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Review

Nanotechnology: A reality for diagnosis of HCV infectious disease

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SUMMARY

Hepatitis C virus (HCV) is the primary etiologic agent of liver cirrhosis or hepatocellular carcinoma. HCV elevated infection rates are mostly due to the lack of an accurate and accessible screening and diagnosis, especially in low- and middle-income countries. Conventional HCV diagnostic algorithm consists of a serological test followed by a nucleic acid test. This sequence of tests is time consuming and not affordable for low-resource settings. Nanotechnology have introduced new promising tests for the diagnose of infectious diseases. Based on the employment of nanoparticles and other nanomaterials which lead to highly sensitive and specific nanoscale tests, most of them target pathogen genome. Implementation of nanoscale tests, which are affordable, portable and easy to use by non-specialized personal, would improve HCV diagnosis algorithm. In this review, we have summed up the current emerging nanotechnology tools, which will improve actual screening and treatment programs, and help to reach HCV elimination proposal.

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Introduction

Hepatitis C virus (HCV) is the primary etiologic agent of liver cirrhosis or hepatocellular carcinoma. Up to eight major genotypes (1–8) and 86 subtypes [1,2] have been described so far. Among them, the type 1 the most common genotype worldwide, followed by type 3 [3]. Besides, HCV genotypes are differently distributed worldwide, with China and South-East Asia showing the most diverse genotypes [4].

Despite the introduction of new Direct-Acting Antiviral Agents (DAAs) hepatitis C virus (HCV) infection remains as a global health problem. It represents one of the principal cause of deaths related to liver cirrhosis and hepatocellular carcinoma [5] and the World Health Organization (WHO) has recently estimated around 71 million viraemic chronic infections and 400.000 related deaths, and a prevalence of 1·1% in 2017 [6]. Number of deaths caused by Hepatitis C is still increasing, despite the fact that highly effective medicines already exist to cure chronic hepatitis C. WHO objective to eradicate HCV for 2030 could be reached [7] but for this purpose it is required more efficient screening and treatment programs.

DAAs can satisfactory deal with more than 95% of HCV-infected people, reducing the risk of death from liver cancer and cirrhosis as a result. However, since these drugs are too expensive there is a socioeconomical barrier for large scale treatment in particular in low- and middle-income countries (LMICs). Thus, less than 1% of patients infected with HCV worldwide can access to DAAs treatment [6]. On the other hand, an efficient and cheap diagnostic policy is also instrumental to achieve HCV eradication. Underdiagnosis of HCV remains a serious challenge, so fast and reliable diagnosis tests are the main objective to improve treatment access. Current diagnostic methods for HCV detection are based on serological and molecular tests. However, they are time-consuming and expensive; they require modern laboratory infrastructure and expert personnel that avoid their implementation in difficult-to-reach population with no access to hospitals and in LMICs. Of note, genotyping of HCV may no longer be necessary shortly as future pan-genotypic HCV DAAs will simplify the treatment strategy [4].

It is critical to develop reliable alternative diagnostic tools for HCV infection in order to overcome the diagnostic barriers and therefore to reach those undiagnosed HCV-infected people. New diagnostic devices should be fast, affordable, time-saving, and capable to reduce the window period for HCV detection, with high rates of efficacy and sensitivity as well.

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The aim of this review is to provide an overview of the new promising alternative tools related to the screening and diagnosis of HCV infection, based on the emerging area of nanotechnology.

Conventional systems for HCV diagnosis

Conventional diagnostic systems for HCV infection involved serological biomarkers and molecular tests. Serological biomarkers may detect anti-HCV antibodies or HCV antigens, while molecular tests detect HCV RNA. Currently the use of serological assays to detect anti-HCV antibodies is the first step within HCV testing algorithm, because they are easy to handle and more affordable than molecular assays. However, the detection of anti-HCV antibodies cannot confirm active HCV infection, since HCV specific immunoglobulins remain in the organism longtime after viral clearance. Thus, a molecular test for HCV RNA detection is required as a final step [4]. Another drawback of serological tests is that they can not detect HCV infection in early stages, since detection of antibodies requires at least two weeks after primo-infection [8].

The gold standard serological test to detect anti-HCV antibodies are the third generation of *enzyme immunoass*ays (EIA) as ORTHO HCV Version 3.0 ELISA Test System (Ortho Diagnostic Systems, USA) and Murex anti-HCV 4.0 (Murex Diagnostics, UK) multiplex that detect anti-HCV antibodies employing different core, NS3, NS4, and NS5 recombinant antigens. Their high specificity and sensitivity are around 99·4–100% in both EIA commercial systems [9,10] have boosted their use in industrialized countries.

