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Label-free optical monitoring of surface adhesion of extracellular vesicles by grating coupled interferometry

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ABSTRACT

In this proof-of-principle study a label-free optical sensor is demonstrated to monitor the surface adhesion of extracellular vesicles secreted by live cells on to various extracellular matrix proteins.

1. Introduction

Sensitive monitoring of the presence and interactions of biological objects at the nanometer length scale is increasingly important for both basic research and industrial applications. Following refractive index variations caused by the biological object itself is an emerging strategy to create optical sensors where any further labeling of biomolecules or living cells is unnecessary. This approach may open the way for a natural, reliable and cost-effective investigation of nanosized structures [1].

Most of the label-free optical sensors are exploiting a surface bound *evanescent wave* to follow the refractive index variations [2]. The evanescent wave is localized in the close vicinity of the sensor surface, making it ideal for in situ monitoring of surface adhesion processes. A relatively well-known evanescent wave sensor is the surface plasmon resonance (SPR) using a gold film. However, planar optical waveguides offer higher sensitivities [3,4]. The typical depth of penetration for a waveguide mode at optical frequencies is 80–300 nm. In contrast to SPR, using waveguides the penetration depth can be easily tuned through waveguide design, matching in this way the sensing depth to the size of the adsorbed object [5–7].

An emerging area where the label-free surface sensitive sensors could be exploited is the interaction of extracellular vesicles (EVs) with model surfaces, biomolecules, polymers and receptors. Extracellular vesicles are cell-derived membrane surrounded structures of various sizes. The two most extensively characterized types of EVs are the exosomes (50–100 nm vesicles generated by the exocytosis of multivesicular bodies) and microvesicles (MVs, plasma membrane-derived 100–1000 nm in diameter, often also referred to as shedding microvesicles, microparticles or ectosomes). The field of EVs represents one of the most rapidly emerging research areas in current biomedicine. These membrane bound structures are recently recognized conveyors of intercellular communication mediated either by membrane proteins or by their mRNA and microRNA cargo [8,9]. The size range of EVs does not match that of the detection of instruments used in conventional cell biology and clinical diagnostic laboratories. Thus, there is a sudden demand and an intensive methodology development worldwide to meet these unmet needs. The evanescent field-based highly sensitive sensors may provide an exciting detection system for EVs.

We have recently introduced grating coupled interferometry (GCI), a label-free evanescent-field based optical sensing concept

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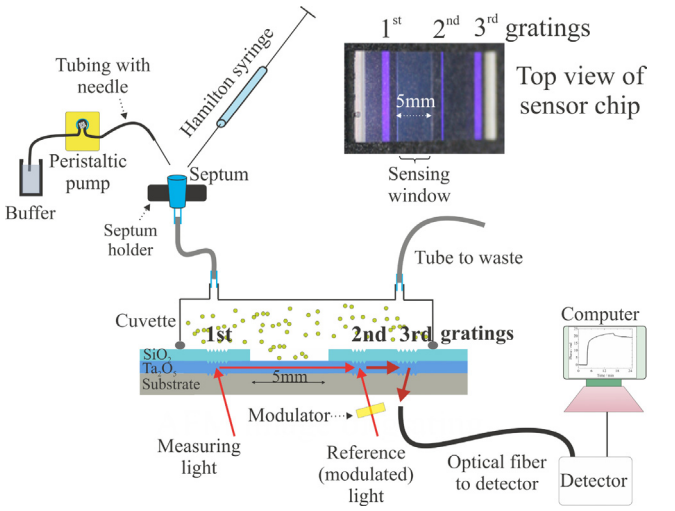

Fig. 1. Schematic cross-sectional illustration of the GCI sensor arrangement. The cuvette positioned above the optical waveguide sensor is equipped with a septum injection port for sample introduction using a Hamilton syringe. The washing off the irreversibly bound vesicles from the sensor by pure buffer is also done through the septum using a peristaltic pump equipped with needle ended tubing. The top view photograph of the GCI sensor chip is also shown. The two incoupling and the single outcoupling grating, as well as the 5 mm long sensing window, are clearly visible.

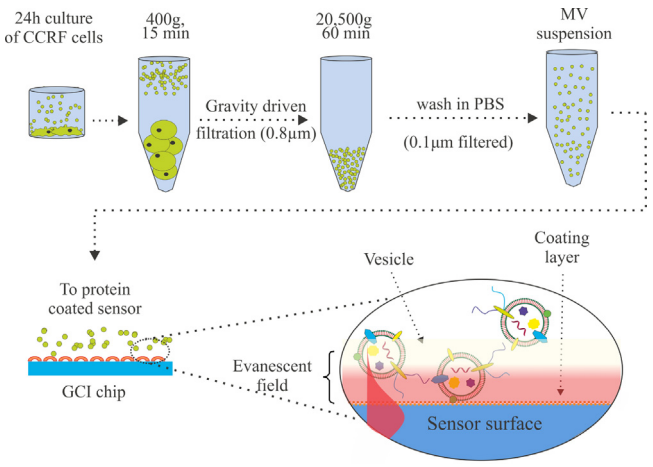

Fig. 2. Steps of isolation of MVs that were analyzed for their adhesion to the protein pre-coated GCI sensor surface. The surface adsorbed microvesicles are detected by the evanescent field of the propagating waveguide mode.

