal, termed response unit here, to a surface concentration change in standard area of 1 μg/mm^2. A promising benefit of SPR technique, in comparison with other biosensor approaches, is being label-free and real-time system with high sensitivity grade in picomolar levels (Davis and Wilson 2000; Katsamba et al. 2006; Mariani et al. 2013). The most typified range application of SPR method is devoted to the evaluation of kinetics, thermodynamic indices and the affinity constant of ligand–analyte bilateral interactions (Banères-Roquet et al. 2009; Fathi et al. 2017; Frostell-Karlsson et al. 2000; Hu et al. 2013; Karlsson and Fält 1997; Pope et al. 2009; Sharifi et al. 2017; Torreri et al. 2005).

" Fig.2 "

In line with potency of SPR, new emerging applications related to SPR technique are being defined to illuminate a variety of multicellular dynamics, such as physiological and biochemical behavior, intra- and intercellular interactions notably with special focus on cell surface modulations, cell volume and morphology changes and cell crosstalk with different agents such as factors, hormones and *etc.* (Horii et al. 2011; Rezabakhsh et al. 2017; Shanehbandi et al. 2017; Yanase et al. 2007b; Ziblat et al. 2006). In addition to vast arrays of cell analysis approaches provided by SPR method, plausible unique advantages have been however touted so far. In spite of most famous current cell analyzing methods via different convenient techniques, a low concentration of label-free cells could be interestingly examined through short-time period without cell loss during sample preparation, analyzing and result acquisition. These features make SPR as an appropriate approach for the elucidation and characterization of scarce cell population among mixed cell populations (Fathi et al. 2017; Rich and Myszka 2000; Sharifi et al. 2017; Wittenberg et al. 2014). Previous works have revealed that SPR method is capable to monitor cellular response and adhesion, and detect trivial amount of metabolites (Shabani and Tabrizian 2013; Yanase et al. 2007a). To the best of our knowledge, few numbers of experiments have been conducted for the application of SPR in field of cell biology and stem cell research until now. Two main approaches have been used by immobilizing distinct cell phenotypes on chip or counteracting analytes. Quinn et al. first described SPR-based bio-sensing system for investigating the interaction of red blood cells (RBCs) with anti-A IgG antibody from cell

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supernatant. They hypothesized that RBCs could react with chip-bounded anti-A IgG antibody during a one minute period which was applicable for the analysis of real human samples (Quinn et al. 2000). In addition to ligand-analyte interaction, SPR provides various step-wise evaluations of cytoskeletal-associated intracellular signaling and cell shaping following incubation with different cytokines, peptides, and factors. The mass and evanescent filed adaptations and light angle (θ) alteration could be recorded and simulated for different physiological and pathological conditions (Cuerrier et al. 2008b). In an experiment done by Cuerrier and colleagues, they attached HEK-293 cell line on Au-coated slide and monitored morphological remodeling and actin cytoskeleton re-organization after the exposure to angiotensin II. In some circumstances, no evident SPR signal variations were detectable; for example when cells are exposed to intracellular calcium mobilizer carbachol. Cell volume fluctuation was also measured by SPR technique after removing cellular fluid or diluting cytosolic components in single monolayer MDCK II cells in the presence of hyper- (410 mOsmol) and hypotonic (135 mOsmol) solutions. These modifications resulted in inherent deviation of refractivity indices per time resolution (Robelek and Wegener 2010). Even, a substantial physiologic function of cell transport via paracellular (intercellular) or intracellular (transcellular) routes indicated a variation of taken SPR signals in an *in vitro* model of canine renal cell line monolayer (Viitala et al. 2013). Calling attention, cell activity and response to analytes (hormones, drugs, and especially effective cells, etc.) and transport through cell barrier could be used as promising tool to predict, diagnose and evaluate the real status of many circumstances and diseases. For example, a preliminary allergic response of mast cells, as effective cellular part participating in onset of anaphylaxis allergic reaction, was previously described by Hide et al. (Hide et al. 2002). They assessed the interaction of an allergen, dinitrophenol-conjugated human serum albumin, with IgE-sensitized living mast cell layered on gold surface. Unbelievably, they reported an unexpected 1000-fold in SPR response unit alteration rather than theoretical calculation. Therefore, a highly precise monitoring of cell response to different external stimuli could be investigated in different milieu as well. According to a few number of studies, it, however, conceived that SPR technique could be augmented in the field of tumor biology and oncology science (Su et al. 2008). Notably, a universally validated precise results observed during the application of anticancer agents on different typified cancerous cell lines (Ladd et al. 2009; Liu et al. 2011b). Various underlying oncostatic and cytotoxic effects such as apoptosis, especially on hepatic and pancreatic

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carcinoma, and the disturbance of mitochondrial membrane's potential integrity (ΔΨm) have been reported post-drug treatment (Kosaihira and Ona 2008). For instance, Nishijima and colleagues revealed a differential SPR angle rate of apoptotic and healthy cancer cells following priming with anti-cancer drugs such as Roscovitine and D-allose. They also notified the rate of cells showing apoptotic changes after the accumulation of p53 factor and cell cycle arrest (Nishijima et al. 2010).

4.2. SPR imaging

SPR imaging (SPRi) is imaging a typical and modified model of classical SPR analysis of ligand-analyte (Jordan and Corn 1997). Similar to classical SPR technique, SPRi method is based on same theory, but an extra facility is developed by using a high-resolution video charge-coupled device (CCD) camera that enable it to visualize continuously the whole steady-state of procedure on gold chip while giving simultaneously information on molecular binding, bio-molecular interactions and kinetic processes of different objects. In better word, the reflected beams are recorded by CCD camera. Also, numerous spots could be performed in an array format of different interactions by continuous flow microfluidic (CFM) spotter or an Omnigrid robotic arrayer® with total number of 90 regions or more area. The active interaction of analyte-ligand was illuminated as numerous white dots due to weak resolution and relatively deformed paradigm. One of the most prominent advantages of SPRi compared to conventional SPR is parallel evaluation of multiple hundreds analyte-ligand interaction at the same time during entire set of even one experiment (Foley et al. 2008; Jordan et al. 1997; Kyo et al. 2005; Shabani and Tabrizian 2013).

