Enzyme-mediated synthesis of ceramic materials

Hidero UNUMA,[†] Yuta MATSUSHIMA and Takahiro KAWAI

Graduate School of Science and Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510

Some enzymes catalyze the formation of precipitants of metal ions, and some others directly interact with metal-containing precursors. Such enzymes allow the synthesis of inorganic solids under mild conditions. Due to the nature of enzymatic reactions, enzyme-mediated ceramic synthesis techniques can facilitate (1) site-selective ceramic deposition, (2) low temperature ceramic synthesis, (3) intact combination of ceramics with heat-sensitive materials, and sometimes (4) synthesis of metastable phases. The morphology of the resultant ceramics can be tailored in many ways by immobilizing enzymes onto various templates. So far, successful cases have been reported in preparing thin film coatings, hollow micro- or nanospheres, multi-layer stacking, nanotubes, 2D patterns, honeycombs, 3D replicas of biominerals, and so on. Enzyme-mediated ceramic synthesis provoked the emergence of a new type of processing technique which employs proteins or peptides as the mediators. Practical applications of enzyme- or peptide-mediated ceramics are on the horizon in many fields, such as biomaterials, enzyme immobilization supports, and enzyme sensors. This article presents a comprehensive survey of the recent advances of enzyme-mediated ceramic synthesis and related techniques as well as future outlook.

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1. Introduction

Enzymes are protein catalysts that show both substrate specificity and reaction specificity and catalyze specific chemical reactions, including hydrolysis, reduction-oxidation, structural transfer, elimination of specific functional groups, isomerization, and bond formation, mainly in organic compounds. Enzymatic reactions naturally take place in living organisms; however, nowadays enzymes are also utilized in bioreactors to manufacture industrial products such as drugs, medicines, and agricultural chemicals, or as components of tooth pastes and detergents. Almost all enzyme-mediated chemical reactions are organic reactions, although inorganic reactions involving enzymes do exist.

The first attempted enzyme-mediated ceramic synthesis goes back to 1961, when carbonate apatite was synthesized with the mediation by carbonic anhydrase.¹⁾ During the following four decades, enzyme-mediated ceramic synthesis was explored by a number of scientists and unique products were reported; however, the technique did not become a major area of ceramic science.

It was the discovery of a silicate synthesis enzyme,²⁾ silicatein, which enabled the synthesis of SiO₂ or TiO₂ from inorganic reagents, that attracted much attention to the advantages of enzyme-mediated ceramic synthesis. The number of research papers in this area has been mounting rapidly during the last decade. The mediation of enzymes in ceramic synthesis has brought in a number of unique advantages, such as, site-selective ceramic deposition, low temperature ceramic synthesis, or intact combination of ceramics with heat-sensitive materials. In some cases, metastable crystalline phases have been synthesized by the mediation of enzymes.

Enzyme-mediated ceramic synthesis stimulated the occurrence of a new stream of processing techniques utilizing

proteins or artificial peptide sequences that are capable of precipitating ceramic materials out of aqueous solutions; there have been a number of comprehensive review articles in this regard.³⁾⁻⁸⁾

In this article, we will focus on the individual enzymes employed in enzyme-mediated ceramic synthesis, the resultant ceramic products, attempts to tailor morphology and function, and potential applications using enzyme-mediated techniques.

2. Enzymes and ceramic products

Enzyme-mediated ceramic synthesis techniques fall into two main categories: category I, in which precipitants of metal ions are produced by enzymatic reactions, and metal ions in solutions precipitate into oxides, hydroxides, carbonates, or phosphates; and category II, in which enzymes interact directly with metal-containing substrates to produce ceramic materials (**Fig. 1**). Enzymes and ceramic products are listed in **Tables 1** and **2**. The most easily understandable example of category I is urease-mediated precipitation of CaCO₃.²⁷⁾ When a small amount of urease is dissolved in a solution containing CaCl₂ and urea, the urea is hydrolyzed to ammonia and H₂CO₃, then Ca²⁺ reacts with CO₃²⁻, and CaCO₃ precipitates out at room temperature.

