Nanomaterial-functionalized Cellulose: Design, Characterization and Analytical Applications

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Cellulose-nanomaterial hybrid systems are promising platforms for the development of portable devices that can be used for fast and inexpensive analysis in the clinical, environmental and food monitoring fields. By combining the chemical and physical properties of the cellulosic network with the unique optical, electrical and catalytic functions of nanomaterials, it is possible to create versatile devices with engineered sensing functions. This review describes the most commonly used types of nanomaterials, their unique properties and assembly in hybrid structures in conjunction with cellulose paper and provides an overview of the most commonly used detection methodologies and their performance for selected applications. Finally, future perspectives and challenges to the implementation of these devices for real world applications are discussed, with focus on method optimization, validation and regulation in order to reach consumers.

Keywords Cellulose paper, nanoparticles, portable sensors, printable paper sensors, optimization, validation

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composed primarily of porous networks of interconnecting cellulose pulp fibers consisting of β-anhydroglucose units with pendant hydroxyl (OH) groups which enable facile modification, imparting additional functionality for expanding the range of applications. The biodegradability of cellulose paper and its abundance in nature continue to drive research and applications of this material in various fields. Despite these general features, the properties of cellulose-based papers are diverse and can have varying flow rates, sizes and retention times which can all influence the performance of analytical devices. Additionally, it is possible to modify the intrinsic properties, porosity, size and composition of the native cellulose structure by chemical (e.g., solvent treatment) or thermal treatment resulting in cellulose-derived materials with entirely new properties and functions. A summary of the different types of paper utilized for microfluidic devices has been reviewed by Mace et al. The majority of analytical applications are based on Whatman paper (Fig. 1), while the use of treated cellulosic material is scarcely reported, mainly in the form of pyrolyzed paper. This paper will focus primarily on cellulose-based analytical devices that incorporate nanomaterials for generating signal, their performance and applications.

Nanostructures can be diverse in elemental composition, size, morphology, oxidation state or shape which allow them to be tuned in order to maximize reduction/oxidation, optical or catalytic properties. A variety of nanomaterials can be used, with the most prevalent being: i) metals such as silver (Ag), platinum (Pt), gold (Au) and palladium (Pd), ii) metal-oxides like silica (SiO\textsubscript{2}), titania (TiO\textsubscript{2}), ceria (CeO\textsubscript{2}) and iron oxides (Fe\textsubscript{3}O\textsubscript{4}) as well as iii) carbon, semiconducting and polymer-based materials and composites such as carbon nanotubes, graphene and quantum dots. A summary of their main properties is provided in Table 1. Integration of nanomaterials with cellulose fibers provides additional functionality to paper substrates enabling new properties such as conductivity, fluorescence, catalytic, antibacterial and redox functions (Fig. 2). The combination of cellulose with nanomaterials can also increase mechanical and thermal stability of the native cellulose material. For example, a 50% improvement of tensile strength was achieved using cellulose/graphene composite fibers fabricated with only 0.2% loading of graphene. Two general strategies can be used to fabricate nanomaterial-based devices. One approach is to use pre-synthesized nanomaterials and interface them with paper, or, alternatively it is possible to perform in situ synthesis, a method that has been demonstrated recently with AgNPs. In addition, chemical or biological receptors can be integrated within the platform to provide selectivity. The main challenge to create functional analytical devices is to maintain both the recognition properties of the immobilized molecular receptors as well as the transduction functions of the nanomaterials. Fabrication of such devices requires the development and characterization of an appropriate nanostructured assembly, attachment of selective receptors, generation of response and sensing functions.

Ideally, these devices should incorporate all reagents needed for analysis (e.g., reagent-less), be used without external equipment and require only low sample volumes.

2 Device Fabrication

2.1 Classes of nanomaterials for paper-based analytical devices

The majority of analytical devices on paper platforms with NP detection are utilizing metal NPs, primarily Ag and Au, which provide unique optical features for color-based detection as well as conductivity and catalytic properties. These NPs have attracted considerable attention for optical detection because they both have a wide range of colors and can be tailored by changing properties of the particles such as size and shape. Au-based detection has also been reported with surface-enhanced Raman scattering (SERS) on surface functionalized with biomolecular receptors. Development of SERS-based detection on paper is a recent development illustrated by a few examples of lateral flow strips. Detection of neuron-specific

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Table 1 Outlines properties from different types of nanomaterials that can be utilized with a cellulose-paper sensing platform

| Application | Property | Nanomaterial |
|-------------|----------|--------------|
| Optical     | Visible color | AuNPs, AgNPs, CeO\textsubscript{2}NPs |
|             | Fluorescence  | Quantum dots (QDs) |
| Medicinal   | Biocompatibility | Polyethylene glycol coated NPs |
|             | Low toxicity  | SiO\textsubscript{2}NPs |
|             | Anti-bacterial | AgNPs, CuNPs |
|             | Anti-fungal   | TiO\textsubscript{2}-chitosan nanocomposites |
| Physical    | Electrical conductivity | Carbon nanocomposites |
|             | Thermal conductivity | Silicon carbide nanowire |
|             | Insulating ability | SiO\textsubscript{2}NPs |
|             | Redox activity  | CeO\textsubscript{2}NPs |
|             | Catalytic activity | PtNPs, PtNPs, CoNPs |
|             | Magnetic activity | Fe\textsubscript{3}O\textsubscript{4}NPs |
|             | High adsorption capacity/surface chemistry | CeO\textsubscript{2}NPs, ZrO\textsubscript{2}NPs |
|             | High thiol affinity | SiO\textsubscript{2}NPs |
|             | pH dependent sensitivity | AuNPs, PEI-coated NPs |

