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INVITED REVIEW PAPER

Recent developments and applications of bioinspired silicification

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Abstract−Bioinspired synthesis of silica has attracted attention from a wide range of researchers as novel route for fabrication of various nanomaterials. Proteins including silaffins and silicateins as well as polyamines from marine diatoms and sponges are key biomolecules in these biomimetic silicification processes. These methods allow silica mineralization from various silica precursors under mild, biologically compatible conditions in an unprecedentedly fast and facile manner. Notably, the silica polycondensation entails the concomitant encapsulation of other molecules in the reaction solutions. Due to the efficient encapsulation and synergetic effects brought by the encapsulated molecules and the characteristics of biomimetic silica synthesis as well as the mechanical and chemical properties of silica itself, the silica-biomolecule nanocomposites have broad applications in biocatalysis, biosensor, and biomedical areas. Introduction and combination of novel template, precursors, inorganics, or enzymes with the previously used strategies will allow construction of more efficient, purpose-optimized silica nanomaterials with controlled size, composition, and morphology.

Keywords: Biosilica, Biosilicification, Silaffin, Silicatein, Polyamine

INTRODUCTION

Silicon (Si), as a member of the metalloid family, is one of a few elements having both metallic and non-metallic properties. It is one of the most abundant elements in the universe and also composes the second most dominant component of the earth crust. Silicon is an essential part of our lives--from window glass, brick, and mortar in construction industries to semiconductors, optical data transmission fibers, castings, and health care devices in high-tech industries. Silica (SiO_2), an oxidized form of silicon, is synthesized at high temperature or extreme pH in current industries [1,2]. As silica has tremendous uses in many industries, bringing harsh processing conditions to moderate conditions can possibly offer advantages in cost, energy, and safety perspectives.

Interestingly, many marine living organisms produce amorphous biosilica with various morphologies at moderate physiological conditions to maintain lives [3-5]. Among many biosilica forming organisms, sponges and diatoms are known for their intensive utilization of biosilica; they uptake silicic acid from the environment and build external biosilica structures to protect themselves from incoming threats. Their secrets in manipulating biosilica at physiological conditions, unlike the harsh conditions found in modern industries, are ascribed to biomolecules and the underlying mechanisms [4].

Sponges and diatoms use different types of biomolecules to precipitate silica ions; sponge uses enzymes called silicatein and diatoms use long-chain polyamines (LCPAs) and polypeptides called silaffins [6-9]. These marine organism-derived molecules are the most widely studied biomolecules in biosilicification due to their outstanding performance, though other molecules, such as lysozyme, papain, pepsin, trypsin, bovine serum albumin, and PEG-PEO polymer-based materials, are also able to form silica at physiological conditions [10-13]. In addition to forming biosilica at physiological conditions, these organisms demonstrate their strong points in control of particle sizes and pore sizes from nano to micro scales, in construction of structures and morphologies, and in control of silica condensation rates [14,15]. These silica-forming molecules have different origins and structures; however, they share common features: they assemble into large network of zwitter ionic structures in which their negatively charged residues repel silicic ions so that their positively charged residues can efficiently interact with the ions and nucleate silica particle through facilitating siloxane bonds [16,17]. The efforts for deeper understanding of mechanisms and processes of biosilicification are being unfolded by many researches and still on-going.

Silicatein takes a key role in making silica-based exoskeletal matrix and spicule in sponges. Silicatein is expressed in a sponge's sclerocytes and released to catalyze biosilica formation. Silicatein has three isoforms (silicatein -α, -β, -γ) that are categorized into cathepsin L and papain protease families [5,6]. Ser/Cys, His, and Asn residues are characteristic amino acids that act as the catalytic triad for interacting with silicic acid substrates [18]. Interestingly, silicatein plays roles not only in enzymatic silica polycondensation reaction but also in structural formation; silicatein forms fibril-like structure as mature silicatein self-assembles into aligned bundles, which eventually becomes spicules [19,20].

The key features in diatom biosilicification are silaffins and LCPAs, which synergistically interact with silicic ions and polymerize them into amorphous silica structure [21,22]. Representatively, four silaffin precursor genes were identified from *Cylindrotheca fusiformis* and *Thalassiosira pseudonana*, which are translated and posttrans-

