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Editorial

Chemistry and physics of metal oxide nanostructures
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Low-temperature ZnO atomic layer deposition on biotemplates: flexible photocatalytic ZnO structures from eggshell membranes

Seung-Mo Lee,‡a Gregor Grass,b Gyeong-Man Kim,c Christian Dresbach,d Lianbing Zhang,a Ulrich Göselea and Mato Knez*ad

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Macroporous ZnO membranes with a strong photocatalytic effect and high mechanical flexibility were prepared from inner shell membranes (ISM) of avian eggshells as templates after performing low-temperature ZnO atomic layer deposition (ALD). In order to evaluate the potential merits and general applicability of the ZnO structures, a comparative study of two membranes with coatings of either TiO2 or ZnO, processed under similar conditions, was performed. The study includes crystallographic features, mechanical and thermal stability and bactericidal efficiency. Both, the ZnO and the TiO2 coated membranes clearly exhibited bactericidal effects as well as mechanical flexibility and thermal stability even at relatively high temperatures. The ZnO membranes, even though prepared at fairly low temperatures (~100 °C), exhibited polycrystalline phases and showed a good bactericidal efficiency as well as higher mechanical flexibility than the TiO2 coated membranes. This study shows the benefits of low-temperature ZnO ALD i.e., the thermally non-destructive nature, which preserves the mechanical stability and the native morphology of the templates used, together with an added functionality, i.e. the bactericidal effect.

1. Introduction

During the evolution of biological creatures, numerous micro- and nanostructures with specific functionalities developed for adaptation to environmental conditions. Adoption of such structures by mimicking or templating came into focus of science in recent years.1 Functionalization of structures by coating biological templates is one of the methods to produce more stable organic or inorganic micro/nanostructures. So far those coatings have been performed mainly by chemical vapor deposition and sol-gel strategies on various biotemplates such as cellulose,2 wool,3 butterfly wings,4 superhydrophobic plant leaves5 and pine wood.6 However, these methods have some limitations in processing, such as occasional non-uniform coating of large templates or demanding film thickness control.6,7

As a promising method to overcome these processing limitations, atomic layer deposition (ALD) has recently attracted attention. Advantages of ALD are the conformal replication of 3D morphologies, large area uniformity, precise film thickness control on the nanometer scale and a wide range of operation temperatures.8–10 The feasibility of ALD for biological templates,11–13 as well as for organic materials14–16 has already been proven. However, to the best of our knowledge, so far ALD researchers have mainly focused their interest on the perfect coating of the fine structures of biotemplates with functional metal oxides such as TiO211 and Al2O3.11–13 Research, focusing on the optimal combination of the original functionality of the biotemplate itself and an appropriate metal oxide which can maximize additional functions of the resulting templated structures, has rarely been undertaken. Moreover, mechanical stability as a guarantee for easy handling and practical use has hardly been considered. Here, we present an example which satisfies the above requirements.

As an example for a temperature sensitive biotemplate an avian eggshell membrane (ESM, Fig. 1) was processed. Those membranes were already previously used as templates for sol-gel17–19 or further deposition methods.20–22 In our studies we used the macroporous inner shell membrane (ISM) which is a part of an avian ESM (Fig. 1). It prevents bacterial invasions, thus protecting the embryo.23–25 We deposited TiO2 or ZnO by ALD on this ISM, both of which show bactericidal photocatalytic effects under UV illumination (ISM/TiO2 and ISM/ZnO).26,27 We investigated the bactericidal properties of those membranes and characterized them quantitatively using a photocatalytic reaction which inactivated Escherichia coli (E. coli) bacteria. Both resulting membranes showed successful photocatalytic functionalization of the original ISM structure in line with good bactericidal effects. ZnO membranes, even though prepared at fairly low temperatures (~100 °C), showed polycrystalline phases and exhibited stronger bactericidal effects than TiO2 coated membranes. In addition, an improved mechanical stability of the ZnO coated membranes was observed.
3. Experimental

3.1 Preparation of the inner shell membrane (ISM) from a hen’s egg

Hen’s eggs were purchased from a grocery store. They were gently broken and the ISM around their air cell portion was carefully cut out and collected (Fig. 1a). The ISM was washed several times with deionized water in order to thoroughly remove the thin albumin layer, and were subsequently dried at room temperature for 4 h.

