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Blueprint of quartz crystal microbalance biosensor for early detection of breast cancer through salivary autoantibodies against ATP6AP1

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ABSTRACT

Breast cancer represents a significant health problem because of its high prevalence. Tests like mammography, which are used abundantly for the detection of breast cancer, suffer from serious limitations. Mammography correctly detects malignancy about 80–90% of the times, failing in places when (1) the tumor is small at early stage, (2) breast tissue is dense or (3) in women of less than 40 years. Serum-based detection of biomarkers involves risk of disease transfer, along with other concerns. These techniques compromise in the early detection of breast cancer. Early detection of breast cancer is a crucial factor to enhance the survival rate of patient. Development of regular screening tests for early diagnosis of breast cancer is a challenge. This review highlights the design of a handy and household biosensor device aimed for self-screening and early diagnosis of breast cancer. The design makes use of salivary autoantibodies for specificity to develop a noninvasive procedure, breast cancer specific biomarkers for precision for the development of device, and biosensor technology for sensitivity to screen the early cases of breast cancer more efficiently.

Contents

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1. Introduction and epidemiology of breast cancer

Breast cancer is the abnormal growth of cells occurring in breast tissue. The term "breast cancer" refers to a malignant tumor that either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Less commonly, breast cancer can begin in the stromal tissues, which include the fatty and fibrous connective tissues of the breast (Lavigne Jc Fau–Desaive and Desaive, 1975). So far, several etiological factors have been associated with occurrence of breast cancer, such as age at menarche and menopause (Oeser and Fuchs, 1975), childbearing (Litton and Theriault, 2010), breastfeeding (Anderson, 1975), hormonal status (Lippman, 1975), consumption of alcohol (Berkey et al., 2012; Seitz et al., 2012) and type of diet (Smith-Warner et al., 2001), obesity (Cleary and Grossmann, 2009), radiation (Lagadec et al., 2012), and genetic susceptibility (Roy et al., 2012).

Breast cancer represents a significant health problem because of the numbers of individuals affected by this disease. It is the most common cancer in women worldwide and constitutes about 23% of all female cancers (Jemal et al., 2011). There is an estimated 4.4 million women alive globally, who within the past 5 years were diagnosed with breast cancer (Parkin et al., 2005), and it now appears that incidence and mortality rates of breast cancer are increasing. Breast cancer in Asian women occurs at a younger age and unlike in west, it is usually diagnosed at later stage of development (Kwon et al., 2011). And so, in Europe and US, the mortality is declining probably due to availability of better screening and diagnostic facilities, and adjuvant therapy, thus allowing cancers to be successfully treated at earlier stages (Chu et al., 1996; Stockton et al., 1997). Despite the high incidence rates, 89% of women in Western countries with breast cancer are still alive 5 years after their diagnosis, which is due to detection and treatment strategies (Parkin et al., 1999). Screening of breast cancer involves the use of tests or examinations on asymptomatic individuals, for early diagnosis of the disease (before it becomes clinically apparent) in order to lower the risk of death, or complications of treatment (Knutson and Steiner, 2007).

2. Current methods for diagnosis of breast cancer

The only proven effective method of breast cancer screening is mammography. X-ray mammography is the "gold standard" for breast cancer screening (Miller, 2001). Mammography is a low-dose X-ray procedure that allows visualization of the internal structure of the breast. On average, mammography will detect about 80–90% of the breast cancers in women without symptoms (Michaelson et al., 2002) and accounts for a reduction in breast cancer mortality of about 20–30% for women of age 50–69, and of about 17% in women of age 40–49 (Bailey et al., 2010). Despite being a highly accurate method for diagnosis of breast cancer, mammography is associated with few limitations: (i) mammography screening detects only 70% of breast cancers (Wang, 2003), (ii) currently, high- risk populations, such as women with benign breast disease, have an increased risk of developing cancer (relative risk 1.568), with false positive mammograms leading to unnecessary biopsies (Hartmann et al., 2005), (iii) tumors less than 7.5 mm in size are not easy to be detected (Michaelson et al., 2003), and also mammograms of women with dense breast tissue are difficult to read (Graham–Rowe, 2012), (iv) low energy X-rays used in mammograms are themselves cause of mutation (Lokate et al., 2011), (v) mammography is only done for women between 40 and 49 (Peres, 2010), and (vi) compressing, squeezing, or otherwise disrupting tumors may result in trauma-induced micro metastases. These dislodged cancer cells are called "traumets" (Rosser, 2001).