Variants of the EIA include *chemiluminescent immunoassays* (CLIA), as it is the case of Foresight HCV EIA test (ACONLabs, US) (99·8% specificity and > 99·9% sensitivity) [11], enzymatic CLIAs as luminol-H_2O_2-horseradish peroxidase (HRP) [12], and chemiluminescent microparticle immunoassays (CMIA), as ARCHITECT (Abbott Diagnostics, USA) which is also able to detect viral infections by HBV and HIV-1.

Fluorescence immunoassays, like VIDAS test (BioMerieux, Marcy l'Etoile, France), also detects anti-HCV IgGs. Of note, this brand is more sensitive (99·8%) and specific (99·61%) for low titer samples than Ortho HCV [9,13]. As ARCHITECT CMIA, VIDAS test was first developed for detection of similar viruses such as HAV, HBV, and HIV, and since 2017 it also detects the 6 different HCV genotypes [13,14]. Fluorescence detection methodology has also been implemented to develop protein chip microarrays, which are also widely used in diagnostic. Zhang et al. (2005) developed a protein chip for anti-HCV antibody detection based on ELISA methodology, which improves ELISA specificity and sensitivity: 100% coincidence rate between ELISA and protein chip for negative results, and 99·5% or 97·4%, in regard to the recombinant antigen used, for positive results (ELISA shows a higher number of false positive results) [15], which reinforce that new methodologies are better than conventional ELISA.

Confirmatory serological tests are hardly used nowadays. Ortho-HCV RIBA-2 (Ortho Diagnostic Systems, Raritan, N.J., USA), was one of the most confirmatory tests used. However, false negative or negative results have been reported using this test [16]. Matrix Immunodot HCV Assay (Abbott Laboratories, USA) and new generation assays that include recombinant antigens, such as Murex anti-HCV 4.0 (Murex Diagnostics, UK), are more specific, and therefore they have had a broader use [17].

However, the determination of HCV viremia must be finally confirmed through molecular methods in order to identify an active HCV infection, as recommended by WHO [4]. This detection of HCV RNA is the only way to determine an active HCV infection, since anti-HCV antibodies remain in the organism long-time after virus clearance [4,8,18]. Different quantitative molecular methods to determine viremia have been developed so far. To highlight, the use of real time RT-qPCR technology like COBAS Amplicor HCV Test (Roche, US) [19], and Abbott RealTime HCV assay (Abbott assay, Abbott Labs Illinois, US). Despite the robustness of this technology, one of the most important drawbacks of molecular methods is the complexity of the laboratory equipment required, for instance the variety of lasers for detection of fluorescence dyes in RTqPCR [20,21].

It is noteworthy that WHO recommend testing for bloodborne viruses like HIV, HBV, and infections such as TB [4] among HCV risk populations. Thus, multiplex assays are required in viral detection in these groups. Yang et al. (2014) proposed to develop a multiplex assay by coupling new specific probes against HIV or HBV to their bead array test [22]. Conventional molecular methods can detect HBV, HCV, HIV-1, and HIV-2 in a single test, such as qPCR COBAS TaqScreen MPX test v2.0 (Roche Molecular Systems Inc., Pleasanton CA, US).

Alternative molecular methods include the *Transcription-Mediated Amplification* (TMA), which employs acridinium labelled probes and magnetic microparticles to form detectable complexes [23]. TMA has a better sensitivity than PCR assay amplification (<9·6 IU/mL) as determined by Morishima et al. (2006) for the commercial test VERSANT HCV RNA Qualitative Assay (Siemens Healthcare Diagnostics, Eschborn, Germany) versus conventional PCR AMPLICOR HCV Test, v. 2.0 assay (Roche Diagnostics, Germany) [24]. VERSANT HCV bDNA 3.0 Assay is a quantitative version developed in order to determine viral load, and has a very similar capability to quantify RNA compared with COBAS AMPLICOR HCV Monitor assay version 2.0 [25]. Regarding multiplex analysis, Procleix Ultrio Elite Assay (Grifols Diagnostic Solutions, Inc., USA) is a qualitative test based on TMA technology developed to detect HIV-1, HIV-2, HCV (genotypes 1–6), and HBV (genotypes A-H) in serum or plasma samples at the same time [26]. However TMA is a technique that still requires exclusive and expensive instrumentation, not affordable for developing countries.

Current multiplex assays are expensive and time consuming, so standardization of them still remains a challenge. In order to reduce time and cost of multiplex assays, Sing et al. (2017) developed a single-step multiplex RT-qPCR to discriminate among different HCV genotypes. Currently, single-step multiplex assay requires two separate assays to detect the 6 genotypes: one reaction for genotypes 2, 3, 4, and 5, and another reaction for genotypes 1 and 6. Their study showed that single-step multiplex RT-qPCR has 100% specificity and 94·89% sensitivity. Cost-effective and time-saving genotyping tests are needed until pangenotypic DAAs become more established [27] highlighting the value of this new test.

