Diagnosis of prostate cancer via nanotechnological approach

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Abstract: Prostate cancer is one of the leading causes of cancer-related deaths among the Caucasian adult males in Europe and the USA. Currently available diagnostic strategies for patients with prostate cancer are invasive and unpleasant and have poor accuracy. Many patients have been overly or underly treated resulting in a controversy regarding the reliability of current conventional diagnostic approaches. This review discusses the state-of-the-art research in the development of novel noninvasive prostate cancer diagnostics using nanotechnology coupled with suggested diagnostic strategies for their clinical implication.

Keywords: bioassay, nanomaterial, nanodevice, PSA, non-PSA biomarker, bodily fluid

Introduction

As the name implies, prostate cancer originates from a gland in the male reproductive system found near the bladder. It is one of the leading causes of cancer-related deaths among Caucasian males in the USA, and it is the most commonly diagnosed form of cancer in both Europe and the USA.1,2 A statistical report predicts new cases and deaths in the USA to be 220,800 and 27,540, respectively, for 2015. As shown in Figure 1, the disease can advance to a more aggressive malignant form, which can be stratified into four discrete stages: I, II, III, and IV. Upon biopsy examination, the stages are determined and stratified according to Gleason’s score method.3,4 Stages I and II are localized in the prostate gland, whereas stages III and IV exhibit regional spread to the nearby bladder and distant spread to other organs, such as liver and bone, which are far away from the prostate gland.5–11 The prevalence of local, regional, and distant forms of the cancer is known to be 81%, 12%, and 4%, respectively. No apparent symptoms appear in stage I, but they start to show and become apparent as the disease progresses. Patients diagnosed with prostate cancer at stage I, II, or III have a high 5-year survival rate, but patients with stage IV cancer have a low 5-year survival rate of <27%, highlighting the importance of early detection.

Currently available clinical diagnostic methods for prostate cancer include biochemical assay,12–20 biopsy,21–27 digital rectal examination (DRE),28–32 and transrectal ultrasonography33–39 as described in Figure 2. Among these methods, the biochemical assay is used for initial screening. The biochemical assay measures serum – a term meaning the processed medium from whole blood – prostate-specific antigen (PSA) level where a concentration >4 ng/mL is considered to indicate a risk of prostate cancer.12–20 Since approval by the US Food and Drug Administration (FDA) 25 years ago, it has been used as the gold standard for the initial screening of the disease.

The patients with >4 ng/mL PSA level undergo further DRE. DRE is performed in order to inspect the prostate gland condition by examining its texture and size.
Combined with a PSA screening result, the decision is made whether to do a biopsy for further examination. However, DRE is not useful for the early detection of prostate tumor because of a possibility that a tumor could originate from the ventral or other untouchable sides of the gland. In addition to the poor sensitivity of the examination, it is an unpleasant procedure for a patient to undergo.

After DRE patients undergo transrectal ultrasonography. It can offer a visualization of the gland for examination and can be used to guide immediate, subsequent biopsies. Upon detecting a suspicious portion of the gland, specimens are collected. Typically, 12 specimens are collected and evaluated according to the Gleason scoring system, and the most reliable, accurate diagnosis is finally made. Patients may undergo radical prostatectomy or hormone therapy depending on the extent of malignancy. The major drawback of a biopsy is the possibility of a potential infection caused by microbes that have migrated from the rectum which can cause inflammation in the diseased gland.

In addition to the traditional diagnostic methods, a bone scan is also carried out to scan the whole body for the presence of metastatic prostate cancer. ProstaScint- scan, positron emission tomography (PET) scanning, and computer-aided tomography (CT) with PET have all been used for prostate cancer detection. Prostate-specific membrane antigen (PSMA) has been selected as a target in the detection of prostate cancers with these techniques.

A notable molecular imaging technology is 68Ga-PSMA PET/CT, which targets PSMA as the biomarker. The FDA has recently approved a clinical trial of this technology. It is the first time in the history of the development of prostate cancer diagnosis, prognosis, or monitoring, where a diagnostic tool is used to target PSMA. This technique uses an agent that is a monoclonal antibody to detect the intracellular domain of PSMA. It is possible that this agent may only capture the dead cells of the prostate cancer cells.
Moreover, an earlier diagnosis also demands a more accurate efficacy of subsequent therapies for the diagnosed patients. Not only minimize the complications but also maximize the accurate diagnosis even at the biochemical assay level will of the methods and accurately diagnose prostate cancer. An novel diagnostic strategy that will eliminate the drawbacks to an appropriate therapy. Thus, it is inevitable to develop a tribute to bringing the best clinical decision to match a patient in Figure 2. Improvement of the diagnosis accuracy will con the drawbacks of the biochemical assay and DRE described inaccuracy of conventional, initial screening is largely due to difficult, and remains a persistent clinical problem.