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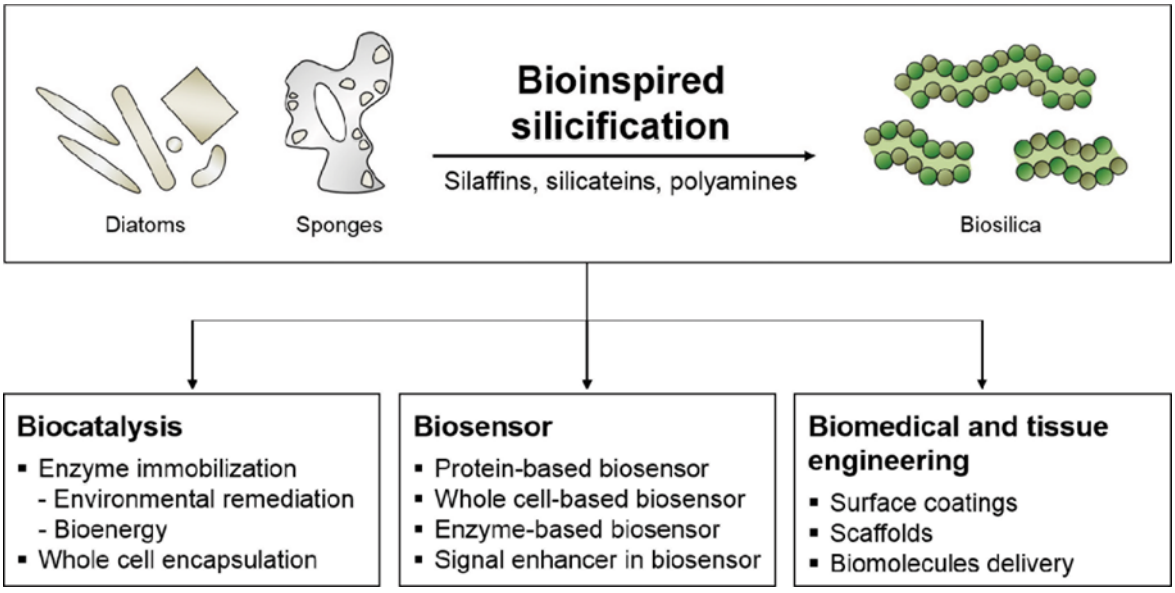

Fig. 1. Schematic representation on diverse applications of bioinspired silicification.

lationally processed into various types of silaffins, such as silaffin-1A, silaffin-1B, or silaffin-2 [8,22-24]. Silaffins commonly share zwitter ionic structure with rich lysine and serine residues [16,17]. However, the amino acid sequences seem to be diverse among diatoms. The short part of silaffin's repeating units, such as R5 in silaffin-1, has been synthesized and widely used as silica forming peptides in many *in vitro* experiments [25,26], including the first example of the technological application of R5 peptide in the holographic silica nanopatterning [27].

LCPAs are other molecules utilized for biosilicification by diatoms. Most diatoms use a cocktail of silaffin and LCPAs, but some of them only use LCPAs for building their frustules [8]. Depending on the species of diatom, these molecules are post-translationally attached on silaffin's lysine residues or exist as free molecules [9]. Even though silaffin alone without any post-translational modification can precipitate silica, the presence of LCPAs can accelerate silica formation or can even precipitate silica under acidic condition where the unmodified synthetic R5 peptide cannot precipitate silica [9,15,17,25]. In addition to the biosilica formation, LCPAs are alleged to affect the morphologies of biosilica structure in combination with silaffin [22,28,29].

Understanding biosilicification inspires biomimetic approaches to where sensitive and intricate control of silicification is beneficial. Interestingly, biomimetic silicification encapsulates or entraps adjacent substances into the silica matrix during the formation so that this method is considered very useful in immobilization of various materials, such as enzymes [21]. As aforementioned, silicatein and silaffin peptides are widely used for biomimetic silicification. This technique is particularly attractive over the conventional sol-gel silica immobilization because organic solvents or other harsh conditions are avoided which often ruin or debase the performance of the encapsulated molecule. In addition, silica provides the encapsulated molecules with mechanical or biological protection. In this review, we present general insights and report progress in biomimetic silicification approaches over biocatalysis, biosensor, and biomedical applications (Fig. 1).

1. Bioinspired Silica Encapsulation for Biocatalysis

Since Luckarift et al. demonstrated enzyme immobilization in a biomimetic silica support [21], encapsulation of enzyme has been the main subject of subsequent studies on the application of biosilicification. The silica formation and the simultaneous enzyme encapsulation occur under mild condition, which cannot be achieved without such a biomimetic method. The conventional chemical processes for the synthesis of inorganic materials generally cannot be applied to the encapsulation of living cells because of the harsh reaction conditions that are lethal to cells. Following are recent developments of biosilica- and biomimetic silica-based catalysts. We included biomimetic silica encapsulations of cells because of their potential importance as whole-cell biocatalyst and biosensor.

1-1. *In Vitro* Immobilization of Enzyme in Biomimetic Silica

In vitro biomimetic silicification has been widely used mostly for enzyme immobilization since the method provides a rapid, facile and mild route that allows effective enzyme immobilization with high activity retention and excellent stability. Readers are encouraged to refer to the previous review [30] for the early studies (before 2008) on this subject. Especially, most enzymes immobilized in biomimetic silica are those important in energy and environmental applications.