For certain women at high risk for breast cancer, screening through magnetic resonance imaging (MRI) is recommended along with a yearly mammogram. MRI utilizes a magnetic field to visualize the features of soft tissues with highly detailed resolution, even in cases of dense breasts or implants (Enriquez and Listinsky, 2009). Unfortunately, not all cancers are visible on MRI and some cancers, such as ductal carcinoma in-situ and lobular carcinoma, are more difficult to detect using MRI (Friedman et al., 2006). In addition, magnetic resonance imaging is not a cost-effective technique for routine screening for breast cancer (Sarvazyan et al., 2008).

A breast biopsy can also be done for breast cancer detection and it is the only way to determine if a potential trouble spot is cancerous or benign. A biopsy is the removal of cells or tissue from a suspicious mass. The tissue or cells are then examined under a microscope to check for breast cancer cells (Van Phuc et al., 2010). Medical guidelines say that about 90% of biopsies should be needle biopsies – the least invasive procedure. Still, research has shown that about 70% of breast biopsies are surgical biopsies. This means that many women who do not have cancer are having unnecessary surgery. It also means that women who are diagnosed with breast cancer have to have a second operation to remove the cancer. The major disadvantages of this technique are that biopsies may lead to metastasis of cancerous tumor (Ashbeck et al., 2007), these molecular tests also require experienced and skilled lab workers, and they are not cost effective and are performed in late stages after detection of cancer.

A painless and free of exposure-to-radiation technique is breast ultrasound. Ultrasound, also known as sonography, is an imaging method in which sound waves are used to look inside a part of the body. For this test, a small, microphone-like instrument called a transducer is placed on the skin. It emits sound waves and picks up the echoes as they bounce off body tissues. The echoes are converted into a black and white images that are displayed on a computer screen (ODLE, 2007). Although ultrasound is less sensitive than MRI (that is, it detects fewer tumors), it has the advantage of being more available, non-invasive and less expensive (Kuhl et al., 2005).

For molecular imaging, tests specific to breast imaging such as Breast Specific Gamma Imaging (BSGI)/ Molecular Breast Imaging (MBI) (also known as breast scintigraphy) reveal tumors in dense or surgically altered breasts (Schillaci and Buscombe, 2004; Tadwalkar et al., 2012) BSGI/MBI uses a small injection of radioactive material in which breast cancers are revealed as "hot spots" in the image (O'Connor et al., 2009). As cancerous cells have a higher rate of metabolic activity than normal cells, so they absorb more of the tracing agent at a faster rate than healthy tissue. Although BSGI is a very useful imaging modality, like all other tests, it has some limitations. First, this test also experiences false-positive findings and secondly, unlike MRI, it cannot see the extension of cancers through the pectoralis muscle. So, MRI is a better choice for patients with very large tumors and for patients with aggressive cancers near the chest wall (Brem et al., 2008).

Other tests may be useful for some women, but they are not used often and have not yet been found to be helpful in diagnosing breast cancer in most women. These include scintimammography (Liberman et al., 2003), thermography (Dodd, 1977), ductogram (Cardenosa et al., 1994), nipple discharge exam (Cabioglu et al., 2003), nipple aspiration (Shao and Nguyen, 2001), and ductal lavage (Dooley, 2003).

