A brief review on the nanomaterials-based detection of CKD biomarkers

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Abstract. Chronic kidney disease (CKD) imposes a significant worldwide medical burden, exacerbated by the often limited efficacy of current treatments. The future prevention and management of CKD critically rely on early detection and effective intervention. Nanomaterials (NMs), such as fluorescence carbon dots (CDs), quantum dots (QDs), and metal-based NMs, emerge as unique and highly sensitive probe materials expected to play a substantial role in the precise identification of CKD biomarkers. NMs' design makes it possible to manage properties including size, shape, charge, and targeting ligands, which enhances the biological compatibility and availability of medicines. Consequently, the rise of NMs in medicine has brought about fresh approaches to CKD diagnosis. This review explores the utility of the NMs for the rapid identification of CKD biomarkers.

1 Introduction

In terms of the evolution of kidney function, kidney disorders can be broadly categorized into acute kidney injury (AKI) and chronic kidney disease (CKD) [1]. AKI is characterized by an elevation in serum creatinine levels beyond the optimal concentration, which is typically between 0.7 and 1.3 $mg.L^{-1}$ for adult males and 0.5 to 1.1 $mg.L^{-1}$ for females [2]. Common causes of AKI include urinary blockage, hypovolemia, and medication toxicity [3]. On the contrary, CKD signifies the enduring decline of kidney function, characterized by a low glomerular filtration rate (GFR) (much less than 60 mL per min per 1.73 m^2) or albuminuria (greater than 30 mg/24 hours) persisting for greater than three months [4,5]. Nearly 10% of people across the globe have been diagnosed with CKD with higher prevalence in the elderly [6]. Consequently both the affected people and society are put under a heavy financial burden [7]. Therefore, early CKD diagnosis and prompt disease progression prevention have emerged as top priorities in the management of CKD.

CKD biomarkers are essential for the diagnosis and follow-up of kidney diseases. These biomarkers include (a) conventional CKD biomarkers like serum creatinine and proteinuria and (b) Molecular biomarkers includes N-acetyl-D-glucosaminidase (NAG) [8], kidney injury molecule-1 (KIM-1) [9], neutrophil gelatinase-associated lipocalin (NGAL) [10], cystatin C (Cys C) [11], and so on. Molecular biomarkers have garnered significant

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recognition in recent years for their precise and early diagnostic capabilities in identifying kidney disease.

NMs can offer reliable and consistent monitoring of kidney shape and function by means of enhanced renal targeting, retention, and localization of medications [12]. Consequently, this field is developing into an advantageous tool for medical applications like the detection of biomarkers, renal imaging, drug delivery, gene therapy, chronic diseases treatment, tissue engineering, and regenerative drugs [13]. The conventional diagnostic techniques, however, have a lot of drawbacks, including insensitivity and discomfort [14]. The detection of CKD biomarkers has traditionally relied on methodologies like mass spectrometry, electrophoresis, and immunoassays [11]. While these methods demonstrate outstanding sensibility and accuracy, they are often time-consuming, costly, complicated, and bulky [15]. Recognizing the need for innovative approaches in CKD diagnosis, NMs have emerged as a promising tool for constructing a highly tunable detection platform [14]. NMs present several advantageous properties for CKD biomarkers detection, including tunable surface characteristics, ultrafine dimensions, unique optical and luminescent spectra, as well as distinctive catalytic, thermal, magnetic, and electrical aspects [16]. Their modular nature allows for easy incorporation into sensor arrays, capitalizing on their chemical versatility and simple fabrication, ultimately enhancing sensitivity for target analytes [17]. NMs may therefore be crucial for the extremely specific rapid diagnosis of CKD. This review describes the importance of NMs in the identification of CKD biomarkers that will help in the early diagnosis of CKD. The article will offer a path forward for further research studies into immunoassay development for the determination of CKD-specific molecular markers in the context of clinical trials.