1-1-1. Environmental Remediation Including Carbon Sequestration

Biocatalysts can be used for environmental remediation, including carbon capture with high catalytic efficiency, specificity, and environmental friendly process. Forsyth et al. reported on bioinspired silica encapsulating carbonic anhydrase (CA), an enzyme that catalyzes CO_2 hydration, for carbon capture [31]. The silica-encapsulated CA, synthesized using diethylenetriamine (DETA) and Na-orthosilicate, exhibited enzyme loading, activity retention, and thermostability higher than CAs immobilized by conventional meth-

ods. The CO_2 removal efficiency was 86%, showing a similar level to that by free CA. Similarly, an alkaline-active CA was encapsulated in spermine-mediated bioinspired silica [32]. The CA-silica showed high thermo- and pH-stability and was even better catalyst for CO_2 hydration than bovine CA, a CA with very high turnover number, under alkaline condition that is expected for practical CO_2 sequestration processes. A more robust catalyst was developed by immobilizing CA in silica via autoencapsulation mediated by R5 peptide fused to the CA [33]. Encapsulation efficiency was dependent on enzyme concentration, and the leaching of encapsulated enzyme was almost negligible due to tight binding of R5 to silica. Thermostability and thermoactivity were also highly enhanced compared to the DETA-based CA, demonstrating the efficiency of R5-mediated autoencapsulation in biomimetic silica.

The enzymes organophosphate hydrolase (OPH) and phosphodiesterase sequentially degrade toxic organophosphate compounds such as paraoxon found in chemical warfare agents and pesticides. These enzymes were fused to R5 and subsequently autoencapsulated in biomimetic silica support [34]. The enzyme loading was more efficient than that by separate R5 peptide. Michaelis constant rather than maximal velocity was lowered, implying that substrate diffusion was limited by the encapsulation. Interestingly, fusion with three tandem repeats of R5 peptide resulted in smaller silica particles, suggesting that nanoscale dimensions of the silica matrix may be artificially controlled.

Laccase, an enzyme with its potential use in bioremediation of pollutants and phenolic compounds, was immobilized in ultrathin alginate/protamine/silica hybrid membranes with an immobilization yield of 100% [35]. Thermo- and pH-stability were significantly enhanced by the immobilization. Even though the encapsulation reduced the maximal enzyme activity to 37% of that of free enzyme, the outermost inorganic totally inhibited swelling of alginate carriers, which can prevent the leakage of laccase.

1-1-2. Bioenergy Production

Biocatalysts are being intensely studied and utilized for the production of biofuels, which are clean and renewable. Lipase is a kind of esterase that hydrolyzes ester bonds in lipids. This enzyme can be used as a green catalyst in transesterification reaction for biodiesel production from oils or fats. Lipase from *Pseudomonas cepacia* was immobilized in biomimetic silica using polyallylamine and TMOS [36]. Even though the catalytic activity decreased ~50% after encapsulation, the activity was recovered and even exceeded the free enzyme activity when the silica was synthesized using TMOS doped with methyl-substituted methoxysilanes. Emond et al. encapsulated *Candida antarctica* lipase or *Pseudomonas fluorescens* esterase within biomimetic silica by R5 fusion strategy [37]. The autoencapsulation showed greater efficiencies than polyethyleneimine (PEI)-mediated silicification even though the relative activity was quite low compared to free enzyme. Intriguingly, the esterase within silica support exhibited altered enantioselectivity. These studies demonstrate that silica not only serves as enzyme support but also participates in catalysis itself. In another study, Forsyth and Patwardhan immobilized *C. antarctica* lipase on biomimetic silica using various amine additives [38]. They found that the relative enzyme activity on silica, which was correlated with the size of silica aggregates, could be controlled by using the different kind of amines. The performance of the immobilized enzymes was comparable to Novozym 435, the commercially available lipase immobilized on an acrylic resin.

Methanol can be produced from CO_2 via the sequential catalysis of formate dehydrogenase (FDH), formaldehyde dehydrogenase, and alcohol dehydrogenase using NADH as electron donor. A group of Chinese researchers immobilized the enzyme(s) in hybrid microcapsules fabricated by layer-by-layer assembly comprising the biomimetic formation of silica shell [39-41]. The production yields of methanol and formaldehyde (the precursor of methanol) were greatly increased by the encapsulated multienzyme systems, probably due to the proximity channeling of the intermediates before diffusive equilibrium [42]. Also, the ratio of methanol (or formaldehyde) production to NADH consumption was also improved.

