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Ultra-Rapid and Efficient Immunomagnetic Separation of Listeria Monocytogenes from Complex Samples in High-Gradient Magnetic Field Using Disposable Magnetic Microfluidic Device

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Abstract : The incidence of infections caused by foodborne pathogens such as Listeria monocytogenes (L. monocytogenes) poses a great potential threat to public health and safety. These issues are further exacerbated by legal repercussions due to “zero tolerance” food safety standards adopted in developed countries. Unfortunately, a large number of related disease outbreaks are caused by pathogens present in extremely low counts currently undetectable by available techniques. The development of highly sensitive and rapid detection of foodborne pathogens is therefore crucial, and requires robust and efficient pre-analytical sample preparation. Immunomagnetic separation is a popular approach to sample preparation. Microfluidic chips combined with external magnets have emerged as viable high throughput methods. However, external magnets alone are not suitable for the capture of nanoparticles, as very strong magnetic fields are required. Devices that incorporate externally applied magnetic field and microstructures of a soft magnetic material have thus been used for local field amplification. Unfortunately, very complex and costly fabrication processes used for integration of soft magnetic materials in the reported proof-of-concept devices would prohibit their use as disposable tools for food and water safety or diagnostic applications. We present a sample preparation magnetic microfluidic device implemented in low-cost thermoplastic polymers using fabrication techniques suitable for mass-production. The developed magnetic capture chip (M-chip) was employed for rapid capture and release of L. monocytogenes conjugated to immunomagnetic nanoparticles (IMNs) in buffer and beef filtrate. The M-chip relies on a dense array of Nickel-coated high-aspect ratio pillars for capture with controlled magnetic field distribution and a microfluidic channel network for sample delivery, waste, wash and recovery. The developed Nickel-coating process and passivation allows generation of switchable local perturbations within the uniform magnetic field generated with a pair of permanent magnets placed at the opposite edges of the chip. This leads to strong and reversible trapping force, wherein high local magnetic field gradients allow efficient capture of IMNs conjugated to L. monocytogenes flowing through the microfluidic chamber. The experimental optimization of the M-chip was performed using commercially available magnetic microparticles and fabricated silica-coated iron-oxide nanoparticles. The fabricated nanoparticles were optimized to achieve the desired magnetic moment and surface functionalization was tailored to allow efficient capture antibody immobilization. The integration, validation and further optimization of the capture and release protocol is demonstrated using both, dead and live L. monocytogenes through fluorescence microscopy and plate- culture method. The capture efficiency of the chip was found to vary as function of listeria to nanoparticle concentration ratio. The maximum capture efficiency of 30% was obtained and the 24-hour plate-culture method allowed the detection of initial sample concentration of only 16 cfu/ml. The device was also very efficient in concentrating the sample from a 10 ml initial volume. Specifically, 280% concentration efficiency was achieved in 17 minutes only, demonstrating the suitability of the system for food safety applications. In addition, flexible design and low-cost fabrication process will allow rapid sample preparation for applications beyond food and water safety, including point-of-care diagnosis.

Keywords : array of pillars, bacteria isolation, immunomagnetic sample preparation, polymer microfluidic device
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