Recent Advancements in Nano-Based Biosensor for Early Detection of Prostate Cancer

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ABSTRACT
Cancer is deadly disease, especially "Prostate cancer" ranks as third most common cancer among men (Damber &Aus, 2008) between the age group of 55-80 years. In India, every year 241,740 new prostate cancers are diagnosed and estimated deaths are 28,170 in 2012-2013. However early diagnosis of disease is not a simple process. The advancement in Technology and Science has introduced "Biosensors" which helps in early detection of tumours and reducing the mortality rate of men. These sensors are applied in various methods for diagnosis and treatment purpose. With the proper awareness about prostate cancer and its consequences the increased mortality rate of this deadly disease can be reduced up to an extent. In this review we are discussing about some of the recent advancements in the field of Biosensors used for Prostate cancer detection.

KEYWORDS: *Prostate Cancer, Biosensors, PSA, Sol-gel.*

I. INTRODUCTION

Venetian anatomist Niccolo Massa was the first person to describe prostate cancer in 1536 and illustrated by Flemish anatomist Andreas Vesalius in 1538, which was not identified until 1853. The 'Medieval Latin Prostate' and ' Medieval French Prostate' is the origin of word prostate. Prostate means formation of tumour in prostate gland. Prostate cancer is a slow growing disease and there were some aggressive cases also (ACS, 2010). Prostate cancer is the sixth leading cause for death in men even though there is advancement in science (Baade and Youlden, 2009). It is one of the most common cancers in U.S. In 2008, 186,000 new cases were found, among that 35,000 were diagnosed and 28,600 deaths were recorded (Scott *et al.*, 1975). The causes of disease are dietary pattern, lifestyle, genetic factor etc. The symptoms are observed in earlier stage and screening is done to prevent further more onset of cancer.

II. BIO SENSORS

Bio sensor device is used to detect the biological analyte such as proteins, nucleic acid, glucose etc. It converts the biological analyte in to electrical signal which may in turn helps for the quantitative and qualitative study. When a specific target molecule interacts with the biological component, a corresponding signal is produced in proportional to concentration of substance. (Pier Andrea Serra). Biosensors play a vital role in cancer detection as a "Bio markers" because of its advantages over other. The fast and accurate detection, monitoring and treatment of various problems make its worthier in the field of science. It is like a chip scaled devices placed or inserted in to human body for monitoring (Declan A. Healy *et al.)* Different types of biosensors are electrochemical, mass based and calorimetric biosensors (Brain Bohunicky and Shaker A mousa., 2010). It finds various applications in fields of environmental study for the detection of harmful bacteria, pesticides in air, water and food. In medical field, to monitor blood glucose levels in diabetics and pathogen detection.

In military, biosensors are used as counter Bioterrorism devices and wide variety of applications in the field of nanotechnology.

III. PROSTATE SPECIFIC ANTIGEN (PSA)

Prostate secretes the fluid substance called prostate, which are present in human blood and semen. The PSA concentration is measured by blood test. It is measured in Nanograms per millilitre of blood (ng/mL). Normal value of PSA is 4.0 ng/mL; above 4-10 it represents 25% of PCa and more than 10 represents 50% and above PCa.

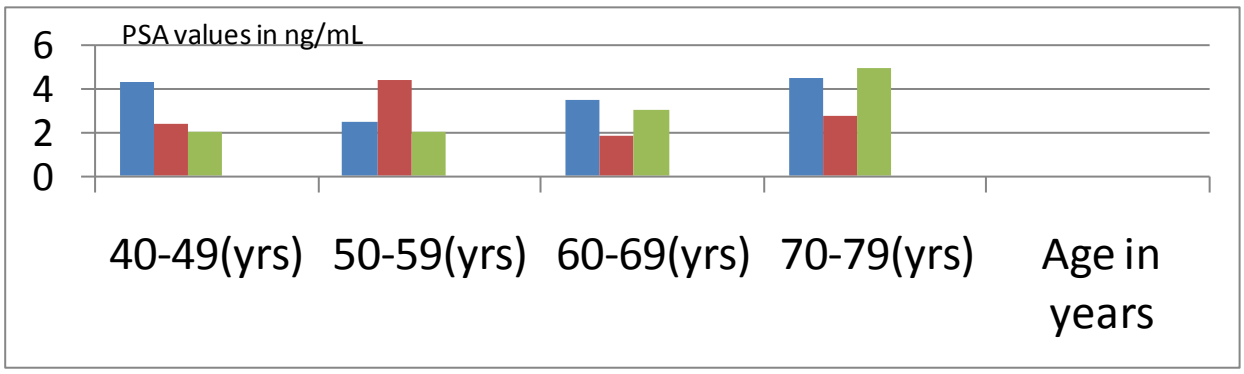

Fig: 1- PSA values according to ages in ng/mL

Proposed by Oesterling *et al ;* Dalkin *et al ;* De Antonl *et al.*
Factors that influence PSA levels in human serum are, it increases according to age, non cancerous enlargement and inflammation of Prostate gland.