1-2. *In Vivo* Enzyme Encapsulation in Living Diatom

Apart from the *in vitro* silicification, Poulsen et al. described a method for *in vivo* enzyme encapsulation in diatom silica [43]. A gene coding for hydroxylaminobenzene mutase fused to the C-terminus of silaffin-3 was incorporated into the genome of *Thalassiosira pseudonana*. The enzyme was functionally expressed and localized (encapsulated) in the diatom cell wall, showing high stability. This concept was also demonstrated to be applicable for more complex enzymes requiring oligomerization, redox cofactors, or post-translational modifications [44]. This approach is particularly valuable because enzymes to be immobilized do not need to be separately produced and purified and more importantly, the whole process including enzyme production and encapsulation entirely depends on oxygenic photosynthesis that only requires sunlight, water, CO_2, and minerals.

1-3. Biomimetic Silica Encapsulation of Whole Cell

Microbes are promising biofactories for useful materials as well as efficient biocatalyst due to their high growth rate, versatility, and ease of manipulation. Encapsulation of microbial cells may confer stability, reusability, and unnatural functionality for whole-cell biocatalytic application. Self-encapsulation of living microbes in silica has been achieved by genetic engineering of bacterium *Escherichia coli* with silicatein-α [45] and yeast *Pichia pastoris* with cationic lysozyme fused with a signal peptide for secretion [46]. In these novel approaches, viscous poly(silicate) covers are formed around the cells after incubation with a silica precursor Na-orthosilicate. Notably, the growth kinetics remains unaffected by the silica, implying that the silica capsules formed by the two biomolecules are relatively soft and flexible.

As well as the self-encapsulation by the genetic transformation of microbes, single-cell encapsulations with silica have been also demonstrated via cell surface coating using cationic polyelectrolytes or peptide. Individual yeast or bacterial cells were encapsulated with silica by alternative layer-by-layer assembly of cationic and anionic polyelectrolytes [47]. The outermost cationic polyelectrolyte could catalyze biomimetic silicification from TMOS, resulting in single-cell encapsulation with a nanometer-scale control of thickness [48]. The encapsulated yeast cell showed higher viability under nutrient deprivation and no growth under nutrient rich condition, demonstrating stabilization of the encapsulated cell and designed control of cell division. By introducing (3-Mercaptopropyl)trimethoxysilane (MPTMS) along with TMOS in the silica-

forming reaction, the encapsulating silica was thiol-functionalized that could be subsequently conjugated to fluorescein or streptavidin, extending the applicability of the biomimetic whole-cell silica encapsulation [49]. The encapsulation process was facilitated by one-step templating of yeast cell using a designed peptide selectively adsorbed onto the cell surface [50].

With similar strategies described above, photosynthetic cyanobacteria (*Synechocystis* sp. PCC 6803) and microalgae (*Chlorella*) were encapsulated in nanoshell made of silica and silica/titania composite, respectively, under mild conditions [51,52]. The nanoshells greatly improved thermo-tolerance of the encapsulated microalgae [52] or significantly alleviated the photo-inhibitory effects of cyanobacterial cells under high light stress conditions [51]. These studies deserve more attention because oxygenic photosynthetic microbes are ideal biocatalytic factories not only for bioproduct (including biofuel) but also for CO_2 mitigation.

Biomimetic silicification has been also applied to encapsulation of mammalian cells and even viral particles. HeLa cells were coated with PEI that was used as a catalytic template for inorganic silica formation [53]. The silica-coated HeLa cells showed enhanced viability and resistance to trypsin and poly(allylamine hydrochloride) that are normally lethal to the cells. Wang et al. recently demonstrated successful silicification of human enterovirus type 71 using Na-orthosilicate by pH adjustment to slightly acidic condition [54]. They found that hydrated silica exterior conferred thermostability on the virions without affecting other biological characteristics. This silicification strategy was further used to develop silicified polio viral vaccine that could be stored at ambient temperature owing to its improved thermostability, providing an efficient route for vaccine distribution.

2. Biosilica-based Biosensor

2-1. Biosensors Based on *In Vivo* Silicification of Protein

Diatoms have some advantages as scaffolds in biosensors: they have a large surface area matrix to functionalize higher amounts of target biomolecules within biosilica pores, they stabilize the immobilized biomolecules against external environment, and their porosity facilitates mass transfer of analytical sample into detector, allowing effective detection [55-57]. Recent advances in genetic modification of diatoms have made it possible to localize recombinant proteins in the biosilica wall [43,44]. Marshall et al. developed label-free biosensor by expressing a bacterial periplasmic ribose binding protein flanked by cyan and yellow fluorescent proteins in diatom *Thalassiosira pseudonana* for detection of ribose [56]. Based on changes in Förster resonance energy transfer (FRET) of the fusion protein upon binding of ribose, the biosensor exhibited EC_{50} of 23.3 mM.