In a work done by Cortes and et al. a panel of monoclonal antibodies, comprising anti-CD86 and anti-CD11b, was applied against murine macrophage spotted on 1 cm surface of the biochip and compared to control negative cells such as human promyelocytic HL60 cell line (Cortès et al. 2011). They even were able to define a different mixed population of macrophage/HL60 cells ratio ranging from 0 to 50% co-plated at same region to detect the correlation of SPR signals with the number of positive cells. A high throughput correlative regression was determined when the results of SPRi were compared with flow cytometry values. Apparently, a directly proportional relationship between the intensity of analyte-ligand interaction and response units were recorded in association with visualized white dots (Peelen et al. 2006). It was previously exhibited a deposition of incremental concentration of epithelial cell

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adhesion molecule (EpCAM) antibody on chip, including 3, 6 and 13 µg/ml, resulted in high degree of response unite and imaged white dots during cancer cell association of three different breast cancer cell line HS578T, SKBR3 and MCF7 with 80 µl/sec flow rate at period of 30 min (Stojanović et al. 2014). Collectively, it is assumed that SPRi assay, a sophisticated form of conventional SPR could be broadly exploited in vast array of biological fields.

4.3. SPR application in regenerative medicine and stem cell biology

Based on the available literature and published papers on PubMed database, a few number of SPR-associated stem cell sensing works have been reported up to 21th February 2016, (Outlined in Table 1). Increasing data confirm the application of SPR assay on stem cell biology related to cell phenotype identification, differentiation, and multipotentiality. In line with exquisite capacity of SPR analysis on non-stem cell biology, Lee and colleagues recently described potent advantages of this technique on stem cell during osteoblast differentiation of human mesenchymal stem cells (hMSCs) (Kuo et al. 2011). Various conventional *in vitro* assays, notably histochemistry, immunofluorescence, immunocytochemistry and molecular analyses such as flow cytometry, western blotting, and real-time PCR assays, are currently used to evaluate the potency of stem cells differentiation into different lineages (Panetta et al. 2009). However, most of them are likely time-consuming and can only provide a set of preliminary qualitative and descriptive data where the data are not in the form of quantitative obtained by SPR analysis. It is believed that osteoblast cadherin (OB-cadherin) expression increased with alkaline phosphatase activity during differentiation of MSC to osteoblast-like cells. Lee and colleagues monitored osteogenic differentiation of human MSCs on Au chips pre-coated with anti-human OB-cadherin antibodies. The intensity of SPR curve revealed close relation with attachment of OB-cadherin positive cells to slide. They showed that OB-cadherin expression level was increasingly raised as human MSCs exposed to osteoinduction medium in a time-dependent manner. By concurrent analysis of OB-cadherin dynamics via Western blotting, real-time PCR, and immunocytochemistry analyses, they confirmed much higher sensitivity of SPR-based method to measure target protein at nomogram levels at early developmental stage of osteogenic differentiation by day 6 whereas measurable values in western blotting was not detectable until the 9th day of inductive differentiation (Kuo et al. 2011). Another unique application of SPR was intra-cytoplasmic detection of mouse embryonic stem cells biomarkers such like Oct4, Sox2, Nanog, Rex1, and Lin28 (Tyagi et al. 2015b). Also in our experiment, we

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recently developed SPR method for early stage detection of MSCs differentiation into the endothelial-like cells (Fathi et al. 2017). Our results exhibited that SPR signals could detect early changes in the amount of VE-cadherin during the first 3 days after MSCs entering to differentiation procedure as compared to flow cytometry and immunofluorescence imaging.

A high-throughput, rapid, validated and an accurate characterization of induced pluripotent stem cells (iPSCs) biomarkers was successfully determined by introducing the cell lysate to a fabricated antibody arrays of pluripotency factors as mentioned above (Tyagi et al. 2015a). Tyagi et al. introduced embryonic stem cells lysate with various types of salt, blocking solution to antibody array fabricated on three different surfaces coated on gold chips to heighten the specificity with minimization of nonspecific adsorption rate (35). They acclaimed that a higher salt concentration of running buffer elicited a shelter to limit electrostatic charges and thereby reduced nonspecific adsorption.

Among the most interesting uses of SPR on cell biology, detection of cultured neural stem cells from mature neurons expressing neuronal cell adhesion molecule (NCAM) was performed by anti-NCAM antibodies pre-coated on binary polystyrene monolayer of SPR slides (Auer et al. 2010). Regarding the ability of neurons to express NCAM factor, they could easily attach to pre-coated surface, enabling them to interact with ligand antibody.

The application of SPRi has been extended to stem cell research in addition to somatic cells biology. For instance, a typical glycomic analysis microarray imaging of murine pluripotent stem cells, including mouse embryonic stem cells (mESCs), mouse-induced pluripotent stem cells (miPSCs), was done in response to eight spotted lectins DBA, MAL, PHA-E, PHA-L, EEL, AAL, PNA, and SNA (Nand et al. 2014). It was found that only MAL, PHA-E, PHA-L, EEL, AAL, and PNA attached to mouse embryonic fibroblasts. DBA and SNA were recognized by O-linked glycoproteins (Oct-4 and Sox-2) and had tendency to counteract with both mESCs and miPSCs. These results were concurrently affirmed by lectin microarray analysis of mouse stem cells. The recent use of SPR application on stem cell biology was to investigate the signaling pathway playing key role on cell fate and destination (Kawazoe 2007). For example, Ding et al. applied small molecules like retinoic acid (RA) and synthetic inhibitor nominated as TWS119 (Potent cell-permeable recombinant glycogen synthase kinase-3β [GSK-3β inhibitor]) that were able to induce neuronal differentiation of embryonic stem cells (Ding et al. 2003). In addition, to calculate TWS119 affinity with GSK-3β factor, they described the optimal doses of TWS119 to

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trigger neuronal differentiation. They concluded that GSK-3β has a potent role in the induction of mammalian neurogenesis of ESCs.