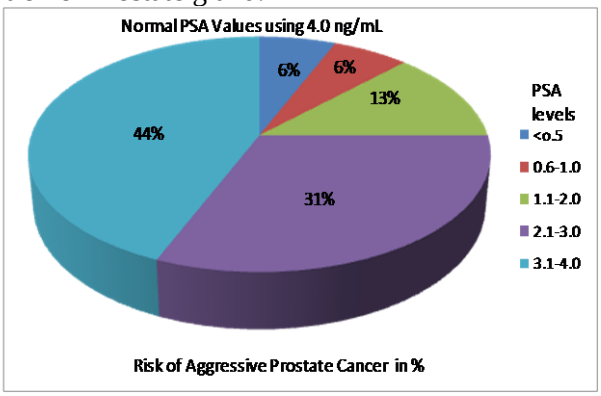

Fig 2: Normal PSA value using 4.0 scales

IV. ROLE OF PSA AS BIOMARKER

PSA(Prostate Specific Antigen) has a remarkable role in the history of oncology as the Tumour markers. From early 1970's to 2013 it plays a major role in treatment of prostate cancer. Fishman and Lerner developed an assay called acid Phosphatase produced by the prostate gland(prostatic acid phosphate [PAP]), hoping thus to increase the specificity of biochemical detection of prostatic disease. Prostate specific antigen (PSA) was initially isolated from prostate tissue extracts and can be used as a serum marker for prostate cancer detection by Chu *et al*. The same protein had earlier been purified from seminal fluid and called gamma-seminoprotein, E1, and P30.

S.No.	Various compounds and their year of invention	Reference
1.	Gamma semi protein from seminal plasma, 1971	Hara *et al.*
2.	Purification of the protein from seminal plasma, 1973	Li and Beling
3.	Purification of the protein from prostate tissue. 1979	Wang *et al.*
4.	Association of PSA and prostate cancer, 1981	Nadji *et al.*

5.	Function and characteristics of PSA, 1985	Lilija *et al.*
6.	Reference range of PSA, 1986	Myrtle
7.	Clinical study about effectiveness of PSA as tumour markers, 1987	Stamey
8.	Three legged stool application to detection of PCa, 1990	Cooner
9.	Concept of PSA velocity, 1992	Carter
10.	PSA density, 1992	Benson
11.	Utility of PSA alone in the detection of CAP, 1993	Brawer *et al. & Catalona et al.*
12.	Age specific reference ranges, 1993	Oesterling *et al.*,Palkin *et al.*
13.	Application of PSA to final pathologic stage, 1997	Partin

Table 1 History of PSA

V. TECHNIQUES APPLIED IN PSA BIOSENSOR

5.1 Ionic Liquid Carbon Nanotubes (ILCNT)

Prostate antigen based on ILCNT modified electrode is used as cancer Biomarker for prostate cancer detection due to its high sensitivity, which was proposed by (Abdollah Salimi *et al*., 2012). The Prostate Specific Antigen (PSA) is a reliable tumour marker for early diagnosis of prostate cancer (Panini *et al.*, 2008). Electrochemical immunoassay and sensors are used for clinical diagnosis for its variable properties (Wang *et al*.,2006, Okuno *et al*.,2007, Mantzila *et al*.,2008, Zhou *et al*., 2009, Du *et al*.,2010, Huang *et al*.,2010, Tung and Ren.,2010). Different materials like organically modified Sol gel, Titania sol gel and gold alumina derived sol gel (Tan *et al*., 2009), colloidal gold particles modified electrode (Wang *et al*., 2004), and self assembled monolayer of thiol and dendrimer (Namgung *et al*., 2009) and polymeric film (Dong *et al*., 2006)are used for clinical diagnosis. The ILCNT have shown potential application in the fabrication of electrochemical sensor and biosensor (Zhu *et al*., 2010). The steps followed in Diagnosis of disease involves preparation of prostate cancer cells and modified CNT's thionine with composite modified electrode and Fabrication of Immunosensor for PSA detection

After the steps followed in clinical diagnosis the sample or serums are analyzed for various determinations. First, ILCNT Immunosensor is characterized and amplification of bio catalyzed anti PSA is done. Then the optimizing conditions such as electrochemical response, reproducibility, regeneration of the Immuno-sensor and stability of PSA are categorized. At last the Human serum samples are used for detection of prostate cancer biopsies.

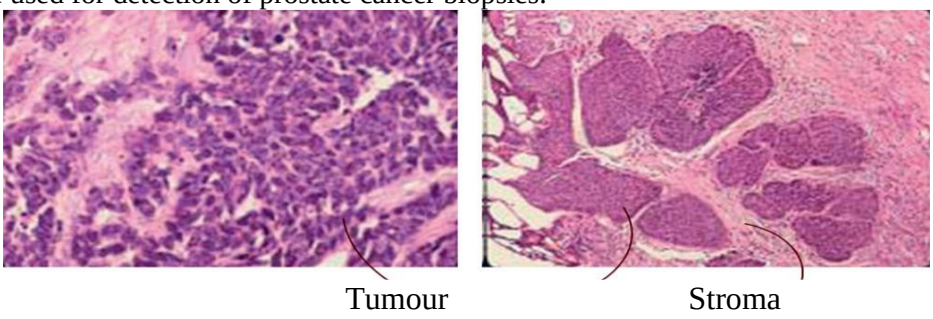

Fig 3. Micrograph of Prostate Cancer Biopsy

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5.2 Impedance based miniaturised biosensor