3.2 TiO$_2$/ZnO atomic layer deposition (ALD) on ISM

The prepared ISM was placed in the ALD chamber (Savannah 100, Cambridge Nanotech) and dried at 70 °C for 20 min in vacuum (1 × 10$^{-2}$ torr) with a steady Ar stream (20 sccm). For the TiO$_2$/ZnO deposition, well established ALD processes were applied. Titanium(IV) isopropoxide (Ti(OiPr)$_4$, TIP) and water$_2^{11,28}$ and diethylzinc (ZnEt$_2$, DEZ) and water$_2^{29,30}$ were used as precursors, respectively. The Ti(OiPr)$_4$ and ZnEt$_2$ were purchased from Sigma Aldrich. Each cycle was composed of a pulse, exposure and purge sequence for each precursor. For the TiO$_2$ deposition, for example, the TIP vapor was injected into the ALD chamber for 1.5 s (PULSE). Subsequently, the substrate was exposed to the TIP vapor for 30 s (EXPOSURE). The excess TIP was purged from the ALD chamber for 30 s (PURGE). In the same manner, the PULSE (1.3 s)/EXPOSURE (30 s)/PURGE (30 s) processes of H$_2$O were repeated. The thickness of the TiO$_2$ and ZnO films was adjusted by the number of cycles to 30 and 55 nm, respectively. For the preparation of diverse samples of TiO$_2$ and ZnO, the substrate temperature was varied between 70 and 300 °C. More detailed information on the applied ALD processes and sample denotations is given in Table 1.

3.3 Characterization

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were applied to investigate the size and morphology of the samples. The investigations were conducted using a JSM-6340F at 15 kV (SEM) and JEOL 2010 at 200 kV (TEM), respectively. The crystallographic features of the metal oxide membranes were investigated by X-ray diffraction (XRD, Philips X'Pert MRD) with Cu K$_\alpha$ ($\lambda = 1.5421$ Å) radiation. The transfer of the samples was done in air. For $\theta$ – 2$\theta$ measurements the samples were suspended on a silicon wafer as a convenient substrate.

3.4 Microbiology

As a test strain for all bactericidal effect studies, *Escherichia coli* (E. coli) strain W3110 was used. A single colony was inoculated from a Luria-Bertani (LB) agar plate (Carl Roth

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Fig. 1  Structure of an avian egg and scanning electron micrographs of an inner shell membrane (ISM) of a hen’s eggshell membrane (ESM). (a) Details of an avian egg (redrawn from ref. 23) and photograph of the ISM from ESM around the air cell of the egg. (b) Low magnification SEM of an ISM viewing from the direction of the blue arrow shown in (a). (c) Magnification of several ISM fibers, showing their highly interwoven and conglutinate feature.
Taking a 100 volume of solution 1 from the prepared stock solution and pippetted carefully into the reactor. The cell concentration of the Escherichia coli logarithmic growing culture for bactericidal effect experiments (E. coli solution 2). The resulting logarithmic growth phase population of E. coli ranged approximately from 10^5 to 10^6 colony forming units (CFU)/ml.

A reactor for the photocatalysis experiments was designed (Fig. 2). The Escherichia coli suspension was exposed to UV light (Osram UVC-LPS 9, peak: 365 nm, power: 2W, UV light including visible blue light) from the lower part of the reactor (polytetrafluorethylene). The sterilization was performed at high temperatures for each experiment. In order to reduce the UV absorption through the supporting part as well as to support the ISM, a PMMA (polymethyl methacrylate) sheet (1 mm thick) was used. Through the ring shaped gasket and the mechanical clamping (spring and bolt/nut type) the leakage of E. coli solution was effectively prevented.