Screening is currently being challenged. Inaccurate diagnoses lead to overdiagnosis or undertreatment, which exacerbates the physiological state of the tissue, makes treatment more uncertain. Its poor sensitivity and specificity has led to false-negative (15%) and false-positive (66%) diagnoses, which leads to a loss of confidence in the biomarker as an effective tool for initial screening. As a consequence, almost two-thirds of prostatic biopsies made annually in the USA and Europe are unnecessary.

To circumvent the aforementioned procedures, an accurate biochemical assay is desperately needed. However, the accuracy and preciseness of the prostate cancer initial screening is currently being challenged. Inaccurate diagnoses lead to overdiagnosis or undertreatment, which exacerbates the physiological state of the tissue, makes treatment more difficult, and remains a persistent clinical problem. The inaccuracy of conventional, initial screening is largely due to the drawbacks of the biochemical assay and DRE described in Figure 2. Improvement of the diagnosis accuracy will contribute to bringing the best clinical decision to match a patient to an appropriate therapy. Thus, it is inevitable to develop a novel diagnostic strategy that will eliminate the drawbacks of the methods and accurately diagnose prostate cancer. An accurate diagnosis even at the biochemical assay level will not only minimize the complications but also maximize the efficacy of subsequent therapies for the diagnosed patients. Moreover, an earlier diagnosis also demands a more accurate probe that requires highly sensitive and specific sensors to detect highly reliable biomarkers. Thus, just like for any other diseases, an accurate diagnosis is very important for delivering the appropriate treatment to the right patient. In this regard, nanotechnology with a new biomarker for prostate cancer has been chosen as a promising tool for future noninvasive diagnosis of prostate cancer.

Nanotechnology has been employed in the development of various biomedical applications such as drug development. On the other hand, nanotechnology is also being employed for diagnostic development.

The rationale for the employment of diagnostics development is to achieve a higher accuracy of diagnosis. Improvements in the sensitivity and specificity of nanotechnology are warranted and promising. Recent studies have demonstrated such improvements and suggested it as a promising tool for next-generation diagnostics, because such improvements could contribute to making a more accurate diagnosis.

In addition to employing nanotechnology, an accurate initial screening also requires a highly reliable prostate cancer biomarker. Since biomarkers reflect the physiological state of tissue from which they are secreted, it is best to use one that is both tissue and cancer specific. PSA itself is only

<table>
<thead>
<tr>
<th>Current challenges for new diagnosis</th>
<th>Application of nanotechnology as the solution</th>
</tr>
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<tbody>
<tr>
<td>• High percentage of</td>
<td>• Noninvasive</td>
</tr>
<tr>
<td>• False positive (~67%)</td>
<td>• Patient friendly</td>
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<tr>
<td>• False negative (~15%)</td>
<td>• Highly sensitive</td>
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<tr>
<td>• The main driver leading to</td>
<td>• Accurate</td>
</tr>
<tr>
<td>unnecessary biopsy</td>
<td>• Safe</td>
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<tr>
<td>• Unpleasant procedure</td>
<td></td>
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<td>• Subjectivity</td>
<td></td>
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<td>• Inter- and intrapersonal</td>
<td></td>
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<tr>
<td>variation of examiners</td>
<td></td>
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<tr>
<td>• Biased diagnosis</td>
<td></td>
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<tr>
<td>• Not patient-friendly method</td>
<td></td>
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<tr>
<td>• Exhausting procedure</td>
<td></td>
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<tr>
<td>• Unwanted side effects</td>
<td></td>
</tr>
<tr>
<td>• Infection caused by biopsy</td>
<td></td>
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<tr>
<td>• Erectile dysfunction</td>
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</table>

Figure 2 Current conventional approach of prostate cancer diagnosis.

Notes: Current conventional diagnostic methodologies for the patients with prostate cancer hold several drawbacks. Patients with PSA levels 4–10 ng/mL are subjected to further examinations. The assay gives a significant number of false positives, which raises a question of its reliability. Concurrently, DRE is performed in an initial screening for prostate cancer but introduces subjectivity to the examination. The last method is TRUS, which takes a visual image of the tumor tissue in the gland, and, in most cases, biopsies are performed with a risk of potential infections from the rectum. Due to the drawbacks of current diagnosis, a novel, noninvasive, effective, initial screening prior to diagnosis is in high demand with excellent sensitivity and accuracy.

Abbreviations: DRE, digital rectal examination; PSA, prostate-specific antigen; TRUS, transrectal ultrasonography.
tissue specific rather than cancer specific which has resulted in controversy regarding its reliability for initial screening of prostate cancer. In order to achieve a more reliable biochemical assay other biomarkers that are specific to prostate cancer beyond PSA are highly favored. Detecting biomarkers that are specific to prostate cancer from a body fluid would make novel diagnosis less invasive and more accurate.