2-2. Biosensor Based on *In Vivo* Biomimetic Silicification of Whole Cell

By mimicking *in vivo* silicification of sponges, Adanyi et al. constructed silicated recombinant *Escherichia coli* [58,59]. Silicatein, which is involved in the formation of amorphous hydrated silica on sponges [6], was expressed on the surface of *E. coli*. To develop a microbial biosensor combined with optical waveguide light-mode spectroscopy, the recombinant *E. coli* was immobilized onto SiO_2-containing sensor chip via physical adsorption followed by the silica polycondensation after addition of hydrolyzed TMOS solution. The biosensor was used to investigate the inhibitory effect of oxidative stress and environmental pollutants in real time.

2-3. Biosensors Based on *In Vitro* Biomimetic Silicification of Enzymes

In vitro biomimetic silicification has been used to stabilize enzymes and cells in biosensors because the method is capable of forming silica layer around enzymes and cells at mild condition [60-69]. Silaffin or silicatein-derived peptides have been widely used because the peptides play an important role in formation of nano-structured silica precipitates on diatoms and sponges [25]. Luckarift et al. have developed a biosensor system based on immobilized enzyme reactors (IMERs) integrated with an impinge-based aerosol sampling system for detection of organophosphate aerosols [60]. The IMERs was composed of enzyme-entrapped silica nanoparticles (NPs) prepared by R5 peptide-templated silicification: a solution of hydrolyzed TMOS and one of two target enzymes, OPH and butyrylcholinesterase (BuChE) was added into metal affinity column that was combined with His_6-R5 peptide, followed by the formation of the silica NPs. OPH-IMER biosensor showed detection limit of ~0.52 μmol/m^3_{AIR} for paraoxon and ~21 μmol/m^3_{AIR} for both demeton-S and malathion, whereas BuChE-IMER biosensor had lower detection limit for paraoxon (~0.21 μmol/m^3_{AIR}). The biosensors also showed stability and reproducibility with similar response over two successive changes in paraoxon concentration. Choi et al. constructed glucose oxidase (GOx) genetically fused with R5 peptide for GOx-entrapped silica-based amperometric biosensor [69]. The biosensor consisted of an Ag/AgCl reference electrode, a platinium counter electrode, and a working electrode that was prepared by self-immobilization of the fused enzyme on poly(neutral red)-formed graphite rod electrode and then, treating with Nafion. This biosensor specifically detected glucose in mixed samples containing ascorbic acid and acetaminophen with detection limit of 0.67 mM glucose.

Apart from R5 peptide, lysozyme-mediated silicification of enzymes has been shown to encapsulate them within biomimetic silica and stabilize them against denaturation [10,70]. Ramanathan et al. proposed a rapid protein immobilization method for development of OPH-based array biosensor in which OPH was encapsulated into silica by immobilized lysozyme on a waveguide surface [12]. The biosensor showed a detection limit of ~35 μM paraoxon and storage stability retaining 60% of original activity after 2 months.

Several synthetic amine templates have also been used to stabilize enzymes in biosensors via biomimetic silicification, including poly(L-lysine) (PLL) and PEI [61-66]. The Chaniotakis group developed a highly stable electrochemical biosensor using biomimetic silica-carbon nanofiber composite [62]. This composite was prepared by incubating acetylcholine esterase (AChE)-immobilized carbon nanofiber with silicic acid solution. The biosensor showed operational stability with a remaining activity of ~70% under continuous polarization for 3.5 months at 25 °C, whereas AChE-immobilized carbon nanofiber without the silicification was decreased to ~50% of original activity within 25 days. Along with good thermal stability with 50% retaining activity for 150 min at 50 °C, the biosensor showed 100% of original response for 12 h when exposed to Pronase solution. Furthermore, researchers have studied the mobil-

ity and interaction of AChE in the carbon nanofiber-silica composite using micro-Raman spectroscopy and electrochemical impedance spectroscopy [63]. They found that the AChE was in a thermodynamically stable state in the composite, and the carbon nanofiber acted as efficient nanochannel for electron transfer. Silicated CdSe/ZnS quantum dots (QDs)-based optical biosensor was also developed via PLL-templated silification [66]. AChE-immobilized QDs was capped by PLL, and the outer nanoporous silica shell was subsequently formed by adding freshly prepared silicic acid solution. The silica shell contributed to the enhancement of stability of the immobilized AChE without alteration of optical property of QDs, which led to good storage stability of the biosensor showing 65% residual activity after 45 days. In addition, Buiculescu et al. used PLL-based biomimetic silicification to stabilize amperometric biosensor based on Au NP-AChE nanocomposite [67]. While the normal AChE biosensor lost 50% of original activity after operation for 42 days, the silicified biosensor showed excellent operational stability for four months without reduction of activity. The biosensor showed initial sensitivity of 27.58 nA/mM and quantitative detection range of 0.04-0.4 mM. Tian et al. fabricated adenosine biosensor utilizing PLL-based biomimetic silicification [68]. Three enzymes (adenosine deaminase, nucleoside phosphorylase, and xanthine oxidase) were entrapped into a continuous transparent silica layer formed on the top of PLL-immobilized Pt electrode through the catalytic action of PLL. The biosensor could detect an adenosine level as low as 40 nM with a sensitivity of 153.0±2.4 μA/(mM·cm^2), and exhibited a favorable response time of 25±2 sec. Along with good selectivity, the biosensor retained more than 90% activity after 1 month.

