

NANOASSEMBLY OF GRAPHENE OXIDE FOR CIRCULATING TUMOR CELL ISOLATION

H.J. Yoon, K. Lee, Z. Zhang, T.M. Pham and S. Nagrath*
Department of Chemical Engineering, University of Michigan, USA

ABSTRACT

Integrating nanotechnology with established cancer research provides extraordinary opportunities to find essential breakthroughs in the fight against cancer. Nanowires and nanotubes are among the leading approaches under development for biological applications. Till now, the nanodevices face a key challenge to develop surfaces and structures that can be chemically modified for specific conjugation of proteins with limited non-specific adsorption. Here we demonstrate a microfluidic device with graphene oxide (GO) for selective and sensitive detection of circulating tumor cells aimed at early diagnosis of cancer. The functionalized GO can self-assemble on patterned gold surfaces via electrostatic interactions between functional groups on GO and gold surfaces.

KEYWORDS: Graphene oxide, Nanoassembly, Circulating tumor cells, Cancer detection, Cell capture

INTRODUCTION

Technological obstacles in isolation and capture of circulating tumor cells (CTCs) from blood continue to stymie the progress in quantitating CTCs for early diagnosis and effective monitoring of therapeutic response in patients. CTCs are extremely rare cells (about 1 tumor cell per 1 billion blood cells) that are known to circulate in the peripheral blood of patients with metastatic cancer. Previously, Nagrath et al. demonstrated the efficient and reproducible microfluidic chip, 'the CTC chip' to isolate CTCs from the blood of metastatic patients [1]. CTC-Chip has demonstrated high clinical utility for detecting genetic mutations in advanced lung cancer patients [2]. Since in early stage patients, it is expected not only CTCs could be lower frequency but also EpCAM expression and other cancer related biomarker expression could be heterogeneous. Hence, the CTC isolation technology needs to be more sensitive and specific for detecting CTCs in early stage cancer patients.

Graphene has attracted much attention in recent times because of its unique properties and potential applications in a variety of fields. Graphene is suitable for physical devices like electronic devices and sensors, whereas, GO is a promising material for the biological application. It has very high specific surface area and is highly amenable to functionalization and self-assembly [3-5]. In the literature, there are only two reports about the assembly of GO on metal surfaces [4-5]. But none of them showed the application of nanoassembly technique for live cells. Here we have fabricated functionalized GO nanoassemblies on the patterned gold layer through a microfluidic silicon device as an effective tool to isolate, capture, identify and characterize extremely rare CTCs aimed at the early diagnosis of metastatic disease.

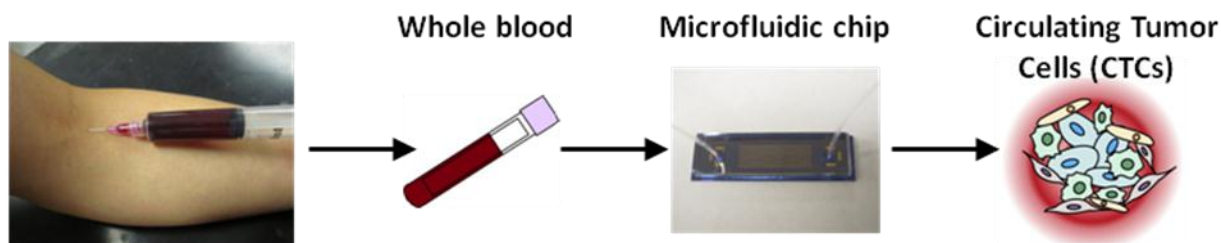


Figure 1: Overview of one-step integrated nano and microfluidic technology for point-of-care isolation of CTCs from peripheral blood [1].

EXPERIMENTAL

Figure 2 shows the schematic diagram of the integrated nano microfluidic device for capturing circulating tumor cells on the functionalized GO/gold posts. The single layer graphene oxide (SLGO) powder was used as a starting material. The product of SLGO was followed by a modified hummer's method [6]. The colloidal suspension of GO from SLGO was prepared by tip sonication in water. Tetrabutylammonium (TBA) hydroxide was added for intercalation and complete exfoliation of GO. To functionalize GO, polyethylene-glycol (PEG) was added and the mixture was bath sonicated. After flowing the GO suspension through the microfluidic device, antibody against surface proteins on the circulating tumor cells, namely epithelial cell adhesion molecule (EpCAM) is immobilized on the PEG-functionalized GO.