3. Diagnostic media in diagnostic techniques

Blood is the most commonly employed medium for serological tests, it contains almost all the chemical components present in the body. Disease diagnosis involves the detection of certain molecular markers that may be raised in the blood serum in case of a certain ailment, like sugar levels in diabetes, etc. (Van Hove et al., 1993). The level of proteins in serum is approximately 60–80 mg/ml, (Merrell et al., 2004) and there is a need for the processing of blood samples before any diagnosis procedure. This is achieved by clotting or the enzymatic activity during the process which can cleave relevant pathway related proteins (Teisner et al., 1983).

Use of plasma as a diagnostic media has specific requirements such as that of selecting an appropriate anticoagulant. Blood specimens need to be collected in a specific sequence and under-filling tubes with additives; the results of protein analyzes may be altered if not done so. Moreover, collection of blood is an invasive technique requiring pricking of skin using syringes which poses a threat of disease transfer such as HCV from one person to another (Streckfus and Guajardo–Edwards, 2011).

These associated disadvantages gave rise to a need for selecting an alternative for the purpose. Use of saliva for diagnostics was one of the remarkable ideas proposed in the second half of the 20th century. The advantages associated with it are easy, non-invasive sample collection technique, early detection of certain diseases and monitoring of disease course in conjunction with therapy (Pink et al., 2009).

It has been demonstrated through extensive research that oral mucosal transudate (OMT), one of the serum-derived fluid that enters saliva from gengivial crevice and across oral mucosal surfaces, can yield detectable levels of immunoglobulins (i.e. IgG and IgM antibodies) against various bacterial and viral diseases. Assays based on OMT analysis can aid in the disease diagnosis and management of therapeutic drugs (George and Fitchen, 1997). Saliva-based antibody testing has been used for diagnosis and screening of viral infections such as HIV (Archibald et al., 1986; Johnson et al., 1988) and hepatitis A, B, and C viruses (Parry et al., 1988; Siddiqi and Abdullah, 1988; Thieme et al., 1992), bacterial infections such as *Vibrio cholerae* (Jertborn et al., 1986) and *Haemophilus influenzae* (Gildorf and McDonnell, 1991). Lesser risk of inadvertent transmission of blood-borne pathogens, convenient use in pediatric and geriatric populations, and blood-free sample collection are some inherent features of OMT technology (George and Fitchen, 1997).

Numerous studies validate the utility of saliva for the detection of malignancies remote from the oral cavity. With current detection techniques, it is possible to readily measure proteins present at femto-molar concentrations, providing more treatment options to the patient, with better recovery chances. Saliva, thus, can play a role as an adjunct diagnostic test for systemic cancers (Streckfus and Bigler, 2002).

4. Salivary biomarkers for detection of breast cancer

Biomarkers serve as molecular indicators of a biological status of a living organism and to evaluate the presence of cancer and therapeutic interventions (Tainsky, 2009). The identification of biomarkers by a sensitive assay using non-invasively collected clinical specimens is ideal for breast cancer detection. Zhang, et. al. in 2010 performed a de novo biomarker discovery approach using saliva for breast cancer detection. The group evaluated the potential of transcriptomic and proteomic biomarkers in saliva for breast cancer detection. The profiling of tumor markers revealed a statistically significant difference in the level of biomarkers present in the saliva of healthy controls and breast cancer patients. An accuracy of 92% was obtained (83% sensitive and 97% specific) for eight mRNA and one protein biomarker in saliva, unaffected by other factors. Hence, the potential for using salivary sample for the specific detection of breast cancer has been recorded previously, the results of which may be confirmed by subsequent tests (Zhang et al., 2010b).

In another study, levels of vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and carcinoembryonic antigen (CEA) in the saliva were measured using enzyme-linked immunosorbent assay (ELISA) in 49 healthy individuals and 49 breast cancer patients. These salivary fluid protein levels were significantly elevated in cancer patients (Brooks et al., 2008). Other breast cancer antigen, CA15-3 and tumor marker, c-erB-2 also shows higher levels in the saliva and serum of breast cancer patients as compared to the healthy controls. This shows a positive correlation between the levels of biomarkers in saliva and serum (Agha-Hosseini et al., 2009). However, these protein markers are used primarily to monitor advanced disease and do not have sufficient clinical sensitivity for early detection of breast cancer (Harris et al., 2007).

