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Application of Carbon Nanomaterials in Human Virus Detection

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Abstract

Human-pathogenic viruses are still a chief reason for illness and death on the globe, as epitomized by the COVID-19 pandemic instigated by a coronavirus in 2020. Multiple novel sensors have been invented because diseases must be detected and diagnosed as early as possible, and recognition methods have to be carried out with minimal invasivity. Sensors have been particularly developed focusing on miniaturization by the use of nanomaterials for fabricating nanosensors. The nano-sized nature of nanomaterials and their exclusive optical, electronical, magnetical, and mechanical attributes can enhance patient care through the use of sensors with minimal invasivity and extreme sensitivity. Amongst the nanomaterials utilized for fabricating nano-sensors, carbon-based nanomaterials are promising as these sensors respond better to signals in various sensing settings. This review provides an overview of the recent developments in carbon nanomaterial-based biosensors for viral recognition based on the biomarkers that arise from the infection, the nucleic acids from the viruses, and the entire virus. The role of carbon nanomaterials is highlighted by the improvement of sensor and recognition functionality. The Dengue virus, Ebola virus, Hepatits virus, human immunodeficiency virus (HIV), influenza virus, Zika virus and Adenovirus are the virus types reviewed to illustrate the implementation of the techniques. Finally, the drawbacks and advantages of carbon nanomaterial-based biosensors for viral recognition are identified and discussed.

Keywords: Virus; Carbon; Nanomaterial; Sensor; Viral Recognition; Graphene.

Abstract

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1. Introduction

Viral pollution of resources is one of the major causes of diseases leading to several hundred thousands of demises per annum. Such health-related problems continue to be unsolved as is evinced by a plethora of individuals that suffer from multiple diseases. Currently, the incidence of infective diseases that can importantly affect human life has risen outstandingly. A number of communicable diseases influence a lot of individuals and continuously cause substantial health issues[1]. Viruses are obligatory intracellular parasites that require the host cell system and resources for replication and propagation. Mammalian cells have developed defense machineries elaborately for detecting and inhibiting viral reproduction. This, in turn, has resulted in emerging viral strains being able to manipulate and subvert host immune reactions, leading to a boosted virus-mediated pathogenesis[2]. These human-pathogenic viruses are still a chief reason for illness and death on the globe, as epitomized by the COVID-19 pandemic instigated by a coronavirus in 2020. In the absence of primary diagnostics, it is not possible to adequately and promptly make proper disease treatments. Sensitive, specific and rapid diagnostic tests not only provide the basis towards effectual therapies but also have a major contribution to prevent the transmission of infective diseases[3].

The approach conventionally applied to detect viruses is the cell/tissue culture process, in which permissive cells are inoculated with a virus or a deactivated virus solution. The method creates cytopathic impacts and, subsequently, comprises titration of a 50% tissue culture infectious dose of the virus into the tissue culture. Although virus titration with the observed cytopathic impacts has a protracted history as the golden rule in research of detecting viruses, still several multiple drawbacks continue to exist[4]. For virus detection, a manifold of studies has applied alternative approaches to the conventional cytopathic impacts technique. Electron Microscopy, Serological Methods and Nucleic Acid based approaches are the major alternative techniques[5]. The polymerase chain reaction, enzyme-linked immunosorbent assay and reverse transcription polymerase chain reaction are utilized for the detection of viruses. Whereas key clinical laboratories propose sensitive and specific tests, these are time-taking and laborious, expensive and rely on complex devices and skilled operators. Figure 1 presents the "ASSURED" criteria according to the World Health Organization (WHO) and addresses the infectious disease control needs. Based on that, research has focused on novel detection components to promote detection in biosensing[6] as there is a need for rapid

diagnosis and improvements in biosensor technologies with more selectivity, stability, and cost-effectiveness.
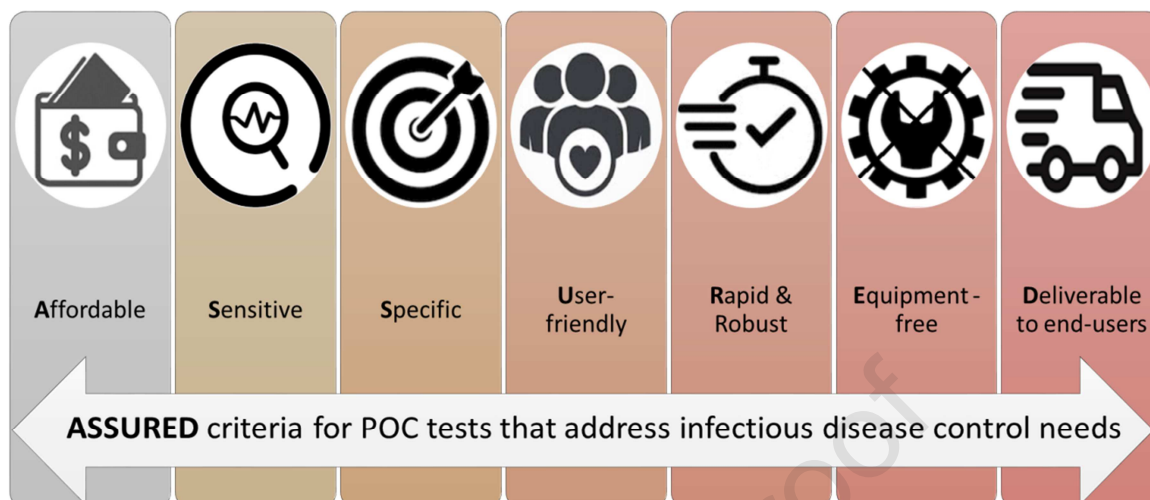

Fig.1 According to the World Health Organization (WHO), POC tests that address infectious disease control needs, especially for the developing countries, should follow "ASSURED" criteria.

Multiple novel sensors have been invented because diseases must be detected and diagnosed as early as possible and recognition methods have to be carried out with minimal invasivity. Sensors have been particularly developed focusing on miniaturization by the use of novel materials for fabricating sensors. The interesting characteristics of nanomaterials are illustrated in their combined advantages of a tiny size with an extreme increase in the surface area, rendering them a tremendous potential for versatile applications [7-10]. The nano-sized nature of nanomaterials and their exclusive optical, electronic, magnetic, and mechanical attributes can enhance patient care through the use of sensors with minimal invasivity and extreme sensitivity. Amongst the nanomaterials utilized for fabricating nano-sensors, carbon-based nanomaterials are promising as these sensors respond better to signals in various sensing applications[11-13]. In addition to their high surface area, carbon-based nanomaterials are biocompatible materials and are advantageous in terms of simplicity, rapidity, and sensitivity rendering them a high position to be considered in up-to-date technologies for viral recognition[14]. To the extent of authors' knowledge, no review can be found concerning the use of carbon nanomaterial structures for viral sensing and detection. The present paper, therefore, reviews existing carbon nanomaterial-based biosensors for viral recognition at the level of the virus type. Dengue virus, Ebola virus, Hepatits virus, human immunodeficiency virus (HIV), influenza virus (H5N1 and H1N1), Zika virus and Adenovirus are the virus types reviewed here and presented in Figure 2. The review is

divided according to the analyte type into three main groups based on what could be detected from the virus: the biomarkers that arise from the infection, the nucleic acids from the viruses and the entire virus. The role of carbon nanomaterials is highlighted in the improvement of the sensor and recognition functionality.
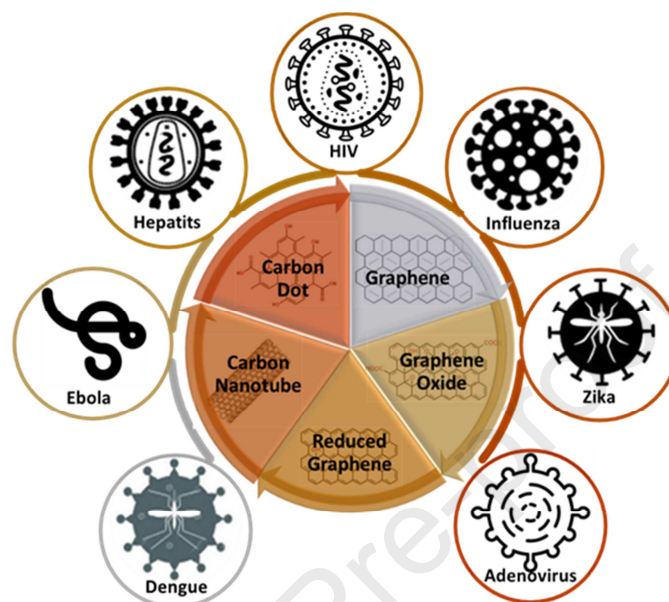

Fig.2 Carbon nanomaterial applications for the detection of different human viruses.