Alternatives to molecular tests

Most of LMIC use anti-HCV antibody screening tests and only a reduced percentage of these countries have access to HCV RNA tests (between 5 and 30%, as determined by Reipold E.I. et al., 2017) [28]. The requirements of molecular tests for high specific and expensive analytical equipment make difficult their implementation in LMIC. However, serological tests cannot detect HCV infection at early stages or in immunosuppressed patients [29,30]. Because of that, detection of HCV core antigen is becoming an alternative option in resource-limited settings: as an indirect marker of HCV RNA, it can correlate with viremia values and determine an active infection. HCV core antigen could be detected in those platforms that detect anti-HCV antibodies, making easier their implementation in LMIC. HCV antigen tests are more expensive than antibody tests, but they reduce the costs associated with instrumentation compared to molecular methods [31,32].

HCV core antigen test could be used as an alternative confirmatory assay instead of HCV RNA tests. However, since its sensitivity is lower than molecular tests, only a positive result of HCV core antigen makes the HCV RNA test unnecessary [32]. Moreover, core

antigen disappears later than RNA so it can be used to detect later phases of HCV infection when HCV RNA is undetectable [31].

Examples of HCV core antigen tests include HRP-based CLIA systems as described by Lui et al. (2015) with high specificity (96·7%) and sensibility at low concentrations (0·6 pg/mL) [12]. CMIA test (Abbott HCV cAg assay, Abbott Diagnostics, USA) can be also used to detect HCV Core antigen. Its sensitivity is about 93% and shows higher specificity (98·8% versus 96·7%) with the system described by Lui et al. (2015). Nevertheless, it is not yet systematically used in these LMIC countries because of its costs [28].

In summary, LMICs only have routinely access to serological tests. Moreover, just a reduced population group (between 5 and 30%) [28] have access to HCV RNA tests, which ends up in an important percentage of undiagnosed population, due to immunosuppression or screening in pre-seroconversion stages. In addition, there are people who are diagnosed as HCV positive and thus they start receiving treatment when they present anti-HCV antibodies, but could have spontaneously cleared HCV infection [28,33]. Thus, implementing HCV molecular or antigen tests in resource-limited countries will reduce the unnecessary medical treatment. For this purpose, affordable nucleic acid amplification tests (NAAT) or HCV core antigen point-of-care (POC) tests have to be developed.

Rapid diagnostic tests

Rapid diagnostic tests (RDTs) are designed for its use at the POC, and can be adapted in low-resource settings. Under-diagnosis of HCV remains a serious challenge in order to achieve HCV elimination. Development of new diagnostic tools that do not require laboratory infrastructure and expertise in their operation, reduce the economic cost and time of diagnosis, are essential particularly in difficult-to-reach population and in LMICs.

Serological rapid POC tests already detect anti-HCV antibodies. The most widely used test is OraQuick-HCV, a rapid test for anti-HCV antibody detection that consists on a finger stick. Preferred samples are whole blood and plasma or serum, but OraQuick-HCV can also detect antibodies in oral fluid although with lower sensitivity, 97·6% versus 99·4%, with whole blood [34]. This test can detect all HCV genotypes and gives the results in 20 min: a pink colour change that can be read at naked eye. It is a reliable and easy-to-use test, perfect for diagnosis at point-of-care [35]. However, OraQuick-HCV test price remains still high for its implementation in large-scale screening.

INNO-LIA HCV Score (INNOGENETICS, Ghent, Belgium), using a line immunoassay (LIA), can detect anti-HCV antibodies on a nylon strip, to get 6 different lines according to the recombinant antigen detected in a sandwich reaction. It is possible to automatize this method, reducing the laboratory personal needed, and with higher sensitivity than OraQuick-HCV (100%) either in serum and in blood samples [36].

Less known methods have been developed, such as SD Bioline (Standard Diagnostics, South Korea), a rapid immunochromatographic test approved by WHO [37], developed to detect anti-HCV antibodies from whole blood, serum, or plasma samples (100% sensitivity and 99.4% specificity) (Standard Diagnostics). For HCV screening, SD Bioline employs HCV recombinant antigens Core, NS3, NS4, NS5, and this test has been developed for HAV, HBV, and HIV as well [38]. In this line we find Assure HCV Multisure rapid test (MP Biomedicals, Santa Ana, CA, USA) (99% sensitivity and 99·8% specificity), and VIKIA® anti-HCV, (100% sensitivity and 99·7% specificity), but they have not been approved by WHO yet. Tri-Dot (J. Mitra, India) is a rapid membrane based test similar to ELISA for anti-HCV antibodies detection from serum or plasma samples. With a 100% sensitivity and 98·9% specificity, Tri-Dot test gives a visual result in 3 min [39].

