Ferromagnetic Resonance Biochip for Diagnosing Pancreatic Cancer

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ABSTRACT

The objective of the paper is to develop a "lab-on-a-chip" device for early disease (pancreatic cancer) diagnosis by using ferromagnetic resonance (FMR). Magnetic microbeads, which are functionalized for target molecules (antigens), are immobilized by antigen-antibody reactions on the surface of a microwave circuit. These magnetic labels are detected inductively using FMR, which detects a single bead with a sensitivity of 1-10 μV/V. This method has distinctive advantages compared to other conventional immunoassay techniques; it requires a small sample volume, is non-invasive, cost effective, and easy to implement. It also does not alter the native properties of the antigen and antibody complex.

Keywords: biomarker, cancer, microbead, immunoassay

1 INTRODUCTION

Modern medicine's detection of pancreatic cancer comes at a stage when preventative medicine is no longer a viable option. Several methods exist for early stage detection such as tomography and invasive biopsy. These solutions, however, are expensive, dangerous, and/or unreliable [1,2].

A non-invasive method exists by detecting the associated antigen in a blood sample from the patient. Currently several techniques are available to achieve this goal and they can be categorized into two groups: label required and label-free. Label-free methods have several advantages, but are notoriously difficult to implement. There are three label required techniques: radioactive, luminescent, and magnetic [3-10]. They are typically used to detect the target molecules. Luminescent labels, specifically fluorescent or Chemi-luminescence, covalently link to antibodies and function as labels to create a visually detectable signal proportional to the amount of antigen in the sample. This method, however, presents several problems. One of these is the confirmation of the antigen and antibody bonds, where visual detection can be unreliable, bulky, and expensive. The samples used must be large to ensure a detectable signal. Also, this method often alters native properties of the antigens, and puts constraints on the size of the equipment that prevents rapid detection due to transportation of analytes to the test site. Radioactive labels are not suitable for the application due to requirements of the radioactive waste disposal and limited shelf life.

On the other hand, magnetic labels, in the form of micron-sized beads, allow for a cost effective and deterministic alternative toward detecting biomarkers using novel immunoassay principles. The protein sensor device would provide a high-throughput operation, use small sample sizes, be highly transportable, and provide higher process control over interactions, resulting in rapid and accurate detection of diseases. Bead-based detection also has the desirable trait of offering a high surface area to volume ratio for the attachment of the antigens.

Not all magnetic detection methods offer the same benefits as FMR detection. Electromagnetic induction uses a similar "sandwich" assay, measures changes in inductance in its micro-patterned coil, and has the advantage of a rather simple integration with CMOS technology, but suffers from a lack of sensitivity [11, 12]. Similarly, the low sensitivity of devices based on Anisotropic Magnetoresistance (AMR) outweighs the simplicity of its fabrication [13]. AMR, like Tunneling Magnetoresistance (TMR) and Giant Magnetoresistance (GMR) have risen in popularity of late due to their thin-film design, which is conducive to Integrated Circuit (IC) integration. All three technologies detect the presence or absence of beads, similarly, by changes in the resistance of the sensor based on fluctuations in the magnetic field caused by the immobilized beads. While they may integrate well and have sufficient sensitivity, the fabrication of the multiple layers of films is more costly and complicated than the processes already developed for FMR detection. In addition, TMR sensors lose their sensitivity as the functional area is increased [14-18].

Ferromagnetic resonance (FMR) detection is characterized by its high sensitivity and simple, inexpensive fabrication [19]. The functionalized beads are commercially available and become immobilized when they flow over an antibody-activated sensor. Detection with FMR uses frequencies in the gigahertz range and at resonance; the effective permittivity is increased in the magnetic material. Leveraging both of these observations increases the signal strength associated with the detection event. Our aim is to use the principle of ferromagnetic resonance in the detection of magnetically labeled pancreatic cancer biomarkers in order to offer an alternative diagnostic solution that lowers the cost, and increases the sensitivity, specificity, portability, and reliability of the test through simpler sample processing and the low cost of integrated circuit manufacturing technology.

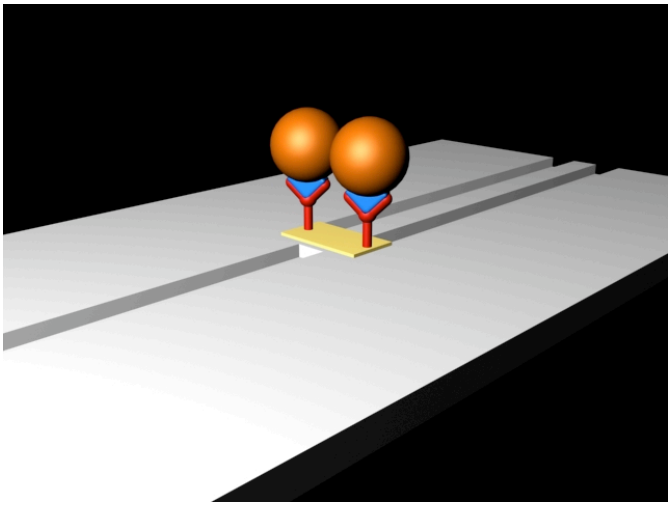

Fig. 1: Representation of beads immobilized on functional area of FMR device.

