Tuberculosis 127 (2021) 102055

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://www.elsevier.com/locate/tube

Point-of-care detection of tuberculosis using magnetoresistive biosensing chip

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ARTICLE INFO

Keywords:
Biosensor
Giant magnetoresistance (GMR)
Tuberculosis
ESAT-6

ABSTRACT

In this paper, a highly sensitive and specific technique based on the principle of giant magnetoresistance (GMR) has been proposed for the early stage Tuberculosis (TB) diagnostics. This GMR biosensing assay employs monoclonal antibodies against *M. tuberculosis* specific ESAT-6 antigen with the use of magnetic nanoparticles (MNPs) as labels. MNPs bind to the GMR sensor in presence of ESAT-6 and the binding is proportional to the ESAT-6 protein concentration leading to the change in overall resistance of GMR sensor. GMR biosensor simulation showed that ESAT-6 concentration can be detected in the range of pg/mL in comparison to the other transduction techniques available for ESAT-6 detection and further, the signal strength increased with the increase in the concentration. This work has shown that the GMR biosensing strategy is pertinent for the TB detection at the primitive phases when compared with other magnetic techniques used for TB diagnostics.

1. Introduction

Tuberculosis (TB) is a strikingly contagious disease caused by the bacterium named *Mycobacterium Tuberculosis* or *M. tuberculosis*. Mainly lungs are impaired by TB leading to pulmonary disorder. Extrapulmonary TB is resulted if other organs are damaged such as genito-urinary tract TB, osteo-tuberculosis, skin TB, neuro-tuberculosis, etc. Multiple organs can also be affected by *M. tuberculosis* causing Miliary or Disseminated TB [1,2]. WHO's (World Health Organization's) Global TB report of 2017 [2] has stated that among the worldwide chief killer diseases, TB is on the ninth number making it a fatal infectious menace. Furthermore TB, malignant or benign, is also the preeminent mortality cause from a single virulent agent, even ranking above AIDS (Acquired Immune Deficiency Syndrome) caused by HIV (human immunodeficiency virus) [1–3]. In the last few decades for the thorough and ubiquitous management of this TB epidemic, constant efforts have been made globally for the development of point-of-care TB detection techniques which are sufficiently accurate, efficient, rapid and cheap.

Various techniques based on nanotechnology approach have been adapted for specific, sensitive and speedy detection of TB [3]. To date, chip-based giant magnetoresistance have gained much popularity and have become a persuasive tool for high specificity, sensitivity, rapid biorecognition molecule detection with real-time electrical read-out [4]. The mass production of these GMR biosensors is economical as their integration and fabrication is compatible with the current technologies like large multiplex, VLSI (Very Large Scale Integration), microfluidics etc. [5,6]. GMR based biosensors are robust and matrix-insensitive as their performance is not affected by the environmental factors [7].

In the present work, we have demonstrated sensitive detection of *M. tuberculosis* specific ESAT-6 (Early secreted antigenic target-6) protein using GMR biosensor. It has also been discussed that in the future, the same concept can be used to detect drug-resistant gene mutations such as *rpoB* gene related to rifampicin resistance, *katG* and *inhA* gene related to isoniazid resistance etc.

2. Materials and methods

2.1. GMR chip structure

The proposed GMR biosensor is made up of an insulator layer and silicon substrate and two wires are sandwiched between these two as shown in Fig. 1. One of these wires behaves as GMR and other wire has the conducting properties. The GMR sensor wire is a multilayer structure consisting of a non-magnetic layer between ferromagnetic layers. This multilayer structure is sandwiched between a substrate and an adsorptive insulating layer in order to fabricate a biosensing chip platform. An

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https://doi.org/10.1016/j.tube.2021.102055
Received 1 June 2020; Received in revised form 19 January 2021; Accepted 22 January 2021
Available online 29 January 2021

immobilized layer of ESAT-6 specific primary antibodies is coated over the insulator layer. Also, other ESAT-6 specific antibodies referred to as secondary antibodies are labelled with magnetic nanoparticles (MNPs).

