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Title: Restored endothelial dependent vasodilation in aortic vessels after uptake of ceria coated silica nanoparticles,ex vivo.

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Keywords: nanoparticles; silica; ceria; free-radical scavenging; vasodilation; artery.

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Abstract: Ceria nanoparticles (CeNPs) have attracted considerable interest in the treatment of a number of conditions associated with increased production of reactive oxygen species (ROS), due to their unique antioxidant properties. We have previously demonstrated the attenuation in vasodilation after uptake of silica nanoparticles (SiNPs). Hence, we investigated whether ceria coating of SiNPs would improve the magnitude of vasodilation. Ceria coated SiNPs (CeSiNPs) were fabricated and fully characterised and their direct influence on vasodilator responses of aortic vessels examined, ex vivo. We demonstrate that while SiNPs significantly attenuate endothelial-dependent (acetylcholine-ACh) vasodilation, their surface modification with CeNPs leads to the significant improvement in dilator responses ($n=5$, $p<0.001$, at most ACh concentrations). These findings have implications in the fabrication of biocompatible nanoparticles for medical intervention. Furthermore, CeSiNPs may represent novel therapeutic tools for the protection and treatment of conditions where attenuated dilator responses are observed.

**Cover Letter**
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The Editor
Nanomedicine:NBM

20th June 2013

Dear Sir/Madam

Please find attached a paper entitled: 'Restored endothelial dependent vasodilation in aortic vessels after uptake of ceria coated silica nanoparticles, *in-vitro',* by Asima Farooq, Debra Whitehead, May Azzawi, for consideration as a '**Short Communication'** in your journal: Nanomedicine; Nanotechnology, Biology, and Medicine.

Our paper lies within the scope of your journal for 'preclinical studies addressing the diagnosis of disease'; specifically, it addresses a key issue related to the use of silica nanoparticles in imaging diagnostics and therapeutic intervention, namely that of nanotoxicity and their effects on vascular function. The work presented is novel and interdisciplinary involving vascular function studies using synthesised silica and ceria coated silica nanoparticles.

We demonstrate, for the first time, that ceria coated silica nanoparticles will restore dilator function which is reduced after exposure of aortic vessels to silica nanoparticles alone. Our findings have implications in the fabrication of biocompatible nanoparticles for medical intervention. Furthermore, CeSiNPs may represent novel therapeutic tools for the protection and treatment of conditions where attenuated dilator responses are observed.

We believe that our work would be of great interest to readers of Nanomedicine; Nanotechnology, Biology, and Medicine, as it lies within three of the preferred topics:

- improve imaging, diagnostics, and therapeutics;
- bioavailability, and toxicological assessment of nanomedicines;
- interactions of nanomaterials and nanodevices with cells, tissues, and living organisms.

I certify that this manuscript, or any part of it, has not been published and will not be submitted elsewhere for publication while being considered by the journal Nanomedicine: Nanotechnology, Biology, and Medicine.

Financial and competing interests disclosure : The authors have no other relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilised in the production of this manuscript.

**Conflict of interest:** None

I look forward to hearing from you soon.

Regards
May

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## Graphical abstract text:

We exposed aortic vessels to silica nanoparticles and ceria coated silica nanoparticles and examined their effects on vascular function. We show that these nanoparticles are taken up by endothelial cells lining the blood vessel. While silica nanoparticles reduced endothelial dependent dilator responses, their surface modification with ceria nanoparticles lead to improvement in dilator responses. This may be due to the unique antioxidant properties of ceria nanoparticles. Our findings have implications in the synthesis of biocompatible nanoparticles for medical intervention.