2 MATERIALS & METHODS

The substrate used for experimentation was a gold sputtered silicon wafer. Streptavidin and biotin solutions were prepared using concentrated solutions from Vector Labs®. Two drops of each added to 0.5 ml of 1X Phosphate Buffered Saline (PBS) in individual eppendorf tubes.

2.1 Characterization of Beads

In order for FMR detection to be possible, there are certain magnetic and compositional properties that must be met, namely that the magnetic particles possess a magnetic susceptibility of at least 1.6 and a ferrite content of at least 27% by weight. We chose the Dynabeads® M-450 Epoxy produced by Invitrogen®, which are uniform, super-paramagnetic polystyrene beads with a diameter of 4.5 µm. The 4.5 µm beads have a surface area of 6.287e-11[m^2] and a volume of 4.666e-17[m^3]. While the magnetic properties of the beads are required for the FMR detection, other properties, such as their diameter were chosen due to product availability.

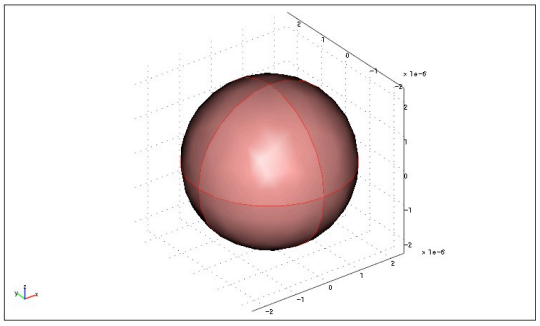

Fig 2: Simulation of 4.5µm diameter bead

2.2 Surface coating and functionalization

The setup for the experiment entailed the independent functionalization of the gold sensing area and the magnetic beads in order for the immobilization of the beads on the surface to occur. The gold substrate was saturated with 50 µl of a streptavidin solution for 30 minutes at room temperature, allowing for physi-chemical adsorption to the surface. Similarly, a 1 µl solution of Dynabeads M-450 in distilled water with a bead density of 4 x 10^8 beads/ml was added to a 50 µl solution of saturated biotin and then agitated for 15 seconds using a vortex in order to re-suspend the particles. The solution was then allowed to incubate at room temperature for 30 minutes, before the beads were introduced to the streptavidin-coated surface. The streptavidin-biotin coupling was left undisturbed for an additional 30 minutes in order to ensure covalent bonding between the proteins before a washing step was conducted with 50 µl of 1X PBS solution. Excess PBS wash was removed using chemical wipes.

For the control experiments the 50 µl of saturated biotin was replaced with 50 µl of 1X PBS solution. The 1X PBS solution was chosen in order to demonstrate that a lack of specific ligand coupling would preclude the immobilization of magnetic particles to the gold surface.

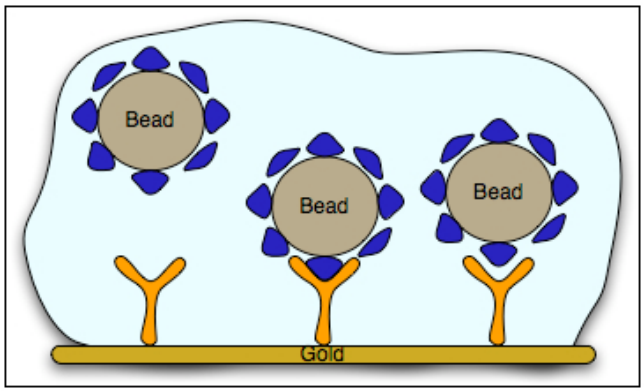

Fig. 3: Symbolic representation of bead immobilization through the coupling of streptavidin, orange, and biotin, blue.

3 RESULTS

The experimental results were quantified using optical detection of the presence of magnetic particles on the surface of the gold substrate after the washing step had concluded. A representative viewing area was chosen and the number of beads present counted and recorded. The results were as expected, the functionalization of the magnetic beads with biotin enhanced their immobilization on the surface, purportedly due to the coupling with the streptavidin saturating the chip. On average, trials with biotin-coated beads resulted in 70 beads remaining in a viewing window at 40x magnification, whereas the control experiments, using naked beads, averaged about 30 beads in the same viewing space. The results are illustrated in Fig. 4(a) and 4(b). A green filter in the microscope was used to improve image clarity.