PEI-based biomimetic silica was applied into an amperometric biosensor [64,65]. Zamora et al. constructed a biomimetic silica-based amperometric biosensor in which chemically derivatized cationic horseradish peroxidase was deposited onto anionic PEI-modified gold electrode, and silica precipitates were formed on the surface of electrode by adding silicic acid solution [65]. The biosensor exhibited analytical performance with a quantitative detection range of 0.50 μM-0.35 mM hydrogen peroxide and storage stability that retained 60% activity after 1 month at 4 °C. However, each electrode showed a response with high deviation for the same substrate concentration. In addition, thiol-functionalized silica NP was constructed for the development of amperometric biosensor [64]. The silica particle was produced by the condensation of MPTMS after addition of 25 kDa PEI into a mixture of GOx enzyme, and the particles were immobilized into gold electrode. The biosensor exhibited a linear detection range of 0 mM to 4 mM glucose along with sensitivity of 109 nA/mM.

2-4. *In Vitro* Biomimetic Silicification as Signal Enhancer in Biosensor

In addition to the use of biomimetic silicification for stabilization of enzymes and cells, silaffin peptides, including R5, R4, R2, and R1 peptides, were utilized as signal enhancer in gravimetric biosensor [71]. Distinct from green fluorescent protein (GFP), silaffin peptides-tethered GFPs showed significant changes in resonance frequency when hydrolyzed TMOS solution was supplied, demonstrating the possibility of the silaffin peptide as biomolecular signal enhancer for gravimetric biosensor.

3. Medical and Tissue Engineering Applications of Biosilica

In the past decade, biosilica has been diversely applied to biomedical and tissue engineering areas as a natural and morphogenetically active inorganic polymer. Particularly, biosilica-based novel biomaterials have been designed for bone tissue engineering, inspired by the natural biosilica formation for protection or mechanical support in various living organisms such as sponges, diatoms, and mammals [72]. It has been experimentally studied that the biomimetic silica generated by silicatein induces hydroxyapatite crystallite formation in human osteoblast-like SaOS-2 cells [73,74], and these cells showed upregulated expression of bone morphogenetic protein-2 (BMP-2), a key inducer of osteoblast differentiation, on a silicatein/silica matrix [75]. In particular, biomimetic silica is capable of increasing the gene expression ratio of osteoprotegrin (OPG) to receptor activator of NF-κB ligand (RANKL) in human osteoblast-like cells. OPG inhibits the function of the osteoclastogenic ligand RANKL, as a decoy receptor for RANKL, and the increased OPG/RANKL ratio promotes inhibition of osteoclast differentiation [76]. Recent results also revealed that the morphogenetic activity of silica has been narrowed down to the Wnt and the sonic hedgehog (SHH) signaling pathways [77]. Thus, strategies for the therapy and prophylaxis of osteoporosis and related bone diseases have been proposed using biomimetic silica as an anabolically-acting polymer [78].

Bioinspired silica has been also attractive for dental applications such as a protective dental layer to prevent caries formation. The silica layer was generated by binding silicatein to the dental hydroxyapatite surface, thus enabling to prevent caries formation and to reduce dental hypersensitivity through sealing of dental pits, fissures, and dental tubules [79]. Biosilica-based materials strongly upregulate the expression level of the genes encoding amelogenin and enamelin, ultimately increasing β-glycerophosphate-induced mineralization [80]. Silica has also been used as an environmentally friendly antifouling material with bioinspired approaches to prevent fouling. Inspired from biofouling of sponges by a dynamic surface coating with biosilica shield, it has been demonstrated that biosilica-based antifouling surfaces inhibit the growth of biofilm-producing bacteria [81]. Biosilica-based applications have been widely used in bone and enamel mineralization, biofouling, and also medical therapy of diseases such as cancer. In this section, we summarized and discussed how the biosilica-based applications for medical and tissue engineering have been developed with the type of biosilica-based materials such as surface coatings, scaffolds, and biomolecule delivery vehicles.