**Graphical Abstract: Image**

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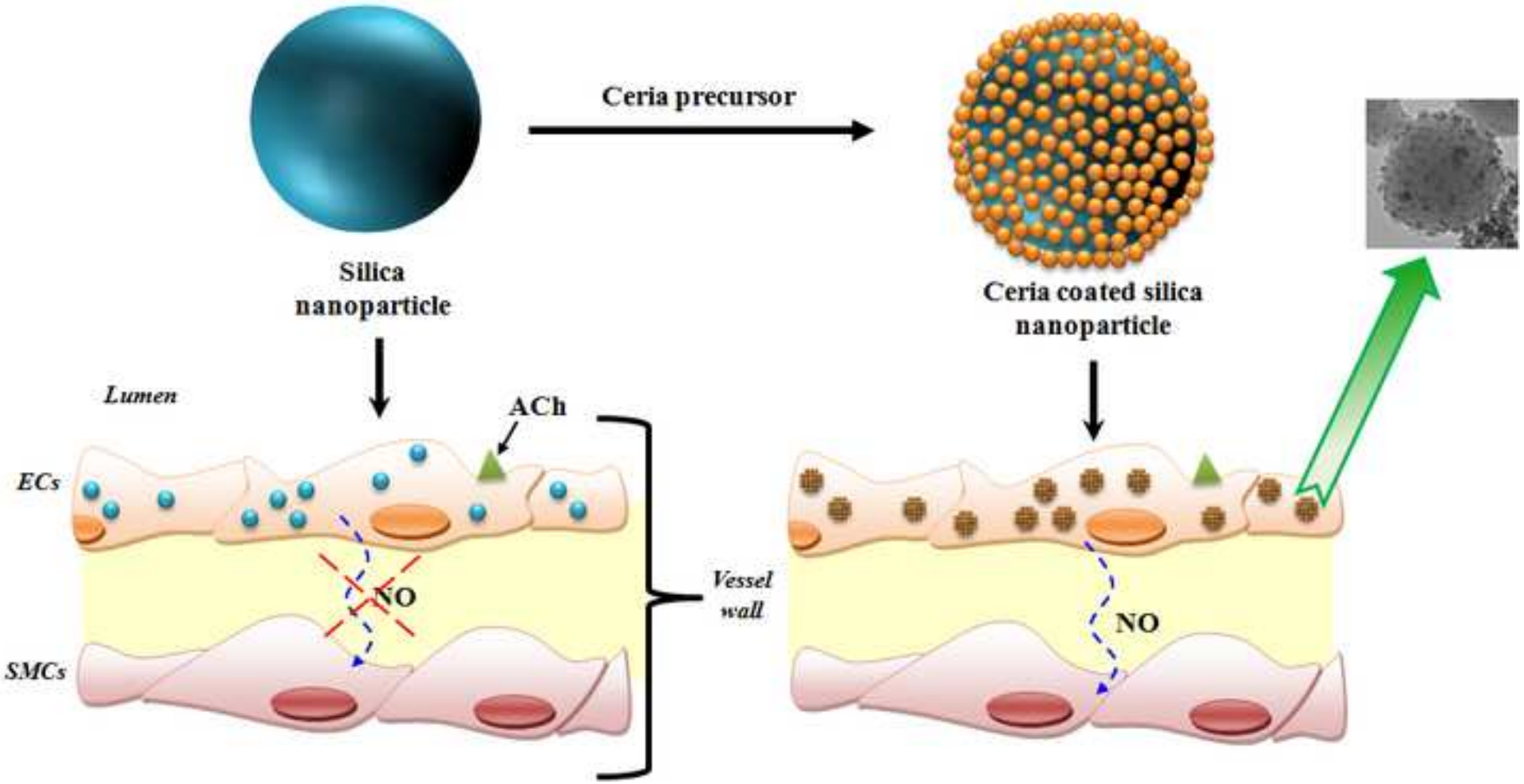

**TITLE**: Restored endothelial dependent vasodilation in aortic vessels after uptake of ceria coated silica nanoparticles, *ex vivo.*

**Short running title**: Influence of ceria coated silica nanoparticles on vessels

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Abstract= **142 words**.

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Number of references= **16**; Number of figures= **3**.

[*Entire manuscript including abstract, references= 1,904*]

## ABSTRACT

Ceria nanoparticles (CeNPs) have attracted considerable interest in the treatment of a number of conditions associated with increased production of reactive oxygen species (ROS), due to their unique antioxidant properties. We have previously demonstrated the attenuation in vasodilation after uptake of silica nanoparticles (SiNPs). Hence, we investigated whether ceria coating of SiNPs would improve the magnitude of vasodilation. Ceria coated SiNPs (CeSiNPs) were fabricated and fully characterised and their direct influence on vasodilator responses of aortic vessels examined, *ex vivo*. We demonstrate that while SiNPs significantly attenuate endothelial-dependent (acetylcholine-ACh) vasodilation, their surface modification with CeNPs leads to the significant improvement in dilator responses ($n=5$, $p<0.001$, at most ACh concentrations). These findings have implications in the fabrication of biocompatible nanoparticles for medical intervention. Furthermore, CeSiNPs may represent novel therapeutic tools for the protection and treatment of conditions where attenuated dilator responses are observed.