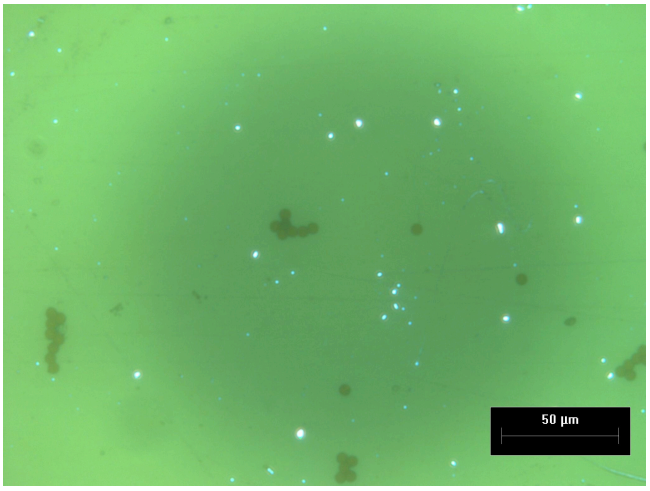

Fig. 4(a): Control Trial: Optical micrograph at 40x magnification of naked beads immobilized on gold surface.

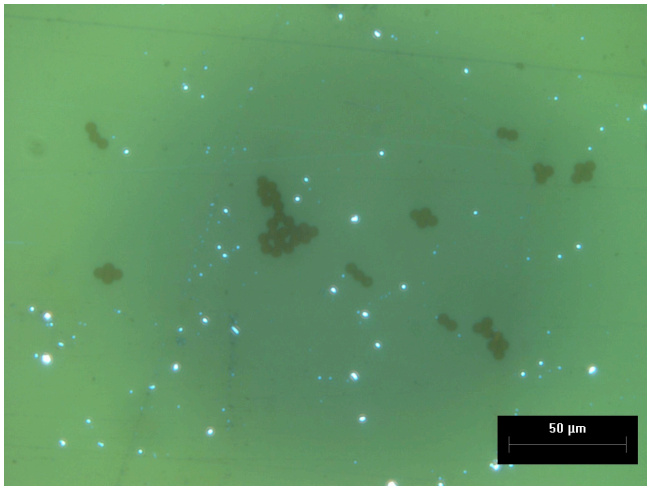

Fig. 4(b): Experimental Trial: Optical micrograph at 40x magnification of biotin-coated beads immobilized on gold surface.

4 DISCUSSION

Our results are demonstrative of the concept that magnetic particles can be immobilized with the current protein chemistries and can presumably be quantified using FMR detection. Beads with alternate surface reactive groups are being explored in order to reduce the incubation period necessary for the covalent coupling of the cancer protein biomarker, versus the current physi-chemical adsorption, to the bead's surface.

Currently, only optical techniques are being employed to quantify the results, which are not as precise, nor quick as the proposed magnetic detection scheme. Trials need to be conducted that incorporate FMR detection on samples that contain beads immobilized by ligands.

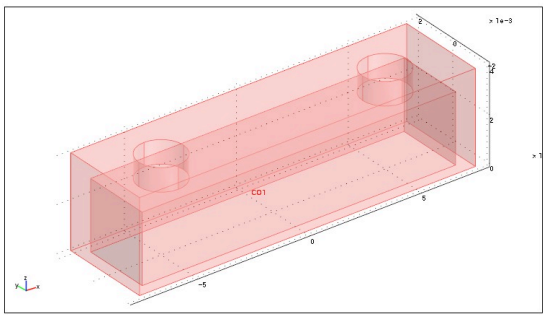

Fig. 5: Model of proposed microfluidic device, simulated dimensions are - exterior: 15x4x4 mm, thickness: 0.5 mm, & channel diameter: 2 mm.

We intend to incorporate a microfluidics device in order to optimize the wash steps and to ensure precise, localized fluid placement onto the functional area of the device. The microfluidic device is a rectangular-parallelepiped-shaped with an inlet and outlet for the deposit and removal of solutions during trials, such as the one shown in Fig. 5. The design of the proposed device is currently being evaluated through the development of a Comsol Multiphysics® model in order to optimize fluid flow and the deposition of magnetic material.

We are applying the incompressible Navier-Stokes equations to simulate Laminar flow of the solutions. The microfluidic device must be small enough to prevent turbulent flow, but big enough to cover the functionalized area (50µm x 100µm), and long enough to apply relatively consistent horizontal force on the functionalized area.

Lastly, the focus of future experimental trials will be to optimize the protein concentration needed to meet the goal of minimizing the potential patient sample volume. This will be accomplished by using a proven, reproducible protocol over a range of antigen concentrations. The expectation is that there will be a minimum concentration necessary to reliably immobilize a sufficient number of beads, for FMR detection, to the antigen-binding site of the biotinylated antibody present on the surface.

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