The first feature unique to enzyme-mediated ceramic synthesis is that ceramic precipitation takes place at low temperatures, such as room temperature, and the apparent rate of ceramic precipitation can easily be controlled. Since enzyme-mediated ceramic products can be synthesized at low temperatures, such ceramic products can readily be combined with organic or heat-sensitive substances such as proteins. For example, enzyme-mediated ceramics can be suitable inorganic supports of other types of enzymes.³⁾ Such encapsulated enzymes in enzyme-mediated ceramics sometimes showed higher activity than free-enzymes.^{59),67)} Most enzyme-mediated precipitates are hydroxides, carbonates, or hydrated oxides, rather than non-hydrated oxides. Therefore, the precipitates sometimes need to be heat-treated to convert to non-hydrated oxides.

[†] Corresponding author: H. Unuma; E-mail: unuma@yz.yamagata-u.ac.jp

Fig. 1. Schematic representations of the ceramic formation processes mediated by enzymes in categories I and II.

Table 1. Enzymes in category I and the resultant ceramic products

Enzyme	Precipitant	Ceramic product
Carbonic anhydrase	Carbonate ion	Carbonate hydroxyapatite ¹⁾
Alkaline phosphatase	Phosphoric acid	Calcium phosphates ⁹⁾⁻¹⁷⁾
Urease	Ammonia, carbonate ion	Magnetite, ¹⁸⁾⁻²⁰⁾ iron hydroxide, ^{21),22)} basic aluminium sulfate, ²³⁾⁻²⁶⁾ CaCO_3 , ²⁷⁾⁻²⁹⁾ SrCO_3 , ³⁰⁾ BaCO_3 , ³⁰⁾ calcium phosphates, ³¹⁾⁻³⁸⁾ yttrium hydroxide, ³⁹⁾ ZnO ⁴⁰⁾
Glucose oxidase and catalase	Gluconic acid	Hydrated tin oxide ⁴¹⁾
Lipase	Oxalic acid	Calcium oxalate 2.25 hydrate ⁴²⁾
Artificial enzyme mimicking carbonic anhydrase	Carbonate ion	CaCO_3 ⁴³⁾

Table 2. Enzymes in category II and the resultant ceramic products

Enzyme	Substrate	Ceramic product
Silicatein ⁴⁴⁾⁻⁴⁶⁾	TEOS , ^{47),48)} sodium metasilicate ⁴⁹⁾	SiO_2
	Ti-BALDH ⁵⁰⁾⁻⁵²⁾	TiO_2
	Hexafluorozirconate ⁵¹⁾	ZrO_2
	Gallium nitrate ⁵³⁾	GaOOH , $\gamma\text{-Ga}_2\text{O}_3$
	BaTiF_6 ⁵⁴⁾	BaTiOF_4
Lysozyme	Sodium silicate ^{55),56)}	SiO_2
	TMOS ⁵⁷⁾⁻⁵⁹⁾	SiO_2
	TEOS ⁶⁰⁾	SiO_2
	Hexafluorotitanate ⁵⁷⁾	TiO_2
	Ti-BALDH ⁶¹⁾	TiO_2
	$\text{Bi}(\text{NO}_3)_3$ and thiourea ⁶²⁾	Bi_2S_3 , Bi_2O_3
Lipase or pepsin	TEOS ⁶³⁾	SiO_2
	Titanium lactato- and glycolato-complexes ⁶⁴⁾	TiO_2
Papain or trypsin	Ti-BALDH ⁶⁵⁾	TiO_2
Papain	TMOS ⁶⁵⁾	SiO_2
Catalase or glucose oxidase	Titanium sulfate ⁶¹⁾	TiO_2
Hydrolases	Alkoxysilanes ^{66),67)}	SiO_2

In category I synthesis, the rate of production of metal ion precipitants follows well-established Michaelis–Menten kinetics as expressed in Eq. (1):

$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad (1)$$

where K_m , $[S]$, and $V_{\max}$ represent the Michaelis–Menten constant, the substrate concentration, and the maximum rate, respectively. When the substrates are present in large excess compared to the enzyme, which is the general case, the rate of precipitant production can be regarded as being linearly proportional to the enzyme concentration, as in Eq. (2):

$$v = V_{\max} = k[E_0] \quad (2)$$

where $[E_0]$ and k represent the enzyme concentration and the rate-determining rate constant with respect to the production of precipitant, respectively.

By changing the enzyme concentration, therefore, the apparent rate of ceramic precipitation may be controlled. When a product precipitates out of a homogeneous solution, the homogeneous nucleation theory tells that a small number of nuclei are formed if the precipitant is supplied slowly. It should be noted, however, that when the precipitate is supplied by enzymes, the enzymes themselves sometimes act as nucleation sites³⁹⁾ and the homogeneous nucleation theory is not always followed. In other words, when an enzyme is dissolved in an apparently homogeneous solution, the solution may no longer be homogeneous.

