One more step against nanotoxicity: Hierarchical particles designed to antifungal properties

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HIGHLIGHTS

- Synthesis of hierarchical ZnO generates flower-like structures by a formation mechanism based on oriented aggregation
- The obtained ZnO hierarchical structure improves the antifungal activity
- The improved antifungal activity is due to low participation of chemical interactions favouring a purely physical mechanism

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:

Received 21 June 2017

Received in revised form 16 August 2017

Accepted 17 August 2017

Available online 19 August 2017

Keywords:

ZnO

Hierarchical structure

Antifungal activity

Abrasion interactions

ABSTRACT

The interest that awakens the ZnO as material is greatly extended both by its unique properties as by its wide variety of applications. Within these applications, antimicrobial activity is gaining relevance. Especially, the bactericidal activity of ZnO nanoparticles is outstanding but little is known about antifungal activity. In addition, the recent discussion about the potential toxicity of nanoparticles and their massive use places them in the spotlight. In response to this emerging problem, a simple and general approach to synthesize *hierarchical structure* of ZnO is reported. A formation mechanism of these surprising structures is proposed. Moreover, the obtained zinc oxide hierarchical particles improves the antifungal activity towards *Aspergillus niger* in contrast to ZnO micro or nanoparticles. Due to surprising results, the different action mechanisms of ZnO hierarchical particles against fungi are studied. Thus, we pose a novel physical mechanism by which ZnO hierarchical particles achieve excellent fungicidal results.

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1. Introduction

The great interest in research on ZnO like smart and versatile material, despite not being a new material, is because of its unique properties and wide range applications. Among its important properties stand out II–VI semiconductor due to a wide band gap energy of 3.37 eV and a large exciton binding energy of 60 meV [1,2]. Mainly, zinc oxide

presents a hexagonal *wurtzite* structure at ambient conditions. This unit cell has two lattice parameters, $a = 3.250 \text{ \AA}$ and $c = 5.207 \text{ \AA}$ and belongs to the space group $P6_3mc$ [3].

Another important factor as useful material is the morphological variety. ZnO has one of the greatest structural collections [4] among all known materials and can be classified in one- (1D), two- (2D) and three-dimensional (3D) structures. Some examples of 1D structure are nanorods [5,6], nanoneedles [7], nanowires [8] and nanobelts [9]. Most common 2D structures are nanosheet [10] and nanopellets [11]. Regard to 3D structures include cauliflower [12], snowflakes [13] and

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nanocages [14] among others. This assortment of morphologies is possible thanks to diversity synthesis processes reported in the literature such as aqueous solution growth, chemical vapour deposition, hydrothermal synthesis, sol-gel method and microwaves assisted synthesis [15,16].

Modulate size and shape of ZnO structures allows for adapting the product to the intended application. ZnO plays an important role in an extensive range of fields of technology like photocatalysis [17,18], solar cells [12,15], rubber additives, sensors [15,19] and cosmetic [20]. Moreover, ZnO presents an antimicrobial activity which catches the attention of researchers in recent times. The antimicrobial activity can be broken down into antibacterial and antifungal activity. About antibacterial activity that has been widely reported [21–23], one of the most important factors is the particle size. Yamamoto et al. [24] studied the influence of ZnO microparticles (ZnO MPs) on the antibacterial activity, while Padmavathy and Vijayaraghavan [25] reported the antimicrobial behaviour of ZnO nanoparticles (ZnO NPs) with different particle size, both proved that the antibacterial activity increased with decreasing particle size due to the higher surface area to volume ratio. Applerot et al. [26] confirmed the particle size dependence with efficient antibacterial activity, relating it to reactive oxygen species (ROS) and particle defects. However, antifungal activity has not been intensively studied. Recently, Lipovsky et al. [27] reported the effective antifungal activity of ZnO nanoparticles compared to micron sized that may be attributed to the reduced size and increase surface area to volume ratio. Meanwhile, Lin's group [28] investigated the effect of ZnO nanoparticles on fungal growth by SEM and Raman spectroscopy. To date, there are only a few studies about if size plays a relevant role in the antifungal mechanism. Therefore, there is no well-established reaction mechanism yet.