As is shown, strip format is one of the preferred chosen for POC systems, because it makes the test accessible for decentralized settings**.** Sensitivity and specificity data show that if LMIC policies contributed to promote screening programs, rapid POC tests would be great options for diagnosis in decentralized settings. Rapid POC tests do not need high qualified personal or specific instrumentation; they are usually easy to use, avoiding sample transport to specialised settings. However, faster tests have not accomplished yet specificity and sensitivity of conventional immunoassays (>99·4% specificity and >99·8% sensitivity) [4].

New molecular rapid POC tests using NAAT and core antigen are the most desirable ones, and fast POC versions have been developed. HCV Quant Assay [40] is a new HCV diagnostic test for HCV RNA detection from plasma samples. It is an automated system that uses paramagnetic particles for RNA extraction, and then performs a RT-qPCR to detect and quantify RNA. Using the new technology described by Kelso et al. (2017), all the process can be done in a single device, with 100% sensitivity and 100% specificity. However, this system has not been developed yet to be used by untrained personal, so it is difficult to implement HCV Quant Assay in low-resources settings.

Xpert HCV Viral Load (Cepheid, Sunnyvale, CA) is another automated HCV RNA test. It consists on a cartridge where RNA extraction and conventional RT-PCR amplification are done from 1 mL of serum or plasma samples; hence it does not require the usually expensive NAAT equipment. This POC system has 100% specificity and it can quantify HCV viral load, comparable to the widely used Abbott Real*Time* HCV assay [41]. Xpert HCV VL Fingerstick (Cepheid, Sunnyvale, CA) is another version of the assay to quantify HCV RNA from drop blood samples [42]. This method consists on a fingerstick which detects and quantifies RNA in less than an hour. Xpert HCV VL Fingerstick assay allows patients to get their results in a single visit. Besides it needs less sample preparation compared with Xpert HCV Viral Load [43], but both tests still suppose a high cost for low-resources settings.

TaqMan Array Cards (TAC, Life Technologies, Grand Island, NY) is a rapid test that has been developed for detection of HAV, HBV, HCV, HDV, and HEV RNA simultaneously (Kodani et al., 2014) which has 96% sensitivity and 98% specificity. It has recently been applied to multiplex detection of HIV-1 and HIV-2, additionally to all hepatitis virus (Granade et al., 2018). Nevertheless, these multiplex assays has lowest sensitivity and specificity compared to single RT-qPCR, but reduced costs, so they are not yet replacing conventional methods but they are an important option to consider when further studies were done. Improving sensitivity and specificity, these tests could be used in LMIC and for large-scale screening where rapid tests are necessary [44,45].

A rapid new affordable POC diagnostic system based on detection of HCV core antigen is being developed by Daktari diagnostics (Boston, USA), a system that could perfectly fit in the requested methods for LMIC. Daktari system is based on microfluidic technology, and it would be able to analyse a blood drop in a single cartridge in 30 min [46]. This technology is also in development for HIV. Again, as Xpert HCV VL Fingerstick [42], this test would allow patients to get their results in one visit, facilitating their access to screening and diagnosis, maybe for both HIV and HCV at the same time, allowing co-infection diagnosis.

Nanotechnology in theragnosis

Nanotechnology is becoming a larger important field in theragnosis, specially referring to POC systems, since they require less sample volume and laboratory equipment, are more sensitive, time-affordable, and cost-effective. Most of them are paper-based systems which makes them easier to transport and storage [47–51].

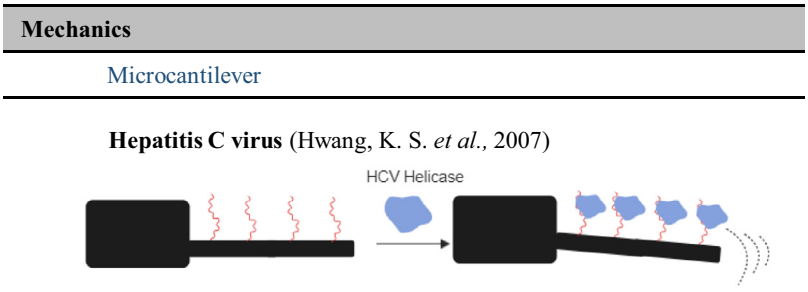

Fig. 1. *Scheme of a functionalized microcantilever for detection of HCV Helicase.* Binding of HCV Helicase to the aptamers generates oscillations on the microcantilever.