Herein, this review introduces the current state of nanotechnology applied toward the development of diagnostics for prostate cancer. From the standpoint of diagnostic development for prostate cancer, the relevant research trend is discussed along with clinical implications of nanotechnology-based prostate cancer detection in initial screening.

**From bodily fluids to nanotechnology-based bioassays**

**Bodily fluids for noninvasive diagnosis**

Semen has become a promising proximal fluid for prostate cancer diagnosis by detecting related biomarkers and monitoring the disease’s pathological process. Due to the fact that seminal fluids are directly transported from prostate glands, they contain more biomarkers than other fluids such as serum and urine.

Serum is plasma after the removal of blood cells. Since all cells require oxygen that is delivered through the circulatory system, serum contains biomarkers of diseased tissues from any location of the body. Thus, serum is an outstanding source for diagnosis. However, the environment of serum is likely to hinder detection of biomarkers due in part to proteolytic degradation activity and a high concentration of albumin, which takes up to 50% of the weight of the whole serum proteome. Low-level protein biomarkers are subjected to degradation by proteases and peptidases, which divide into different classes. Typical protease classes are aspartic, cysteine-, serine-, and metallopeptidases. Peptidases belong to one of the three classes, including endo-, exo-, and carboxypeptidases. High-abundant proteins can create a masking effect by noncovalently attaching to the low-level protein biomarkers.

Urine is rich in proteome, including the biomarkers of various diseases. Since urine comes from blood through glomerular filtration, urine is in contact with the genitourinary tract that releases biomarkers of the disease. Urine has gained interest as a source or medium for diagnosis due to it being a noninvasive procedure. Many reports have demonstrated that urine contains a variety of biomarkers that could indicate diseases. Urine contains both urinary and systemic information since urine is in direct contact with urogenital organs and gets filtered from serum via glomerular filtration. Considering genitourinary organs are in direct contact with the body fluid, it is appealing to use urine for prostate cancer diagnoses in a noninvasive manner. However, some challenges lie in using urine as the source because of the low pH level, high salt concentrations, and interference from other biomarkers of higher abundance.

**Reliable biomarkers and emerging nanotechnology for noninvasive diagnostic strategies**

As a source for diagnosis, various bodily fluids have been exploited because they contain metabolized biomolecules that are biomarkers of diseases. Figure 3 illustrates the typical body fluids of semen, serum, and urine. The wide variety of biomarkers from the bodily fluids indicates the body’s physiological or pathophysiological state. For example, diseased tissues alter the physiological metabolisms and produce nonphysiological levels of biomolecules, enzymatic activity, or a new biomolecule species such as TMPRSS2:ERG that does not exist in the physiological state otherwise. The fusion gene is a result of a rearrangement on chromosome 21, and the TMPRSS2:ERG fusion protein is an oncogene that deregulates cytological metabolisms. The biomolecules are diverse in types, which are divided into carbohydrates, metabolites, nucleic acids, and proteins.

The current FDA-approved biomarker PSA shows poor sensitivity and specificity and often leads to negative biopsies indicating its poor specificity in prostate cancer diagnosis. In order to compensate for the limitations of PSA as the effective biomarker for prostate cancer, novel biomarkers beyond PSA have been proposed and are under investigation. The main idea with novel biomarkers is exploiting them in conjunction with PSA. For instance, the currently FDA-approved prostate cancer gene 3 test requires urinary PSA level information so that it can generate a prostate cancer gene 3 score that would aid the diagnostic decision making. Thus, in addition to the biomarker discovery, a previous study has used panels of biomarkers for prostate cancer detection and evaluated their specificity toward prostate cancer detection. The trend with use of a panel of biomarkers for prostate cancer detection indicates the realization that there is no such thing as a single perfect biomarker.

The more reliable biomarkers they are, the more effective prognosis or initial screening will be. Aspiration for noninvasive patient-friendly prostate cancer detection is one of the main drivers of novel biomarker discovery from
bodily fluids. These features will add an enormous benefit by minimizing the number of unnecessary non-patient-friendly DRE and invasive biopsies for prognosis and diagnosis. The conventional methods, PSA assay and DRE, for initial screening put a huge burden on the patients considering the high false-positive rate of initial screening leading to unnecessary repeated biopsies.

