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Impact of cell adhesion and migration on nanoparticle uptake and cellular toxicity

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ABSTRACT

In vitro cell-nanoparticle (NP) studies involve exposure of NPs onto the monolayer cells growing at the bottom of a culture plate, and assumed that the NPs evenly distributed for a dose-responsive effect. However, only a few proportion of the administered dose reaches the cells depending on their size, shape, surface, and density. Often the amount incubated (administered dose) is misled as a responsive dose. Herein, we proposed a cell adhesion-migration (CAM) strategy, where cells incubated with the NP coated cell culture substrate to maximize the cell-NP interaction and investigated the physiological properties of the cells. In the present study, cell adhesion and migration pattern of human breast cancer cell (MCF-7) and mouse melanoma cell (B16-F10) on cell culture substrate decorated with toxic (cetyltrimethylammonium bromide, CTAB) and biocompatible (poly (sodium 4-styrenesulphonate), PSS) gold nanoparticles (AuNPs) of different sizes (5 and 40 nm) were investigated and evaluated for cellular uptake efficiency, proliferation, and toxicity. Results showed enhanced cell adhesion, migration, and nanoparticle uptake only on biocompatible PSS coated AuNP, irrespective of its size. Whereas, cytotoxic NP shows retard proliferation with reduced cellular uptake efficiency. Considering the importance of cell adhesion and migration on cellular uptake and cytotoxicity assessment of nanoparticle, CAM strategy would hold great promises in cell-NP interaction studies.

1. Introduction

Biomedical application of organic and inorganic nanoparticles (NPs) as a drug delivery vehicle for therapeutic, bio-contrast, and theranostic agents has increased in recent years (Agulla et al., 2014; Arunkumar et al., 2015; Chen et al., 2015; Li et al., 2016; Nguyen et al., 2016; Passeri et al., 2015; Sasidharan and Monteiro-Riviere, 2015). Its unique physicochemical properties induced by nanoscale dimension such as optical, thermal, and conductivity, their exploitation in day-to-day life is unavoidable (Agulla et al., 2014; Liu et al., 2015; Shi et al., 2011). At the same time, NP-induced toxicity and the environmental safety are the challenging problems in addition to its promising applications. Therefore, the study elucidating the interaction of nanomaterial with living cells has gained much attention (Chanana et al., 2005; Ginzburg and Balijepalli, 2007; Smith et al., 2014). Among various inorganic NPs, gold NP (AuNP) has been widely used and gained abundant interest due to its optical, ease of quantification in a biological system, and size/shape dependent photothermal properties (Sasidharan and Monteiro-Riviere, 2015; Venkatesan et al., 2013). Moreover, due to its biocompatibility and flexibility in surface functionalization, AuNP has been proposed and studied as a biomaterial, therefore study related to its interaction with cells is highly desirable (Cheng et al., 2013; Venkatesan et al., 2013).

Cellular uptake is a preliminary requirement used to determine the toxicological phenomena of a broad range of nanomaterials (Alkilany and Murphy, 2010). Reports clearly explain that the cellular uptake of the NP depends on the size, shape, surface charge, nature of nanomaterials, and the properties like concentration, density, sedimentation, and diffusion in the medium (Cho et al., 2011; Dasgupta et al., 2014; Jiang et al., 2008; Kim et al., 2012; Lu et al., 2014; Mahmoud et al., 2010). The properties of NP in culture medium also tend to change with the incubation time, where NPs attains biomolecular corona (Tenzer et al., 2013). The formation of biomolecular corona significantly affects the interaction of the NP with cell resulting in the variation of NP uptake (Jeynes et al., 2013). Although physiological properties of NP was widely studied to understand cell-NP interaction (Cho et al., 2011;

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Abbreviations

AuNP	gold nanoparticles
PSS	poly (sodium 4-styrenesulphonate)
CTAB	cetyl trimethyl ammonium bromide

Jiang et al., 2008), only less attention has given to the cellular properties like cell adhesion and migration, which largely alters the NP uptake.