From early days, there were two approved screening methods for the detection of prostate cancer. They are Prostate Specific Antigen and Digital Rectal Examination blood test (T.Steuber *et al*., 2008). Later ELISA -Enzyme Linked Immuno Assay for PSA detection. It is based on Chromatography

technique. The new method that's introduced was called Miniatured Biosensor based on Impedance due to its Ultra sensitivity and fast detection of PSA (Ganna Chornokur *et al*., 2011). In this method EIS- (Electrochemical Impedance Spectroscopy) used as an identifying technique (A.J.Bard *et al*., 2003). This is type of electrochemical biosensor used due to its various properties and applications in Miniatured platforms. It is used to characterize surface modified electrodes for the investigation of electrochemical system and process (E.Barsoukov, J.R.Mac Donald., 2005). It is better than ELISA in sensitivity and time consumed is less for detection.

Methods followed in PSA detection: The PSA samples are collected and added with reagents and chemicals. Then it is stored in cancer center or in some clinical diagnosis. EIS is used to characterize the Anti PSA/EA to analyze PSA concentration then the measurements are carried out. Using standard photolithography techniques, the test chip are fabricated on an oxidized 4" silicon wafer (K.E.Sun *et al*., 2008) (S.K.Arya, *et al*., 2010). The chips were pre cleaned and analysed for monolayer preparation, formation and antibody immobilization. Testing with PSA solutions in PBS (Phosphate buffered saline) and Human plasma with Biosensor are the steps to analyze and it helps in early diagnosis of Prostate cancer.

5.3 Micro-fluidic Chips

For prostate specific antigen detection in biochemical sensing platform, the micro fluidic chip based on nano electrode array were used (Napat Triroj *et al*., 2010). These chips are made up of three electrode configuration which is embedded in Nanoelectrode array. Because of its high sensitivity and portability it's used for the development of point of care testing devices (POCT devices). Micro fluidic chip is used as a diagnostic tool, for manipulation and for bio molecular processes. Immunoassay method and biosensor complex are used for PCa detection.

5.4 Nano Particle Sensors

Rapid and sensitive detection of PSA is done by a Nanoparticle sensor label/ Immuno chromatographic electrochemical biosensor which are developed by (Ying-Ying *et al*., 2008). PCa in human serum were detected by Nanoparticle sensor. It consists of strips with the electrochemical detector for transducing signals. It is made up of Cd Se @ ZnS and used because of its distinguishable properties and is compared with detection of 0.02 ng m/L.

For PSA detection, Nanomaterials and nanostructures based biosensor are used. PSA diagnosis can also be done by Immunosensor which is made up of Enzyme, DNA, Nanoparticle, CNT (Healy *et al*., 2007). It is used as a sensitive diagnostic tool. This method is good for detection purpose. Like Diaminoheptane, Phosphate buffer saline, bovine serum albumin etc are the chemicals, and materials used to prepare the QD-anti PSA conjugates by QD conjugation Kit protocol are used for preparation of sensor. (Wu *et al*., 2007; Wang *et al*., 2008). The Analyzation of anti PSA antibodies on the surface of the test zone membrane, blocking of lateral flow membranes of the strip is done by sensor. It is also observed by anti PSA -QD conjugate on glass fiber pad preparation. It is detected by Lateral flow Immunoassay, Electrochemical Detection and Enzyme linked Immunosorbent assay. Determination by principle of PSA assay and optimization of experimental parameters are used for earlier detection of PCa and mortality rate is reduced.

5.5 PDA Molecule

Polydiacetylene (PDA) supramolecules is enhanced for sensitivity using hybrid stimulus and used for diagnosis of prostate cancer proposed by (Ilk young kwon *et al*., 2010). PDA vesicle chip is given with stimulus and primary response is noted by antigen-antibody reaction and secondary response is noted by mechanical pressure of PAb conjugated micro beads. Detection of cells, proteins, DNA for sensitive bio sensing based on PSA supramolecules is done by a PDA molecule (Ilk young kwon *et al*., 2010). The various forms of PSA, are either PSA is free or in complex with two major proteinase inhibitors, α 1 anti chymotrypsin (PSA - ACT, MW 90 k Da) and α 2 Macroglobulin (PSA-AMG) which is found in human serum(Armbruster, 1993; Lilja *et al*., 1991; Savage and waxman., 1990).

The major protease inhibitor occurring at a cone from 4.0 to 8.0 ng/mL is called ACT (Sarkar *et al*., 2002). Efficient measurement of ACT and f-PSA can be used to diagnose PCa. The ratio of 0.25 was defined as the cut off value for 90-95% of PCa (Catalona *et al*., 1998). For disease recognition, the value ranges are observed between 4.0-20ng/mL and it referred to as a "Diagnostic gray zone" (Kim

et al., 2009). Biosensor uses a Bio chromic property for various stimulus detection. PDA molecule is formed using materials like (10, 12 penta cosadiynoic acid (PCDA), BSA, Human IgG etc. The PDA vesicle is prepared by addition of interlinker and immobilization of PDA vesicles on amine coated glass. For immunoreactions and fluorescent analysis of the PSA-ACT complex the conjugation of PSA-ACT mAb on to PDA vesicles is performed and this preparation is performed by using PSA PAb-conjugated magnetic beads. The interpretation of results is done by optimization of PSA-ACT mAb and analysing of non specific binding on the PDA vesicles. The detection of low concentration of PSA-ACT complex is done by using the PDA vesicle sensors chip. Then it is followed by amplification of fluorescent signals using the PDA vesicle sensors chip. These PDA molecule interactions are helpful in early detection of PCa and simple a method to analyze.

