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Aptamer-based hydrogel barcodes for the capture and detection of multiple types of pathogenic bacteria

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ABSTRACT

Rapid and sensitive diagnosing hematological infections based on the separation and detection of pathogenic bacteria in the patient's blood is a significant challenge. To address this, we herein present a new barcodes technology that can simultaneously capture and detect multiple types of pathogenic bacteria from a complex sample. The barcodes are poly (ethylene glycol) (PEG) hydrogel inverse opal particles with characteristic reflection peak codes that remain stable during bacteria capture on their surfaces. As the spherical surface of the particles has ordered porous nanostructure, the barcodes can provide not only more surface area for probe immobilization and reaction, but also a nanopatterned platform for highly efficient bioreactions. In addition, the PEG hydrogel scaffold could decrease the non-specificity adsorption by its anti-adhesive effect, and the decorated aptamer probes in the scaffolds could increase the sensitivity, reliability, and specificity of the bacteria capture and detection. Moreover, the tagged magnetic nanoparticles in the PEG scaffold could impart the barcodes with controllable movement under magnetic fields, which can be used to significantly increase the reaction speed and simplify the processing of the bioassays. Based on the describe barcodes, it was demonstrated that the bacteria could be captured and identified even at low bacterial concentrations (100 CFU mL^{-1}) within 2.5 h, which is effectively shortened in comparison with the "gold standard" in clinic. These features make the barcodes ideal for capturing and detecting multiple bacteria from clinical samples for hematological infection diagnostics.

1. Introduction

Bacteremia caused by bacterial bloodstream infections, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and so on, can lead to vascular leakage, tissue damage, and multiorgan failure; these are associated with one-third of global mortality (Angus and van der Poll, 2013; Kailasa and Wu, 2012, 2013; Rocheteau et al., 2015). Identifying bacteria rapidly in the early stage of infection is significant to decrease high mortality (Yealy et al., 2014). However, current "gold standard" in clinical diagnosis of bacteremia usually needs 3–5 days of incubation and at least 12 h of growing on solid media to identify the bacteria (Sarkar et al., 2006). Various approaches have been devised to improve the sensitivity for bacterial identification, such as real-time polymerase chain reactions (Ottesen et al., 2006), fluorescent in situ hybridization, surface enhanced Raman scattering, and fluorescent probes (Kang et al., 2014). In spite of the improvement, these methods still require long-term blood culture and expensive equipment, which limit their widespread use in clinical applications. As an alternative, some simple cell capture platforms have been constructed to shorten the time needed for pathogen detection as well as identification. However, most of these methods were only carried out with single target test, and could not effectively distinguish between different bacteria simultaneously in a simple detection. Therefore, the development of a new platform that can capture and distinguish multiple types of bacteria simultaneously in a short time is highly desired.

Barcodes(also called as encoded microcarriers), which encode information about their specific compositions and enable simple identification, have attracted increasing interest for multiple bioassays (Liu et al., 2014; Meng et al., 2015; Shi et al., 2013; Xu et al., 2017; Yang et al., 2008; Zhang et al., 2016; Zheng et al., 2014). Many kinds of encoding strategies have been proposed for the barcodes, including fluorescent molecules, quantum dots, photonic crystals, or graphical or shape-encoded microplates, and so on (Ge and Yin, 2008, 2011; Kanai et al., 2010; Lee et al., 2015; Mao et al., 2010; Shang et al., 2013; Sim et al., 2015; Song et al., 2015; Xu and Chen, 2015; Yu et al., 2009; Y.Q. Zhang et al., 2013, Y.S. Zhang et al., 2013; Zhao et al., 2014, 2015).

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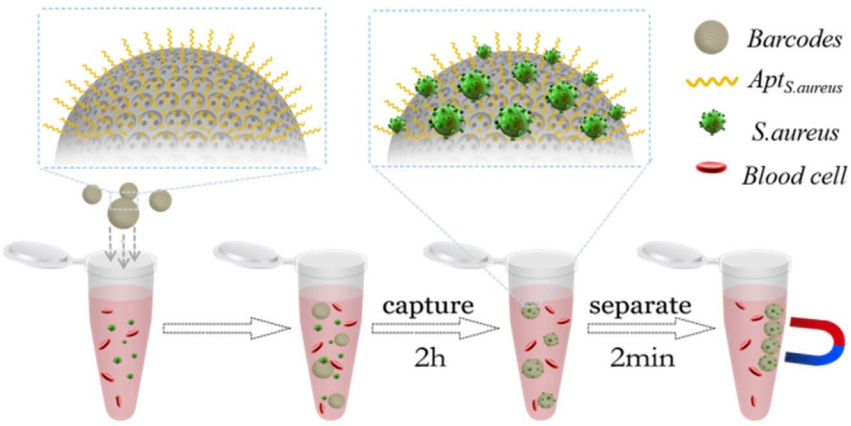

Scheme 1. Schematic diagram of the inverse opal structured magnetic hydrogel barcodes with aptamer probes for the bacteria capture.