3.5 Photocatalytic experiments of ISM/TiO2 and ISM/ZnO

A reactor for the photocatalysis experiments was designed (Fig. 2). The Escherichia coli solution was exposed to UV light (Osram UVC-LPS 9, peak: 365 nm, power: 2W, UV light including visible blue light) from the lower part of the reactor and was continuously shaken at 250 rpm to obtain a logarithmic growing culture for bactericidal effect experiments (E. coli solution 2). The cell concentration of the E. coli solution 2 was determined by the spread plate method.32 The initial logarithmic growth phase population of E. coli ranged approximately from 10^5 to 10^6 colony forming units (CFU)/ml.

Fig. 2 Schematics of the reactor for Escherichia coli (E. coli) photocatalysis experiments. The whole body was made from PTFE (polytetrafluoroethylene). The sterilization was performed at high temperatures for each experiment. In order to reduce the UV absorption through the supporting part as well as to support the ISM, a PMMA (polymethyl methacrylate) sheet (1 mm thick) was used. Through the ring shaped gasket and the mechanical clamping (spring and bolt/nut type) the leakage of E. coli solution was effectively prevented.

4. Results and discussion

4.1 Film quality, crystallographic features and bactericidal efficiency

Since the crystallinity of the deposited coating is temperature dependent,28,33 the composite membranes were prepared at various temperatures ranging from 70 to 300 °C. The resulting...
membranes, both ISM/ZnO and ISM/TiO₂, show unchanged morphological features of the original ISM. As representative SEM and TEM micrographs of the resulting ISM/ZnO and ISM/TiO₂ membranes, the images of ISM/ZnO/100 and ISM/TiO2/275 are shown in Fig. 3 (sample denotations can be found in Table 1; the reason that we choose these two samples as representative samples will be discussed in a further section). Both images show, as expected, good quality of the metal oxide deposition. The films were conformally deposited over the whole collagen membrane without any distortion and shrinkage, as can be confirmed from Fig. 3a, c and e showing fibers of ISM/ZnO/100 and Fig. 3b, d and f showing fibers of ISM/TiO2/275.

Apart from the deposited film quality, the bactericidal properties, as a function of the crystallographic features of the resulting macroporous membranes, are of interest. Fig. 4a and b show X-ray diffraction (XRD) patterns of the ISM/TiO₂ and ISM/ZnO prepared at temperatures ranging from 70 to 300 °C. The TiO₂-coated membranes (ISM/TiO₂) processed at 70 and 160 °C did not show any obvious diffraction peaks, whereas in the higher temperature range they showed reflections from the anatase phase (ICDD card No. 21-1272), anatase (101) being the strongest peak among them. The deposition temperature of 225 °C shows an onset of crystallization. In

| Table 2 | Maximum stress (MPa) and strain (%) value of Native ISM, ISM/TiO2/275 and ISM/ZnO/100 |
|-------------------------------------------------|
| Maximum stress ($\sigma_{\text{max}}$)/MPa | Maximum strain ($\varepsilon_{\text{max}}$)/% |
| Native ISM | 6.21 ± 0.62 | 6.18 ± 0.52 |
| ISM/TiO2/275 | 6.02 ± 0.25 | 3.45 ± 0.43 |
| ISM/ZnO/100 | 9.09 ± 0.71 | 9.02 ± 0.83 |

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Fig. 3 Electron micrographs of ISM/ZnO/100 and ISM/TiO2/275. Parts (a) and (b) display a macroscopic view of the ISM/ZnO/100 and the ISM/TiO2/275 membrane, respectively, showing the morphology of the native ISM (their highly interwoven and conglutinate feature). Parts (c) and (d) show the composite membranes of collagen fibers/metal oxides at high magnification. Parts (e) and (f) display the corresponding transmission electron microscope (TEM) images of the ISM/ZnO/100 and ISM/TiO2/275 membrane, respectively, showing the coating of the fibers with the metal oxide.
contrast, the ZnO coated membranes (ISM/ZnO) showed diffraction peaks already after the 70 °C ALD deposition process. The positions and the intensities of the individual peaks are in good agreement with the hexagonal wurtzite structure of ZnO according to JCPDS card No. 36-1451.