**Key words:** nanoparticles; silica; ceria; free-radical scavenging; vasodilation; artery.

## 1. INTRODUCTION

Ceria (Cerium oxide; $CeO_2$) nanoparticles have attracted considerable interest in the treatment of conditions associated with increased production of reactive oxygen species (ROS), due to their unique antioxidant properties.[1,2] Ceria nanoparticles (CeNPs) have been shown to protect against ischaemic stroke[3], prevent vision loss due to light induced degeneration of photoreceptor cells[4] and protect normal cells against radiation damage[2]. The redox property of CeNPs is due to the large number of surface oxygen vacancies giving it a radical scavenging property which has been described to be more efficient than the biological antioxidant, superoxide dismutase (SOD).[5] Silica nanoparticles have potential biomedical applications,[6] but may themselves generate ROS upon cellular uptake.[7-9] Indeed, we have demonstrated that exposure of aortic vessels to silica nanoparticles (SiNPs) attenuated vasodilation,[10] which improved after co-incubation in SOD, suggesting a role for ROS in quenching nitric oxide. [11] In the present study, we fabricated and characterised ceria coated SiNPs (CeSiNPs) and compared their influence on vasodilator responses of aortic vessels with SiNPs *ex vivo*. We suggest that ceria coating of SiNPs will restore vasodilator responses, hence increase their biocompatibility for use in therapeutic intervention.

## 2. EXPERIMENTAL

### *2.1. Materials and solutions*

All reagents and agonists were purchased from Sigma-Aldrich (UK). Millipore water was used for all experiments. Physiological Salt Solution (PSS, 60 mM) was prepared as previously described.[10]

### *2.2. Synthesis and Characterisation of nanoparticles*

SiNPs were synthesised via a sol-gel precipitation method as previously described,[11] The ceria precursor and ceria nanoparticulate shell was grown on the SiNPs surface as

previously described by Oh et al. (2010).[12] NPs were prepared for transmission electron microscopy (TEM) as previously described.[11]

### *2.3. Vascular functional studies*

The thoracic aortic arteries of male Wistar rats (150-250 g) were utilised for the *in vitro* studies (n=11 animals; one vessel from each animal). The rats were humanly killed by stunning followed by cervical dislocation following institutional approval and in accordance with guidelines issued by the European Commission Directive 86/609/EEC. Approximately 3-4 mm aortic rings were mounted in an organ-bath system (gassed PSS in 95 % $O_2$: 5 % $CO_2$; 35 $^{o}$C), as previously described and tension recorded using Labchart 6 (Powerlab, AD Instruments, UK).[10] Vessels were pre-constricted with high $K^+$ (60 mM, KCl). Responses to endothelium-dependent dilator agonist were examined by adding cumulative doses of acetylcholine (ACh; 0.01-100 μM), before and after incubation with NPs for 30 min. The final concentration of SiNPs, and CeSiNPs placed in the organ-bath experiments were 1.96 $10^{12}$ NPs $mL^{-1}$. Vessels were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at *pH* 7.3. and processes for TEM, as previously described.[10]

### *2.4. Statistical analysis*

Data are expressed as mean ± standard error of mean (SEM) with 'n' representing the number of vessels. Dilator responses are expressed as percent relaxation. Concentration response curves were assessed using statistical package for the social sciences (SPSS; version 19). The difference between groups at a given concentration was tested by one way analysis of variance (ANOVA) with Bonferroni corrections. Statistical significance is taken as $P<0.05$.

## 3. RESULTS

### *3.1. Characterisation of nanoparticles*

The SiNPs were monodispersed with an average diameter of 47±8 nm as demonstrated by TEM (Fig. 1A). The addition of ceria shell around the SiNPs increased the diameter to 50±4 nm with the individual CeNPs size of 3.8±1.2 nm (Fig. 1B, C). The energy dispersive spectroscopy (EDS) of SiNPs confirms the presence of silica (Fig. 1D). The CeSiNPs contain both the silica and ceria (Fig. 1E).

### 3.2. Influence of nanoparticles on endothelium-dependent vasodilator responses

All vessels constricted to high potassium physiological salt solution (n=11). Incubation in SiNPs (3.03±0.22 g tension and 2.58±0.24 g tension, before and after incubation respectively), or CeSiNPs (3.03±0.27 g tension and 2.96±0.32 g tension, before and after incubation respectively) had no influence on the magnitude of the constrictor response. All pre-constricted vessels dilated to ACh in a dose dependent manner. Incubation in amorphous SiNPs at 1.96 $10^{12}$ NPs $mL^{-1}$ caused a significant attenuation in dilation as compared to PSS alone (Fig. 2). Incubation in CeSiNPs (50±4 nm) at 1.96 $10^{12}$ NPs $mL^{-1}$ led to a significant improvement in dilator responses (Fig.2). TEM transverse sections demonstrated uptake of both SiNPs and CeSiNPs into the cytoplasm of ECs (Fig.3)