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Fig. 1 The most commonly used commercial papers used for microfluidic analytical devices (permission from Ref. 5).
functionality and catalytic properties. Oxides such as TiO$_2$ known mainly for their adsorption properties, rich surface platforms for detection of enzyme substrates and electrostatic interactions which when combined with their potential to increase stability of enzymes through strong analytical sensing applications. Optical and catalytic properties can provide a powerful sensing analytical devices. Oxides of Mg, Zn and Zr have shown porosity of the paper had a determinant role on the distribution of the Ag nanostructures along the paper fibers and that using a well patterning technique it was possible to achieve good signal uniformity for paper-induced aggregation. In addition to Au and Ag, other metal particles such as those based on platinum, palladium, cobalt and nickel can be also utilized primarily for their catalytic properties and can be incorporated primarily for paper for analytical sensing applications.

Other nanomaterials are those based on metal oxides that are known mainly for their adsorption properties, rich surface functionality and catalytic properties. Oxides such as TiO$_2$ and Fe$_3$O$_4$ have high adsorption capacity for heavy metal ions and small molecules and can be used as capture and concentration sites for increasing detection sensitivity of analytical devices. Oxides of Mg, Zn and Zr have shown potential to increase stability of enzymes through strong electrostatic interactions which when combined with their optical and catalytic properties can provide a powerful sensing platform for detection of enzyme substrates and inhibitors. Some oxides such as those based on cerium are redox active at the nanoscale and can function both as oxidation and reduction catalysts. These oxides are present in dual oxidation states (Ce III/IV) and can replace oxidase and peroxidase enzymes in sensing schemes for detection of hydrogen peroxides and oxidase enzyme substrates on filter paper functionalized with these materials through silane bounds. In addition, CeO$_2$ NPs are able to react selectively with catechol-containing molecules which makes them interesting sensing materials for detection of polyphenol antioxidants on paper, in the absence of exogenous materials or soluble redox dyes. Another popular oxide for biosensing is MnO$_2$ which has found many applications in the development of optical sensors for antioxidant detection in serum, and of enzymatic sensors for detection of glucose using redox dyes like 3,3',5,5'-tetramethylbenzidine (TMB). In a recent example, MnO$_2$ nanosheets have been used as an oxidase-mimetic material that can oxidize TMB in the absence of horseradish peroxidase and H$_2$O$_2$ to develop a paper-based strip for detection of acetylcholinesterase (AChE) activity. In the presence of AChE, the enzyme substrate, acetylcholine triggered the decomposition of the nanosheets, decreasing the absorbance of TMB.

Large classes of nanomaterials are those based on carbon, polymers, fluorescent quantum dots and composites of these materials. These are widely available or can be synthesized on demand for niche applications. Carbon based materials in the form of single wall and multiwall carbon nanotubes (SWCNTs and MWCNTs), and graphene have been largely used in electrochemical applications. Polymer based nanomaterials are valuable for ion chelation and detection. These platforms can be used separately or in conjunction with metal or metal oxide NPs for enhancing performance of sensing devices. Examples of such hybrid materials include MWCNT-ZnO, Au/Pd nanocubes-SWCNTs, and gelatin dispersed MWCNT-GOx which have been used in glucose oxidase based sensing for detection of glucose. Liu et al. created MWCNT-cellulose films by combining cellulose with MWCNTs, sodium hydroxide and urea followed by treatment with 5% sulfuric acid. The resulting films were used to determine percent water content in water-ethanol solutions. The hygroscopic swelling caused by the hydrophilic cellulose was measured by monitoring relative resistance change when the composite was immersed in water and dried. These films are reversible and can find applications as water sensors or water-smart materials. Meyyappan et al. demonstrated two techniques for preparing CNT-cellulose. The first method involves depositing a CNT solution on the surface of the cellulose in a layer by layer (LbL) assembly technique. The second preparation involved soaking of cellulose in CNT solution with ultrasonication to form a CNT-cellulose composite. Both approaches were used to determine ammonia using resistance produced by ammonia adsorption on the surface of the paper. The authors could determine concentration of ammonia down to 5 ppm levels. It was found that the LbL modified CNT-cellulose had a higher sensitivity because of the

![Fig. 2 Summary of potential applications of nanomaterial-functionalized cellulose analytical devices.](image)
concentration of the CNT on the surface of the cellulose which created a larger surface for reaction with urea. Merkoçi et al. have introduced graphene quantum dots (GQDs) embedded into a nitrocellulose matrix for chemical screening. The GQDs were synthesized by pyrolyzing citric acid at 200 °C and lowering the pH below 9. The GQDs were dropped onto wax contained nitrocellulose to create the GQD-cellulose. The fluorescence of the GQDs-cellulose was quenched by 4-nitrophenol and paraaxon with limit of detections 40 and 44 μM respectively. The quenching was monitored by a mobile phone camera and fluorescent response was produced by both UV LEDs and UV lamp with comparable sensitivity.

