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$(Ti,Sn)O_2$ Solid Solution Self-Aligned into "Sandwich" Array on Grafted Modification Collagen Matrix**

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Monolayer and multilayer films with morphology-controllable structures are promising for applications in building novel sensor devices, light-emitting diodes, biomedical coatings, and in creating organically based nonlinear optical materials.[1] Various approaches, including spin-coating, the Langmuir–Blodgett technique, electrostatic adsorption of oppositely charged polyelectrolytes, and covalent attachment of polymers using conventional coupling chemistry, have been used to produce complex nanostructures and have been developed for preparing different hybrids with diversified morphologies.[2] The general utility of these methods has been further extended by incorporating nanocrystals into polymer matrices.[3] The versatility and complexity of these polymeric composites provide new opportunities in the semiconductor, photovoltaic, and molecular electronic fields.[4] However, it is still important to study thin-film preparation in various media and with a variety of modification agents.[5]

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Incorporating metal nanocrystals in biological systems is a widespread, yet incompletely understood, procedure, involving complex interactions at the biomacromolecule–metal nucleus interface.[6] Studying this process may help us to understand and control the formation of metal nanocrystals in a generalized peptide–amphiphile chain system. Two major approaches have been developed to organize metal nanoparticles into polymer substrates.[7–12] The first method involves the self-coding of nanoparticle building blocks that can be coupled via interparticle connectors with specific recognition properties based on, for example, DNA duplex formation,[7] antibody–antigen specificity,[8] streptavidin–biotin coupling,[9] electrostatic matching,[10] or shape-directed hydrophobic forces.[11] Another method, the template-directed approach,[12] utilizes porous solid substrates or discrete liquid droplets as patterned or shaped interfaces for the assembly of preformed nanoparticles. In this process capillary, drying, and swelling forces, or other chemical interactions, are used to implant, position, and immobilize nanoparticles irreversibly within the template or around the surface of individual latex beads pre-coated with a layer-by-layer shell of oppositely charged polyelectrolyte macromolecules. However, to the best of our knowledge, there has been no previous example of incorporating bimetal solid solutions into a biological system in order to form a well-defined morphology without using any template or specific recognition interparticle connectors.

Herein, we present a versatile procedure, based on the self-assembly process, for preparing a large-area nanoscale "sandwich" layer array of tailored composition, with a controllable crystalline phase and defined morphology. The idea for creating this composite film was stimulated by Yang and Rubner,[13] who developed a novel assembly method by using hydrogen-bonding interactions to fabricate a polyelectrolyte multilayer film. Differing from their work, we employed a protein with a unique tertiary structure, collagen—whose stalks consist of right-handed supercoils of three left-handed polyproline II-type helices with major sequences of $(Gly–Pro–Hyp)_n$[14,15]—to design and synthesize an ordered biological scaffold, and to control the growth of various metals in an exact arrangement, with high reproducibility and accuracy, by using two uniform interactions of both hydrogen bonding and covalent bonding between the biomatrix and the nanocrystals.[16] This strategy combines organic modification with metal-assisted stabilization of collagenous triple helices in order to achieve an ordered assembly architecture, which is distinctly different from the previous methodologies, such as those used by Koide and co-workers[14,17] and Babu and Ganesh, to modify collagen.[15] A model of multiple spatial interactions (covalent bonding and hydrogen bonding) existing in this system is shown in Scheme 1. These two tethering forces allow a certain degree of mobility and an enhanced long-term periodicity. In particular, this synthetic procedure was carried out without heating or adding any organic surfactant or direction-guiding agent. The film was formed in an aqueous system at a relatively low temperature, in a simple and rapid process. Unlike ordinary Langmuir–Blodgett (LB) films, the prepara-

