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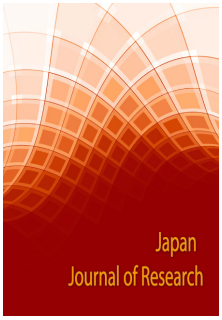

Hydrogel particles for biomarker detection in liquid biopsies

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Abbreviations
NIPAm: N-isopropylacrylamide; GCE: glassy carbon electrode; PAni: polyaniline; AFM: atomic force microscopy; SEM: scanning electron microscope; TEM: transmission electron microscope; NP: nanoparticles; PEG: polyethylene glycol; HCR: hybridization chain reaction; QD: quantum dot; LSPR: localized surface plasmon resonance; NS: nanoshells; PNM: poly(N-isopropylacrylamide-co-methacrylic acid); CEA: carcinoembryonic antigen; DPV: differential pulse voltammetry; BSA: Bovine serum albumin; AβO: amyloid-beta oligomers PrPC: cellular prion protein LOD: limit of detection

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Abstract
In this brief review we assessed the recent progress of hydrogel materials design and processing for applications in biomarker sensing for early diseases diagnosis and potential clinical use. Three types of hydrogel platforms were identified that can be classified in the following categories: (i) fluorescence hydrogel biosensing platform, (ii) integrated electrochemical biosensor systems and (iii) synthetic core-shell hydrogel for biological specimen manipulation. In each type of these approaches, hydrogel materials were functionalized with features suitable for liquid biopsy application. These features can comprise high conductivity, biocompatibility, ease-of-use, robustness, but also anti-fouling properties. The properties of these nanomaterials will enable them into promising tools in precision medicine.

Introduction

In this mini-review, the criteria of inclusion of the literature reports comprised searches of the online databases from 2015 to 2020. They were searched in PubMed, SciFinder, Scopus, and Web of Science as databases in English. Search words include "biomarker", "hydrogel", "biosensing", "enzyme", "core-shell" and "particle". Initially, 279 studies met the criteria but then only significant papers were selected that described potential application suitable for the detection of biomarkers in relevance to liquid biopsies.

Early diagnosis is well needed in clinical medicine (Figure 1). Many approaches have been attempted to make diagnosis more accurate and quicker. However conventional techniques of diagnosis usually need expensive monoclonal antibodies, fluorescent dye, or sophisticated instruments. Furthermore, most of them are time-consuming [1].

Hydrogel materials are highly biocompatible substrates for biosensors. They can provide superior electrochemical biosensing platform with high conductivity by using metal nanoparticle-properties. They also exhibit a large surface-to-volume ratio, which can provide some unique stimuli-responsive behavior for enhancing the sensitivity and specificity of biological interactions in complex biological systems, such as biofluids.

Below, we reviewed the studies reported in the literature which used approaches that were more specifically directed toward early diagnosis using liquid biopsy biomarkers (Figure 2). We also assessed these studies in view of their potential for scaling up manufacturing for future validation in clinical settings.

Discussion

Hydrogel preparation

Typical preparation of *N* -isopropylacrylamide (NIPAm) hydrogel particle is described in reference [2] with NIPAm prepared with closslinker and functional monomer in water and then deoxigenized. The solution was polymerized with heating by catalyst in nitrogen atmosphere. The polymerized suspension was stirred to break down particles into smaller entities. A polyaniline/platinum nanoparticle-modified glassy carbon electrode (GCE) was also prepared and demonstrated for application as an electrochemical biosensing platform [3]. Drops of aniline monomer solution and polymerization initiator solution were mixed and coated onto the GCE. The coated solution was gelated to form a hydrogel thin film within 3 min. After purification and drying, the thin film was put into contact with a solution of chloroplatinic acid and formic acid to generate platinum nanoparticles on the hydrogel matrix. A polyaniline/Pt nanoparticle-modified glassy carbon electrode could also be prepared as blow for the substrate to be used for the adsorption of enzymes. Polyaniline (PAni) hydrogels were synthesized by directly mixing of two solutions (A) and (B) together. Solution A was prepared by dissolving phytic acid in deionized water, and then aniline monomer was added to the solution. Solution B was prepared by dissolving ammonium persulfate in deionized water. Solution A and B were stored in a refrigerator (3°C) until the temperature of the solutions became stable. Next, the mixed solution was coated onto GCE. The mixed solution containing 1,3,5-benzenetricarboxylic acid and ferric nitrate was then gelated to form the hydrogel, which was ultimately obtained by freeze-drying [4].

