NanoImpact 1 (2016) 55–59

Contents lists available at ScienceDirect

NanoImpact

journal homepage: www.journals.elsevier.com/nanoimpact

Real-time toxicity testing of silver nanoparticles to *Salmonella* Enteritidis using surface plasmon resonance imaging: A proof of concept

Florian Mallevre [a], Vincent Templier [b,c,d], Raphael Mathey [b,c,d], Loic Leroy [b,c,d], Yoann Roupioz [b,c,d], Teresa F. Fernandes [a], Thomas J. Aspray [a,*], Thierry Livache [b,c,d]

[a] School of Life Sciences, NanoSafety Research Group, Heriot-Watt University, Edinburgh EH14 4AS, United Kingdom
[b] Univ. Grenoble Alpes, INAC-SPRAM, F-38000 Grenoble, France
[c] CEA, INAC-SPRAM, F-38000 Grenoble, France
[d] CNRS, INAC-SPRAM, F-38000 Grenoble, France

ARTICLE INFO

Article history:
Received 25 November 2015
Received in revised form 15 February 2016
Accepted 17 February 2016
Available online 28 March 2016

Keywords:
Surface plasmon resonance imaging
Salmonella
Nanoparticle
Silver
Ecotoxicology
Biosensor

ABSTRACT

In this paper we report for the first time on the suitability of surface plasmon resonance imaging (SPRi) for performing ecotoxicity testing of nanoparticles (NPs). Specifically, the impact of silver NPs (using Ag NM-300K) and ions (using $AgNO_3$ salt) on *Salmonella* Enteritidis growth was assessed in Luria Bertani medium using the culture-capture-measure (CCM) based SPRi method. Clear effects were observed at 10 mg L^{-1} Ag NPs characterised by shifted SPRi detection times (T_D) by ca. 2.6 h compared to the control. Comparable results were obtained using 1 mg L^{-1} Ag ions. No clear effects were observed at 1 mg L^{-1} Ag NPs and 0.1 mg L^{-1} Ag ions. Overall results match the current trend in nanoecotoxicology using bacteria (e.g. impact of Ag NPs between 1 and 10 mg L^{-1} and higher toxicity of Ag ions compared to Ag NPs). The dose dependent patterns of toxicity were coherent with those obtained using a standard plating method; however, the SPRi approach was faster (i.e. results within a few hours) and generated kinetic data (i.e. real-time monitoring). In addition, SPRi presents many valuable intrinsic advantages (e.g. label-free, multiplex, bespoke and robust) over current approaches. Consequently, a plethora of opportunities for future developments and applications of SPRi in NP testing is associated with the proof of concept reported herein.

1. Introduction

The risk assessment of engineered nanoparticles (NPs) is essential in supporting the development of nanotechnologies (Krug, 2014; Seal and Karn, 2014). Nanoecotoxicology is quickly growing but the toxicity testing of NPs remains overall a challenging task (Garner and Keller, 2014; Juganson et al., 2015). Although bacteria are widely used as model organisms (Holden et al., 2014; Juganson et al., 2015), methods based on plating and spectrophotometry have clear limitations. Plating is long to perform and lacks accuracy (Pan et al., 2014) while spectrophotometry is rarely suitable for analyses in complex and/or coloured matrices (Oostingh et al., 2011). Approaches exploiting the advantages of luminescent genetically modified bioreporters (GMB) have emerged (Deryabin et al., 2012; Li et al., 2013; Mallevre et al., 2014; Mallevre et al., 2016a) offering additional perspectives, but regulations and concerns on applicability of GMB limit their broad use and relevance (Weimer, 2010).