" Table.2 "

5. Impedance-based method

The term "impedance" refers to the intricate-valued generalization of resistance used commonly in electric cell-substrate impedance sensing (ECIS) and electrochemical impedance spectroscopy (EIS). ECIS is a non-invasive biophysical approach for analyzing the morphological and electrophysiological characteristics of living cells in a laboratory environment (Kuo et al. 2011; Seriburi et al. 2008). In these systems, cells are grown and attached on the gold surface after leaving planar gold electrodes into the cell culture dish, (Cuerrier et al. 2008a). Cell plated on gold films can have the similar role like dielectric particles because of having the shielding layer of cell membrane (Hug 2003). The impedance changes depend on coverage area on the electrode–cell interface, cell size and morphology and inherent dielectric properties of cells (Atienza et al. 2006; Lo et al. 1995). Thus, by recording time-resolved impedance ranges, cell shape changes like growth, migration and cell death can be monitored in real-time and label-free manner for bio-analytic purposes (Arndt et al. 2004; Linderholm et al. 2006).

The other impedance-based method is electrochemical impedance spectroscopy (EIS) bio-sensing that belongs to electrochemical biosensor. EIS displays the shifting of the charge transfer resistance (R) and properties of distinct molecules on an electrode surface immersed in redox solution (Fathi et al. 2017). Another biosensors based-electrochemical methods like voltammetric technique measures the currency and potentiometric sensors, showing the potential of an electrochemical vessel. Among these methods, EIS is a powerful and advantageous assay for cell sensing because of detecting label-free form, having high sensitivity and enabling of electron transfer at great rate and mass transfer at small frequency unlike the most of electrochemical-based biosensors (Feng et al. 2011; Yang et al. 2011). The theories of ECIS and EIS methods are similar, but in EIS method the existence of potassium ferrocyanide (K4[Fe(CN)6]) and potassium ferricyanide (K3[Fe(CN)6]) is necessary for measuring impedance and all electrodes after attaching cell samples immersed in electrochemical solution. While in the ECIS systems, cells and culture media were seeded on gold electrode array without any electrochemical solution for impedance measuring (Fig. 3). The use of impedance-based cell

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sensor offers several advantages like noninvasive monitoring of cell attachment, spreading, and proliferation and real-time cellular analysis of manipulations and treatments. As an example, for direct and label-free detection of liver cancer cells, EIS biosensor has been developed with good sensitivity and selectivity sensed glycoproteins on the hepatic cancer cell surface with a detection limit of 234 cells/ml (Hu et al. 2013). Johari and colleagues previously developed a novel nanostructured immune-biosensor for early detection of cancer antigen 125 (CA-125) in intestine cancer cells by using impedimetric methods with a limit of detection (LOD) of 0.0016 U/mL (Johari-Ahar et al. 2015). To improve the efficiency of cell assessment and test substances, the use of cell on chip methods are being increasingly used. For instance, Cho et al (2008) examined the electrical characterization of stem cells after growing on the electrode surface (Cho and Thielecke 2008). During the long-term cultivation of these cells, they showed that impedance spectra had an extracellular resistance with increasing of adhesion and growth rate. In another work done by Khalilzadeh et.al, a peptide-based electrochemical biosensor was modified with gold nanoparticles and used for sensitive detection of caspase-3 activity. This method was ability to calculate the apoptosis rate during hematopoietic stem cell differentiation (Khalilzadeh et al. 2016). Interestingly, they recorded the activity of caspase-3 at femtomolar level and effectively applied for early stage detection of stem cell differentiation over a period of 21 days (Khalilzadeh et al. 2016).

"Fig. 3 "

By using powerful noninvasive ECIS analytical method, cell morphological and volume changes, migration and differentiation of stem cells can be assessed (Mitra et al. 1991; Reitinger et al. 2012; Wegener et al. 2000). In ECIS method, the differentiation of mesenchymal stem cells (MSCs) into adipocytes, osteoblasts, myocytes and other cell types was studied. It is hypothesized that these cells have distinct dielectric properties in various cell lineages. In this regard, the stem cell differentiation to osteogenic, adipogenic and neural cells was done by some research groups (Kramer et al. 2014; Park et al. 2011; Reitinger et al. 2012). For instance, Stephan Reitinger and colleagues cultured bone marrow mesenchymal stem cells for several days and ECIS showed changes after seeding upon MSC spreading and adhesion (Reitinger et al. 2012). They exhibited that differentiation of human MSC into adipogenic and osteogenic lineages show significant impedance signal differences in ECIS (Reitinger et al. 2012). This

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reusable sensor was further confirmed appropriate for continuous live cell monitoring up to several days and can be applied for quality control of stem cells in medical cell production.

In the similar work of Bagnaninchi group the adipose-derived stem cell differentiation to osteoblast and adipocyte cells was performed (Bagnaninchi and Drummond 2011). They showed that differences in cell lineage dielectric properties can be recorded sensitively by impedance measurements. Also, time-course measurement of mean impedance demonstrated that osteogenesis of MSCs have more resistance compared with adipogenesis (Bagnaninchi and Drummond 2011). In the similar work for adipocyte differentiation of murine 3T3-L1 pre-adipocytes, this system can reproducibly screen the primary morphological changes during differentiation using adipogenic factors like insulin, dexamethasone, and rosiglitazone (Kramer et al. 2014).

Neural differentiation of MSCs examined after culturing on ECIS electrode array and the time-course of impedance changes was obtained (Park et al. 2011). This work indicates that the relatively slow change in resistance values of ECIS method can be detected due to the decrease of growth capacity and the morphological changes in differentiation of MSCs to neural cells. Also, the Immunocytochemical analysis of differentiated MSC to neuronal cells and ECIS data show that MSCs cultured in neural differentiation media were more effectively induced to differentiate into neuronal cells compared with normal growth media (Park et al. 2011). Moreover, osteogenic differentiation of human-derived MSC in 2D and 3D cultures studied by Hildebrandt et al confirmed that impedance increases during osteogenesis (Hildebrandt et al. 2010). ECIS can act as a high clinical relevance method for predicting the drug-induced cardiotoxicity in pharmacological researchers. As an example, human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were integrated into an impedance-based sensor to display the morphology and adhesion changes induced by cardiomyocyte rhythmic beating in real time (Hu et al. 2015). In addition, the effect of pesticides like chlorpyrifos was investigated by impedance-based measurement system on the differentiation of human mesenchymal stem cells (hMSCs) to adipocytes (Cho et al. 2009). A rapid fall in resistance in the cell layer was observed after adding chlorpyrifos to adipogenic differentiation medium (Cho et al. 2009). Therefore the use of electrical impedance spectroscopy provides appropriate and new data in the exploration of drug effects, cellular processes and differentiation of stem cells.

