Real-Time Nanoparticle–Cell Interactions in Physiological Media by Atomic Force Microscopy

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Real-Time Nanoparticle–Cell Interactions in Physiological Media by Atomic Force Microscopy

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Supporting Information

ABSTRACT: Particle–cell interactions in physiological media are important in determining the fate and transport of nanoparticles and biological responses to them. In this work, these interactions are assessed in real time using a novel atomic force microscopy (AFM) based platform. Industry-relevant CeO₂ and Fe₂O₃ engineered nanoparticles (ENPs) of two primary particle sizes were synthesized by the flame spray pyrolysis (FSP) based Harvard Versatile Engineering Nanomaterials Generation System (Harvard VENGES) and used in this study. The ENPs were attached on AFM tips, and the atomic force between the tip and lung epithelia cells (A549), adhered on a substrate, was measured in biological media, with and without the presence of serum proteins. Two metrics were used to assess the nanoparticle cell: the detachment force required to separate the ENP from the cell and the number of bonds formed between the cell and the ENPs. The results indicate that these atomic level ENP–cell interaction forces strongly depend on the physiological media. The presence of serum proteins reduced both the detachment force and the number of bonds by approximately 50% indicating the important role of the protein corona on the particle cell interactions. Additionally, it was shown that particle to cell interactions were size and material dependent.

KEYWORDS: Nanoparticles, Nanotoxicology, Protein corona, Atomic force microscopy, Cerium oxide, Iron oxide, Nano-EHS, Nano–bio interactions

INTRODUCTION

The use of engineered nanoparticles (ENPs) in many commercial products and their involvement in many industrial processes makes environmental, occupational, and consumer exposure inevitable. Nano enabled technologies are currently in use for various biomedical applications ranging from preventing the transmission of infectious diseases to theranostic applications. Nanoparticle mediated therapies have been introduced which can either enhance current diagnostic methods like MRI and X-rays or introduce new methods, such as photo acoustic tomography (PAT).

Both the potential adverse health effects and the efficacy of theranostics are directly related to the nanoparticle–cell interactions and particle uptake from cells. There is a plethora of published literature documenting the ability of ENPs to penetrate biological barriers and initiate a cascade of events, possibly leading to adverse health effects.

It is also recognized that when nanoparticles enter physiological media, there is an instant formation of a protein coating, widely known as the protein corona. The protein corona dictates to a great degree the behavior and the fate of the nanoparticles in biological systems; it influences their agglomeration potential, the nanoparticle adhesion to the cell membrane, and potential cell-uptake and possible toxicity. Due to the importance of the corona in the nanoparticle–cell interactions, many studies have focused on the identification of (a) parameters influencing the adsorption of proteins on the surface of nanoparticles in various physiological fluids and (b) the role of the corona on the nanoparticle cell uptake. Although these studies aim to investigate the nanoparticle–cell interactions, they do so indirectly by observing secondary features such as the cell adhesion/viability, morphology, metabolic activity, oxidative stress, and particle uptake, which are later related to nanoparticle properties such as size, shape, and surface chemistry/modifications. Among them, the most commonly used metric is the quantification of particle uptake. Currently the leading method for the nanoparticle uptake quantification is the flow cytometry, which requires fluorescent ENPs. However, only a limited number of industry
relevant ENPs possess intrinsic fluorescent properties and possible ENP surface modification with fluorescent dyes may alter the chemistry and affect the nanoparticle–cell interactions. In an alternative approach, Wang et al. used the plasmonic properties of gold nanoparticles to study the intracellular localization of nanoparticles and recreate a three-dimensional mapping of their distribution, which again is limited to a small number of ENPs with intrinsic particle properties. Other researchers have used more conventional methods like ICP-MS to quantify the nanoparticle uptake. James et al. used a very sophisticated method employing X-ray fluorescence microscopy to map ZnO particles distribution in THP-1 cells. Recently, there have been attempts to utilize molecular dynamic simulations to investigate these interactions. Although insightful, there are still inherent limitations of this approach including the finite number of atoms that can be added to the simulations and the inability to accurately simulate an entire cell. In summary, although all these aforementioned methods can provide some information on nanoparticle–cell interactions, they have major drawbacks: (a) they do not provide a direct quantification of the nanoparticle–cell interactions; (b) they depend on intrinsic particle properties (e.g., fluorescence, plasmonic resonance, etc.) which limits their applicability to only a few particle systems; and (c) they require highly specialized equipment.