There is no nanotechnological approach made in clinical settings, yet. Attempts are currently underway to develop bioassays with high sensitivity and specificity that could analyze bodily fluids, and nanotechnology has emerged as a promising tactical tool. However, there are challenges in detecting the novel biomarkers in body fluids. Urine, especially, has a range of different pH levels, high concentrations of salts, and interferences by a variety of high abundance biomolecules, which all contribute to the obstruction of detecting the desired biomarkers with low sensitivity. Therefore, the major tasks in the development of bioassays would be achieving a high sensitivity as well as specificity in the detection of biomarkers through nanotechnology.

Highly sensitive PSA detection through nanotechnology

As Table 1 shows, the vast majority of studies have developed various nanotechnology-based bioassays for the detection of PSA in serum, whereas only a few studies have utilized the other body fluids. The larger number of studies with serum may be attributed to the longer history of serum as a bodily fluid for diagnosis. The first nanotechnological approach for prostate cancer screening was serum detection of PSA with a cantilever. The sensitivity was recorded in terms of the limit of detection, and it was as low as 200 pg/mL. The principle of this bioassay method is by measuring the difference in oscillation frequency between the PSA bound and unbound cantilevers.

Of all the developed nanotechnology-based bioassays, the electrochemical assay is the most popular method and has also achieved the highest sensitivity with <0.9 fg/mL in its detection limit and 1 ng/mL being the highest. The most common material for this method is carbon nanotubes (CNT), which is the typical material for electric sensing systems. CNT is an excellent signal transducer for achieving a high sensitivity. Biomedical functionalization with the PSA antibody (anti-PSA) conjugation onto the surface of the material allowed for the detection of protein PSA in serum samples. The captured PSA alters the current that runs through the CNT giving rise to detection.

Electrochemiluminescence assays took advantage of this in conjugation with the nature of titanium nanotubes to achieve a low detection limit of 1 fg/mL. Electrochemiluminescence assays took advantage of this in conjugation with the nature of titanium nanotubes to achieve a low detection limit of 1 fg/mL. The most popular nanomaterial is gold either as nanoparticle or as a nanopore. Only this material has been utilized...
Table 1 PSA and related nanotechnology-based bioassays

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Method</th>
<th>Nanomaterial</th>
<th>Limit of detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen</td>
<td>ICG</td>
<td>CGP</td>
<td>1/200 dilution³</td>
<td>167–169</td>
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<td>Urine</td>
<td>ICG</td>
<td>GNP</td>
<td>1 ng/mL</td>
<td>170</td>
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<td>Serum</td>
<td>BBA</td>
<td>GNP</td>
<td>330 fg/mL</td>
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<tr>
<td></td>
<td>RCIA</td>
<td></td>
<td>30 pg/mL</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>CLA</td>
<td>TiO₂/MWCNT</td>
<td>800 fg/mL</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>ODI-CL</td>
<td>Fe₃O₄ GONP</td>
<td>500 pg/mL</td>
<td>177</td>
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<tr>
<td></td>
<td>EA</td>
<td>CNT</td>
<td>1 ng/mL</td>
<td>178</td>
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<tr>
<td></td>
<td>ECA</td>
<td>AgNP</td>
<td>0.9 fg/mL</td>
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<td></td>
<td></td>
<td>NPG</td>
<td>750 pg/mL</td>
<td>156</td>
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<tr>
<td></td>
<td></td>
<td>Ag@MSN</td>
<td>15 pg/mL</td>
<td>147</td>
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<tr>
<td></td>
<td></td>
<td>SNP</td>
<td>760 pg/mL</td>
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<td></td>
<td></td>
<td>STV-CdSe/ZnS QD</td>
<td>20 pg/mL</td>
<td>150</td>
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<tr>
<td></td>
<td></td>
<td>Pol-Si NW</td>
<td>&lt;5 fg/mL</td>
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<td></td>
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<td>MWCNTs-IL-TH</td>
<td>20 pg/mL</td>
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<tr>
<td></td>
<td></td>
<td>CNT</td>
<td>1 ng/mL</td>
<td>152</td>
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<td></td>
<td></td>
<td>MWCNT-GCE</td>
<td>1 ng/mL</td>
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<td></td>
<td></td>
<td>SWCNT</td>
<td>1 ng/mL²</td>
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<td></td>
<td></td>
<td>GNP/PSA/MWCNT-CAS</td>
<td>7 pg/mL</td>
<td>157</td>
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<td>MWCNTs/IL/Chit, GNP–PAMAM</td>
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<td>151</td>
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<tr>
<td></td>
<td>ECLA</td>
<td>Fe₃O₄ @SiO₂ NP, Den/GNP</td>
<td>300 fg/mL</td>
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<td></td>
<td></td>
<td>CdS–TiO₂ NT, CdTe–MWCNT</td>
<td>1 fg/mL</td>
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<tr>
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<td></td>
<td>PEDOT/Gr, CNS@CdTe</td>
<td>800 fg/mL</td>
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<td></td>
<td>FA</td>
<td>QD</td>
<td>250 pg/mL</td>
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<tr>
<td></td>
<td>INPA</td>
<td>ENP</td>
<td>830 fg/mL</td>
<td>181</td>
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<td></td>
<td>MCA</td>
<td>GF</td>
<td>200 pg/mL</td>
<td>146</td>
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<td></td>
<td>NABD</td>
<td>GNP</td>
<td>1 fg/mL</td>
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<td></td>
<td>RLS</td>
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<td>32 pg/mL</td>
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<tr>
<td></td>
<td>SPR</td>
<td></td>
<td>10 ng/mL</td>
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<tr>
<td></td>
<td>LSPCF-FOB</td>
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<td>−100 fg/mL²</td>
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<tr>
<td></td>
<td>SERS</td>
<td></td>
<td>1 pg/mL</td>
<td>163</td>
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</table>