6. Quartz crystal microbalance (QCM) biosensor

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The QCM (Quartz Crystal Microbalance), as a piezoelectric mass-changing biosensor, measures the resonant frequency shifts of the gold-plated quartz crystal electrodes. Therefore, any mass changing on the surface of the crystal yields variation in the frequency of crystal that it is detectable by transducer (Fig.4) (Anderson et al. 2007; Auge et al. 1994). Given the existence of mega-gravity and inertial field on the surface of the quartz resonator, the QCM became a highly sensitive mass and viscosity dependent device in biosensor technologies (Mecea 2006).

QCM biosensor-like SPR method is label-free and real-time technique commonly used for investigation of the interaction between proteins like antibodies, binding, and kinetic features. This technique also has potency to monitor immunological reactions and cell-substrate interactions in a dynamic and non-invasive system. In this regard, the differences between aptamer-based and antibody-based QCM biosensors were used by Yao et al for the detection of immunoglobulin E (IgE) in biological samples (Yao et al. 2010). Results confirmed the effectiveness of the QCM sensing in cell detection by antibody array chip. Vaughan et al designed piezoelectric immune sensors for the detection of *Listeria* monocytogenes on the gold surface of the piezoelectric crystal. The crystals were modified by a self-assembled monolayer (SAM) of thiosalicylic acid for immobilization of specific antibody. They stated that this system can detect *Listeria* monocytogenes to 10^7 bacteria/ml of solution in real time manner while the similar methods with SPR technology are able to detect 10^3 bacteria/ml (D'Urso et al. 2008; Vaughan et al. 2001).

"Fig.4"

Progress in on knowledge about the QCM biosensor has gained a lot of attention in monitoring cellular responses and cell-surface interactions. The occurrence of active cytoskeletal remodeling in human oral epithelial cells (cell line H376) was investigated after culturing these cells on quartz biosensors and their response to microspheres using the QCM technique (Elsom et al. 2008). In an aptamer-based QCM biosensor, leukemia cells were detected after the attachment to gold nanoparticles (Au-NPs) on cell membrane. Signals were intensified by silver enhancement (Shan et al. 2014). Pei and colleagues studied the kinetic parameters and molecular interactions between lectin Concanavalin A and the surface of epidermoid carcinoma cell using a QCM biosensor. They suggested that this approach provided a real time monitoring with accurate data in the field of cell surface glycosylation, such as changes associated with cancer progression and development (Pei et al. 2012). In SCs research, this method can present

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significant information about cell function, physical properties, and cellular morphology during differentiation and self-renewal activity. The efficacy of the QCM system for measuring minute variations in mass has been contributed to multiple works in cellular behaviors like cellular adhesion. These features are thought to be essential in differentiation and proliferation process (Modin et al. 2006; Soumetz et al. 2008; Wegener et al. 2001). Modin et al. studied the binding and spreading capacity of osteoblastic cells by QCM biosensor. They found a direct and positive correlation between QCM frequency shift and the area of spread cells (Modin et al. 2006). The use of QCM biosensors in the cell-substrate interaction seems to open new avenues in regenerative medicine and tissue engineering (Soumetz et al. 2008). For instance, early adhesion capacity and the morphology of MSCs in response to biopolymers such as hyaluronic acid and chitosan were investigated during regeneration of various tissues. Chung et al. studied the adhesions of MSCs on the surface of QCM electrode modified with chitosan (CS), hyaluronic acid (HA) and fibronectin (FN) layers (Chung et al. 2011). They displayed the numbers or mass of attached MSCs on the FN layer of the QCM electrodes were significantly higher than those on HA and CS surfaces (Chung et al. 2011). These data suggest that QCM approach could distinguish the tendency of specific cell to target protein in different milieu. The adhesion of MSCs onto various functional end groups including –COOH, –OH or $–NH_2$ onto gold electrodes was checked by real-time shifts in resonance frequency of QCM cell biosensor. This capacity is useful for determining MSCs–drug interactions. In line with this statement, SCs were injected on the quartz crystal surfaces after exposure to various concentrations of anti-microtubule drug vinblastine. The decrease in frequency was achieved after cell adhesion onto surface with chemical functional groups of the modified QCM electrodes (Şeker et al. 2016). Moreover, QCM based biosensor is able to detect insignificant changes in the viscosity/density value of biological environments for *in situ* differentiation of MSCs growth like lab-on-a-chip devices. It was shown that the addition of various components, such as antibiotic, stem cells or supplements, had strong impact onto sensor signal of QCM biosensor (Esmeryan 2015). As a matter of fact, QCM system is an invaluable approach for detecting cell adhesion and spreading thereby provides useful information about the interaction between cells and modified surfaces of QCM electrodes, especially in stem cell studies.

7. Lateral flow biosensors

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Lateral flow biosensors (LFBs) are conceived as the simplest bio-sensing devices and special types of paper-based biosensors used to detect the presence and/or absence of target analytes in matrix of cellulose substrate without the need for specialized and costly equipment (Polito et al. 2000). This technique is based on a series of capillary beds as seen in porous papers (Yetisen et al. 2013). Low cost–benefit ratio, portability, less time-consuming in preparation and stable capability are inherent advantages of LFBs. Notably, this technique possess wide range of applications and high potential of commercialization with sensitivity range in the μM to mM concentrations, which is significantly less sensitive than other sensing approaches and molecular techniques such as SPR, EIS and enzyme-linked immunoassays (ELISAs). Nonetheless, some authorities report a high rate of sensitivity. Thus, this technique is not proper for detecting early changes in a specific disease course due to low levels of analytes or antigens in microfluidic sample (Qin et al. 2012). Technically, LFBs can operate either competitive or sandwich assays.

