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Novel QCM-based method to predict in vivo behaviour of nanoparticles

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Abstract

In biological fluids, proteins and other biomolecules bind to the surface of nanoparticles to form a coating known as the protein corona which in turn becomes primary determinant of the nanoparticles' fate and behaviour. Here we develop a QCM-based platform and methodology to obtain data from real-time interactions of nanoparticles with selected human plasma proteins. Polystyrene particles coated with transferrin are immobilized on QCM sensor chips and by means of a 'sandwich' format binding assay, specific epitopes on the particles can be quantified as measured by the increase of the sensor's resonant frequency. Cell binding experiments where adherent cells are directly grown on the sensor surface are also performed. Interaction of nanoparticles injected over the cell surface is observed only in the case of particle-transferrin complexes demonstrating that it is the nanoparticle-corona complex, rather than the native nanoparticle, "what the cell sees", with the corona being the interface between the nanoparticle and the cellular system. Our data highlight the potential of the proposed QCM-based platform and methodology for characterization of the bio-nano-interface and tracking the interaction of nanoparticles with biological cells in the presence of a realistic milieu.

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Keywords: QCM; nanoparticles; protein corona, biochemical assays; cell-based assays; interactions

1. Introduction

Increased understanding of nanoparticles' (NPs) behaviour in vivo is necessary to recognise their impact on humans and ecosystems. While their physicochemical properties can be characterized accurately under idealized conditions, this is not the case in complex biological milieu where biomolecules rapidly bind to NPs forming a well-

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organised corona. This corona also explains why many nanoparticle targeting strategies which seem promising when tested on cells under non-physiologically relevant conditions, fail when tested in vivo, where multiple competitive interactions occur, and where non-specific binding in many cases blocks the targeting functionality of the nanoparticles, rendering them inactive. So far, coupling of nanoparticles with various QCM-based platforms has been applied in a few examples mainly looking at nanoparticle amplification of binding events between antibody – antigen [1] or nanoparticle molecular recognition on immobilised surfaces [2,3]. Some have also demonstrated the growing potential of QCM in investigating molecular recognition on supported lipid bilayer membranes [4]. However, the proposed approach is novel in the sense it can return kinetic data from real time interactions and it provides the ability to grow cells directly on the sensor surface allowing direct assessment of nanomaterial-receptor interactions in realistic milieu.

Nomenclature

NP	nanoparticle
QCM	quartz crystal microbalance
Tf	holo-transferrin (transferrin)
TfR	transferrin receptor
PBS	phosphate buffered saline
HEK	human embryonic kidney

2. Biochemical studies

To analyse kinetics of protein adsorption on nanoparticle surfaces, several different methods to immobilize various types of nanoparticles on sensor surfaces were explored, providing in that way the possibility to test a range of nanoparticles with different physicochemical properties. In this study, polystyrene nanoparticles with and without surface carboxyl groups, 200 nm and 100 nm of diameter respectively, were coated with human holo-transferrin (Tf) by physical adsorption. Holo-transferrin is an abundant human plasma glycoprotein, which transports iron and binds to the Tf-receptor (TfR). Tumour cells generally overexpress the TfR since they have higher demand for iron, thus Tf is largely used to functionalise nanoparticles due to its potential to target cancer cells [5,6]. After formation of the NP-Tf complexes, the surface of a QCM chip was functionalized with anti-transferrin monoclonal antibodies via carbodiimide chemistry. Through antibody-antigen interactions, the polystyrene nanoparticles were successfully immobilised on the chip surface and binding assays were performed by subsequent injection of the same anti-transferrin monoclonal antibodies over the immobilized NPs. This type of 'sandwich' format binding assay allowed us to quantify the number of epitopes exposed on the surface of the particles. These results were validated with previous results obtained for the same model system by titrating immunogold labels against the transferrin-coated NPs and observing the change in size of the complex by differential centrifugal sedimentation [7].

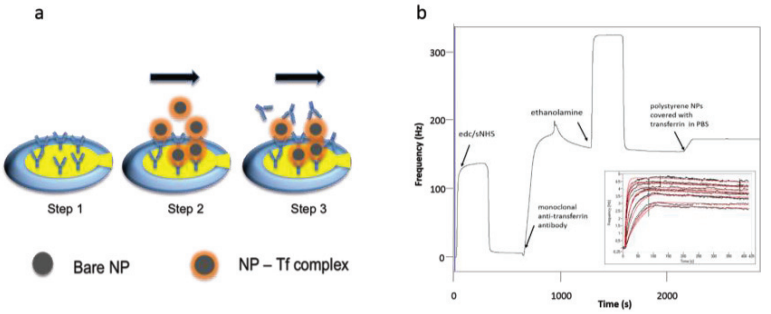

Fig. 1 (a) Schematic illustration of a QCM biochemical assay in a 'sandwich' format. Surface is functionalised with monoclonal anti-transferrin antibodies (step 1), followed by injection of NPs-Tf complexes (step 2). An injection of the same monoclonal antibodies follows directly after immobilization of the complexes (step 3); (b) Sensorgram showing the change in frequency obtained during each step of the biochemical assay. In the inset, representative sensorgrams for both biochemical and cell based assays showing the interaction between NPs and the respective sensor surface.