Notes: ¹Diluted the sample to measure limit of detection. ²Multiplex detection. ³Used AUC to measure performance of test.

Abbreviations: AgNP, silver nanoparticle; AUC, area under the curve; Ag@MSN, silver hybridized mesoporous silica nanoparticle; BBA, bio-barcode assay; CGP, colloidal gold particle; CLA, chemiluminescence; CNS@CdTe, cadmium telluride-coated carbon nanosphere; CNT, carbon nanotube; EA, enzymatic assay; ECA, electrochemical assay; ECLA, electrochemiluminescence assay; ENP, europium(III) nanoparticle; FA, fluorescent assay; GF, gold film; GNP, gold nanoparticle; GNP/PSA/MWCNT–CAS, gold nanoparticles encapsulated starch-cross-linked multiwalled carbon nanotube; GNP–PAMAM, gold nanoparticles–incorporated polyamidoamine dendrimer; GONP, graphene oxide nanoparticle; ICG, immunochromatography; INPA, immunometric nanoparticle-based assay; MCA, microcantilever arrays; MWCNT-GCE, multiwalled carbon nanotube-modified glassy carbon electrode; MWCNTs/IL/Chit, multiwalled carbon nanotubes/ionic liquid/chitosan; MWCNTs-IL-TH, multiwalled carbon nanotubes-ionic liquid-thionine; NPG, nanoporous gold; ODI-CL, 1,10-oxa-diimidazolode; Pd@rGO, palladium nanoparticle decorated-reduced graphene oxide; PEDOT/Gr, poly(3,4-ethylenedioxythiophene)/graphene; poly-Si NW, polycrystalline silicon nanowire; PSA, prostate-specific antigen; QD, quantum dot; RCIA, reverse colorimetric immunoassay; RLS, resonance light scattering; SERS, surface-enhanced Raman scattering; SNP, silicon nanoparticle; STV-CdSe/ZnS QD, streptavidin conjugated CdSe/ZnS quantum dot; SWCNT, single-walled carbon nanotube; TiO₂/MWCNT, TiO₂ nanoparticles coated multiwalled carbon nanotubes; Den, dendrimer; NABD, nucleic acid barcode dot; SPR, surface plasmon resonance; LSPCF-FOB, localized surface plasmon coupled fluorescence fiber-optic biosensor.

across all the different body fluids, indicative of its popularity in nanotechnology applications. ¹⁵¹,¹⁵⁶,¹⁵⁷,¹⁶¹–¹⁷² However, its most popular form of use is as a nanoparticle. These nanomaterials are fabricated to obtain a high surface area to volume ratio to maximize the amount of antibodies loaded onto the material.

Although gold nanoparticles (GNPs) have been used the most in the electrochemical assay, the trend for PSA detection is to use CNT. ¹⁴⁸,¹⁵¹–¹⁵⁴,¹⁵⁷,¹⁷³–¹⁷⁵ This trend is most likely for proof of principle of the developed bioassays because CNT is primarily used for academic purposes rather than for practical use. GNP was the second most used nanomaterial, an indicative of effort to develop more sensitive PSA detection methods in serum. ¹⁵¹,¹⁵⁷,¹⁶¹–¹⁶⁶,¹⁷¹,¹⁷² Unlike theCNTs, GNPs are used across various methodologies (Table 1). GNP is an excellent material for conjugation via various methods, thus it can load a high amount of targeting moieties that capture biomarkers, dye conjugates, or catalysts using its high surface area to volume ratio.