Over the past few years, the potential toxicity of NPs has provoked public and scientific discussions, and attempts to develop generally accepted handling procedures for NPs are under way. More concretely, the common utilization of ZnO NPs results in an increasing environmental release (estimated ~500 tons in 2009 in the U.S.) and may lead to adverse health effects [29]. The impact of nanotechnologies on everyday products could be more tremendous than expected. Within the nearly limitless diversity of the nowadays commercialize NPs, some happen to be toxic to biological systems, others are relatively benign, while others confer health benefits [30]. The question that nobody can answer today is related to *how the massive use nanoparticles will affect the living organism in a medium-large period?* Right now it is mandatory to think of how to avoid or minimize the harmful future impact and to strengthen the enormous benefit that nanoparticles could contribute to the society. Thus, we believe that the solution of the harmful collateral effect of nanotechnology must be addressed by hierarchical nanostructured particles, in which provides the advantages of nanoparticles would be preserved but harmful effect eliminated.

Herein, we pose a strategy for the in situ self-assembly of ZnO nanoparticles to build “*hierarchical structures*” with flower shaped, which are favoured by subsequent heat treatments. Using this *safe hierarchical structure* allows for improved antifungal activity without being required nanoparticles with high surface area to volume ratio and therefore its efficiency of use. To our knowledge these ZnO hierarchical particles are unique in two properties: size and SSA sufficient to be away from potential toxicity and an improvement of the fungicidal activity based on a physical mechanism.

2. Experimental section

2.1. Sample preparation

All the chemicals were directly used without further purification. To start the procedure, 3.6 mol urea ($\text{CO}(\text{NH}_2)_2$) was added to 5 M zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) solution under stirring at room temperature. The reaction was heated in an oil bath at 100–120 °C

and stirred at 300 rpm for 2 h. After naturally cooling to room temperature, hot water was added to obtaining a white suspension. Subsequently, the precipitate was isolated by filtration and washed with water and ethanol several times to remove impurities. At the end, the white powder product was dried at 80 °C for 24 h. To study the effect of temperature on the microstructure and application performance, annealing treatment at different temperatures between 350 °C and 700 °C was realized. The thermal treatments were performed under air atmosphere. The heating ramp has a rise of 1 °C/min and a cooling rate of 10 °C/min with a short dwelling time, 5 min, to the desired temperature. The experimental details are schematically shown in Fig. S1 of the ESI.

2.2. X-ray diffraction (XRD)

Crystalline phases were characterized by X-ray diffraction (XRD, X'Pert PRO Theta/2theta of Panalytical, Cu K_α radiation, PANalytical, The Netherlands), using CuK_α radiation. The patterns were recorded over the angular range 15–80° (2 θ) with a step size of 0.0334° and a time per step of 100 s, using Cu K_α radiation ($\lambda = 0.154056$ nm) with working voltage and current of 40 kV and 100 mA, respectively. The lattice parameters were refined by a global simulation of the full diagram using the *fullprof* program employing crystallographic information obtained from the International Center for Diffraction Data (ICDD) PDF-2 database in the form of card 36–1451 for ZnO. The crystallite size is calculated from the full width at half-maximum of the diffraction peaks by using Scherrer's equation.

2.3. Electron microscopy

The morphology of the powders was evaluated using primary electron images of field emission scanning electron microscopy (FE-SEM, Hitachi S-4700). The composition of the powders was estimated using Energy Dispersive Spectroscopy, EDS. In the specific case of ZnO particles with fungi evaluation by SEM, the samples were prepared under the same conditions as antifungal activity tests, except for the change in the culture medium, using distilled water. After that, an aliquot is deposited in a sample holder for analysis. The average particle size was determined from FE-SEM micrographs by an image processing and analysis program (Leica Qwin, Leica Microsystems Ltd., Cambridge, England) considering >200 particles in each measurement. To further understand the detailed morphology and crystal structure of the as-synthesized ZnO nanostructures, a 400 °C sample was evaluated using a JEOL 2100F transmission electron microscope (TEM/HRTEM) operating at 200 KV and equipped with a field emission electron gun providing a point resolution of 0.19 nm. For TEM sample preparation, the particles were carefully suspended in ethanol. The suspension was dropped on a copper TEM grid with carbon film support. The particles were kept at the grid after evaporation of ethanol.

