REVIEW

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 ultrasonography[33–49] 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.
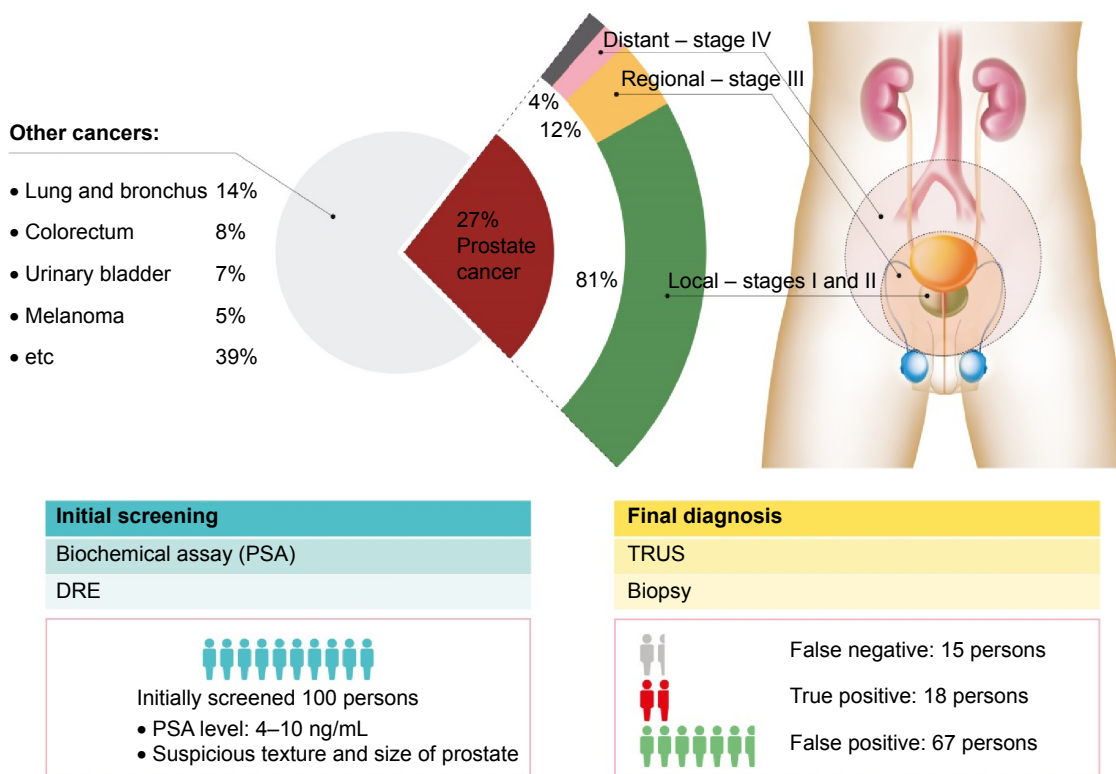
Figure 1 Prostate cancer diagnosis statistics.

Notes: Prostate cancer accounts for 27% of all male cancer cases meaning every one out of four men is diagnosed with prostate cancer in the USA. The localized, regional, and metastasized tumor tissue accounts for 81%, 12%, and 4%, respectively, of all prostate cancer diagnosis with the unknown stage remaining. Typical subjects with prognoses to have prostate cancer are 85 persons per 100 persons after the initial screening, while the other 15 persons are not predicted to have prostate cancer. Only 18 persons of the 85 persons with positive initial screening result are diagnosed with prostate cancer. Surprisingly, the other 15 persons, who are believed to have no prostate cancer based on the initial screening result, are turned out to be diagnosed with prostate cancer. Data from Siegel et al.[1]

Abbreviations: DRE, digital rectal examination; PSA, prostate-specific antigen; TRUS, transrectal ultrasonography.

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.[28–32] 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.[33–49] 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.[21–27,50–56] Patients may undergo radical prostatectomy[57] or hormone therapy depending on the extent of malignancy.[58–65] 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.[66–72]

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.