Surface plasmon resonance imaging (SPRi) is a biosensor technology suitable for biomolecular interaction assessment (Kodoyianni, 2011) and analyte detection (Abadian et al., 2014a) using a microarray format. SPRi has proven to be robust over the years via the emergence of various instruments, dedicated studies and companies worldwide (Hill, 2015; Nguyen et al., 2015; Rich and Myszka, 2011). Due to an appealing portfolio of advantages (e.g. real-time, label-free, rapid, multiplex, GMB-free, using small volumes and generating little waste) and on-going refinements (e.g. increasingly operator friendly and compatible with various types of biological materials, matrices and configurations), applications of SPRi are expanding. SPRi applications with bacteria have emerged recently (Abadian et al., 2014b; Bouguelia et al., 2013; Bulard et al., 2015; Mondani et al., 2014; Mondani et al., 2016). The notion of real-time monitoring of the bacterial growth by SPRi was first reported by Bouguelia et al. (2013) proposing a culture-capture-measure (CCM) method using the advantages of specific interactions between monoclonal antibodies microarrays and bacteria (e.g. *Salmonella enterica*, *Escherichia coli*). Additional applications with various bacteria followed using the same method (Bulard et al., 2015; Mondani et al., 2014; Mondani et al., 2016). Concomitantly, Abadian et al. (2014b) reported the use of SPRi for the monitoring of *E. coli* and *Pseudomonas aeruginosa* biofilm attachment, formation and removal.

From a technical viewpoint (Hill, 2015; Nguyen et al., 2015), a surface plasmon is an electro-magnetic wave propagating along the surface of a thin di-electric metal film (e.g. gold). The resonance of the surface

* Corresponding author.
E-mail addresses: T.J.Aspray@hw.ac.uk, thomasaspray@gmail.com (T.J. Aspray).

http://dx.doi.org/10.1016/j.impact.2016.02.004
2452-0748/

plasmons can be achieved by a polarised light beam undergoing total internal reflection at the film and medium interface. The resonance state is highly sensitive to changes (e.g. stresses, interactions) in the medium adjacent to the surface and modifications at this level can be quantified in terms of variation of reflectivity via the monitoring of the reflected light intensity. As such, SPRi is simply the characterisation of the SPR signals on the whole surface of a biochip (e.g. a gold coated prism) using a video camera (Fig. 1A). The CCM based SPRi method per se uses biochips functionalised with a bespoke series of specific and non-specific antibody bearing spots. In this particular case, the surface plasmon alteration at the spot level is generated by occurring interactions bacteria-antibodies (Fig. 1B). As originally demonstrated (Bouguelia et al., 2013), the method is specific (i.e. a selected bacterial model will interact first and mainly with its specific antibody) and quantitative to some extent (i.e. the higher the number of cells, the larger the surface interactions and therefore the corresponding output signal). Consequently, by monitoring the variation in reflectivity over time one can derive the growth curve of one or several selected models in a microarray format. Using independent chambers (i.e. four herein, Fig. 1C) one biochip can be exposed to different conditions of growth (or different exposure conditions) at the same time.

The use of SPRi with bacteria has been initiated in ecotoxicology for the testing of antibiotics (Abadian et al., 2014b) and for the impact assessment of thermal stresses (Mondani et al., 2014). However, no evidence of workability in nanoecotoxicology has yet been reported. In light of this, we aimed to extend the CCM based SPRi method to nanoecotoxicological studies to assess its suitability and potentially to propose new options for performing toxicity testing of NPs and related ions when using bacteria as model organisms.

2. Experimental procedure

2.1. Model bacterium

S. enterica subspecies *enterica* serovar Enteritidis (hereafter referred to as *S.* Enteritidis) from the Scientific Institute of Hygiene and Analysis (ISHA, Massy, France) was used as a model bacterium in this study.

2.2. Antibodies

An *S.* Enteritidis monoclonal antibody (hereafter referred to as SE103, kindly provided by Dr. H. Volland, CEA-Saclay, France) was used as a specific probe. A keyhole limpet hemocyanin (KLH) specific monoclonal antibody (kindly sourced by Dr. L. Bellanger, CEA-Marcoule, France) was used as a negative control probe (Bouguelia et al., 2013).

2.3. SPRi apparatus and biochips

Experiments were performed in a polyether-ether-ketone (PEEK) reactor containing four independent chambers in combination with a SPRi PlexII instrument (Horiba Scientific). The SPRi PlexII was housed in a temperature controlled incubator maintained at 37 °C. Gold coated glass prisms were used as biochips (Horiba Scientific). All the material is commercially available. The biochips were arrayed with pyrrolylated SE103 and KLH antibodies by electrochemical directed pyrrole-based polymerisation (Cherif et al., 2006). The biochips were freshly made for each experiment.