2. Carbon Nanomaterials for diagnostic applications

As an amply occurring element, carbon is widely applied in scientific and technological areas. It is possible to synthesize a variety of carbon allotropes by changing the combinations of sp, sp^2, and sp^3 hybridization and an array of carbon structures and nanostructures has been presented up to the present time[15]. The specifications of carbon nanomaterials are exclusive, including high electrical conductivity and chemical stability as well as a vast functional surface area[16,17]. This section discusses the specifications of carbon nanomaterials for diagnostic applications.

2.1 Carbon Nanomaterials

The three major classes of carbon nanostructures, viz. zero, one and two–dimensional, are widely used in biosensing. For example, carbon dots and graphene quantum dots fall into the class of zero-dimension carbon nanostructures. Nanotubes comprise one-dimensional carbon nanostructures. Graphene and its derivatives are two-dimensional carbon nanostructures with fascinating specifications[15]. Carbon and graphene quantum dots (CDs and GQDs), denoted as zero-dimensional (0D) nanomaterials, have gained increasing ground in the past years.

Their exclusive electronic, fluorescent, photoluminescent, chemiluminescent and electrochemiluminescent features are those providing them with sensing potentiality[18-21]. Other merits of CDs include simple synthetic paths, cost-effective synthesis, inexpensive preparatory materials, water-solubility, low toxicity level, chemical stability and facile functionalization. CD-based sensors and biosensors function by different modes of action, namely: fluorescence quenching, static quenching, dynamic quenching, energy transfer, inner filter effect (IFE), photo-induced electron transfer (PET) and fluorescence resonance energy transfer (FRET)[22]. Carbon nanotubes (CNTs) are carbon molecules with a cylindrical hollow structure whose walls are made by sheets of sp^2-hybridized carbon. Two main types of CNTs are: single walled CNTs (SWCNTs) and multi walled CNTs (MWCNTs), with the former composed of a sole layer of graphene and diameters in the range of 0.7-1.4 nm, whereas their length can vary from a few hundred nm up to several μm. MWCNTs comprise multiple concentric cylinders of rolled-up graphene sheets forming tubes with diameters up to 100 nm [23-25]. CNTs are interesting options for elechtrochemical sensing applications. The electronic specifications need to be taken into consideration in the manufacture of CNT-based electrodes as an essential factor in the electron transfer rate. The CNT modified electrodes are primarily advantageous owing to their minor diameter and long length allowing their plugging into an analyte, their electro-activity being apparently similar to or better than all other carbon-based electrodes and the high surface area of the nanotube modified electrodes[26]. By definition, graphene is a single-atom-thick sheet of hexagonally arrayed, sp^2-bonded carbon atoms that occurs inside a carbon material construct. The thickness of a graphene film with 100 μm of lateral size is regarded as a stack of carbon planes joined together by van der Waals forces that act over a distance of ca. 0.335 nm. Graphene possesses notable thermal and electrical conductivity and a striking mechanical strength. In particular, it has the potential for sensing because of its exceptionally high conductivity and vast surface area. The charge transfer and electronic features of graphene result from its exclusive electronic band structure. Particularly, graphene is an available nanomaterial that possesses a vast surface area (2630 m^2/g) by which it can directly interact with a variety of biomolecules[27-30]. As excellent members of the graphene family, graphene-derived materials, such as graphene oxide (GO) and reduced graphene oxide (RGO), are widely used as biosensors, the applications of which are greatly influenced by their faults, dysfunctions and chemical functionalization on their surfaces. GO, a result of chemical exfoliation and oxidation of layered crystalline graphite (normal or synthetic), is a single atom carbon layer in which both surfaces have undergone modification with oxygen

bearing functional groups. Despite the fact that GO, smilar to grapheme, is a 2-D carbon material, its features highly differ from those of graphene. It is characterized by a plethora of exclusive chemical, optical, and electronic specifications. GO is not capable of absorbing visible light, possesses a low electric conductance relative to that of graphene and exhibits a considerably greater chemical reactivity. There are a lot of epoxide, carboxyl and hydroxyl groups on the basal plane and edges of GO, showing a high potential for binding with biomolecules through covalent, electrostatic, and hydrogen interplays[31-32]. With a wide utilization, RGO is typically manufactured byoxidizing/exfoliating graphite to GO, after which it is reduced to graphene through various chemical, thermal or electrochemical paths. The likely different features of fabricated RGO in terms of defects (vacancies and holes) and carbon to oxygen ratio (C/O) depend on the applied reduction procedure, which has a critical contribution to the electrochemical features of the material. In comparison to graphene, there is even a small number of functional groups on the RGO surface enabling chemical functionalization for immobilization of the molecular receptors onto its surface. Within the last decade, the development of RGO-based sensors have aimed at detecting a variety of intended analytes owing to their brilliant electrochemical features and electrocatalytic functions[33-35].

2.2. Diagnostic techniques based on carbon nanomaterials

As an analytic instrument, a biosensor consists of a biologically active substance that is utilized and closely combined with an apparatus that converts a biochemical signal into a measureable signal. Typically, a biosensor conformation possesses a three-component system: a bioreceptor involved in the selectiveness of the apparatus, a transducer translating the physical or chemical alteration by recognition of the analyte, and a signal-processing unit (signal output) (Figure 3). Since the biomarker oftentimes has an extremely low concentration and detects diseases selectively, its sensibility and selectiveness are critically important. Further needs are its reproducibility, stability, cost-effectiveness, and disposability. Transducing components integrated commonly in the biomarker biosensor scaffold, hitherto comprise components with electrochemistry, optic or mass-sensitivity properties capable of generating quantifiable current, light or frequency signals[36,37].
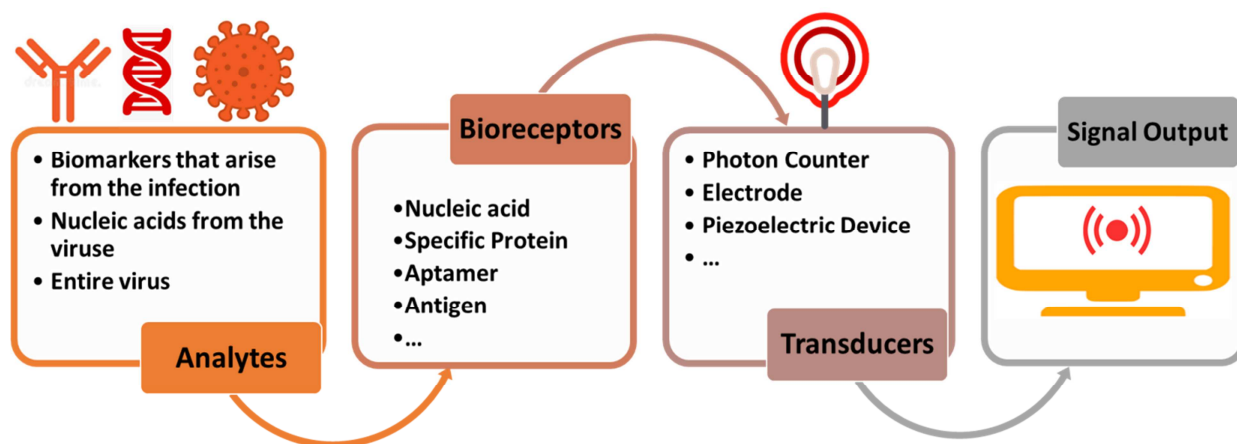
Fig.3 Schematic diagram of biosensor for detection of human viruses as an analytes.

2.2.1. Electrochemical Biosensors: The basis of these biosensors consists of electrochemical methods in which the analyte is sensed by determining the electric response due to the electrochemical reaction of the analyte with the surface of the functional electrode of the sensor. Analyte concentrations and sensor responses generally need to be linearly correlated in order to be applied practically. Amongst a variety of biosensors, the electrochemical biosensors show the promise that they are highly sensitive, have a high signal-to-noise-ratio, are relatively simple and have a fast response time. As carbon has a wide-ranging potential, is chemically inert and affordable, it has been for long time of interest as an electrode substance of importance in electrochemical biosensors. Despite the variable eminent features of CNs, they share attributes causing their attractiveness for the production of electrochemical biosensors including electrochemical activity, electric conductance, wide surface area, easiness of functionalization and biocompatibility. Some CNs and their derived products are able to display electrochemical responses, as they are electrochemically active, intrinsically or capably. The high electric conductance of CNs assures their function as a perfect electron transfer agent in electrochemical biosensors. A wide surface area of CNs makes it possible to assemble extra necessary elements in electrochemical biosensors. Modification of CNs is simple, which will improve and uplift the operations of CNs in electrochemical biosensors. As CNs are biocompatible, the use of CN-based instruments are facilitated in biologic areas. Owing to the above specifications, CNs can serve either as nanoprobes, depending upon their predominant electrochemical features, or as nanocarriers depending upon their additional manifold of specifications. Utilization of CNs in electroanalytical analyses allows a substantial improvement of their analytic functioning[36,37].