Despite the fact that PSA has been the gold standard for the initial screening, whether PSA screening has provided a major contribution in early prostate cancer detection remains
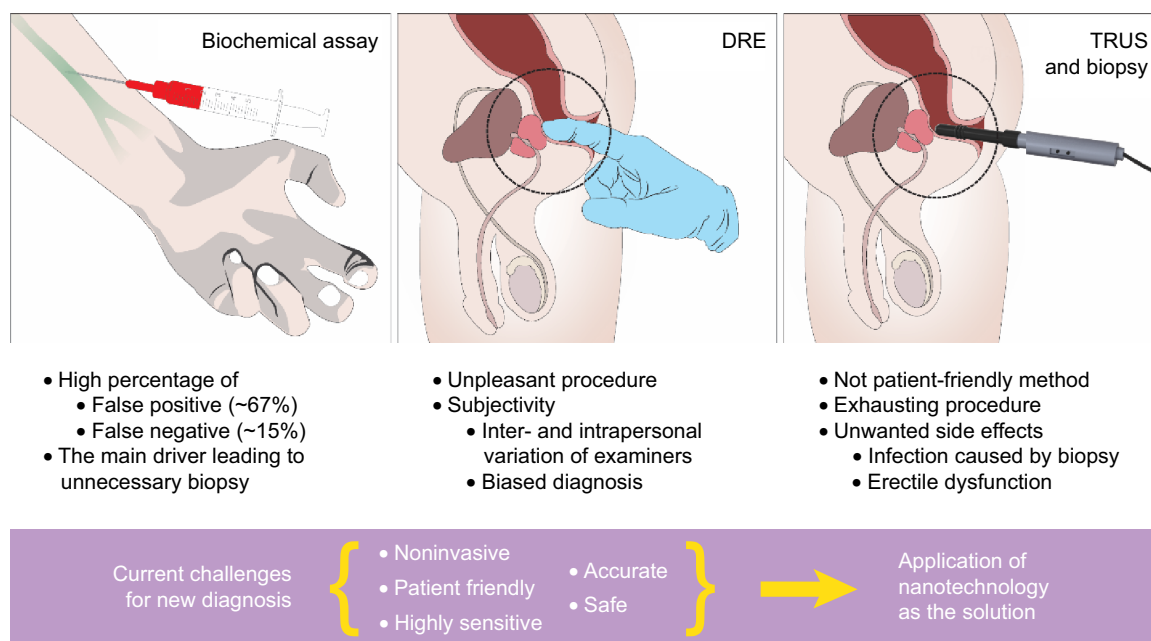
Figure 2 Current conventional approach of prostate cancer diagnosis.
Notes: Current conventional diagnostic methodologies for the patients with prostate cancer hold several drawbacks. Patients with PSA levels 4–10 ng/mL are subjected to further examinations. The assay gives a significant number of false 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.

uncertain.[73,74] 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.[75–79] As a consequence, almost two-thirds of prostatic biopsies made annually in the USA and Europe are unnecessary.[1,2]

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.[78–82] 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.[83–104] On the other hand, nanotechnology is also being employed for diagnostic development.[101,103] 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.[105,106] 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.[86,107–115]

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

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.[116] 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.[116]

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.[117–121] 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.[122–126] 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.[127] 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.[128]

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,[116] serum,[129] and urine.[127,129] 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.[130–144] The biomolecules are diverse in types, which are divided into carbohydrates, metabolites, nucleic acids, and proteins.[127]