For the detection of ESAT-6 antigens in our case, we have considered the basic trilayer structure of GMR for simulation. This trilayer geometry having cross-sectional area of 100 nm × 100 nm comprises a non-magnetic Cu (copper layer) between two ferromagnetic layers made up of NiCoFe alloy. The dimensions of these layers are shown in Fig. 2. Further, for the simulation purpose, the primary antibody-antigen-secondary antibody sandwich product is depicted as a block of 15 nm thickness in Fig. 2 because the average height of antibody-antigen product is around 6.6 ± 0.3 nm [8]. The secondary antibody is labelled with an iron-oxide nanoparticle (IONP) as IONPs have super-paramagnetic properties leading to the increase in the strength of induced magnetic field, thus enhanced detection can be achieved [9,10].

Giant magneto-resistance based biosensor follows the principal that in the presence of an external magnetic field, stray magnetic field induced by the MNPs (which are bounded onto the sensor surface) will alter the magnetization in GMR sensor layers which will in-turn shift the total resistance of GMR biosensor [11,12]. Higher the number of MNPs bounded to the surface, higher will be the signal strength and better the detection [13].

2.2. Detection principle

The detection follows the following steps:

1. Initially, the resistance of GMR biosensor is measured by passing a current through the conducting wire.
2. The ESAT-6 specific primary antibodies work as capture antibodies for ESAT-6 present in the blood sample.
3. In the next step, MNPs labelled ESAT-6 specific secondary antibodies are introduced which get attached to the ESAT-6 bounded on primary antibodies and thus form a sandwich structure as shown in Fig. 1.
4. Finally, an external magnetic field is applied which forces the MNPs to induce a stray magnetic field which alters the overall resistance of sensor as measured initially. The induced magnetic field strength is proportional to the concentration of ESAT-6 as shown in Fig. 4.

Moreover, in addition to protein biomarkers, TB specific gene mutations like *rpoB* gene related to rifampicin resistance, *katG* and *inhA* gene related to isoniazid resistance etc. can be detected using the proposed GMR biosensor by analyzing the DNA. However, in this work, ESAT-6 protein detection has been simulated and validated using GMR biosensing technology. But in the future, similar procedure described above for the TB specific protein detection can be utilized for DNA detection and analysis as represented in Fig. 3 [14]. The following steps are needed to be performed:

1. Firstly, the probe DNA samples are spotted onto the surface of sensor. These samples are immobilized using epoxy groups embedded into the top layer of polymer.
2. After this, the sensor is exposed to analyte DNA (target) labelled with the biotin which is then hybridized to complementary probe DNA.
3. Final step concludes with the introduction of streptavidin-coated magnetic markers which bind to the biotin of the hybridized (target) analyte DNA specifically. Magnetic markers induce the stray magnetic field due to the effect of magnetic field applied externally which eventually causes the resistance change in GMR sensor embedded underneath the spot of probe DNA.