5. ATP6AP1 autoantibodies biomarker in early detection of breast cancer

ATP6AP1 is an ATPase and a component of a multi-subunit enzyme of 1 mDa. It takes part in the body′s intracellular processes such as protein sorting, zymogen activation, and receptor-mediated endocytosis involving VATPase dependent organelle acidification. Vacuolar ATPase (V-ATPase) comprises of a cytosolic V1 (site of the ATP catalytic site) and a transmembrane V0 domain. It may assist in the V-ATPase-mediated acidification of neuroendocrine secretory granules. ATP6AP1 is expressed in normal tissues such as brain marrow, blood, embryonic tissue, heart, kidneys, nerves, and skin. Its expression is also found to be associated with several tumors such as adrenal tumor, head and neck carcinoma, leukemia, lung tumor, retinoblastoma, and various other cancers. However, its prevalence in breast cancer is far greater than in the rest of these cancers. Tumor cells exhibit this ATPase system to maintain an acidic pH level for their survival in a hypoxic microenvironment. It was hypothesized that vacuolar H+-ATPases (V-ATPases) that normally reside in acidic organelles are also located at the cell surface, thus regulating cytosolic pH and exacerbating the migratory ability of metastatic cells. Lower extracellular pH may cause the degradation of extracellular matrix (ECM) and its remodeling through proteolytic enzyme activation, and hence may contribute to cancer invasion and metastasis (Hernandez et al., 2012).

ATP6AP1 Autoantibodies (AAb) to tumor-derived proteins are spontaneously generated in cancer patients These autoantibodies are induced and secreted in the serum of patients due to changes in protein glycan expressions or structure. These AAbs are detected in early stage when cancer is not prominent and detection at this phase has more survival chances. A study titled as ‘Protein microarray signature of autoantibody biomarkers for the early detection of breast cancer′ was performed using probed novel high-density custom protein microarrays (NAPPA) expressing 4988

candidate tumor antigens with sera from 102 patients with stage I–III breast cancer, was used to measure levels of bound auto-antibodies IgG. The result suggested 28 potential biomarkers for early diagnosis of breast cancer in which ATP6AP1 ranked first. It showed that AAbs to ATP6AP1 were significantly higher amongst breast cancer patient ($p < 0.0002$, $p < 0.0034$) with a specificity of 95% and sensitivity of 30% (Anderson et al., 2010). Therefore, it is concluded that ATP6AP1 can serve as an efficient biomarker for early detection of breast cancer which may be used to develop a biosensor device aimed for screening and early detection for breast cancer by means of salivary specimen.

6. Early detection of cancer through autoantibodies in saliva

The detection of autoantibodies in saliva can lead to very specific detection of various cancer types. It is now being reported with greater frequency that cancer biomarkers generate antibody production against themselves in the patients. In the early phases of colorectal cancer, an increase of ecPKA- expression occurs and the presence of auto-antibodies generated against ecPKA highly correlate with cancer. Scientists in Austria have used autoantibody biomarkers against extracellular Protein Kinase A for the detection of colorectal cancer via ELISA. The anti-ecPKA auto-antibodies serum levels were shown to be markedly up-regulated in patients with cancer (Stefatić et al., 2008). Similarly, p53 autoantibodies are found in the serum of cancer patients which are not found in healthy people. Waymakulasuriya et al. developed a protocol for the detection of p53 autoantibodies in the case of oral squamous cell carcinoma, using saliva as the sample fluid. Based on their work and literature survey, they found that 25–30% of head and neck cancer patients develop p53 antibodies in their serum and saliva (Warnakulasuriya et al., 2000).

