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Array-based sensing using nanoparticles: an alternative approach for cancer diagnostics

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Abstract

Array-based sensing using nanoparticles (NPs) provides an attractive alternative to specific biomarker-focused strategies for cancer diagnosis. The physical and chemical properties of NPs provide both the recognition and transduction capabilities required for biosensing. Array-based sensors utilize a combined response from the interactions between sensors and analytes to generate a distinct pattern (fingerprint) for each analyte. These interactions can be the result of either the combination of multiple specific biomarker recognition (specific binding) or multiple selective binding responses, known as chemical nose sensing. The versatility of the latter array-based sensing using NPs can facilitate the development of new personalized diagnostic methodologies in cancer diagnostics, a necessary evolution in the current healthcare system to better provide personalized treatments. This review will describe the basic principle of array-based sensors, along with providing examples of both invasive and noninvasive samples used in cancer diagnosis.

Keywords

array-based sensing; chemical nose sensing; invasive; noninvasive; personalized diagnostics; selective sensing

Early cancer detection can drastically improve survival rate, allowing the use of less invasive treatment options at earlier stages of the disease. An important goal in cancer diagnosis is to determine cancer localization and progression of patients. As important as this information is, it still does not address the critical fact that cancer genotype and phenotype can drastically influence the choice of therapeutic option for an individual. In many cases, even patients with the same cancer type can respond differently to the same drug, making the role of personalized medicine even more critical. Therefore, studying

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individual responses to drugs in pharmacogenomics has driven much of the move towards personalized therapeutics [1].

Personalized diagnostic methodologies are essential to the development of personalized medicine. Among a variety of cancer diagnosis methods, nanoparticle (NP)-based sensors have shown to be promising for cancer detection. NPs provide useful platforms for diagnostic design by exploiting the physical and chemical properties of NPs [2]. Most NP-based sensors for cancer detection mirror traditional diagnostics, utilizing lock and key specific interactions between the sensor and known biomarkers [3-7]. This strategy can be quite powerful, however, identification of an ideal early biomarker can be challenging. Such a biomarker needs to be specific to the cancer type, and its level of expression should correlate to the cancer progression. At present, there is no such ideal biomarker in cancer diagnosis.

In general, current biomarker assays either lack specificity in their diagnostic capabilities or the targeted biomarkers have widely different baseline expressions in the population. As a result, biomarker panels have been used to improve predictive performance of cancer diagnostics over single marker strategies. Several predictive models that range from six to eight specific biomarkers have proved to improve the diagnostic rate in some cancer types using diagnostic samples. Recently however, these models have been re-evaluated using prediagnostic samples (specimens collected before an individual is diagnosed with cancer). The results have shown that these biomarker panels might not be suitable for early cancer diagnostics since they are only effective when the disease has become more advanced [8]. Indeed, using specific biomarkers singly or in a multiplex fashion for early cancer detection is a challenging task. When utilizing this approach, prior knowledge of the biomarker is usually required. However, biomarkers for cancer diagnosis are often poorly characterized due to the unpredictable transformation of the disease progress. Therefore, the search for effective biomarkers presents a considerable challenge to personalized medicine approaches. In addition, genetic analysis of tumors can provide important diagnostic information. Nevertheless, if there are multiple genetic alterations that lead to similar health outcomes, a diagnostic test that can pinpoint the specific phenotype rather than a genetic mutation would be more useful for physicians. Unfortunately, the correlation between genotype and phenotype is not well established for many genetic variants and current gene expression-based tests cannot reflect the whole spectrum of phenotypic variations.

Array-based profiling of cancer phenotype provides an attractive complement to specific biomarker-focused strategies. Array-based sensing can either combine recognition of multiple specific biomarkers or use a selectivity-based modality known as chemical nose sensing. An array of specific biomarker-based sensors is used when a single biomarker is inadequate for cancer-type detection [9,10]. On the other hand, chemical nose array-based sensing uses selective interactions of sensor elements and the analyte mixture. This approach can facilitate diagnosis when a specific biomarker is not available. Chemical nose sensors have been successfully trained to recognize a wide range of analytes, such as volatile organic compounds [11], amino acids [12], proteins [13], carbohydrates [14], mammalian cells [15] and bacteria [16]. Combined responses from the sensor array, either specifically or selectively, create a characteristic pattern for each analyte [17].

In this review, we will highlight recent investigations on array-based sensors that use the physical and chemical properties of NPs to provide the recognition and transduction required for biosensing. Through the integration of these functions, nanomaterials provide promising approaches for new cancer diagnostics. We will provide examples of strategies that use cell surfaces and tissues, as well as noninvasive approaches that use urine and breath samples.