[10–12]. The sensing strategy combines cost-effectiveness, simplicity and reliability of grating-coupled planar optical waveguides with the high resolution of interferometric measurements. The heart of the GCI technology is a high quality planar optical waveguide shown in Fig. 1. In order to fabricate the sensor chip, 3 gratings are etched into a glass support, and the glass is coated with a Ta_2O_5 waveguide film of 155 nm thickness. Next, a relatively thick layer of SiO_2 is used to coat the entire waveguide surface, except for a 5 mm long aperture (see Fig. 1). This aperture serves as a sensing window of the device where the evanescent field of the waveguide mode can interact with the surface-adsorbed analytes. The present waveguide design with TE polarization gives a penetration depth of 80 nm into the cover media. The bulk refractive index sensitivity is 0.072, and the final refractive index resolution is approximately 10^{-7}.

In this work we demonstrate the use of GCI in the field of EV research. The biological question we addressed was whether MVs, secreted by the human CCRF-CEM T cell line (ECACC), bind to components of the extracellular matrix. As yet, there is very limited data available on the adhesion of EVs to matrix proteins [13–15]. Several types of EVs carry adhesion molecules on their surface (for a meta-analysis see Ref. [16]). Most interestingly, the presence of integrins and proteins involved in integrin-mediated signaling are characteristic for the surface of MVs [16]. Therefore we hypothesized that EVs bind to extracellular matrix molecules (such as type I collagen or fibronectin), enabling them to participate in tissue regeneration and repair, cell migration, inflammation and blood clotting.

2. Materials and methods

The methodology developed in the present work to monitor the interaction of MVs with matrix proteins is summarized schematically in Fig. 2. One million CCRF-CEM cells/mL were cultured in serum-free RPMI for 24 h. The serum-free culture condition did not induce a significant apoptosis as confirmed by the absence of annexin V-FITC binding using flow cytometry (data not shown). The 24 h conditioned medium was pelleted at 400 × *g* for 15 min to remove cells, and the supernatant was filtered through a 800 nm pore-sized filter (Millipore, Billerica, CA) by gravity. The filtered supernatant was centrifuged at 20,500 × *g* for 60 min to pellet MVs, as described previously [17–21]. MVs were washed once in PBS filtered through a 0.1 μm pore-size filter. Protein concentration of the MV preparations was measured by Micro BCA Protein Assay Kit (Pierce Biotechnology, Inc.). The average protein concentration in the samples used for GCI analysis was 15 μg/mL. Samples were stored at −80 °C until use, but for not longer than 2 weeks. Finally, the GCI sensor surface, coated with the biological molecule of interest, was exposed to the MV-containing samples.

To investigate the quality of the vesicle solution, shortly after centrifugation the supernatants were carefully removed, and the pellets were fixed at RT for 60 min with 4% PFA in 0.01 M PBS. After washing with PBS, the preparations were post-fixed in 1% OsO_4 (Taab, Aldermaston, UK) for 30 min. Following rinsing with distilled water, the pellets were dehydrated in graded ethanol, including block-staining with 1% uranyl-acetate in 50% ethanol for 30 min, and embedded in Taab 812 (Taab). After overnight polymerization at 60 °C and sectioning for EM, the ultrathin sections were analyzed with a HITACHI 7100 electron microscope equipped by Megaview II (lower resolution, Soft Imaging System) digital camera. Fig. 3(a) demonstrates the excellent quality of the MV preparation. The size distribution of MVs was further analyzed using a scanning ion occlusion sensing approach (qNano, Izon Science Ltd., Christchurch, New Zealand) [22].

Samples were counted for 5 min using 7.15 mbar pressure. Voltage was set in between 0.1 and 0.25 V in order to achieve a stable 100 nA current. Particle size histograms were recorded when RMS noise was below 12 pA, and particle rate in time was linear. Using the 200 and 400 nm pore membranes successively, most of the MVs were around 200–300 nm, but a smaller fraction of vesicles with larger diameters was also present (see Fig. 3(b)). This size range is in accordance with EM images in this study and previous findings [17,23].

3. Results and discussions

In order to monitor MV adsorption on to the GCI sensor, the cleaned sensor chip was first placed into an optical arrangement shown schematically in Fig. 1. Here, two beams of a linearly polarized He–Ne laser illuminate the 1st and 2nd gratings, which couple the waves into the planar optical waveguide. The second beam is phase modulated using a Liquid Crystal Modulator (LCM), creating a time-dependent interference signal after the 2nd grating. This signal is outcoupled at the 3rd grating, and picked up by a fiber-coupled optical detector. The interference signal is analyzed by a personal computer, which calculates the phase shift the waveguide