2-2 Modification of cellulose paper

Modification of cellulose paper can improve homogeneity of the paper surface and is a necessary step for adding functionality for further grafting of molecular receptors. However, modification of cellulose is not limited to building onto its structure; cellulose’s structure can also be degraded which allows for more applications. One type of destruction cellulose modification is pyrolysis. Heating cellulose paper to high temperatures (1000 °C), without the presence of oxygen, creates a dense carbon material. Garcia et al. have demonstrated that pyrolyzing paper, under reductive conditions, produces carbonized cellulose fibers. These fibers have been decorated with CuNPs and used as electrodes. The CuNPs composite paper electrodes showed an increased electrochemical signal for detection of glucose compared to a bare carbon electrode. The sensor displayed a LOD of 5 μM for glucose and had the capability to selectively detect glucose in beverages even in the presence of dyes and chemical preservatives. Additional modifications can be achieved using different types of solvents. There is substantial research on the derivation of cellulose with ionic liquids (ILs). IIls can also be used to partial dissolve cellulose and chemically modify the surface of the paper. Takahashi et al. produced cellulose triacetate demonstrated by adding isopropenyl acetate (IPA) and 1-ethyl-3-methylimidazolium acetate (EmimOAc) to cellulose. This type of modification does not severely change the surface of the cellulose and allows for direct reactions with the modified cellulose surface. In addition to conventional cellulose papers like the widely used Whatman filter paper, there are number of other forms of cellulosic materials that have potential and can be used for analytical applications. An example is nanofibrillated cellulose composed of nanosized fibers instead of microfibrils.

This type of cellulose can be extracted from lignocellulosic biomass. Fibril size, conformation and structural packaging differences cause nanofibrillated cellulose to have a significantly higher tensile elasticity and optical transparency compared to other forms of cellulose paper. These properties could provide many advantages for flexible and optical sensing applications in the future. Moreover, there is increased interest in using nanocellulose in various applications and interest in analytical applications of these materials continues to grow. Although many such materials are not yet fully explored for fabrication of paper based analytical devices, nanoscale cellulose or thermally chemically-treated cellulose can offer a unique form of cellulosic material with potentially useful functionalities and added properties.

With respect to conventional cellulose paper, there are a variety of surface chemistry approaches that can be used graft or covalently immobilize nanomaterial and/or biomolecules onto cellulosic filter paper, through functionalization of the hydroxyl groups of its backbone. Figure 3 shows an example of a simple approach of ultrasound assisted coating of paper with ZnONPs (~20 nm) without the aid of a binder. The ZnO coated paper has shown antibacterial activity against Escherichia coli 11634. Figure 4 provides an example of a fabrication procedure of a microfluidic paper-based analytical devices (μPADs) that involved treatment of the paper chip with a nanomaterial colloidal solution of Fe3O4NPs, MWCNT and graphene oxide (GO) to create a biocompatible layer with high catalytic activity.

Evans et al. modified μPADs with SiO2NPs to improve color intensity and uniformity of a colorimetric device applied to three different enzymatic reactions with clinical relevance (lactate, glucose, and glutamate). Sharpe et al. developed novel chemical sensing arrays based on redox active metal oxide NPs as a portable and inexpensive paper-based colorimetric method for polyphenol detection and field characterization of antioxidant containing samples (Fig. 5). The metal oxides used to fabricate the array have activated hydroxyl surface functionalities that can attach hydroxyl rich polyphenols by forming surface stabilized complexes with enhanced charge transfer properties.

Attachment of biomolecules to paper can be achieved by modifying the paper surface with silanes. This method facilitates protein immobilization and prevents leaching. In a typical procedure, a silane ((3-aminopropyltriethoxysilane (APTES)) coupling reaction is carried out to introduce amine groups onto the surface. A TiO2 silane modified filter paper (STCP) was
used to attach bovine serum albumin capped gold nanoparticles (BSA-AuNPs), leading to the fabrication of BSA-AuNPs decorated membrane (BSA-Au NPs/STCP) that was further used to detect chromium(VI) ions. Other examples of modification include: gold (Au) amalgam layer formation for dual signal amplification detection and fast removal of mercury(II), thin film coatings for detection of peroxide vapors, particle impregnation of cellulose for antimicrobial packaging materials, AuNPs affinity towards-SH functionality for heavy metal detection and dip coating of PdNPs for the development of recyclable supported catalysts.

