# ZnO nanoparticles supported on mesoporous silica hosts as efficient antibacterial agents for nanocoating applications

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## 1. Introduction

Infectious diseases pose a significant threat to human health and the global economy, accounting for more than 20% of global mortality, while viruses are responsible for about one-third of these deaths.[1,2] To tackle with these threats, antimicrobial coatings are widely used, presenting however certain draw-backs related to: (i) the release of the active compounds that might slowly enter the ecosystem, (ii) the loss of their efficiency in short time, (iii) the resistance of the microbes to the commonly used agents and (iv) the high human toxicity of some antimicrobial agents (e.g., Ag). Addressing these challenges, the present study is focused on the developement of novel active nanomaterials (ANMs) based on ZnO nanoparticles (NPs) supported on mesoporous silica featuring different pore geometry. Specifically, mesoporous SBA-15 and KIT6 were used as non-toxic ZnO NPs hosts. Inclusion of ZnO NPs within the mesoporous networks provides various benefits related to higher NPs dispersion,[3] limitation of growth phenomena, as well as reduction of zinc amount and of the harmful release of zinc cations. To achieve a geometrically restricted deposition of ZnO NPs within mesoporous silica hosts special attention must be given to the employed metal loading method, since most of them usually lack control over particle size and distribution. Thereby, a novel dendritic polymer templating strategy called assisted impregnation (A.I.) was employed.[4] This technique relies on the use of hyperbranched polymers as metal entrapping and templating agents by taking advantage of their chemical and chelating properties. Particularly, in this study, the water soluble, low cost and commercially available hyperbranched polyethyleneimine (PEI) of 5,000 Da molecular weight was exploited. After the structural characterization of the synthesized nanomaterials, their antibacterial activity was investigated against Gram-positive Staphylococcus Aureus bacteria, while their biocompatibility was investigated through cytotoxicity on mammalian cell lines.

## 2. Experimental section

SBA-15 and KIT-6 mesoporous hosts were produced through the synthetic routes described Kosuge et al.[5] and Kleitz et al.[6] Inclusion of ZnO within mesoporous silicas was

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performed via assisted impregnation (A.I.) technique consisting of the following synthetic steps (Figure 1). The initial step involved the introduction of PEI within silicas' mesochannels through wet impregnation. Following PEI addition, a washing procedure was performed to remove the free polymer. Then metal sorption was carried out by adding the obtained organically modified mesoporous silicas into aqueous solution of $Zn(NO_3)_2{\cdot}6H_2O$ ( with initial concentration 10,000ppm, pH ≈ 5) and left stirring for 24h. During this period, anchoring of $Zn^{2+}$ ions in specific sites (tertiary and primary amino groups) supplied by the hyperbranched polymer took place. Finally, solids were obtained by filtration, dried and calcined at 550°C for 5h at a heating rate of $1^{o}Cmin^{-1}$ under air flow for the PEI decomposition and the acquisition of ZnO loaded silicas. The obtained nanomaterials were characterized by TGA, XRD, SEM, EDX and $N_2$porosimetry.

The antibacterial activity of ZnO NPs supported on mesoporous SBA-15 and KIT6 was investigated against Gram (+) Staphylococcus Aureus bacteria as model microorganism Their minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of theses nanomaterials were determined by the broth dilution and colony counting methods according to M07-A9 and M26-A protocols issued by the Clinical Laboratory Standards Institute (CLSI), respectively.[7,8] On the other hand, their toxicity was evaluated on DU145 and PC3 human prostate adenocarcinoma cell lines, A549 human lung carcinoma cell line, and HEK293 normal human kidney cell line by MTT assays after 24h incubation time.

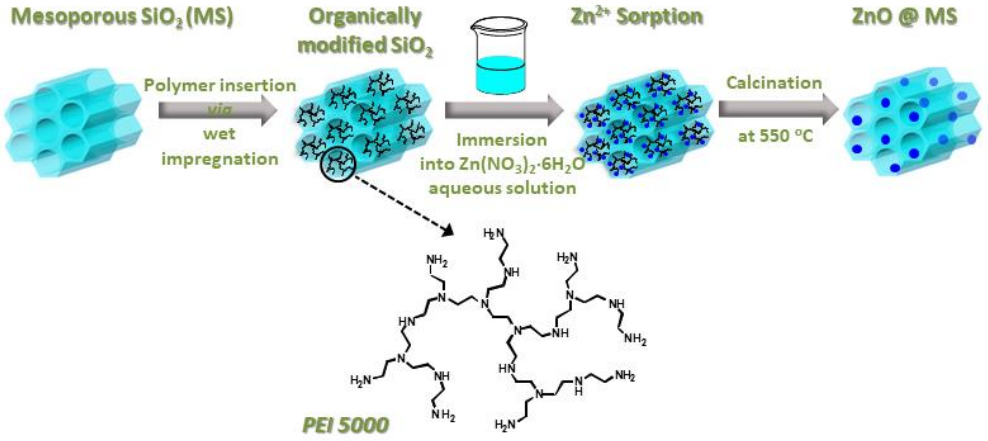


*Figure 1: Schematic illustration of ZnO NPs supported on mesoporous $SiO_2$ through Assisted Impregnation*