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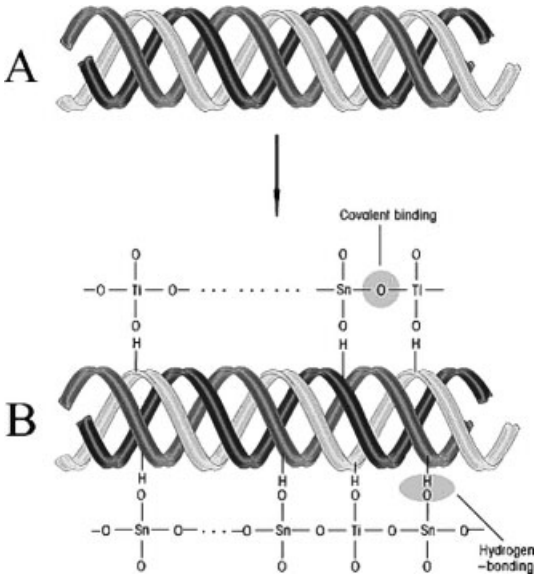


Scheme 1. A) Grafted collagen triple helices, and B) multiple spatial interactions (covalent bonding and hydrogen bonding) model of $(Ti,Sn)O_2$ self-assembly into grafted collagen matrix. The strong attachment of nanoparticles onto the substrate fragment is caused by interactions between the hydrogen atom of the hydroxyl group in the peptide chain and the oxygen atom in the $(Ti,Sn)O_2$ molecules. The covalent bonding promotes the linkage of adjacent TiO_2 or SnO_2 molecules.

tion of this hybrid nanocomposite film did not need any special apparatus (see Experimental). We propose that the structure described herein, assembled with a peptide–amphiphile backbone, is a novel way to produce metal solid solutions and provides new opportunities for their applications in areas such as photonics, photoelectronics, and optics.

The high-resolution transmission electron microscopy (HRTEM) image of $(Ti,Sn)O_2$ nanoparticles (Fig. 1A) shows small particles with a fine spherical shape, good dispersion, and uniform size, with an average diameter of 6 ± 2 nm (see Supporting Information). After the nanoparticles were incorporated into the grafted collagen matrix by refluxing for six hours, the obtained hybrid composite showed a "sandwich" layer arrangement. In the control—in absence of the grafted collagen matrix—only agglomerated nanoparticles with disordered shapes were obtained (Fig. 1B). When the collagen was replaced with other proteins, such as human serum albumin (HSA), bovine hemoglobin (Hb), and myoglobin from horse heart (Mb), to prepare the grafted matrix, the TEM images of the obtained composites also showed random, agglomerated structures (Fig. 2). These results demonstrated that the grafted collagen matrix played an important role in the formation of the ordered array structure, due to its unique and uniform interaction with the $(Ti,Sn)O_2$ nanoparticles. The spacing between the obtained nanoscale layers was as small as 20 nm, and the lateral distance was about 3–4 μm. The electron diffraction (ED) pattern (inset in Fig. 1A), taken from the [111] surface, indicates an ordered arrangement at the axis, which is strong evidence of the ordered structure of the sample.

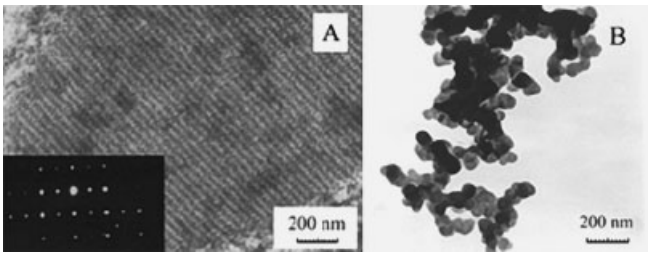


Figure 1. HRTEM micrographs of the "sandwich" array of the multilayer film prepared A) in the presence of a grafted collagen matrix, with refluxing for 6 h; B) the composite prepared without the grafted collagen matrix. Inset: the corresponding ED pattern.

The X-ray diffraction (XRD) pattern shows remarkable reflection associated with the formation of small-sized particles (Fig. 3). The peaks at 2θ values of 21.1°, 28.2°, 45.1°, and 60.6° correspond to the reflection of Sn [101], [111], [311], and [220], respectively; the peaks at 2θ values of 30.0°, 34.9°, and 50.8° are attributed to Ti [200], [220], and [222], respectively. These features indicate the presence of a two-dimensional lamellar structure of bimetal crystals, which is consistent with the electron diffraction result. The Ti [200] peak gave an interplanar spacing of $d = 3.04$ Å. The crystallite size of $(Ti,Sn)O_2$ particles was determined to be 6.2 nm from the full width at half maximum of each reflection using Scherrer's formula. This value is in good agreement with that estimated from HRTEM image.