Currently, methodologies used to identify such autoantibodies include microarrays, ELISA, and other serological techniques. These technologies may be improved for direct application in clinical setting by the integration of multiple assays and approaches (Dudas et al., 2009) and sensors. A biosensor, as a diagnostic device, detects changes in biological response in patients and converts these into detectable and measurable signals. The development of a biosensor is an one-time investment, because a single device will be able to screen many people due to its reusable nature (Choi and Chae, 2009). Moreover, this technology has shown great promise as a diagnostic device previously (Wang, 2008) and has opened up a new horizon in detection of cancers (Bohunicky and Mousa, 2011). Nano-bio-chip sensors have been designed to detect important proteomic and transcriptomic cancer biomarkers in saliva previously (Jokerst et al., 2009; Zhang et al., 2010a). Onen, Kruk et al. have reported a biosensor that detects anti-apoptotic protein Bcl-2 in pico grams and requires no invasive procedure for sampling (Onen et al., 2011). Another biosensor reported by Liang, Cha et al., detects Carbohydrate Antigen (CA 15-3) in saliva of breast cancer patients and is very sensitive (Liang et al., 2012). A summary of similar biosensors based on breast cancer have been reported (Table 1). These biosensors have shown a great promise for use in future for easy detection of cancers. However, these biosensors are to be tuned for early detection of cancer especially breast cancer.

7. Blueprint of ATP6AP1 biosensor for early detection of breast cancer

Here, we want to propose the blueprint of a biosensor which could become a possible candidate for early detection, as well as the monitoring of disease progress and reoccurrence, of breast cancer (See Fig. 1). The components are as follows.

7.1. Analyte

Analyte is the biological molecule under investigation that needs to be recognized by the biocomponent of the sensing device (Vo–Dinh and Cullum, 2000). In our case, this part will be played by autoantibodies (AAbs) that are generated spontaneously against protein biomarkers in cancer tissues. These immunoglobulins are found to appear in the serum (Anderson et al., 2010) but we propose that these may also appear in oral secretions like saliva, mucosal transudate, etc., because antibody molecules from serum have been found to be excreted into oral cavity previously as well, probably around the same time as they appear in serum (Kaufman and Lamster, 2002).

7.2. Biocomponent

The biocomponent is a biological part which interacts and recognizes the analyte specifically and is immobilized on the transducer (Vo–Dinh and Cullum, 2000). The natural choice for biocomponent is the ATP6AP1 protein against which AAbs are found to be specifically raised in breast cancer patients (Anderson et al., 2010).

7.3. Signal transducer

The transducer component serves to transfer the output of the biological component to the detection system employed in the sensor. Hence, it is also called as a detector, sensor, or an electrode (Thévenot et al., 2001).

The reasons for selecting a piezoelectric biosensor for the purpose are its sensitivity to detect even a small amount of auto-antibodies produced, and simplicity. The theory is to get a quartz crystal microbalance, a stable oscillator, which changes its frequency in response to mass accumulation, and hence it quantitatively registers and reports the mass deposited on its surface (Bunde et al., 1998). Quartz crystal microbalance systems designed commercially for use in mass measurements can measure changes upto $\sim 100\ \mu g$ accurately, whereas the minimum detection limit for the system is usually $\sim 1\ ng\ cm^{-2}$ (O'sullivan and Guilbault, 1999). An ethanol sensor employing QCM, as demonstrated by Bello et al., has been shown to have $1.73 \times 10^{-2}\ \mu g$ limit of detection and $3.69 \times 10^{-2}\ \mu g$ as the limit of quantitation, which can be designed for use for monitoring ethanol levels during bakery production process (Bello et al., 2007). It has also been used for the detection for volatile chemical compounds, like toluene and xylene (Matsuguchi and Uno, 2006) and toxins such as 2.3.7.8-tetrachlorodibenzo-p-dioxin (TCDD) which was detected by anti-TCDD monoclonal antibodies (Kurosawa et al., 2005).