### 2.3 Scalable manufacturing of paper-based nanostructured devices by printing

Large scale production of paper-based analytical devices with high reproducibility and low cost is an essential step for advancing implementation of this technology in everyday life. Printing enables rapid, cost-effective, and simple large-scale production of functional patterns or arrays. Different printing techniques are available to fabricate nanoscale assemblies and create functional paper based devices. These include ink-jet printing, screen-printing, direct-writing of inks and more recently nanodrop and 3D printing. The benefits of inkjet printing for paper based analytical devices have been reviewed by Citterio et al. Printing can be used to modify paper, create channels on paper, add reagents and even perform reactions on paper. Eventually, the analytical device can be prepared entirely by printing. In many cases, printing requires development of pastes containing linkers or dispersing agents to stabilize NPs and prevent aggregation, maintaining uniformity of the sensing surface. Sensors that involve the use of biomolecules, high temperatures, complicated processing, and toxic elements should be avoided to prevent deactivation. A low-cost, SERS substrate was produced by modifying the surface chemistry of cellulose paper and patterning NPs arrays using a consumer inkjet printer. Fabrication of lateral flow immunochromatographic devices from a single piece of filter paper by patterning microfluidic channels and dispensing immunosensing inks (including colloidal gold-labeled anti-human IgG) was also demonstrated with a commercial inkjet printer. With the resulting paper-fluidic immunosensing device, human IgG concentrations down to 10 μg/L could be detected within 20 min. A simple, low cost and user-friendly method for the fabrication of a paper electrode (PE) was “printed” by direct writing onto photo paper using a ball-point pen filled with nanoink. The developed PE exhibited a wide linear calibration range from 1.7 μM to 30 nM for the determination of H₂O₂ with a detection limit of 0.5 μM. Metallic NPs with different physical properties have been screen printed as authentication tags on different types of paper. The combined signals can be used to obtain a user-configurable label, providing a high degree of security in anti-counterfeiting applications using simple commercially-available sensors. More recently, a functional proof-of-concept relative humidity sensor was fabricated on paper by inkjet-printing a commercially available AgNP colloidal ink, to create an interdigitated electrodes configuration directly on a paper substrate, which was used as sensing material. Viscoelastic inks composed of anisotropic cellulose nanocrystals (CNC) that enable patterning of 3D objects by direct ink writing have been formulated can be used for such applications. Citterio et al. have demonstrated the inkjet printing of microfluidic paper sensors for determining glucose concentration, protein content and pH. The sensors were fabricated by soaking cellulose paper in 1% polystyrene in toluene and then inkjet printing a layer of toluene and chemical sensing inks on the surface. The authors demonstrated multianalyte sensors exclusively by printing. Andreescu et al. fabricated a paper sensor for phenol detection utilizing a layer by layer (LbL) printing technique. The authors sandwiched an enzyme, tyrosinase, between layers of alginate and chitosan in order to stabilize and protect the enzyme, and generate an optical signal through a chemical reaction between the product on the enzymatic process and printed layers. This methodology could easily substitute the enzyme functionality with nanomaterials in order to create a stable cellulose-nanomaterial.
3 Detection Methodologies for Nanomaterial-Cellulose Based Paper Sensors

3-1 Optical and fluorescence based detection

The most common methods of detection using cellulose/nanomaterials based sensors are optical since many nanomaterials display spectral changes that can vary with size, shape, surface chemistry and surface binding. Here, we summarize the detection mechanism of main types of paper-based nanomaterial analytical devices and their most relevant applications. The low cost and robustness of the cellulose platform allow for quick and facile testing which is desirable for many applications. Cellulose also allows for reagents to be immobilized on the surface creating a "reagentless" sensing platform. The benefits of paper as an optical sensing platform and examples of nanopaper configurations have been reviewed by Merkoci et al.64 Lateral flow devices that involve migration of reagents and analyte samples through specifically designed channels have been largely used in applications in the clinical field. Figure 6 shows an example of fabrication and operation procedure of a lateral flow device obtained by creating wax barriers on paper.65 Patterning paper with hydrophobic barriers enables controlled migration of soluble reagents through capillary action across these channels.66 Multiple layers of patterned papers that contain multiple functionalities can be assembled in separate stacked layers that are then folded together for analysis.5 Merkoçi et al. optimized a cellulose-AuNPs lateral flow immunoassay on conjugate and sample pads which yielded an eight time increase in sensitivity.67 Lin et al. demonstrate a portable, fluorescent LFIA using quantum dots instead of the traditional gold nanoparticles. The authors tested the assay with nitrated ceruloplasmin with a detection limit of 8 ng/mL in undiluted plasma and 0.4 ng/mL for spiked plasma which demonstrates a greater sensitivity compared to gold immunoassays. The development of microfluidic paper-based analytical devices with colorimetric detection have recently been reviewed by Carrilho et al.68

In addition to Au and AgNPs, CeO2NPs that contain dual oxidation state at their surface (Ce4+/Ce3+)27 have been used as sensing particles. These NPs have rich surface hydroxyl functionalities when placed in an aqueous environment which allow for facile modification and complex formation.25,26 CeO2 can serve as a colorimetric agent independently,28 a mechanism that has been demonstrated for detection of H2O2, phenolic antioxidants and enzyme activity.27,28 Enzymes such as glucose oxidase and horseradish peroxidase can be utilized in addition to CeO2NPs to impart selectivity and expand the range of analytes that can be detected.24b,72 Recently, detection schemes based on CeO2NPs have been extended to quantify DNA recognition processes.73

Colorimetric measurements can be achieved by tailoring the affinity and color based properties of the substrate for binding specifically the target analyte. A well-known example is the use of AuNPs that can easily detect compounds with thiol groups through the affinity of Au for sulfur.74 This method was utilized for detection of cysteine and homocysteine amino acids which play an important role in cardiovascular disease and immune deficiency syndrome.75 To enhance selectivity, NPs can be modified with selective ligands or biomolecular recognition receptors like antibodies (Ab) or DNA. Monoclonal Abs were used to detect twenty types of mycotoxins using their unique binding ability.76 A similar strategy was also utilized to detect human Immunoglobulin G.14 Zhang et al. demonstrated a colorimetric response for white blood cells. AuNPs were attached to white blood cells with CD45 antigen and Ab which produced a purple hue.77

Color based detection has been demonstrated for detection of metal ions for assessing environmental toxicity.7 Molecules that have high affinity for metal ions like ethylenediaminetetraacetic acid (EDTA),78 dithiothreitol (DTT)78 and polyethyleneimine (PEI)79 and some proteins like bovine serum albumin (BSA)27 can be patterned to create capture sites and facilitate analysis on paper substrates through complexation coupled with color-based reactions. Another strategy for colorimetric detection is exploiting NP aggregation. Au and AgNPs exhibit changes in their surface plasmon band upon change in particle size and aggregation status. Target-induced NP aggregation may occur in response to ion concentration, covalent attachment, binding or charge attraction. As an example, detection of glutathione (GSH) was achieved by measuring aggregation of AgNPs upon GSH binding to the NPs, turning orange particles, to a reddish brown color.76 In other examples, Cr6+ induced AuNP aggregation with a change in color from wine red to blue.67 Rodriguez et al. utilized streptavidin-biotin affinity to aggregate AgNPs and produce a colorimetric signal.80 However, in the development of these methods it is important that the resulting
aggregation is a response to only the analyte of interest and it is not a result of nonspecific aggregation.

