Detection of Food-Borne Pathogens Using Nanozyme Technology

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ABSTRACT

The recurrent outbreak of *E. coli* necessitates the need of rapid and sensitive technology to detect bacteria in the food samples. *E. coli* O157:H7 is infectious at very low CFU counts (10-100 viable cells). Herein, we report a unique combination of magnetic and plasmonic properties in a single nanoplatform, which have superior peroxidase-like activity. This new nanosensor platform, magneto-plasmonic nanosensor (MPnS), is composed of superparamagnetic iron oxide nanoparticles (IONPs) and gold nanoparticles (GNPs) and stabilized with polyacrylic acid polymer, providing surface -COOH functional groups. By using EDC/NHS bioconjugation chemistry, the surface of MPnS is decorated with *E. coli* O157:H7-specific antibodies. We compared the catalytic activities of MPnS with that of GNPs, IONPs and traditional HRP and calculated Michaelis-Menten kinetics, which showed highest catalytic activity for MPnS. The ELISA-like experiments were performed using MPnS to detect E.coli within 30 min with higher sensitivity. We extended this detection study using milk and spinach samples. Various spectrophotometric and colorimetric experimental results in the specific detection of E.coli will be detailed in this presentation.

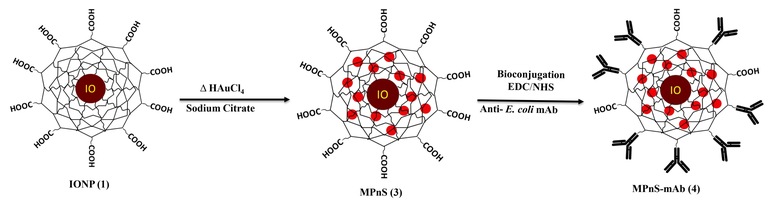
INTRODUCTION:

Escherichia coli (*E. coli*) O157:H7 is a pathogenic bacterium which is capable of contamination food and water causes bloody diarrhea, food poisoning and occasionally kidney failure. According to CDC, around 2,65,000 Shiga toxin producing *E. coli* (STEC) infections occurs in the United States and 36 % of infection occurs with STEC O157. CDC estimate that the around 3600 hospitalization and 30 deaths occurs each year because of STEC infection.

Conventional methods for the detection of pathogen includes PCR based analysis, surface plasmon resonance, culturing techniques, and enzyme-linked immunosorbent assay (ELISA). Among this techniques ELISA is the most common and effective method for the detection of pathogen. Conventional ELISA uses natural enzyme such as HRP, however these enzymes have limited catalytic activity to environmental condition, high-cost demand and low stability (digestion and denaturation). To overcome these limitations, recent studies have developed enzyme mimetics. Researchers have constructed nanomaterials which possess intrinsic peroxidase-like activity and it can be used in ELISA by replacing natural enzymes such as cerium oxide nanoparticle, gold nanoparticle, iron oxide nanoparticle, carbon nanomaterial, silver nanoparticles and many more.