The photocatalytic efficiency (bactericidal effect) was evaluated from the E. coli survival ratio with respect to the illumination time with UV light for the two different types of metal oxide membranes. It is generally known that semiconducting metal oxides, such as ZnO and TiO2 generate conduction electrons (e−) and valence band holes (h+) on the surface upon illumination with an energy higher than the band gap energy (Eg,ZnO = 3.37 eV, Eg,TiO2 = 3.2 eV) in an aqueous solution.26,27 Subsequently, holes react with the water adhering to the surface of the ZnO and TiO2 to form highly reactive hydroxyl radicals (OH•). Oxygen acts as an electron acceptor forming super-oxide radical anions (O2•−) which are an additional source of hydroxyl radicals upon subsequent formation of hydrogen peroxide (H2O2).26,27 The generated OH•, O2•− and H2O2 can attack the cell walls in E. coli, which will finally be damaged. After eliminating the protection of the cell wall, oxidation of the underlying cytoplasmic membrane and the intracellular contents takes place and eventually leads to the death of the E. coli.34 As illustrated in Fig. 4c and d, in the dark without ZnO and TiO2, the survival ratio was constant or slightly increased due to the natural replication of the E. coli. Under UV illumination both membranes clearly showed the capability to inactivate E. coli in aqueous solution. In agreement with previous literature, it was observed that the bactericidal effect of ZnO and TiO2 has a stronger dependence on the crystallinity than on the film thickness.35,36 Specifically, the bactericidal efficiency of the ISM/TiO2 with anatase/rutile phases and the ISM/ZnO with the hexagonal phase revealed a proportional relationship to the ALD deposition temperature and the relative intensity of the (100) crystal direction, respectively.

Fig. 4 X-Ray diffraction (XRD) patterns and E. coli survival curves related to ISM/TiO2 (upper row) and ISM/ZnO (lower row). (a) and (b) XRD patterns of both membranes illustrating the effect of the deposition temperature. (c) and (d) E. coli survival curves in aqueous solution over time corresponding to each membrane after the UV illumination for various deposition temperatures of TiO2 and ZnO, respectively.
The membrane itself has a strong impact on photocatalytic behavior. From the respective E. coli survival curve, it can be seen that, in terms of bactericidal efficiency under UV illumination, the ISM without metal oxide coating is more effective than just the suspension only, i.e., without a membrane. The metal oxide coating enhances the efficiency drastically. Comparing the bactericidal efficiency of the coated membranes with TiO$_2$ particles (Degussa P25, average particle size: 30 nm$^{37}$ or home-made TiO$_2$ films$^{38}$ reported in the literature, the ISM/TiO$_2$/300 is much more efficient when considering the irradiation intensity and the area exposed to UV light. Similar to TiO$_2$, the ISM/ZnO/100 also shows higher efficiency, as compared to ZnO powder$^{39,40}$ with a much larger surface area. Presumably, this is caused by the macroporous structure of the ISM. Probably, E. coli bacteria can easily adhere to the macroporous ISM, leading to an increased concentration of bacteria close to the UV source. Thus more bacteria are destroyed in the same time period. It is noteworthy that ISM/TiO$_2$ reveals a higher bactericidal efficiency than ISM/ZnO. This result is consistent with a previous publication comparing the photocatalytic efficiency of TiO$_2$ and ZnO$^{41}$; however, opposite results have also been reported.$^{42,43}$ The relative photocatalytic behavior of TiO$_2$ and ZnO still seems to be ambiguous.