2.2.2. Optical Biosensors: The basis of these sensors consists of the detection of alterations in the emanation of light upon the target-detection element interplay. Carbon nanomaterials, in particular graphene–derived products, are effective fluorescence quenching agents. In the last few years, CNTs have widely been a matter of concern for developing biosensors due to their exclusive optical features. CDs hold optical specifications with regard to optical absorption, fluorescence, chemiluminescence, electrochemiluminescence, phosphorescence, up-conversion photoluminescence and photo-stimulated electron transfer activity. CNTs are highly luminosity intensive and have outstanding features, which can ideally be used for optical biosensing. Semiconductive CNTs are able to serve as quenching agents for the fluorophores and can exhibit distinct near-infrared (NIR, wavelength ~0.8–2 µm) photoluminescence emitting from the band-gap fluorescence. GO possesses recombined electron–hole pairs positioned inside sp^2 carbon clusters imbedded inside a sp^3 matrix and displays light absorption from UV to near-infrared (NIR). The π-electrons of GO with large dislocations result in a high fluorescence quenching capability, which is helpful in optical-based biosensors. Besides, graphene is usable as a SERS substratum to improve Raman signals of absorbed biomolecules. Another type of optical biosensor is Surface plasmon resonance (SPR) that utilizes surface plasmon polaritons to probe interplays between an analyte in solution and a biomolecular detection component subjected to immobilization on the SPR sensor surface. Depending on the binding-induced refractive index alteration of the solvent near the surface, SPR biosensors can accomplish a real-time, label-free recognition for the target analyte with high sensitivity. Carbon nanomaterials are good candidates for these types of optical biosensors tools [39-42].

2.3, Field-Effect Transistor (FET) Biosensors: The field-effect transistor is a semiconductor instrument, where the current flows from an electrode (source) on one side to the electrode (drain) on the other side. The semiconductor channel between source and drain is controlled by the electric field generated by a voltage at a third electrode named gate, which is capacitively attached via a thin dielectric layer. The electric FET biosensor specifications can be altered by the adhesion of biomolecules onto the biosensor. CNTs can be metallic or semiconductive contingent on the helicity. Semiconductive CNTs are usable for fabricating FET-based biosensors. The electric CNTFET specifications can be altered by the adhesion of biomolecules onto the CNTs. In recent years, graphene-based FET biosensors have been of great interest as they are highly sensitive to electric disturbances and have a high carrier mobility[39,41].

3. Application of carbon nanomaterials for viral diagnosis

In this section, the recent developments in carbon nanomaterial-based biosensors for viral recognition are reviewed based on what could be detected from the virus. Detection of the antibodies that arise from the infection, detection of the nucleic acids from the viruses and detection of the entire virus are the subdivisions of this section.

3.1. Detection of the biomarkers that arise from the virus infection

The dengue virus of the *Flavivirus* genus in the family Flaviviridae, is an arthropod-borne virus consisting of four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), all of which are encased, spherical viral particles with a diameter of approx. 500Å20(?) [43]. The WHO declares dengue as a main challenging worldwide public health problem in the tropical and subtropical populations [12]. This viral infection results in a variety of pathogenic indications including febrile, fever, and hazardous life threatening situations. Hence, it is not only a major peril to the public but also a main issue for personnel healthcare. Scarce data are available regarding the pathognomonic characteristics of Dengue infection from other febrile ailments. Thus, it is critical to diagnose this viral strain in the initial phases of the disease[44]. The non-structural (NS) proteins of the dengue virus are NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5[45]. NS1 is a secretive protein abundantly present in the acute phase of diseases linked to hemorrhagic fever. Dias et al. invented an immunosensor for the NS1 of the dengue virus on the basis of carbon nanotube-screen printed electrodes (CNT-SPE). A homogenous mixture comprising carboxylated carbon nanotubes was disseminated in carbon ink for preparation of a screen printed working electrode. Anti-NS1 antibodies were connected by a covalent link to CNT-SPE using an ethylenediamine film approach. Evaluation of the matrix effect and the performance of the tests were achieved through spiked blood serum samples yielding superb recovery levels in the outcomes. CNTs integrated into the carbon ink led to improvements in the duplicability and sensitiveness of the CNT-SPE immunosensor[46]. Silva et al. designed a sensitive nanostructured immunoelectrode according to a poly(allylamine) (PAH) sandwich for NS1 of the dengue virus. Anti-NS1 antibodies undergo immobilization on the electrode surface by a thin layer of PAH amassed on carboxylated CNTs. PAH is a cationic polymer serving as a bi-functional material for tight attachment of CNTs to the electrode surface and anti-NS1 antibodies through their Fc(?) terminal, which prevents them to be immobilized randomly. Electrochemical responses of the immunoassay are created at a controlled level by a reaction between H_2O_2 and the peroxidase

enzyme coupled to the anti-NS1 antibodies. Assayed serum samples revealed an accurate and specific process as well [47]. The whole of laboratory and commercial techniques for diagnosing the Dengue Virus NS1 needs the drawing of blood for sample assays, which limits the point-of-care diagnostics and lowers the patient compliance. Instead, NS1 identification in human saliva has the potential to diagnose the Dengue Virus infection earlier. The saliva can be collected simply, non-invasively, painlessly, and inexpensively, even with a staff of minimal training. Wasik et al. offered a label-less chemiresistive immunosensor for detecting the Dengue Virus NS1 protein through a network of SWCNTs subjected to the functionalization with anti-dengue NS1 monoclonal antibodies. Sensitive and selective NS1 detection was successful in contaminated synthetic human saliva within concentration ranges of clinical relevance[48]. An electronic nanobiosensor using a SWCNT network chemiresistor transducer was functionalized with antidengue NS1 monoclonal antibodies to rapidly detect the dengue NS1. Wasik et al. discovered NS1 in a spiked adult *Aedes aegypti* homogenate in a sensitive and selective way over a vast dynamic scope. The biosensor has compatibility with "gold-standard" adult mosquito field-collection instructions and produces electronic data capable for storage or wireless transmission. It has, therefore, the potential for monitoring remotely and real-time[49]. Palomar et al. represented an impedimetric immunosensor for detecting the dengue virus antibody (Figure4(a)). The system takes the advantage of forming CNT deposits on electrodes in a controllable and reproducible way. Their simple functionalization through electrogeneration of a polypyrrole-NHS (N-hydroxysuccinimido 11-(pyrrol-1-yl) undecanoate) film makes it possible to immobilize the Dengue Virus 2 NS1 glycoprotein, the receptor unit, on the porous CNT layer by covalent amide joining to offer the required selectivity toward the Dengue NS1 antibody. The entire fabrication stages of this immunosensor and the functionality of this system were scrutinized by impedance spectroscopy and cyclic voltammetry. The resultant impedimetric dengue biosensor was examined in bovine blood plasma besides measuring conventionally under a controlled environment[50].

The human immunodeficiency virus (HIV) belongs to the genus Lentivirus within the family of Retroviridae and the subfamily Orthoretrovirinae. According to genetically characterized variations in the virus antigens, HIV is grouped into the types HIV-1 and HIV-2. The HIV genome contains two equal single-stranded RNA molecules enveloped inside the core of the viral particle [51]. CD4$^+$ T cells are mostly targeted by HIV. Following a spreading event, HIV occupies the mucosal tissues, and during days is dispersed to the lymphoid organs. At