Basics of array-based sensing using NPs

Biosensing consists of three requisite processes: analyte recognition, transduction and signal analysis. Recognition elements are used to identify and bind to the target analytes, with specific recognition used for biomarker-based approaches and selective binding used in array-based strategies [18]. The recognition process must then be read-out through a transduction process to generate a signal that can be then processed through a variety of mechanisms. NPs can provide both of these functions, providing scaffolds for presentation of multivalent recognition elements and transduction through the physical properties of the particles.

In an array-based sensing strategy, each analyte produces a response from each of the sensor elements. A multivariate data matrix is obtained from different analytes and analyzed using multivariate chemometrics methods (Figure 1). These methods consist of a collection of techniques that can be used when several measurements are done on each individual analyte. Among these techniques, principal component analysis (PCA) and linear discriminant analysis (LDA) are most commonly used. These methods reduce the dimensionality of the data sets by extracting the most useful information into new and simpler components called principal components or canonical factors for LDA.

PCA is one of several multivariate methods that explore patterns in these data. PCA can determine the general relationship between these data by indicating which analytes behave similarly (in another words, which analytes belong to a similar group). While it is useful to know the pattern of these data, identifying which group a new unknown analyte belongs to is necessary. LDA as a classification (supervised pattern recognition) method is used for this purpose. In the classification methods, samples with known identities are used to define the groups (classes). These known sets of samples are referred to as a training set. The unknown analytes can then be assigned to the predefined groups using the appropriate classification algorithm [19,20].

Array-based cancer diagnostics

Cell surface sensing

Genotypic and phenotypic differences in cells result in different levels of polysaccharides, proteins and lipids on the cell surface [21,22]. This cell surface signature was used to differentiate cell genotype. Rotello and coworkers used an array of gold NPs (AuNPs) with varying headgroups to differentiate the cell surfaces. This strategy employed the strong quenching ability of AuNPs [23] to enable the use of fluorescent displacement assays, where

displacement of a quenched fluorophore from the recognition element signals the binding event [24].

In an earlier study, Rotello *et al.* developed an array of fluorescent polymer-conjugated AuNPs to detect different cancer cell types. Cationic AuNPs were used as recognition elements to noncovalently bind negatively charged poly(p-phenylene-ethynylene) polymer (PPE-CO₂) as the transducer [25]. The electrostatic interaction between PPE-CO₂ and AuNPs quenched the fluorescence of the polymers, which was recovered to varying degrees upon incubation with cells. Distinct and differentiated patterns were observed with human cancerous (MCF-7), metastatic (MDA-MB-231) and normal (MCF10A) breast cell lines. These cell lines, however, came from different individuals, making it possible that differentiation was based on individual genetic background. To avoid individual variation, isogenic cell lines derived from BALB/c mice (CDBgeo [normal], TD [cancerous], and V14 [metastatic]) were used to demonstrate the ability of the sensor array. In a further study, the author demonstrated that green fluorescent protein (GFP) could also be used as a transducer with high quantum yield, low aggregation and high sensitivity. This GFP–AuNP array sensor was able to discern different cancer cell types with as low as a 5000 cells (Figure 2) lower limit of detection, compared with the 20,000 cells used in AuNP–PPE-CO₂ [26]. Recently, quantum dots were used in combination with AuNPs to provide a sensor array [27].

Biomolecules, such as antibodies and/or synthetic aptamers, are typically used for specific recognition. They can, however, also be used for selective recognition [28]. Nonspecific aptamers were used to protect bare AuNPs from salt-induced aggregation. Upon addition of target cells, the competition between citrate-capped AuNPs and the cells will detach various amounts of aptamers from the AuNPs. Depending on the target cell line, different aggregation levels generated color change patterns that were used to differentiate cell lines. Through the use of two thrombin aptamers (Tro-1 and Tro-2) and one human IgE aptamer (HIgE-1), human cancer cells (Junkat, Reh and Raji) and normal human cells (WIL2-S) were differentiated [28].

Aptamer-conjugated magnetic NPs (ACMNPs) were developed for cancer cell sensing using magnetic signal transduction [9]. This magnetic NP-based sensor was fabricated by conjugating streptavidin-coated iron oxide NPs with biotin-labeled aptamers. ACMNPs bind to the target cells through specific and nonspecific interactions between the aptamer ligands and the membrane receptors. The spin–spin relaxation time (T₂) read out was measured by nuclear magnetic resonance spectroscopy. Using the magnetic property of their NPs, the authors were able to detect ten cancer cells in buffer and 100 cancer cells in other biological complex media, such as fetal bovine serum, plasma and whole blood.