A thermogravimetry–differential thermal analysis (TG-DTA) study of the obtained hybrid composite with a 14.60 % $(Ti,Sn)O_2$ content is shown in Figure 4. The TGA curve indicates that the composite possesses outstanding thermal stability: no appreciable mass change was observed until 160 °C, whereas commercial collagen is stable to only 39 °C.[18] The improved thermal stability results from the reduced mobility of the collagen peptide chains in the nanocomposite, which inhibited the chain transfer reaction, thus slowing the degradation process.[19] The DTA curve showed a single endothermic peak at about 385 °C. This result clearly indicates a strong and uniform interaction between the grafted collagen matrix and the $(Ti,Sn)O_2$ nanoparticles.

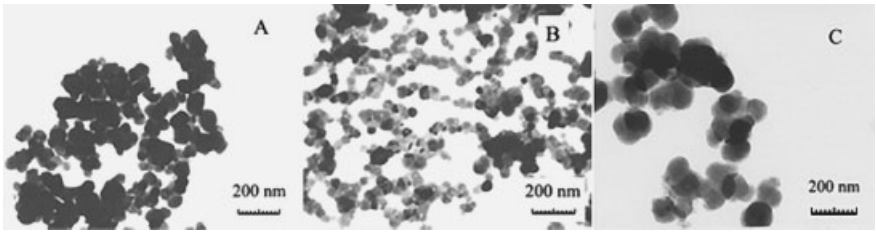


Figure 2. TEM micrographs of the composites prepared with $(Ti,Sn)O_2$ nanoparticles and grafted A) HSA, B) Hb, and C) Mb (matrices with reflux for 6 h).

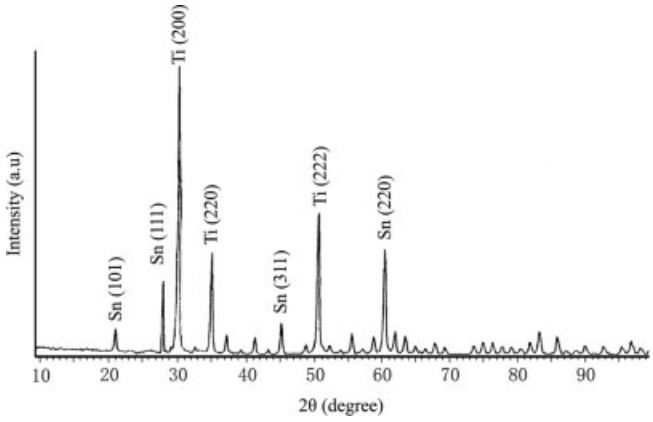


Figure 3. XRD pattern of the ordered array composite.

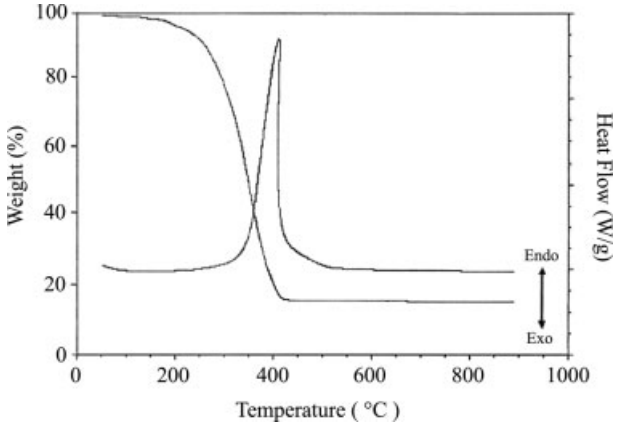


Figure 4. TG-DTA curves of the hybrid composite with a $(Ti,Sn)O_2$ content of 14.60 %.

