Lateral Flow Capillary Concentration for Rapid Bacterial Identification

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Abstract

Lateral flow capillary concentration (LFCC) immunoassays are being developed as a replacement for lateral flow immunoassays (LFI), which are used for rapid, point-of-care bacterial identification and antibiotic resistance determination. They employ a two nanoparticle system: a superparamagnetic capture particle and a reporter particle. Each particle is conjugated via crosslinker with antibodies specific to the amplified bacteriophage being detected. In the presence of phage the capture and reporter particles are bound through the antibody phage linkage, and are concentrated on a magnet for subsequent analysis by Raman spectrometry.

Introduction

LFI coupled with phage amplification was a milestone in the rapid detection and identification of bacteria. However, a more reliable, time efficient, and cost effective method is necessary for rapid identification of bacteria and determination of antibiotic resistance. LFCC immunoassays are simple devices which employ a two particle system: the reporter particle (either dyed polystyrene particles or particles with a Raman active organic layer) and the capture particle (super-paramagnetic microparticles). Both particles are conjugated with antibodies specific to a phage. In the presence of the phage the particles will be bound while the absence of the phage will cause no binding. This system is then run through an LFCC immunoassay which consists of a capillary running perpendicular to a magnetic strip. The capture particles are arrested by the magnet which forms a line in the capillary. If the reporter particles are dyed and phage is present, the line will be the color of the reporter particle. If phage is not present the line will be the color of the capture particle. If the reporter particle is Raman active, the color is not significant. The test line can be analyzed with a Raman spectrometer to determine the presence of phage as an indirect biomarker for the of bacteria.

Materials and Methods

Particle Preparation:
For Carboxylated Particles [1]

- Prepare antibody solution at 0.5 to 1.0 mg/mL
- Combine antibody solution with particles
- Add crosslinker (EDC: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide)
- React for 3 – 5 hours
- Wash particles and suspend in a storage buffer

For Thiolated Particles

- Mix protein solution at 0.5 to 1.0 mg/mL with crosslinker (sulfo-SMCC: Sulfosuccinimidyl-4-(N-maleimidomethyl)cyclo-hexane-1-carboxylate)
- Remove unreacted crosslinker and add particles
- React for 2 – 3 hours.
- Wash particles and suspend in a storage buffer.

PDMS Test Strip Creation

- Silanate test strip mold
- Mix base and curing agent until completely homogenized and pour into mold
- Place the mold into a vacuum chamber for one hour or until all bubbles are gone
- Transfer mold to oven at 80 C for 3 hours
- Cut mold to desired specifications
- Bind PDMS test strip to a glass slide using Plasma Cleaner

Nanoparticle Constructs

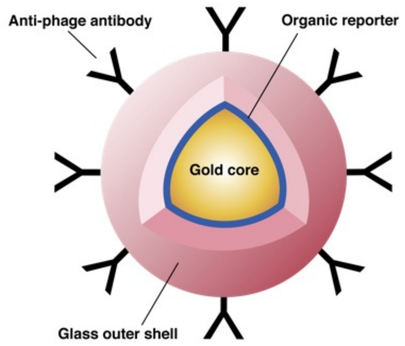

Figure 1: SERS Oxonica Nanoplex Biotags have a gold core surrounded by an organic reporter which has a unique Raman profile. There are a variety of reporters that give different Raman spectra. The glass outer shell is thiolated, which allows for antibody conjugation to the surface of the particle.[2]

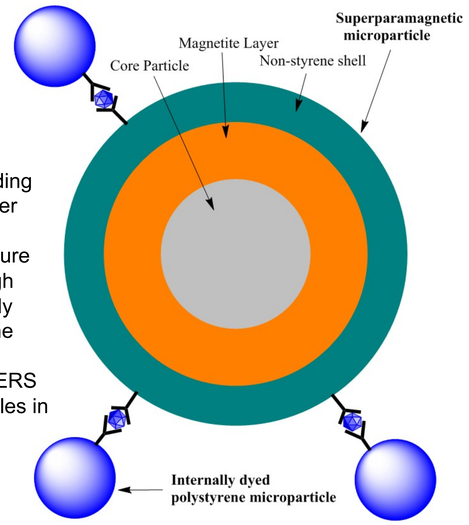

Figure 2: Binding of dyed reporter particles to a magnetic capture particle through phage antibody interaction. The same binding occurs with SERS reporter particles in figure 1.