Magnetic glyco-NPs were also used for array-based sensing. In this approach, a variety of carbohydrates were coated as ligands on magnetic NPs to explore the interactions of carbohydrate–cell membrane receptor upon cell incubation (Figure 3) [29]. Similarly with the ACMNPs, different degrees of aggregation upon incubation with cancer cells generated different local magnetic field gradients and altered the transverse relaxation time. Magnetic

glyco-NPs were able to differentiate several cancer cell types with a sensitivity of 10^5 cells/ml (Figure 4).

Cancer-cell sensing using array-based sensors provides a proof of concept for rapid cancer diagnosis. However, moving from cancer cell sensing *in vitro* to real cancer cells from patients will need extensive development to transform the methodology into a useful and meaningful clinical screening tool.

Tissue biopsy sensing

The above studies demonstrate the use of NP array-based sensing for cell surfaces in cell suspension. Solid tissues are much more complex systems, making cell surface sensing impractical for identification of solid tumors. Currently, excisional biopsy is the standard approach for cancer diagnosis with solid tumors. In this method, a tissue specimen is obtained from the patient to be examined by a pathologist. Although this method detects meaningful phenotypic differences, it is very time consuming, low throughput and requires high expertise.

As a potential alternative for point-of-care diagnosis, chemical nose sensors have been applied to tissue sensing. Given the complexity of tissues, Rotello and coworkers have focused on sensing of intracellular proteins using an array of GFP–AuNP conjugates. Protein sensing approaches using chemical nose sensors have been developed, facilitating this strategy [13,28,30]. In their studies, a mouse metastasis model was generated by inoculating NCI-H1299 non-small-cell lung cancer cells that metastasize to multiple organs. Tissue lysates were prepared from tumor tissue microbiopsies (~1000 cells) using lysis buffer that contained protease inhibitors. By focusing on unique proteomic profiles, GFP–AuNP sensors were able to detect site-specific metastatic tissues from healthy ones with high sensitivity, providing a potentially rapid and effective tool for clinical diagnostics [31].

Urine samples

Many cancer diagnoses are accomplished through excisional biopsy. Despite the usefulness of this method, these techniques are unpleasant for patients. Therefore, efforts have been made to achieve sensing methodologies using noninvasive biofluids. Noninvasive biofluids are easily-accessible biological samples, which usually do not involve instrument insertion into the patient's body. Besides breath, urine, saliva and sweat, other minimally invasive procedures such as nipple aspiration along with ductal lavage can provide many cells collected from the milk ducts [32].

Currently, NP array-based sensors have focused on the use of urine [10] and breath [33] as samples in cancer diagnosis. Although urine is an attractive noninvasive biofluid for analysis, concentrations of naturally occurring biomarkers are typically low. To compensate for this limitation, an array of sensors with the ability to amplify biomarker concentration in urine has been developed [9]. For this purpose, a class of engineered mass-encoded peptides with specific protease-sensitive moieties conjugated to iron oxide NPs were synthesized. These synthetic biomarkers passively accumulate in the cancer tissue after administration. Aberrantly active proteases in the tumor subsequently cleave the protease-sensitive agents of

these NPs, with the resulting fragments excreted in the urine (Figure 5) [34]. Using a library of different substrates as the protease-specific mass signatures, differentiation between different proteases was possible. The unique profiles of the isobar-coded reporters for each protease were determined by Pearson's correlation analysis.

Breath samples

Breath sensing is perhaps the least invasive of all diagnostic strategies. Metabolic reactions in the body generate different volatile organic compounds (VOCs). These VOCs include hydrocarbons, alcohols, aldehydes, ketones, esters, nitriles and aromatic compounds. VOCs can be detected in diverse biosamples, such as cancer cells, blood, urine, skin/sweat [35,36] and breath. In fact, besides cancer detection, VOCs are being used as new diagnostic-based biomarkers for detection of different diseases such as diabetes [37], Alzheimer's, Parkinson's [38], and chronic kidney disease [39].

Compared with healthy individuals, cancer patients express different VOC compositions in their breath due to the different activities of cancer cells [33]. These activities can generate very subtle changes in the concentration and composition of VOCs in the blood stream. Through constant exchange between the lung and bloodstream, these subtle changes in VOC compositions can be transported to the patient's breath, creating distinct breath signatures for each cancer type [40]. In a healthy breath, the concentration of several VOCs are normally in the range of 1–20 p.p.b. [11]. However, they can be detected in the levels of 10–100 p.p.b. in some cancer types. These changes in concentration and composition mixture of VOCs have made it possible to not only distinguish between the breath of healthy individuals and cancerous patients, but also differentiate between different types of cancer [11,33]. Previously, VOCs have been detected using gas sensors such as gas chromatography [41], and ion mobility spectrometry [42]. However, the downsides to these methods are that they are time consuming and require large size, expensive instrumentation and an expert operator. Moreover, to improve the detection in some of these devices, capturing and preconcentrating the breath sample is a prerequisite step [43,44].