The general definition, designs, applications and other specifications of LFBs were previously discussed in a work by Sajid and colleagues (Sajid et al. 2015). A LFB is composed of four parts; a sample pad, a conjugate pad, a strip of nitrocellulose membrane and an absorbent pad with potency to transport fluid such as nucleic acid, protein or cell (Fig. 5).

“Fig.5 “

For biological recognition of target molecules, different analytes of protein, DNA or cells carry easily on the cellulose pad and attach easily to related labeled-tagged ligands (Quesada-González and Merkoçi 2015; Sajid et al. 2015). Regarding these comments, the most significant part of LFBs is the nitrocellulose membrane pieces with marked lines for test groups and positive control. Usually, the first line on nitrocellulose membrane is designated to test zone, containing a specific capture molecule that only attaches to flowing analytes and appeared as red color after interaction. The second line is used simultaneously as positive control. The last part of absorbent pad comes after three former zones and acts as a waste container (Fig.5). Based on literature, LFBs have been clinically used for the detection of any analytes in biological fluids such as plasma urine, water, milk, meat, agriculture samples and cells (Baeumner et al. 2003; Byzova et al. 2010; Lie et al. 2012; Liu et al. 2011a; Mao et al. 2009; Tang et al. 2011; Xu et al. 2008; Zhang et al. 2006a; Zhang et al. 2006b). An LFB-based aptamer-functionalized gold nanoparticle

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was developed by Xu et al. in order to detect thrombin in complex biological matrixes such as plasma samples (Xu et al. 2008). A rapid immune-chromatographic lateral flow test strip was designed in a competitive format with the gold-conjugated monoclonal antibody to specifically determine the residues of Clenbuterol, a β-adrenergic agonist, in swine urine with detection limit of as low as 0.1 ng/ml (Zhang et al. 2006b). The work of Baeumner et al. revealed another interesting application of LFBs. They developed a highly sensitive and specific RNA biosensor for the rapid detection of viable Escherichia coli in drinking water with a detection limit of 5 femtomole per sample (Baeumner et al. 2003). In other experiments, LFBs were applied for detecting the existence of antibiotic chloramphenicol in milk and the 1-aminohydantoin (AHD), as the metabolites of nitrofurans, in meat samples with limit of detection 10 ng/ml and 1.40 ng/ml, respectively (Byzova et al. 2010; Tang et al. 2011). Interestingly, femtomolar concentrations of nucleic acids were measured by LFB by applying isothermal strand-displacement polymerase reaction and gold nanoparticles (Lie et al. 2012). There are a few numbers of LFB-based experiments targeting whole cell or cell lysate. In one of these works done by Fang et al. they used HeLa cells lysate in fluidic section of sample pad to form DNA and c-jun proteins binding in test line (Fang et al. 2011). Three components including DNA probe containing the c-Jun binding site, gold nanoparticle (Au-NP) and cell lysate or purified c-jun protein in binding buffer incubated on conjugated pad. C-jun proteins can form both homo and heterodimers and therefore are capable of binding to DNA by themselves. By moving sample buffer in stripe, c-jun protein attached to DNA probe can be captured by the antibody against the DNA-binding protein (c-jun antibody) immobilized on the test zone to form a red line for visual detection of the target protein. This is a positive response for the detection of c-jun protein in lysate cell and indicates the presence of active c-jun protein with streptavidin as positive control zone. Interestingly, it was acclaimed that this technique is capable to detect c-jun activity in 100 µg of crude cell lysate protein. By the application of specific antibody directed against c-jun, they also could exclude any non-specific immune-reactivity (Fang et al. 2011).

LFBs were also applied for the rapid, specific and sensitive detection of circulating cancer cells (Mao et al. 2009). An aptamer-nanoparticle strip biosensor was developed to specifically recognize human lymphoblastic Ramos cells (Mao et al. 2009). For this propose, a thiolated aptamer was attached on the Au-NPs and the immobilization of a biotinylated aptamer was done on the test zone. Ramos cells positively interacted with aptamer part of the Au-NP-

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aptamer molecules to form the Au-NP-aptamer-cell complex .They showed a large number of Au-NPs collected on the test region and produced a distinctive red band, which can be used for either qualitative, i.e., visual assessment, or quantitative calculation of cells via a strip reader. Notably, this biosensor could sense a lowest of 4000 cells without tool (visual detection) and 800 cells through a strip reader instrumentation in 15 min (Mao et al. 2009).

Finally, there are quite few works studying stem cell detection using LFBs developed up to now (Wu et al. 2015; Wu et al. 2013). The surface marker of human pluripotent stem cells (hPSCs), SSEA-3 and SSEA-4, were used for detection of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Different stem cell associated antibodies such as SSEA-4 and -3 are routinely used. For instance, the antibody of SSEA-4 was attached onto gold nanoparticles for the detection of hSCs. The SSEA-3, antibody against hPSCs surface antigen, was immobilized on the test region of the nitrocellulose membrane. The complex of nanoparticles stem cell with moving in strip of LFBs finally was captured by SSEA-3 antibody immobilized on the test zone to form a red line for visual or quantitative detection of cells by a portable strip reader. It is estimated that this biosensor can detect a minimum of 10,000 ESCs by the naked eye and 7000 cells with a portable strip reader within 20 min. LFBs not only have shown excellent specificity to distinguish cell types but also display great potential for specific and handy detection of hPSCs (Wu et al. 2013). In another work by the same author, LFB was used for ultrasensitive detection of hPSCs via amplified single strand DNA (ssDNA) (Wu et al. 2015). In this study, the magnetic beads were coated with antibodies against SSEA-3 and SSEA-4 with carboxyl-modified tDNA sequence used as template for strand displacement amplification (SDA). In comparison with previous biosensor, this approach was able to detect 100 hPSCs, displaying high sensitivity than nanoparticles–stem cells approach (Wu et al. 2015).

