A DNA aptamer for detection of *Haemophilus influenza type b* by cell SELEX assay

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Introduction

Acute bacterial meningitis (ABM) is a syndrome involving bacterial infection and the associated inflammation affecting the central nervous system (CNS) leptomeninges, manifesting over hours to several (1) . ABM kills or maims about a fifth of people with the disease(2) .

Haemophilus influenza type b is a common causative pathogens of ABM in children(3) .

In recent years, the biosensors have been widely used as a rapid, sensitive, highly specific, low-cost detection technique for the detection of pathogens(4) .

Aptamers can be considered as valid alternatives for biosensors and other analytical methods in diagnostic and detection . Aptamer is a kind of synthetic short oligonucleotides discriminated by in vitro screening and systematic evolution of exponential enrichment technology (SELEX) is a high-flux screening technique(5) .

Materials & Methods

Cell SELEX was applied for the selection of target-specific aptamers(6) .

ss DNA aptamers prepared by lambda exonuclease were incubated with with *Haemophilus influenzae type b*(ATCC 10211) as the   target cell (7).

Nonbinding sequences were washed off and bound sequences were recovered and were used for *H.parahaemolyticus*(ATCC 10014), *Neisseria meningititis type b* (ATCC 13090), *Ecoli o1:K1:H7*(ATCC 11775), *Staphilococus aureus*(ATCC 25923), *Acinetobacter baumanii*(ATCC 19606), *Listeria monocytogene*(ATCC 7644), *streptococcus pneumoniae* (ATCC 33400) , as counter SELEX on another bacterial meningitis to eliminate the aptamers binding *Hib*.

 This leads to the enrichment of specific *Hib* binders. *Hib* bound sequences were amplified by PCR.

 For the second and subsequent rounds of SELEX, incubation time were decreased while increasing the washing time and volume of wash buffer(8) .

Results

The most abundant PCR product was obtained using an annealing temperature of 58°C, and a single 80-bp band was observed after DNA gel electrophoresis (Fig.1) .

A total of 7 rounds of selection were performed until the ssDNAs binding to the target cells dominated the DNA pool.

The percentage of ssDNA binding to the target cells gradually increased with each SELEX round except for a slight drop in elution yield after the 7th round(Fig.2) .

During SELEX, two counter selection processes were examined to enhance the selectivity of aptamers to the target cells using a mixture of other bacterial meningitis after the 3th and 5th rounds of selection.

Flow cytometry analysis after the 7th round of selection, the evolution of the ssDNA pool was stopped(Fig.3,4), and selected ssDNAs were amplified with unmodified primer sets to identify their sequences by TA-cloning and sequencing.

From the sequenced transformants, 64 unique aptamer sequences were selected for preliminary screening of binding affinity.
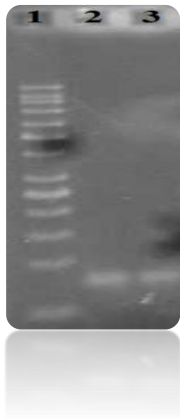

Fig.1: PCR-amplified nucleic acid fractions of initial DNA library. Lane 1: is the DNA ladder (50 bp). Lane 2: is the initial DNA library.Lane 3:is the seventh round of aptamer pool.
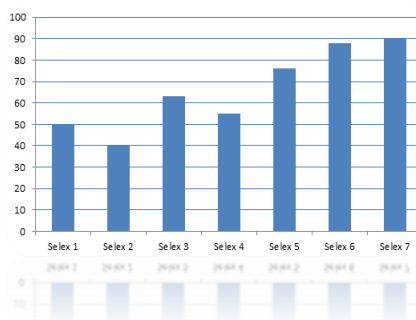
Fig.2:Monitoring the progression of the selection process by evaluating the portion of ssDNA bound to the target cells in the DNA pool.
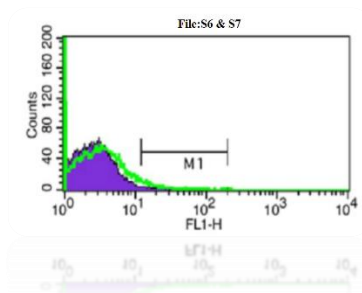
Fig.3: Binding affinity identical of selecte aptamer after 6th and 7th rounds of SELEX.
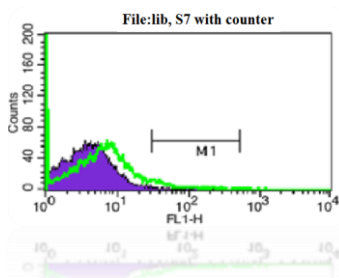
Fig.4: reduced binding affinity aptamer selected after 7 selex of the counter to the library.

Discussion & Conclusion

Specific aptamers against pathogens have been used as affinity reagents to develop sandwich assays, to label and to image cells, to

bind with cells for flow-cytometry analysis, and to act as probes for development of whole-cell biosensors.

Future applications of aptamers to pathogens will benefit from recent advances in improved selection and new aptamers containing

modified nucleotides particularly slow off-rate modified aptamers(6) .

This study is the first report on the use of whole-cell SELEX to identify DNA aptamers specific for *H.influenzae* .

We believe that these probes could be further developed for early pathogenic bacteria detection that would cause meningitis .

References

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