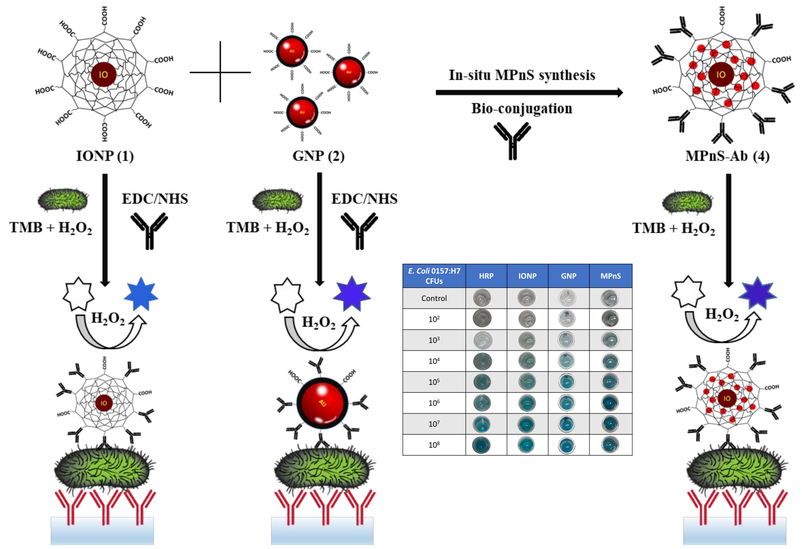
In this study we have developed a hybrid nanoparticle with the combination of magnetic and plasmonic property. Magneto plasmonic nanoparticle (MPnS) is combination of iron oxide nanoparticle and gold nanoparticle. We report a rapid and specific detection of *E. coli* 157:H7 in less than 30 mins. The *anti-E. coli* O157:H7 monoclonal antibody conjugation on MPnS with EDC-NHS chemistry is specific to *E. coli* O157:H7. MPnS possess the peroxidase like activity and can oxidize the TMB in the presence of H_2O_2. In the H_2O_2 TMB oxidation gives blue color which is observe by naked eye and can be measure by the UV-absorbance at 652 nm. In the present study MPnS can detect pathogen in real sample like milk and spinach rinse even in low CFUs. Nanozyme based ELISA detection of pathogen is rapid, sensitive, specific and cost-effective method.

Synthesis of Magneto Plasmonic Nanoparticle



Scheme 1: Synthesis of Magneto Plasmonic Nanoparticle and bioconjugation of anti-E. coli O157:H7 antibody

Schematic representation of ELISA



Characterization of MPnS

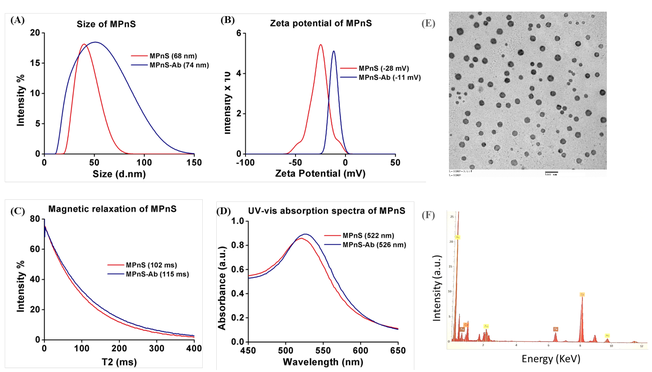


Figure 1: Characterization of MPnS and antibody conjugated MPnS: (A) Hydrodynamic radius of MPnS before and after conjugation, (B) Zeta potential of MPnS and MPnS-Ab, (C) Magnetic relaxation (T2) of MPnS and MPnS-Ab, (D) UV-vis absorption spectra of MPnS and MPnS-Ab further confirming successful conjugation, (E) TEM of MPnS and, (F) EDS of MPnS.

Optimization of peroxidase-like activity of nanozymes

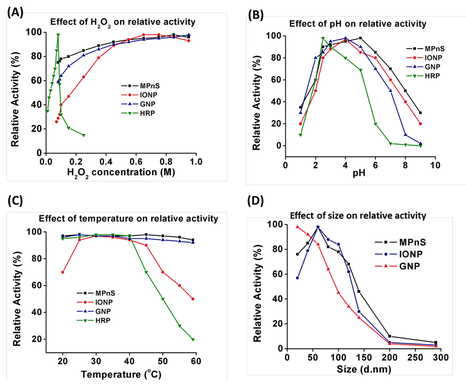


Fig 2: : Peroxidase-like activity of nanozymes and natural enzyme with (A) varied H_2O_2 (B) pH, (C) Temperature and (D) size.

