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RESEARCH PAPER

Investigation on Properties of Collagen Nanowires Quasiepitaxially Grown on Mica Lattice Plane

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Abstract: Collagen fibrils and hydroxyapatite might recognize each other at the mesoscale by multiple cooperative interactions due to their intrinsically repetitive structured surfaces, and thus effectively directing the biomineralization, a biological process involving regulating the growth of bones, teeth and other organs. In this work, a simple technique was developed to prepare nanowire arrays of biological macromolecules by reversely using the biomineralization mechanism, with results similar to the 'hot wall epitaxy', a molecular beam deposition technique under vacuum. With this technique, we successfully cultured rat tail type I collagen monomers adsorbed on the mica (001) lattice plane into collagen nanowire arrays along one unique direction across the whole cleavage surface. Based on the characterization results of AFM, EBSD, and TEM, the quasiepitaxial growth mechanism of collagen nanowires was verified in detail. With low experimental requirements, this technique could precisely manipulate the collagen nanowire arrays despite its simple operations, which potentially provided a highly efficient method to manufacture hydrophilic cell culture vessels, produce biological probes with high specificities, and synthesize novel micro/nano optoelectronic materials.

Key Words: Collagen; Epitaxial growth; Biomineralization; Nanowire; Electron diffraction

1 Introduction

In the field of materials science, epitaxy often refers to the growth of one mineral crystal on the surface of another mineral crystal so that the same crystal structure orientation occurs at the interface of two kinds[1]. For more than half a century, the investigation on controlling the structure and growth dynamics of organic molecular thin films on inorganic substrates has become a popular field by using the principle of epitaxial growth, with lots of amazing accomplishments achieved so far[1–13]. In the late last century, many kinds of inorganic light emitting diodes on the market were manufactured by a new crystal growth technology, i.e., liquid-phase epitaxy[6]. During the past twenty years, by combining the promise of tunable molecular structures and properties brought by organic synthetic chemistry, as well as a wide range of microscopic structural selection of substrates, the ultrahigh vacuum technique of organic molecular beam deposition provided many possibilities for obtaining desirable micro-nano optoelectronic materials and devices with predictable functions[7–9]. For example, the 'hot wall epitaxy' technique could create small molecule-based blue light-emitting materials with high quantum efficiency, such as orderly thin films of para-sexiphenyl (PSP) nanowire arrays on the substrates of mica, TiO_2, ITO[10–13]. The thin films exhibited strong optical anisotropy due to their long-range ordered structures, and high optical gain as well, with great potential to produce the random laser and prepare electronic luminescent devices with polarization characteristics[12,13].

Epitaxy might not be a term familiar to many biochemists. However, during the biomineralization process in nature, the collagen fibrils could act as scaffolds to grow mineral crystals. The same proteins could act as both specific growth inhibitors and nucleation templates by the multiple cooperative interactions with inorganic mineral crystals due to their intrinsically repetitive structured surfaces, so as to regulate the

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growth of bones, teeth and other organs in size and orientation[14,15]. On the other hand, it should also be feasible to reversely control the self-assembly of biomacromolecules on the surface of mineral crystals in the physiological environment through the recognition between crystals and macromolecules[16−19]. Understanding this epitaxial growth of ordered protein arrays on inorganic substrates might be of great significance to further study the function of biomacromolecules, synthesize new micro-nano materials, and prepare highly efficient biological probes[20−22].

Recently, by taking rat type I collagen monomers and muscovite mica as raw materials, we developed a 'reverse-phase biomineralization' technique in liquid phase to synthesize biomacromolecular nanowire arrays epitaxially grown on the inorganic crystal surface with periodically distributed charges. This technique could be easily implemented, without the requirements of the 'hydrodynamic flow'[16], or preparation of complex buffer solution[17−19]. In this work, we preliminarily investigated the properties of collagen nanowire arrays based on the characterization results of various imaging and analysis methods.