In order to optimize the reaction conditions, the arrangement of $(Ti,Sn)O_2$ nanoparticles on the grafted collagen matrix was first investigated as a function of the component ratio (matrix/nanoparticle = 4:1, 1:1, 1:4, and 1:10, w/w), temperature (50–70 °C), and reaction time (4–12 h). A component ratio of 4:1 resulted in a disordered, heterogeneous nucleation of $(Ti,Sn)O_2$ on the matrix chain. As shown in Figure 5A, a compactly and disorderly stacked sphere structure was formed after refluxing at 70 °C for only 4 h. These particles displayed a slight aggregation phenomenon, with a diameter of about 10 nm and a narrow particle size distribution. At a low component ratio of the grafted collagen matrix (1:1), a similar thick film could be obtained after a longer reaction time (6 h, Fig. 5B) at 70 °C. The image shows ordered morphology in local areas. Further decreasing the matrix content resulted in a highly ordered multilayer structure being obtained after 6 h, at a temperature as low as 60 °C, similar to the growth of TiO_2 on an α-MoO_3 substrate (Fig. 5C).[20] At doping quantities of $(Ti,Sn)O_2$ of more than 8:1, the deposition of $(Ti,Sn)O_2$ on the grafted collagen matrix again became poorly ordered (not shown here). Thus, it can be concluded that the ratio of matrix to $(Ti,Sn)O_2$ is a key factor in the self-alignment process. A plausible reason for this is that the substrate offered a limited density of binding sites; the immobilization of $(Ti,Sn)O_2$ was initiated at these limited locations. At a low composition of $(Ti,Sn)O_2$ nanoparticles, these particles could not be arranged onto all the neighboring positions in the peptide chain, leading to a disordered structure. Furthermore, when there were more $(Ti,Sn)O_2$ nanoparticles than binding sites, the redundant nanoparticles resulted in a disordered deposition.

The self-alignment that occurs during the reflux process for the growth of the lamellar thin film is shown in Scheme 2. At the beginning, $(Ti,Sn)O_2$ nanoparticles self-assemble on the surface of the one-dimensional collagen peptide chain to form

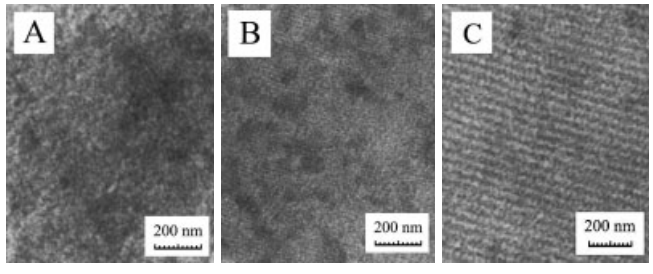
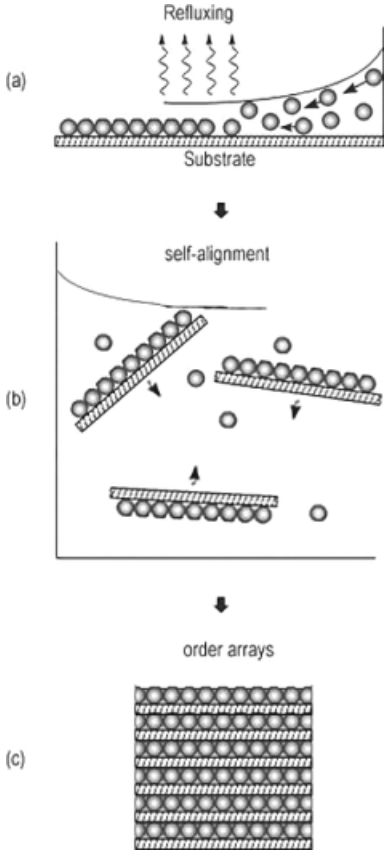


Figure 5. Deposition of $(Ti,Sn)O_2$ nanoparticles on the grafted collagen matrix with matrix/nanoparticle ratios of: A) 4:1 at 70 °C for 4 h, B) 1:1 at 70 °C for 6 h, and C) 1:4 at 60 °C for 6 h.



Scheme 2. Three-step self-alignment procedure that occurs on refluxing at 60 °C. The array formed, with its multiple interactions, is shown in Scheme 1.