The microfluidic device consists of a PDMS layer with a microchannel and a silicon substrate with gold nanoposts. The PDMS structure is fabricated using standard SU-8 mold. Gold nanoposts on a silicon substrate are fabricated by evaporating and patterning Cr/Au layers (10/100 nm). The silicon substrate and the PDMS layer are bonded by oxygen plasma treatment. Figure 3 shows a photograph of the microfluidic device. The diameter of gold patterns in the microchannel is 20 μm , and the overall size of the microfluidic device is 35 mm \times 10 mm \times 3 mm.

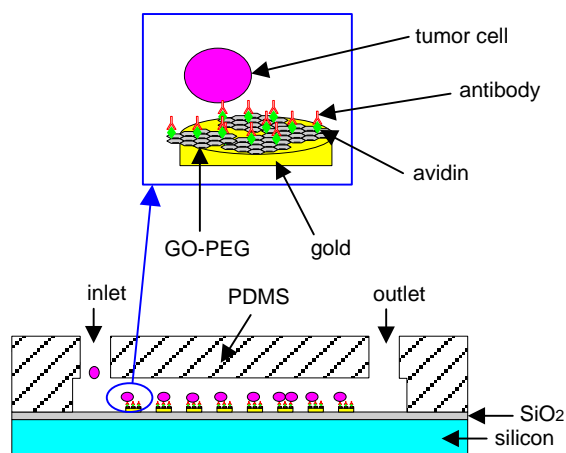


Figure 2: Schematic diagram of the microfluidic device and captured tumor cells on the functionalized GO/gold nanoposts.

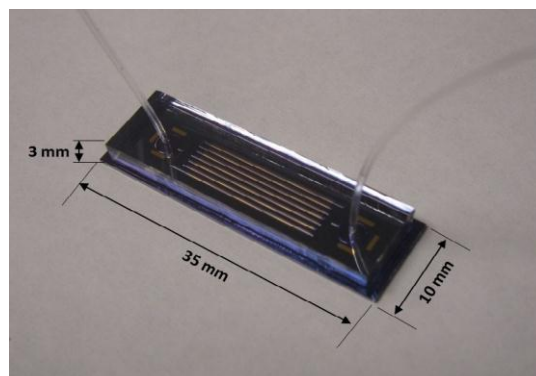


Figure 3: Photograph of the microfluidic device.

RESULTS AND DISCUSSION

SEM images in Figure 4(a) reveal that gold nanoposts are covered with functionalized GOs. By self-assembly, GOs were directed to bridge arrays of gold electrodes on the substrate, allowing large numbers of on-substrate GO devices. This will help remove the coatings on GO, increasing the electrical conductance of the GO device which is called an “electrical annealing” method. As a result, the functionalized GOs are attached to the sides of the posts and as well as to the top of the posts. It shows the high selectivity of GO deposition on gold rather than onto the SiO₂ substrate and the uniform assembly and saturation density of GO on the gold surface. We used atomic force microscopy (AFM) to characterize the materials deposited on the substrates from the supernatant and observed most of the GOs were SLGO of various shapes and sizes. For the hundreds of GO measured, we found that average size of SLGO was around hundreds nanometer and the topographic height was about 1 to 1.5 nm as shown in Figure 4(b).

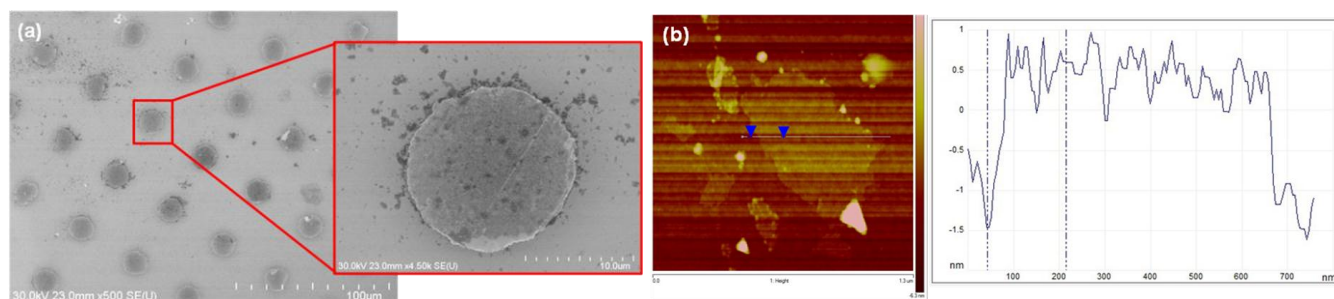


Figure 4: Self-assembled GO-PEG on gold nanoposts: (a) SEM images; (b) AFM images.