around day 10, the virus can be detected in the circulation after which it keeps spreading at an exponential rate within the subsequent few weeks, which mostly peaks around day 30, when it is possible to detect the HIV antibody levels[52]. Developing more dependable approaches for detecting and quantifying HIV is highly interesting and worthwhile. A simple one-stage approach for preparing hydrophilic and peptide-functionalized upconversion nanoparticles was presented by Wu et al. (Figure 4(b)). The technique was utilized for designing a biosensor to sensitively and selectively determine HIV antibodies in human serum according to FRET from the upconversion nanoparticles to the graphene oxide. The sensor is usable for anti-HIV-1 gp120 antibody sensing both in an aqueous buffer and in a serum matrix with equivalent performances, demonstrating that the biosensor is able to overcome background interference from complicated biologic samples. The sensor was employed for determining antibody concentrations in human sera[53]. The development of a GO-based fluorescence biosensing platform by Zhang et al. aimed at detecting HIV-1 protease, where fluorescent labeled HIV-1 protease substrate peptide molecules underwent a covalent linking to GO. Without the use of HIV-1 protease, fluorescein quenching was efficiently achieved by GO. On the contrary, the use of HIV-1 protease could split the substrate peptide into short segments, thereby creating fluorescence. The sensor was able to identify HIV-1 protease in human serum[54]. In the last decade, the HIV-1-related capsid protein p24 has gained ground due to the necessity for screening approaches with simplicity, rapidity, sensitivity, specificity, and affordability for diagnosing HIV infection. A competitory electrochemical immunosensor for detecting p24 in unprocessed human serum was designed as a facile and convenient device to screen serum for ahead-of-time discovery of HIV contamination. The immunodevice was employed on throw-away gold-free SWCNT-functionalized screen-printed electrodes. The competitory sensor depends upon immobilizing the target protein on the electrode surface by a chitosan/glutaraldehyde crosslinking system, capable of ensuring, under modrate settings, a strong immobilization and a good exhibition of p24 to interact with a mouse anti-p24 IgG1[55].
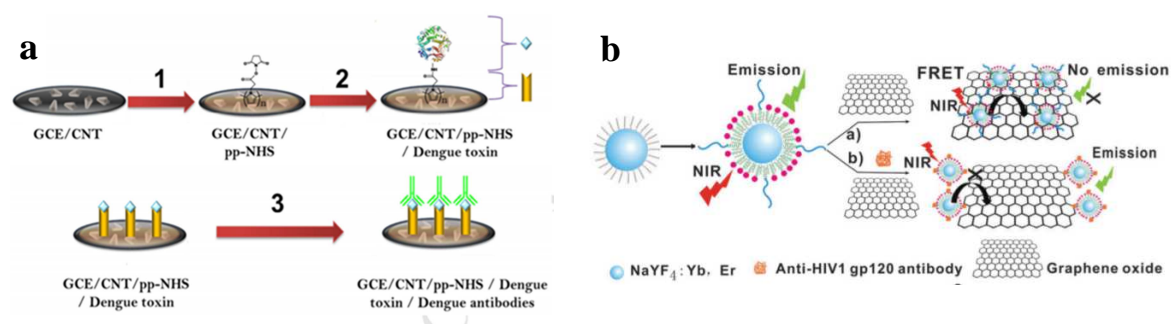
Fig.4 a) Construction steps of the impedimetric immunosensor for the dengue virus antibody detection using functional CNT. Reprinted with the permission of Ref [50]. b) Schematic illustration of the upconversion FRET-based biosensor for the detection of anti-HIV-1 gp120 antibody. Reprinted with the permission of Ref [53].

Influenza viruses are among the utmost prevalent causative agents of human respiratory infections, and one of the most important ones as they induce elevated illness and fatality rates. Influenza viruses of the family *Orthomyx-oviridae* are encased negative-strand RNA viruses with fragmented genomes consisting of seven to eight gene fragments[56]. Of the four existing genera of this family, viz. types A, B, C and Thogotovirus, only the genera A and B are of clinical relevance for humans[57]. Fowl influenza viruses in aquatic birds function as the natural source for all identified subtypes of influenza A viruses and may be the final source of human pandemic influenza strains[58]. Influenza A viruses are further divided by antigenic classification of the hemagglutinin (HA) and NA surface glycoproteins projecting from the virion. There are 16 HA and 9 NA known subtypes[59]. A challenge is to diagnose Influenza A viruses accurately and rapidly as several strains circulate in humans and animal communities and new strains are emerging. An electrochemical immunosensor with a sandwich-type immunoassay format was fabricated for quantification of the fowl influenza virus H7 (AIV H7) with the help of silver nanoparticle-graphene (AgNPs-G) as trace label in clinical immunoassays. The instrument possesses a gold electrode covered with gold nanoparticle-graphene nanocomposites (AuNPs-G), the gold nanoparticle surface of which can additionally be subjected to modification with H7-monoclonal antibodies (MAbs). The immunoassay was done with H7-polyclonal antibodies (PAbs) that were coupled to the AgNPs-G surface (PAb-AgNPs-G). Such a technique of applying PAb-AgNPs-G for detecting antibodies presents a high signal intensification[60].

3.2. Detection of the Nucleic Acid from the Virus

A single viral particle typically consists of either an RNA or a DNA genome. The basis of a DNA biosensor is to immobilize a single stranded oligonucleotide on a transducer surface to identify its complementary DNA sequence because of (due to?) surface hybridization. Then, the hybrid created on the electrode surface undergoes a transformation into an analytic signal through a transducer. This section covers the detection of a nucleic acid from the virus using carbon nanomaterials[61]. The genome of each serotype of the dengue virus contains ca. 11 kb of positive-sense, single-stranded RNA, encoding ten proteins. Jin et al. formulated a composite of 3-Aminopropyltriethoxysilane (APTES) functionalized graphene oxide

(APTES-GO) twined on SiO_2 particles (SiO_2@APTES-GO) through self-assemblage. An impedimetric biosensor was made for detecting dengue DNA and dengue RNA through hybridizing primers by a variety of oligonucleotide sequences. It was observed that the SiO_2@APTES-GO electrode material could enhance the dengue RNA recognition sensitivity with selectivity and an LOD of 1 femto-Molar as opposed to both APTES-GO and APTES-SiO_2. The three-dimensional structure, higher contact area, electrical specifications and the capability of fast hybridization displayed by the SiO_2@APTES-GO resulted in the design of a dengue biosensor[62]. Ebola, is a seriously fatal disease mainly affecting humans and nonhuman primates. The occurrence of the Ebola virus disease (EVD) results from a viral infection belonging to the genus *Ebolavirus* of the family Filoviridae in the order Mononegavirales, viruses whose genome comprises a single-strand RNA with negative polarity[63,64]. Facile, facilitated tests are required to detect and manage the Ebola endemics earlier tests which are capable of detecting the virus in blood at highly little quantities. The WHO announced rapid, sensitive, safe, and simple EBOV diagnostic assays in November 2014[65-67]. Wen et al. developed a GO assisted rolling circle amplification platform for simple and sensitive identification of the Ebola virus. No rolling circle amplification products were produced when the Ebola virus gene was absent, and the adsorption of the fluorescein amidate labeled recognition probe on the surface of GO resulted in quenched fluorescence of the fluorescein amidate. Adding the Ebola virus gene led to the occurrence of an amplified rolling circle, and a double-stranded DNA formed between the rolling circle amplification products and the fluorescein amidate labeled recognition probe, by which the fluorescein amidate labeled detection probe was desorbed from the GO surface associated with the fluorescence retrieval. The ebola virus gene can be identified both in aqueous and 1% serum solutions [68].

By definition, hepatitis is an inflammatory state of the liver. Oftentimes, it has a viral cause defined as viral hepatitis[69]. The hepatitis B virus (HBV) is a hepatotropic virus capable of establishing persisting and prolonged contamination in humans via immunity anergy. HBV is an encased viral DNA belonging to the Hepadnaviridae family[70]. Assays of HBV paves the ground for providing therapy and preventive instructions to positively detected people (and their contacts) and for recommending vaccination to those diagnosed negatively but of persistent risk[71]. Research is mostly focusing on the leading issues of HBV screening and diagnosing infective HBV[72]. Given the robust interplay between single-stranded DNA and graphene material, Xiang et al. have produced a simplistic but intelligent electrochemical

instrument for detection of HBV-DNA with the help of a GQD modified glassy carbon electrode connected to a specific sequence of DNA molecules as probes. The probe DNA is produced such that it is complemental to the HBV-DNA. The strong coupling of the probe DNA to the GQD modified electrode surface makes it difficult for an electron to convey from the electrode to the electrochemically active species $K_3[Fe(CN)_6]$. Even so, when the target HBV-DNA is present in the assay solution, the probe DNA will attach to the target HBV-DNA rather than to GQDs. Consequently, the resultant peak currents of $K_3[Fe(CN)_6]$ will elevate differently with varying concentrations of the target HBV-DNA[73]. Hepatitis C is an infective illness induced by the hepatitis C virus (HCV), which is a viral RNA of the family Flaviviridae. When HCV infection is screened by simple and rapid techniques with high sensitivity and specificity, it can assist to control the encumbrance on HCV health care globally[74]. Fan et al. established an experiment to ultra-sensitively identify HCV RNA according to the RGO nanosheets (rGONS) and hybridization chain reaction amplification technique (Figure 5(b)). The recognition system possesses a pair of single fluorophore-labeled hairpin probes capable of free existence in the solution in the lack of target RNA. By introducing target RNA, a robust hybridization chain reaction is triggered with the two probes which produces long nanowires having a double-stranded construct. The poor adsorption to rGONS induces the long nanowires for emitting a robust fluorescence. Additionally, the technique is selective for discriminating complemental and incompatible sequences. Lastly, the innovative technique was utilized as a HCV RNA test in biologic samples with a robust anti-interfering ability in complex settings[75]. In an introduced DNA aided magnetic rGO-copper nanocomposite (mrGO-CuNCs), copper ions can expedite the oxidation of o-phenylenediamine. The electrochemical signals of the oxidized product, 2,3-diaminobenazine, are applied for characterizing the HCV DNAs. In a critical manner, it was capable of effective discrimination of the 1b and 6 k subtypes of HCV[76]. Based on the host-and-guest interplay between cucurbit[7]uril(CB[7]) and methylene blue, a CB[7]-graphene nano-composite(CB[7]-N3-GO) is publicized to detect HCV DNA electrochemically. The introduced identification approach has the ability of discriminating the 1b and 6k subtypes of HCV and is promising in screening the blood for HCV in clinical diagnostics[77].