2.4. Textural characterization

The specific surface area was determined by the BET method (SSA, Monosorb Surface Area Analyser MS-13, Quantachrome).

2.5. Chemical analysis

Zn^{2+} released was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Iris Advantage, Termo Jarrel Ash). For sample preparation, 10 g/L of ZnO (as-prepared and references) was added in 25 mL of peptone water (PW). The suspension was centrifuged and decanted obtaining a colourless dissolution. Data are expressed in concentration in oxide form.

2.6. Antifungal activity tests

As for antifungal activity, the pathogenic fungus *Aspergillus niger* (CECT 2807) was used for testing. Antifungal tests were performed by the Kirby-Bauer method (or agar diffusion method) with some modifications. First, the culture of *A. niger* (initial concentration of 5.20×10^7 spores/ml) was inoculated on the surface of Petri dishes. After, filter paper disks were impregnated with a suspension of different ZnO products with a concentration of 0.01% each one. The Petri dishes with the inoculums were then incubated at 37 °C for 3 days. The effectiveness of ZnO samples was evaluated by measuring the inhibition diameter of the grown fungus in the Petri dish and was compared with commercial references of ZnO. All tests were performed in triplicate and the values were expressed in millimetres. Finally, two commercial references of ZnO were chosen based on their size, micro and nanosized. MicroZnO was purchased from Asturiana de Cinc and nanoZnO was purchased from Evonik. To compare the results between synthesized samples and the reference materials, both commercial ZnOs were thermally treated at 500 °C. The morphology of the MicroZnO materials shows a typical ZnO morphology, consisting mainly of elongated prismatic particles and nearly hexagonal particles, with sizes of 0.2–1.0 µm and an average particle size 0.5 µm, see ESI Fig. S2a. Meanwhile, the nanoZnO is characterized by small spherical particles of 15–30 nm, which form globular agglomerates, see Figs. S2(b–c) of the ESI.

3. Results and discussion

3.1. Design and characterization of hierarchical structure

After annealing treatment of ZnO “hierarchical structure”, hereinafter ZnO hierarchical particles, a basic identification of the structure was performed using X-Ray Diffraction, XRD, (see Fig. S3 in the ESI). The position and intensity of diffraction peaks match to hexagonal wurtzite structure ZnO (JCPDS Card No. 36–1451), Fig. S3a (ESI). No other peaks are detected, which means no residual reactants or intermediates are present. No significant changes are detected in the XRD pattern after such thermal treatment. From the Fig. S3b (ESI), we can observe that the ZnO crystallite size grows moderately as temperature increases. In the case of lattice parameters, both *a* and *c*, remains practically constant throughout the temperature range between 350 °C and 700 °C.

Moreover, their values are in good agreement with the space group $P6_3mc$ confirming the ZnO hexagonal structure. Summarizing, these results indicate the successful synthesis of crystallized ZnO by *soft chemistry* without any secondary products.

