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学　位　論　文　内　容　の　要　旨

博士の専攻分野の名称　　博士（工学）　　氏名　Kasun Shameera Godigamuwa Maheem Bandara Mudiyanselage

学　位　論　文　題　名

Biological fabrication of silica-based hybrid materials using silica-polymerizing enzymes
(シリカ重合酵素を用いたシリカハイブリッド材料の生物学的作製)

Inorganic-organic hybrid materials are being extensively used in many fields including electronics, energy generation, water treatment, and biomedical applications because they potentially possess the characteristics of both organic and inorganic materials. Silica is a promising inorganic matrix that can be used in inorganic-organic hybrid materials due to its chemical and physical stability, biocompatibility, and nontoxicity. Currently, scientists are focusing on the immobilization of biomolecules in the silica or organic matrix to develop novel inorganic-organic biohybrid materials with excellent properties. However, conventional methods used for the synthesis of silica and coating of materials by silica require high temperatures, harsh pH conditions and toxic chemicals that are not compatible with live cells and biomolecules such as enzymes.

In the detailed studies related to spicules of sponges, a group of enzymes called silicateins that can catalyze the silica formation under mild conditions have been found. However, silicateins show aggregation properties in aqueous media which makes them difficult to handle in applications. This aggregation property could be solved by fusing a soluble protein tag. In this study, silica-based biohybrid materials were fabricated in physiological conditions using novel interfacial catalysts composed of silicatein, soluble proteins, and solid-binding proteins.

In Chapter1, the research background, literature review, research scope, and originality of the research were presented.

In Chapter 2, cellulose has been used as the organic matrix to fabricate silica-cellulose hybrid materials. Cellulose is the most abundant polysaccharide on the earth and is used in cosmetics, pharmaceuticals, drug delivery systems, and tissue engineering. The biological route for the fabrication of silica-coated cellulose hybrid materials with biocompatibility is a novel tool that can be used in biomedical applications. In this study, two tags were fused with silicatein (Sil). They were ProS2, a soluble protein from *Myxocuccus xanthus*, and a carbohydrate-binding module (CBM), a domain of cellulase from *Clostridium thermocellum*. ProS2 and CBM enhance the solubility of silicatein and help to immobilize silicatein on cellulose material, respectively. The fusion silicatein (ProS2-Sil-CBM) containing both tags was successfully expressed in *Escherichia coli*. Solubility, enzymatic activity, adsorption on cellulose, and formation of silica on cellulose were investigated for ProS2-Sil-CBM. The results showed that ProS2-Sil-CBM maintained its solubility in aqueous media for 24 h and showed almost the same activity as ProS2-Sil. ProS2-Sil-CBM adsorbed on cellulose within 1.5 h, and the adsorbed ProS2-Sil-CBM successfully catalyzed silica formation on cellulose.

In Chapter 3, chitin was studied as another organic matrix to fabricate a silica hybrid material since chitin is used in agriculture, water and wastewater treatment, packaging materials, cosmetics, and biomedicine due to its biocompatibility and biodegradability. In this study, a novel fusion silicatein was designed by fusing silicatein with two tags, which were InakC, a truncated protein from ice nucleation protein (InaK) from *Pseudomonas syringae* as a soluble protein tag, and a chitin-binding domain (ChBD) from *Bacillus circulans* as a binding domain. Solubility and silica polymerization activity of fusion silicatein (InakC-ChBD-Sil) were investigated. After adsorption of InakC-ChBD-Sil on chitin, it was subjected to silica formation. Silica formation on chitin was investigated using SEM-EDS analysis. InakC-ChBD-Sil showed the almost same activity as ProS2-Sil. SEM-EDS results revealed that the adsorbed InakC-ChBD-Sil has successfully catalyzed the formation of silica on the chitin material.

In Chapter 4, as an advancement of the previous chapters, sphere-shaped chitosan gel-silica hybrid material was fabricated, and horseradish peroxidase (HRP) was successfully immobilized in the hybrid material as a model enzyme. Here, chitosan gel was the organic matrix used to form the silica-based hybrid material. Chitosan gel is used in many biomedical applications such as tissue engineering, drug delivery, and wound healing due to its biocompatibility. InakC-ChBD-Sil was investigated to form chitosan gel-silica hybrid material since chitosan is derived from chitin. According to the adsorption studies, InakC-ChBD-Sil showed significant adsorption on chitosan gel. InakC-ChBD-Sil adsorbed on chitosan gel and formed silica on chitosan gel successfully. During the preparation of chitosan gel beads, HRP was immobilized in the gel matrix, and they were subjected to silica formation by adsorbed InakC-ChBD-Sil. According to the enzyme activity test, it was found that HPR can be successfully immobilized in the chitosan gel-silica hybrid material while exhibiting its activity. These findings suggested that the chitosan gel-silica hybrid material can be used to encapsulate enzymes in biomedical and environmental applications.

In Chapter 5, research outcomes presented in each chapter were summarized along with some prospects for future works in the above research area.