The HIV genome contains two equal single-stranded RNA molecules enveloped inside the core of the viral particle[51]. A sensitive impedimetric DNA biosensor for determining the HIV-1 gene was designed by the use of electrochemical RGO (ERGO) as a sensing tool. Gong et al.

manufactured the DNA biosensor by drop-coating GO on a glassy carbon electrode and by covalent immobilization of the fabricated single-stranded DNA probe onto the GO by carbodiimide chemistry. Then, GO was exposed to electrochemical reduction to produce ERGO and utilized for genosensing. The incidence of hybridizations between the surface-confined single-stranded DNA probe with the target DNA in solution for forming double-stranded DNA at the electrode surface led to changes in the negative charge in the electrode/electrolyte interface, and, in turn, to the electron transfer resistance of the electrodes towards the [Fe(CN)6]3–/4– redox couple. Tthe change was utilized for impedimetric DNA biosensing. According to reported findings, the application of ERGO as an immobilization platform resulted in an efficient acceleration of the electron transfer and in an enhancement of the EIS response of the DNA biosensor. This approach excluded the need for DNA labelling, which significantly simplified the process[78]. Wang et al. produced an electrochemical biosensor on the basis of an one-stage ultrasonic manufactured graphene stabilized gold nanocluster (GR/AuNC) modified glassy carbon electrode with an exonuclease III (Exo III)-assisted target recycling amplification approach for detecting HIV DNA. GR/AuNCs have been utilized as biosensor instruments and as aptamer with a cytosine-rich base set as capture probe for constructing the biosensor. By combining the cytosine-rich capture probe, the proper conductivity and the high surfaces of GR/AuNCs, and Exo III-assisted target recycling amplification, they achieved the recognition of target HIV DNA sensitively and selectively [79]. A nanocomposite of polyaniline/graphene (PAN/GN) was formulated by reverse-phase polymerization. The nanocomposite material was dropcasted onto a glassy carbon electrode. Next, a single-stranded DNA probe for detecting the HIV-1 gene was exposed to immobilization on the modified electrode, and the negatively charged phosphate framework of the HIV-1 was coupled to the modified electrode surface through π-π^* stacking interplays. The hybridization between the single-stranded DNA probe and the target HIV-1 formed double-stranded DNA, and the electron transfer resistance of the electrode was determined by impedimetric examinations with a $[Fe(CN)_6]^{3-/4-}$ redox couple[80]. SWCNT-based sensors proved to be capable of optical recognition of small, free oligonucleotides in biofluids and in vivo, although proteins reduced the sensitiveness. Harvey noticed a phenomenon in which the CNT optical response to nucleic acids could be improved by denatured proteins. Mechanistic investigations revealed that hydrophobic patches of the denatured protein chain interacted with the unbound nanotube surface following hybridization, leading to improved shifting of the nanotube emission. They utilized this machinery to discover an untouched HIV in serum, giving rise to particular responses within minutes(Figure 5(c)). The present survey

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foreshadows the application of carbon nanotubes to optically detect viruses at the point-of-care by measuring viral nucleic acids[81].

Influenza viruses of the family *Orthomyx-oviridae* are encased negative-strand RNA viruses with fragmented genomes consisting of seven to eight gene fragments[56]. The development of a microfluidic incorporated rGO transistor was also reported for recognition of the H5N1 influenza virus gene through a flow-through approach. Both the fluorescence determination and the electrical recognition were executed for evaluating the functionality of rGO transistors in a flowing environment. This microfluidic incorporated rGO transistor with its expanded capture probe immobilization technique was able to offer a tool for sensitive and stable recognition of nucleic acid and can potentially be applied for flow-through chips[82]. Jeong et al. produced a fluorometric system to detect influenza subtype viral genes by GO. A fluorescent DNA probe corresponding to the hemagglutinin gene of the influenza virus is broken by the 5'-3' exonuclease function of *Taq* polymerase throughout PCR. After adding GO, the emitted fluorophore maintains its fluorescence while not being adsorbed onto GO, while the unchanged fluorescent DNA probe undergoes adsorption onto GO with quenched fluorescence. The multi-well plate system can identify 3.8 pg of the influenza viral RNA[83]. Binary-NP-decorated CNTs (bNP-CNTs) were manufactured via a facile two-stage technique and utilized as a biosensing tool (Figure 5(b)). Gold (Au)/iron-oxide magnetic NP-decorated CNTs (Au/MNP-CNT) were employed for influenza sensing channels. The biosensing was demonstrated by initial magnetic alignment of the Au/MNP-CNTs on a Pt-interdigitated electrode, after which a thiol-group-functionalized probe DNA was connected to the Au NP surface on the bNP-CNT hybrid structure through thiol chemistry. DNA hybridization between the target influenza and probe DNA was determined for monitoring changes in electrical conductivity of the Au/MNP-CNTs. The specificity was demonstrated by differing mismatched DNA sequences, presenting specificity[84].
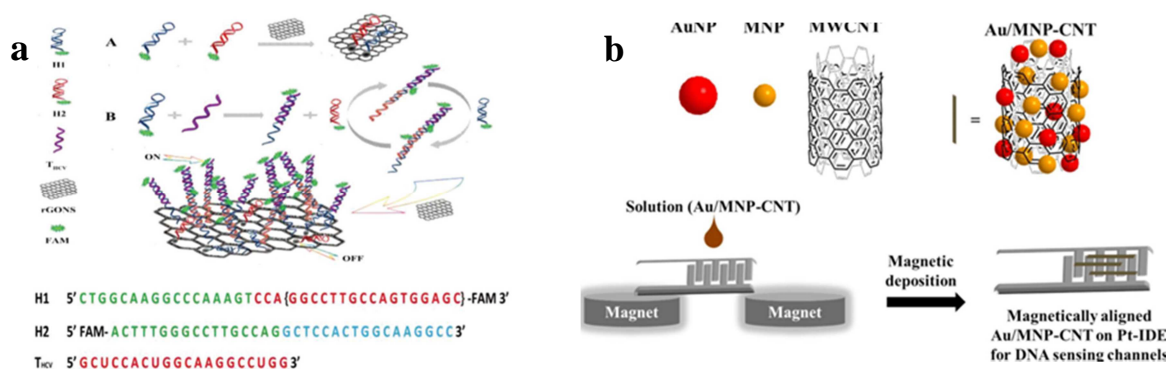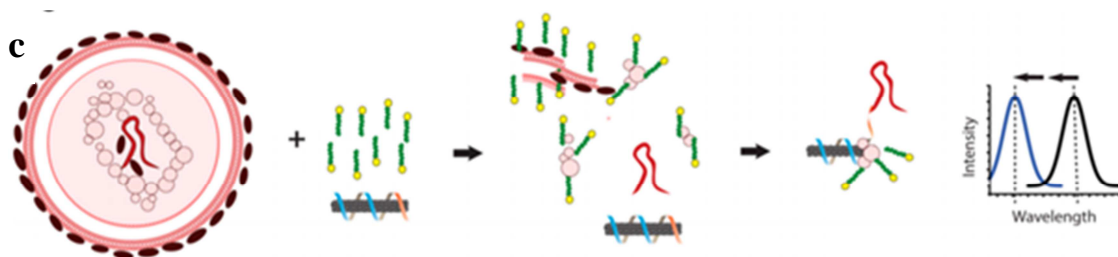
Fig.5 a) Schematic of the proposed rGONS-HCR platform for HCV detection. Reprinted with the permission of Ref [75]. b) Illustration of the preparation of the magnetically aligned Au/MNP-CNTs on the Pt-IDE for DNA sensing channels. Reprinted with the permission of Ref [84]. c) Model depicting HIV RNA detection via a CNT RNA sensor. Reprinted with the permission of Ref [81].