2 Applications of nanomaterials in the identification of advanced molecular biomarkers of CKD

It is thought to be essential to evaluate early and particular markers in order to forecast the swift development and rise of nephritis. Employing efficient intervention therapies to reduce CKD and mitigate associated consequences, e.g., infection, hyper stress, cardiac failure, and anaemia, is imperative. Notably, NMs can be utilized for detecting molecular CKD biomarkers through diverse signal readout methods, including electrochemical method, surface-enhanced Raman scattering (SERS), colorimetric method, fluorescence spectroscopy, and various others. Albuminuria constitutes a perilous factor influencing both the initiation and advancement of CKD [18]. Micro-albuminuria can be identified through various specialized antibody techniques or specific urine albumin dipsticks [19]. However, these techniques are often both harsh and inconvenient. Consequently, SERS has been proposed as a highly sensitive, non-destructive, and selective method for detecting CKD biomarkers [20]. In this context, SERS entails the inflexible dispersion of incoming laser beams on metal surfaces, includes copper, silver, and gold nanoparticles (NPs). Excellent sensibility, easy preparation of the samples, quick analysis, and the ability to bought accessible Raman spectrometers are just a few benefits that come with SERS [21]. Stefancu and colleagues documented a robust correlation between SERS peaks corresponding to anticipated and actual albumin levels, yielding a root-mean-square error for predicted value of 2.82 [22]. This discovery underscores the reliability of the SERS technique in identifying albuminuria. The remarkably lowest limit of detection (LOD) value of 3×10^3 ng·ml^{-1} for albumin quantification highlights the superior performance of SERS methodology compared to conventional strategies in detecting microalbuminuria. Presently, commercially available tools facilitate the convenient screening of micro-albuminuria at the point-of-care [23]. A recently developed point-of-care evaluation approach for micro-albuminuria provides a

portable electrochemical-based immunosensor that is simple to use. To improve the physiological compatibility and conductive properties of the electrodes, the immunosensor incorporates polystyrene/silver/anti-human serum albumin antibody (PS/Ag/HAS-Abhas) nanoclusters (Figure 1) [24]. The sensor demonstrated the capability to detect albumin within the range of 1×10^4 - 3×10^5 ng·ml^{-1}, achieving a LOD of 97×10^2 ng·ml^{-1}.

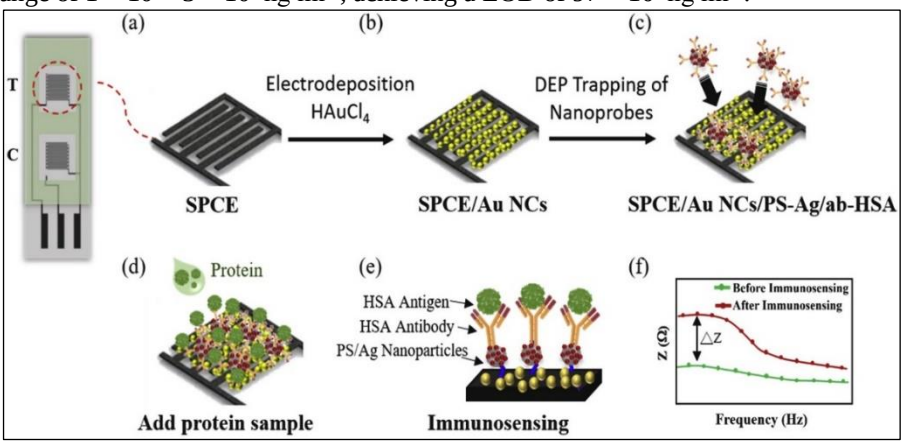

Fig. 1. A schematic showing a systematic process for the creation and use of immunosensors. With permission from [24]. Copyright © 2018 Elsevier B.V.

CKD could be predicted accurately by promising molecular biomarkers including NAG, Cys C, NGAL, and KIM-1 [25]. According to a growing body of research, NMs may be employed to boost an immunosensor's response while looking for these biomarkers [26–28]. The biomarker KIM-1 which represents the development of renal damage and recovery was quantified utilizing an electrochemiluminescence (ECL) biosensor [28]. Further, an environmentally friendly amperometry immunosensor was created to use a sandwich-style test to find Cys C in human serum [26]. These authors created an enhanced response by layer-by-layer building using gold NPs, enabling the immunosensor to assess Cys C with high sensitivity (as shown in fig. 2).

