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Biosensors to diagnose Chagas disease: A review

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Abstract: Chagas disease (CD), which mostly affects underprivileged people, has turned into one of Latin America’s main public health problems. Prevention of the disease requires early diagnosis, initiation of therapy, and regular blood monitoring of the infected individual. However, the majority of the infections go undiagnosed because of general mild symptoms and lack of access to medical care. Therefore, more affordable and accessible detection technologies capable of providing early diagnosis and parasite load measurements in settings where CD is prevalent are needed to enable enhanced intervention strategies. This review discusses currently available detection technologies and emerging biosensing technologies for a future application to CD. Even if biosensing technologies still require further research efforts to develop portable systems, we arrive to the conclusion that biosensors could improve diagnosis and the patients’ treatment follow-up, in terms of rapidity, small sample volume, high integration, ease of use, real-time and low cost detection compared to current conventional technologies.

Keywords: Chagas disease, biosensors, detection technologies, diagnosis, neglected diseases.

1. Introduction

Chagas disease (CD), discovered in 1909 by the Brazilian physician Carlos Chagas (1), nowadays has turned into one of Latin America’s main public health problems (2). Based in disability-adjusted life-years as a measure of disease burden, CD figure as the most important parasitic vector-borne illness in the Region of Americas, seven times higher than Malaria and up to three times more than Dengue (3), and yet it is still absent in the agenda of the public health policies and practices of many endemic countries (4, 5). More generally, ranks fourth in mortality and eighth in morbidity...
among world neglected tropical diseases (6), and is estimated that between eight and eleven
million people are infected, while 100 million are at risk of acquiring the disease; the main cause
being to live in proximity with disease vectors (7). CD is caused by the parasitic presence of the
Trypanosoma cruzi in the organism, which is mainly transmitted by contamination with infected
feces of blood-sucking triatomine vectors during a human blood meal. Nevertheless, it can also be
transmitted through blood transfusions, organ transplants, infected mothers to their unborn
children and ingestion of contaminated food (i.e. Oral transmission; WHO, 2002). Although disease
progression can be associated with the mechanism of infection, with oral transmission causing the
most severe outbreaks (9), people living at risk regions are susceptible to polyparasitism (i.e.
Coinfections and superinfections with different strains of T. cruzi), with unknown effects in the
variability of the disease progression and response to treatments (10).

CD mostly affects underprivileged people and the majority of the T. cruzi infections go undiagnosed
because of general mild symptoms and lack of access to medical care (11). Due to this fact, CD is
considered as a Neglected Tropical Disease whose improvement in diagnosis and treatment today
requires research and development efforts with non-profit interests. The highest prevalence of
Chagas disease has been reported in Bolivia (6.75–15.4 %), followed by Paraguay (0.69–9.3 %),
Panama (0.01–9.02 %), Brazil (0.8–1.30 %), Mexico (0.5–6.8 %) and Argentina (4.13–8.2 %)
(12). Citing a case, this disease causes almost 6% of the annual deaths in Mexico and the
seroprevalence can roughly be estimated at least in 3% due to a lack of active epidemiological
surveillance (most cases are detected during blood screening procedures in blood banks). Yet, less
than 0.5% of the infected individuals have access to treatment in this country as a result of
anachronisms in the normativity, among other failures in the public health system (13).

Even if CD mainly affects tropical countries, with nowadays ease of traveling and migration, other
countries are also being affected by this infection (14). Several cases have been reported in USA,
Canada, Europe and in Western Pacific regions like Japan and Australia (14–16). Notably, CD
continues to be an inconspicuous public health problem, with limited medical awareness, either
because it is commonly targeted in people with relatively low medical access or because it can
currently occur in unexpected regions. Thus, the treatment of CD urgently needs to generalize and
standardize diagnostic procedures.