It is evident that there is a lack of a methodology that is independent of the particle properties, cell type, and media that can directly measure the nanoparticle–cell interactions. We recently developed a methodology that allows for the direct measurement of nanoparticle–nanoparticle interactions using atomic force microscopy (AFM). AFM is a state-of-the-art surface sensitive technique that has the ability to characterize in real time the interaction forces on a molecular level. While it has been used extensively in material science for imaging and atomic force measurements, only recently has the AFM been employed for understanding the nanoparticle-to-nanoparticle interactions in physiologic fluids. The high reproducibility of the method in terms of preparation of ENP coated AFM tips and atomic force measurements was showcased in our recently published study by Pyrgiotakis et al. It was also shown in this study that the agglomeration potential of CeO2 nanoparticles in water was inversely proportional to their primary particle diameter, but for Fe2O3 nanoparticles, that potential is independent of primary particle diameter in these media. In RPMI (Roswell Park Memorial Institute Medium no. 1640) + 10% Fetal Bovine Serum (FBS), the corona thickness and dispersibility of the CeO2 is independent of PP diameter while, for Fe2O3, the corona thickness and dispersibility were inversely proportional to primary particle (PP) diameter.

**Research Strategy.** In this companion study, our recently developed AFM platform was utilized to investigate nanoparticle–cell interactions in two relevant physiological media. To the best of our knowledge, this is one of the first systematic studies to determine in real time the nanoparticle–cell interactions and atomic force profiles and link them to nanoparticle properties and biological media using AFM.
Figure 1a describes the overall research strategy. Industry relevant ENPs of controlled size were synthesized in house using the FSP based Harvard Versatile Engineering Nanomaterials Generation System (Harvard VENGES) system and were attached on the surface AFM tips, as described in detail in the Materials and Methods section. The interaction force between the ENP functionalized tips and the cells was measured. It is worth noting that FSP made ENPs are highly relevant as they account for 90% by volume of ENPs currently on the market. Typical examples of FSP made ENPs are, among others, carbon black, pigmented titanias, and fumed silica, as well as other novel metal and metal oxide ENPs currently in use as catalysts, gas sensors, biomaterials, and even nutritional products.

Two ENP systems were used as test materials in the study, CeO$_2$ and Fe$_3$O$_4$. These ENPs were synthesized in two different sizes, small (S) and large (L), and more specifically, approximately 5 and 50 nm for CeO$_2$ and 10 and 100 nm for Fe$_3$O$_4$. Both ENPs are extensively used in many applications. Cerium oxide is employed in many industrial and commercial applications such as a catalyst, additive in fuels, oxygen storage in fuel cells, pigment in cosmetics, and abrasive medium in chemical mechanical polishing (CMP). Recent toxicological evidence suggests that in the nanoparticle form there might be adverse health effects and environmental implications. Iron oxide is widely utilized as pigment and has attracted considerable attention due to its promising potential in biomedical applications for its superparamagnetic properties and its use in nutritional applications. In addition both ENPs have also been investigated in our recently published nanoparticle−nanoparticle interactions AFM study.

As test cells, the A549 cell line (lung epithelia cells) were used. Epithelial cells constitute the first line of defense against ENPs in the lung. RPMI 1640 (Roswell Park Memorial Institute formulation 1640) and RPMI containing 10% Fetal Bovine Serum (FBS) were used as biological media in the study. The aforementioned media are commonly employed in the preparation of nanoparticle suspensions in toxicological studies.