Several other types of colorimetric based detection have been reported. Sadollahkhani et al. demonstrated an ion exchange mechanism for detection of Cu(II) in which Cu(II) changes the color of zinc particles from white to brown due to formation of a copper sulfide shell. The reaction mechanism is shown below:

\[ \text{ZnS}(s) + \text{Cu}^{2+}(aq) \rightarrow \text{CuS}(s) + \text{Zn}^{2+}(aq) \]

Luckham et al. demonstrated a method for paraoxon detection in the range of 500 nM to 1 mM by measuring the production of thiocholine by acetylcholinesterase which reduces Au(III) into gold particles which can then be visually detected on paper. In other examples, pH changing polyaniline-poly(sodium 4-styrenesulfonate) (PANI:PSS) NPs have been used to detect concentrations of amine vapors by monitoring the change in color to blue for basic pH environments. Chaiyo et al. detected Cu(II) by measuring the change in color of Ag nanoparticles in the presence of thiosulfate from pink to colorless on paper due to catalytic etching. This method provided a LOD of 1 ng/mL for Cu(II) ions.

In addition to sensors based on optical detection, there are a number of devices with fluorescence detection in which either the nanomaterial used produces a fluorescent signal or nanomaterials are used in conjunction with a fluorescent compound. Fluorescent signals can be visualized with a UV lamp by the naked eye which provides convenience for site analysis. A commonly used fluorescent agent is rhodamine B which can be used by itself or alongside AuNPs. Another commonly used fluorescent agent is 2,3-diaminophenazine which can be oxidized from o-phenylenediamine in the presence of Ag(I) or Au(II) ions to create fluorescent light. Lanthanide series ions such as Tb or Dy have been utilized to generate a fluorescent response for detection of soluble uPAR (an important tumor marker) or dipicolinic acid (DPA), an anthrax biomarker. Fluorescent nanospheres of tris 8-hydroxyquinoline aluminum (Alq3), a complex between aluminum and three 8-hydroxyquinoline molecules have been used as fluorescent probes to detect 2,4,6-trinitrotoluene (TNT) and 2,4,6-trinitrophenol (TNP) explosives through fluorescence quenching.

Quantum dots (QD) are another category of fluorescent, semiconducting nanomaterials that can be tuned to alter their emission response. Compared to fluorescent dyes, semiconducting QD have higher extinction coefficients, broader excitation spectra, narrower emission peaks and higher fluorescent yield. An emerging type of QD is a carbon dot (CQD) that are carbon based materials which exhibit similar activity to semiconductor QD but are biocompatible, water soluble, and have low toxicity, high photostability and low photobleaching. Both of these materials provide a measurable response directly from the intrinsic fluorescent properties of the material without the need of an exterior fluorescent agent. Quantum/carbon dot hybrids or two color carbon dot hybrids can also be combined or covalently attached to achieve a more sensitive fluorescent response. By quenching one dot with the target analyte, a broad range of fluorescent response is produced with increasing analyte concentration (Fig. 8). This technique can be integrated with paper-based technology to facilitate sample handling and analysis. Merkoci et al. demonstrated a photoluminescent-based sensor comprising CdSe@ZnS quantum dots conjugated on paper and showed modulation of plasmonic or photoluminescent properties for detection of various biologically-relevant analytes from small sample volumes.

### 3-2 Electrochemical detection

Devices with electrochemical detection have gained popularity due to ease of analysis and the availability of miniaturized and low cost electrochemical instrumentation. Modification of cellulose with conductive materials or thermal treatment can be used to create flexible, conductive cellulose derived materials. Ghosale et al. used sintered AgNPs capped with octylamine (AgNPs-OA) as a working electrode material for detection of H2O2 in wastewater samples through cyclic voltammetry (CV) measurements. Cunningham et al. assembled an “immosandwich” assay consisting of AgNPs modified with a ricin α chain Ab to detect ricin. In this configuration, a magnetic microbead modified with ricin Ab was also used to facilitate immunomagnetic separation and detection as shown in Fig. 9. An origami slip paper analytical device (oSlip) was then assembled to electrochemically measure the concentration of ricin with a detection limit of 34 pM. Wang et al. demonstrated immunodetection of microcystin-LR (MC-LR) using modified SWNTs and monitored the difference in conductance of the SWNTs upon MC-LR. The electrochemical sensor had a LOD of 0.6 ng/mL which is lower than the World Health Organization limit for drinking water.
Although not as commonly utilized as optical or electrochemical methods, Raman Scattering can also be used as a detection modality for paper based platforms. AgNPs modified cellulose paper has been used as a platform for Raman scattering for determining low amounts of melamine in milk\(^{96}\) and HPV in human samples.\(^{97}\) AgNPs were printed onto cellulose paper along with \(p\)-aminobenzenethiol and used to detect trinitrotoluene (TNT). Wang \(\textit{et al.}\) demonstrated detection capability for cloth, leather, paper and soil and the method serves as a powerful TNT screening tool.\(^{98}\)

### Applications

Due to their versatility, devices based on cellulose-nanomaterials hybrid platforms have a wide range of applications. These sensors can be used as simple yes/no devices or as fully integrated analytical tools ideally suited for field analysis. In the following sections, we provide representative examples for major application categories and discuss their performance. Table 2 summarizes cellulose-nanomaterial based analytical platforms reported in literature.