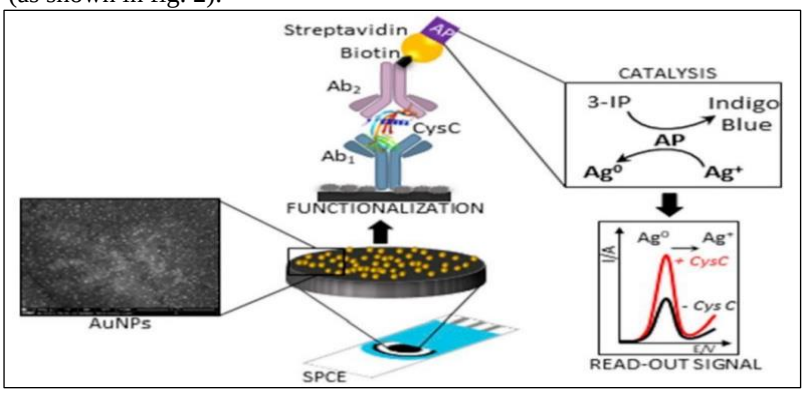

Fig.2. Diagrammatic representation of the CysC immunosensor design and working principle. With permission from [26]. Copyright © 2019 Elsevier B.V.

The present clinical assessment procedures for CKD could therefore be improved and supplemented by NMs, thereby facilitating early detection of CKD biomarkers. In this context, Desai et al. employed papain (a cysteine protease) to immobilize carboxyl-enriched multi-walled nanotubes made of carbon on a screen printed electrode display. [29]. The amino acid segment of papain was covalently immobilized onto the electrode surface through a carbodiimide coupling reaction. Following this, Cys C and papain interacted within a 10-

minute timeframe, resulting in an electrochemical response. It was found that the LOD for the Cys C estimate was 5.8×10^{-4} ng mL^{-1}. Recently, Yin and colleagues introduced a method using europium nanoparticles (Eu NPs) and linked monoclonal antibodies (mAbs) (1G1 and 2F4) to develop a rapid and reliable lateral flow sandwich immunoassay for the early identification of NGAL in urine [30]. A mAb (2F4) was employed for detection of Eu-NPs-1G1-antigen mixture on the T-line after conjugation of mAb 1G1 and Eu-NPs. It was determined that the LOD for the NGAL determination was 0.36 ng mL^{-1}. Later, SERS was used to construct a molecular-based specialised immune assay for determining the presence of NGAL in human serum and urine [31]. NGAL antibodies were captured by means of amide bonds using SERS-activated base materials that were modified silver chips containing 4-mercaptobenzoic acid. The LOD and LDR of the assay was computed as 10 ng·mL^{-1} and 0–1000 ng·mL^{-1}, respectively. Additionally, it was claimed that an electrochemical immunosensor with higher specificity could detect NGAL in human urine samples. [32]. The transducer used in this investigation was a screen-printed electrochemical cell with two miniature working electrodes. In terms of NGAL estimation, the synthesised biosensor obtained a minimal LOD of 0.096 ng·mL^{-1}. Furthermore, by immobilising the rabbit lipocalin-2 antibody (polygonal) on the gold NPs attached to subsequent generations-1 polyamidoamine (PAMAM) dendrimer molecules (LA2/AuNPs/PAMAM)-modified with gold electrodes, a electrochemical-based immunosensor for identifying amounts of NGAL in samples of human serum and urine was developed [33]. The synthesised immunosensor demonstrated a broad range (50-250 ng·mL^{-1}), extended sustainability, and outstanding sensitiveness (1 ng·mL^{-1}). Further, the up-converting phosphor (UCP) NPs (lanthanide-doped NPs) were combined with LFIA diagnostic strip to determine the amount of NGAL in urine and serum samples of human [34]. This UCP technology-based LFIA displayed a rapid response to NGAL with detection limit and range of 7.68 ng·mL^{-1} and 7.68 to 1000 ng·mL^{-1}, respectively.