The strategy is to coat the proteins onto the surface of the crystal, which will subsequently bind antibodies. The quartz crystal is made to oscillate at a particular frequency by externally applied alternating current. According to the piezoelectric effect – where electricity is produced by deformation in mass and vice versa – the alternating fields will produce alternating mass conformations in the crystal, and will be further calibrated to produce a stable oscillation. Upon the binding of autoantibodies, the slightest mechanical stress is expected to provide a detectable change in frequency that can be recorded (O'sullivan and Guilbault, 1999) and related to the amount of marker molecules that are highly specific for breast cancer (Anderson et al., 2010).

Table 1
Biosensors for detection of breast cancer. The components such as sensitivity, transducers, type, analyte and medium used for detection of these biosensors have been shown.

Medium for detection	Sensitive	Type	Bio-component	Analyte	Transducer	References
Serum	30 pg/ml	Optical	Human monoclonal 27.B1 IgM	GIPC-1 protein	Fiber optic immune sensor	(Salama et al., 2007)
Urine	Sub pg/ml	Piezo-electric	Bcl-2 antibodies	Anti-apoptotic protein Bcl-2	ST cut Quartz wafers	(Onen et al., 2011)
Serum	10 fM–1 nM	Piezo-electric	Micro-RNA	Micro-RNA	Au nanoparticle	(Johnson and Mutharasan, 2012)
Biopsy sample	0.05–25 fmol	Electrochemical	cDNA complementary to BRCA1	BRCA1	cDNA/CHIT-*co*-PANI/ITO electrode	(Tiwari and Gong, 2009)
Serum	>2 ng/mL	Piezo-electric	Anti-HER2 PEMS	HER-2	Piezoelectric microcantilever sensors	(Loo et al., 2011)
Cell sample	50 nM	Optical	Complementary DNA	Mutated DNA	Surface Plasmon Resonator	(Carrascosa et al., 2009)
Biopsy sample	70 nm	Optical	Molecular beacon probe	BRCA1	Miniature biochip system	(Culha et al., 2004)
Serum	6–60 ng/ml (or 0.06–0.6 nM	Piezo-electric	H3 single-chain variable fragment (scFv)	Human epidermal growth factor receptor 2 (Her2)	Lead zirconate–lead titanate (PZT)/glass piezoelectric microcantilever sensor (PEMS)	(Capobianco et al., 2011)
Serum	>2 ng/mL	Piezoelectric	Anti-HER2 PEMS	HER-2	Piezoelectric microcantilever sensors	(Loo et al., 2011)
Human serum	13–100 ng/mL in 30 min	Optical	–	HER2 extra-cellular domain	Opto-fluidic ring resonator (OFRR)	(Gohring et al., 2010)
Culture medium	–	Piezo-electric	Anticancer drug doxorubicin	Human breast cancer cells (MCF-7)	Magnetic silica composite (DOX-Fe_3O_4@SiO_2)	(Zhou et al., 2012)
serum	2810.25 m^2/kg at 8.704 pg/Hz	Piezo-electric	Streptavidin/biotin-based five-layer immunoassay	Mammoglobin (hMAM)	Surface acoustic wave (SAW) biosensor in complementary metal-oxide semiconductor (CMOS)	(Tigli et al., 2010)
Saliva	2.5–20 U/mL	Optical	Au/ZnO thin film	Carbohydrate antigen 15.3 (CA15-3)	Surface plasmon resonance (SPR)	(Liang et al., 2012)
Serum	26 cells/mL	Electro-chemical	Hydrazine and aptamer-conjugated gold nanoparticles (AuNPs)	SK-BR-3 breast cancer cells	Quantitative stripping voltammetry	(Zhu et al., 2012)
Serum	3–400 ng ml^{-1}	Optical	CEA antigen	Carcinoembryonic antigen (CEA)	Surface plasmon resonance (SPR)	(Altintas et al., 2011)
Cell lysate of total RNA	6 pmol	Electro-chemical	Capture probe	mir21	Pencil graphite electrode	(Kilic et al., 2012)