**Nanotechnology toward non-PSA biomarker detection**

Non-PSA biomarkers are not only protein biomolecules but also nucleic acids and other metabolites. Nucleic acids are the building blocks of DNA or RNA strands. Studies listed in Table 2 show that the nucleic acids are microRNAs, a type of RNAs. ¹⁸²,¹⁸³
Table 2 Non-PSA biomarkers and related nanotechnology-based bioassays

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Biomolecule type</th>
<th>Biomarker</th>
<th>Method</th>
<th>Nanomaterial</th>
<th>Limit of detection</th>
<th>Note</th>
<th>References</th>
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<tbody>
<tr>
<td>Serum</td>
<td>Nucleic acid</td>
<td>miR-200c, -21, -210, -205, -20a, -143*, -143, and -16 miR-141</td>
<td>ECA</td>
<td>SWCNT</td>
<td>8 fM</td>
<td>Polymer</td>
<td>183</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>GNP</td>
<td>1 fM</td>
<td>Multiplex</td>
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<td>Protein</td>
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<tr>
<td></td>
<td>Protein</td>
<td>OPN, PSMA, PF-4, IL-6</td>
<td>ECA</td>
<td>SWCNT</td>
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<td></td>
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<td>ANXA3</td>
<td>QCM</td>
<td>QD</td>
<td>750±10 pg/mL</td>
<td>Sensitivity: 50%, specificity: 90%; WB</td>
<td>184</td>
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<td>AMACR</td>
<td>FA</td>
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<td>N/A</td>
<td>Biomarker discovery</td>
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<td>DLSA</td>
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<tr>
<td></td>
<td></td>
<td>PAP</td>
<td>ICG</td>
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<td>250 pg/mL</td>
<td>Binding to prostate cancer cell</td>
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<tr>
<td></td>
<td></td>
<td>N/A</td>
<td>ICC</td>
<td></td>
<td>IC_{50}=2.62 µg/mL</td>
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<td>Urine</td>
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<td>MFC</td>
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<td></td>
<td>189</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-OHdG</td>
<td>ECA</td>
<td>PMP</td>
<td>5 pg/mL</td>
<td>Microparticle</td>
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</tr>
<tr>
<td></td>
<td>Protein</td>
<td>PSMA</td>
<td>M13-PEDOT</td>
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<td>100 µM</td>
<td>Polymer</td>
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<tr>
<td></td>
<td></td>
<td>M13-PEDOT NW</td>
<td></td>
<td>56 nM</td>
<td>Polymer</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Endoglin</td>
<td>GMRA</td>
<td>MNP</td>
<td>83 fM</td>
<td>Unprocessed urine</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANXA3</td>
<td>QCM</td>
<td>QD</td>
<td>750±10 pg/mL</td>
<td></td>
<td>184</td>
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</tbody>
</table>

Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; AMACR, alpha-methylacyl-CoA racemase; ANXA3, annexin A3; CALD1, caldesmon 1; DLSA, dynamic light scattering assay; ECA, electrochemical assay; FA, fluorescent assay; GMRA, giant magnetoresistive assay; GNP, gold nanoparticle; GN, gold nanorod; GO, graphene oxide; GN, graphene nanosheet; ICC, immunocytochemistry; ICG, immunochromatography; IL-6, interleukin-6; LEDGF/PSIP1, lens epithelium–derived growth factor/PC4- and SF2-interacting protein 1; M13-PEDOT, bacteriophage M13-poly (3,4-ethylenedioxythiophene); M13-PEDOT NW, bacteriophage M13-poly (3,4-ethylenedioxythiophene) nanowire; MFC, microfluidic chip; miR, microRNA; MNP, magnetic nanoparticle; MRI, magnetic resonance imaging; OPN, osteopontin; PAP, prostatic acid phosphatase; PARK7, parkin protein 7; PF-4, platelet factor-4; PMP, paramagnetic particle; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; QCM, quartz crystal microbalance; QD, quantum dot; SWCNT, single-walled carbon nanotube; TARDBP, transactive response DNA binding protein-43; TLN1, Talin-1; WB, western blot; N/A, not available.
These nanotechnology-based bioassays for non-PSA biomarker detection point toward a practical use. None of these biomarkers are a good reference point for the proof of principle. The only thing that matters with these new prostate biomarkers is whether the bioassays are capable of capturing the biomarkers for detection. Effective detection ability with sufficient sensitivity and specificity in urine requires nanotechnology to achieve noninvasive initial screening. Notably, there are more studies about the detection of new non-PSA markers in urine. In response to clinical researchers, a number of studies have tried to detect non-PSA biomarkers in urine with some of the recently discovered prostate cancer biomarkers.\textsuperscript{184–189} PSMA, endoglin, and ANXA3 are the most promising biomarkers among the new ones, and the fact that they are tested with bioassays indicates how the studies are intended to show the practicality of the bioassays for initial screening.