The XRD technique gives structural information of the ZnO but yields no morphological information. In order to determine the morphology of the ZnO hierarchical particles, field emission scanning electron microscopy (FE-SEM) is made, Fig. 1. At lower magnifications (first row, Fig. 1a–e), we can see well dispersed agglomerates. These agglomerates have a spherical shape and a diameter about ca. 5 µm. From the high resolution scanning electron microscope (HR-SEM) images (second row of the Fig. 1f–j), we can see that the ZnO agglomerates consist of well-defined flower shaped particles with equivalent diameters of ~3 µm, which remains almost constant on the selected temperature range. Each flower is formed by a combination of 5 or more petals plus a centre that can be called “pistil”. A keen observation at higher magnifications (third row, Fig. 1k–o) shows in detail the centre of the flower. Pistil is composed of hexagonal platelets of 500 nm, approximately, typical of the wurtzite structure; see Figs. 1k–o. Moreover, some isolated nanoparticles around these hierarchical organizations can also be observed in Fig. 1, especially at low thermal treatments (see Figs. 1k and l). In the temperature range between 350 °C and 500 °C, these nanoparticles increase in size with the temperature, 30, 50 and 100 nm respectively (Fig. S4). As the temperature increases to 600 °C and 700 °C, the presence of nanoparticles is not observed. Therefore, the thermal treatment provokes the nanoparticles coalesce to build larger nanoparticles, which end up forming part of the flower-like structure. These facts allow us to build a mechanistic pathway for the formation of flower-like ZnO nanostructured.

3.2. How is it possible to form hierarchical micrometric flowers?

To further understand the detailed morphology and crystal structure of the ZnO hierarchical particles, a 400 °C sample was analysed by transmission electron microscope (TEM/HRTEM, 2100F, Jeol). Fig. 2a shows a TEM image of isolated flower shaped ZnO, selecting one of the multiple petals for its study. At high-magnification TEM images of an individual petal (see Fig. 2b–c), and a nanoparticle can be clearly found. Increasing magnifications, Fig. 2d displays a nanoparticle deposited on petal with very well-faceted crystals. In petals, their side faces have exposed

Fig. 1. Morphological characterization of the ZnO hierarchical particles with flower shaped at different temperatures: Low-resolution scanning electron microscope, LR-SEM, (a–e) and HR-SEM (f–o) micrographs of obtained ZnO after the annealing treatment at 350 °C (a, f, k), 400 °C (b, g, l), 500 °C (c, h, m), 600 °C (d, i, n) and 700 °C (e, j, o). Additionally, on the top of each image, the annealing temperature value is indicated. Scale bar of the panels a–e is 10 µm, panels f–j is 1 µm and k–o is 200 nm.

Fig. 2. Revealing the growth mechanism of the ZnO hierarchical structures with flower shaped: TEM (a–b) and HR-TEM (c–d) images of the thermally treated samples at 400 °C. From HR-TEM image (d), we can observe that one nanocrystal is fused to another larger nanocrystal (petal) during the crystal growth process. In addition, the interplanar distance and the crystallographic orientation of both, nanoparticle and petal, can be measured. Schematic view of a particle supported by the ZnO [0001] surface, in which is represented the stages of the growth mechanism based on orientation attachment. Scale bar of the panel a is 1 μm , panel b is 100 nm, panel c is 20 nm and panel d is 5 nm.

{1010} facets and are very flat, in good agreement with previous works [31,32], confirming that they are single crystalline and elongated along the [0001] axis. While on the nanoparticle, measured lattice spacing of adjacent lattice planes is about 0.26 nm, corresponding to the {0002} plane of wurtzite hexagonal ZnO, indicating different crystallographic

orientation between nanoparticle and petal. Fig. 2(d) further demonstrates oriented aggregation and a coarsening process whereby one nanocrystal is fused to another larger nanocrystal, in this case the petal, during the crystal growth process. The isolated nanoparticle collides and coalesces in the same crystallographic orientation that it

Fig. 3. Possible formation mechanism of flower-like ZnO hierarchical particles based on growth assisted by nanoparticles: the scheme illustrates the formation process of the ZnO hierarchical structures with flower shaped, which are characterized by four steps: (1) deposition stage, (2) coalescence stage, (3) growth stage, (4) stacking stage and (5) flower-formed stage. (A detailed explanation of the scheme can be followed by the text of the main body).

possesses the larger nanocrystal, [0001], which is the most thermodynamically stable direction on ZnO structure. This behaviour is favoured by the thermal treatment, which is in agreement with the results obtained in Fig. 1n–o for annealing temperatures between 600 and 700 °C. Based on the above results, we pose a simple model of hierarchical structures growth with flower shaped by orientation attachment (OA) [33,34] of nanoparticles and petals, where petals play as an orientation template (Fig. 2e). Thanks to this mechanism is possible to demonstrate oriented aggregation and a growth process whereby nanoparticles irreversibly attach to “template” in slightly oriented fashion to produce the petals.