5.6 Piezo Electric Crystal

The Analyzation of sensitive piezoelectric immunoassay of prostate specific antigen by cell based immobilization strategy technique which was proposed by (Yanjun ding *et al*., 2008). Yeast cell strategy is used in this method for immobilization of antibodies. The fabrication and coupling process of anti PSA immunoassay results in production of microbalance quartz crystal sensor. This crystal was modified with cysteamine to deposit yeast cells on which PSA are immobilized. A PSA serum values are in range of 5.0-604.0 ng m/L can be interpreted using this system (Yanjun ding *et al*., 2008). Bio films are used as a carriers using cell surface of micro organisms. These intrinsic systems are useful to immobilize heterologous proteins on cell surface of micro organisms, segregation of produced polypeptides, construction of microbial bio catalysts, whole cells adsorbents and live vaccines etc. (flickinger *et al*., 2007; Ueda and Tanaka, 2000).

The immobilization of enzymes and green fluorescent proteins, the surface yeast can be produced (Ueda and Tanaka, 2000). They were termed as "arming yeasts" of biocatalysts and functional proteins. The yeast technique is applied to various fields such as biotechnology, environmental science and health care (Junter and Jourenne 2004; Peinado *et al*., 2006). The reaction between yeast cells and substrate is mainly based on Physico-chemical interactions (Smit *et al*., 1992; Hui *et al*., 2005; Bowen and Cooke, 1989) and specific biochemical interactions (Boonaert *et al*., 1999; Bowen *et al*., 2001).

Crystal is formed using Quartz crystal microbalances (QCM, AT-cut, 9MHz, gold electrode) and materials like Yeast Saccromyces cerevisiae, HSA, GLU etc. The immobilization of yeast cell binding is carried out by GLU cross linking procedure. The monolayer of cysteamine formation and unbound aldehyde groups on the surface of crystals is observed for QCM measurement (Frequency is measured). Realization of QCM crystal is by yeast cell based binding procedure. The optimization of assay medium, comparison of immunoactivity of antibody immobilization. Analytical performance characteristics, regeneration of sensor are the steps followed in sample analysis. Yeast cell is simple eukaryotic and used in various fields of science. This method is simple, cost effective and proposed for easy identification of PCa.

5.7 Surface Plasma Resonance (SPR)

Surface plasma resonance technique is proposed due to its high sensitivity and specificity enhancements in detection of prostate specific antigen α1- antichymotrypsin (Cuong cao *et al*., 2005). A Biochip is fabricated with various materials to improve the sensitivity, specificity, to reduce the non specific binding and steric hindrance effect of immunoassay. It uses a Sandwich strategy for detection of PCa. The compound, α 33-k Da serin protease can be used to detect PCa in early stages. PSA is recognized as the premier tumour marker of PCa (Armbruster, 1993; Savage and Waxman; 1998). The conventional assays for PSA detection involves a preparation of monoclonal or polyclonal antibody of PSA tagged with an enzyme or a flurophore or a radioactive isotope (Armbruster, 1993).

SPR overcome the disadvantages of earlier methods. The changes in mass concentration at a bio specific interface increases affinity of optical sensor (Cuong coa *et al*., 2005). The detection limit range of PSA is approximately 1-10nM for 20kDa molecule and is even higher for smaller molecule (Gomes and Andreu, 2002). The detection limit range or sensitivity of SPR are improved by sandwiching immunoassays using Au or Ag Nanoparticle or (Gu *et al*.,1998) or ,latex spheres(Homola,2003), or Liposome's(Wink *et al*.,1998), or streptavidin biotinlyated antibody complexes (pei *et al*.,2001) or an enzyme precipitation strategy(Kim *et al*.,2005).Different self

assembled monolayer (SAM) surfaces have been introduced to improve the sensitivity of SPR biosensors. Oligo Ethylene Glycol(OEG) is recognized, for its ability to prevent non specific adsorption of proteins(Chapman et al.,2000; Benesch *et al*.,2001; Frederix *et al*.,2004; Chen *et al*.,2005) Biotin streptavidin chemistry are routinely used as a biomaterial immobilization device(Green *et al*., 1971; orth *et al*.,2003).