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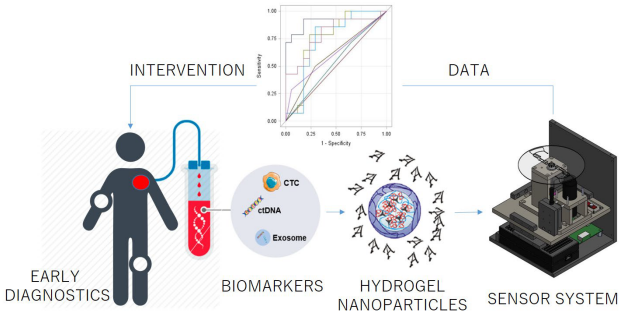

Figure 1. Patients demographic characteristics, clinical presentation and treatments

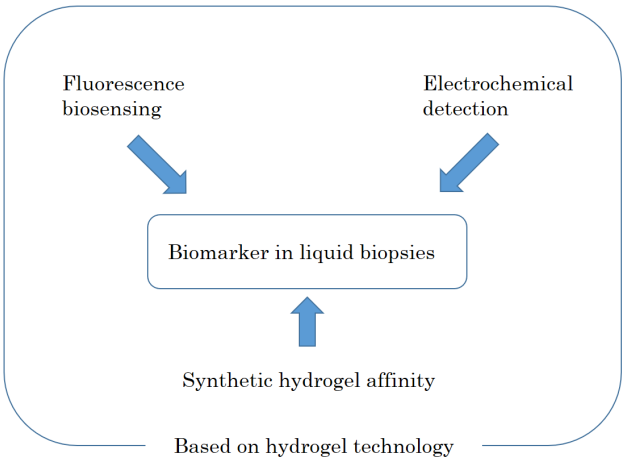

Figure 2. Technical approaches of nanoparticles in early disease detection using biofluids or liquid biopsies

Typically, hydrogel nanoparticles sizing was characterized by atomic force microscopy (AFM) and photon correlation spectroscopy. For example, NIPAm hydrogel particle size and size dispersion was determined as 200-300 nanometer [2]. A common technique for the characterization of the nanoparticles is microscopic inspection by electron microscopy such as SEM and TEM imaging. Several literatures report the characterization of the nanostructure in the hydrogels by these high- resolution imaging and spectroscopic techniques. For example, Yu and colleagues show the morphology of PANI hydrogel and PANI/PtNP hybrid films by high resolution TEM prior to test the materials for their electrochemical properties in the configuration of electrodes revealing very sensitive amperometric response to the addition of hydrogen peroxide [3].

Fluorescence hydrogel biosensing platform

Biosensing often require targeting biomarkers which can be from the family of molecular species such as nucleic acids, proteins or other biomolecular components. In the case of miRNAs detection, a method of choice for the detection is to use the gold standard method of Northern blotting. In this configuration, a fluorescent readout is combined with an optical setup to a fluidic chip for running the assay chemistry revealing the presence of the miRNAs. For example, Causa and colleagues have developed fluorescence biosensor for detecting miRNA in biological matrices. Molecular beacon probes were used for such a biosensor. In this experimental approach, the probes needed to be conjugated to the hydrogel interface with a controlled and well spread distribution to avoid crowding effects. Moreover, the authors conducted detection test with human serum samples to confirm not to be disturbed by other components upon antifouling property of hydrogel [5].

In a different study, Liu and colleagues could conduct circulating miRNA detection in serum [6]. Biomarker detection from serum may be disturbed with that color and fouling of other components. They compared serum samples of gastric cancer patients and healthy donors, then confirmed the up- regulated expression level of miRNA-21 in cancer samples. In experimental details, the carboxylate polyethylene glycol (PEG) hydrogel array was fabricated on glass slide via photo polymerization and modified with miRNA capture probe. In that target miRNA binding triggered hybridization chain reaction (HCR) in hydrogel with biotin labelled DNA probes to generate numerous DNA polymer chains where biotinylated CdS QDs were subsequently conjugated with a streptavidin bridge.

Interfacial cation exchange amplification was triggered in hydrogel upon the introduction of Ag+ and Rhod-5N, and abundant Cd2+ was released from CdS to bind with Rhod-5N for substantial fluorescence enhancement.