From the perspective of the microstructure and keeping in mind the stages of posed growth in the Fig. 2, the experimental results observed here can be interpreted as follows. Firstly, a group of nanoparticles are sintered to form a nanoplatelet (see stage marked as 1 in Fig. 3). Through the diffusion mechanism, a new group of nanoparticles is deposited on the platelet to build another hexagonal layer (stage 2 of the Fig. 3). This behaviour can be seen at low temperatures micrographs (Fig. 1k and l), where nanoparticles are hierarchical organized. The stages marked as 2.1–2.3 on the Fig. 3 show, in detail, the formation of nanoplatelet by orientated attachment (OA). Nanoparticles are disoriented among them, so they rotate to match each other, (stage 2.1). An increase in the annealing temperature provokes that the agglomerates of the nanoparticles consolidate and coalesce (stage 2.2), leading to the formation oriented of larger nanoplatelets with the same crystallographic orientation of [0001]; stage represented by the points 2.3 and 3 in the Fig. 3. This crystallographic orientation, [0001], must be the same as formed nanoplatelet for correct aggregation, which is the most thermodynamically stable direction in ZnO. The growth continues with annealing treatments allowing the formation of new sheets becoming smaller (stage 4). Finally, this platelet stacking is part of the flower-shaped structure (stage 5). Since each platelets stack can be oriented differently from a common origin, i.e., vertical stacks build the pistil and horizontal stacks represent the petals. We can call this mechanistic pathway as epitaxial growth mediated by nanoparticles due to similarity with this stepwise growth and the main role of nanoparticles during the process. However, platelet distribution is not aligned growing, each level is randomly rotated to its predecessor as Fig. 1k–o displays at high magnifications getting screwed-like petals. Some authors attribute this screw character to spiral growth, which occurs during orientated attachment [35,36].

3.3. To obtain fungicidal activity, avoiding the potential toxicity of the nanoparticles

Keeping in mind the potential toxicity that implies the NPs utilization; an important aspect to consider, related to size, is the specific surface area (SSA). So, we calculated the minimum theoretical SSA of different nanoparticles morphologies (detailed explanation of calculation in ESI). Selected nanostructures are sphere, fibre and plate (see Fig. S5). The calculations allow dividing into two behaviours the specific surface area: Microscale and Nanoscale, depending on position above or below with respect to theoretical structures (see Fig. 4a). Then, specific surface area of samples is classified in these ranges. SSA of ZnO hierarchical particles is calculated by Brunauer-Emmett-Teller (BET) method. For comparison, two different sized commercial products are used in this study: microZnO and nanoZnO. These data are represented in Fig. 4a. As expected, SSA nanoZnO (23.9 m²/g) reference is located in the nanoscale behaviour. SSA of microZnO reference (4.6 m²/g) is placed close to microscale behaviour. To our surprise, SSAs of ZnO hierarchical particles (from 1.8 to 1 m²/g) are even lower than commercial microZnO. These samples are sited in microscale behaviour. In addition, the SSA evolution with the annealing treatment (blue arrow of Fig. 4a) reveals that at higher thermally treatment, specific surface area decreases. Thus, the results suggest that the platelets can be very compacted with each other, blocking some pores which are created on the synthesis. Hence, annealing temperature play an important role in

the compaction process of platelets, which allows tune SSA in samples. Another factor that may indicate the potential toxicity of ZnO is the Zn²⁺ cation released, so a chemical analysis of ZnO in solution was performed. Fig. 4b shows concentration of Zn²⁺ cation in dissolution for synthesized ZnO and references. NanoZnO presents the highest value (8 mg L⁻¹). Conversely, microZnO (0.7 mg L⁻¹) and as-prepared ZnO (<1 mg L⁻¹) have lower values than nanoZnO. A keen observation of flower-like ZnO annealed shows a Zn²⁺ released concentration with values close to the microZnO reference (see Fig. 4c). All these data are indicative that the nanostructured ZnO is potentially safer than the nanoparticles, regarding these parameters analysed.