3.3. Detection of the Entire Virus

This part covers the detection of the entire virus using carbon nanomaterial. The three structural proteins of the dengue virus encoded by the genome are the membrane (M) protein, envelope (E) protein and capsid (C) protein. It was reported that a biofunctionalized tapered optical fiber based sensor with the incorporation of GO could be utilized to detect Dengue virus II E proteins. The narrowed site was amassed with GO and underwent functionalization with anti-DENV II E protein IgG antibodies for testing with various concentrations of DENV II E proteins. The sensor was found to be precise, selective, and affinitive towards E proteins [85]. Omar et al. reported the development of a SPR sensor on the basis of a self-assembled monolayer/rGO-polyamidoamine dendrimer (SAM/NH$_2$rGO/PAMAM) thin film to identify DENV II E proteins. They then assessed the SPR sensor in terms of specific, sensitive, binding affinity and selective activities. The researchers found that variations in the sensing layer because of differing spin speed, time incubation, and concentration could provide an improved interplay between the analyte and the sensing layer. Selective activity of the SPR sensor toward the DENV II E proteins was obtained with the use of other competitors[86]. Heparins, an analogue of the heparan sulfate proteoglycans, are receptors for the dengue virus during infection of Vero cells and hepatocytes. Wasik et al. proposed an electronic biosensor based on a SWCNTs network chemiresistive transducer, which was subjected to functionalization with heparin for recognition of the entire dengue virus (Figure 6(a)). Recognition of the dengue virus in a viral culture supernatant is as sensitive as the corresponding viral titer in a phosphate buffer in spite of using growth media and Vero cell lysate. The biosensor exhibited sensitivity within a range of clinical relevance for humans and infected Aedes aegypti[87]. A report indicates a novel procedure for detecting, classifying and antibody screening of the dengue virus on the basis of electrochemical impedance

spectroscopy . Navakul et al. discovered that the charge transfer resistance of a gold electrode covered with a GO strengthened polymer was affected by virus strains (and amount subjected to the surface?). Such a finding could explain the ability to recognize molecularly (proven?) while preparing the GO-polymer composite. This technique can demonstrate that DENV is present at an initial stage of the infectious condition. This technique could distinguish between DENV and the other sub-types, besides H5N1[88].

Ebola virus glycoprotein is the only protein capable of expression on the viral surface. The Ebola glycoprotein has crucial contributions to the viral entrance into cells and to the escape of the immune system. Maity et al. offered an electronic resonance frequency modulation to identify Ebola glycoprotein within a dielectric-gated rGO field-effect transistor. The sensitiveness of Ebola recognition can be considerably improved by evaluating the device electronic-resonance frequency, including its inflection frequency, in which the phase angle approaches a maximum. Utilizing charge-relaxation dynamics, a bio-FET sensing platform is achieved for healthcare and bioelectronic uses via resonance shifting[89]. In a further research, Jin et al. reported a field effect transistor based immunoassay for detecting the deactivated Ebola virus. An (equid?) antibody versus the Ebola virus glycoprotein underwent immobilization on the surface of the field effect transistor T modified earlier with RGO. The antibody versus Ebola virus was subjected to immobilization on the modified field effect transistor and the response to Ebola virus was assessed as a function of the shift of Dirac voltage. The test is specific satisfactorily and was utilized to quantize the deactivated Ebola virus in spiked serum[90].

A composite comprising GO and gold nanorods (GO-GNRs) was invented by Liu et al. for determining the trace of hepatitis B surface antigen (HBsAg) through surface enhanced Raman spectroscopy (Figure 6(b)). The antibody on the GO-GNRs couples HBsAg with a high specific activity, resulting in a brilliant selectivity. The immunoassay determines the sensitivity and selectivity of HBsAg in serum and develops the potential of GO-GNR based surface enhanced Raman spectroscopy tag to be utilized in clinically related examinations[91]. Zhao et al. manufactured a composite of graphene oxide-ferrocene-chitosan (GO-Fc-CS) and utilized it for developing an electrochemical immunosensor with a GO-Fc-CS/Au-nanoparticle layer film for HBsAg recognition. The modified layer film demonstrated not only an improved electron conductivity, but also a robust reversible redox signal for current changes, superb biocompatibility and good film-making capability to bind a plethora of antibodies. The functionality in HBsAg recognition implies that the introduced

immunosensor can potentially be applied in clinical diagnostics[92]. A hybrid biosensor on the basis of a graphene resistor functionalized with self-assembled Graphene-AuNPs (Gold Nanoparticles) is established for the instantaneous recognition of HBsAg. The hybrid biosensor contains an ssDNA sequence coupled to a graphene resistor instrument through π–π stacking interplays combined with an ssDNA functionalized AuNP. The ssDNA has complemental sequences that produce the graphene-AuNP hybrid biosensor via hybridization[93]. The HCV genotype dispersal varies by region[94]. The asymmetrical and heterogeneous virion has a buoyant property and protein content to the same level as low-density lipoparticles. The core protein is amorphous and consists of the two envelope glycoproteins, E1 and E2[95]. Valipour et al. discovered a simple green method to apply silver nanoparticle (AgNPs) and thiol GQD (GQD-SH) as the nanomaterial to detect the HCV core antigen. The AgNPs/GQD-SH was applied as a substratum to load antibodies for detecting the HCV core antigen. AgNPs were subjected to immobilization on SH groups of GQDs through the forming of the Ag-S and anti-HCV bond and were laden on the electrode surface by the interplay between the –NH$_2$ group of the antibody and AgNPs. Riboflavin was utilized as a biologic molecule with intrinsic features as the redox probe for developing a HCV core antigen electrochemical immunosensor. This immunosensor was utilized for the analysis of a serum sample[96].
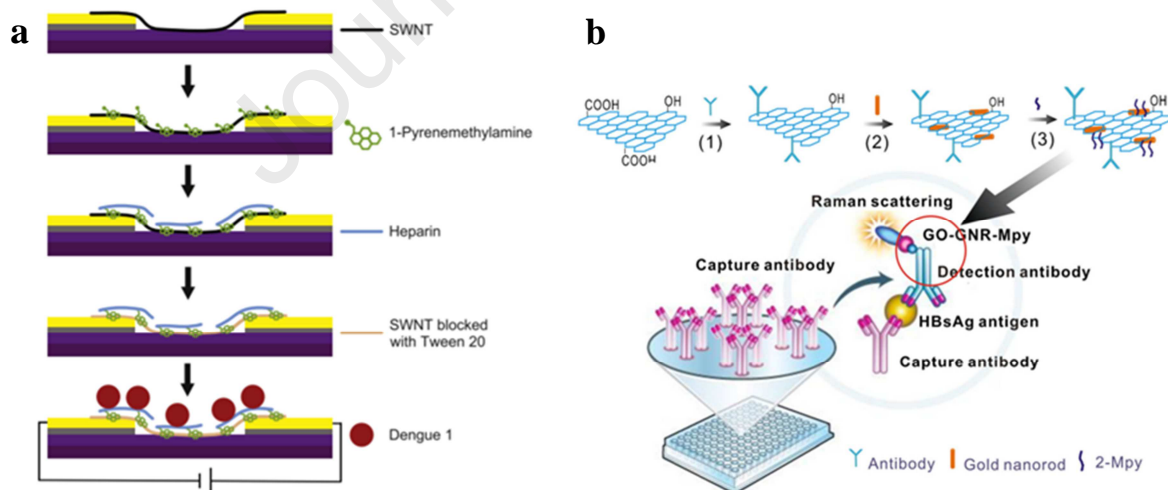
Fig.6 a) Functionalization schematic representation of the fabrication of the chemiresistor used for detection of Dengue virus. Reprinted with the permission of Ref [87]. b) Schematic illustration of Raman immunoassay based on GO-GNRs. Reprinted with the permission of Ref [91].

Yeh et al. described an exclusive CNT size-tunable enriched microdevice (CNT-STEM) that can effectively enrich and concentrate viruses sampled from field strains. The channel sidewall in the microdevice was prepared by mounting orders of vertical alignments of

nitrogen-doped multiwalled CNTs, in which the intertubular distance between CNTs could be designed in the scope of 17-325 nm for accurate matching the size of various viruses. The CNT-STEM led to a significant improvement of LODs and virus isolation rates by at least hundred times. They used this device for identification of an emergent fowl influenza virus and a new virus strain[97]. Veerapandian et al. described the design of an electrochemical-based dual-sensor platform consisting of methylene blue-electroadsorbed GO nanostructures modified with monoclonal antibodies towards the HA proteins of H5N1 and H1N1. Bio-functional layers composed of chitosan and protein-A molecules were executed at the interface of the sensor element and the antibodies, which synergistically promoted the bio-activity of the immobilized antibodies to form the immune complex[98]. Graphene-FET detects the targets electrically with high sensitivity due to the high carrier mobility of graphene. A report by Ono et al. indicates that the sialoglycan-functionalized G-FET can selectively detect the possible pandemic virus. Initially, sialoglycan underwent modification on the graphene channel by a π-stacking cross-linker. Subsequently, the sialoglycan-functionalized G-FET was utilized to discover the target. Two lectins obtained from *Sambucus sieboldiana* (SSA) and *Maackia amurensis* (MAM) were applied as targets in place of the human and fowl influenza virus, respectively(Figure 7(a)). Finally, G-FETs were used to selectively detect the targets, one is modified by the human type sialoglycan and the other by the avian-type sialoglycan[99]. Singh et al. offered a microfluidic chip incorporated with an RGO-based electrochemical immunosensor for label-free detecting an influenza virus, H1N1. Three microelectrodes were manufactured on a glass substrate by the photolithographic method, whereas the working electrode was subjected to functionalization with RGO and monoclonal antibodies specific to the virus. These chips were incorporated with polydimethylsiloxane microchannels[100]. An effective electrochemical influenza A biosensor was designed based on a graphene–gold (Au) hybrid nanocomposite modified Au-screen printed electrode, which principally works in dependence of the measured neuraminidase activity. The experimental factors, including the impacts of the bovine serum albumin inclusion and the immobilization times of fetuin A and PNA lectin, were optimized to investigate the analytical characteristics of the influenza A biosensor. The invented biosensor was utilized for detecting the actual influenza virus A (H9N2)[101]. A magnetic/plasmonic-assisted fluoro-immunoassay system is designed to detect the influenza virus by magnetic-derivatized plasmonic molybdenum trioxide quantum dots (MP-MoO$_3$ QDs) as the plasmonic/magnetic agent and fluorescent graphitic carbon nitride quantum dots (gCNQDs) as the monitoring probe. A specific antibody towards the influenza A virus was coupled onto the surface of the MP-

