Molecular-Beacon-Conjugated PEG Nanohydrogels for Nucleic-Acid Detection

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Statement of Purpose: Drug-resistant bacteria have become an increasingly important clinical issue. Since the identification of genetic changes that cause multi-drug resistance is critical for treatment as well as for drug development, there is an urgent need for rapid, sensitive and reliable detection methods. One widely used method to identify genes employs a real-time Polymerase Chain Reaction using molecular beacons (MBs)[1]. Molecular beacons are hairpin-shaped fluorescent nucleic acid hybridization probes, which are highly specific and only fluoresce when bound to their complementary target nucleic acid sequence. They possess a fluorophore and quencher pair, which are in close proximity when the probe is not bound to a target, resulting in total quenching of the probe's fluorescence. However, when the probe is bound to a target, the probe undergoes a conformational change causing the fluorophore and quencher to separate, thereby restoring the fluorescence of the probe. The signal to background ratio (SBR) of an MB is a measure of probe sensitivity and varies from ~50 to 900 in aqueous solutions[1]. Like other array technologies there is great interest in binding MBs to surfaces to: (1) use less reagent; (2) associate spatial position with a specific probe sequence; and (3) explore a large number of probes simultaneously. Attaching MBs to solid surfaces has, however, been a challenge due to difficulties in maintaining their structural properties. Whether on particle carriers, dissolved in solution, or directly on bulk solid surfaces, the resulting SBRs are much lower than those of MBs in solution. The lack of high SBR with surface-bound MBs impedes development of new solid-surface MB-based applications. Here, we hypothesize that the SBR can be improved if MBs are bound to highly hydrated gels mimicking an aqueous environment. This approach minimizes non-specific interactions between a substrate and an MB. Furthermore, if the MBs are concentrated on a submicron-sized surface-bound hydrogel particle, the amount of sample needed also dramatically decreases.

Methods: Biotin-PEG-Biotin (MW=5000 g/mol) was purchased from Nanocs Inc., NY. A LEO 982 DSM field-emission scanning electron microscope (FEG-SEM) was used to generate the PEG hydrogels on Si wafers using established methods[2]. The patterned gels were subsequently functionalized with streptavidin (SAv). To test the performance of molecular beacons on the PEG hydrogels, we adapted a molecular beacon design from a study to identify the mecA gene in the methicillin resistant *Staphylococcus aureus*[3]. The molecular beacon (mecA-MB) was labeled with Texas Red and Blackhole Quencher 2, and further modified to contain a biotin moiety for conjugation to the SAv-functionalized PEG gels. In addition, we synthesized a perfect complementary target sequence (mecA target) and two more oligonucleotides, designed to mimic target sequences (spa and bac target) in genes present in different bacterial species. To characterize the performance of the platform, fluorescence images were collected utilizing a Nikon E1000 microscope and quantified by ImageJ (NIH) before and after incubation of a mecA-MB conjugated gel with a 10^{-6} M mecA target solution (Fig. 1 and 2). To explore the specificity of the mecA-MB conjugated PEG nanohydrogels, we incubate the hydrogel with different mixtures of target sequences (Fig. 3).

Results:

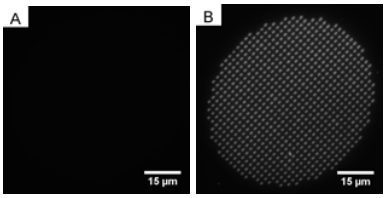

Figure 1. Fluorescence images of patterned PEG gels: (A) MecA functionalized; (B) fluorescence increase after exposure to a mecA target oligonucleotide solution.

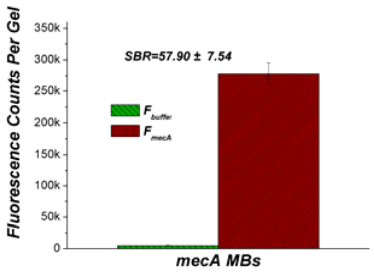

Figure 2. Determination of the Signal-to-Background ratio.

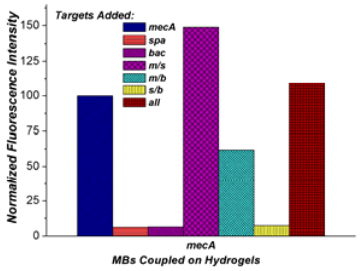

Figure 3. Specificity of mecA-MBs conjugated surface-patterned PEG hydrogel particles.

Conclusions: Molecular beacons conjugated to PEG hydrogels are as effective in detecting and discriminating target nucleic acids with high sensitivity and specificity as molecular beacons in aqueous environments. Quantitatively, immobilized MBs reached high SBR comparable to those of MBs dissolved in solution. These attractive properties can enable construction of an easy, fast and reliable bacteria identification platform.

References: (1) Tyagi, S. Nat Biotechnol 1996, 14, 303-308. (2) Krsko, P. Langmuir 2003, 19, 5618-5625. (3) Sinsimer, D. J Clin Microbiol 2005, 43, 4585-4591.