In order to understand the behaviour of flower-like ZnO against fungus growth, Kirby-Bauer method is used. *A. niger* is cultivated on petri dishes containing ZnO hierarchical particles thermally treated and incubated at 37 °C for 3 days. As shown in Fig. 4d, ZnO affects the development of fungus. To better know how much more effective is prepared ZnO, different sized commercial references are used again. Fig. 4e shows the reduction of fungal growth for ZnO hierarchical particles, microZnO reference and nanoZnO reference. The averages of sporulation inhibition diameters are 24 mm, 27 mm and 34 mm for micro, nano and synthesized ZnO respectively (see ESI Fig. S6). The above results indicate that flower-like ZnO nanostructured increases the antifungal activity about 42% respect to microZnO and 26% to nanoZnO. For this reason, the ZnO hierarchical particles significantly inhibit the growth of *A. niger* being more effective than ZnO commercial products.

3.4. Looking for the main factors of antifungal activity

The detailed mechanism for the antifungal activity of ZnO is still under debate. However, previous studies show several factors that can affect the antimicrobial activity [37–39]. These factors can be divided into physical, chemical and a combination of them, shown schematically in Fig. S7. The most important physical interactions are electrostatic effect, abrasion of membrane envelope and penetration of particles through the cell. As to chemical interactions, there are two possibilities: reactive oxygen species (ROS) production and Zn²⁺ release. This led us to investigate what factors delimit antifungal activity of ZnO hierarchical particles. Some authors report the higher resistance of fungi to ZnO that may come from their less sensitivity to ROS [40,41]. However, the presence of Zn²⁺ cations can alter the respiratory chain of cells, causing their death [42]. A chemical analysis also serves to check the antifungal importance of Zn²⁺ released (Fig. 4b). The highest value of nanoZnO suggests a strong participation of Zn²⁺ in antifungal activity, and therefore, in this case the chemical interactions govern the antifungal activity. On the other hand, the low values of microZnO and as-prepared ZnO pose other action mechanisms like physical interactions. Nanostructured ZnO annealed below 500 °C displays a Zn²⁺ release slightly higher than the microZnO reference. Accordingly, at 350–400 °C there may be a very small contribution of these cations to the antifungal mechanism, as in the case of nanoZnO. Broadly, these results discarded chemical interactions like principal mechanism of flower-like ZnO. To know if physical interactions are present in the antifungal mechanism of ZnO hierarchical particles, a FE-SEM study is performed. The contact between spores and flower-like ZnO is observed in Fig. 5. As shown, the ZnO hierarchical particles favour the cracking (Fig. 5a), punctures (Fig. 5b) and rupture of the spore membrane (Fig. 5c). A possible explanation of this effect is the abrasive surface texture of ZnO caused by the uneven surface texture due to rough edges and corners [25]. These properties fit with the morphology of flower-like ZnO, giving rough surfaces which contributes to the mechanical damage of the membrane. To ensure correct allocation of spores, an EDS study is performed. The EDS mappings, represented in Fig. 5d–e, display the main atom of each structure, i.e. carbon for spores and zinc for flower-like ZnO. Turning to other physical factors, penetration of particles through the cell is not possible due to large size differences between synthesized ZnO and the pores of the membrane, as shown in Fig. 5.