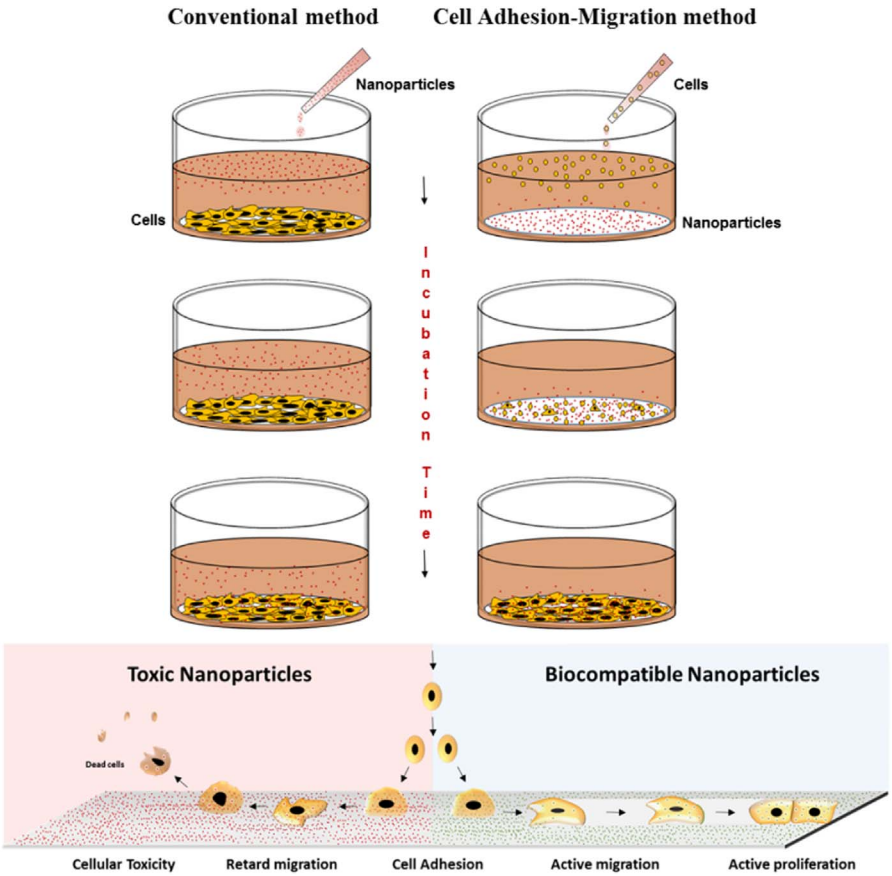
Cell adhesion and migration are the most common cell physiological events that play a significant role in cell progression, differentiation, and metastasis (Stroka and Konstantopoulos, 2014; Zhang et al., 2007). The adhesion and migration of cell depend on its microenvironment, which differs from cell to cell. The process is regulated by the various matrix adhesion molecules like cadherin, integrin, selectin, etc., (Xin et al., 2015). In cancer, metastasis starts with a loss of cell-cell adhesion from the primary tumor that enables the cells to invade into the circulatory system and distributed throughout the body (Xin et al., 2015). Both cell adhesion and migration interplayed in many biological events, and failure to this results to develop abnormalities like cancer progression and metastasis (Stroka and Konstantopoulos, 2014; Xin et al., 2015).

Conventional routine in vitro toxicity analysis of NP involved exposure of NP suspension to the monolayer of adhered cells and assumed that the cells exposed to the predetermined concentration of NP for dose-responsive effects. However, NP does not behave as a soluble molecule, only a few proportion of the total dose reaches to the monolayer of the cells depending on density gradient sedimentation, which is much lower than the administered dose, thus leading to false dosages. Recently, studies with the cellular disparity of AuNP under gradient sedimentation was well explained by terms of transport zone, interaction zone, and uptake zone, in a cell culture environment (Cho et al., 2011). However, to ensure complete interaction of nanomaterials with cells, we hypothesized that during adhesion and migration, cells could take up NP along the way of migration and its cytoplasmic extension. Such migration would favor rapid uptake of NP along it migrating zones thereby enhancing the NP uptake, which would provide an ideal scenario for the assessment NP-induced cytotoxicity. To test our hypothesis, we designed a cell adhesion and migration (CAM) strategy as demonstrated in Scheme 1. In a typical experiment, cells were allowed to adhere to the surface of cell culture substrate fabricated with different AuNPs and investigated its cell adhesion and migration pattern along with its cellular uptake efficiency and the dose responsive toxicity in comparison with the conventional method (CM).

2. Materials and methods

2.1. Chemicals and cell lines

Gold (III) chloride trihydrate ($HAuCl_4.3H_20$, 99.99%), sodium citrate (≥ 99%), sodium borohydride (≥ 99%), cetyl trimethyl ammonium bromide (CTAB, ≥ 99%), poly(sodium 4-styrenesulphonate) (PSS; Mw ~ 70,000), were purchased from Sigma-Aldrich (Milwaukee, WI, USA). (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)-MTT, methylene blue, and Pierce™ LDH cytotoxicity assay kit were purchased from Fisher. All chemicals were of analytical grade and used as received. Milli-Q water was used for the preparation of all reagents and chemicals. The human breast cancer cell line MCF-7 (ATCC® HTB-22™) and mouse melanoma cell line B16-F10 (ATCC® CRL-6475™) was purchased from ATCC, Manassas, USA. MCF-7 cells were maintained in Eagle's minimum essential medium (EMEM) supplemented with 10 μg/mL human recombinant insulin; B16-F10 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) and, both were additionally supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin 100 U/mL and streptomycin (100 μg/



Scheme 1. Experimental setup of the conventional method (CM) of cellular uptake study and the modified method based on cell adhesion and migration (CAM). In CM, NPs were exposed to the pre-cultured monolayer of cells and the administered dose is divided into transported dose and uptake dose due to various factors like size, charge, density, sedimentation, and diffusion, etc. In the CAM method, cell culture substrate was pre-coated with NP, and the cells were cultured over the surface of NP to investigate its physiological activities like cell adhesion and migration, and also evaluated its cellular uptake and cellular toxicity in in vitro conditions.