The second feature of enzyme-mediated ceramic synthesis is that the precipitates sometimes exhibit different crystalline phases, morphologies, or phase evolution behaviors than those prepared in conventional, enzyme-free methods. For example, Kato et al.⁴²⁾ reported that calcium oxalate precipitated with lipase-derived oxalic acid was a 2.25 hydrate with fibrous morphology, while that precipitated under a lipase-free condition was a monohydrate with granular shapes. It has also been reported that the crystalline phases and the phase evolution upon heating of enzyme-mediated TiO_2 vary depending on the types of substrate and enzyme employed.^{50),61),64)} For example, glucose oxidase catalyzes the precipitation of rutile from a titanium sulfate solution at room temperature, while catalase precipitates anatase, and lysozyme mediates the precipitation of a mixture of anatase and β - TiO_2 in a Ti-BALDH [titanium bis-(ammonium lactato)-dihydroxide] solution.⁶¹⁾ Katagiri et al. reported that the crystallization behaviors of amorphous titania precipitates obtained with the aid of lipase and pepsin were not identical.⁶⁴⁾ In addition, when native silicatein filaments were immersed in a $\text{Ga}(\text{NO}_3)_3$ solution, GaOOH precipitated at 289 K, while a metastable γ - Ga_2O_3 phase, oriented along the filaments, precipitated at 298 K.⁵³⁾ The reasons for these peculiar phenomena are still unclear, but one hypothesis is that the amino acid residues or 3D structure of the enzyme offers preferential nucleation sites to specific crystalline phases, thereby affecting the crystalline phase and phase evolution behavior.

The third feature of enzyme-mediated ceramic synthesis is that the morphology of the ceramic products can easily be controlled by immobilizing enzymes onto various templates. In some cases the enzymes themselves act as such templates. Templates of various types and shapes have been used to synthesize thin film coatings, hollow micro- and nano-spheres, multi-layer stacking, nanotubes, 2D patterns, honeycombs, and 3D replicas of biominerals. As well as chemical immobilization using glutaraldehyde or carbodiimides, physical immobilization within gels of alginate or chitosan has also been used. Since ceramic

Fig. 2. Alkaline phosphatase-mediated collagen/calcium phosphate composite sheet in which calcium phosphate was deposited only in the enzyme-loaded area. Reprint from Ref. 12 with permission from Elsevier.

precipitation takes place in the vicinity of the immobilized enzymes, the enzymes are buried by the precipitates as precipitation proceeds; eventually, precipitation terminates unless the enzyme immobilization process is repeated. In other words, ceramic synthesis mediated by immobilized enzymes is site-selective and self-terminating.

2.1 Alkaline phosphatase

Alkaline phosphatase catalyzes the hydrolysis of phosphate esters into inorganic phosphate ions and alcohols, diols, or glycols under alkaline conditions, as expressed in Eq. (3):

where R represents an alkyl group. When reaction 3 takes place in the presence of calcium ion for instance, calcium phosphates are precipitated. Under alkaline conditions, the calcium phosphate phase is usually hydroxyapatite.

In the early work of Banks and co-workers,⁹⁾ alkaline phosphatase was immobilized onto reconstituted collagen tape with dimethyl suberidiimide, and hydroxyapatite was biomimetically precipitated onto the tape in a solution of calcium glycerophosphate. They observed that the *c*-axis of the hydroxyapatite crystals aligned parallel to the collagen fibers. Although their work was not intended to manufacture materials but to try to verify a proposed mechanism of calcification, the technique was utilized by others to produce collagen–calcium phosphate composites for biomedical applications.^{10)–14)} Figure 2 shows an example. The alkaline phosphatase-mediated collagen–calcium phosphate composites were shown to support the attachment and growth of fibroblast cells¹²⁾ and to promote the differentiation of preosteoblast cells.¹³⁾ They also showed bioresorbability when implanted in the backs of Wistar rats,¹¹⁾ and promoted the formation of bone-like tissue in the root canal of a dog.¹⁴⁾

These are good examples of applications which make full use of an advantage unique to enzyme-mediated ceramic synthesis: the use of alkaline phosphatase enables the precipitation of hydroxyapatite under ambient temperatures thereby avoiding unnecessary heat damage to reconstituted collagen.