2 Experimental

2.1 Instruments and reagents

Bruker Dimension Icon Atomic Force Microscope and FESP silicon probes, with the cantilevers plated by gold and chromium, a force constant of 2.8 N m^{-1}, a tip height of 15 μm, a tip cone angle of 25°, and a tip radius of curvature of 12 nm were purchased from Bruker, Germany. Kruss DSA100 Drop Shape Analyzer was from Kruss, Germany. Auriga Focused Ion Beam Scanning Electron Microscope with 50 nm spatial resolution, equipped with an EBSD detector was purchased from Zeiss, Germany. FEI Tecnai G2 20 LaB6 High Resolution Transmission Electron Microscope was from FEI, USA.

Rat tail tendon collagen type I monomer solution (5 mg mL^{-1}) was purchased from Beijing Solaibio Science & technology Co., China, and dissolved in acetic acid (0.006 M). Na_2HPO_4, NaH_2PO_4, K_2HPO_4 and KH_2PO_4 were purchased from Sinopharm Chemical Reagent Co., China. $2M_1$ muscovite Mica substrate was fromm Beijing Zhongjingkeyi Technology Co., China. Adhesive single- and double-sided tapes (3M, USA), and deionized water with a resistivity of 18 MΩ·cm were used in this experiment.

2.2 Experimental method

Rat tail type I collagen monomer solution was diluted to a specific concentration with phosphate buffer. The substrates, square-shape mica pieces (1 cm × 1 cm) were placed in the center of the slides with the double-sided tape. A single-sided adhesive tape was used to peel mica upper layers off to expose the fresh mica (001) cleavage plane for 15 min. In this way, the mica cleavage plane was covered with the collagen monomer solutions. And then the substrate was rinsed twice with the buffer solution to remove loosely bound collagen and incubated in this buffer for 12 h at room temperature. Immediately after the incubation, the substrate was dried by a hot air gun before imaging.

AFM imaging was carried out in the Tapping mode at amplitude of around 75 nm and a drive frequency of 192 kHz (close to the resonance frequency of the cantilever in air). Before the static contact angle test by the Drop Shape Analyzer, the sample was placed in a vacuum oven to degas for 30 min. Before the TEM/SAED test, the mica substrate was cleaved into a thin piece (< 100 nm), and then punched into a sample with a diameter of 3 mm. As soon as the collagen nanowires were detected in TEM mode, NBD (Nano-beam electron diffraction) mode was applied to obtain the electron diffraction pattern of a fixed point, followed by nanowires' orientation analysis. For the SEM/EBSD imaging, the mica surface covered with collagen nanowires was plated with gold of 5 nm in thickness. The parameters of EBSD were set as acceleration voltage of 20 kV, and working distance of 13 mm.

3 Results and discussion

3.1 Self-assembly of collagen monomers on mica surface

Collagens are the major constituents of the connective tissues of multicellular animals. As the most important extracellular matrix (ECM) proteins found in a wide range of vertebrates and invertebrates, collagen monomers can self-assemble into fibril scaffolds to regulate the tissue architecture and support organs. Type I collagen is distinguished by its abundance among all of the collagens[14]. Tropocollagens, also called collagen monomers, consist of three polypeptide chains coiled around each other to form a super triple helix, with long rope of 1.5 nm in diameter and 300 nm in length. Although the single polypeptide chain exhibits a periodic supercoil pitch around 85.5 Å, the super triple helix, however, has a helix rise of only 8.6 Å along its axis for both residues and the charge distribution. Under the guidance of the telopeptide, the collagen monomers can self-assemble into protofibrils. During the biomineralization process, hydroxyapatite is first nucleated at specific locations of protofibrils, and then grows into mineral platelets within the collagen fibrils in a highly organized staggered manner.

In this experiment, Na_2HPO_4/NaH_2PO_4 and K_2HPO_4/KH_2PO_4 buffer solutions with different pH values were used to prepare collagen monomer solution, and the same buffer was applied to incubate proteins on substrate for self-assembly. In the range of pH 6.8−8.0, the collagen monomers in the sodium