The non-PSA biomarkers vary in type and require a different way of capturing the molecules. As with most nanomaterials, immunoassays are the most popular form of assay that is functionalized with specific antibodies against targeting biomarkers. All the studies used traditional nanomaterials as shown in Table 2. However, a novel material was used for the detection of PSMA. This nanomaterial is a nanowire that is conjugated with engineered bacteriophage M13. The unconventional nanomaterials are also used to develop new alternative assays. Rather than using traditional nanomaterials such as gold nanoparticles, quantum dots, and magnetic nanoparticles, some studies have utilized and exploited graphene and virus-based nanomaterials to achieve a more sensitive detection.\textsuperscript{186,187} The two interesting studies demonstrated the use of M13-PEDOT polymer nanowires to detect PSMA using an electrochemical assay. Another interesting study used a polymer-based nanomaterial which was a single-walled CNT. The nanotube detected miR-141, a microRNA known as a novel prostate cancer biomarker. Like the assay method used in the studies with M13-PEDOT polymer, the electrochemical assay is used for miR-141 detection in serum.\textsuperscript{183,186,187}

A unique study performed multiplex detection by using a microarray method with GNPs. This study does not present a limit of detection with the biomarkers but instead presents a unique aspect of the assay with the sensitivity and specificity for prostate cancer diagnosis. Unlike all the other studies that employed nanotechnology shown in Tables 1, 2, and 3, it has a clinical implication on how it can be a promising tool for future prostate cancer diagnoses.\textsuperscript{190}

Another notable fact from Table 2 is that nanotechnology-based assays can be applied for multiplexed detection. Nanotechnology is capable of detecting multiple biomarkers simultaneously. The use of a panel of prostate cancer biomarkers has been suggested for future diagnosis of prostate cancer. However, no single study has demonstrated multiplexed detection of other body fluids.\textsuperscript{148,182,190}

Considering there are many DNA biomarkers for prostate cancer, more studies are warranted for a nanotechnological approach for biomarkers. Since almost all known DNA biomarkers exhibit epigenetic alteration, a sensitive nanoprobe is required. Such as alteration as a biomarker candidate for prostate cancer would add another layer of reliability to biomarker detection. Having diversified biomarkers would lead to a more accurate initial screening.

**Nanotechnology into device platform**

Nanotechnology-based bioassays of PSA and non-PSA biomarkers have been developed to achieve a higher sensitivity and specificity. The aforementioned techniques are built into devices to materialize new sensing platforms (Table 3). The development of the device is a convergence of nanotechnology and other sensor technologies. The main focus is to utilize nanomaterials in conjugation with traditional sensors to develop a new nanomaterial-bearing sensing device platform. This approach has led to higher sensitivity and specificity toward the detection of the prostate biomarkers, which have not been achieved without nanomaterials. The efforts to improve the properties of the devices that are intended for the detection of biomarkers from body fluids other than serum is because other body fluids – such as urine – require a higher sensitivity. However, serum is the most tested bodily fluid. One of the device platforms that yielded one of the highest sensitivities is the field-effect transistor, which mainly utilized CNT as sensing nanomaterial.\textsuperscript{148,152,178,183,191}

It is interesting to note that the sensitivity differs dramatically depending on the sensing nanomaterial used. Compared to CNT, polysilicon nanowires demonstrated outstanding sensitivity of ~5 fg/mL of limit of detection.\textsuperscript{149} A similar device, microgapped interdigitated enzymatic assay, achieves even higher sensitivity with a 0.9 fg/mL limit of detection.\textsuperscript{155} Electrical sensing devices seem to be the most popular sensing method. An interesting aspect of this device is that the distance between the two electrodes is in the micrometer range, and there is a report of a nanogapped microelectrode array.\textsuperscript{155,198} Optical-based sensing devices such as immunochromatographic strips and quartz crystal microbalance.
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(NCM) exhibit a fair sensitivity in comparison to the electrical-based sensing devices.1,4,9,167–169

**Conclusion**

Beginning from the early 2000s, nanotechnology has emerged as a therapeutic and diagnostic tool for the treatment and diagnosis of prostate cancer, and more studies have been accumulating since. Complications from unreliable initial screenings gave a need for researching the development of panels of novel prostate cancer biomarkers for better initial screening. Many reports have been published about the discovery of novel biomarkers. However, for the detection of some of the novel biomarkers, the initial screening requires a bioassay with a higher sensitivity and specificity while keeping it noninvasive. In response to these needs, studies have employed nanotechnology to bring forth novel bioassay development. Each of the studies has demonstrated noninvasive detection of their bioassays in one of the body fluids.

Nanotechnology is of particular interest because when we shrink the sizes of the existing materials down to the nanoscale, new distinct physical properties start to appear. As we have harnessed the properties of bulk materials, the new physical properties of nanomaterials can also be harnessed for various applications such as biomedicine. The studies aforementioned have exploited these new properties to overcome the limitations that we face with prostate cancer initial screening by significantly improving the sensitivity and specificity of biomarker detection.