The graphs in Fig. 4c and d show that, as expected, ISM/TiO$_2$/300 has the strongest bactericidal effects. However, with decreasing processing temperature, the efficiency of the TiO$_2$-coated membrane also decreases. Already at 275 °C (ISM/TiO$_2$/275), the efficiency is comparable to that of the ZnO-coated one, processed at much lower temperatures (ISM/ZnO/100). Therefore, for the strongest effects, one has to coat the membrane at a high temperature, thereby somewhat damaging the morphology of the original ISM. Since most biological templates have a tendency to decompose or deform at high temperatures (pyrolysis temperature $\approx$240 °C)$^{44}$, a deposition at such temperatures is not suitable in most cases. A good compromise can be found if the sensitive membranes are coated with ZnO at 100 °C, showing reasonably efficient photocatalytic behavior. Thus, in terms of bactericidal efficiency as well as preservation of the original morphology, the ISM/ZnO is more beneficial than ISM/TiO$_2$.

4.2 Mechanical flexibility and thermal stability

Even though the ESM is stable against the reaction byproducts of the ALD process (e.g., isopropanol)$^{24,25}$ as stated above, upon heating (around 240 °C) it undergoes pyrolysis.$^{44}$ For coating of the ISM with ZnO, the pyrolysis is not a critical issue, the highest photocatalytic efficiency and preservation of the original structures of ISM can be assured by virtue of low processing temperatures ($<240$ °C). In contrast, in the case of TiO$_2$, due to the required higher processing temperature ($>240$ °C), even though the photocatalytic efficiency can be assured, the mechanical stability was reduced by the pyrolytic damage to the original structures of the ISM.

The results from our experiments are shown in Fig. 5 and Table 2. The resulting ISM/TiO$_2$/275 membranes deposited at 275 °C were more brittle and stiffer than the native ISM (decreased maximum strain $\epsilon_{\text{max}}$ and increased initial Young's modulus, $E_{\text{init}}$(ISM/TiO$_2$/275)). In contrast, the ISM/ZnO/100 membrane showed an even higher flexibility and mechanical stability against external load than the native ISM (increased maximum stress $\sigma_{\text{max}}$ and strain $\epsilon_{\text{max}}$) together with increased initial Young’s modulus $E_{\text{init}}$(ISM/ZnO/100)). Considering that most of the metal oxides are generally brittle even at nanometer thicknesses$^{45}$, the ISM membranes after ALD are expected to show lower flexibility and stability to external load, if the contribution of the metal oxide layer itself and thermal effects are considered. However, this is not the case. It is known that the collagen based ESM contains functional groups, such as amines, amides and carboxylates$^{24,25}$ which may interact with the ALD precursors (TIP or DEZ/water) during the deposition. During the alternating exposure/purge sequence of the ALD precursor pairs, the highly reactive precursors chemically interact with the ISM fiber surface as well as the bulk of the protein structures (results will be published elsewhere). Hence the flexibility of those composite membranes can presumably be ascribed to anchored precursors containing metal compounds, such as Zn or Ti, similar to the mechanical properties enhancing effects of insect’s cuticles by small amounts of impregnated metals.$^{36}$

5. Conclusions

In conclusion, eggshell membranes were used as templates for coatings with TiO$_2$ and ZnO, respectively, via ALD. The resulting structures satisfy the optimal combination of original functionality of the biotemplate and appropriate metal oxides which can improve the functionality of the resulting structures. The membranes show good mechanical flexibility for practical use. Depending on the deposition temperature of the metal oxides, the resulting films were either amorphous or polycrystalline. Upon UV illumination, the ISM/TiO$_2$ and
ISM/ZnO membranes clearly exhibited bactericidal effects. Above all, it was found that polycrystalline ISM/ZnO membranes can be prepared at a fairly low temperature (100 °C) and nevertheless show bactericidal efficiency which is competitive with that of ISM/TiO$_2$ membranes prepared at a much higher temperature (275 °C).Furthermore, the ZnO-coated membranes are mechanically more stable than the TiO$_2$-coated ones. We conclude that for coatings of diverse temperature sensitive templates (such as biological materials or polymers), low-temperature ALD of ZnO is more suitable than the deposition of TiO$_2$, i.e. thermally less destructive and photocatalytically competitive.

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