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the primary units of the ordered structure. The tight interfacial covalent binding and hydrogen bonding ensure a good order is arranged between the nanoparticles and the grafted collagen matrix. These primary units then agglomerate together in a directional, ordered fashion due to the hydrogen bonding interaction between them.

In summary, we have developed a method well-suited to the synthesis of hierarchical nanoscale arrays in which the distance between each layer is within mesoscopic dimensions (about 20 nm), whereas the lateral distance is within macroscopic dimensions (about 3–4 μm). The whole process gains profit from the high reproducibility and the accuracy of the collagen matrix control of the packing density and the conformation of the monodisperse $(Ti,Sn)O_2$ nanoparticles. Although only the $(Ti,Sn)O_2$ solid solution self-alignment process is shown here, we believe this method can be easily extended to other monodisperse bimetal nanometer-scale systems. Hence, this method establishes an alternative route for the preparation of multilayer structures based on multiple spatial interactions. Such hybrid materials may spawn new interest in both fundamental nanoscience research and the development of nanodevices.

Experimental

Preparation of $(Ti,Sn)O_2$ Nanometer-scale Solid Solution: A precursor solution containing tin tetrachloride ($SnCl_4$; 0.1 mL) and titanium tetraisopropoxide (TTIP; 1 mL) was mixed with *n*-propanal (15 mL) under stirring for 5 min. The resulting mixture was added to distilled water (75 mL) and concentrated nitric acid (0.35 mL) in a reciprocal shaking bath at 30 °C for 36 h. The precipitate produced was then dipped into nitric acid at 50 °C for 16 h in a reflux system. The bimetal solid solution was obtained by evaporating the solvent at 70 °C for 24 h.

Preparation of Grafted Protein Matrices: A 1:1 mixture of 0.01 mol L^{-1} ceric ammonium nitrate (CAN) in 1 M nitric acid and 0.01 mol L^{-1} azobisisobutyronitrile (AIBN) in methanol was used as the polymerization initiator. Collagen powder (2 g) was soaked in water/methanol (75:25; 100 mL) for 2 h. As a control, HSA, Hb, or Mb powder (200 mg) was soaked in water/methanol (75:25; 10 mL) for 2 h. The required amount of methyl methacrylate (MMA; 0.5 mol L^{-1}) was then added, followed by the initiator. The reaction was carried out at 35 °C in a nitrogen atmosphere for 2 h. Ammonia (0.05 mol L^{-1}) was slowly dripped into the system to maintain the pH at 6.0–6.5 during this process. After the reaction, the system was separated by filtration, and the obtained product was then washed with distilled water and extracted with acetone to remove the loosely bound homopolymer. This process was continued until no more homopolymer was observed.

Preparation of the Multilayered Assemblies: $(Ti,Sn)O_2$ solid solution and the grafted collagen were dispersed in ethanol and stirred overnight. After a reflux process at 60 °C for 6 h, the product was refrigerated for 2 h to remove impurities, and was then separated at room temperature for further characterization.

Characterization: HRTEM was performed on a JEM-2010F electron microscope (JEOL, Japan) with a point resolution of 0.23 nm operated at 200 kV. TEM micrographs were obtained using a Hitachi H-600 operated at 80 kV with a 35 μm objective. Micrographs were recorded at a magnification of 125 000 using Kodak SO163 film that was developed in D19 for 5 min. Specimens for sectioning were embedded in white resin and cured prior to sectioning 70 nm thick samples. Sectioning samples were picked up on a carbon coated copper grid. XRD on the dried precipitate was performed on a Rigaku D/MAX-R with a copper target at 45 kV and 40 mA. Powder samples, each 100 mg, were evenly dispersed onto glass slides. Scattering patterns were collected from 10 to 90° with a scan time of 5.0 s per 0.02° step. The sample was analyzed by thermogravimetry using a TG-DTA apparatus (TMDSC, TA Q-600, TA instrument) operated at a heating rate of 20 °C min^{-1}, to determine simultaneously the correlation of temperature and weight loss and the reaction heat of the materials.

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