In a similar way Saeed et al. constructed an electrochemical detection system for sensing of albumin that relies on nanorods made of cobalt-tellurium (CoTe) [35]. The albumin present in urine specimens of CKD patients was quantified using a CoTe-customized glass carbon electrode (CoTe-GCE). The electrochemical device demonstrated linearity, detection, and quantification limits of 63.91 ng·mL^{-1}, 5.985 ng·mL^{-1}, and 0.19 ng·mL^{-1}, respectively. The sensor exhibited exceptional stability, with minimal changes in response observed over the course of 100 cycles. Additionally, individuals with diminished glomerular filtration rate (GFR) exhibit elevated serum uric acid (UA) levels [36]. UA represents the ultimate oxidation product of purine metabolism and is excreted through renal processes [37]. However, a contemporary perspective suggests that UA may not only be a consequence but also an active contributor to the pathophysiology of CKD and potentially acute kidney injury [38]. Recently, UA was quantified by nitrogen-doped carbon dots (N-CDs) in human serum and urine specimens through a ratiometric fluorescent (FL) and colorimetric strategy [39]. The FL excitation and emission maxima of the as-prepared N-CDs were reported at 330 and 430 nm, respectively. The synthesized biosensor relies on the uricase-catalyzed oxidation of UA to generate hydrogen peroxide (H_2O_2), subsequently converted to hydroxyl radical (·OH) by I- (iodide ions). The ·OH species then oxidize o-phenylenediamine to form 2,3-diaminophenazine (DAP), resulting in distinct yellow emissions at 580 nm. The presence of DAP leads to the quenching of the FL of N-CDs at 427 nm due to the inner filter effect. Consequently, the FL emission of the N-CDs decreases with the concentration of UA. The synthesized biosensor demonstrates strong linearity for UA over a range of 84.05×10^3 to 25.22×10^6 ng·mL^{-1}, with a detection limit of 10.09×10^3 ng·mL^{-1}. Further, Rezaei *et al.* reported chemically synthesized gold NPs-based sensor for quantification of UA [40]. The sensor response was optimized in terms of various parameters, such as pH, solution

concentration, and time. Under optimized conditions, the calibrated plots showed linear stability and LOD values as 500 - 10000 ng·mL^{-1} and of 200 ng·mL^{-1}. These findings demonstrated the high accuracy and flexibility of this NMs based approach for UA measurement in urine samples (Figure 3).

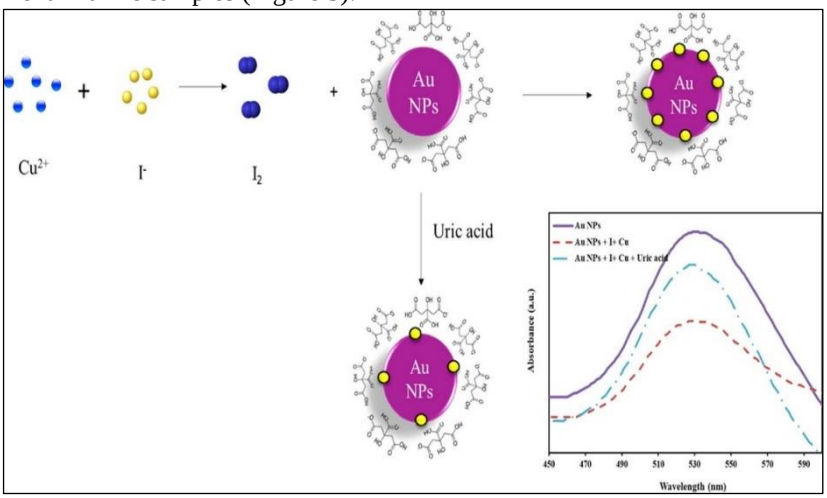

Fig. 3. Schematic representation of sensing of uric acid in human urine samples by the chemically synthesized gold NPs. With permission from reference [40]. Copyright © 2022 Elsevier B.V.

3 Conclusion and future perspectives

NMs have facilitated the detection of biomarkers of CKD, especially in the laboratory scale. The combination of NMs with CKD biomarkers (as mentioned previously) may enhance the sensitivity and selectivity of the bioassays by various techniques including SERS, fluorescence, and colorimetric recognition. Nonetheless, the widespread acceptance of NMs-based diagnosis of CKD biomarkers is still in infancy as majority of the reported data requires strict validation under clinical settings. The feasibility of such biosensors for commercial and mass scale monitoring of CKD biomarkers still remains a puzzle owing to numerous fundamental drawbacks of this technology. These include – highly expensive synthesis of NMs, poor recovery in pure form, loss of activity of the biological molecules upon interfacing with NMs, and many more. As a result, a great deal of efforts is still needed to move the NMs-based biosensors from lab to industry.

Acknowledgement

This work is supported by University Center for Research & Development (UCRD), Chandigarh University.

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