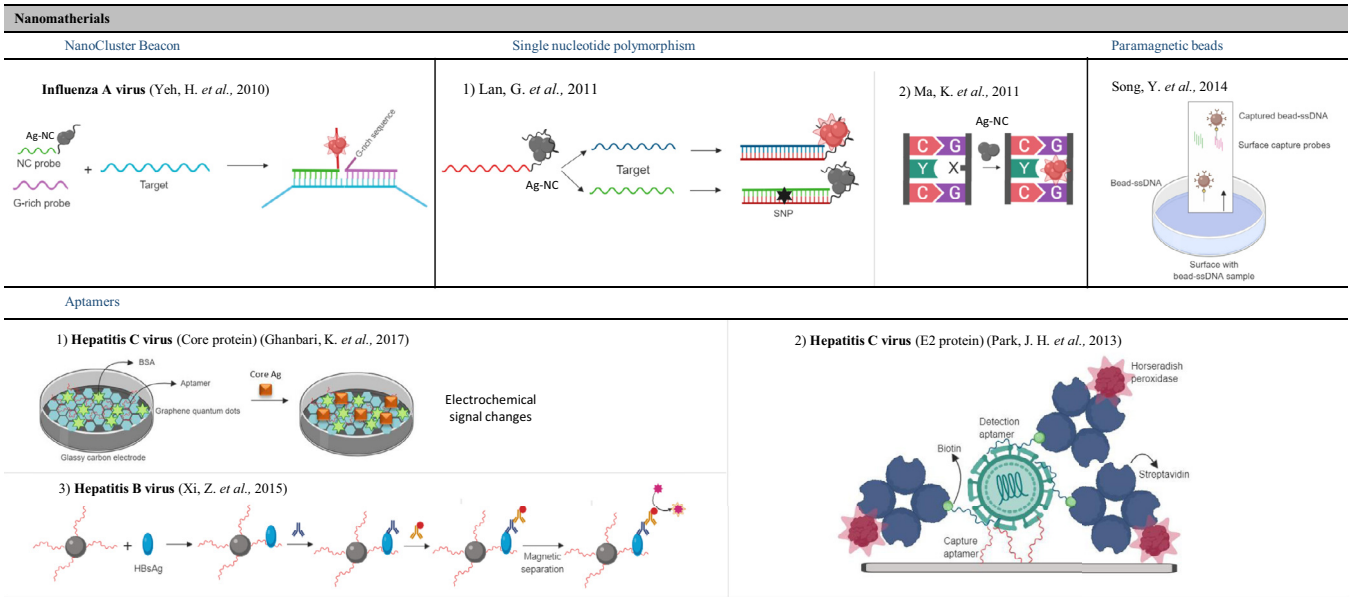

Fig. 2. *Schematic representation of diagnostic systems using nanomatherials.* Ag-NC: silver nanocluster; SNP: single nucleotide polymorphism; Core Ag: Core antigen; HBsAg: Surface antigen of Hepatitis B virus.

Nanoparticles are usually the preferred chosen system, but also mechanics are making a place in diagnostics within the nanoscale field. For instance, the oscillations generated when a target binds a microcantilever generate a signal that can be measured, allowing the identification of the pathogen of interest [52,53]. Hwang et al. (2007) developed a microcantilever diagnostic system for HCV, employing HCV Helicase as target [54].

Concerning the therapy, nanoparticles are an useful drug delivery tool: nanoparticles can be functionalized with the drug or they can be functionalized with linkers that can recognise the target such as different DNA molecules [55]. Once the drug is encapsulated in the nanoparticle, drug stability, solubility, and absorption are enhanced [56]. The drug could be released thanks to combination of diffusion and desorption mechanisms [57].

Nanotechnology also includes the use of dendrimers [58], liposomes [59], or carbon nanotubes [60]. Liposomes have been successfully used for delivering AZT, a drug against HIV: its biodisponibility in the target tissues was higher than administered in the soluble conventional version [61].

Nanoparticles have been studied for treatment of infectious diseases such as those caused by *Escherichia coli* and *Staphylococcus epidermidis*. These systems are sequence-specific, since they use DNA-functionalized silver nanoclusters targeting specific regions on the bacteria genome and thus inhibiting its growth [62].

DNA-functionalized nanoparticles or nanoclusters have been employed for diagnosis. Using silver nanoclusters, detection as fluorescence emission of nanoclusters is enhanced when DNA-functionalized nanoclusters bind to the target. Different systems have been developed based in this technology, as the NanoCluster Beacon designed by Yeh et al. (2010) for detection of influeza A virus [63] and other similar systems for detection of single nucleotide polymorphism or SNPs [64], as this p53 gene mutation related with cancer [65]. Song et al. (2014), have functionalized streptavidin-coated paramagnetic beads and studied their ability to detect PCR fragments immobilized in filter paper by capillarity: paramagnetic beads can hybridize with PCR fragments and detection at naked eye takes just 2 min [51]. This kind of systems for DNA detection would be suitable for detection in early stages of HCV infection; because of their reduced incubating time this technology can facilitate the access of patients to diagnosis in LMIC since they could get their results in a single visit.