Biosensors are relatively new analytical devices that can help to detect the presence of specific
compounds and pathogens in liquid environments and complex mixtures like: water and blood
serum. Although these devices have been formerly used in the alimentary industry (mostly to
detect toxins and infectious pathogens), they are being increasingly used to diagnose human
diseases (17). Therefore, these devices can be employed for the diagnosis of CD. The development
of biosensors requires a biological active component to be immobilized onto the surface of a
transducer. The selective recognition layer, towards *T. cruzi* specific antigens present in patients’
blood serum, can selectively detect the target analyte generating a signal response in the sensor
(see Figure 1). Depending on their transducing principle biosensors can be electrochemical,
asoustic or optic.

In this work, we firstly introduce a brief description of the disease. Secondly, we present a review
of biosensor technologies whose applicability to diagnose CD has been investigated. Finally, we
mention the benefits and drawbacks of applying biosensors as solutions to this major public health
issue and the infrastructure required to conduct biosensor experiments for this application.

2. Brief description of CD and current needs

CD passes through two successive stages: an acute phase and a chronic phase. The acute phase
occurs at the following 6-8 weeks after infection. The acute phase is followed by the chronic phase
of CD, which lasts for the rest of the life of the infected individual, and has different forms. In the

Figure 1. General scheme of a biosensor detection strategy. A biosensor is composed of a biochemical
interface where specific bio-species are absorbed; a transducer which translates the recognition event to
another physical response that can be measured and an electronic system which acquires and records the
signal.
indeterminate form, an equilibrium between the parasite and the immunological response of the infected individual is reached and most infected patients appear healthy, with no evidence of organ damage that could be found by current standard methods of clinical diagnosis (8). About 50–70% of infected individuals will remain in this condition for the rest of their lives. However, several years after the chronic phase has started, 10–40% of infected individuals will pass to the cardiac form of the disease and will develop injuries of various organs, mainly the heart, the digestive system, and occasionally, the peripheral nervous system (8, 18). These important symptomatic changes occur 10–20 years after the acute phase of the disease and include a broad range of types of damage.

The clinical manifestations vary from mild symptoms to heart failure and, frequently, sudden cardiac death (18). The acute phase of CD is recognized only in an estimated 1–2% of all individuals acquiring the infection (8) due to a lack of access to sufficient medical care. Thus, more than any other parasitic disease, CD is closely related to social and economic development. Paradoxically, acute phase is the most appropriate period for drug treatment, showing relatively high levels of sero-conversion, while organ damage is prevented (19). Conversely, it has been shown that drug treatment with Trypanocidal therapy in patients with established Chagas cardiomyopathy, can cause seroconversion, but does not stop cardiac clinical deterioration (20).

Given that no vaccine is currently available to prevent CD, vector control, diagnosis tests, opportune drug treatment, and clinical follow-up are the most effective methods to fight against the disease (21). Nevertheless, all these measures suffer of several hindrances imposed by the synergistic negative effects of diverse vulnerability components of CD risk, such as ecological factors (i.e. land-use changes) -that are broadening the contact zones between humans and parasites (Lopez-Cancino et al., 2015)-, the chronic failure of health care policies hindering the reduction of CD incidence (5), the limited awareness of physicians (22), and several socio-cultural practices that perpetuate CD exposure in endemic regions (23). Health policies to control/reduce vectorial exposure to CD in Latin America are challenged for the relatively high diversity of vectors that shows a broad environmental tolerance. Likewise, a wide territory of America is suitable for CD vectorial transmission (24–27).

In order to face the epidemiological challenges due to the increasing complexity of interactions among the transmission routes of T. cruzi in endemic and non-endemic countries, access to early diagnostic and treatment seems as the most cost-efficient ways to reduce the CD burden (11).

Several paths of scientific advances and discoveries envisage an optimistic future to reduce CD
burden coming from a better understanding of CD transmission and management toward its interruption (28). The fact that the World Health Organization (WHO) has made a commitment with Bill and Melinda Gates Foundation and other stakeholders to control the most neglected diseases by 2020, including CD among them, opens an opportunity window to orientate research priorities. This commitment, launched in 2012, was called the London Declaration (http://www.unitingtocombatntds.org/). However, claims have been raised to urge implicated organizations to set the needed measures to reach these goals, among which improved diagnosis (i.e. precision and accessibility) seems as one of the most important first steps (5).