Using the advantages of NPs, a nanoscale artificial nose has been designed by Haick and co-workers [44]. This simple, cost effective and portable sensor is able to detect cancer by analyzing the VOCs using pattern recognition methods. The nanoscale artificial nose is capable of identifying different odors even at very low concentrations and subtle differences [44]. The gas sensor is based on an array of highly cross-reactive chemiresistors made of AuNPs with different organic capping layers [11]. In the resulting sensor, electrical conductivity was provided by the metallic particles and the organic capping layers create sites used to capture the analytes (Figure 6) [11].

Due to the chemical diversity of sensor materials, each sensor of the array shows a unique response to a certain group of VOCs. This means that the characteristic signal (electrical resistance) of each sensor in the array changes specifically when exposed to a specific VOC, which could be the cancer specific odor (Figure 7) [44]. Consequently, for each cancer type, a distinct fingerprint is produced from the array of cross-reactive sensors. Using this gas sensor along with pattern recognition methods, it is possible to discriminate different cancer types and stages (Figure 8) [33,45]. It is also worth mentioning that these sensor arrays have

detection limits of 1–5 p.p.b. or even down to approximately 10 p.p.t. [33]. Breast, lung, colon, gastric, colorectal, head-and-neck and prostate cancer are the cancer types that have been detected using this sensor [33,44]. Carbon nanotube arrays have been used in a similar fashion [46,47].

It should also be mentioned that although breath sensing is a novel method for cancer detection, the approach has some limitations. First of all, there are not dramatic changes in VOCs in the early stages of cancer development, only certain stages will cause the expression of these VOCs. Second, the conditions and type of foods and drinks consumed by patients can influence results. The results can also be affected if the patients have other diseases and are using other medicines [33]. Therefore, having sufficient controls over sample collection is essential when using this type of noninvasive sample.

Conclusion

The challenges in the fight against cancer in the last century have revealed a pressing need for personalized screening devices. In this review, we have discussed a variety of array-based sensing strategies employing NPs in cancer diagnosis. In theory, all sophisticated sensors should be able to provide detailed geno- and pheno-typic information. One of the major advantages of these strategies is their versatility. The versatility of an array-based strategy enables the sensor to recognize a wide range of analytes, but at the same time, it comes with a challenge: the sensor needs to be retrained and validated for each new analyte. Careful examination of baseline profiles for known samples is also a vital step in training the sensor to ensure accuracy in identification of unknown samples.

Future Perspective

Array-based sensing provides a valuable partner to traditional biomarker sensing. These chemical noses provide rapid and effective bioprofiling, an important tool in personalized diagnostics. The use of selective sensing also eliminates the need for complex (and unstable) biomolecular systems used in traditional immunosensing, providing a potential new tool for global medicine.

Array-based sensing strategies face challenges before they are accepted in the clinic. First and foremost, these sensor systems are essentially hypothesis free, an approach that makes many researchers uncomfortable. This issue will presumably be ameliorated by the continued successes generated by this strategy. Array-based sensors also share some of the challenges in reproducibility faced by traditional strategies. Inconsistencies in dealing with human specimens can significantly alter their molecular composition and interfere with the reproducibility of the scientific results. Therefore, several guidelines have been established in the clinical community to better address the consistency of human biospecimen handling, such as Biospecimen Reporting for Improved Study Quality (BRISQ) and Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) [48].

Taken together, the success of array-based sensor development will require the convergence of interdisciplinary knowledge in biology, chemistry and statistics. Advances in pattern

recognition sensing will then be truly transformative, making rapid and highly personalized diagnosis a reality.

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- of interest;
- of considerable interest.

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Executive summary

Background

- Detection of cancer in its early stages can improve survival rates by allowing the use of more favorable treatment options.
- Cancer geno- and pheno-type can drastically influence therapeutic options for an individual. Therefore, traditional medicine will be shifted to highly personalized therapy.
- To promote personalized medicine, development of personalized diagnostic methodologies is essential. Among a variety of cancer diagnosis methods, nanoparticle (NP)-based sensors have shown to be promising for cancer detection.
- At present, there is no ideal biomarker in cancer diagnosis to be effectively used in conventional single specific biomarker-focused strategy.
- By combining sensor responses to the analyte (either specific or selective inputs), NP array-based sensors do not have to rely on single specific biomarkers in cancer diagnosis.