RNA aptamers: applications for HCV diagnosis

RNA aptamers are single-stranded RNA oligonucleotides that specifically bind to a target molecule, which allow their employment for detection and even identification of different pathogen and tumour markers. Thus, aptamers are emerging as a key tool for analytical diagnosis, that can be implemented in small POC devices [37]. RNA aptamers have been used in the development of HCV serological tests. Enzyme Linked Aptosorbent Assay (ELASA), is a new generation of aptamer-based assay developed by Park et al. (2013) for detecting HCV E2 protein. In fact, two different aptamers recognize two regions of E2 protein, through the capture

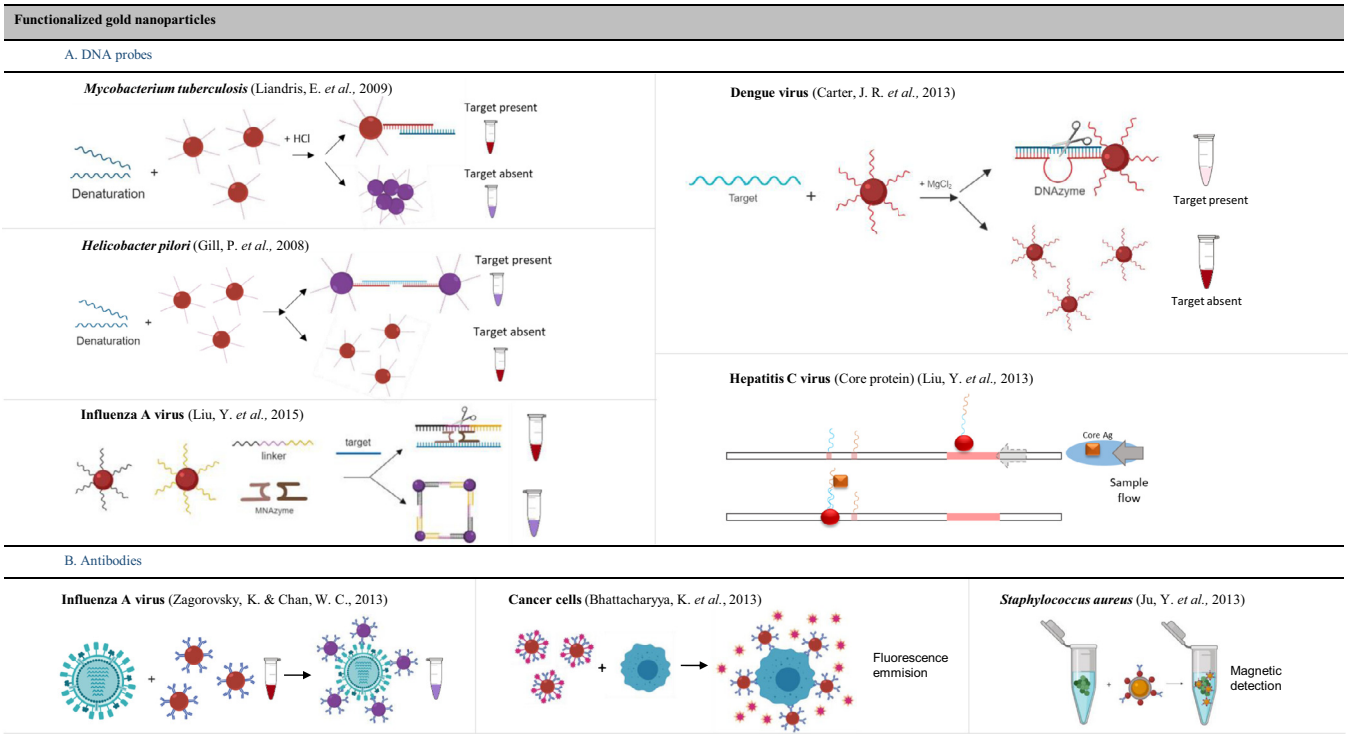

Fig. 3. *Schematic representation of diagnostic systems using functionalized gold nanoparticles.* Two different functionalization methods are represented, based on (A) DNA probes, and (B) antibodies.

of the aptamer and a biotin-labelled detection, with a $3{\cdot}13 \times 10^2$ FPU/mL as detection limit [66].