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8. Conclusion and future prospective

The application of label-free biosensors with great emphasis on stem cell detection extends the scope of this review paper. In this field, SPR technique has been exhibited to be one of the most sensitive and fast biosensors for detecting cell surface marker in stem cell areas with providing qualitative and quantitative data. Meanwhile, to get more knowledge about the differentiation of MSCs into other lineages, impedance spectroscopy is beneficial to assess spreading and proliferation of stem cells by monitoring the resistance of cell layer on electrode

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arrays. In stem cell studies, QCM systems, compared with other sensing methods is a valuable approach for detecting cell adhesion and spreading; thereby it provides useful information about the viscosity value of biological environments for *in situ* differentiation of stem cells. Finally, paper based approaches in LFB were used to detect the presence or absence of target molecules in cell surface without the need for specialized and costly equipment. Also this review article focuses on the reliable and possible future application of label free bio-sensing methods in human medicine required for the analysis and diagnose of specific cell type and abnormal molecules in various human diseases with tumors, biochemical defects and etc. It seems that focusing on diverse application of these useful methods in medicine along with classical laboratory evaluations could yield better outcomes health care system.

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Table legend

Table 1: An overview to label free biosensors.

Table 2. Examples of biosensors for stem cell studies.

Figure legends

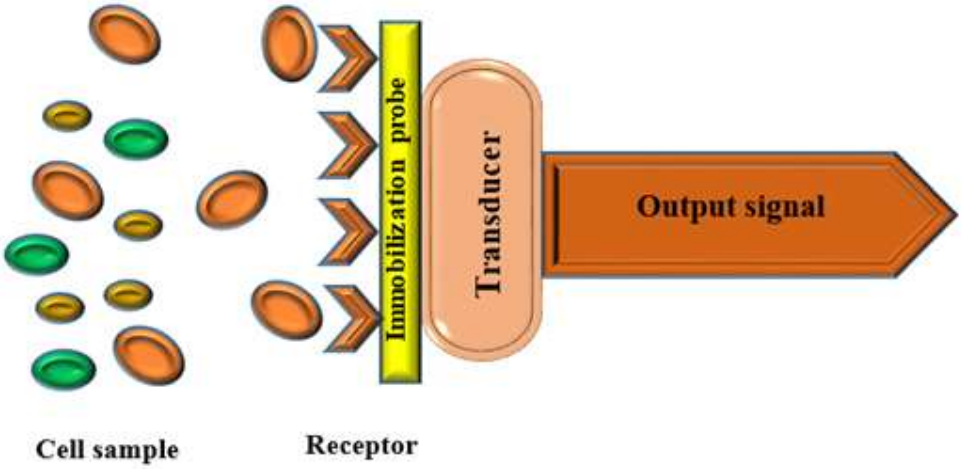
Figure 1: Schematic image of biosensor mechanism.

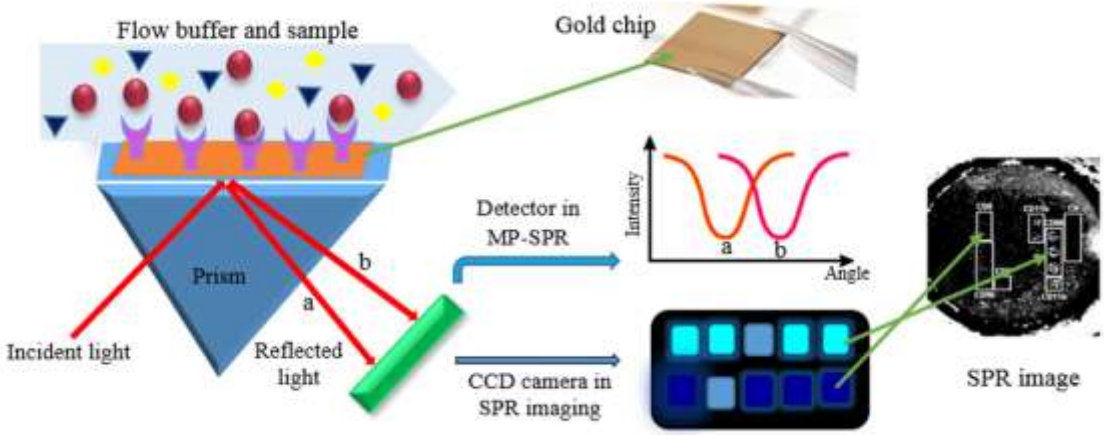
Figure 2. Representative image of SPR and SPRi. SPR: Surface Plasmon Resonance; MP-SPR: Multi-parametric Surface Plasmon. Resonance; SPRi: Surface Plasmon Resonance imaging; CCD: Charge Coupled Device.

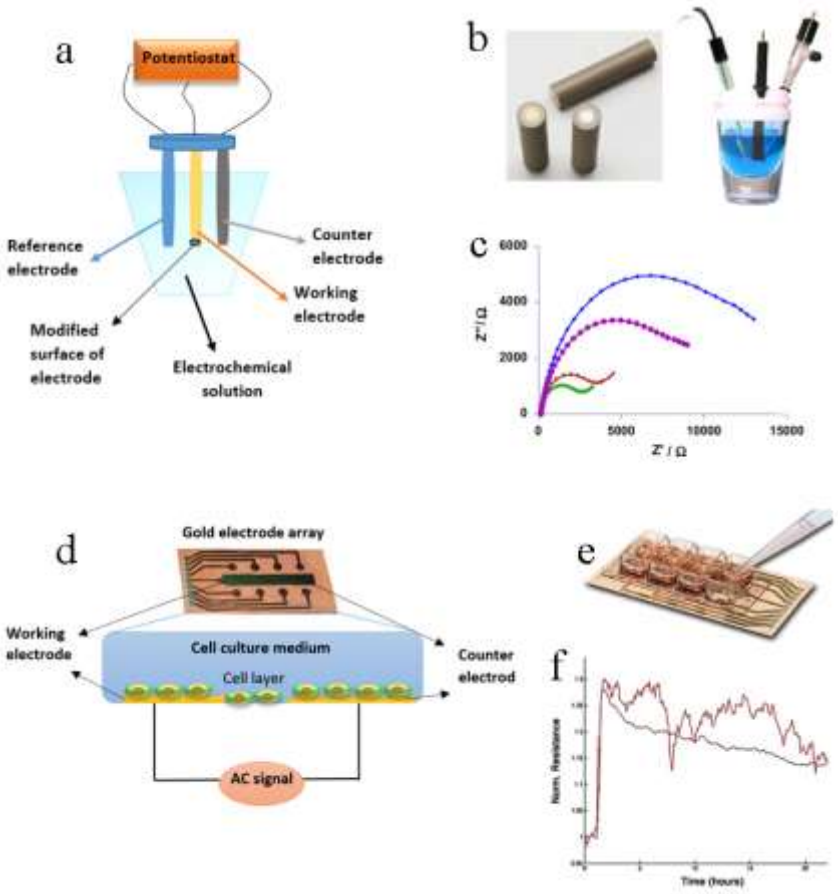
Figure 3: Representative picture of electrochemical cell in EIS system (a) real image of EIS electrodes (b) Output data of EIS (c) Schematic image of ECIS (d) Specialized slide of gold electrode arrays with 8 individual wells for cell culturing in ECIS (e) and output data of ECIS.EIS: Electrochemical impedance spectroscopy; ECIS: Electric cell-substrate impedance sensing.