#### 4-1 Clinical/medicinal field

The clinical/medicinal field can benefit from the availability of inexpensive devices for biomarker detection and point of care diagnosis. Utilizing Ag nanoplates, Yen \(\textit{et al.}\) have designed a paper assay which can detect biomarkers for dengue fever, yellow fever, and the ebola virus.\(^{99}\) Ortega \(\textit{et al.}\) also designed an assay targeting dengue fever utilizing magnetic NPs.\(^{100}\) Using a similar approach, Abe \(\textit{et al.}\) demonstrated immunoglobulin (IgG) detection down to a concentration of 10 \(\mu\)g/L.\(^{14}\) Kim \(\textit{et al.}\) detected human papillomavirus (HPV) using SERS with AgNPs on paper.\(^{97}\) Using paper modified with AuNPs, Rodriguez \(\textit{et al.}\) were able to extract, purify and detect Influenza A (H1N1) RNA.\(^{80}\) Tsai \(\textit{et al.}\) detected tuberculosis using single strand DNA and AuNPs on a paper platform.\(^{101}\) Zhang \(\textit{et al.}\) created a method of quantifying the number of white blood cells in human blood cells which can be used to assess the health of an individual.\(^{77}\) The sensor response was visible with the naked eye and the device required only a prick of blood (15 – 20 \(\mu\)L). Liang \(\textit{et al.}\) have developed a AuNPs based sensor for detection of NADH. The device can also provide information about NAD\(^{+}\) enzymatic reactions of dehydrogenase with and without inhibitors.\(^{102}\) Cellulose-nanomaterial based paper sensors have also been reported for measurement of antioxidant molecules such as GSH in human serum using AgNPs immobilized on cellulose.\(^{65}\)

#### 4-2 Environmental detection

There are a number of paper sensors that have been reported for detection of environmental samples such as toxic metal ions, pesticides and other environmental toxins such as cyanobacterial blooms. Most detection methods are by fluorescence and colorimetric mechanisms. Many groups propose a cell phone application\(^{8,10,63,103}\) for simple sensor calibration as well as reporting concentrations of harmful analytes.\(^{9}\) Chen \(\textit{et al.}\) introduced a new method of capture and detection for \(\text{Hg}^{2+}\) using AuNPs modified filter paper (Fig. 10). Mercury(II) ions form an amalgam with AuNPs that allows for the reduction of rhodamine B (RhB) in the presence of sodium borohydride.

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Fig. 9 A paper-based assay platform for detection of ricin. (a) The paper platform is assembled by simple origami paper folding. The pre-formed ricin immunocomposite sample is injected into the oSLip inlet (b), driven down by capillary action (c), concentrated under the first carbon electrode by the magnetic field (d), the oxidant diffuses across the hollow channel to the magnetically sequestered ricin immunocomposite and oxidizes the AgNPs (e). Finally, the dissolved Ag\(^{+}\) ions are electrodeposited on the electrode as metallic Ag for 200 s (f), and then stripped off (g) (permission from Ref. 94).
Examples of analytical applications of cellulose-nanomaterial hybrid platforms