Moreover, aptamers are used in new diagnostic methods for detection of HCV antigens**.** Lee et al. (2007) developed a new diagnostic tool based on the use of RNA aptamers, to detect HCV core antigen in serum samples. The system consists on a sol-gel based protein-chip where aptamers are immobilized. Once they bind the core antigen Cy3-labelled human antibodies are added for detection. Optimization of the system sensitivity is still needed before starting using it for screening [67]. Chen et al. (2009) developed a similar method to detect HCV E2 protein with biotinylated ssDNA aptamers [68]. As well, Ghanbari et al. (2017) have developed a electrochemical biosensor to detect HCV core antigen [69]. Aptamers that target the Hepatitis B surface antigen has been used in HBV diagnosis, a method that significantly improved the sensitivity of conventional methods [70].

Gold nanoparticle-based biosensors for the diagnosis of infectious diseases

Among the different types of nanoparticles, gold nanoparticles are an emerging tool in POC diagnosis, and different tests are in development. Gold nanoparticles-based assays have already been employed for detection of infectious diseases and other harmful biological agents, increasing and improving existing test's options. Gold nanoparticles have not been yet applied to HCV diagnosis but they have been used for diagnosis of different infectious diseases, such as *Mycobacterium tuberculosis* [71,72], *Helicobacter pilori* [73], Dengue virus [74], or influenza A virus [75]. Usually, gold nanoparticles are functionalized with monoclonal antibodies or DNA probes. Regarding DNA-modified gold nanoparticles, sDNA probes like for *H. pilori* [73] are used or even include enzymatic systems as DNAzyme (DNA oligonucleotides with catalytic activity in presence of a specific target) like for Dengue virus [74], or the system described by Zagorovsky and Chan (2013) [76] potentially applicable for multiple targets. DNAzyme system is also used in either biosensors for metal detection, to detect environmental contamination or in toxicology assays [77]. With respect to antibody-modified gold nanoparticles, they have been used for detection of influenza A virus [75], or even circulating tumour cells [78]. Sung et al. (2013) described a colorimetric biosensor based in the combination of antibody-modified gold and magnetic nanoparticles to enhance the detection signal for *Staphylococcus aureus* and to reduce the assay time compared to ELISA [79].

As already mentioned before, nanoparticles can be functionalized with aptamers as small biosensors and using them especially at POC. The usefulness of gold nanoparticles functionalized with aptamers has allowed the development of nucleic acid lateral flow strips for detection of HCV core antigen. These nanoprobes specifically bind to the HCV core protein and detection can be determined at naked eye [80].

However, it has been described that gold nanoparticles functionalization is not always necessary since the presence of free nucleic acids can change gold nanoparticles aggregation state and modify their wavelength absorption. Specific probes can hybridize with target DNA. This change from free ssDNA to dsDNA alters gold nanoparticles stability and generates a wavelength change in the visible spectrum, observable at naked eye. This principle has been employed for detection of HBV [81] and also bacteria such as *Chlamydia trachomatis* [82], *Lysteria monocytogenes* [83], and *Salmonella spp.* Of note, this system shows both a high sensitivity (89·15%) and specificity (99·04%) for detection of Salmonella spp [84]. In all of these examples mentioned for un-modified gold nanoparticles, DNA amplification is necessary as a first step before detection, and gold nanoparticles are employed instead of gel electrophoresis techniques. Indeed, Shawky et al. (2010) have developed a method that detects non-amplified HCV RNA with non-functionalized gold nanoparticles [85,86].

Nevertheless, nucleic acid extraction is a limiting step in molecular diagnostics. Thus, if this step could be obviated it would

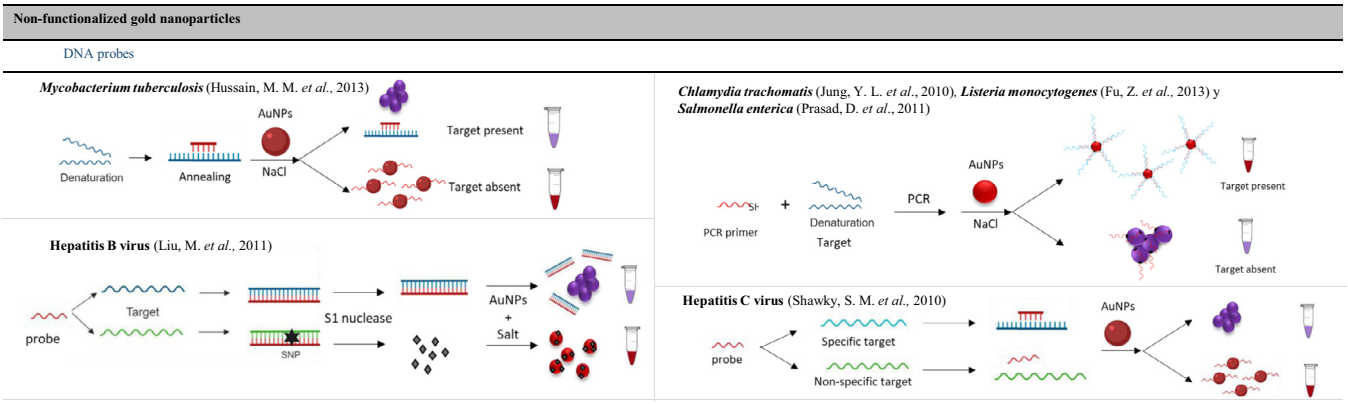

Fig. 4. *Schematic representation of diagnostic systems using non-functionalized gold nanoparticles.* AuNPs: gold nanoparticles; SNP: single nucleotide polymorphism; Core Ag: Core antigen.