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.[17,145] 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.[127] 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
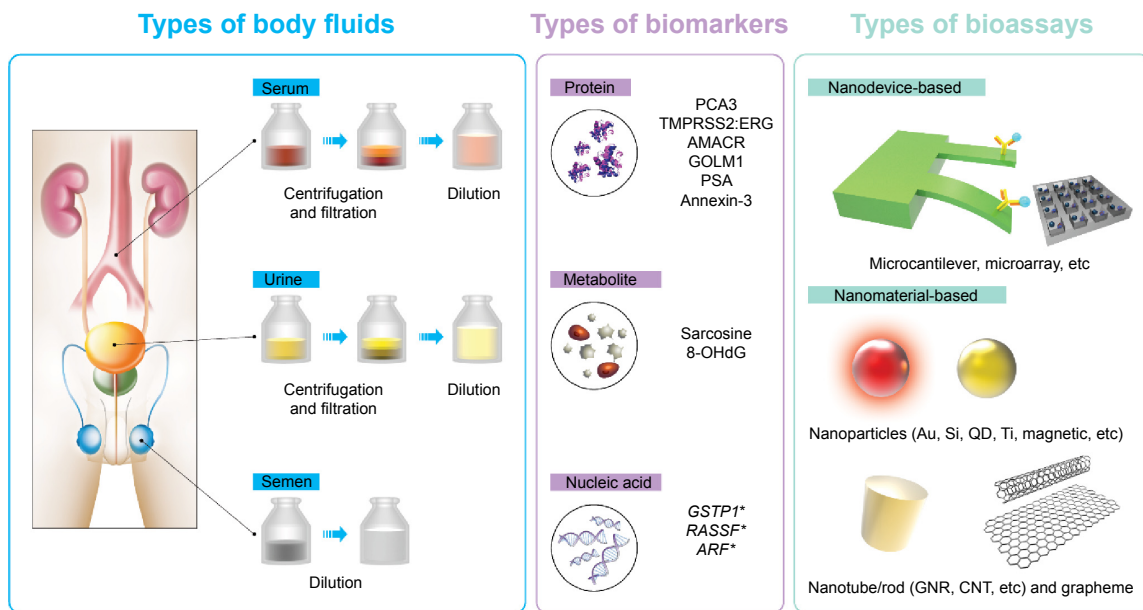
Figure 3 New diagnostic strategy based on nanotechnology (bioassay) with non-PSA biomarkers in various body fluids.
Notes: Different body fluids are subjected to appropriate sample preparations for subsequent analysis. The sample preparations are carried out to create the best environments for the nanoprobe's function. These specimens may be centrifuged and/or diluted. Various biomarkers can be collected or detected by the nanoprobes from the body fluids. Nanotechnology-based bioassays detect various prostate cancer biomarkers. The *denotes the detection of epigenetic change.
Abbreviation: PSA, prostate-specific antigen.

bodily fluids. These features will add an enormous benefit by minimizing the number of unnecessary nonpatient-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.[128] 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.[146] 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.[147–159] Electrochemiluminescence assays took advantage of this in conjugation with the nature of titanium nanotubes to achieve a low detection limit of 1 fg/mL.[160]

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

Body fluid	Method	Nanomaterial	Limit of detection	References
Semen	ICG	CGP	1/200 dilution[a]	167–169
Urine	ICG	GNP	1 ng/mL	170
Serum	BBA	GNP	330 fg/mL	172
	RCIA		30 pg/mL	162
	CLA	TiO_2/MWCNT	800 fg/mL	176
	ODI-CL	Fe_3O_4 GONP	500 pg/mL	177
	EA	CNT	1 ng/mL	178
	ECA	AgNP	0.9 fg/mL	155
		NPG	750 pg/mL	156
		Pd@rGO	10 pg/mL	158
		Ag@MSN	15 pg/mL	147
		SNP	760 pg/mL	159
		STV-CdSe/ZnS QD	20 pg/mL	150
		Poly-Si NW	<5 fg/mL	149
		MWCNTs-IL-TH	20 pg/mL	154
		CNT	1 ng/mL	152
		MWCNT-GCE	1 ng/mL	153
		SWCNT	1 ng/mL[b]	148
		GNPs/MWCNT–CAS	7 pg/mL	157
		MWCNTs/IL/Chit, GNPs–PAMAM	1 pg/mL	151
	ECLA	Fe_3O_4@SiO_2 NP, Den/GNP	300 fg/mL	171
		CdS–TiO_2 NT, CdTe–MWNT	1 fg/mL	160
		PEDOT/GR, CNS@CdTe	800 fg/mL	179
	FA	QD	250 pg/mL	180
	INPA	ENP	830 fg/mL	181
	MCA	GF	200 pg/mL	146
	NABD	GNP	1 fg/mL	166
	RLS		32 pg/mL	164
	SPR		10 ng/mL	165
	LSPCF-FOB		~100 fg/mL[c]	161
	SERS		1 pg/mL	163

Notes: [a]Diluted the sample to measure limit of detection. [b]Multiplex detection. [c]Used 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; GNPs/MWCNT–CAS, gold nanoparticles enwrapped starch-cross linked multiwalled carbon nanotube; GNPs–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-oxalyldiimidazole chemiluminescence; Pd@rGO, palladium nanoparticle decorated-reduced graphene oxide; PEDOT/GR, poly(3,4-ethylendioxythiophene)/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; TiO_2/MWCNT, TiO_2 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 the CNTs, 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