Figure 4: Representative picture of QCM based biosensor (a) and a QCM electrode (b). QCM: Quartz crystal microbalance.

Figure 5. Real image of lateral flow biosensor (LFB) (a) schematic representation of LFB (b).


Immobilization probe
Transducer
Output signal
Cell sample
Receptor


Flow buffer and sample
Gold chip
Prism
Incident light
Reflected light
a
b
Detector in MP-SPR
Intensity
Angle
CCD camera in SPR imaging
SPR image


a
Potentiostat
Reference electrode
Counter electrode
Working electrode
Modified surface of electrode
Electrochemical solution
b
c
6000
4000
2000
0
Z'' / Ω
0
5000
10000
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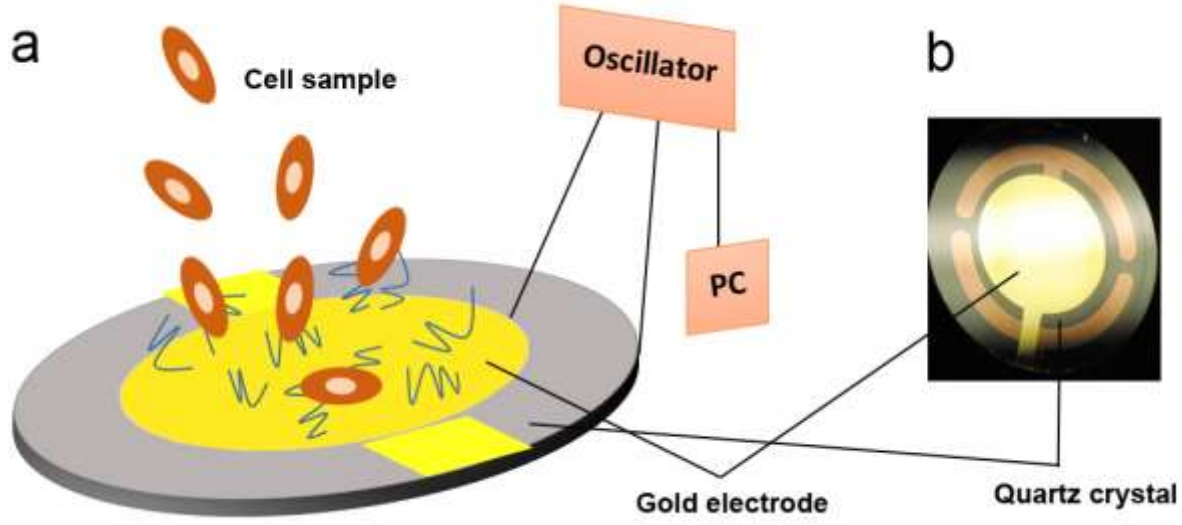
d
Gold electrode array
Working electrode
Cell culture medium
Cell layer
Counter electrod
AC signal
e
f
Norm. Resistance
Time (hours)