The PEG-GO surfaces are further modified with NeutrAvidin to immobilize the biotinylated epithelial-cell adhesion molecule antibody (anti-EpCAM). EpCAM is a transmembrane glycoprotein that is frequently overexpressed in a variety of solid-tumor cells and is absent from hematologic cells. To confirm the active functionalized surface, we used fluorescently labeled NeutrAvidin. As seen in the Figure 5(a), the gold nanoposts with modified GO specifically showed the presence of fluorescence, whereas the negative controls without NeutrAvidin showed no fluorescence at the same exposure, indicating high sensitivity and specificity, which is expected to exhibit high cell capture efficiency when employed to isolate viable cancer cells from whole blood samples. To further test the capture of real cancer cells, fluorescently labeled breast cancer cells (MCF-7, ATCC) were spiked into buffer and captured on the GO assemblies. Figure 5(b) presents captured MCF-7 cells on gold nanoposts. These results show the feasibility of that GO can be an effective nanomaterial biological application, especially for isolation and capture of circulating tumor cells. Most importantly, the high surface-to-volume ratio of the GO

on gold can generate 3D electrical surfaces that significantly enhance detection limits, highly reproducible detection of clinically important cancer markers.

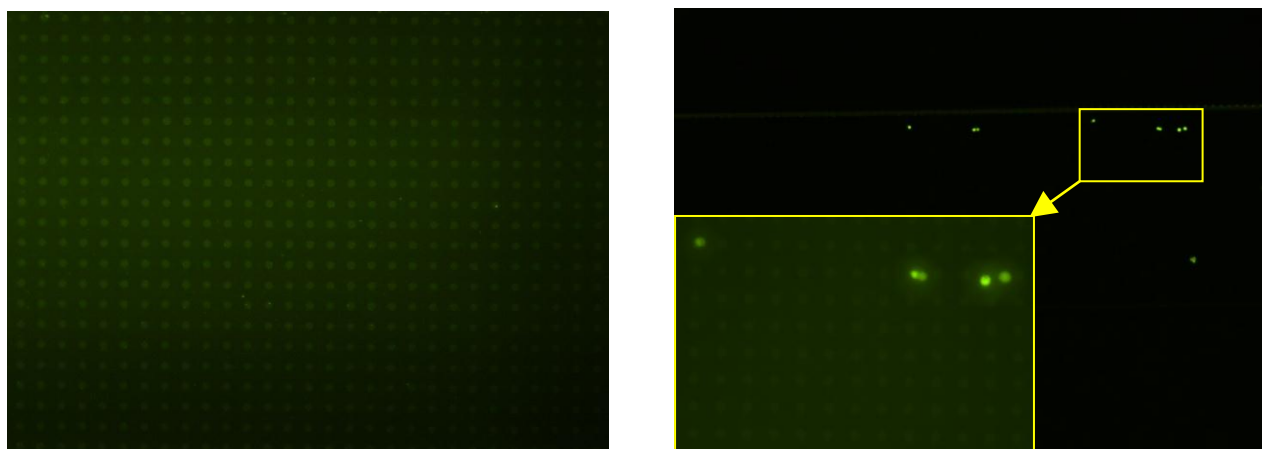


Figure 5: Fluorescence microscopy images; (a) fluorescently labeled NeutrAvidin on gold posts; (b) Captured MCF-7 cells.

CONCLUSION

We have demonstrated an integrated nano microfluidic device with functionalized GO/gold as an orthogonal integrated translational approach using bioengineering tools to CTCs using breast cancer cell line as a model system. The approach utilizes self assembly of GO in a unique way for enhanced sensitivity and specificity of detection tool. Graphene oxide has attracted significant attention due to its unique properties, such as higher 2D and 3D electrical conductivity, large surface area, superb mechanical flexibility, and high chemical and thermal stability. This chemically derived and noncovalently functionalized GO has the ability to overcome the limitations of carbon nanotubes (CNTs) such as variations in electrical properties of CNT-based devices and the limited surface area of CNTs. Till now, very limited efforts have been made to explore their potential uses against cancer. It is critical to develop nanoscopic graphene-based biosensors that are simple in device structure and small in size to detect key biomarker proteins for cancer.

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CONTACT

*S. Nagrath, tel: +1-734-647-7985; snagrath@umich.edu