Body fluid	Biomolecule type	Biomarker	Method	Nanomaterial	Limit of detection	Note	References
Serum	Nucleic acid	miR-200c, -21, -210, -205, -20a, -143*, -143, and -16	Scano-miR	GNP	1 fM	Multiplex	182
		miR-141	ECA	SWCNT	8 fM	Polymer	183
	Protein	OPN			30 fM		191
		PSMA, PF-4, IL-6			PSMA: 10 ng/mL, PF-4: 1 ng/mL, IL-6: 30 pg/mL	Multiplex	148
		ANXA3	QCM	QD	750±10 pg/mL	Sensitivity: 50%, specificity: 90%; WB	184
		AMACR	FA		N/A		192
		Serum protein	DLSA	GNP		Biomarker discovery	193
		TARDBP, TLNI, PARK7, LEDGF/PSIP1, CALD1	Microarray			Sensitivity: 95%, specificity: 80%; multiplex	190
	N/A	PAP	ICG		250 pg/mL		194
		N/A	ICC	GNR	IC_{50} =2.62 µg/mL	Binding to prostate cancer cell	195
			MRI	MNP	N/A	MNP with MRI	196
Urine	Metabolite	Sarcosine	PMME	GO, GN	1 ng/mL		197
			MFC	MNP	1 µM		189
	Protein	8-OHdG	ECA	PMP	5 pg/mL	Microparticle	188
		PSMA		M13-PEDOT	100 pM	Polymer	187
				M13-PEDOT NW	56 nM	Polymer	186
		Endoglin	GMRA	MNP	83 fM	Unprocessed urine	185
		ANXA3	QCM	QD	750±10 pg/mL		184

Abbreviations: 8-OHdG, 8-hydroxy-20-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; GNR, 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, parkinson protein 7; PF-4, platelet factor-4; PMME, polymer monolith microextraction; 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; TLNI, 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.[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 nanomaterials, quantum dots, and magnetic nanoparticles, some studies have utilized and exploited graphene and virus-based nanomaterials to achieve a more sensitive detection.[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.[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.[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.[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.[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.[149] A similar device, microgapped interdigitated enzymatic assay, achieves even higher sensitivity with a 0.9 fg/mL limit of detection.[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.[155,198] Optical-based sensing devices such as immunochromatographic strips and quartz crystal microbalance

Table 3 Device platforms for the nanotechnology-based bioassays

Device	Body fluid	Nanomaterial	Limit of detection	References
ICGS	Semen	CGP	1/200 dilution	167–169
ICGS	Serum	GNP	250 pg/mL	194
Cantilever		GF	200 pg/mL	146
CNT network TR		SWCNT	1 ng/mL	178
CNT 4-electrode array			8 fM	183
			PSMA: 10 ng/mL, PF-4: 1 ng/mL, IL-6: 30 pg/mL	148
CNT FET		CNT	30 fM	191
			1 ng/mL	152
Poly-Si NW FET		Poly-Si NW	<5 fg/mL	149
MGIDEA		AgNP	0.9 fg/mL	155
Microarray		GNP	N/A	190
			1 fM	182
QCM		QD	750±10 pg/mL	184
QCM	Urine	QD	750±10 pg/mL	184

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.

(QCM) exhibit a fair sensitivity in comparison to the electrical-based sensing devices.[1,4,9,167–169,184]

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.

Nanotechnology has proven its potential in prostate cancer biomarker detection. Nanotechnology-based bioassays have demonstrated a much higher detection sensitivity compared to the conventional enzyme-linked immunosorbent assay (ELISA) method. The higher sensitivity has much benefit because it means a stable detection – even of a trace of a biomarker. Higher sensitivity, therefore, allows for biomarker detection via urine instead of serum. The current initial screening of prostate cancer requires a blood sample for the PSA assay. It usually takes more than a drop to obtain and requires a syringe with a needle to acquire. Since this kind of operation needs a professional to carry out, the conventional method is still not enough to be patient friendly. Furthermore, the conventional method needs to be carried out by a professional, and thus, it cannot be a point-of-care for the general population to use. If we are able to take advantage of what nanotechnology can offer, it would be a great advancement in patient care because it is highly anticipated to minimize both overdiagnosis and underdiagnosis of cancer – due to its excellent specificity and sensitivity, respectively – and could monitor the disease for people at risk of recurrence after recovery.

To overcome the limitations of conventional initial screening methods, a significant number of studies proposed their nanotechnology-based bioassays. Most of the bioassays have measured biomarkers – PSA and beyond-PSAs – from serum. They outnumber the studies tested from other body fluids. Of the studies with serum, the majority of them chose PSA as the targeting biomarker. It is quite interesting to

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*".

Disclosure

The authors report no conflicts of interest in this work.

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