In the early days, the deposition of calcium phosphate took a long time, as its rate was determined by the diffusion of reactants through a network of reconstituted collagen sponge. The reaction took several days, until ca. 60 mass % of calcium phosphate was deposited in the composite.¹¹⁾ The time required was shortened to one day by continuous replenishment of the reactants under mechanical stirring of the calcium glycerophosphate solution.¹⁴⁾

2.2 Urease

Urease catalyzes the hydrolysis of urea into ammonia and carbonic acid (Eq. 4):

Fig. 3. Urease-mediated CaCO_3 thin films deposited (left) in line patterns and (right) on polystyrene beads. Reprint from Ref. 28 with permission from American Chemical Society.

The reaction 4, which is well known as a homogeneous precipitation technique utilizing the spontaneous hydrolysis of urea above ca. 340 K, can be made to take place at room temperature by employing urease.

The optimum pH range of urease is relatively wide from 3 to 8. Either ammonia or carbon dioxide can act as a precipitant. Urease-mediated ceramic products reported so far include hydroxides, carbonates, hydroxyapatite, and oxides.

Unuma et al. immobilized urease in alginate gel, W/O emulsion, and polymer microspheres to precipitate basic aluminum sulfate, iron hydroxide and hydroxyapatite on or in the templates, to prepare porous alumina,²⁶⁾ hollow alumina microspheres,²⁵⁾ and hollow particles of magnetite,²²⁾ and hydroxyapatite.³⁸⁾ With the aim of developing materials for cancer treatment, Kawashita et al. prepared porous particles of magnetite²¹⁾ and Y_2O_3 ³⁹⁾ by using immobilized urease in gel template spheres. Enzyme-mediated precipitation has facilitated the preparation of hollow ceramic microspheres several tens of microns in diameter, which was previously found difficult.

Mediation by immobilized urease can also be used to prepare ceramic coatings. Unuma et al. immobilized urease onto the surface of silk fabric³⁴⁾ and the inner surface of poly(L-lactic acid) sponge³²⁾ with water soluble carbodiimide (WSC) and precipitated a layer of hydroxyapatite onto the immobilized urease. The hydroxyapatite coatings were shown to enhance the proliferation of osteoblast-like cells.³⁵⁾

Yeom et al.²⁸⁾ prepared physically immobilized urease multilayers by alternately coating negatively charged urease and positively charged poly(diallyldimethylammonium chloride) (PDAC) on a silicon wafer, polystyrene beads, and polyurethane, and deposited amorphous CaCO_3 layers on them (Fig. 3). They reported that, after conversion of the amorphous CaCO_3 to calcite in a humidity-controlling chamber, the calcite adopted a (104) orientation. Urease activity was found to increase as the number of alternately stacked urease layers increased.

An interesting application of urease was reported by Hamaya et al.,^{18)–20)} who prepared magnetite particles by urease mediation from a solution containing FeCl_2 , KNO_3 , and urea. The formation of magnetite was based on the pH dependence of the oxidation potential of nitrate ions; in a certain pH range, Fe^{2+} was oxidized to Fe^{3+} by nitrate ions, and fine particles of magnetite precipitated spontaneously.

The physical and chemical properties of urease-mediated ceramic products sometimes differ from those of products prepared under urease-free conditions. Sondi et al.^{27),30)} reported that urease-mediated CaCO_3 precipitated in the amorphous phase at first, and spontaneously transformed to calcite via vaterite, while the morphology of urease-mediated SrCO_3

Fig. 4. Urease-core ZnO-shell nanoparticles. Reprint from Ref. 40 with permission from Wiley-VCH Verlag GmbH&Co.KGAA.

spontaneously evolved from spherical to rod-like. De la Rica and Matsui⁴⁰⁾ reported that, on addition of zinc nitrate hexahydrate solution to a urease/urea solution, particles with a urease-core and a ZnO-shell, 18 nm in diameter, precipitated at room temperature (Fig. 4). Although Zn(OH)_2 is the product of the reaction of Zn^{2+} and NH_3 in an ambient condition, it was ZnO that came out around urease. The investigators ascribed the formation of ZnO to an interaction between Zn ions and the urease surface.

A smart method in which urease was used in two ways at the same time was reported by Gutiérrez et al.³⁶⁾ They added urease to a solution containing urea, chitosan, calcium ions and phosphate ions. When the pH was raised, gelation of a weakly acidic chitosan solution and precipitation of calcium phosphate took place concurrently. After uniaxial freezing and freeze-drying, they prepared chitosan–calcium phosphate composites with a hierarchical pore structure. The urease they used showed activity even at 273 K, and the composite prepared at 273 K contained dispersed amorphous calcium phosphate.