During a typical AFM force measurement, the interaction between the ENP modified tips and the cells is divided in two subsequent modes, trace and retrace modes. “Trace mode” is referred to the approach of the tip to the cell, while “retrace mode” is referred to the retraction of the tip away of the cell. The atomic force is obtained as a function of the distance between AFM tip and cell surface for both modes. Additionally, the AFM allows the regulation of the contact time (dwell time) of the tip with the cell surface (see Figure 1b for more details). During the approach of the AFM tip toward the cell surface, the nanoparticles will interact with the cell membrane (lipids, proteins, receptors etc.) and will compress the cell wall. This is an elastic deformation of the cell wall that will contribute to the force measured by the AFM tip.

At some point, the compression will stop, and the tip cantilever will start bending, resulting in a force which is linearly increasing with the distance from cell surface (Figure 1c). During the retrace, different force curve is observed as several phenomena occur: (a) multiple small detachment forces shown as a “pee-saw” pattern in the atomic force curve and (b) a final detachment force, which is distinctly larger than the small detachment forces and indicates the complete detachment of the tip from the cell surface (Figure 1d). The typical see-saw pattern is characteristic of the multiple events of adhesive bonds between the ENPs and the cell surface, breaking sequentially.

During the retrace there are three important parameters that define the ENP-cell interactions: (a) The average magnitude of the force to break these individual bonds which is defined as atomic force per bond (AFB); (b) the number of these breaking bonds (number of bonds (NB)); and (c) the detachment force (DF) which is defined as the final larger single event force for the complete detachment of the ENPs. These three aforementioned parameters were used as metrics for the data analysis in this study.

### MATERIALS AND METHODS

The utilized AFM methodology has been fully described and characterized in our previous publication. In brief, the ENP synthesis and characterization, the AFM tip preparation, the cell substrate preparation, and the force acquisition and analysis were performed as follows:

#### Synthesis of ENPs.

The nanoparticles were synthesized using Harvard VENGES, which is based on flame spray pyrolysis (FSP). The exact procedure is described in detail in our previous publication. Flame aerosol technology accounts for more than 90% of the total volume of all nanomaterials produced in the gas phase worldwide. Among the advantages of this method is its precise control of the nanoparticle properties (i.e., composition, dimensions, shape, etc.); the high yield (g/h), the ease of scaling, and the reproducibility with regard to nanoparticle properties.

In brief, during the FSP synthesis, a precursor solution, which contains dissolved organometallic compounds in a high enthalpy solvent, is pumped through a stainless-steel capillary tube at a controlled flow rate. Oxygen flow disperses the liquid precursor solution into fine droplets, which in turn are combusted by a small pilot flame. This results in the full conversion of the liquid precursor’s organic constituents into metal oxide nanoparticles. The nanoparticle diameter is fully controlled by the operational parameters, and the results are consistent and reproducible. The nanoparticles are collected on a water-cooled glass fiber filter (Whatmann, 25.5 cm Ø) for off-line characterization and further use.

#### ENP Dispersion Preparation.

ENP dispersion was used for the AFM tip modification as described in detail by Pyrgiotakis et al. The ENP dispersions in deionized water (18.1 MΩ/cm) were prepared according to the protocol developed by Cohen et al., which includes calibration of sonication equipment to ensure accurate application of delivered sonication energy (DSE) in joules per milliliter in order to break agglomerates that might have formed. This method is currently standardized and widely used for preparation of nanoparticles for toxicological studies. According to the protocol, in order to achieve stable nanoparticle suspensions over time, the delivered sonication energy (DSE) should exceed a critical value (DSE$_{cr}$). The DSE$_{cr}$ for various ENPs has been previously experimentally determined, and the values varied from 161 to 242 J/mL. The required sonication was done with a Branson Sonifier S-450A (Branson Ultrasonics, Danbury, CT, USA) fitted with a 3 in. cup.

#### ENP Characterization.

The ENPs were characterized by transmission electron microscopy (TEM) regarding their morphology and by X-ray diffraction (XRD) regarding their crystal structure and size. BET N$_2$-adsorption was used to measure their surface area and the equivalent diameter. In more detail:

- **TEM.** ENP dispersions were prepared as described before. After sonication, the nanoparticle suspension was diluted down to 100 μg/mL. TEM grids (Ted Pella Inc., Redding, CA) were submerged in the solution and were let to dry. The particles were imaged with the Libra 120 (Carl, Zeiss Oberkochen, Germany).