In general, biosensing system without expensive molecular reagent is a preferred approach for developing point of care platforms. Peppas and colleagues utilized localized surface plasmon resonance (LSPR) to detect low concentration of two protein biomarkers, i.e. lysozyme and lactoferrin. Upon binding lysozyme or lactoferrin, silica gold nano-shells combined poly (N-isopropylacrylamide- co-methacrylic acid) (AuNS@PNM) exhibits large, concentration-dependent red shift in LSPR wavelength, which enabled the detection of the concentration of biomarkers [7].

Hydrogel biosensor with electrochemical detection method

As it was previously mentioned, hydrogel can work as particular good electrode compatible with biomolecules and metal nanoparticles, so that it enables organizing specific biosensing platforms. Recently, attempt to get higher conductivity have been investigated by using immobilized metal nanoparticles. In Yu and colleagues' work, Pt nanoparticles immobilized hydrogel were utilized with enzyme reaction to measure current dependency on quantity of biomarkers [3]. This system can detect different metabolites selectively depending on different immobilized enzymes. The authors have checked selectivity of this system by adding other metabolites like urea, lactic acid, and glucose. From this experiment, it can be applied to blood sample with good anti-interference ability.

Hamachi and coworkers challenged interesting sensing mechanisms for high sensitivity [8]. Their H2O2 signal amplification system consist of supramolecular hydrogel, an enzyme, and an amplifier. When a multicomponent hydrogel was added with the biomarker, the hydrogel was destroyed revealing a colorimetric signal that could be seen with naked eye. Even though this system can apply to blood plasma sample, it can only show on/off signal and the threshold of sensing cannot be controlled.

From another point of view, robustness of the biosensing system is very important. Pang and colleagues applied self-healing hydrogel by room temperature preparation to electrochemical biosensing platform [9]. They utilized

guanosine-based G4 hydrogel. That hydrogel was easily recovered from destruction and keeps its high conductivity. Rong and colleagues created highly conductive hydrogel/Au composites with large surface nanostructure for electrochemical biosensing platform [10]. This embodiment could detect carcinoembryonic antigen (CEA) to bind with anti-CEA to decrease current in differential pulse voltammetry (DPV) response. Typically, Gold nanoparticles increased electron conductivity of hydrogel. This sensing system was blocked by 1% BSA but interfering substances of other components of serum for two order magnitude of CEA's didn't disturb CEA sensing. There is also specific binding between amyloid-beta oligomers (AβO) and thiolated cellular prion protein (PrPC) probes. It could selectively differentiate oligomers from monomers and fibril [11]. The reason why PrPC probes have high selectivity only for oligomers is the short peptide with 16 amino acids located in the N-terminal region strongly binds to Aβ oligomer.

Concentration of biomarker with synthetic hydrogel affinity for mass spectroscopy

Other kinds of materials technologies are related to molecularly imprinted synthetic hydrogels. Molecularly imprinted hydrogels are synthesized by incubating template analytes with functional monomers prior to polymerization to create specific 3D binding sites. Although the potential of imprinted hydrogels still remains promising, there has been minimal success in achieving truly selective hydrogels, largely due to challenges associated with protein size and structural complexity [12-14]. On the other hand, non-imprinted hydrogels capable of semi-selective protein recognition are appealing alternatives as they could also be easier to prepare. Luchini and colleagues have developed core-shell hydrogel particles which have specific affinity with proteins in liquid biopsies [2,15,16]. That recognition mechanism based on size exclusion property of hydrogel shell and electronegative affinity of abit ore an exhibit promising functions. After separation with the hydrogel, high abundant components can be removed, and it makes easy to distinguish low abundant target biomarkers by mass spectroscopy. Peppas and colleague compared these nonimprinted hydrogels to biomolecular-containing hydrogels [14,16].

Conclusion

This brief review reported a list of the most relevant biosensing platforms for the detection of many types of molecular biomarkers. They could detect miRNAs, proteins (e.g. alpha-fetoprotein, carcinoembryonic antigen, amyloid-beta oligomers, platelet derived growth factor, human growth hormone), and low molecular metabolites (e.g. lysozyme, lactoferrin, urea, lactic acid, glucose, H_2O_2, uric acid) in blood plasma, serum, urine, tear, and cerebrospinal fluid. Their limit of detection (LOD) can go to the order of fMmL-1. Electrochemical biosensors have especially quick response time like seconds [3]. It is also important to emphasis the robustness of self-healing hydrogel sensor for practical clinical use [9]. In the near future, it is also expected that better hydrogel biosensing platforms which don't contain expensive bioassay reagents with low LOD, quick response, and high robustness will be developed.

Conflict of Interest

The authors have no conflict of interest to report.

Acknowledgments

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