| Application       | Detection          | Approach                                                                 | Characteristics                                                                 | Ref. |
|-------------------|--------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------|------|
| Clinical/medicinal| Colorimetric       | Combines both an AChE and a signaling element in a sol-gel-derived entrapped AuNPs coating that is deposited on a hydrophobically modified paper substrate. | Detection of Paraaxon over the concentration range of 500 nM to ~1 mM             | 82   |
|                   | Colorimetric       | Paper-based device based on NADH-mediated inhibition of AuNPs dissolution. The device consisting of a mixed cellulose ester paper, AuNP-coated film sandwiched between two plastic cover layers. | The device exploits capillary force-assisted vertical diffusion with a LOD of 12.5 μM | 102  |
|                   | Colorimetric       | Fabrication of lateral flow immunochromatographic devices made from a single piece of filter paper by patterning microfluidic channels and dispensing immunosensing inks. | Detection of human IgG concentrations of at least down to 10 μg/L within 20 min.   | 14   |
|                   | Colorimetric       | Poly(ether sulfone) paper matrix-based assay that incorporates all three RNA extraction, amplification, and detection steps directly from human clinical specimens without the need sample preparation of any kind. | Immediate visual detection of Influenza A (H1N1) RNA on lateral flow strips. It has a clinically relevant viral load DL of 10^6 copies/mL. | 80   |
| Environmental     | Fluorescence       | "Magnetic paper-based ELISA" using core-shell magnetite@polydopamine NPs supported on a Whatman paper-like new solid immunoassay platform. Ag and Cu NPs are also used as detection inks. | IgM-dengue antibodies detection system with two orders more sensitive and with a 700-times lower LOD than traditional ELISA. | 86   |
|                   | Fluorescence       | Paper-based visual sensor designed by using filter paper based on terbium(III)/gold nanochuster (Tb³⁺/AuNCs). | Detect Hg²⁺ as low as 0.1 μM observed by the naked eyes.                           | 50   |
|                   | Fluorescence       | Paper electrode (PE) using AgNPs capped with octylamine (AgNPs-OA) used as a working electrode. | A linear range from 1.7 μM – 30 mM for the determination of H₂O₂ with a LOD of 0.5 μM, selective in wastewater samples. | 93   |
|                   | Electrochemistry   | Paper platform based on quantitative, electrochemical detection of AgNPs labels linked to a magnetic microbead support via a ricin immunosandwich. | Detection of ricin at concentrations of 34 pM, the device is easily remediated after use. The total assay time is 9.5 min. | 94   |
|                   | Fluorescence       | Integration of AgNPs-Tb³⁺ onto nitrocellulose membrane for detection of anthrax biomarker (dipicolinic acid, DPA). | LOD by naked eyes ~1 μM, which is 10 times < Tb³⁺ alone (~10 μM).                | 88   |
| Security          | Fluorescence       | Filter paper soaked with luminescent polymer-coated nanocomposites for visual detection of 2,4,6-trinitrophenol (TNP) | The sensor demonstrate rapid and convenient visual detection of TNP in aqueous solution | 89   |
|                   | Electrochemistry   | Paper platform based on quantitative, electrochemical detection of AgNPs labels linked to a magnetic microbead support via a ricin immunosandwich. | Detection of ricin at concentrations of 34 pM, the device is easily remediated after use. The total assay time is 9.5 min. | 94   |
|                   | Fluorescence       | Integration of AgNPs-Tb³⁺ onto nitrocellulose membrane for detection of anthrax biomarker (dipicolinic acid, DPA). | LOD by naked eyes ~1 μM, which is 10 times < Tb³⁺ alone (~10 μM).                | 88   |
| Point of care     | Colorimetric       | Fabrication of plasmonic (Ag₄Au)NP-containing test paper based on the laser-induced annealing method. | Rapid fabrication time (a few seconds), large-area throughput, selectivity in the positioning of the NPs, and the capability of preparing NP arrays in high density. | 75   |
|                   | Colorimetric       | Paper based microfluidic device embedded with (+) AuNPs for detection of uric acid (UA). | Detect as low as 8.1 ppm of UA within < 20 min.                                  | 127  |
|                   | Colorimetric       | Silica NP-modified μPAD produced on a Whatman grade 1 filter paper and using a CO₂ laser engraver. | Detection of lactate, glucose, and glutamate in clinically relevant concentration ranges with LODs of 0.63, 0.50, and 0.25 mM. | 128  |
| Field analysis    | Colorimetric       | μPAD using only polystyrene and a patterned screen, with CrO₃ NPs as colorimetric probes to analyze H₂O₂. | The analysis of H₂O₂ in real samples produced the same results at 95% confidence level. | 129  |
|                   | Colorimetric       | TiO₂NPs in a hydroxypropyl cellulose matrix produced and were utilized to prepare films on polycarbonate slides and coatings on cellulose papers. | Detection of H₂O₂ with LOD of 90, and 1.5 ppm after a 1-min and 1-h integration. | 130  |
| Food              | Electrochemistry   | An antibody to the microcystin-LR (MC-LR) and single-walled carbon nanotubes (SWNTs) were used to dip-coat the paper and rendering its conductivity. | The linear detection range up to 10 nM/mL with the LOD of 0.6 nM/mL. | 95   |
|                   | Colorimetric       | AuNP-based semi-quantitative and quantitative ultrasensitive multi-immunochromatographic (ICA) strip (paper sensor) system for the detection of twenty types of mycotoxin | Using a hand-held strip scan reader, the LOD for ZEAs, DONs, T-2s, AFs and FBs was 0.04 – 0.17, 0.06 – 49, 0.15 – 0.22, 0.056 – 0.49 and 0.53 – 1.05 μg kg⁻¹, respectively. | 76   |
|                   | Colorimetric       | Polyvinyl-poly(sodium 4-styrenesulfonate) (PANI·PSS) colloid were homogenously deposited on filter paper and used to detect triethylamine (TEA). | The green color changed to blue at a TEA concentration as low as 188 ppm. | 83   |
Both the gold amalgam and the NaBH₄ must be present to reduce the RbB which decreases the fluorescent response. The cellulose/AuNP paper created can serve as a tool for remediation and detection of toxic ions with a detection limit of less than 5 nM.⁶⁸

Chen et al. have developed a colorimetric sensing platform for Hg(II) ions through thymine-mercury coordination chemistry on AuNPs, with a limit of detection of 50 nM.⁶⁹ Zhou et al. have created a method of detecting As(III) with carbon dots (CQD) which produce two unique fluorescent responses and operate similar to a pH paper. The red CQD is modified with dithiothreitol (DTT) which specifically binds to As(III), causes aggregation of the CQD and quenches the fluorescent response. The cyan CDs used in the system remain unchanged however, contribute to the background fluorescent response which allows for a wider range of detection, down to 5 pph.⁷⁰ Choudhary et al. have demonstrated fluorescent detection of Pb(II) utilizing cube-shaped nitrogen-doped CD. The yellow emission of the CDs changed to red with the addition of Pb(II) ions. The proposed mechanism suggests formation of a coordination complex between the Pb²⁺ and the CD. The sensor has a reported LOD of 10.0 μg/L for Pb²⁺.⁷¹ Rajeshwari et al. used citrate-capped AuNPs to detect chromium contamination on filter paper. Cr³⁺ causes the NPs to aggregate which produces a visible shift in color from 526 to 714 nm with a detection limit of 109 nM.⁷² Li et al. have fabricated a functionalized cellulose paper which detects Fe³⁺ ions through catalytic oxidation of AuNPs.⁷³ Cellulose-TiO₂NPs were functionalized with amino and sulfide groups, which bind strongly to AuNPs. The addition of Fe³⁺ ions changes the color of the paper from pinkish to white.⁷⁴ Sadollahkhani et al. use ZnSNPs with a ZnO core in order to measure toxic levels of Cu²⁺. The Cu²⁺ ion exchange with the Zn²⁺ of the particle ZnS shell; the color of the resulting particles are yellow while the original zinc particles do not display a significant color visible by the naked eye enabling detection.⁷⁵