- **XRD.** The X-ray diffraction pattern was measured from 2θ 15°−70° with a Bruker AXS D8 Advance (Bruker, Karlsruhe, Germany). The

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The ENPs were attached on the AFM cantilever tips (BL-AC40TS) using a Rietveld method to determine the nanoparticle phase and crystalline size.

**Specific Surface Area.** BET N$_2$-adsorption of the nanoparticles allowed for the determination of the specific surface area. Approximately 100–200 mg of the nanoparticle was flushed with a N$_2$ gas at 150 °C for >1 h with the Flow Prep 060 (Micromeritics, Norcross, GA). The specific surface area was measured with TriStar (Micromeritics, Norcross, GA).

**Cell Culture and Cell Substrate Preparation.** For these set of experiments, the A549 lung epithelia cells were used (ATCC; cell line number CCL-185). They were selected due to the relevance to experiments, the A549 lung epithelia cells were used (ATCC; cell line number CCL-185). They were selected due to the relevance to nanoparticle respiratory exposures and their resilience to the AFM conditions.

The growth media is made of 90% RPMI-1640 with L-glutamine (from Cellgro; cat. no.: 25-053-CL) and 10% Fetal Bovine Serum (four times filtered through 0.1 μm filter, from Hyclone; cat. no.: SH30070.03). The cells were cultured and plated according to the suggested ATCC protocols. For all the experiments the cells were used between passages 5–10.

For these experiments, the cells were seeded on a on cover glass bottom sterile culture dish (70674-02, Electron Microscopy Sciences, Hatfield, PA) at approximately 5 x 10$^5$ cells by seeding 100 μL of cell suspension to cover the glass bottom of the culture dish. The substrates were placed in the incubator for 45 min, sufficient time for the cells to attach on the glass bottom of the culture dish. Following attachment the cells were washed and 1 mL of growth media was added and the cells were incubated at 37 °C and 5% CO$_2$ for a minimum of 24h before conducting AFM measurements. Prior to the usage of the AFM the media was removed, the cells were rinsed with PBS (Phosphate Buffer Saline) and the appropriate media was added.

**Modification of the AFM Tips. AFM Tip Selection.** It is critical for the AFM cantilever to yield before the tip punctures the cell. In order to ensure that, the AFM tips with small spring constants should be used (0.1 N/m). The tip type was optimized by trial and error. Several types of tips were tried, and the tip producing consistent and reproducible results was selected. It is worth noting that long cantilevers, although have small spring constant, which is appropriate for this study, are hard to functionize as they are very wobbly. The selected tip was the BioLever Mini (Olympus BL-AC40TS, Asylum Research, Santa Clara, CA).

**Attachment of ENPs on AFM Tips.** The ENPs were ex situ attached on the tips from aqueous suspensions according to the method developed by Pyrgiotakis et al. The ENPs were attached on the AFM cantilever tips (BL-AC40TS) with a fine coordination of Leica micromanipulators (Micro-

Figure 2. Method for tip preparations. (a) The tips are coated with the creation of a fine droplet on the edge of a fine capillary. (b) The tip is brought in contact with the capillary and is dunked several times. (c) A micro sized droplet is formed at the edge of the tip. (d) The droplet is left to dry to create a small particle aggregate. (e) A photograph that depicts the process with the key elements illustrated.
between two parameters that resulted in statistically significant $p$-values. The comparison between values was based on ANOVA and FPB parameters, the values of each parameter were averaged, and used to estimate the confidence intervals and significance. Comparison between two parameters that resulted in $p$-value $< 0.05$ was considered statistically significant.

**RESULTS AND DISCUSSION**

**Particle Synthesis and Characterization.** These particular ENPs have been already rigorously characterized in our previous publication.\(^{29}\) The CeO$_2$ and Fe$_2$O$_3$ nanoparticles were synthesized in two distinctly different primary diameters of 5–10 and 50–100 nm. Figure 3 shows collectively the structural characterization of CeO$_2$ (Figure 3a) and Fe$_2$O$_3$ (Figure 3b) nanoparticles for both diameters. Figure 3c–f shows the TEM images of the same particles. Table S2 (Supporting Information) summarizes the results of the particle characterization including the diameter based on the XRD patterns (Rietveld analysis) and the Brunauer–Emmett–Teller (BET) N$_2$ adsorption specific surface area.