Blood banks in endemic countries require fast and secure screening method for small and medium health facilities, since screening for CD is mandatory in some endemic countries (8). Several countries do not have an active program for CD detection and depend only for the blood bank reports. The most important reason of this fact is the low feasibility to detect Chagas in patients. Since, CD do not produce particular symptoms, there are not incentives from society. Nevertheless, the bug bite is quite notorious and people can suspect that they are infected with *T. cruzi* when a *chinchoma*\(^1\) appears. In such cases, people can search medical assistance and be subjects of a blood test.

Climate change and global warming increase the risk of rising CD burden in some regions. Climate change impacts on vector-borne diseases (29) and is undoubtedly detonating variables that make the CD transmission become potentially dangerous, as the WHO points out (30). Nevertheless, the incidence of CD can be greatly reduced by residual insecticide-based vector control programs that decrease the populations of the transmitting vectors and by improving housing (31).

### 3. Current detection technologies and their limitations

Currently, laboratory methods are employed to diagnose CD. Depending on the patients’ phase of infection some are more convenient than others. During the acute phase of CD, a large number of parasites are present in the peripheral blood and can be diagnosed by direct microscopical observation of fresh blood (parasitological test). However, for the chronic phase of CD the diagnosis is not possible, due to the scarce parasitemia. Therefore, the immunodiagnosis is widely used since nearly all *T. cruzi*-infected individuals in the chronic phase develop antibodies against the complex antigenic mixture of the parasite (8).

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\(^1\) Name of the inflammatory injury after a bug bite.
Several immunodiagnosis tests are available, but mainly three conventional tests are widely used: indirect haemagglutination (IHA), indirect immunofluorescence (IIF) and Enzyme Linked Immunoassay (ELISA). These tests present several limitations such as: (i) cross-reactivity with other parasites; (ii) not 100% of sensitivity; (iii) the need to be performed in a laboratory; and (iv) a long time is required to obtain the results. IHA test results can be obtained in about two hours, whereas IIF results can be obtained after numerous steps in two hours and ELISA takes several hours to carry out, including prior sensitization of microplates with *T. cruzi* antigens for about 12 hours (6). All these tests have to be performed in centralized laboratories; some of them require sophisticated equipment and skilled technicians. Since none of these tests have a sensitivity of 100%, the WHO recommends conducting at least two conventional tests for a definitive diagnosis of *T. cruzi* infection (8).

More recently, non-conventional tests, like rapid lateral flow (RLF) tests, are commercially available in the market to detect *T. cruzi* infection using whole blood, serum or plasma (Sánchez-Camargo et al. 2014). These tests are based in different tests principles: immunochromatography, particle agglutination, immunofiltration or immunodot. They provide fast results (between 5 to 60 min reading times) without the need of electrical equipment and they require low volume samples (5 to 150 μl). However, the sensitivities and specificities of such tests are lower than that of conventional tests and they only provide qualitative or semi-quantitative results, which prevents obtaining important test information like genetic lineage of the *T. cruzi* (32) and the immunoreaction kinetics.

From a prospective point of view, it is important to discuss about another kind of technology, which could be deployed for Chagas Disease diagnosis in the near future. Over the last decade, Shear-Horizontal (SH) Surface Acoustic Wave (SAW) immunosensors (Love-SAW with a guiding layer) have been developed for the diagnosis of various diseases (33). Such immunosensors exhibit a high sensitivity and a very low limit of detection (in the order of pg/μl of blood serum). This technology give very relevant results for the detection of antibodies, specific to certain diseases (34). The only drawback of this technology is that a residual frequency or phase shift always remains, which is induced by non-specific mass effects, i.e. a shift which does not correspond to specific antigen-antibody interactions on the sensor surface (34). In order to drastically reduce or avoid cross-reactions with other type of interactions that may lead to false positives, Rayleigh-SAW generation, on the same piezoelectric substrate, seems to be very promising (35). In addition,
these waves can be used to generate fast fluid actuation to improve mixing and desorption promoting faster molecular interactions. Indeed, Rayleigh-SAW liquid effects can induce intense recirculation, actuation, heating or atomization, depending on the mechanical power conveyed by these acoustic waves (36, 37). This recirculation can allow to re-suspend all non-specific species that could settle and lead to non-specific responses.