MoO$_3$ QDs and gCNQDs in respective order. When influenza A virus is present, a core-satellite immunocomplex forms between the antibody-coupled nanomaterials and their interplay modulates which enhances gradually the fluorescence strength of the detecting probe with an elevation depending on the influenza virus concentrations[102]. A photoelectrochemical immunosensor with sensitivity and specificity was manufactured to analyze subgroup J avian leukosis viruses (ALV-J) according to a dual signal-on approach. Gold nanoparticles (AuNPs) decorated graphitic carbon nitride (AuNPs/g-C$_3$N$_4$) as photoelectrochemical species and primary antibody against ALV-J underwent successive immobilization on the ITO electrode. An ALP-CdTe-Ab$_2$ bio-conjugant was made by the assembly of a second antibody and alkaline phosphatase (ALP) to the CdTe quantum dots surface. The photoelectrochemical immunosensor was produced by attaching the target ALV-J and ALP-CdTe-Ab$_2$ bio-conjugants on the electrode surface through the immune detection[103]. An electrochemical immunosensor for ALV-J was assembled on the basis of mesoporous graphitic carbon nitride (mpg-C$_3$N$_4$) (Figure 7(b)). Mpg-C$_3$N$_4$ was utilized as the sensor scaffold to bind to the primary antibodies (Ab$_1$). The complex of thionine and mpg-C$_3$N$_4$ (Th-mpg-C$_3$N$_4$) was manufactured to function as the electroactive probe and the carrier of secondary antibodies (Ab$_2$). Mpg-C$_3$N$_4$ holds a greater specific surface area, less electrochemical resistance and plentiful active positions relative to bulk g-C$_3$N$_4$. Thus, the introduced electrochemical immunosensor displayed augmented detecting signals that could sensitively detect ALVs-J[104].
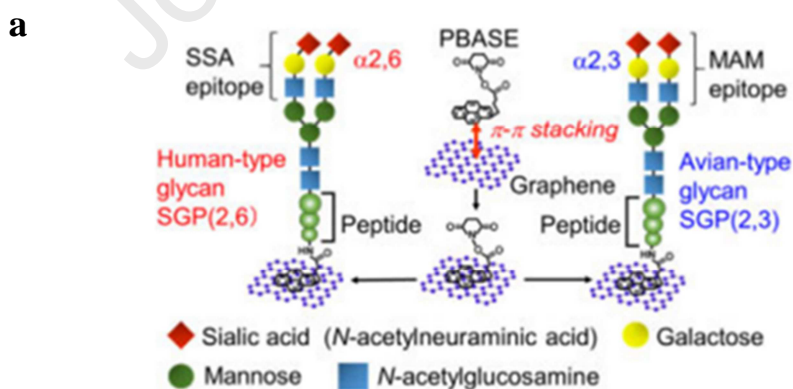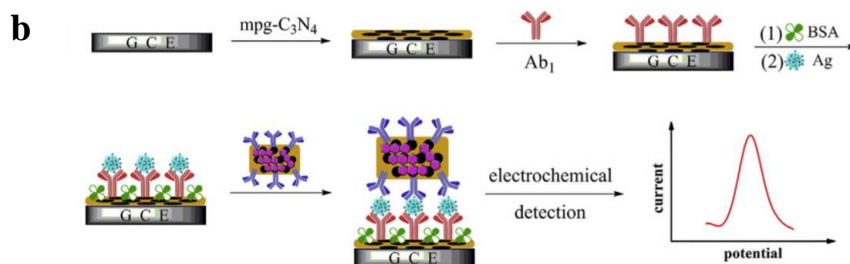
Fig.7 a) Schematics of the immobilization method for SGPs on graphene surface using PBASE linker. Reprinted with the permission of Ref [99]. b) Schematic representation of the fabrication of the iElectrochemical Immunosensor Based on Mesoporous Graphitic Carbon Nitride for Detection of Subgroup J of Avian Leukosis Viruses. Reprinted with the permission of Ref [104].

The Zika virus (ZIKV) is defined as an arthropod-borne virus belonging to the Flaviviridae family comprising the Flavivirus and Hepacivirus genera. It is a positive, single-stranded ribonucleic acid (RNA) virus with a casing, rendering it a close relation to the Spondweni virus. Fast and severe detection platforms are urgently needed for the Zika virus[105-107]. Afsahi et al. fabricaed an economical and movable graphene-enabled biosensor to recognize the Zika virus with a specific immobilized monoclonal antibody (Figure8(a)). Field Effect Biosensing with monoclonal antibodies and covalent linkage to graphene allows the detection of local Zika viral antigens quantitatively and in real-time. Measurement of the Zika antigen in a simulated human serum revealed a promising diagnostic applicability. Validation of its selectivity was achieved by the Japanese Encephalitis NS1, a homologue viral antigen with potential cross-reactivity[108].

Human adenoviruses (HAdVs) are grouped in the genus *Mastadenovirus.* Adenoviruses are non-encased double-stranded DNA viruses infecting various human tissues[109]. The adenoviral structure consists of two main components, the exterior capsid and the interior core, where the viral dsDNA genome is enveloped together with a plethora of histone-like proteins. Viral proteases have a key contribution to the maturation by cleaving progenitor proteins for the capsid and the core. Adenoviruses are oftentimes linked to pediatric disorders of the upper respiratory tract, such as the common cold[110]. Jin et al. proposed a procedure for detecting the HAdV hexon antigen by CNT sensors. An anti-HAdV antibody was exposed to immobilization on the reverse surface of a CNT sensor. As a control, non-specific mouse IgG was subjected to immobilization on another CNT sensor. I-V(gate) curves were determined after various concentrations of recombinant HAdVs hexon antigen (were? subject) incubated with anti-HAdVs antibody-immobilised or non-specific mouse IgG-immobilised sensors. The curves revealed a positive shift that depended upon the hexon antigen concentrations in the anti-HAdV antibody-immobilised sensor, while no such a shift

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was noticed in the non-specific mouse IgG-immobilised sensor[111]. Template-free *In situ* gold nanobundles (Au NBs) were produced on an electrode for optoelectronic sensing of fowl adenoviruses (FAdVs). A Au NB film was manufatured on a carbon electrodes working area by L(+) ascorbic acid, gold chroloauric acid, and poly-*L*-lysine (PLL) via a modified layer-by-layer (LbL) technique. Both Au NBs and GQDs underwent conjugation with the target FAdVs specific antibodies bringing them near to one another with the adding target FAdVs via an antibody–antigen interplay(Figure 8(b)). At close proximity, the light–matter interplay between Au NBs and QDs yields a local electric signal improvement under ultraviolet–visible light radiation allowing the recognition of extremely small concentrations of a target virus even in complicated biologic media[112].