As it is evident from the XRD patterns, both CeO$_2$ and Fe$_2$O$_3$ are crystalline. More specifically, the CeO$_2$ nanoparticles have the characteristic cubic (CaF$_2$ structural type), in agreement with literature,\(^{49}\) and the Fe$_2$O$_3$ nanoparticles are in gamma phase, also in agreement with FSP literature.\(^{58}\) In addition, the TEM showcase the characteristic hexagonal form of the Fe$_2$O$_3$ particles\(^{59}\) and the rhombohedral shape of the CeO$_2$ particles.\(^{49}\) The desired diameter variation is confirmed by both the XRD patterns and TEM images (Table S2, Supporting Information). It is worth noting that there is a nearly self-preserving diameter, as it is true for all flame generated materials.\(^{58}\)

Figure 4a–d shows an SEM image of the AFM tips modified with the CeO$_2$(L), CeO$_2$(S), Fe$_2$O$_3$(L), and Fe$_2$O$_3$(S) nanoparticles, respectively. In both cases, it is evident that the tips are coated with the nanoparticles with a single nanoparticle protruding. In our previous publication, we examined the stability of the tips by successively imaging them with SEM after 200 measurements in air, 200 measurements in water, 200 measurements in RPMI, and 200 measurements RPMI+10% FBS.\(^{29}\) Examination of the tips before and after each measurement in this study also showed that the nanoparticles remain in place after each measurement. Overall, our current data showed that the utilized method results in nanoparticles very well adhered on AFM tips.\(^{29}\)

Supporting Information Figure S2 shows a typical force curve during the cell approach (trace mode) and cell retraction (retrace mode). The three important parameters/metrics that define the ENP–cell interactions, namely, the detachment force (DF), the atomic force per bond (AFB), and the number of bonds (NB) are illustrated in the figure as well. The number of bonds usually follows a normal distribution as shown in Figure S2b.

The results of this analysis are summarized in Figure 5 for both RPMI+10% FBS and RPMI media for a 30 s dwell time. Figure 5a, b, and c show the DF, NB, and AFB metrics, respectively, for both the CeO2 and Fe2O3. Figure 5d–f shows the same parameters for the 180 s dwell time. The related p-values are listed in Supporting Information Tables S3–S10.

Role of Biological Media. DF appears to be greater for the case of pure RPMI compared to RPMI+10%FBS regardless of the material or the size of the nanoparticles, with only exception to the observation the small CeO2, where the p-value is not showing statistical significant difference (Figure 5a). In the case of the 30 s dwell time, the DF values are significantly greater as compared to the RPMI+10%FBS for all the cases with the exception of the small CeO2 nanoparticles, where the difference is not significant due to the relative large statistical error. These observations indicate that the cells have a stronger affinity to the nanoparticles in the absence of serum proteins (no protein corona). These results are in good agreement with the literature. It was shown in previous published studies that protein corona plays a significant role on the cellular uptake of ENPs.18 Tedja et al. showed that the serum proteins result in reduced titania nanoparticle uptake.61 Similarly, Johnstone et al. showed that the surface associated serum proteins inhibit the particle uptake of various polymer nanoparticles.62

Moreover, the number of bonds forming between the nanoparticles and the cell surface (Figure 5b) is also significantly greater for the case of pure RPMI, as compared to the nanoparticles in RPMI+10% FBS, with exception of the case of the small Fe2O3 nanoparticles that the observed difference is within the experimental error. (See related p-values in Supporting Information Table S10). This is in agreement that with our previous hypothesis, stating that the nanoparticles in the absence of serum have higher affinity toward the cells. The nanoparticle surface without the protein corona has more binding sites available from the various constituents of the cell to attach, and therefore the number of bonds is significantly higher.61 The larger particles have higher surface area per particle. They offer more binding sites, which may result to greater DF values.

