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Microfluidic platform for pathogen load monitoring

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ABSTRACT

This paper reports on a microfluidic diagnostic platform prototype to be used for pathogen load monitoring which is compact and can be operated by primary care personnel. The platform combines magnetic separation and optical detection in order to rapidly determine the presence of magnetically labelled pathogens and provide additional clinical information (the pathogen load) in a simple, quick and compact manner without flow. One of the methods used to monitor and initiate treatment of several diseases (e.g. HIV or Influenza) is a pathogen (e.g. viral) count of patients. Knowing the number of pathogens per unit volume of blood is essential in order to determine the initiation and dosage of therapeutic medication and thus increase the survival rate. Preliminary measurements to determine the size of magnetic nanoparticles were performed using dark field microscopy and particle tracking.

1. Introduction

Traditional methods currently used to identify pathogens or infection rely upon conventional clinical tests, e.g., chest X-rays and microbiology monitoring approaches that are well established, e.g., blood tests [1,2]. However, these methods suffer from a number of considerable drawbacks especially when intended to be used in resource-limited settings (e.g., developing countries). Standard susceptibility and culture tests allow pathogen detection but are time-consuming, require well equipped laboratories and are expensive [3–5]. Additionally, such routine tests do not directly monitor the viral load. In order for scientists to quickly identify the presence of pathogens (either in food, water or blood) reliable and sensitive tools are needed. Techniques which use microfluidics for pathogen detection are currently available. To the best of our knowledge these techniques are based on flow, membrane filtration and/or fluorescent labeling [6–9]. The biggest disadvantage of such methods is the limited photostability of the fluorophores with time, their narrow excitation range and broad emission spectra of the fluorescent labels.

In this paper we present the concept for a microfluidic diagnostic platform for pathogen load monitoring which is compact and can be operated by primary care personnel. The platform combines magnetic separation and optical detection in order to rapidly detect the presence of magnetically labelled pathogens and provide additional clinical information (the pathogen load) in a simple, quick and compact manner without flow. The optical detection method is based on the observation of the Brownian motion of magnetic nanoparticles (MPs) utilizing dark field microscopy. MPs are used to label the pathogens under investigation and the Brownian motion is affected by the change of dimensions of the MPs when a pathogen binds to their surface. An appropriate algorithm is then used for quantification of Brownian motion characteristics related to the size of the recorded moving particles. The advantage of this method is that the measurements can be repeated without a time limitation since no fluorescent labeling is required and that even a conventional light source can be used for the illumination of the nanoparticles. The presented method also lacks common disadvantages of existing microfluidic systems such as complicated structures (e.g., micropumps) often required for on-chip microfluidic flow. In our microfluidic chip no flow is required thus simplifying its fabrication and reducing its cost. In addition, most of the newly reported microfluidic techniques do not provide information regarding the pathogen load. Our microfluidic platform however, can be used to monitor pathogen load in addition to pathogen identification.

2. Material and methods

2.1. Microfluidics

Our microfluidic platform consists of a separation channel, the detection chamber (chamber A) and the reference chamber (chamber B) as shown in Fig. 1. The platform was fabricated using a Polydimethylsiloxane (PDMS) and standard photolithography process; a process

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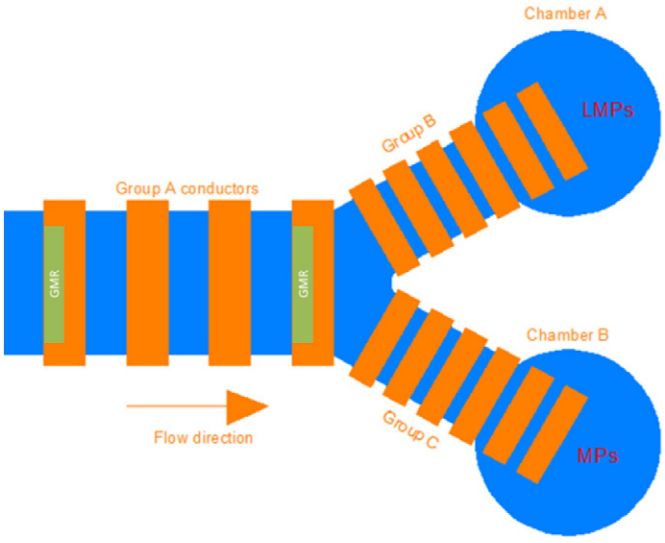

Fig. 1. Schematic of the microfluidic platform; it consists of a filtering (separation) channel (Group A conductors), a detection chamber (chamber A) and a reference chamber (chamber B); two GMR sensors are fabricated underneath the filtering channel for MPs detection (Green structures). Current carrying conductors are lying along the channel acting as magnetic actuators (Orange structures).

described in detail in the literature [10,11]. Fig. 2 shows a photo of the device.