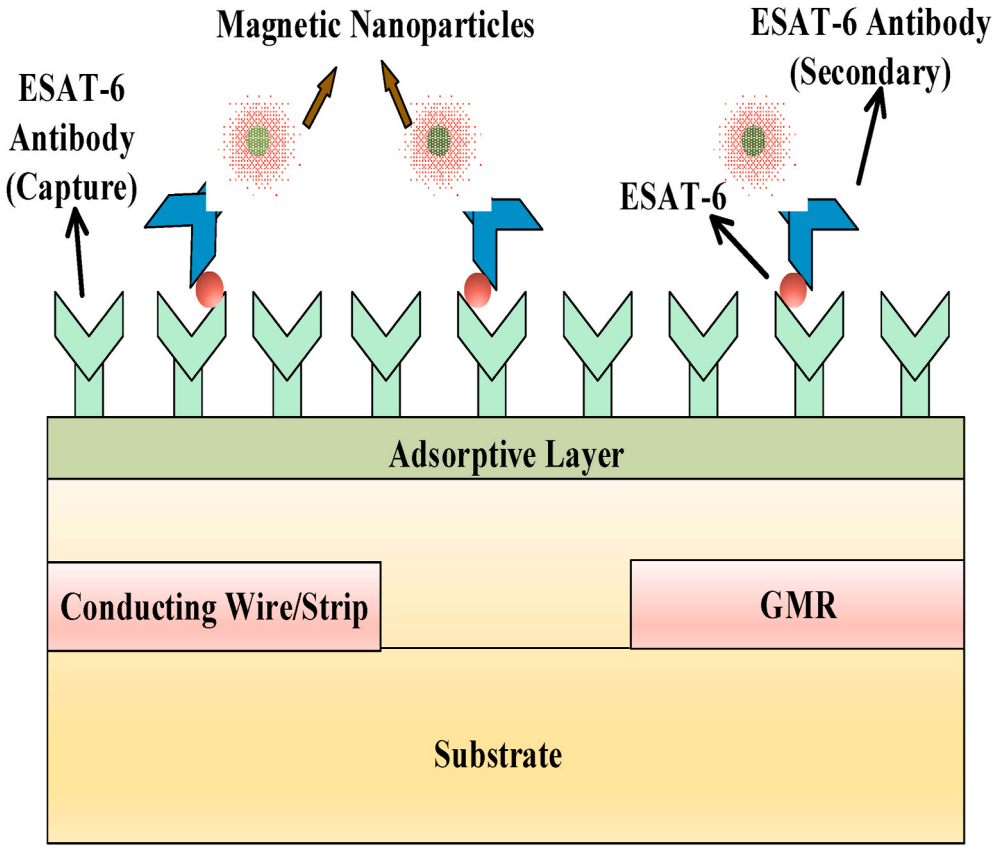

Fig. 1. Schematic Representation of ESAT-6 Detection using GMR Biosensor.

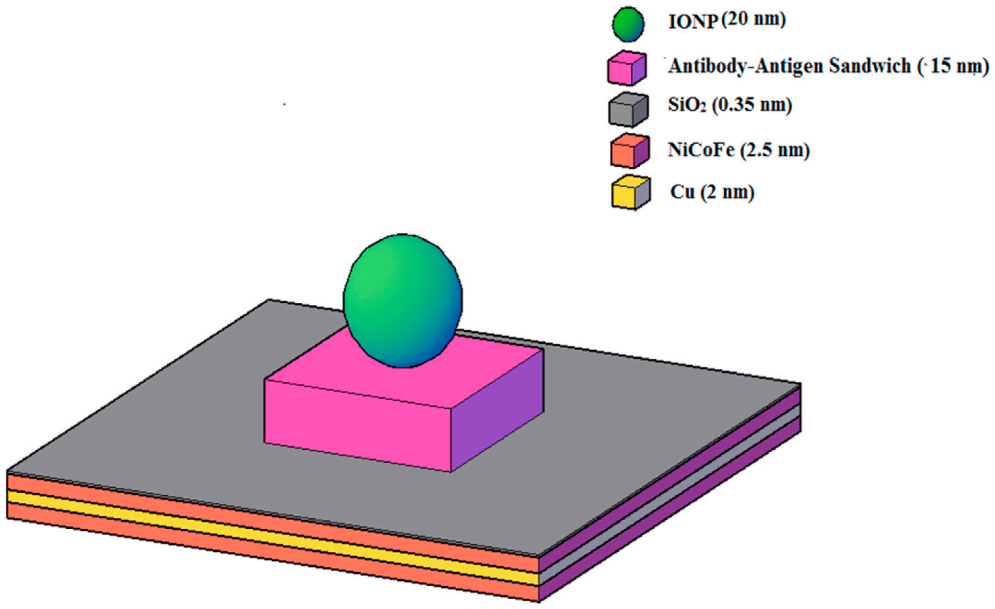

Fig. 2. Multilayer GMR sensor geometry.