The corona formation and characteristics are particle/media dependent and play an important role in nanoparticle cell uptake and biointeractions in general.15,16 In the future, we plan to expand the investigation and characterize the protein coronas on the nanoparticle systems used here in order to better understand the link between corona characteristics and AFM measured interactions.

Role of ENP Size. It is also interesting to examine the effect of the ENP size on the magnitude of the detachment force (DF). For the CeO2 nanoparticles in RPMI media, the nanoparticle size has a significant effect on DF value, while in the case of RPMI+10% FBS, the nanoparticles size does not have a significant effect. However, for the of the Fe2O3 nanoparticles, the smaller size results in larger DF, but the difference is not as strong as for the case of the CeO2 nanoparticles. These differences observed in the material dependency might be explained by the differences previously observed in corona properties.29 As shown in our previously published companion AFM study on nanoparticle–nanoparticle interactions,29 the repulsive layer thickness (RLT, an estimate of the protein corona thickness) of the Fe2O3 nanoparticles decreased with the size, a clear indication that the corona properties were size dependent. These findings reflect differences on the size of the corona and do not necessarily reflect differences in corona composition. It is well documented that the corona composition depends both on the particle size and surface properties.63

In the case of the pure RPMI and the absence of protein corona, the adhesion of both CeO2 and Fe2O3 nanoparticles to cells show stronger dependence on the particle size as indicated by the DF. More specifically, the larger CeO2 nanoparticles have an approximate 2-fold DF compared to the smaller CeO2 nanoparticles. Similar findings were found for the Fe2O3 nanoparticles although the size dependency was found to be less prominent.

Finally, in the case of RPMI, the NB was found to depend on the particle size (Figure 5b and c). Generally larger nanoparticles displayed a higher NB value. Larger nanoparticles offer more absolute surface per single particle and therefore more binding sites.64 It is worth noting that, in RPMI, there was no significant difference observed.

Role of Dwell Time. Figure 5d, e, and f shows the same parameters (DF, NB, AFB, respectively), for the case of the larger contact time (180 s). The calculated p-values are summarized in Supporting Information Table S6. The general trends observed for the DF in the case of 30 s dwell time seem to remain the same for this case. The CeO2 nanoparticles have stronger DF values as compared to the Fe2O3 ones regardless of the media. It should also be noted that the various differences between RPMI and RPMI+10% FBS seem to have been reduced for the longer dwell time. It is also evident that for the CeO2 nanoparticles the longer dwell time results in stronger DF as compared to the case of 30 s, while the opposite is observed for the Fe2O3.

The direct comparison of the DF parameter for the dwell times of 30 and 180 s shows that in pure RPMI the DF at 180 s appears to be greater compared to the 30 s for the CeO2 nanoparticles. The Fe2O3 nanoparticles follow the opposite
trend with DF value to be either small or not changing between the dwell times ($p$-values are summarized in Supporting Information Table S9). For the case of RPMI+10% FBS and 180 s dwell time, DF values are consistently greater as compared to the 30 s dwell time regardless of the material type and size. These observations are in agreement with other published studies indicating the dependency on interaction time of the protein adsorption on the nanoparticle surface.65,66 Similarly, these findings are also in agreement with computer simulations studying the nanoparticle cell interactions.67 It was previously shown that longer dwell time (time scale of minutes) brings the adsorption dynamics closer to equilibrium. Here this is causing the difference in DF between the RPMI and the RPMI+10% FBS to be reduced.68

Moreover, similar trends are observed for the NB parameter. More specifically, the small CeO$_2$ nanoparticles have a significant higher NB while the small Fe$_2$O$_3$ nanoparticles do not show significant differences between the two dwell times. For the large Fe$_2$O$_3$ in RPMI, the NB value is reduced by 50% while for the small Fe$_2$O$_3$ it remains unchanged (see $p$-values in Supporting Information Table S10). It is worth noting that the