Fig. 4. Comparison of ZnO hierarchical particles with respect to commercial references in several properties: SSA, leaching assay and antifungal activity against *Aspergillus niger*. The panel **a** represents specific surface area of commercial references microZnO (purple) and nanoZnO (yellow) with ZnO hierarchical particles (blue), which are marked with triangle, square, and circle symbols, respectively. In addition, there are two regions named as Microscale and Nanoscale that correspond to the different behaviour of the ZnO. Besides, this range is obtained from a theoretical calculation of SSA ideal structures: sphere, fibre and plate. The graphic **b** shows concentration of Zn^{2+} released into peptone water solution for micro- and nano-references and synthesized ZnO after thermal treatments. The panel **c** displays the behaviour of Zn^{2+} concentration against annealing treatments. The panel **d** shows antifungal activity of flower-like ZnO after annealing treatments. Sporulation inhibition diameters (SID) of commercial references and ZnO hierarchical particles are displayed in panel **e**. SID data are shown with mean values and standard errors. Each column represents an average of triplicate measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Therefore, after these results an action mechanism of flower-like ZnO against fungi is outlined. The ZnO hierarchical particle mechanism is fundamentally based on physical interactions. As shown in Fig. 6, the procedure can be divided into four stages. At first stage (Fig. 6.1), the ZnO hierarchical particles are approaching the spore until contact between them is reached. This is achieved because of the affinity between the two species, ZnO and fungi. Second step (Fig. 6.2) displays the first

consequences of ZnO hierarchical particle abrasion. The flower-like ZnO punctures the spore and causes cracking in the fungus membrane. After that, membrane damage like cracking, wrinkling, rupture, etc. causes leakage of intramembrane components (Fig. 6.3). In the last stage (Fig. 6.4), the spore ends “holey” by the abrasive action of ZnO hierarchical particles. All these membrane damage finally causes the fungi death. Moreover, ZnO hierarchical particles saves the flower-like

Fig. 5. Discovering ZnO hierarchical particles attacks to *A. niger* spores. The panel **a** shows cracked spore after contact with flower-like ZnO. The micrograph **b** displays a set of flower-ZnO particles pricking a spore. Panel **c** illustrates how flower-ZnO pulls off a piece of spore. The EDS mappings **d, e, f** correspond to micrographs **a, b, c** respectively. The principal component of spore is carbon (purple) and the Zn element of ZnO is represented by blue dots. Scale bar of HR-SEM micrographs and EDS mapping are 1 μm (**a, d**) and 2 μm (**b, c, e, f**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Revealing action mechanism of ZnO hierarchical particles against *A. niger* spores based on abrasive methods. The scheme illustrates the steps by which flower-like ZnO attaches spores; (1) contact between ZnO and spore, (2) cracking and pricking spore, (3) total or partial rupture of the spore and (4) spore “holey” that prevents the growth of fungus.

structure throughout the mechanism (Fig. 5a–c) keeping the micrometric behaviour. Hence, the *hierarchical structure* is available for new abrasion attacks, allowing a long-lasting antifungal activity.

4. Conclusions

In summary, we have synthesized flower-like ZnO nanostructured by soft chemistry providing an easy route and cost-effective process. The present study reveals a possible formation mechanism of flower shaped ZnO by nanoparticles diffusion with orientation attachment. The obtained ZnO hierarchical particles after annealing treatments show a SSA of micrometric behaviour. The low Zn^{2+} release in solution, is also another indicative of a very different behaviour than the nanoparticles. Furthermore, ZnO hierarchical particles enhance antifungal activity against *A. niger* respect to commercial references using mainly physical mechanisms as abrasion. Therefore, ZnO hierarchical particles generate a new concept of materials with a combination of micro and nanoparticles properties, reducing the potential negative effects of nanoparticles.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO) under projects MAT2013-48009-C4-1-P. The Spanish National Research Council (CSIC) under project NANOMIND CSIC 201560E068. Dr. F. Rubio-Marcos is also indebted to MINECO for a “Juan de la Cierva” contract (ref: JCI-2012-14521), which is co-financed with FEDER funds F. R-M is also indebted to MINECO for a ‘Ramon y Cajal’ contract (ref: RyC-2015-18626), which is co-financed by the European Social Fund. Finally, the authors are grateful to Mercedes Monte-Serrano, Santiago De-Bernardi and Javier Menéndez for antimicrobial activity test.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.matdes.2017.08.038>.

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