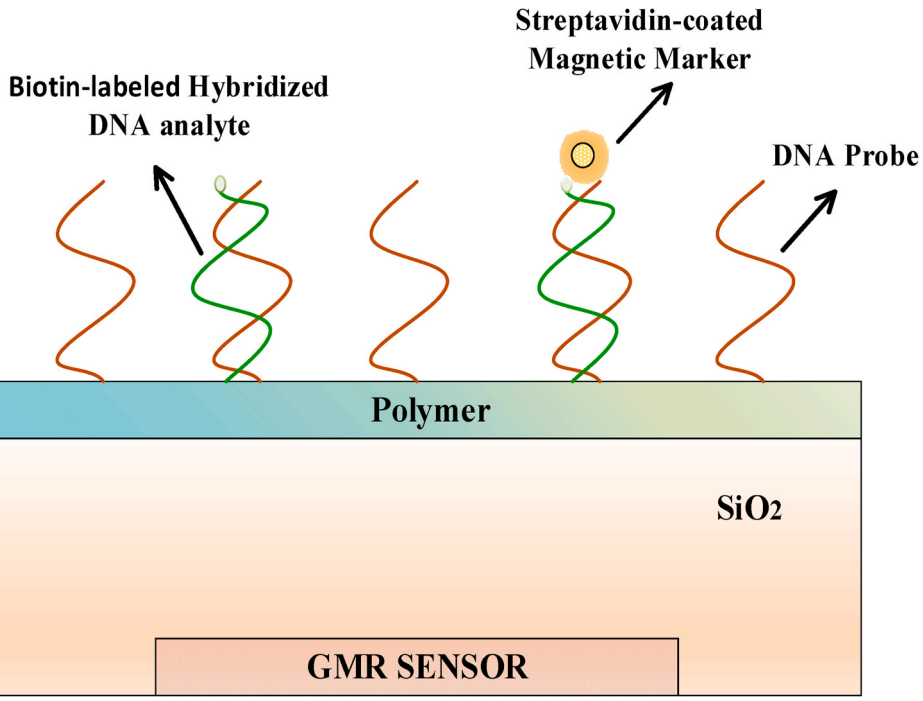

Fig. 3. Detection and analysis of DNA using GMR Biosensor.

3. Results and discussion

Tuberculosis (TB) is a lethal communicable disease which is needed to be controlled in the very initial stages. In this work, we propose to use the concept of GMR for the recognition of *M. tuberculosis* specific ESAT-6 protein and the simulated results shows that GMR biosensor can detect ESAT-6 with the very minimum amount of concentration as low as in pg/mL. With the GMR detection technique, described in the previous section, by applying 50 Oe (Oersted) (1 Oe is equivalent to 100 μT (micro Tesla) of external magnetic field and employing 20 nm IONP, the limit of detection achieved for ESAT-6 is in the range of pg/ml (1 pg/mL = 0.167 pM) as demonstrated in Fig. 4. For 2 pM of ESAT-6, the signal strength achieved is 13.86 μT and this signal strength increases with the increase in the ESAT-6 concentration, as specified in the calibration curve shown in Fig. 4.

Over the last decade, many magnetic detection techniques have been developed for the TB diagnostics which can target different TB specific biorecognition elements. In Table 1, the limit of detection (LOD) of these TB detection magnetic platforms have been compared with the proposed technique. For instance, H. Lee et al. in 2008–2009, have described a DMR (Diagnostic Magnetic Resonance) platform which can target *M. tuberculosis* cells and can also identify by-products from unprocessed sample of sputum in just 30 min. This platform has the limit of detection of 20 CFU/mL [15,16]. Further, in the year 2013, A. Engström et al. have come up with the technique of detecting rifampicin resistance (RR-TB) in *M. tuberculosis* by utilizing padlock probes for targeting rpoB gene