Yu et al.²⁹⁾ prepared urease-containing polypeptide capsules by impregnating mesoporous silica spheres with urease and coating the spheres layer by layer with poly(L-lysine) and poly(L-glutamic acid), before dissolving the silica in a $\text{HF}/\text{NH}_4\text{F}$ solution. The urease-containing capsules were placed in a solution of urea and CaCl_2 to obtain spherical calcite/vaterite particles.

2.3 Other enzymes in category I

Carbonic anhydrase catalyzes the dehydration of carbonate ions and the hydration of carbon dioxide (Eq. 5):

When carbonic anhydrase is added to a solution containing metal ions in a CO_2 -containing atmosphere, metal carbonates or metal carbonate phosphates precipitate in the solution. McConnell et al.¹⁾ reported the formation of carbonate hydroxyapatite when carbonic anhydrase was added to a solution containing sodium phosphate and calcium chloride in a CO_2 atmosphere, or to heat-treated saliva. Abe et al.⁴³⁾ prepared an artificial enzyme mimicking the active center of carbonic anhydrase based on a zinc-imidazole complex, and found that the artificial enzyme mediated the precipitation of vaterite, ca. 1 µm in diameter, from a $\text{NaHCO}_3/\text{Ca(NO}_3)_2$ solution.

Lipase can catalyze the liberation of oxalic acid from oxalate esters, which allows precipitation of metal oxalates.⁴²⁾ To lower the pH of a solution, glucose oxidase, which catalyzes the formation of gluconic acid from glucose, may be used.⁴¹⁾ The hydrogen peroxide by-product must be eliminated with catalase (Eqs. 6 and 7):

Fig. 5. Purified silicatein filaments (A) without and (B) with TiO_2 deposits from Ti-BADLH. Reprint from Ref. 50 with permission from American Chemical Society.

2.4 Silicatein

Silicatein, which is intimately associated with biosilica in the spicules of sponge *Tethya aurantia*, was identified in 1998 by Morse's group. In the next year, the same group found that silicatein was divided into various subunits, which catalyzed the formation of silica from TEOS *in vitro*,⁴⁷⁾ and they claimed that the active sites for silica formation were hydrogen-bonded Ser/His and Cys/His residues. These reports, to a large extent, resulted in the emergence of the field of silicatein-mediated ceramic synthesis.

Thin silica layers were coated on the surfaces of nano-sized $\gamma\text{-Fe}_2\text{O}_3$ or hydroxyapatite particles after the modification of those surfaces with silicatein,^{48),49)} utilizing the site-selective nature of enzyme-mediated ceramic synthesis. In addition, silicatein was found to be active in the synthesis of TiO_2 ,^{50)–52)} ZrO_2 ,⁵¹⁾ Ga_2O_3 ,⁵³⁾ or BaTiOF_4 ,⁵⁴⁾ which is of significance because site-selective precipitation of SiO_2 or TiO_2 has been difficult by conventional techniques. An example of silicatein-mediated TiO_2 is shown in **Fig. 5**. Silicatein-mediated titania precipitated from Ti-BALDH showed different phase evolution behavior from titania precipitated by alkali addition.⁵⁰⁾

2.5 Lysozyme

While investigating substances that promoted the condensation of silicic acid, Coradin, Livage, and co-workers^{68),69)} found that peptides and proteins containing large amounts of lysine and arginine residues are effective in this regard. Based on these findings, they discovered in 2003 that lysozyme and bovine serum albumin (BSA) are especially suitable for silicate condensation.^{55),56)} These reports prompted materials scientists to use lysozyme for ceramic deposition. Lysozyme basically catalyzes the hydrolysis of polysaccharides, with silicate condensation activity as a side-effect. Luckarift et al.⁵⁷⁾ found that lysozyme promotes the formation not only of silica but also of TiO_2 from hexafluorotitanate or titanium lactate complexes. Lysozyme-mediated silica was found to be suitable as a support for enzyme immobilization; Ramanathan et al.⁵⁹⁾ reported that organophosphate hydrolase supported on lysozyme-mediated silica was found to have a longer life time than a conventional support. An interesting application of lysozyme in controlling the morphology of silica was investigated by Shiomi et al.,⁶⁰⁾ who prepared hollow silica microspheres by subjecting a solution of lysozyme and TEOS to ultrasonication (**Fig. 6**). Lysozyme was found to be effective in synthesizing Bi_2S_3 from a solution of bismuth nitrate and thiourea.⁶²⁾ The approach of Coradin and

Fig. 6. Lysozyme-mediated silica hollow particles, scale bar: 3 μm . Reprint from Ref. 60 with permission from American Chemical Society.