**Table 3 Device platforms for the nanotechnology-based bioassays**

<table>
<thead>
<tr>
<th>Device</th>
<th>Body fluid</th>
<th>Nanomaterial</th>
<th>Limit of detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICGS</td>
<td>Semen</td>
<td>CGP</td>
<td>1/200 dilution</td>
<td>167–169</td>
</tr>
<tr>
<td>ICGS</td>
<td>Serum</td>
<td>GNP</td>
<td>250 pg/mL</td>
<td>194</td>
</tr>
<tr>
<td>Cantilever</td>
<td>GF</td>
<td>GNP</td>
<td>200 pg/mL</td>
<td>146</td>
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<tr>
<td>CNT network TR</td>
<td>SWCNT</td>
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<td>178</td>
</tr>
<tr>
<td>CNT 4-electrode array</td>
<td></td>
<td></td>
<td>8 fm</td>
<td>183</td>
</tr>
<tr>
<td>CNT FET</td>
<td>CNT</td>
<td></td>
<td>30 fm</td>
<td>191</td>
</tr>
<tr>
<td>Poly-Si NW FET</td>
<td>Poly-Si NW</td>
<td></td>
<td>&lt; 5 fg/mL</td>
<td>149</td>
</tr>
<tr>
<td>MGIDEA</td>
<td>AgNP</td>
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<td>0.9 fg/mL</td>
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<td>N/A</td>
<td>190</td>
</tr>
<tr>
<td>QCM</td>
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<tr>
<td>QCM</td>
<td>Urine</td>
<td>QD</td>
<td>750±10 pg/mL</td>
<td>184</td>
</tr>
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</table>

**Abbreviations:** AgNP, silver nanoparticle; CGP, colloidal gold particle; GNP, gold nanoparticle; CNT, carbon nanotube; CNT FET, carbon nanotube field-effect transistor; GF, gold film; ICGS, Immunochromatographic strip; IL-6, interleukin-6; MGIDEA, microgapped interdigitated enzymatic assay; PF-4, platelet factor-4; poly-Si NW FET, polysilicon nanowire field-effect transistor; PSMA, prostate-specific membrane antigen; QCM, quartz crystal microbalance; QD, quantum dot; SWCNT, single-walled carbon nanotube; TR, transistor.
note that some studies are not aware of what the clinical community demands and what the exact problem with current initial screening with PSA detection is. This misunderstanding should be avoided to make further research more effective. Thus, the communication between the clinical research and nanotechnology engineering fields must be up to date at all times.

PSA has been a good reference to compare with other bioassays that have a measured PSA level. Using PSA detection as the proof of principle for their developed bioassays will not be as advantageous as before. The complications of the initial screenings turned out to be due to PSA itself being an unreliable biomarker rather than the low sensitivity of the enzyme-linked immunosorbent assay method. As the notion has grown that it is unlikely to find a perfect biomarker to diagnose a disease, PSA might still be used as one of the panel of prostate cancer biomarkers. To establish other prostate cancer biomarkers into the panel, testing the bioassay detection of the novel biomarkers is needed. However, compared to PSA, other biomarkers have less of a chance to get tested in the development of nanotechnology-based bioassays.

Clinical trials are important for the development of the bioassays in order to make them clinically relevant. Finding the cutoff value for a diagnosis or initial screening is another problem. Clinical researcher must have their attention on these nanotechnologies for the development of a more effective initial screening strategy.

Another important clinical implication is that nanotechnology-based bioassays can also contribute to monitoring the stage progression of the disease. Dynamic changes of biomarkers occur as prostate cancer develops and advances. Thus, detecting different biomarkers that represent particular stages of the disease may add an additional benefit to an accurate diagnosis. It has been reported that benign and malignant tumors must be differentiated and subjected to different treatments. Attempting to treat a benign tumor can only exacerbate the diseased state while undertreatment can also bring consequences. In that regard, aggressive stratification of the disease’s stages is one of the urgent needs. To achieve this stratification, developing diagnostic tools capable of discerning the different stages of the disease could significantly improve the quality of life of the diagnosed patients by delivering an accurate treatment.

Finally, the development of nanotechnology-based bioassays is a highly interdisciplinary area that will require a robust multidisciplinary approach. Active collaboration between clinicians and experts in nanotechnology is a must in order to take development toward commercialization. It would be highly favorable for future investors and policy officials to put their interest in this area of research.

Review criteria
Pubmed and Web of Science were used in search, and the keywords were “seminal” OR “serum” OR “urine” AND “prostate cancer” AND “nano*”.

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Disclosure
The authors report no conflicts of interest in this work.

References