Surface Plasma resonance was designed by using Instrumentation of (BIA core 2000 apparatus) and materials like (PSA-ACT, PSA ACT mAb, Goat PSA etc.) are used for the formation of Oligo ethylene glycol layer on the bare gold surface. The Biotinylation of the carboxyl terminated groups of the SAM's and Biotinylation of PSA-ACT mAb occurs. The immobilization of surface and biotinlyated PSA-ACT mAb are used for detection of PSA-ACT complex by Immuno reaction. The PSA-ACT complex signal amplification is performed using intact PSA PAb. The analyzation of Plasma Resonance formation is observed by immobilization of SA and Biotinlyated PSA-ACT mAb on the OEG SAM surfaces. The investigation of non specific binding on the 1:9 surfaces, immune reaction between PSA-ACT complex, biotinlyated PSA-ACT mAb and enhancement assay is performed to analyze the immune reaction of HBS buffer and in Human serum. On the whole, Surface plasma resonance method is easy and better than other techniques and it is effective.

5.8 Localized Surface Plasma Resonance Sensor (LSPR)

Real time detection of PSA is based on label free immunoassay of interferon - γ and Fiber Optic Localized Surface Plasma Resonance Sensor (FO LSPR) technique is published by (Hyeon - Ho jeong *et al*., 2012). Fabrication of FO LSPR is done by using spherical gold Nanoparticle (AuNPs) on a flattened end face of the optical fiber. It follows Turkevich method and used for detection of PSA. Fabricated FO LSPR is used due to its various characteristics and advantages over other. LSPR is the resonance phenomenon of the free electron waves in Metal Nanoparticle (MNPs) and the rough surface of manometer scale in the UV -visible range (Homola, 2003; Homola *et al*., 1999; Mulvaney, 1996; Underwood and Mulvaney, 1994).

LSPR has the various advantages like Label free detection, low sample volume, real time detection and simple optical set up detection (Jeong *et al*., 2011; Jeong and Lee, 2011 and Lee *et al*., 2010). It is generally based on MNPs on an optical fiber. Turkevich method is used because of its fast fabrication process, simple and size of the Nanoparticle can be controlled (Kimling *et al*., 2006; Lee *et al*., 2010). Nowadays MNPs is fabricated using Micro Elector Mechanical Systems (MEMS) technology (Acimovic *et al*., 2009; Barbillon *et al*., 2007; Smythe *et al*., 2007). It is used to detect the interactions of bio molecules such as antigen-antibody reactions etc. (Jeong *et al*., 2011; Sai *et al*., 2009; Shao *et al*., 2010; Stybayeva *et al*. ,2010; Tuleuvo *et al*.,2010). It is used as a Portable sensor for chemical and biological detection. Because it has advantage of lossless of signal delivery, remote sensing, low cost and simple optical set up(Jeong *et al*., 2011; Lee *et al*., 2010; Sharma *et al*., 2007). Detection of PSA can be applied in vivo systems also by combining it with an endoscope or laproscope, because of its miniaturized flattened tip and fast sensitive detection (Hyeon- Ho jeong *et al*., 2012).

Fabrications of FO LSPR sensor by the label free immunoassay technique and optical measurement of system is used for the detection of antigen-antibody reaction of PSA. Results are obtained by measuring the sensing characteristics of LSPR. This method is used because of its various advantages.

VI. CONCLUSION AND FUTURE ASPECTS

In conclusion based on recent research, "it is uncertain whether the benefits associated with PSA testing for prostate cancer screening are worth the harms associated with screening and subsequent unnecessary treatment."(Wilt TJ *et al*., 2008). The treatment of PCa can be done by Radiation therapy, Brachy therapy, Cryosurgery, High intensity focused ultrasound technique and Oral chemotherapeutic drugs (temozolomide) etc. For medical imaging Ultrasound and Magnetic Resonance Imaging is done for PCa detection.

In 2011, PCa is one of the second most frequently diagnosed cancers, sixth leading cause of death in males (Cooper berg MR *et al*., 2006). So, there must be a method to detect PCa at earlier stage easily and 55% of men report discomfort during prostate biopsy (Natarajan *et al*., 2011).

