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Morphological control and assembly of zinc oxide using a biotemplate ☆

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Abstract

Zinc oxide is a wide band gap material that has significant applications in photovoltaics, piezoelectrics and optoelectronics. Traditionally, ZnO has been synthesized using high temperatures and harsh reaction conditions. Recently, benign reaction conditions have been used to synthesize ZnO using amine and citrate additives. In this study, peptide phage display was performed to identify a peptide, termed Z1, that binds to and directs the growth of ZnO hexagonal nanocrystals. By altering the concentration of Z1 peptide, the ZnO nanocrystal morphology can be tailored. Additionally, Z1 peptide was used to direct the growth of ZnO structures on free-standing silk films. The results presented here demonstrate the utility of peptides in controlling the structure and deposition of ZnO.

Keywords: Peptide; ZnO; Silk; Phage display

1. Introduction

Zinc oxide is an important industrial material due to its many inherent properties. It is a wide band gap semiconductor that is piezoelectric, conductive, fluorescent and has catalytic activity (for reviews see [1,2]). These properties make it useful in a variety of applications such as solar cells [3], phosphors [4], piezoelectric transducers and actuators [5], chemical sensors [6] and photocatalysis [7]. The crystal structure and macroscopic morphology of the ZnO crystals dictates their properties and, thus, the applications for which they can be used. The ability to tailor both the crystal structure and morphology (the aspect ratio and the overall crystal shape) would allow ZnO crystals to be tuned for specific applications.

Based on the reaction conditions used in the synthesis of ZnO structures, nanorods, nanowires, nanorings, nanobelts and nanosprings, among others, can be formed [2]. The temperatures for thermal sublimation processes fall roughly between 400 and 1400 °C based on whether a catalyst or other additives are present in the reaction.

A variety of techniques have been used to grow ZnO. Wet chemical methods are used to synthesize crystalline ZnO materials in a variety of morphologies. Using these methods, ZnO crystals can be grown in an aqueous environment in the presence of amines, commonly 1,3-hexamethylenetetramine (HMTA) [8,9] or poly(alcohol)-amines [10]. These reactions have been performed both in solution [8,10] and on flexible polymers or films [9,11], and are characterized by their relatively mild reaction conditions, including low temperatures (less than ~70 °C) and near neutral pH. The addition of citrate to ZnO synthesis reactions tailors the growth of the resulting nanocrystals from nanorods to hexagonal microplates [9].

In the past decade or so, significant efforts have been focused on the directed mineralization of inorganic materials using biological templates. The paradigm for "biomineralization" has been based on the study of diatoms and marine sponges, which are marine organisms that form intricate silica structures using families of proteins. Kroeger and colleagues have led the way on the study of

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the diatom protein family sillafins [12], and Morse and co-workers focused on the silicatein protein family from marine sponges [13]. Both these groups and others have shown that isolated proteins, and even peptides based on those proteins, precipitate silica from silicic acid precursors [12–16].

Many groups have subsequently used phage peptide display to identify peptides that bind to and precipitate inorganics under benign reaction conditions, including neutral pH, low temperature (4–70 °C) and aqueous solutions [17–21]. Inorganics as diverse as silica [17], titanium dioxide [22], cobalt–platinum [23], gold [21], silver [18], iron oxide [24] and calcium molybdate [25] have been templated using peptides and proteins.

Peptide-directed growth of zinc nanostructures has been reported [26]. Umetsu and co-workers used phage peptide display to identify a dodecamer peptide from a combinatorial library that bound specifically to ZnO crystals. That study concluded that the identified peptide assisted the growth of flower-like ZnO structures. In our study, phage peptide display also was used to screen for peptides that bind to ZnO crystals and we identified a nearly identical peptide to the previous report [26], which we have termed Z1. Instead of using zinc hydroxide as the precursor material [26], we used zinc nitrate and HMTA as precursors in combination with Z1 peptide to tailor ZnO crystal growth. The result is the biodirected growth of twinned, hexagonal ZnO nanoplatelets of variable aspect ratio that retain both the crystal structure and fluorescence character of the control ZnO hexagonal bipyramid nanoparticles. The Z1 peptide was used to direct the growth of ZnO on a biopolymer film.