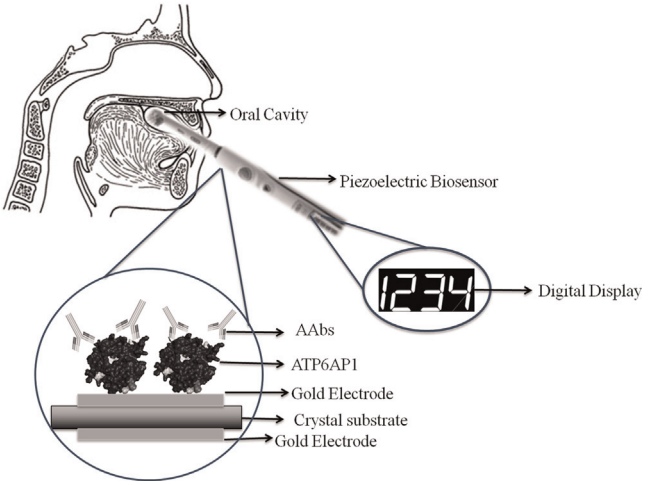

Fig. 1. Detection of breast cancer utilizing the salivary autoantibodies against ATP6AP1 through the biosensor. The hypothetical design of piezoelectric biosensor in the form of a toothbrush has been shown. The surface of the sensing interface has immobilized ATP6AP1, in which the quartz crystal is being sandwiched between gold electrodes.

7.4. Signal processing

Small circuit components for the removal of noise, and refinement and amplification of the signal can also be incorporated for the better functioning of the device.

In the construction of this biosensor, ATP6AP1 is proposed to be immobilized on the surface of a quartz crystal, which will act as transducer by converting biological signal i.e. the presence of autoantibodies in the saliva, into a change in electric signal. This electric signal then can be displayed on the screen as a digital signal (see Fig. 1).

8. Handy device for household's usage

In most of under developing countries especially Pakistan, where breast cancer is targeting every 9th women (Junaidi, 2012), regular checkups are not carried out in general population, especially women, either because of busy routines or Islamic ethical issues. This scenario is further worsened by the fact, that most of the women reach hospitals when it is too late. Early detection of cancer should be a priority because early detection ensures better survival chances. So, a diagnostic device, a handy version that can be easily used by everybody conveniently, will ensure maximum chances of survival by establishing the need for an individual to see a doctor when the threshold concentration of autoantibodies in saliva is crossed. Since the detection route is oral, hence, the instrument can be molded into a toothbrush form, based on a previously patented device, in which the bristles can be replaced by suction pumps that will suck the saliva up and let it run on the sensor inside (Kuo, 2002). This device may be coupled with microfluidic systems accordingly for regeneration and washing steps (Vagdama, 2004). The regeneration solution removes the analyte from the biocomponent to make the device reusable (Wijesuriya et al., 1994). For this purpose, a regeneration buffer needs to be designed that will remove the bound antibodies to make space for new ones for the next round of detection.

9. Limitations and concerns

A lot needs to be done before the idea can take a full form and shape. Therefore, some prospects may be explored before this biosensor can be made into a practical, hand-held device from a theoretical illustration. The literature review establishes its diagnostic potential by clearing the following aspects:

9.1. Do AAbs against ATP6AP1 appear in the saliva of breast cancer patients?

To date, it is not reported that AAbs against ATP6AP1 appear in saliva, however it has been well documented that these AAbs do exist in serum of breast cancer patients (Anderson et al., 2010). Literature review suggests that those AAbs belonging to class IgG present in serum, also appear in saliva. Most of IgG immunoglobulins in saliva are derived from serum (mainly via gingival crevices), whereas some proportion is locally produced (Brandtzaeg, 2007). AAbs for various immunological conditions are being detected in the oral secretions. For instance, AAbs against glutamic acid carboxylase in saliva of children having type 1 Diabetes have been detected (Markopoulos et al., 1997). Anti-mitochondrial autoantibodies in saliva and sera from patients with primary biliary cirrhosis have also been reported (Ikuno et al., 2001). p53 autoantibodies can be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection and screening of this tumor (Warnakulasuriya et al., 2000).