## 4. DISCUSSION

In the present study, we demonstrate that while SiNPs significantly attenuated endothelial-dependent vasodilation, their surface modification with ceria lead to a significant improvement in dilator responses. Both SiNPs and CeSiNPs were rapidly internalised by ECs. There was no evidence of translocation through the elastic lamina or SMC layer. Jin *et al.* (2007) were also able to demonstrate that the majority of NPs that rapidly entered cells

took place within the first 5 min and reached maximum uptake after a 30 min incubation period.[13]

The attenuated dilation due to SiNP uptake, observed in the present study, may be related to the NP's generation and stimulation of intracellular ROS. These can act as scavengers of nitric oxide, the major vasodilator in aortic vessels. SiNPs have a high concentration of surface $OH^-$ groups on their surface and this may play an important role in ROS generation. ROS generation by SiNPs has previously been demonstrated in a number of cell types *in vitro*, including HUVECs,[7] lung submucosal cells [14] and human keratoinocyte cells [15]. The resultant oxidative stress has been shown to be a major cause of nanotoxicity, leading to endothelial dysfunction, DNA damage and apoptosis.[15,16] For example, Duan *et al* (2013),[7] were able to demonstrate that SiNP incubation with HUVECs led to a dose and time dependent cytotoxic influence on these cells (both necrosis and apoptosis) through DNA damage and cycle arrest, as well as inhibition of SOD and glutathione peroxidase enzymes.

We demonstrate the rapid uptake of SiNPs and CeSiNPs in live viable aortic vessels, *ex vivo*. We show that while SiNPs of ~50 nm attenuate endothelial-dependent relaxation, ceria coating of these SiNPs leads to significant improvement in dilator responses. This study will inform the future fabrication of biocompatible SiNPs for use in imaging diagnostics. Furthermore, CeSiNPs may represent novel therapeutic tools for the protection and treatment of conditions where reduced dilator responses are observed.

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**FIGURE LEGENDS**

**Figure 1**: TEM image of A) SiNPs (47±8 nm), B) ceria coated SiNPs (50±4 nm ) , C) enlarged image of ceria coated SiNP; EDS analysis for D) SiNPs and E) ceria coated SiNPs.

**Figure 2**: The influence of amorphous silica nanoparticles (SiNP) and ceria coated silica nanoparticles (CeSiNPs) on endothelium-dependent vasodilator responses. The control is incubation in PSS alone. '**n' is number of vessels**. *=$p<0.05$, **=$p<0.01$ and ***=$p<0.001$, error bars=SEM.

**Figure 3:** Representative TEM images showing uptake of A) CeSiNPs (9300mag) and B) CeSiNPs (30000mag), by endothelial cells lining aortic vessels.

