DEVELOPMENT OF AN OPTICAL BIOSENSOR FOR DIAGNOSIS OF TUBERCULOSIS

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INTRODUCTION.

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis*. TB is the leading cause of morbidity and mortality in the developing world. Early and accurate diagnosis of TB would greatly enhance the treatment and prevention of the disease. Current methods of TB detection suffer from various limitations such as low specificity and sensitivity, being too complex and expensive. In the present work, we aim to develop an optical biosensor based on DNA aptamers, quantum dot (QD) crystals and magnetic nanoparticles (MNP) for detection of MPT64 protein, specific to *M.tuberculosis*. Aptamer-MNP conjugate is used for separation of MPT64 from solution, while aptamer-QD is used to detect its presence afterwards using fluorometer.

Systematic evolution of ligands through exponential enrichment (SELEX)

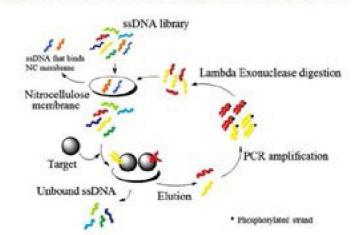


Figure 1. SELEX procedure

MATERIALS AND METHODS.

In order to select DNA aptamers against MPT64 protein, SELEX (systematic evolution of ligands by exponential enrichment) was carried out (Fig. 1).

The affinity of selected DNA aptamers was assessed by ELISA, Dot-Blot, gel shift and SPR assays.

MNPs were synthesized from iron oxide, with coating from polyethylene glycol and oleic acid.

RESULTS AND DISCUSSION.

Single stranded DNA aptamers against MPT64 protein have been selected. Their interaction with the target protein is under study. MNPs with size ranges from 10 to 100 nm were synthesized for future use as separation tools. Binding of QDs to MPT64 was observed by fluorometric ELISA. Further studies will include using MNP-aptamer conjugates to effectively separate MPT64 protein from the sample and QD-aptamer conjugates to detect the target. Streptavidin-coated MNPs and QDs will be conjugated to biotinylated aptamers. The format of the assay may also incorporate antibodies as an alternative to aptamers.

CONCLUSION.

All the necessary components of the biosensors have been acquired. The biosensor is being tested using both recombinant MPT64 and sputum samples obtained from patients of TB dispensary.

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