4. Biosensing research efforts for Chagas diagnosis

Biosensors that have been investigated for the diagnosis of CD can be classified into electrochemical -where amperometric (38–41) and impedimetric (42) sensors can be found- and optical -where mainly Surface Plasmon Resonance (SPR) transducers (6) are found. Biosensors could provide the benefits presented in Table 1 in comparison with other currently employed techniques for the diagnosis of CD.

Pumpin-Ferreira et al., in 2005, reported a biosensor for the diagnosis of CD (38). It consisted on an amperometric immunosensor. This biosensor required an electrochemical interaction and, therefore, a potensiostat-galvanostat was required to conduct the measurements. Potensiostats are powerful equipment, but they are large and heavy for a final portable biosensing system. Hence, other electronics for biosensors characterization should be developed which provide higher miniaturization and integration capabilities for portable systems.

Recently, Luz et al. (2015) presented the first biosensor for the diagnosis of CD based on SPR transducers (6). They obtained the parameter related to the presence of antibodies anti-T. Cruzi found in human serum in approximately 20 min. SPR transducing principle requires an optical source for the laser generation and the integration of this source to the equipment, currently, leads to high volume and heavy apparatus, only suitable for laboratory tests. Moreover, even if optical biosensors can be very sensitive, the cost of SPR equipment is higher than USD $50,000 and for this reason, not many researchers can afford such systems (43).
Table 1. Some methods for the diagnosis of CD.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Drawbacks</th>
<th>Benefits</th>
<th>References</th>
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</table>
| **Selective Media** | - Microorganism needs to overgrowth fast  
                     - Long time to yield results  
                     - Needs a laboratory  
                     - Needs an aseptic work area  
                     - Needs trained personnel  
                     - Tedious procedure | + Cheap  
                     + Easy to perform | (44, 45) |
| **ELISA** | - Requires highly qualified personnel  
                     - Consumes a lot of time  
                     - Needs a laboratory  
                     - Expensive | + High selectivity and sensitivity  
                     + Improves the time required to yield results  
                     + It works well for samples without interfering molecules | (44–49) |
| **Quantitative PCR** | - Expensive  
                     - Needs trained personnel  
                     - Needs a laboratory  
                     - Difficult to perform | + High selectivity and sensitivity  
                     + Improving the time required to yield results | (45) (47–49) |
| **Rapid test** | - Specificity 96.8%.  
                     - Just qualitative results.  
                     - The method needs a tube, a measured volume of sample and reagent  
                     - Can present false positive | + 15-25 min  
                     + High sensitivity of 99.5 %  
                     + Low cost (less than $2 to the end user) | (7, 50) |
| **Biosensors** | - Not commercially available | + No need of an aseptic working area |
|               | - Large dimensions (currently) | + Fast (real-time) |
|               | - Needs a laboratory | + Easy to perform. Not need of trained personnel |
|               | - Further research and development is required for portable systems | + In situ simple preparation |
|               | - High research cost | + High analytical specificity |
|               |                       | + Reduction of reagents consumption |
|               |                       | + Reduced analysis time |
|               |                       | + High reliability |
|               |                       | + High sensitivity |
|               |                       | + Integration of multiple processes in a single device |
|               |                       | + Possible automation |
|               |                       | + Low cost of fabrication |

(6)
(38–42)
(17)
5. Biosensors and their contribution to reduce CD burden

An ideal serological test should be easy to perform in a single step, fast, cheap, require no special equipment or refrigeration reagents and should have a sensitivity and specificity of 100%. Such a test does not exist currently for the diagnosis of CD. Hence, new technologies, which combine, robustness, simplicity, portability and rapidity with an effective sensitivity and selectivity could contribute to more efficiently diagnose CD. There are evidences that show that biosensors could meet most of these attributes for this application (6, 38). Biosensors could improve the diagnosis and the patients’ treatment follow-up, in terms of rapidity, real-time and low cost detection compared to current detection technologies like Polymerase Chain Reaction (PCR) and ELISA. In addition, the use of biosensors offers significant advantages like: small fluid volume manipulation, a high integration capability that facilitates the development of portable devices and ease of use. This should allow their use by non-specialized personnel in non-centralized laboratories (17). Nevertheless, further research efforts are needed to achieve a biosensing portable device for CD diagnosis.