a
Cell sample
Oscillator
PC
Gold electrode
b
Quartz crystal

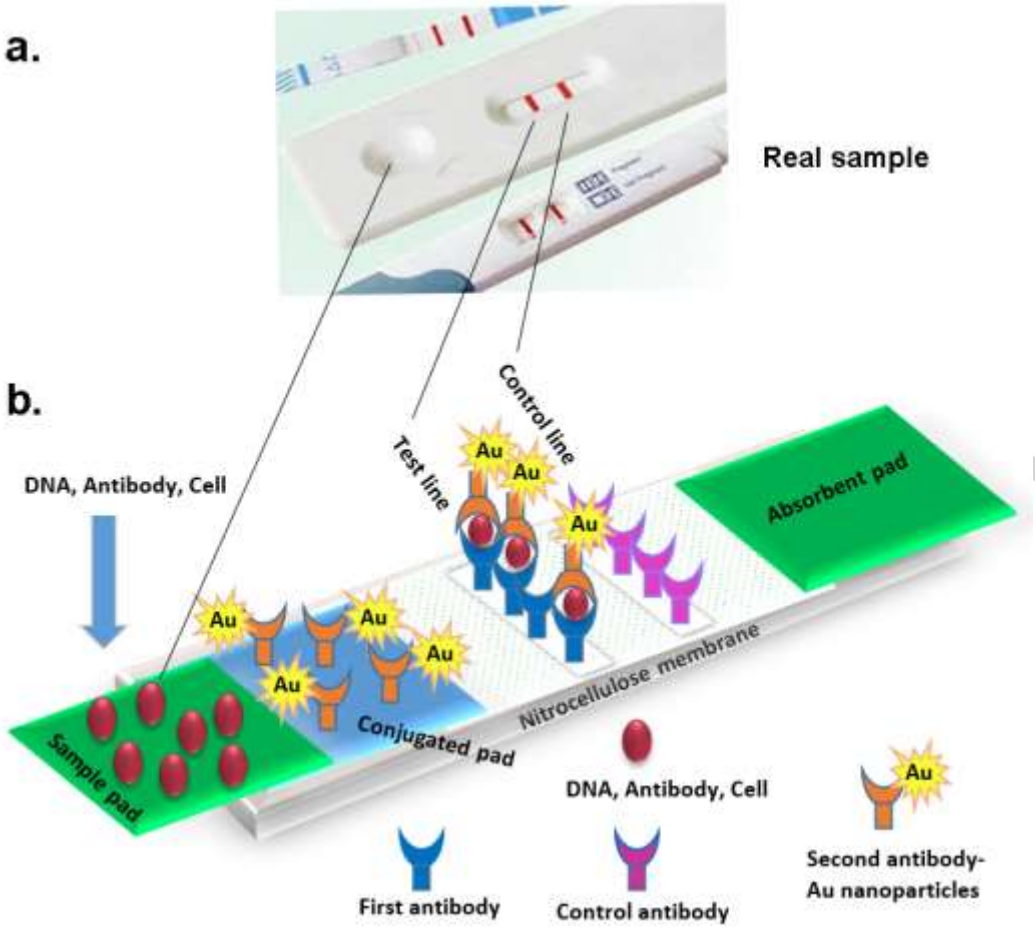

a.
Real sample
b.
DNA, Antibody, Cell
Test line
Control line
Absorbent pad
Au
Nitrocellulose membrane
Conjugated pad
Sample pad
DNA, Antibody, Cell
First antibody
Control antibody
Second antibody-
Au nanoparticles

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Table 1: An overview to label free biosensors.

Biosensor	Immobilization probe	Detection feature	Transducer	Output signal	Device image	Ref
SPR & SPRi	Gold chip (modified chip: CMD, St)	Refractive index change	Photonic	Angle shift		(Cuerrier et al. 2008a; Viitala et al. 2013)
EIS and ECIS	Electrode (au electrode)	Mass and electron transportation	Electrochemical	Impedance		(Feng et al. 2011; Seriburi et al. 2008)
QCM	Quartz crystal plate	Mass variation	Piezoelectric	Resonant frequency		(Elsom et al. 2008; Shan et al. 2014)
LFB	Nitrocellulose paper	Color change	Optic	Visual		(Wu et al. 2013; Zhang et al. 2006a)

SPR: Surface Plasmon Resonance, SPRi: Surface Plasmon Resonance imaging, CMD: carboxy methyl dextran, St: streptavidin, EIS: Electrochemical impedance spectroscopy; ECIS: Electric cell-substrate impedance sensing, LFB: Lateral flow biosensor, QCM: quartz crystal microbalance.

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Table 2. Examples of biosensors for stem cell studies.

Sensor surface	Immobilized fragment	Detected Stem Cell	method	Performance	Ref
Antibody on chip	VE- cadherin antibody	hAMSCs	SPR	Early stage detection of endothelial differentiation	(Fathi et al. 2017)
Antibody array on gold chips	Anti-human OB-cadherin	Mesenchymal stem cells (MSCs)	SPR	Detectin of osteogenic maturation of MSCs in a live cell	(Kuo et al. 2011)
Antibody array on gold chips	Anti- Oct4 , Sox2, Rex1,Lin28 and Nanog	m ESC lysate biomarkers	SPR & SPR i	Minimizing NSA and Identification iPSCs	(Tyagi et al. 2015b)
Antibody–polymer on gold chip	Neural cell adhesion molecule antigen to antibody–polymer layers	Human embryonic stem cells	SPR	Neural differentiation of hESC	(Auer et al. 2010)
Lectin microarray on gold chip	DBA, MAL, PHA, AAL lectins	Pluripotent mouse stem cells	SPR i	Determination of stem cell pluripotency based on lectin reactivities	(Nand et al. 2014)
Enzyme immobilization on gold chip	GSK-3 β	TWS	SPR	Control stem cell fate by small molecules in ESCs	(Kawazoe 2007)

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Quartz crystals gold electerod	Mercaptoundecanoic acid, mercaptoethanol	Mesenchymal stem cell	QCM	Monitoring of adhesion MSCs on modified crystal surfaces	(Şeker et al. 2016)
Quartz crystals gold electerod	Stem cells	Endometrial Stromal and Mesenchymal Stem Cell	QCM	Detection of negligible changes in the density of biological environments	(Esmeryan 2015)
Antibody on nitrocellulose membrane	Human SSEA-3 monoclonal antibody	Human pluripotent stem cell	LFB	Detection of 10,000 stem cells within 20 min	(Wu et al. 2013)
Nitrocellulose membrane	DNA immobilization	Human pluripotent stem cell	LFB	Using magnetic beads enhance detection of hPSCs to 100 cells	(Wu et al. 2015)

SPR: Surface Plasmon Resonance, SPRi: Surface Plasmon Resonance imaging, DBA: *Dolichos biflorus agglutinin* , MAL: *Maackia amurensis lectin* , PHA: Phytohaemagglutinin , AAL: *Aleuria aurantia lectin* , Oct4, Sox2, Nanog, Rex1, and Lin28 biomarkers present in mouse embryonic stem cell (mESC), NSA: nonspecific adsorption, iPSCs: Induced *pluripotent stem cells,*GSK-3 β : glycogen synthase kinase-3β, TWS: inducer of neurogenesis and GSK- 3β inhibitor, LFB: Lateral flow biosensor, hPSCs: human pluripotent stem cell, QCM: quartz crystal microbalance

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Highlights

1- Noninvasive and label free monitoring methods are described for stem cell detection.

2- Bio-sensing techniques for stem cell studies, including surface plasmon resonance, impedance-based method**,** quartz crystal microbalance and paper based detections are reviewed.

3- Label free systems are compared with each other in stem cell sensing researches.

4- This review article focuses on the reliable and possible future bio-sensing application for monitoring and identification of rare and specific cell populations in various human diseases with tumors, biochemical defects and etc.

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