2.4. Nanoparticles

Representative Ag NPs (Ag NM-300K, recently JRCNM03000a) were obtained from the European Commission's Joint Research Centre (Ispra, Italy). Ag NM-300K NPs (negatively charged and polydisperse nanoparticles with a primary size ca. 15 nm) were characterised previously (Klein et al., 2011; Losasso et al., 2014). We reported further information on this material (e.g. hydrodynamic size, zeta potential, spectrum of absorption, and dissolution rate) in various media (including Luria Bertani, the medium used herein) using dynamic light scattering (DLS), UV–visible spectrophotometry (UV–vis) and atomic absorption spectroscopy (AAS) elsewhere (Mallevre et al., 2014; Mallevre et al., 2016a). Silver nitrate ($AgNO_3$, Fisher Scientific) salt was used as a source of Ag ions. All concentrations are in Ag terms (i.e. mg Ag L^{-1}).

2.5. SPRi based assays

This application extends the principle of the CCM method (Bouguelia et al., 2013). Prior to the assays, *S.* Enteritidis was pre-cultured in Luria Bertani (LB) medium (10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 5 g L^{-1} NaCl) at 37 °C under agitating conditions (150 rpm) then used diluted at 10^2 CFU mL^{-1}. Ag NPs were freshly prepared in LB (bath sonicated twice for 8 min), as previously described (Mallevre et al., 2014), prior to addition for testing at 0.1, 1, 5, 10 and 100 mg L^{-1}. Ag ions were tested at 0.01, 0.1, 1 and 10 mg L^{-1}. A typical template for an assay included at least three replicated spots per antibody across four different conditions of exposure which involved a

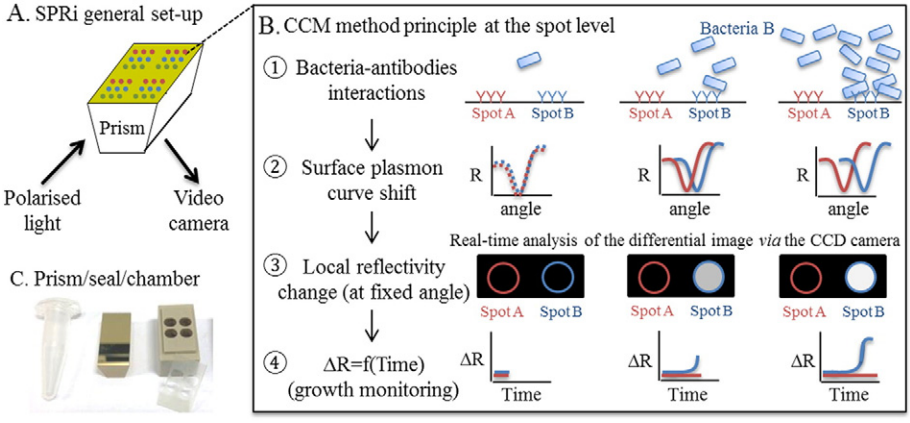

Fig. 1. Introduction to bacterial growth monitoring using the CCM based SPRi method. The general SPRi set-up used herein (based on functionalised prisms with bespoke series of antibody bearing spots) is shown in A. The principle of the CCM method is schematised in B. An example of two spots, bearing non-specific (in red) or specific (in blue) antibodies to the bacterial model of interest, is presented. R is the reflectivity for a defined spot as determined optically using a CCD camera and ΔR is the variation of this reflectivity (due to specific interactions bacteria-antibodies occurring at the surface of the prism) registered over time for a selected (fixed) measurement angle. An example of a used prism, silicon seal and PEEK reactor of four chambers (altogether constituting the biochip when assembled), is shown in C; a 1.5 mL Eppendorf tube provides a perspective on the scale of the material. Additional information on the approach can be found in Bouguelia et al. (2013) and in the patent WO 2012073202 A1.