3-1. Surface Coatings

The biosilica coating strategies have been applied to induce hydroxyapatite mineralization in bone and dental layer. A bioengineered recombinant silicatein containing an oligo-glutamate sequence (Glu-tagged silicatein) allowed biomimetic silica coatings by immobilizing the enzyme onto hydroxyapatite bone surface (Ca-P substrate) and subsequent adding silica precursors [74,79]. Glu-tagged silicatein was shown to bind to the trabecular hydroxyapatite surface via the Glu-tag and induce silica formation in the presence of orthosilicate. The bioinspired silica coating on bone surface recruited osteoblasts and facilitated hydroxyapatite mineralization by upregulating OPG expression. In addition, a new polyurethane

dimethacrylate/methacryloxypropyltrimethoxysilane (PUDMA-*co*-MPS) copolymer was functionalized with biomimetic silica to obtain an osteogenic surface coating, through the polycondensation activity of immobilized silicatein [82]. A chitosan-*graft*-polycaprolactone (CHS-*g*-PCL) was functionalized with osteogenic silica via the surface-immobilized silicatein, and SaOS-2 cells showed enhanced osteogenic activity such as mineral deposition and alkaline phosphatase activity [83]. Silicatein and its silicate substrate have been applied in a poly(D, L-lactide)/poly(vinyl pyrrolidone)-based matrix for *in vivo* prototypic bone substitute material [84].

Silica film coating on variety of substrates was fabricated using 3,4-dihydroxyphenylalanin (DOPA) and nanogram amounts per cm^2 of silicatein [85]. DOPA has strong adhesive property that enables surface functionalization by immobilizing a large amount of silicatein with its activity maintained. The mussel-inspired simple biomimetic silica film coating under mild conditions is potentially applicable for the fabrication of silica films on variety of surfaces over multiple length scales.

Another recombinant protein consisting of the spidroin 1 domain (MaSp1) of the spider *Nephila clavipes* silk polymer and R5 peptide was constructed for silica NP precipitation [1]. The osteogenic differentiation of mesenchymal stem cells (hMSCs) was promoted by the silk-silica composite film coating, indicating the potential utility of biosilica for bone tissue engineering [1,86].

Silica coating to the hydroxyapatite surface based on silicatein was also used to mineralize enamel of dental layer [79]. In general, enamel crystals are tightly packed to rod-shape dentinal tubule structures. Insufficient mineralization of dentin causes radiation of dentinal tubules outward to the border between dentin and enamel, eventually resulting in dentin hypersensitivity by exposing nerves at the pulpal aspect. Sealing of dentinal tubules with silica coating could effectively reduce dental hypersensitivity by upregulating expression levels of amelogenin and enamelin. The amelogenins are involved in the initial mineralization process [87], and the enamelin is required for correct mineralization of enamel [88]. Moreover, silica coating supplemented with poly(ethylene glycol) (PEG) acts as a perspective antifouling coating that inhibits the growth of biofilm-producing bacteria [81]. A recent study demonstrated that the biosilica-based coating can be applied to mechanically protect target cells [53]. The target mammalian cells (HeLa, NIH 3T3, and Jurkat cells) were individually coated with biomimetic silica in a cytocompatible manner, enhancing the resistance of the target cells to enzymatic attack and toxic compounds.

3-2. Scaffolds

The aim of a scaffold is to provide a three-dimensional (3D) platform onto which cells can attach, proliferate, and differentiate. In particular, scaffolds for bone regeneration are designed as nanofibrous structure mimicking the natural inorganic/organic 3D bone architecture. The engineered nanofibrous scaffolds have highly porous properties allowing ingrowth of cells, efficient transport of nutrients, oxygen, growth factors, and also wastes, which facilitate bone cells to grow, differentiate, and eventually mineralize hydroxyapatite [89]. Morphogenetically active inorganic silica has been applied as a suitable material to scaffold fabrication of bone replacement. Biosilica-based nanofibrous scaffolds have been prepared by spinning of nanofibers and by 3D printing. A Glu-tagged silicatein has been developed for a targeted application of the silica-precipitating enzyme to hydroxyapatite nanofibrils [79]. In addition, poly(ε-caprolactone) (PCL) nanofiber mats containing tetraethyl orthosilicate (TEOS) were electrospun and subsequently incubated with silicatein to develop soft scaffolds for bone substitution [90]. Biomimetic silica was successfully generated on PCL nanofiber mats through enzymatic precipitation by silicatein, and it provided morphogenetically active matrix for the *in vitro* proliferation and mineralization of SaOS-2 cells.

It also has been shown that the silk-silica binding peptide chimera consisting of spider silk polymer (MaSp1) and R5 peptide can produce structure-controllable silica polymeric fibers with the positive effect of the silica binding peptide on the silicifying properties [1]. The silk-silica binding chimera nanofibers have been further shown to promote osteoblast development with the upregulation of key markers associated with bone formation [86]. Furthermore, a novel protein chimera consisting of the *N. clavipes* spider silk and a silica-binding peptide (KSLSRHDHIHHH), which was determined by phage display, was constructed by genetic approaches to produce scaffolds combining the elasticity and toughness of silk with the hardness of silica [26].