Immunoassay Design and Spectra Results

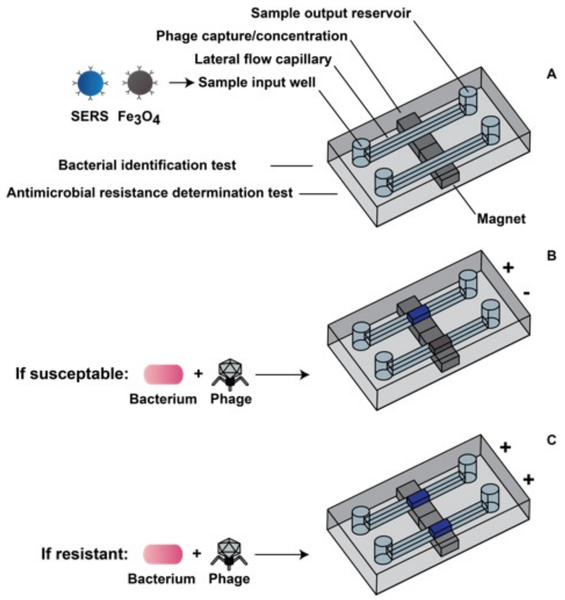

Figure 3: Double channel LFCC immunoassays.
A: Diagram of test strip components and particle types.
B: Test results for bacteria susceptible to phage infection. The top capillary confirms the identity of the bacteria with a positive result. The bottom capillary indicates the bacteria's susceptibility to antibiotics.
C: Double positive confirms identity of bacteria and bacterial is resistance profile. Antibiotics are unable to kill tbacteria, resulting in phage amplification and positive results. [2]

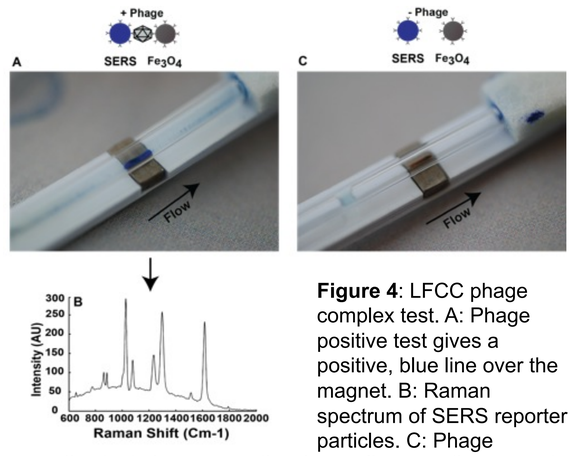

Figure 4: LFCC phage complex test. A: Phage positive test gives a positive, blue line over the magnet. B: Raman spectrum of SERS reporter particles. C: Phage negative test gives a negative, brown line over the magnet.

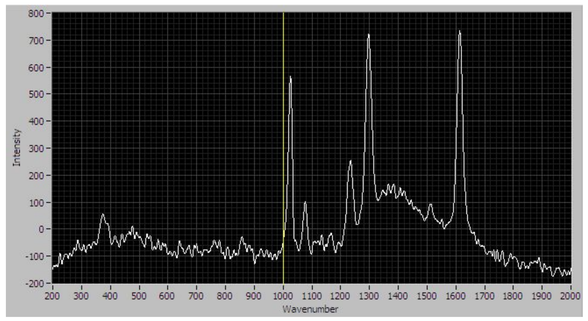

Figure 5: Sera-Mag SpeedBeads (1/6) and SERS M170.1 particles (1/30) diluted in 1xPBS to total volume of 600 µL.

Conclusions

LFCC immunoassays have the potential to replace LFI as a cheap, user friendly, and accurate method for the detection of bacteria through phage amplification. Both kinds of reporter particles have been successfully conjugated to antibodies, as have the magnetic capture particles. However, the reliability of the conjugation methods needs to be greater before LFCC can be prolifically used for rapid bacterial detection and identification.

The use of Raman active SERS particles as a reporter particles shows promise. Figure 3 demonstrates that the Raman profile can be discerned even when mixed with a high concentration of larger magnetic particles. Whether they can be used reliably in LFCC immunoassays will be determined through further experimentation.

Future Work

Eliminate false positives and false negatives

- experiment with particle sizes and conjugation method

Conjugational method experimentation:

- vary antibody and cross linker concentrations to observe effect on particle conjugations

Raman detection of SERS Particles in LFCC Pellet

- vary ratio of capture particles to SERS particles to maximize Raman spectrum profile

Device development:

- create and test different prototypes made from other polymers.

Real sample testing

- test the system on samples from real world problems and compare the results to classical tests.

References

[1] Hermanson, Greg T. *Bioconjugation Techniques.* Rockford, Illinois: Academic Press, 2008. Print.
[2] Used with permission from Christopher Cox

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