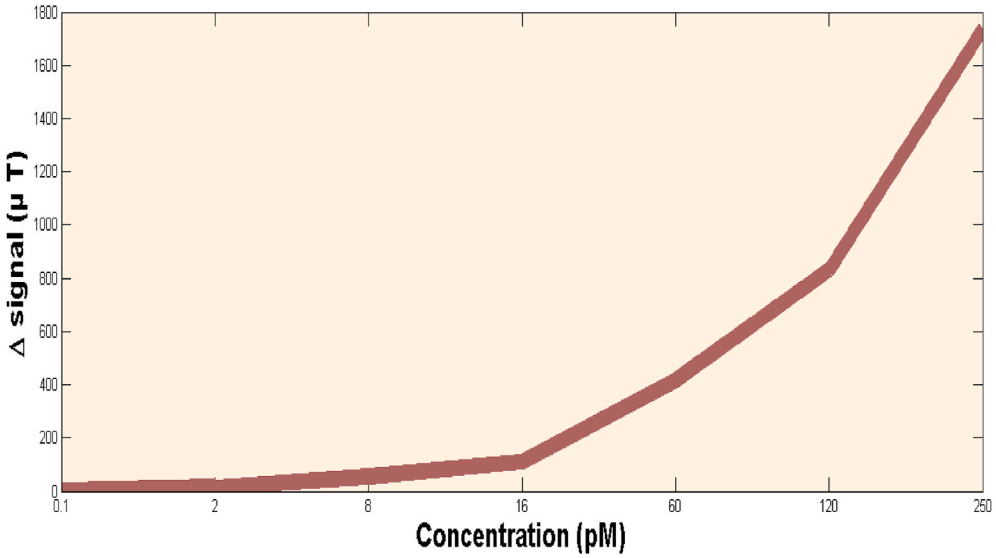

Fig. 4. Signal strength Vs ESAT-6 Concentration.

Table 1
Different magnetic TB detecton techniques.

S. No	Magnetic Techniques	Biorecognition Element	Limit of Detection (LOD)	References
1.	DMR platform	Bacteria (BCG)	20 CFU/mL (30 min)	[15,16]
2.	Magnetic barcode platform	Nucleic Acids (RNA from *M. tuberculosis* cells)	100 CFU/mL [19]	[18]
3.	Molecular technique using padlock probes and magnetic nanobeads	DNA	–	[17]
4.	Proposed GMR technique employing magnetic nanoparticles	TB specific protein/ DNA	12 pg/mL (~2pM)	–

mutations associated with RR-TB. This multiplexed and flexible molecular diagnostic mechanism employs a read-out device based on magnetic nano-beads [17]. A biosensor based on magnetic barcode platform has also been developed in 2013 by Liong, Monty, et al. for the recognizance of RNA from *M. tuberculosis* cells in samples of sputum with the detection limit of up to 100 CFU/mL [18].

Table 1 summarizes the various magnetic techniques used for TB detection with their limit of detection (LOD).

Among the various TB specific biomarkers, ESAT-6 is one of the dominant proteins present in the blood samples of TB infected people as it is secreted early by *M. tuberculosis* in the initial phases of TB and it progresses as the disease enters into advanced phases, making it a suitable candidate for diagnosing TB at early stages. Several biosensing transduction techniques have been developed over the years for the detection of TB specific ESAT-6 protein. A magnetic probe strategy for the detection of recombinant CFP10-ESAT-6 amalgamation proteins from *M. tuberculosis* have been described by Y. Huang et al., in 2011 [20]. This strategy can detect CFP10-ESAT6 proteins within 45 min along with LOD of 1 ng/mL. In the year 2012, H. Mukundan et al. have demonstrated the assays for the direct detection of three specific TB biomarkers (LAM, ESAT-6, Ag85) using optical waveguide-based biosensor with 100 pM of LOD for ESAT-6 [21]. T. Lakshmipriya et al. in 2016, have optimized the conventional ELISA technique by incorporating gold nanoparticles (GNP) in order to detect the ESAT-6 presence up to the LOD of 1 nM [22]. Further, in the year 2017, an electrochemical immunosensor having LOD of 7.1 ng/mL for ESAT-6 antigen has been described by M. F. Diouani et al. for diagnosing TB [23]. Immuno-PCR assay based on magnetic bead-coupled GNP, designed by N. Singh et al. in the year 2018 [24], has LOD in fg/ml as mentioned in Table 2, but since it uses PCR technique, so the chances of false positives and false negatives are also more when compared with the proposed GMR technique [25–27].

Table 2 specifies limit of detection of different transduction techniques available for ESAT-6 detection.

Fig. 5 shows the comparison of LOD in pg/mL among various transduction techniques available for ESAT-6 detection.

Following the erstwhile arguments, the features of proposed methodology can be summarized as mentioned below:

- Specificity: Bio-materials (e.g., food remains, etc.) generally are not magnetic, so the detection by GMR method is specific and insensitive to contaminations.
- Fast time to results: The magnetic actuation of nanobeads/nanoparticles is fast as compared to slow diffusion in other detection methods.
- Homogeneous assay: No washing steps are required [28,29].
- Miniaturized, Integrated and Robust for quick testing.

Table 2
Various transduction techniques for Esat-6 detection.