REFERENCES

[1] "ACS What Is Prostate Cancer?" American Cancer Society Information and Resources for CancerBreast, Colon, Prostate, Lung and Other Forms Web 15 June 2010 Retrieved 9 August 2010
[2] Abdollah Salimi, BegardKavosi , FardenFathi and RahmanHallaj 2012
[3] Abramov MA, Dehaen W and Maes G 2004 Reduced non-specific adsorption on covalently immobilized protein surfaces using poly (ethylene oxide) containing blocking agents *J. Biochem. Biophys. Methods* 58 67–74
[4] Acimovic SS, Kreuzer MP, Gonza´ lez MU and Quidant R 2009 *ACSNano* 3(5) 1231–1237
[5] Armbruster DA 1993 *Clin Chem* 39 181–195
[6] Arya SK, Solanki PR, Singh RP, Pandey MK, Datta M and Malhotra BD 2006 Application of octadecanethiol self-assembled monolayer to cholesterol biosensor based on surface plasmon resonance technique *Talanta* vol. 69 no 4 pp 918–926
[7] Baade PD, Youlden DR and Krnjacki 1971 feb 2009 LJ International epidemiology of prostate cancer geographical distribution and secular trends Molecular nutrition & food research 53 (2) 171–84 PMID 19101947
[8] Barbillon G, Bijeon JL, Plain J, de la Chapelle ML, Adam PM and Royer P 2007 *Surface Science* 601 (21) 5057–5061
[9] Bard AJ and Faulkner LR 1980 *Electrochemical Methods Fundamentals and Application*
[10] Barsoukov E, and MacDonald JR 2005 *Impedance Spectroscopy: Theory, Experiment and Applications* ch 1 *Wiley- Interscience* New Jersey NJ USA 2nd edition
[11] Benesch J, Svedhem S, Svensson SCT, Valiokas R, Liedberg B and Tengvall P 2001 Protein adsorption to oligo(ethylene glycol) self-assembled monolayers experiments with fibrinogen, heparinized plasma and serum *J. Biol. Sci*. 12 (6) 581–597
[12] Benson MC, Whang IS, Olsson CA, *et al*., 1992 The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specificantigen. *J Urol* 147 817–821
[13] Boonaert CJP, Dupont-Gillain CC, Dengis PB, Dufrene YF and Rouxhet PG Cell Separation Flocculation In Flickinger MC Drew SW (Eds.) 1999 Encylopedia of Bioprocess Technology: Fermentation *Biocatalysis and Bioseparation* John Wiley & Sons New York pp 531–548
[14] Bowen WR and Cooke RJ 1989 *Biotechnol. Bioeng.* 33 706–715
[15] Bowen WR, Lovitt RW and Wright CJ 2001 *J. Colloid. Interface Sci.* 237 54–61
[16] Brawer MK, Chetner MP, Beatie J,Buchner DM, Vessella RL and Lange PH 1992 Screening for prostatic carcinoma with PSA. *J Urol* 147 841–845
[17] Carter HB, Morrell CH, Pearson JD *et al*., 1992 Estimation of prostatic growth using serial prostate-specific antigen measurements in men with and without prostate disease *Cancer Res* 52 3323–3328
[18] Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, Richie JP, deKernion JB, Walsh PC, Scardino PT, Lange PH, Subong ENP, Parson RE, Gasior GH, Loveland KG, Southwick PC 1998 and *JAMA* 279 1542–1547
[19] Catalona WJ, Smith DS, Ratliff TL et *al.*, 1991 Measurement of PSA in serum as a screening test for prostate cancer *NEJM* 324 1156–1161
[20] Chapman RG, Ostuni E, Yan L and Whitesides GM 2000 Preparation of mixed self-assembled monolayers (SAMs) that resist adsorption of proteins using the reaction of amines with a SAM that presents interchain carboxylic anhydride groups Langmuir 16 6927–6936
[21] Christensson A, Laurell CB, Lilja H 1990 Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors *Eur J Biochem* 194 755–763
[22] Cooner WH, Mosley BR, Rutherford CL *et al.*, 1990 Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. *J Urol* 143 1146–1154
[23] Cooperberg MR, Broering JM, Carroll PR Risk Assessment for Prostate Cancer Metastasis and Mortality at the Time of Diagnosis Natl J june 2009 *Cancer Inst* 101 (12) 878–87 doi 10 1093/jnci/djp122 PMC 2697208 PMID 19509351
[24] Cuong Caoa, Jun Pyo Kima, Byung Woo Kima, Heeyeop Chaea, Hyun C Yoon, Sang Sik Yang and Sang Jun Sima 2005
[25] Dalkin BL, Ahmann FR, Kopp JB 1993 Prostate specific antigen levels in men older than 50 years without clinical evidence of prostatic carcinoma. *J Urol* 150 1837–1839
[26] Damber JE and Aus G 2008 *Lancet* 371 1710–1721.
[27] Declan Healy A, Conor Hayes J, Paul Leonard, Louise McKenna and Richard O'Kennedy in Biosensor developments: application to prostate-specific antigen detection
[28] Dong H, Li CM, Chen W, Zhou Q, Zeng Z.X and Luong, JHT 2006 *Analytical Chemistry* 78 7424–7431