Table 1
Summary of diagnostic methods for bacterial/viral pathogens, and cancer cells using nanotechnology systems.

Organism	Detection	Reference
Mechanics		
HCV (Helicase)	Microcantilever	Hwang et al. (2007) [54]
Nanomatherials		
Influenza A virus	Nanocluster Beacon	Yeh. et al. (2010) [63]
Cancer cells	Single nucleotide polymorphism	Lan et al. (2011) [64], Ma et al. (2011) [65]
-	Paramagnetic beads	Song et al. (2014) [51]
HCV (E2 protein)	Aptamers	Chen et al. (2009) [68], Park et al. (2013) [66]
HCV (Core protein)	Aptamers	Lee et al. (2007) [67], Ghanbari et al. (2017) [69]
HBV	Aptamers	Xi et al. (2015)
Functionalized gold nanoparticles		
Mycobacterium tuberculosis	DNA probes	Liandris et al. (2009) [72]
Helicobacter pilori	DNA probes	Gill et al. (2008) [73]
Dengue virus	DNA probes	Carter R. et al. (2013) [74]
Influenza A virus	DNA probes	Zagorovsky & Chan (2013) [76]
HCV (Core protein)	Aptamers	Liu et al. (2013) [80]
Influenza A virus	Antibodies	Liu et al. (2015) [75]
Cancer cells	Antibodies	Bhattacharyya (2013) [78]
Staphylococcus aureus	Antibodies	Ju et al. (2013) [79]
Non-functionalized Gold nanoparticles		
Mycobacterium tuberculosis	DNA probes	Hussain et al. (2013) [71]
HBV	DNA probes	Liu et al. (2011) [81]
Chlamydia trachomatis	DNA probes	Jung et al. (2010) [82]
Listeria monocytogenes	DNA probes	Fu et al. (2013) [83]
Salmonella enterica	DNA probes	Prasad et al. (2011) [84]
HCV	DNA probes	Shawky et al. (2010) [85]

remarkably reduce the assay time. In summary, the principal characteristic of gold nanoparticle-based tests is that they are colorimetric, so no final detection reaction is needed to get the result but visualizing at naked eye. Besides they seem to be sensitive enough in preliminary studies. Therefore gold nanoparticle tests are cost-effective and time-saving as well as suitable for LMIC where no specialized personal or laboratory equipment is available.

Conclusion

HCV is still an under-diagnosed infection, especially in low-resource settings, so new diagnostic tests are urgently required. Nowadays, a plethora of new methods are in development, which would greatly overcome drawbacks of current HCV tests. New generation of HCV test should be cost-effective, time-saving, and easy to implement in low-resource settings where no highly specialized personal is available. Nanotechnology-based tests especially meet POC requirements, so it is a highly important emerging field to take into account when looking for solutions in LMIC. Besides, ongoing POC tests usually target nucleic acids, an essential requirement for HCV diagnosis in early stages, reducing percentage of under-diagnosed population. Thus, the use of nanotechnology-based tests for the diagnosis of HCV infection may overcome the diagnostic access and the undiagnosed of HCV infection, leading up to the WHO objective towards the HCV elimination.

Search strategy and selection criteria

In this review, we searched PubMed, Medline, Web of Science and Google Patents databases. Search terms included “Hepatitis C virus”, “HCV”, “HCV coinfection”, “diagnosis”, “diagnostic tests”, “nanotechnology diagnostic methods”, “point-of-care”, and “rapid diagnostic tests” to identify articles published before November, 2018. Reference lists of selected papers where manually searched for additional papers covering topic of interest. We also searched in online websites such as World Health Organisation (WHO) website for HCV guidelines. Articles, patents, and guidelines in English and Spanish were included.

Contributions

V.B. conceived the study. S.A.L. participated in its design and implementation of the research. P.M.R. and I.M.C participated in the search of bibliography related to HCV conventional diagnostic systems. S.A.L. drafted the manuscript. P.M.R., I.M.C, R.M. and V.B.

made a critical revision of the manuscript. S.A.L. R.M. and V. B approved the final version. All authors read and approved the final manuscript.

Figs. 1–4

Table 1

Declaration of Competing Interest

The author(s) declare no competing interests.

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