The expected features of biosensors are high selectivity and sensitivity, real-time label-free monitoring, easy to use, reliability, high miniaturization capabilities and low cost. Biosensors based on optical and acoustic wave sensing technologies could meet these requirements in a near future and seem to be very promising tools for this application. Such devices will lead to more sensitive tests at lower reagent concentrations, allowing biosensing system users to: i) reduce the cost of reagents; ii) obtain valuable quantitative information; and iii) extend the measurement range of the assays.

6. Infrastructure requirements

To develop a portable biosensor system for the rapid diagnosis of CD, first of all, it is necessary to integrate a transducer with a suitable sensitive bio-chemical layer. Some authors have already achieved this milestone, as stated in Section 4. Additionally, the system requires the integration of: i) an electronic read-out system, for the interrogation and signal acquisition; ii) a microfluidic system, to handle bio-fluids; and iii) a thermal control unit, to keep the temperature stable during the sample analysis. This last point could be avoided if it is proven that the temperature sensitivity of the sensor in use is negligible for the experiments we are conducting or if a differential measurement setup for a temperature compensation is employed (51). Furthermore, it is
important to mention, that a fully-automated feature is desirable for the complete system, in order to run the sample analysis as comfortably as possible.

Nowadays, some companies offer commercial solutions for integrated biosensing systems that could be employed to diagnose CD. However, most of this systems are still of considerable size, weight and price, which prevents their wide use for field applications in low income communities. Table 2 shows some integrated biosensing platforms currently available in the market and some of their features. As can be appreciated in the table, all these systems require to be operated in a laboratory due to their dimensions. If researchers choose a non-commercial solution, they require to design and develop a system according to their needs. Nevertheless, this might allow them to pursue a more compact, cost-effective and portable system.

<table>
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<th>Table 2. Different biosensing systems currently available in the market.</th>
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<tr>
<td>Product</td>
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<tr>
<td>Q-Sense Omega Auto®</td>
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<tr>
<td>Biacore X100®</td>
</tr>
<tr>
<td>AWS A20-F20®</td>
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<tr>
<td>OpenPlex®</td>
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7. Conclusions

Since there is not vaccine for CD, currently, the vector control and diagnosis tests are the most effective methods for preventing the disease and apply effective drug treatments. Even if a prophylactic or therapeutic vaccine could be achievable in next years, this would need to be part of integrated efforts that include better diagnostic means, since a vaccine is unlikely to be enough to stop the parasite transmission. Therefore, highly predictive diagnostic tests are required, not only to estimate the real size of CD problem, but also to assess the effectiveness of every action conducted towards a disease burden reduction.

Currently, there are three conventional tests to diagnose Chagas in its chronic phase: IHA, IIF and ELISA. All of these tests have sensitivities under 100%. Therefore, the WHO recommends performing at least two of these tests for a conclusive diagnosis, leading to a bottleneck of parasite detection, caused by limited local availability of laboratories in which such tests can be performed.
In addition, the diagnosis of the disease is generally delayed due to logistic restrictions of potential patients to access diagnostic centers.

Non-conventional qualitative tests, like RLF tests, are currently commercially available. Some of such tests can lead results within minutes, but cannot be considered as conclusive tests by themselves. Biosensors could be employed to support RLF test results in the future, diminishing the overall time to achieve definitive quantitative results. Moreover, biosensors could exceptionally contribute to a fast and secure screening method for blood banks in small and medium health facilities. Hence, biosensors could improve CD diagnosis and the patients’ treatment follow-up, in terms of rapidity, small sample volume, high integration, ease of use, real-time and low cost detection compared to current conventional tests. Pursuing these goals is of considerable importance and interest to diminish CD burden and to reduce the risk of disease spreading intensified due to the climate change. Nevertheless, further research efforts are still needed to develop portable biosensing systems in order to effectively employ this technology for CD diagnosis.

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9. References