Some of the carbon nanomaterial-based biosensors reported in the literature to detect Human Viruses are summarised in Table 1.
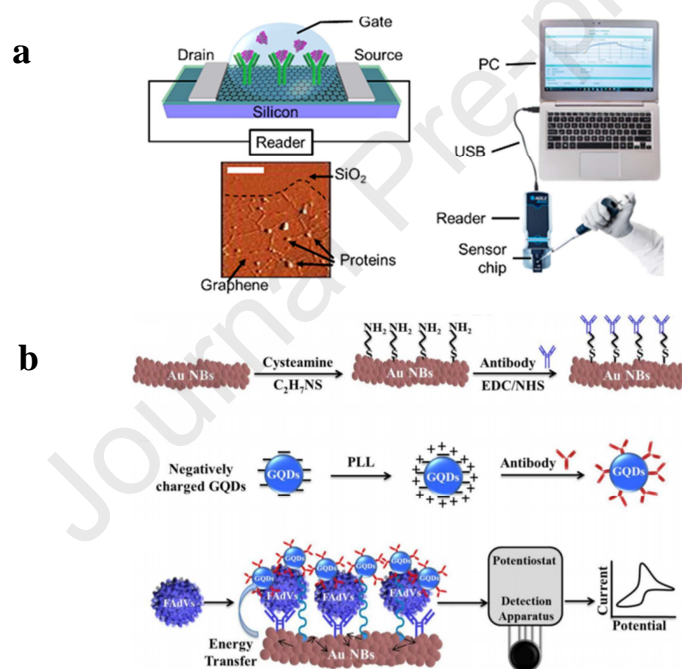
Fig.8 a)Diagram of the movable graphene-enabled biosensor to recognize Zika virus with a specific immobilized monoclonal antibody. Reprinted with the permission of Ref [108]. b) Schematic presentation of optoelectronic Fowl Based on Local Electric Field Enhancement on Graphene Quantum Dots and Gold Nanobundle Hybrid for Adenovirus Detection. Reprinted with the permission of Ref [112].

Table1. Carbon nanomaterial-based biosensor for human virus detections.

Carbon Nanomaterial	Virus	Target	Assay	Detection limit	Detection range	Detection platform	Ref
GQD	Hepatits B	DNA	Electrical	1 nM	(10– 500) nM	Smart	[73]

						electrochemical	
	Hepatits C	Antigen	Electrochemical	3 fg mL^{-1}	(0.05- 60) ng mL^{-1}	Electrodes	[96]
	Fowl adenoviruses	Antigen	Optoelectronic	8.75 PFU/mL	(10 - 10000) PFU/mL	Electrode	[112]
CNT	Dengue	NS1 protein	Amperometrical	12 ng mL^{-1}	-	Screen printed electrodes	[46]
	Dengue	NS1 protein	Electrochemical	0.035 µg mL^{-1}	(0.1 - 2.5) µg mL^{-1}	Electrodes	[47]
	Dengue	NS1 protein	Chemiresistive	-	-	-	[48]
	Dengue	NS1 protein	-	-	-	-	[49]
	Dengue	NS1 antibody	Impedimetric	-	(10^{-13}–10^{-5}) g mL^{-1}	Electrodes	[50]
	HIV	Capsid protein p24	Electrochemical	2 pM	10 pM - 1 nM	screen-printed electrodes	[55]
	HIV	Protein	optical	-	-	-	[81]
	Dengue	Virus	Chemiresistive transducer	-	-	-	[87]
	Influenza	DNA	electrical conductivity change	8.4 pM	(1- 10) nM	Electrodes	[84]
	avian Influenza	-	-	-	-	-	[97]
	human adenovirus	Antigen	-	-	-	-	[111]
Graphene	Influenza H7	Antibody	electrochemical	1.6 pg/mL	1.6×10^{-3}~16 ng/mL	Electrodes	[60]
	HIV	DNA	Electrochemical	30 aM	-	Electrodes	[79]
	HIV	Gene	Impedimetric	1.0×10^{-16} M	(5.0×10^{-16} - $1.0 \times 10^{-10)}$ M	Electrodes	[80]
	Hepatits B	Antigen	Resistance change	50 pg ml^{-1}	-	-	[93]
	Human and Avian influenza	Sialoglycan	Electrical	-	-	-	[99]
	Influenza A H9N2	Neuraminidase	electrochemical	10^{-8} U mL^{-1}	10^{-8} - 10^{-1} U mL^{-1}	screen printed electrode	[101]
	Zika	Antigen	Capacitance change	450 pM	-	-	[107]

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GO	HIV	Antibody	FRET	-	-	-	[53]
	HIV	Protease	Fluorometric	1.18 ng/ mL	-	-	[54]
	Dengue	DNA and RNA	Impedimetric	1 fM	-	Electrodes	[62]
	Ebola	Gene	Fluorometric	1.4 pM	-	Rolling circle amplification	[68]
	Hepatits C	DNA	Electrochemical	160.4 pmol/L	(0.2–10) nmol/L	Electrodes	[77]
	influenza	Gene	Fluorometric	-	-	Multi well plate	[83]
	Dengue	Virus II E proteins	-	1 pM	-	Tapered optical fiber	[85]
	Dengue	DENV-2 E-proteins	SPR	0.08 pM	(0.08 - 0.5) pM	-	[86]
	Dengue	Virus	Electrochemical impedance spectroscopy	0.12 pfu/mL	$(1 - 2 \times 10^{3)}$ pfu/mL	Electrodes	[88]
	Hepatits B	Antigen	Surface enhanced Raman spectroscopy	0.05 pg·mL−1	$(1–1000)$ pg·mL^{-1}	Surface enhanced Raman spectroscopy	[91]
	Hepatits B	Antigen	Electrochemical	0.01 ng/mL	(0.05- 150) ng/mL	Electrodes	[92]
	Influenza Virus H1N1, H5N1	HA proteins	electrochemical	-	25–500 pM	Electrodes	[98]
RGO	Hepatits C	RNA	hybridization chain reaction amplification	10 fM	-	-	[75]
	Hepatits C	DNA	electrochemical	405.0 pM	0.5–10 nM	Electrodes	[76]
	HIV	DNA	Electrochemical	3.0×10^{-13} M	$(1.0 \times 10^{-12} - 1.0 \times 10^{-9})$ M	Electrodes	[78]
	influenza virus H5N1	Gene	-	-	-	Microfluidic Chip	[82]
	Ebola	Glycoprotein	Electronic- resonance- frequency modulation	-	(0.001–3.401) mg/L	Bio-FET sensing	[89]
	Ebola	Glycoprotein	Shift of Dirac voltage	2.4 pg·mL^{-1}	$(2.4 \times 10^{-12} - 1.2 \times 10^{-7})$g·mL^{-1}	FET	[90]
	influenza virus H1N1	Virus	electrochemical	0.5 PFU mL^{-1}	$(1-10^{4})$ PFU mL^{-1}	Microfluidic chip	[100]

	Influenza (H$_1$N$_1$)	Virus	photoluminescence	0.25 pg/mL	(0.001– 100) ng/mL	Probe	[102]
	Influenza (H$_3$N$_2$)	RNA	photoluminescence	45 PFU/mL	(45 -25,000) PFU/mL	Probe	[102]
g-C$_3$N$_4$	J avian leukosis	Virus	photoelectrochemical	85 TCID$_{50}$/mL	-	Electrode	[103]
	J avian leukosis	Virus	Electrochemical	120 TCID$_{50}$/mL	-	Electrode	[104]

4. Conclusion and Future Perspective

Infective diseases continue to be a ubiquitous risk to universal and public health, particularly in numerous countries and rural areas of cities. Basic causes of such severe diseases can be outlined as the scarcity of suitable analytic techniques and consequent therapeutic approaches resulting from the inadequate accessibility of consolidated and fortified healthcare equipments for diagnostics. Biosensors greatly influence, in turn, our existing analytic techniques into diagnostic approaches by rearrangement of their sensing modules for detecting viruses. Indisputably, the existing sensing equipment needs continual updating for addressing the rising challenging issues in the identification of viruses as viruses undergo quick changes and disseminate mainly from individual-to-individual, suggesting the need for urgent diagnosis in the first place. A number of such challenges can fall into biologic barricades, technological limits, and economic facets limiting their application to resource-scarce situations. Biologic barricades include specificity, low number of targets, and biologic media. Detection limit, linear dynamic range, stability, and reliability are technological limits. Although, carbon nanomaterial-based sensor technologies are highly promising, they present many challenges in order to move from the bench to their use in the point of care. Nanotechnologies are offering new means to ease the process of diagnosis based on different platforms by performing direct detection of molecular targets in real time. Different varieties of carbon nanomaterials provide delicate and accurate platforms in this field. Considering all above mentioned benefits, we shall take some more steps to make carbon nano-sensors profitable by the appropriate selection of nanomaterials. The Critical issues are the immobilization methods of the nanomaterials and biological elements to mitigate the risk of accuracy and correctness in virus detection. Another important issue is the economic aspect of the procedure. Carbon nanomaterials are cheaper than the current in use nano-biosensors such as gold. So, it seems pretty handful for investors to think about this field. And the last item we may consider is the lifetime of the assay, which may be considerable. Human beings

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have no choice to provide accessible, accurate, real-time, portable and reusable instruments to study viruses in high sensitivity and selectivity levels. In conclusion, developing the nanotechnologies is a scorecard in the health sector that let us to think about it more seriously in order to provide a better life. At editing my article, the world faces the COVID-19 pandemic and it is obvious that the in-vitro diagnosis virus kit's market will face an inflection in 2020-2021.

Acknowledgements

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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