博士論文

Development and Functionalization of Biomineral Liquid Crystals

（バイオミネラル液晶の開発と機能化）

中山　真成

Chapter 1. Introduction

Liquid crystal is an intermediate state between liquid and crystal, and exhibits ordering and mobility simultaneously. Formation of dynamic and ordered liquid-crystalline (LC) assemblies can lead to various functions. Typically, rod-shaped and disk-shaped organic molecules can form LC states. Inorganic liquid crystals are also expected as new functional materials. However, the number of LC inorganic compounds is limited because it is not easy to design inorganic materials having anisotropic morphologies and colloidal stability suitable for formation of liquid crystals.

In the field of materials science, development of environmentally benign materials has received great attention to realize future sustainable society. Biominerals are structurally controlled biological hard tissues such as nacre of seashells and teeth, which are regarded as a model of sustainable materials. They are composed of $CaCO_3$ or hydroxyapatite (HAp) nanocrystals with well-controlled morphologies, polymorphs, sizes and orientations. In their formation processes, amorphous mineral precursors are formed in the presence of biomacromolecules, and then their crystallization is precisely controlled from the amorphous precursors.

In the present thesis, the author combined knowledge in these different fields of "liquid crystal" and "biomineral" to pioneer new field "biomineral liquid crystal". Introduction about liquid crystals and biominerals is presented in chapter 1. Bio-based inorganic materials having LC properties are developed inspired by formation processes of biominerals. $CaCO_3$-based liquid crystals (chapter 2) and HAp-based liquid crystals (chapter 3) are synthesized using acidic polymers. Material applications of HAp liquid crystal is also pursued to demonstrate the potentials as functional materials. Taking advantage of the unique materials properties, light transmission modulator (chapter 4), bioscaffolds (chapter 5), drug nanocarriers (chapter 6) and photofunctional nanocomposites (chapter 7) are developed. The conclusions and perspectives of this thesis are described in chapter 8.

Chapter 2. Development of Calcium Carbonate Liquid Crystals

This chapter describes development of liquid crystals based on $CaCO_3$ nanocrystals with anisotropic morphologies. Generally, synthetic $CaCO_3$ crystals have a rhombohedral shape, which shows no liquid crystallinity. The strategy to develop $CaCO_3$ liquid crystals is to synthesize amorphous $CaCO_3$ (ACC) colloidal precursors using acidic polymer additives and then find the suitable crystallization conditions for ACC precursors to obtain anisotropic $CaCO_3$ nanocrystals that can form liquid crystals.

Calcite nanorods were synthesized by crystallization of ACC colloidal precursors stabilized by acidic polymers. In addition, vaterite nanodisks were synthesized from the same ACC precursors by tuning the crystallization conditions. The nanorods and nanodisks self-assembled into LC states in their concentrated aqueous colloidal dispersions, forming calamitic and discotic $CaCO_3$ liquid crystals. High-resolution transmission electron microscopy (HRTEM) observation clarified that acidic polymer nanolayer were self-organized with the surface of the mesogenic nanocrystals to provide colloidal stability.

Chapter 3. Development of Hydroxyapatite Liquid Crystals

This chapter describes development of liquid crystals based on hydroxyapatite (HAp) nanocrystals. The strategy to develop HAp liquid crystals is to synthesize amorphous calcium phosphate (ACP) colloidal precursors in the presence of acidic polymers and then find suitable crystallization conditions for the ACP precursors to obtain anisotropic HAp nanocrystals that can form liquid crystal.

HAp nanorods were synthesized by crystallization of ACP colloidal precursors stabilized by PAA. The nanorods self-assemble into LC states in their concentrated aqueous colloidal dispersions. Small-angle X-ray scattering measurements showed that HAp nanorods form nematic phase. HRTEM observation clarified the presence of acidic polymer nanolayer self-organized on the surface of the nanorods. The polymer nanolayer provides colloidal stability with the nanorods.