2. Materials and methods

ZnO nanopowder, zinc nitrate hexahydrate ($Zn(NO_3)_2$) and HMTA were purchased from Sigma–Aldrich (St. Louis, MO). Z1 peptide was synthesized by New England Peptide (Gardner, MA).

Panning using the PhD 12 phage peptide display kit (New England Biolabs, Ipswich, MA) was performed against ZnO nanopowder as described previously [17]. Briefly, ZnO was washed 10× in TBS + 0.1% Tween-20 (Sigma–Aldrich) followed by a 1 h room temperature incubation with 10 μl of phage library in 1 ml of TBS + 0.2% Tween-20 (TBST). The ZnO was washed 10× in TBST and the phage that were bound to the ZnO were eluted in 0.2 M glycine buffer, pH 2.2, and the solution was quickly neutralized with 0.1 vol. of 1 M Tris–HCl, pH 9.0. The eluted phage solution was titrated before and after amplification. Four subsequent rounds of panning were performed, with increasing stringency of Tween-20 in each round. After rounds 3 and 5, individual phage plaques were picked for DNA sequencing. A consensus sequence was identified in the fifth round, and the peptide was named Z1. Following the literature report of a similar peptide [26], a Gly-Gly-Gly-Cys tail was added to the C-terminus of the peptide.

$Zn(NO_3)_2$ and HMTA (100 mM each in water) were used as stock solutions for the mineralization reactions and the Z1 peptide stock solution was prepared at a concentration of 50 mg ml^{-1} in water. Unless otherwise indicated, Z1 was mixed at a final concentration of 0.5 mg ml^{-1} with equimolar concentrations of $Zn(NO_3)_2$ and HMTA. Samples were incubated at room temperature for 24 h, followed by a 72 h incubation at 65 °C [8]. The Z1 peptide did not affect the pH of the reaction solutions. Before the 65 °C incubation, the pH was 6.5 for all Z1 peptide concentrations. After the 65 °C incubation, the pH was 6.2 for all Z1 peptide concentrations. The precipitate was washed 3× in water and resuspended in the original reaction volume. Scanning electron microscopy (SEM) analysis of the samples was performed with a FEI XL30 eSEM field emission gun (FEG) microscope in high-vacuum mode using a secondary electron detector. For transmission electron microscopy (TEM) analysis, samples were cast from suspension onto amorphous carbon-coated grids. A Phillips CM200 FEG microscope operating at 200 kV was used and images were collected on a cooled CCD camera. X-ray diffraction (XRD) was done using a Rigaku CuK_α source, a Statton camera, image plates and a Fuji image plate scanner. Fit2D software (Hammersly) was used to integrate the two-dimensional X-ray patterns. For fluorescence micrographs, the samples were excited with a 100 W mercury lamp (Chiu Technical Corp, Kings Park, NY) and imaged with an Olympus BX51 microscope (Olympus Optical Co. Ltd, Japan) using $\lambda_{EX} = 365$ nm.

As previously described, silk fibroin was purified and dissolved in the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIC; io-li-tec GmbH and Co., Denzlingen, Germany) [27]. Briefly, silkworm cocoons were soaked at 3.3% (w/v) in a solution of 8 M urea, 40 mM Tris–SO_4 and 0.5 M mercaptoethanol and heated to 90 °C for 1 h. The silk fiber was then extensively washed with ultrapure distilled water (18 MΩ cm resistivity) and dried overnight under vacuum. Dried silk fibers were subsequently dissolved in BMIC to form a 10% (w/w) solution. In order to lower the viscosity and melting point of the solution, 25% (w/w) water was added to the solution. The final composition of the solution is 7.5% (w/w) silk fibroin, 25% (w/w) water and 67.5% (w/w) BMIC. Silk films were cast from this solution by spin coating at 3000 rpm for 1 min. The film was immediately crystallized in a bath of methanol for 5 min and freed from the substrates. The free-standing silk films were incubated with 0.5 mg ml^{-1} Z1 and equimolar concentrations of $Zn(NO_3)_2$ and HMTA as described above for the solution reactions. Control reactions contained silk film, but no Z1 peptide. The films were washed extensively in water with perturbation to remove any ZnO crystals not bound to the silk film and were characterized using SEM, TEM, XRD, electron diffraction and optical microscopy as described above.

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