Basics of array-based sensing using NPs

- Biosensing consists of two requisite processes: analyte recognition and transduction.
- A multivariate data matrix is obtained from different analytes and analyzed using multivariate chemometric methods (mostly principal component analysis [PCA] and linear discriminant analysis [LDA]).

Array-based cancer diagnostics

- Detection of a wide range of cancer cell surface, solid tissue and noninvasive samples (such as urine and exhaled breath) has been successfully demonstrated using NP array-based sensors.

Conclusion & future perspective

- A wide range of analytes can be detected due to the versatility of array-based strategy, which requires the sensor to be retrained and validated for each new analyte.
- The success of array-based sensor development will require the convergence of interdisciplinary knowledge in biology, chemistry and statistics.

Figure 1. Chemical nose sensor

A sensor array composed of different recognition elements that possess varying binding affinities to different analytes provides the basis of this method. As a result, distinct patterns of analytes are generated based on different binding affinities of analytes toward the sensor array. These patterns (fingerprints of each analyte) are processed by multivariate analysis methods. After reducing the data matrix dimensions, different clusters of the analytes can be visualized, demonstrating a successful classification.

Figure 2. Mechanism of detecting cancer cell types using green fluorescent protein–gold nanoparticle sensor array
(A) Structures of the NPs with different head groups R used as an array of recognition elements in the sensor. **(B)** Structure of GFP used as the transducer of the sensor array. **(C)** Schematic illustration of GFP–NP complexes for cell surface sensing. The fluorescence from GFP is turned off once GFP–NP complexes are formed due to the strong quenching property of gold NPs. Due to competitive binding between cells and GFP to the NPs, different amount of GFP are being released in the presence of different cell lines based on their binding affinities.

GFP: Green fluorescent protein; MW: Molecular weight; NP: Nanoparticle; pl: Isoelectric point.

Adapted with permission from [26].

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Figure 3. Structures of magnetic glyconanoparticles with different carbohydrate ligands

MGPN: Magnetic glyconanoparticle.

Data taken from [29].

Figure 4. Plot generated from linear discriminant analysis plot for ten cell line differentiation using magnetic glyconanoparticle sensor array

3D linear discriminant analysis plot of T2 patterns generated from the magnetic glyconanoparticle array after different cell line incubations (10^5 cells/ml). T2 patterns of different cell lines were reduced into simpler components, linear discriminants (LD1, LD2 and LD3). Full differentiation of the ten cell lines was achieved.

LD: Linear discriminant.

Adapted with permission from [29].

Figure 5. Use of synthetic biomarker-conjugated nanoparticles for urinary monitoring
(A) Synthetic biomarker library conjugated onto nanoparticles (NPs). **(B)** Accumulation of NPs in disease tissues. Cleavage of the mass-encoded peptides from NPs by active proteases allows their filtration into the urine. **(C)** Detection of biomarker peptides in urine using LC-MS/MS.

h ν : UV light irradiation; LC-MS: Liquid chromatography-mass spectrometry; MS: Mass spectrometry.

Adapted with permission from [10].

Figure 6. Illustration of the diagnosis of lung cancer using breath testing

(A) (i) Photograph of the array of chemiresistors; (ii) Scanning electron microscopy image of a chemiresistor; (iii) Scanning electron microscopy image of gold nanoparticle film located between two adjacent electrodes; and (iv) Transmission electron microscopy image of the monolayer-capped gold nanoparticles. (B) Testing of the exhaled and simulated breaths.

MFC: Mass flow controllers.

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Figure 7. Typical responses of the breath sensor

(A) Schematic representation of the gold nanoparticle sensors (not drawn to scale). Typical resistance responses of sensors functionalized with: (B) 2-ethylhexanethiol; (C) decanethiol; and (D) 2-mercaptobenzoxazole, to the breath of healthy individuals as well as patients with LC, CC, BC and PC.

BC: Breast cancer; CC: Colon cancer; LC: Lung cancer; PC: Prostate cancer.

Reproduced with permission from [33].

Figure 8. Classification results of the sensor array's resistance response

Principle component analysis plots of the sensor responses to: **(A)** LC and healthy controls; **(B)** CC and healthy controls; **(C)** BC and healthy controls; **(D)** PC and healthy controls; and **(E)** all cancer patients and healthy controls together.

BC: Breast cancer; CC: Colon cancer; LC: Lung cancer; PC: Prostate cancer; PC1: Principal component 1; PC2: Principal component 2.

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