The 3D printing technique has been successfully used to prepare scaffolds for bone substitute with the potential to revolutionize regenerative medicine. Biomimetic silica has been impregnated for 3D silica/silicatein scaffold fabrication through a solid freeform fabrication (SFF)/indirect 3D printing technique and a direct 3D printing technique [5,91]. These biosilica-based 3D scaffolds were hardened in the presence of the synthetic polymer PEG. In addition, the silica-containing scaffolds induced the expression of OPG and BMP-2 in SaOS-2 as well as the mineralization of the cells [91].

Bone cells, including osteoblasts and osteoclasts, have been shown to be suspended in an alginate/silica composite hydrogel scaffold to retain ability for proliferation and differentiation of these cells [92,93]. In the silica-containing hydrogels, SaOS-2 cells showed increased levels of growth, expression of the genes encoding BMP-2 and collagen type 1 (Col I), and formation of hydroxyapatite nodule. In addition, a computer-designed and biodegradable alginate/gelatin hydrogel was bioprinted to enhance growth and mineralization of SaOS-2 cells via supplementation with polyphosphate (polyP) and bioinspired silica [94]. The cell-containing scaffolds consisting of a bioprintable, cell-compatible solid inner matrix surrounded by a printable hard and flexible outer matrix containing bioglass, provide a suitable strategy for the fabrication of morphogenetically active and biodegradable implants. Furthermore, biodegradable copolymer PLGA beads (microspheres) encapsulating β-tricalcium phosphate (β-TCP) were supplemented with biomimetic silica [95]. The biosilica-based microsphere scaffolds showed morphogenetic activity on bone-forming cells *in vitro* and an enhanced regeneration of bone tissue around the microspheres *in vivo*.

3-3. Biomolecule Delivery Vehicles

Biosilica structure has attracted considerable attention as a promising delivery vehicle for biomolecules due to high loading capacity, porous architecture, surface chemistry, biocompatibility, and biodegradability [96]. Biomimetic silica has been employed to sup-

port or encapsulate biologically relevant materials such as anticancer drug molecules [97], antimicrobial agents [98], metal oxide NPs [99-101], fluorescent dyes [102], and quantum dots [103].

Recent studies have proposed several methods of silica functionalization for controlled drug release [104-107]. Biosilica-based drug delivery system has been shown to enable tailorable and sustained release of drug molecules with low toxicity [107]. It was reported that biomimetic silica carrier for anticancer drug delivery was autonomously generated as a protein-silica composite upon simple incubation with a silica precursor [97]. A dual-function protein having both silica precipitating activity and anticancer activity showed sustained but slow release of the anticancer protein causing cancer cell death. Additionally, porous silica nanocarriers were bioconjugated with small interfering ribonucleic acid (siRNA), a powerful approach for silencing genes [98]. The bioinspired silica NPs with siRNA were effectively uptaken by human epidermoid carcinoma cells and mainly localized in cytoplasm, thereby enabling gene silencing in cancer cells. In another study, lysozyme was used for the development of nanocarrier delivering antimicrobial agent [70]. Lysozyme played a dual function as a ubiquitous antibacterial enzyme and a template that induces silica polycondensation.

In recent years, bioinspired methods have been used to synthesize metal oxide and bimetallic NPs supported on silica. Surface functionalization of metal oxide nanocarriers has been achieved by immobilizing silicatein via a polymeric ligand carrying catechol groups, which enables silica coatings onto TiO_2 nanowires (TiO_2@SiO_2) [99] and Fe_2O_3 NPs (γ-Fe_2O_3@SiO_2) [100,101]. These metal oxides are known to be suitable for fabrication of microelectronics due to their good semiconductor, dielectric, and/or electro-optic properties [99-101]. Moreover, encapsulation of quantum dots was conducted through synthesis of silica and gold colloid composite materials (Au@SiO_2), and it was demonstrated that the colloids retained their optical activities [103]. These studies show that biosilica-based nanomaterials are also promising candidates for the application to biomedical sensing and imaging.

CONCLUSION

We reviewed and updated on the recent efforts for the development of bioinspired silicification and its applications in the fields of biocatalysis, biosensors, and biomedical engineering. Bioinspired silica materials can be easily fabricated and tailored, and they exhibit stability, sensitivity, and functionality desirable for diverse applications in terms of both mechanical and biochemical interactions. Even though the basic concept of the biomimetic silica synthesis seems to be quite simple, the underlying mechanism has not been fully understood. Also, as examined in the paper, the unexpected or unpredictable results such as the increase of catalytic activity after encapsulation or the alteration of enantioselectivity have been reported. These synergetic effects are likely dependent on what a specific combination of the components (templates, precursors, enzymes, inorganics, and so on) is used during the silica synthesis and how the prepared silica hybrid material is utilized for a specific purpose. Therefore, it will be possible that properties and performances of bioinspired silica materials are extended to meet more specific, complex requirements otherwise unattainable.

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