[29] Du D, Zou Z, Shin Y, Wang J, Wu H, Engelhard MH, Liu J, Aksay IA and Lin Y 2010 *Analytical Chemistry* 82 2989–2995
[30] Du D, Zou Z, Shin Y, Wang J, Wu H, Engelhard MH, Liu J, Aksay IA and Lin Y 2010 *Analytical Chemistry* 82 2989–2995
[31] Fishman WH and Lerner FA 1953 A method for estimating serum acid phosphatase of prostatic origin *J Biol Chem* 200 89
[32] Flickinger MC, Schottel JL, Bond DR and Aksan A 2007 *Biotechnol. Prog.* 23 2–17
[33] Frederix F, Bonroy K, Reekmans G, Laureyn W, Campitelli A,Ganna Chornokur, Sunil K Arya, Catherine Phelan, Richard Tanner and Shekhar Bhansali 2011
[34] Gomes P and Andreu D 2002 Direct kinetic assay of interactions between small peptides and immobilized antibodies using a surface plasmon resonance biosensor *J. Immunol Methods* 259 217–230
[35] Green NM, Konieczny L, Toms EJ and Valentine R.C 1971 The use of bifunctional biotinyl compounds to determine the arrangement of subunits in avidin *Biochem. J.* 125 781–791
[36] Gu JH, Lu H, Chen YW, Liu LY, Wang P, Ma JM and Lu ZH 1998 Enhancement of the sensitivity of surface plasmon resonance biosensor with colloidal gold labeling technique *Supramol. Sci.* 5 695–698
[37] Hara M, Innore T, Fukulyama *et al.,* 1971 Some physicochemical characteristics of gamma-seminoprotein, an antigenic component specific for human seminal plasma Nippon Hoigaku Zasshi 25 322
[38] Hara M, Koyanagi Y, Inogue T et *al.,* 1971 Some physicochemical characteristics of γ-seminoprotein an antigenic component specific for human seminal plasma Jpn *J Legal Med 25 322*
[39] Healy DA, Hayes CJ, Leonard P, McKenna L and O'Kennedy R 2007 Trends *Biotechnol* 25 125–131
[40] Homola J, *Analytical and Bioanalytical Chemistry* 377 (3) 528–539 J,Yee SS and Gauglitz G 1999 *Sensors and Actuators B Chemical* 54 (1–2) 3–15
[41] Huang CJ, Chiu, PH, Wang YH, Chen WR and Meen TH 2006 *Journal of the Electrochemical Society* 153 (8), D129–D133
[42] Huang KJ, Niu DJ, Xie WZ and Wang W 2010 *Analytica Chimica Acta* 659 102–108
[43] Huang L, Reekmans G, Saerens D, Friedt JM, Frederix F, Francis L, Muylder mans S, Campitelli A and Hoof CV *Biosensors and Bioelectronics* 21 (3) 483–490
[44] Huang X, Neretina S and El-Sayed MA 2003 2005 2009 *Advanced Materials* 21 (48) 4880–4910
[45] Hui C, Chew KH, Poenar DP, Zhou XD, Lee AC, Limc TM and Tan SN 2005 *Anal.Chim. Acta* 554 52–59
[46] Hyeon-Ho Jeong, Norov Erdene, Jae-HyoungPark, Dae-HongJeong, Ho-Young Lee and Seung-KiLee 2012
[47] Il Kyoung Kwon, Jun Pyo Kim and Sang Jun Sim 2010
[48] Jeong GH, Park JK, Lee KK, Jang JH, Lee CH, Kang HB, Yang CW and Suh SJ *Microelectronic Engineering* 87 (1) 51–55
[49] Jeong HH and Lee SK, 2010 *Journal of Sensor Science and Technology* 20 (2) 107–113
[50] Jeong HH, Erdene N, Lee SK, Jeong DH and Park JH 2011 *Optical Engineering* 50 (12) 124405–124408
[51] Junter GA and Jouenne T 2004 *Biotechnol. Adv.* 22 633–658
[52] Kim MG, Shin YB, Jung JM, Ro HS and Chung BH 2005 Enhanced sensitivity of surface plasmon resonance (SPR) immuno assays using a peroxidase–catalyzed precipitation reaction and its application to a protein microarray *J. Immunol. Methods* 297 125–132
[53] Kim YH, Kim JP,Han SJ and Sim SJ 2009 *Sens. Actuators B Chem* 139 471–475
[54] Kuriyama M, Wang MC, Papsidero LD, Killian CS, Shimano T, Valenzuela L, Nishiura T, Murphy GP, Chu TM, 1980 Quantitation of prostate-specific antigen in serum by a sensitive enzyme immunoassay *Cancer Res* 40 4658–4662
[55] Lee SW, Kang CD, Yang DH, Lee JS, Kim JM, Ahn DJ and Sim SJ 2007 *Adv*
[56] Li TS, Beling CG 1973 Isolation and characterization of two specific antigens of human seminal plasma. *Fertil Steril* 24 134–144
[57] Lilja H 1985 A kallikrein-like serine protease in prostatic fluid cleaves the predominant seminal vesicle protein *J Clin Invest* 76 1899–1903
[58] Lilja H, Christensson A, Dahlen U, Matikainen MT, Nilsson O, Pettersson and Lovgren T 1991 *Clin. Chem.* 37 1618–1625
[59] Mantzila AG, Maipa V and Prodromidis MI 2008 *Analytical Chemistry* 80 1169–1175
[60] Mulvaney P and Langmuir 1996 12 (3) 788–800
[61] Nadji M, Tabei SZ, Castro A, Chu TM, Murphy GP, Wang MC *et al.,* 1981 Prostatic specific antigen An immunohistologic marker for prostatic neoplasms. *Cancer* 1229–1232