Michaelis-Menten curves of nanozymes

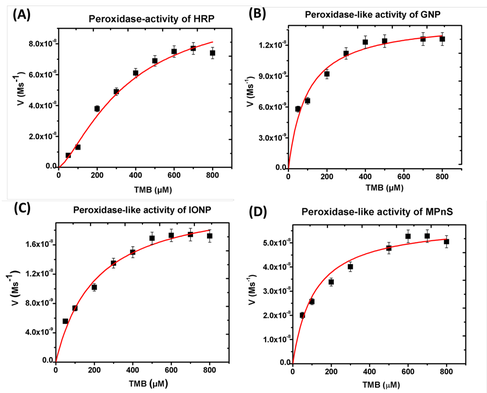


Fig 3: : Kinetic parameters of nanozymes and natural enzyme HRP exhibiting peroxidase activity: Steady state kinetic analysis using Michaelis-Menten Model of (A) HRP (B) GNP (C) IONP and (D) MPnS by varying TMB concentration.

E. Coli O157:H7 detection

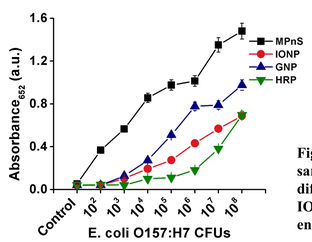


Fig 4: Plot comparing the sandwich ELISA results using different nanozymes (MPnS, IONP and GNP) and Natural enzyme HRP.

Kinetic parameter of nanozymes

Catalyst	K_m (μM)	V_{max} (10^{-8}M/s^{-1})	k_{cat} (s^{-1})
HRP	243	8.7	4.3 x 10^3
IONP	208	2.3	2.5 x 10^4
GNP	96	1.4	2.4 x 10^4
MPnS	111	5.9	5.9 x 10^5

Table 1: Kinetic parameters of IONP, GNP, MPnS and HRP nanozymes obtained from Michaelis-Menten curves. K_m denotes the Michaelis constant, V_{max} is the maximum velocity, k_{cat} is the catalytic constant and is expressed by the formula $k_{cat} = V_{max}/[E]$
The concentration of HRP, IONP, GNP and MPnS are 20pM, 0.9 pM, 0.6 pM and 0.2 pM respectively.

E. Coli O157:H7 detection in real sample

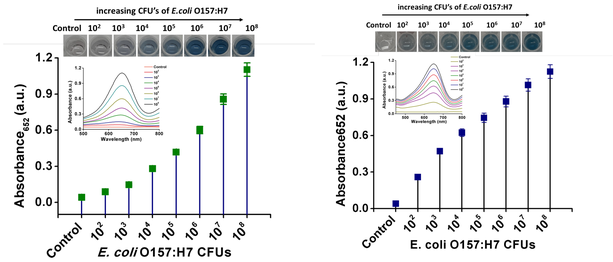


Fig 5: Detection of E. coli O157:H7 using MPnS in real sample,(A) milk and(B) spinach rinse.

Time Dependent Assay

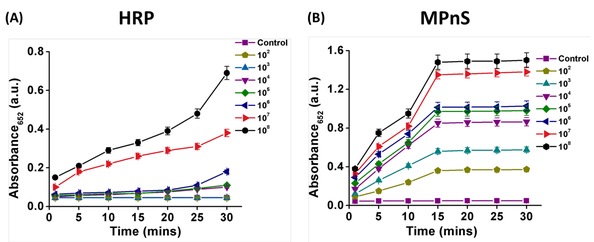


Fig 6: Time dependent assay comparison of (A) HRP and (B) peroxidase-mimetic MPnS.

Conclusion

- In conclusion we have successfully synthesized MPnS which possess intrinsic peroxidase mimetic activity for the rapid and specific detection of E. coli O157:H7 in simple and complex food matrices.
- MPnS enabled ELISA exhibits lower turn around time than conventional ELISA.
- Formulated MPnS customizable and can be tailored for the detection of other food borne pathogens.

References

- **Santra et.al.,** ***ACS App Nano Mat.*** **2019**, 2, 5587-5593.
- **Santra et.al.,** ***ACS Infec Diseases.*** **2018,** 8, 1162.
- **Santra et.al.,** ***JoVE.*** **2017,** 127, e55821
- **Santra et.al.,** ***ACS Infec Diseases.*** **2016,** 2, 667-673
- **Santra et.al.,** ***Scientific Reports.*** **2017,** 7, 7377.

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