S. No	Transduction Technique	Biorecognition Element	LOD	References
1.	Micromagnetic Probe Strategy using home-made optcal sensor	CFP-10 ESAT-6	1 ng/mL	[20]
2.	Waveguide based Optical Sensor	ESAT-6	100 pM (≈598 pg/mL)	[21]
3.	ELISA combined GNP	ESAT-6	1 nM (≈11.8 ng/mL)	[22]
4.	Electrochemical Immunoassay	ESAT-6	7.1 ng/mL (0.6 nM)	[23]
5.	Immuno-PCR assay based on magnetic bead-coupled gold nanoparticle (GNP)	ESAT-6	10 fg/mL	[24]
6.	Proposed GMR technique employing magnetic nanoparticles	ESAT-6	12 pg/mL	–

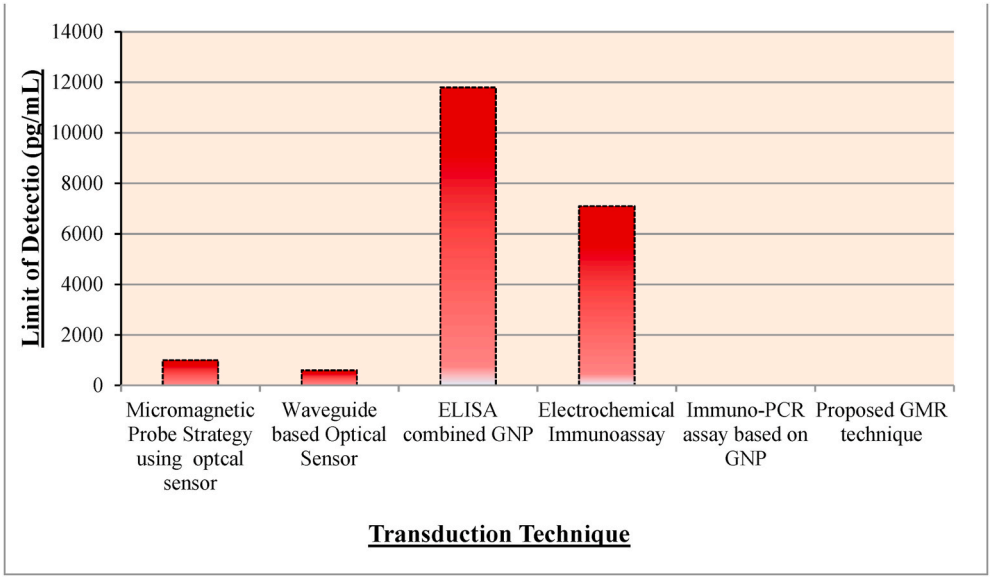

Fig. 5. Comparison of Limit of Detection (LOD) of ESAT-6 concentration in pg/mL among different transduction techniques.

- Sensitive and accurate: GMR technique is based on electronic measurement instead of subjective or less robust optical measurement. Further, with this technique, it is possible to detect the lower concentrations of proteins (in the range of pg/mL) that are specific indicators of individual's health.
- Due to the non-requirement of ionic doping (diffusion and implantation), GMR fabrication involves only low temperature processes and its real integration with many other standard technologies has become possible [30].

4. Conclusion

This work extends the application of GMR-based biosensor for point-of-care detection of Tuberculosis. It can be concluded from this study that the GMR method employing magnetic nanoparticles has the advantage of detecting *M. tuberculosis* specific antigen 'ESAT-6' with high specificity, sensitivity and with lower limit of detection when compared with the other existing TB diagnostic mechanisms. Further, in the future, this GMR biosensing strategy can be explored and validated for detecting the drug-resistant gene mutations in TB patients by analyzing the DNA sequences.

Author contributions

"S.G., P.B., and V.K. - Conceptualization; S.G., P.B., and V.K.- Data curation; S.G., P.B., and V.K. - Formal analysis; S.G., P.B., and V.K. - Investigation; S.G., P.B., and V.K.- Methodology; V.K. -Project administration; S.G. and V.K.- Resources; S.G. and V.K.- Software; V.K. - Supervision; S.G. and V.K.- Validation; S.G., P.B., and V.K.- Visualization; Roles/Writing – S.G.- original draft; S.G., P.B., and V.K. - Writing – review & editing".

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