Chapter 4. Material Application of Hydroxyapatite Liquid Crystals to Optical Modulator

This chapter describes development of magneto-optical modulator based on HAp liquid crystals. Generally, magnetically anisotropic materials can be aligned in magnetic fields, and self-assembly of the materials into ordered LC states promotes the magnetic alignment. In addition, intensity of light transmitted through liquid crystals can be controlled by switching the LC alignment under crossed polarizers. HAp has anisotropic magnetic susceptibility derived from the crystal structure. Therefore, use of magnetic fields is promising for alignment control of HAp liquid crystals and modulation of the light transmission.

HAp liquid crystals were unidirectionally aligned over macroscopic scale in response to applied magnetic fields. Light transmission through the liquid crystals was measured under crossed polarizers. The transmitted light intensity was increased with increasing strength of the applied magnetic fields. Therefore optical switching was achieved by tuning the strength of applied magnetic fields.

Chapter 5. Material Application of Hydroxyapatite Liquid Crystals to Oriented Cell Culture Scaffolds

This chapter describes development of oriented cell culture scaffolds to control cell growth. Oriented assemblies of nanomaterials have been studied as cell culture scaffolds because cells can be aligned in response to the anisotropic surface nanopattern of the scaffolds, leading to enhancement of cell differentiations and gene expressions. Use of liquid crystals is promising for preparation of such oriented scaffolds. However, most liquid crystals are not biocompatible. The HAp liquid crystal with biocompatibility is suitable as a new oriented bioscaffold.

The LC HAp/polymer hybrid nanorods were macroscopically oriented by simple spin-coating processes on the glass substrate covered with poly(vinyl alcohol), which was used as a binder. The spin-coated HAp/polymer hybrid nanorods showed macroscopic radial orientation. Cells were cultured on the oriented scaffolds and glass substrates as a control experiment. The cells cultured on the oriented scaffolds were aligned along the oriented direction of the nanorods. In contrast, the cells cultured on bare glass substrates showed no cellular alignment.

Chapter 6. Development of Drug Nanocarrier for Photodynamic Therapy Based on Hydroxyapatite/Polymer Hybrid Nanorods

This chapter describes development of drug nanocarrier for photodynamic therapy (PDT). The biocompatibility as well as colloidal stability of the HAp/polymer hybrid nanorods are useful properties to deliver drugs to tumors. To functionalize the nanorods as drug nanocarrier for photodynamic therapy, hybridization of photosensitizer with the nanorods was attempted. Photosensitizers produce reactive oxygen species (ROS) upon light irradiation, leading to cancer cell death by oxidative stress. The acidic polymer nanolayer self-organized on the surface of the nanorods are expected to function as a host layer for incorporation of methylene blue (MB), which is positively charged molecule and a typical photosensitizer used as PDT agent.

MB were incorporated into HAp/polymer hybrid nanorods to prepare HAp/polymer/MB hybrid nanorods. Intracellular ROS generation was detected after treatment of the HeLa cells with HAp/polymer/MB hybrid nanorods. The nanorods produced ROS inside the cells upon light irradiation. The cell photocytotoxicity was investigated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In dark conditions, no cytotoxicity was observed, indicating that the nanorod hybrids were highly biocompatible. In contrast, cell viability was significantly decreased upon light irradiation.

Chapter 7. Development of Supramolecular Photofunctional Liquid-Crystalline Hydroxyapatite Nanocomposites

This chapter describes surface modification of LC HAp/polymer hybrid nanorods with functional organic molecules and development of photofunctional LC nanocomposites. Surface of colloidal particles can be modified by physical adsorption of organic molecules onto particle surfaces. For LC HAp/polymer hybrid nanorods, the surfaces are covered with acidic polymer nanolayer with negatively charges. Therefore, the surface of the nanorods can be modified with cationic organic molecules. Photofunctional cationic molecules bearing pyrene and ammonium moieties (PyAm) were designed and synthesized to modify the surface of nanorods and functionalize the HAp liquid crystal.