Livage, which focused on searching for effective biocatalysts based on amino acid sequences, seems to have had a strong impact on peptide-mediated ceramic precipitation, as described in Section 2.7.

2.6 Other enzymes in category II

A series of hydrolases, including lipases and peptidases, were found to catalyze the hydrolysis and condensation of alkoxy-silanes^{63),65)–67)} and titanium complexes.^{64),65)} As may be expected, enzyme activity in the condensation of silanols to form siloxanes strongly depends on the origin of the enzyme.⁶⁶⁾ Usually, enzymes are active in aqueous solutions. However, Kato et al.⁶⁷⁾ found that some enzymes catalyzed silica formation from TEOS in an ionic liquid, and the resultant silica encapsulated the enzymes themselves, forming silica-enzyme composites. Interestingly, these composites showed higher catalytic activity than free enzymes in the hydrolysis and condensation of trimethylethoxysilane.

Glucose oxidase and catalase were found to catalyze TiO_2 formation,⁶¹⁾ but resulted in different crystalline phases: glucose oxidase gave rutile, while catalase gave anatase.

2.7 Peptide-mediated ceramic synthesis and related techniques

Although it does not involve enzymes, we will give a brief overview of a closely related method of peptide-mediated ceramic synthesis. Silaffin, a protein isolated from diatoms, is not an enzyme but acts in a similar manner to silicatein to precipitate SiO_2 or TiO_2 .^{70)–72)} The silica-precipitating ability of silaffin was attributed to the presence of a polycation peptide,⁷¹⁾ $\text{H}_2\text{N-SSKKSGSYSGSKGSKRRIL-COOH}$, named R5. A short time later, the synthesis of silica from a silicic acid solution⁷³⁾ and the synthesis of TiO_2 from Ti-BALDH⁷⁴⁾ were attempted using R5 peptide.

Single amino acids such as lysine and serine, polypeptides such as polylysine and polyserine, and other synthetic oligopeptides were found to be effective in the synthesis of silica from TEOS.⁷⁵⁾ Although the products of these early studies were mostly featureless micron-sized aggregates of nano-sized primary particles, attempts to control the morphology of the products were soon made. Gautier et al.⁷⁶⁾ prepared silica nanotubes by immobilizing poly(L-lysine) (PLL) in the pores of polycarbonate membrane and then depositing silica along the inner walls of the pores. Goldberg et al.⁷⁷⁾ coated the surface of dentin with PLL-mediated silica to modify its permeability. Cha et al.⁷⁸⁾ attempted to control the morphology of silica by changing the atmosphere

Fig. 7. Freestanding TiO_2 replicating (A) a complete and (C) a partial frustule. The picture (D) shows a cross-section of specimen (C) to show the replication of titania struts. Scale bars: $2\mu\text{m}$. Reprint from Ref. 80 with permission from American Chemical Society.

in which certain block copolypeptides interacted with TEOS. Poly(L-cysteine₃₀-b-L-lysine₂₀₀) was found to promote the formation of spherical silica in a nitrogen atmosphere, while column-shaped amorphous silica was formed in air.

Simple molecules including protamine,^{79),80)} cysteamine,⁸¹⁾ cyclic lanreotide,⁸²⁾ and ethyleneamine⁸³⁾ have also been found to catalyze the formation of SiO_2 and TiO_2 . Liang et al.⁷⁹⁾ showed that protamine-mediated titania was a good support for yeast alcohol dehydrogenase (YEAD); the supported YEAD showed excellent stability against temperature, pH, and recycling. Fang et al.⁸⁰⁾ coated diatom frustule (biosilica) with TiO_2 using protamine, and reported that the freestanding TiO_2 , replicating the morphology of the biosilica (Fig. 7), showed electrochemical hydrogen detection properties. Roth et al.⁸¹⁾ fabricated silica patterns by forming a pattern of adsorbed cysteamine on a silicon wafer and then depositing silica (Fig. 8). Pouget et al.⁸²⁾ prepared bundles of silica nanotubes 30 nm thick and several microns long by adding cyclic lanreotide to TEOS; partially hydrolyzed TEOS and cyclic lanreotide self-assembled into nanotubes. Various types of solid, including PbSe ,⁸⁴⁾ BaTiO_3 ,^{85),86)} CaMoO_4 ,⁸⁷⁾ Pt ,⁸⁸⁾ and Ni ⁸⁹⁾ have been synthesized using appropriate peptides during the last decade.