Figure 5. Various metrics of the nanoparticle–cell interactions in RPMI and RPMI+10% FBS for 30 s dwell times: (a) detachment force, (b) number of bonds, and (c) atomic force per bond. The symbol * indicate $p < 0.05$. The various metrics of the nanoparticle–cell interactions in RPMI and RPMI+10%FBS for 180 s dwell times: (d) detachment force, (e) number of bonds, and (f) atomic force per bond. The symbols * indicate $p < 0.05$.
trends are consistent with the observations regarding the DF. In
addition, the differences in terms of NB values between the
RPMI and RPMI+10% FBS seem to decrease for the longer
dwell time. Again, this might be explained on the basis of the
equilibrium of the protein adsorption that is achieved at longer
dwell times.68

Atomic Force Bond (AFB). The magnitude of the AFB was
found to be independent of media, dwell time, size of ENP, and
the material. More specifically, AFB in RPMI does not show
any dependency with either the size or the material (Figure 5c
and f). This is in agreement with literature describing
interactions of various organic molecules with various
substrates (fibrinogen on gold and mica surface).69 Similarly,
in the case of the RPMI+10% FBS, there is no significant
difference of the AFB values either as a function of the
nanoparticle size or the material. Further there is no statistically
significant difference between the AFB in RPMI and the AFP in
RPMI+10% FBS. The magnitude of these forces are in
agreement with similar studies by Ikai et al. describing the
interaction of protein (conA protein) and bacteria (yeast cells
Saccharomyces cerevisiae).38

Overall Nanoparticle Affinity to the Cells. Evaluating the
results collectively, it is clear that both ENPs used in this study
have an affinity toward the A549 cells. In the case of the in
RPMI, the adhesion is governed by the size with the larger
nanoparticles exhibiting higher affinity. In the case of the RPMI
+10% FBS the adhesion is mainly governed by the material
with the CeO2 to exhibit larger DF, regardless of the size and
the dwell time. Although quantification of the nanoparticle
uptake is out of the scope of this manuscript, in numerous
previously published studies, it has been demonstrated that AS49
can uptake both CeO2 and Fe2O3 nanoparticles. Gass et al.
showed that the Fe2O3 nanoparticles can be taken up by AS49
cells and can lead to potential adverse effects.70 Furthermore,
Fe2O3 nanoparticles were shown to be taken up by alveolar cells
during inhalation and can cause adverse health effects. Sotiriou
et al. also demonstrated with animal inhalation studies that
Fe2O3 nanoparticles can cross the air–blood barrier and can
cause oxidative stress in the lung and the heart of the exposed
animals.34 Similarly, the CeO2 nanoparticles were found to be
taken up by cells in vitro35,76 and in vivo studies.35 This has
been documented by Demokritou et al. where it was shown that
CeO2 ENPs were taken up by lung epithelia cells after
animal inhalation exposures.35 These type of interactions
depend on the particle properties, cells, the media, and therefore,
trends observed here cannot be generalized or even
extrapolated to other cell types or particles. Further research is
required to be able to derive more generalized conclusions.

CONCLUSIONS
This study is one of the first attempts to assess in a systematic
manner the role of the protein corona to the nanoparticle–cell
interactions in relevant physiological media using atomic force
microscopy. The AFM platform enables a real time direct
measurement of the ENP–cell interactions. Results from this
study highlight the important role of protein corona in the
particle–cell interactions as indicated by the higher nano-
particle–cell interaction force in the case of the presence of
serum proteins in the biological media. This AFM approach
provides an additional layer of information on atomic force
interactions, which can be valuable in the quest of under-
standing the complex nanobio interactions.

In the future, we plan to use the developed AFM platform to
investigate the forces between nanoparticles and various cell
lines under conditions where certain nanoparticle internal-
ization mechanisms have been blocked. This will allow the
investigation of the specific role of the nanoparticle properties
in the internalization mechanisms.

ASSOCIATED CONTENT
Supporting Information
Summary of the synthesis parameters, particle characterization,
a typical AFB force curve, and the p-values of the statistical
analysis. This material is available free of charge via the Internet
at http://pubs.acs.org/.

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The authors declare no competing financial interest.

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