[62] Natarajan S, Marks LS, Margolis DJ, Huang J, Macairan ML, Lieu P, Fenster may 2011 Clinical application of a 3D ultrasound-guided prostate biopsy system *Urol Oncol 29 (3 Suppl)* 334–42 doi 10 1016/j.urolonc.2011.02.014. PMID 21555104
[63] Oesterling JE, Jacobsen SJ, Chute CG, *et al*., 1993 Serum prostate-specific antigen in a community-based population of healthy men *JAMA* 270 860–864
[64] Okuno J, Maehashi K, Kerman K, Takamura Y, Matsumoto K and Tamiya E 2007 *Biosensors & Bioelectronics* 22 2377–2381
[65] Orth RN, Clark TG and Craighead HG 2003 Avidin–biotin micropatterning methods for biosensor applications *Biomed. Microdevices* 5 29–34
[66] Panini NV, Messina GA, Salinas E,Fernandez H and Raba J 2008 *Biosens and Bioelectron* 23 1145–1151
[67] Partin AW, Subong ENP, Walsh PC, *et al*. 1997 Combination of prostate specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer: A multi-institutional update. *JAMA* 377 1445–1451
[68] Peinado RA, Moreno JJ, Villalba JM, Gonzˇıalez-Reyes J.A, Ortega JM and Maurici JC 2006 *Enzyme Microb. Technol.* 40 79–84
[69] Sam Lister Urine test could speed treatment of prostate cancer London The Sunday Times Retrieved 9 August 2010 February 11 2009
[70] Sarkar P, Pal PS, Ghosh D, Setford SJ and Tothill IE 2002 *Int. J. Pharm.* 238 1–9
[71] Savage P, Waxman J and Eur J 1996 *Cancer* 32A 1097–1099
[72] Scott WW, Johnson DE, Schmidt JE, Gibbons RP, Prout GR, Joiner JR, Saroff J, Murphy GP Dec 1975 Chemotherapy of advanced prostatic carcinoma with cyclophosphamide or 5-fluorouracil results of first national randomized study *The Journal of Urology* 114 (6) 909–11. PMID 1104900
[73] Sensabaugh GF 1978 Isolation and characterization of a semen-specific protein from human seminal plasma: A potential new marker for semen identification. *J Forensic Sci* 23 106–115
[74] Shao Y, Xu S, Zheng X, Wang Y and Xu W 2010 *Sensors* 10 (4) 3585–3596
[75] Sharma AK, Jha R and Gupta BD IEEE Sensors Journal 7 (8) 1118–1129,2007.
[76] Smit G, Straver MH, Lugtenberg BJJ and Kijne JW 1992 *Appl. Environ. Microbiol.* 58 3709–3714
[77] Smythe EJ, Cubukcu E and Capasso F 2007 *Optics Express* 15 (12) 7439–7447
[78] Steuber T, Brien MFO, and Lilja H 2008 Serum for prostate cancer: a rational approach to the literature *European Urology* vol. 54, no. 1 pp 31–40
[79] Stybayeva G, Kairova M, Ramanculov E, Simonian AL and Revzin A 2010 Colloids and Surfaces *B: Biointerfaces* 80 (2) 251–255, 2010.
[80] Sun KE, Ramgir N and Bhansali S 2008 An immunoelectrochemical sensor for salivary cortisol measurement *Sensor and Actuators B* vol 133 no 2 pp 533–537
[81] Tan F, Yan F and Ju H 2006 *Electrochemistry Communications* 8 1835–1839
[82] Tang D and Ren J 2010 *Analytical Chemistry* 82 1527–1534
[83] Triroj N, Lapierre-Devlin MA, Kelley SO and Beresford R 2006 *IEEE Sens J* 6 1395–1402
[84] Tuleuova N, Jones CN, Yan J, Ramanculov E, Yokobayashi Y and Revzin A 2010 *Analytical Chemistry* 82 (5) 1851–1857
[85] Ueda M and Tanaka A 2000 *Biotechnol. Adv.* 18 121–140
[86] Underwood S, Mulvaney P and Langmuir 10 (10) 3427–3430 Zhou J, Ralston J, Sedev R and Beattie DA 2009 1994 Journal *of Colloid and Interface Science* 331 (2) 251–262
[87] Wang MC, Papsidero LD, Kuriyama M, Valenzuela LA, Murphy GP, Chu TM 1981 Prostate antigen A new potential marker for prostatic cancer. *Prostate* 2 89–96
[88] Wang MC, Valenzuela LA, Murphy GP *et al.,1979* Purification of a human prostate specific antigen *Invest Urol* 17 159
[89] Wang Y, Liu N, Ning B, Liu M, Lv Z, Sun Z, Peng Y, Chen C *et al 2012 Biosensor and Bioelectronics* 34 44–50
[90] Wilt TJ, MacDonald R, Hagerty K, Schellhammer P and Kramer BS 2000 Five-alpha-reductase Inhibitors for prostate cancer prevention Cochrane Database Syst Rev (2) CD007091 Doi 101002/14651858 CD007091 PMID 18425978
[91] Wink T, Zuilen SJ, Bult A and Bennekom WP 1992 Liposome-mediated enhancement of the sensitivity in immunoassays of proteins and peptides in surface plasmon resonance spectrometry *Anal. Chem.* 70 827–832
[92] Wu H, Liu G, Wang J and Lin Y 2007 *Electrochem. Commun* 9 1573–1577
[93] Yanjun Dinga, Haixia Lua, Guorong Shib, Jia Liua, Guoli Shena and Ruqin Yua 2008
[94] Ying-Ying Lin, Jun Wang, Guodong Liu , Hong Wu CM , Wai , Yuehe Lin 2008
[95] Zhou Y, Yu R, Yuan R, Chai Y and Hong C 2000 *Journal of Electroanalytical Chemistry* 628 90–96
[96] Zhu Z, Qu L, Li X, Zeng Y, Sun W and Huang X 2010 *Electrochimica Acta* 55 5959–5965