The use of Fe₃O₄NPs aggregated with poly-l-lysine on a LFIA platform decreased the LOD for paraoxon by more than 40-fold, reaching 1.7 ng/mL compared to the unaggregated Fe₃O₄NPs.⁷⁶ Luckham et al. deposited enzyme/NP composites on paper to detect the acetylcholinesterase inhibitor paraoxon which is found in insecticides and nerve gases.⁷⁷ Ying et al. reported an immunosensor for the detection of *Escherichia coli* (*E. coli*). Graphene paper was decorated by AuNPs through one-step electrodeposition and *E. coli* was immobilized on the surface using biotin-streptavidin chemistry. The graphene-AuNP paper was utilized as an electrode for impedance spectroscopy measurements of *E. coli* with a LOD of 1.5 × 10² cfu/mL.⁷⁸

### 4-3 Food safety monitoring

The food industry could greatly benefit from inexpensive sensors that can identify foodstuffs, contaminants, bacteria, and food degradation. Cellulose-nanomerial sensors could provide convenient monitoring tools for regulatory agencies, the food industry and consumers. Sharpe et al. utilized the redox properties of CeO₂ NPs to detect antioxidants in commercially bought tea and medicinal mushrooms. Adsorption of the antioxidants onto the particle surface caused a unique colorimetric response due to formation of charge transfer complexes that vary in color and intensity with the chemical structure of the antioxidants.⁷⁹ Li et al. monitored production of amines, as products of fish degradation, using PANI:PSS composites which change from white to purple at pH 11 because of the release of amines. Eventually this chemistry could be used as freshness indicators for seafood.⁸⁰ Kozlova et al. used cellulose-AgNPs were used to prevent the growth of microorganisms and increase the shelf life of food products.⁸¹ Paper sensors can also detect harmful contaminants in food. Han et al. have created SERS arrays by drawing AgNPs onto cellulose paper. These disposable arrays were used to detect melamine in milk.⁸² Hong et al. have designed a paper strip which can detect 20 different types of mycotoxins through colorimetric detection. The strip utilizes AuNPs conjugated with antigens which inhibit the pink color of AuNPs when the target (mycotoxins) is present. The status of cellulose-nanomaterials NP-based paper sensors for food safety monitoring and their potential utility as multifunctional platforms for capture, detection and inactivation of food pathogens and food packaging applications has been discussed in recent reviews. Despite progress, the number of sensors reported for food applications is relatively small and is expected to grow in the near future, driven by a large interest from industry.

### 5 Conclusions and Future Trends

This review has provided an overview of the various types of hybrid nanomaterials-based cellulose paper platforms reported in literature and discussed their potential as functional analytical devices for field measurement and applications. Combining the low cost and flexibility of cellulose paper with the chemical, optical, catalytic and electrical properties of nanomaterials, new and versatile platforms can be designed that can provide viable alternatives to conventionally used methods. Several strategies have been reported to functionalize cellulose paper and use nanomaterials as transduction platforms; however, efforts to
demonstrate the viability of these devices as robust analytical tools and move away from proof of concept towards real applications are with high demands. To enable implementation of this technology in consumer products, this field would benefit from further fundamental development of flexible nano-sensors suitable for roll-to-roll manufacturing for large scale production. Integration of all sensing reagents, into an all-in-one integrated unit is also desirable for future deployment. Validation, comparability, stability and inter-laboratory studies are also needed to ensure reliability for real world applications. Eventually, application in complex matrices needs to be demonstrated in real samples and the results should be compared side-by-side with widely adopted technologies in order to move paper-based sensors into the market.

Once optimized for specific analyte solutions, (water, serum, etc.) the stability of the device must be examined to ensure accurate and precise measurements regardless of the user. A commercially-viable device must be able to withstand environmental conditions and preserve performance with storage. Although the benefit of printing sensors has been demonstrated, devices that require expensive or delicate reagents (enzymes, aptamers), complex preparation and lengthy operational procedures are not ideal for commercial production. For certain applications, measurement devices must be regulated (e.g. Food and Drug Administration (FDA)) before they can reach consumers. Therefore, these should be designed with such requirements to reach good laboratory practices (GLP) performance criteria in terms of robustness, comparability, standardization, and fulfill additional requirements of biocompatibility, environmental impact, safety and disposal before they can become available on the market. An overview of a typical analytical measurement process from sampling to data analysis and requirements for validation and achieving good laboratory and manufacturing practices (GLP and GPM) criteria.

Fig. 11 Overview of a typical analytical measurement process from sampling to data analysis and requirements for validation and achieving good laboratory and manufacturing practices (GLP and GPM) criteria.

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