The surfaces of HAp/polymer hybrid nanorods were modified with PyAm. The HAp/polymer/PyAm hybrid nanorods showed LC properties in concentrated colloidal dispersions in dimethyl sulfoxide. The color of the colloidal dispersions of HAp/polymer/PyAm nanorods was changed from orange-yellow to orange color by self-assembly of the nanorods into LC states. LC colloidal dispersions of HAp/polymer/PyAm nanorods showed increase of absorbance in the wavelength range 400-500 nm, which suggests the formation of π-π stacking structures for the pyrene moieties on the nanorod surfaces in the condensed ordered LC states.

Chapter 8. Conclusions and Perspectives

The present thesis describes development of new liquid crystals based on biominerals and the material applications to various functional materials.

In chapter 1, general introductions for this study are described. In chapter 2, development of calamitic and discotic liquid crystals based on $CaCO_3$ was reported. In chapter 3, liquid crystals based on hydroxyapatite nanorods were developed. In chapter 4, optical modulator was developed based on alignment control of HAp liquid crystal using magnetic fields. In chapter 5, oriented cell culture scaffolds to control cellular alignment and morphologies were developed based on HAp liquid crystals. In chapter 6, HAp/polymer hybrid nanorods complexed with photosensitizer were used as drug nanocarrier for PDT. In chapter 7, LC HAp/polymer nanorods were functionalized by surface modification with photofunctional molecules.

In the present thesis, new material, “biomineral liquid crystals” was developed by combining knowledge in different fields of “liquid crystal” and “biomineral”. New approaches inspired by the formation of biominerals were used for synthesis of the biomineral liquid crystals. The biomineral liquid crystals have unique materials properties such as stimuli-responsiveness and biocompatibility, which provide interesting material applications. The results shown in the present thesis will give new material concepts for development of next-generation materials with bio-friendliness and functionalities.

List of Publications

Original Papers

[1] "Liquid-Crystalline Calcium Carbonate: Biomimetic Synthesis and Alignment of Nanorod Calcite" M. Nakayama, S. Kajiyama, T. Nishimura, T. Kato, *Chem. Sci.* **2015**, *6*, 6230-6234.

[2] "Bioinspired Synthesis of Liquid-Crystalline Composites: Selective Formation of Discotic and Calamitic Colloidal $CaCO_3$-Based Mesogens" M. Nakayama, S. Kajiyama, A. Kumamoto, T. Nishimura, Y. Ikuhara, T. Kato, *to be submitted*.

[3] "Stimuli-Responsive Hydroxyapatite Liquid Crystal with Macroscopically Controllable Ordering and Magneto-Optical Functions" M. Nakayama, S. Kajiyama, A. Kumamoto, T. Nishimura, Y. Ikuhara, M. Yamato T. Kato, *Nat. Commun.* **2018**, *9*, 568.

[4] "Liquid-Crystalline Hydroxyapatite/Polymer Nanorod Hybrids: Potential Bioplatform for Photodynamic Therapy and Cellular Scaffolds" M. Nakayama, W. Q. Lim, S. Kajiyama, A. Kumamoto, Y. Ikuhara, T. Kato, Y. Zhao, *submitted.*

[5] "Development of Photofunctional Organic/Inorganic Hybrid Colloidal Liquid Crystals through Surface Modification of Liquid-Crystalline Nanorods" M. Nakayama, S. Kajiyama, T. Kato, *to be submitted.*

Reference Papers

[6] "Static Structure and Dynamical Behavior of Colloidal Liquid Crystals Consisting of Hydroxyapatite-Based Nanorod Hybrids" T. Hoshino, M. Nakayama, S. Fujishima, T. Nakatani, Y. Kohmura, T. Kato, *submitted.*

[7] "Development of Superior Fusion Materials through Approaches Inspired by Biominerals" M. Iimura, M. Nakayama, T. Nishimura, T. Kato, *Chikyu Monthly* **2017**, *39*, 57-68. (Review)

[8] "Construction of Organic/Inorganic Fusion Materials through Approaches Inspired by Biomineralization" R. Ichikawa, S. Kajiyama, M. Nakayama, T. Kato, *J. Adhes. Soc. Jpn.* **2018**, *54*, 389-395. (Review)