## 3. Results and Discussion

A hyperbranched polymer uptake of 17 and 27 wt.% in case of SBA-15 and KIT-6 respectively was determined by TGA technique, while polymer's presence was also verified by $N_2$ adsorption analysis. According to the values listed in Table 1, upon PEI addition a drastic drop in specific surface area and total pore volume was induced together with an increase in the average pore diameter, indicating the introduction of the polymer into the channels of the porous hosts. The same conclusion derives by comparing $N_2$ adsorption data of the parent and Zn loaded materials. SEM and EDS mapping analysis (Figure 2) reveals a very high uniformity and homogeneous spatial distribution of Zn species, without the detection of Zn rich domains or large ZnO aggregates. In addition, based on the values listed in Table 1, there is a good correlation between Zn and PEI loadings, with the highest Zn up-take, about 6 wt.%, attained for

KIT-6. Finally, as demonstrated by low and wide angle XRD patterns, Zn addition did not deteriorate the ordered mesostructure of the parent SBA-15 and KIT-6 materials, while the absence of peaks assigned to the ZnO crystal phase in the wide angle area evidences the high dispersion degree and the nanocrystalline nature of ZnO species.

*Table 1: PEI content, Zn loading, pore and structural properties.*

| Samples | PEI content (wt.%) | Zn loading (wt.%) | SSA ($m^2g^{-1}$) | TPV ($ccg^{-1}$) | Average Pore Diameter (nm) |
|---|---|---|---|---|---|
| SBA-15 | – | – | 537 | 0.49 | 3.6 |
| SBA-15_PEI | 17 | – | 223 | 0.28 | 5 |
| ZnO_SBA-15 | – | 2.6 | 511 | 0.56 | 4.4 |
| KIT-6 | – | – | 876 | 1.16 | 5.3 |
| KIT-6_PEI | 27 | – | 297 | 0.57 | 7.7 |
| ZnO_KIT-6 | – | 5.9 | 742 | 1.05 | 5.7 |

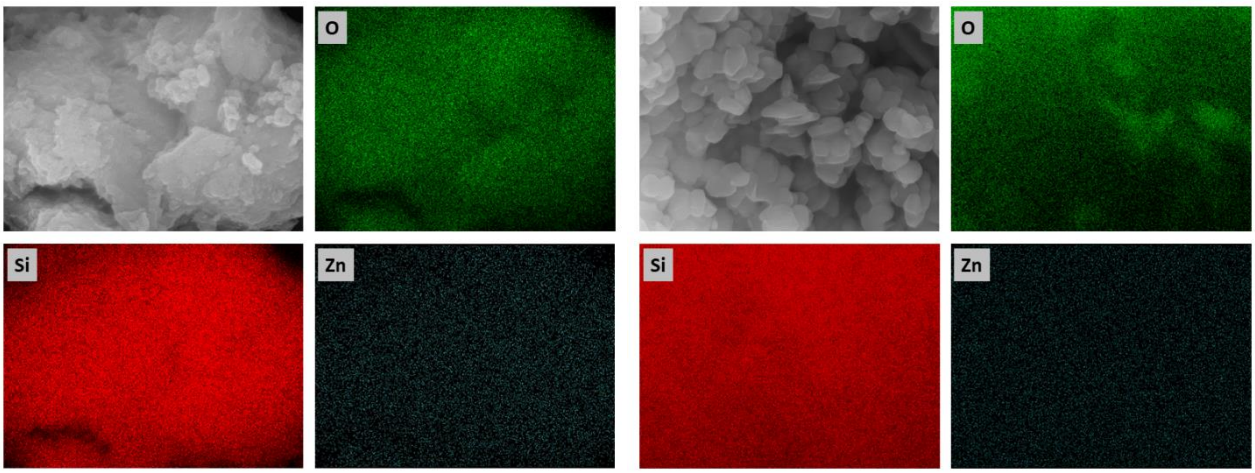


*Figure 2: SEM micrograph and corresponding elemental maps of ZnO_KIT-6 (left) and ZnO_SBA-15 (right)*

## 4. Conclusions

To conclude, a novel dendritic polymer templating strategy, called assisted impregnation, was employed for the development of highly dispersed ZnO species uniformly distributed within SBA-15 and KIT-6 mesoporous networks. As deduced, mesoporous host's pore structural characteristics affect polymer uptake and in turn the final Zn loading. Preliminary assessment revealed that the developed nanomaterials exhibited adequate antibacterial efficancy, at low concentrations (above 200 μg/ml), combined with low cytotocity.

## 5. Acknowledgements

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## 6. References

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