**Figure 1**

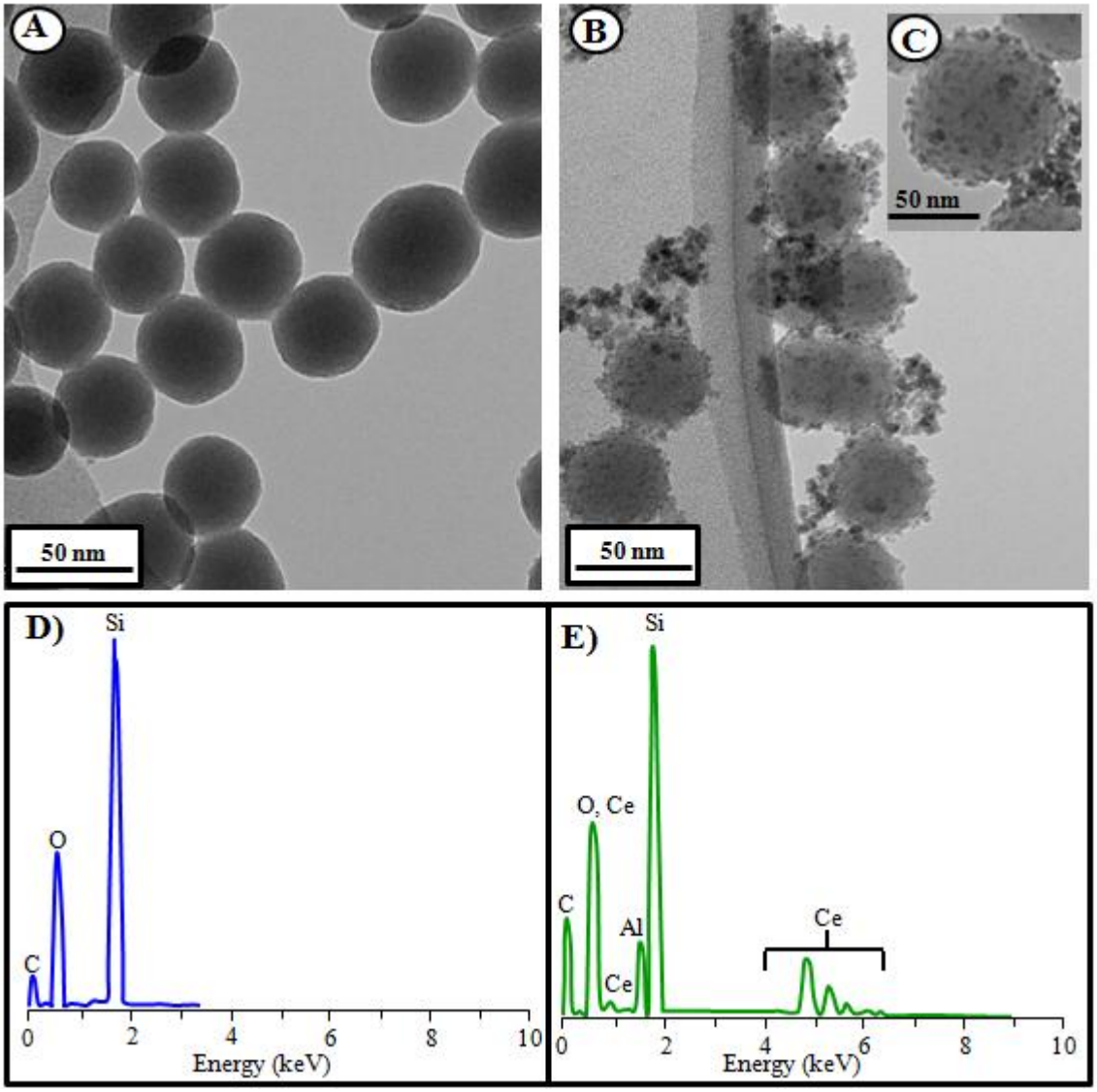
A
50 nm
B
C
50 nm
50 nm
D)
Si
O
C
0
2
4
6
8
10
Energy (keV)
E)
Si
O, Ce
C
Al
Ce
Ce
0
2
4
6
8
10
Energy (keV)

**Figure 2**

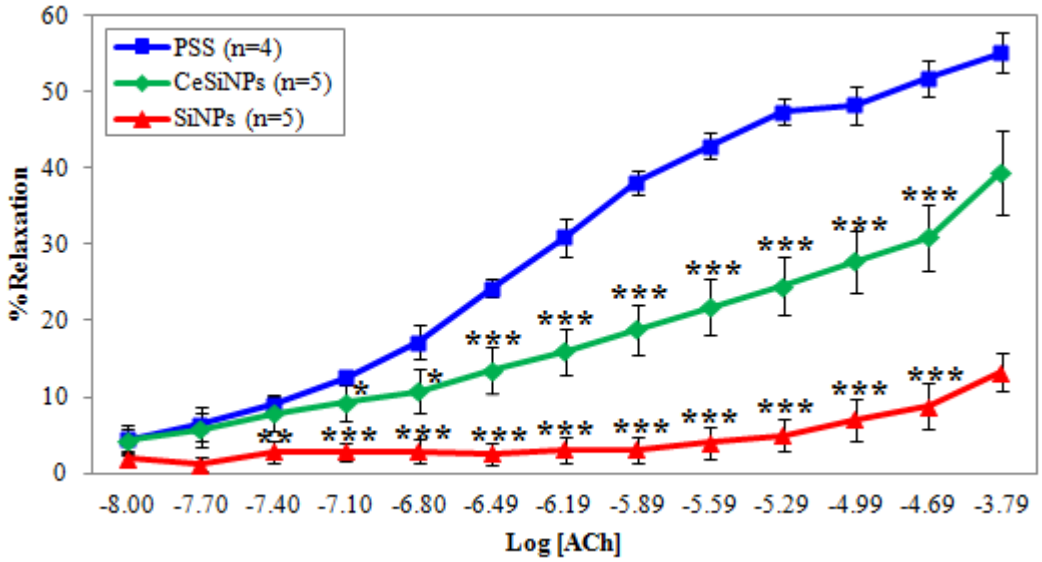
PSS (n=4)
CeSiNPs (n=5)
SiNPs (n=5)
%Relaxation
Log [ACh]
60
50
40
30
20
10
0
-8.00
-7.70
-7.40
-7.10
-6.80
-6.49
-6.19
-5.89
-5.59
-5.29
-4.99
-4.69
-3.79

**Figure 3**