Based on these barcodes, many distinct multiple assays have been carried out for high throughput biomolecule detection, gene function analysis, and clinical diagnosis (Fu et al., 2016). However, the encoded information of most barcodes would be confused or incomprehensible when their surfaces were covered by bacteria, and this could cause false decoding of the barcodes. In addition, the debatable specificity and reliability of their general surface morphology and biochemical modification, as well as the uncontrolled motion, have also limited the application of barcodes for the bacteria detection. Thus, the development of new barcodes-based bacteria capture and detection platform with distinct advantages is still required. Scheme 1

In this paper, we present a new aptamer-functionalized barcodes technology that can simultaneously capture and detect different types of pathogenic bacteria. The barcodes are poly (ethylene glycol) (PEG) hydrogel inverse opal particles with characteristic reflection peak codes that remain stable during bacteria capture on their surfaces (Choi et al., 2009; Hong et al., 2012; Shang et al., 2015; Stein et al., 2013; Y.Q. Zhang et al., 2013, Y.S. Zhang et al., 2013). The decorated aptamer probes in the PEG scaffolds could specifically capture the bacteria, while the PEG hydrogel scaffold could decrease the non-specificity adsorption of other targets. Due to the ordered porous nanostructure on the spherical surface, the barcodes can provide not only more surface area for probe immobilization and reaction, but also a nanopatterned platform for highly efficient bioreactions. In addition, the tagged magnetic nanoparticles in the PEG scaffold could impart the barcodes with controllable movement under magnetic fields, which can be used to significantly increase the reaction speed and simplify the processing of the bioassays. Thus, compared with other reported methods, our barcodes have such advangtages, include low non-specificity adsorption, good efficiency, fast capture speed and multiplex capture capacity. It will be demonstrated that the bacteria with low concentrations could be captured and identified very fast by the barcodes, which fully satisfied the clinical criteria during the hematological infection diagnostics.

2. Experimental section

2.1. Materials

Six kinds of SiO_2 nanoparticles with the size of 211, 260 and 307 nm were purchased from NanJing DongJian Biological Technology Co., Ltd. CY3 labeled rabbit polyclonal anti-human AFP antibody were purchased from Micro Biological Technology Company, Shanghai, China. Two kinds of aptamers ($Apt_{S.aureus}$ and $Apt_{E.coli}$) were purchased from Ruibo Biological Technology Co., Ltd., Guangzhou, China. *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) were purchased from the inquiry network for microbial strains of China. FITC Concanavalin A was purchased from Sigma-Aldrich, Shanghai, China. Poly (ethyleneglycol) diacrylate (PEG-DA) with molecular weights of 700 and 2-hydroxy-2-methylpropiophenone (HMPP) photoinitiator were purchased from Sigma-Aldrich, Shanghai, China. Acrylic Acid (AA) was obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Co., Ltd., Shanghai, China. 2-Morpholinoethanesulfonic Acid (MES) was obtained from AMRESCO LLC, Solon, USA. Phosphate buffer saline (PBS, 0.05 M, pH 7.4) were self-prepared. All buffers were self-prepared using water purified in a Milli-Qsystem (Millipore, Bedford, USA).

30 healthy human blood samples were collected from the affiliated ZhongDa Hospital of Southeast University, Nanjing, China. All the collection and processing of human blood samples were carried out in accordance with the guidelines issued by the Ethical Committee of the Chinese Academy of Sciences.

2.2. Instruments

The microfluidic device used for generating silica colloidal crystal beads was home-made. All reactions were finished in flat-bottom tubes and a constant temperature shaker (Thermomixer comfort 5355, Eppendorf, Germany). The microstructures of silica colloidal crystal beads and hydrogel photonic barcodes were characterized according to a scanning electron microscope (SEM, S-300N, Hitachi, Japan). Photographs of the two kinds of beads were taken by an optical microscope (BX51, Olympus, Japan) equipped with a CCD camera (MP5.0, Media Cybernetics Evolution). The reflectance spectra of the barcodes were recorded by the same microscope equipped with a fiber optic spectrometer (HR2000, Ocean Optics, USA). The fluorescence intensity was detected by a fluorescence microscope (BX53, Olympus, Japan).

2.3. Fabrication of inverse opal hydrogel magnetic barcodes

Silica colloidal crystal beads (SCCBs) used as the template to fabricate this kind of inverse opal barcodes were fabricated using the microfluidic devices. The inverse opal barcodes were replicated from the voids of the template silica colloidal crystal beads. And the pre-gel solution used for the fabrication of inverse opal barcodes was composed of Poly (ethyleneglycol) diacrylate (PEG-DA) and Acrylic Acid (AA). Firstly, the dried silica colloidal crystal beads with different colors were immersed in pre-gel solution (20% PEG-DA, 10%AA and 1% HMPP) for 1 h. The liquid mixed solution could fill the gaps of silica colloidal crystal beads fully. Next, the mixture of beads and pre-gel solution was exposed to UV light for polymerizing the pre-gel solution in and out of the SCCBs. After polymerization, the hybrid beads of different colors could be extracted by stripping the pre-gel on the surface of the beads, and then remove the silica template with 4% hydrofluoric acid to obtain hydrogel inverse opal barcodes. Finally, the magnetic inverse opal barcodes could be obtained by saturating the barcodes with the magnetic nanoparticles.