2.2. Magnetic nanoparticles

Magnetic nanoparticles (MPs) functionalized with ligands (e.g. antibodies) of specific affinity to the pathogens to be detected are mixed with the fluid under investigation. If pathogens are present, they will bind to most of the functionalized MPs. The resulting fluid, containing the magnetically labelled pathogens (called loaded magnetic particles - LMPs) and the remaining MPs that did not attach to any pathogens, is inserted in the microfluidic platform. The fundamental idea behind the magnetic separation method is that the induced velocity of MPs in suspension, while imposed to a magnetic field gradient, is inversely proportional to their volume; the volumetric increase of the MPs due to binding of pathogens onto their surface, changes consequently, their velocity [10–13]. Thus, the LMPs need more time to travel the same distance, as compared to plain functionalized MPs (smaller). This way, the LMPs (slower) can be separated from the plain MPs (faster) without flow and simply by being imposed to a magnetic field gradient, generated by integrated current carrying conductors (Group A) as shown in Fig. 1. The faster MPs are directed to the reference chamber by sequentially applying current to the Group C conductors. The slower LMPs are then directed to the detection chamber by sequentially applying current to the Group B conductors.

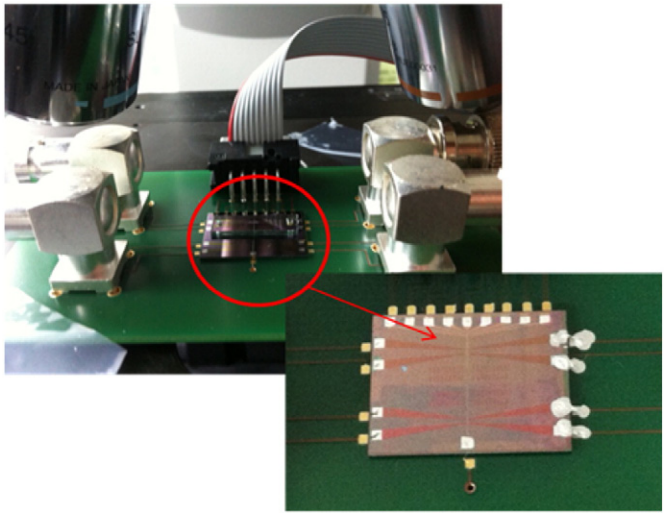

Fig. 2. Photograph of the microfluidic platform prototype.

For the preliminary experiments we used Nanomag-D (250 nm diameter) commercially available MPs, composed of iron oxide nanoparticles and encapsulated into a dextran matrix with protruding amino groups (—NH_2); the MPs can be purchased with different functionalization layers (e.g. antibodies) so that they can be used to label different biological entities (e.g. viruses or microbes).

2.3. Optical quantification and classification

For the pathogen quantification only a conventional optical microscope [14] or custom video microscopy arrangement [15] equipped with a digital camera and a PC are required. Particle tracking programs are widely available and various open source scripts have been described in literature, e.g., ref. [16–18].

The transparent detection and reference chambers are directly illuminated with light in a shallow angle from the same side as the objective, resulting in a reflected dark field arrangement [14]. The objective lens has a long working distance and low numerical aperture to achieve a large focal depth [19]. The height of the chambers is matched to this focal depth and thus continuous observation of all particles over the whole measurement time is possible. The basic dark field microscopy arrangement is shown in Fig. 3. Individual scattering particles appear as bright spots on a dark background, which move randomly due to the Brownian motion. A sequence of time-lapse images is recorded with the camera and analysed with MATLAB. First, the frames are bandpass filtered and particle spots are detected. Then, the centres of the particle spots are determined in every frame and these positions are linked to form trajectories. The mean squared displacement of each trajectory is calculated [20] and fitted in order to obtain the diffusion coefficient D of the respective particle [14,21]. The diameter d is calculated using the well-known Stokes-Einstein relation $d = kT/3\pi\eta D$, with k the Boltzmann constant, T the absolute temperature, and η the viscosity of the medium.

The size of the LMPs in the detection chamber is determined by this tracking procedure and the size of the plain MPs inside the reference chamber is also measured for comparison. An increase in particle diameter would consequently cause reduced diffusive motion, which can be established by particle tracking. This way, the amount of attached pathogens to the surface of the MPs can be calculated from the volumetric increase of the plain MPs.

2.4. Magnetic detection

The platform is relying on the GMR technology to magnetically detect the presence of MPs by detecting the small stray fields produced by the MPs when they pass over the sensor area. During the separation process in the separation channel, two GMR sensors are used to detect the change in velocity of the MPs as described in [11,12]; this step is necessary in order to ensure that the particles entering the detection chamber are only the LMPs (the magnetically labelled pathogens). After recognizing which particles are loaded with pathogens (depending on their velocity change) a set of current carrying conductors (Group B in Fig. 1) will generate the magnetic field gradient needed to move the LMPs to the detection chamber and the rest of the unloaded MPs will be directed to the reference chamber by another set of conductors (Group C) as illustrated in Fig. 1.

To achieve the previously described process, a combination of software and hardware components are used. A microcontroller will trigger the movement of the MPs and LMPs in the platform as well as the operation of the GMRs. Computer software built on LabVIEW in combination