Amines, polyamines,^{90),91)} and poly(ethyleneimine)^{92),93)} are also capable of catalyzing the formation of silica and titania. There is a considerable body of work regarding peptide- and polymer-mediated ceramic precipitation, and comprehensive reviews are available.³⁾⁻⁸⁾ The line between enzyme-mediated and peptide-mediated precipitation techniques is quite obscure, as are the lines between other polymer-mediated techniques. However, we will not delve into the non-enzyme area any further.

Fig. 8. Cysteamine-mediated silica patterned with microcontact printing of cysteamine on a silicon wafer. Reprint from Ref. 81 with permission from American Chemical Society.

2.8 Enzymes in living microbes

Sastry and co-workers have reported that a kind of fungus, *Fusarium oxysporum*, mediates the extracellular formation of CdS from CdSO_4 ,⁹⁴⁾ intracellular Ag nanoparticles from AgNO_3 ,⁹⁵⁾ ZrO_2 from $[\text{ZrF}_6]^{2-}$,⁹⁶⁾ and hollow SiO_2 nanoparticles from sand.⁹⁷⁾ Although the exact enzymes or proteins responsible for ceramic synthesis were not identified, they speculated that a type of reductase or cationic protein might be playing a key role.

3. Concluding remarks and future outlook

The finding that enzymes, amines, and polypeptides can mediate ceramic precipitation has led to the development of a new technique of ceramic synthesis which takes place under mild conditions and allows control of morphology. The nature of enzyme-mediated ceramic precipitates sometimes differs from that of physicochemically derived ceramic precipitates in terms of morphology, crystalline phase, and structural evolution upon heating.

In general, enzyme-mediated ceramic precipitates are aggregates of nanoparticles, have low crystallinity, and contain large amounts of hydration water. The types of ceramic precipitates are limited to those which can precipitate out of aqueous solutions; so far, hydroxides, carbonates, phosphates, sulfides, hydrated oxides and metals have been synthesized. Halides may be precipitated but nitrides or carbides are not likely to be synthesized unless the precipitates are subjected to subsequent nitridation or carbide formation. Enzyme-mediated ceramic synthesis allows us to make coatings and patterns and to fabricate composites of ceramics with heat-sensitive polymers or proteins. When considering the practical application of enzyme-mediated ceramic synthesis, we should make use of the unique characteristics of this method. In particular, enzyme-mediated hydroxyapatite-based biomaterials have been used in animal tests and have achieved successful outcomes. One possible reason for this is that enzyme-mediated hydroxyapatite is readily soluble to body fluids due to its low crystallinity, and the release of calcium ions may cause desirable effects, such as promoting the differentiation of osteoblastic cells as reported by Jung et al.⁹⁸⁾ Enzyme-mediated hydroxyapatite-based biomaterials may become the first successful example of practical application of this technique if the biological safety of the enzymes and reagents used is guaranteed.

Additional potential applications of this technique include the preparation of supports for enzyme immobilization, enzyme sensors, nanoparticles for quantum dots, hollow ceramic microspheres, nanotubes, 2D ceramic patterns, and ceramic coatings. As demonstrated by Fang et al.,⁸⁰⁾ replication of the morphology

and microstructure of biominerals may be facilitated by using enzymes or peptides.

Although the selection of appropriate enzymes for specific ceramic preparations still depends on trial and error or serendipity, the selection of pertinent peptides is easier due to the availability of peptide library screening techniques.^{99),100)}

It should be noted that most papers referred in this article were published after the year 2000. This suggests that enzyme- and peptide-mediated ceramic synthesis techniques are on the frontier of rich vein of exploration. A single example of practical application of these techniques should accelerate exploration considerably. Collaborative and interdisciplinary work among scientists in the areas of chemistry, biology, physics, agriculture, materials science, and others will be important in this regard, allowing the creation of exquisitely designed materials and the establishment of process to develop genuinely smart materials.

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