9.2. Do these AAbs against ATP6AP1 appear in the saliva of healthy people?

Normally, autoantibodies are not in seen in healthy people, (Markopoulos et al., 1997) and if detected, are present in very low concentrations. The level of these antibodies can be eliminated as background noise in the proposed detecting device (Ching et al., 2011; Tiberti et al., 2009).

9.3. Do these AAbs in saliva reflect their corresponding concentrations in serum?

Though titers of AAbs vary in serum and saliva (Ching et al., 2011) but this difference does not outdate saliva to be used as a detection medium (Tiberti et al., 2009).

9.4. How will the device mitigate false positive and false negative errors?

ATP6AP1 offers low sensitivity (30%) and high specificity (95%) for the early detection of breast cancer. Due to its low sensitivity, it is likely to generate false negative results, where the disease may go undetected. However as the cancer progresses the chances of detecting autoantibodies against breast cancer becomes more likely due to more changes in the tissue (Anderson et al., 2010).

Furthermore, ATP6AP1 is recognized as autoantigenic marker in cancers other than breast cancer i.e. melanoma (Nishisho et al., 2011) and ovarian cancer (L'Esperance et al., 2006). Hence, it is likely to raise false positive alarms due to autoimmune diseases or other types of cancers. For this reason, the detection by the device will have to be cross-checked with other techniques and further confirmed. The signal on the device can be thought of more as an alarm, and an alert for a breast cancer check-up, and will allow victims of breast cancer to approach the doctor at a right time.

9.5. Can ATP6AP1 be used an independent marker for the early detection of breast cancer?

According to the literature and inferences from the data posed, autoantibodies against ATP6AP1 can be ideally used for the purpose, once the presence and secretion of these moieties in saliva of breast cancer patients is further confirmed in prospective

studies. A patent suggests that utilizing ATP6AP1 as an independent biomarker can lead to up to 70% accurate diagnosis of breast cancer in stage I or II (Labaer et al., 2013). Although there is still need of conventional methods for confirmation of breast cancer but detection of autoantibodies against this biomarker will help to direct the patients to medical facilities before it's too late.

To enhance the performance of the device, 2 or 3 other autoantigen may be used in conjunction for early detection of breast cancer, as previously in a study (Anderson et al., 2010), which will enhance the specificity and sensitivity of device (Hodi et al., 2002).

9.6. How stable ATP6AP1 Is outside the cell, immobilized upon a quartz crystal?

ELISA kits of ATP6AP1 are commercially available which suggest that ATP6AP1 is stable enough to be immobilized on a sensing support like quartz crystal.

10. Conclusion

Current methods for breast cancer diagnosis require expensive equipment, rely on invasive procedures and often fail to detect cancer at an early stage. Literature review documents that autoantibodies against ATP6AP1 or other candidate autoantigens appear at early stage of the disease that could serve as biomarkers for early detection. Saliva is the non-invasive medium of detection that is becoming a method of choice. A biosensor based on Quartz crystal microbalance ideally detects and quantifies even low levels ($\sim$1 ng cm^{-2}) of antibodies and other chemical compounds. This review focuses on the efficacy of detection of autoantibodies against ATP6AP1 through Quartz crystal microbalance biosensor in saliva. A handy device based on biosensor technology, employing a non-invasive saliva sample, would serve to alert such women who are reluctant for regular check-ups due to emotional, conventional, or religious reasons, so that there may be a proper diagnosis and treatment regime while they have more chances for complete recovery. However, before the device can be made into a prototype, there is a need to address the concern of the secretion ATP6AP1 autoantibodies in the saliva in sufficient amounts, and to validate the specificity and sensitivity of disease detection.

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