Genetically Engineered Polypeptide for Differential Recognition of Platinum on Microfabricated Structures

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Abstract

By evolution, nature has created an astonishing collection of micro and nano scale structures. In this paper, we discuss the development of a "molecular toolkit" which builds the basis for the biomimetic natural processes. As the first step, we have demonstrated the capability to identify polypeptides that specifically bind to an inorganic (platinum) and differentiate between this metal and other inorganic surfaces such as silicon dioxide and gold. We have achieved the integration of the polypeptides with a microfabricated heterogeneous substrate, by selectively immobilizing them on the specific inorganic materials. In the future, this "toolkit", integrating micro-, nano- and biotechnology, can be used for the development of new generation of biomimetic manufacturing process.

1. Introduction

In nature, biological systems have successfully produced a huge variety of materials with distinct structures at nano, micro and macro scales. Compared to the abiotic processes, a number of biological systems can synthesize inorganic materials under environment-friendly, low-temperature conditions. Examples of such processes can be found in magnetotactic bacteria (producing iron oxide), sea sponge spatula (producing silicon dioxide), and even humans (producing calcium compounds of bones) [1, 2]. The primary molecular units responsible for synthesizing these materials and forming them into larger structures are proteins. Biomimetics of these processes at molecular level using genetically engineered proteins can create new opportunities in the material synthesis, structure formation, and device development for micro- and nanotechnology. This approach can potentially enable room-temperature manufacturing and integration of nano and microelectromechanical systems (NEMS/MEMS) structures and devices from aqueous solutions with basic building blocks.

A crucial issue in the biomimetic processes is the development of proteins/polypeptides that can specifically bind to inorganic materials. These polypeptides act as the interface between the biological and inorganic systems, therefore bridging the biomimetic and the traditional micro- and nanofabrication processes. Motivated by this goal, our group has embarked on a project to identify polypeptides that can recognize and specifically bind to inorganic surfaces. We aim to search for polypeptides with selectivity to various inorganic materials, and to develop a molecular toolkit, which uses these polypeptides for nano- and micro-scale biomimetic processes.

Previous research has demonstrated the inorganic binding polypeptides on homogenous substrates, nanoparticles and nanocrystals, and electrochemically modified substrates [3-5]. In this paper, we discuss the development of a molecular toolkit, which can be used to create a generic template for the biomimetic processes. First, we delineate the procedure to select the proper polypeptides. Then, we demonstrate the immobilization of specific polypeptides binding to a substrate patterned with different inorganics, in particular platinum (Pt).

In the following sections, we describe the selection of polypeptides recognizing different materials, the microfabrication technique and experimental setup to integrate the polypeptide with microfabricated structures.

Proceedings of the 2005 International Conference on MEMS, NANO and Smart Systems (ICMENS'05)
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2. Principle

The selection of a polypeptide that specifically binds to an inorganic material is performed *in vivo* by combinatorial biology protocols via different display methods, either phage display (PD) [6, 7] or cell surface display (CSD) [8, 9].

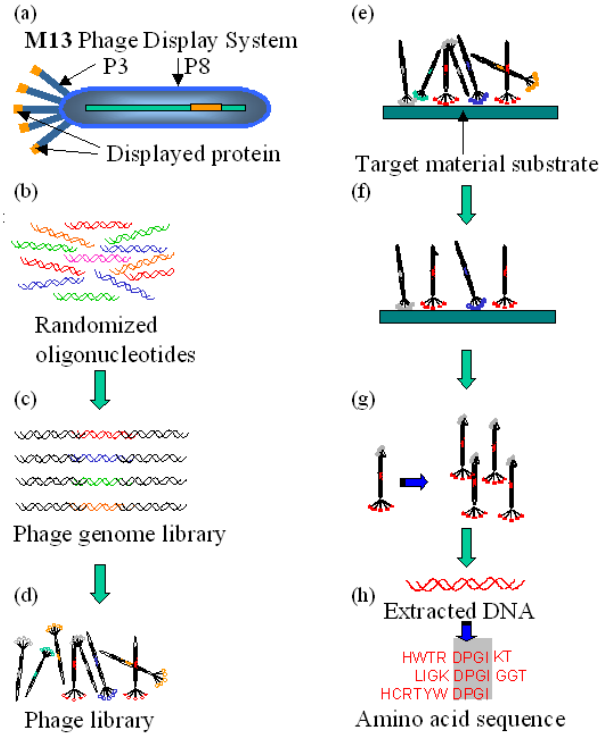

Figure. 1. Genetic selection of polypeptide for specific binding materials by the PD. (a) The PD system of M13. The displayed protein is fused to the P8 minor coating protein at the tip of the phage. (b) Prior to the selection process, randomized oligonucleotides are generated. (c) They are inserted into the phage genome to create the genome library. (d) The phage library can be generated by the protein expression of the different genomes. (e) The biopanning of the phage library to the target material substrate is performed, followed by (f) the removal of weak binding phage by washing. (g) The biopanning process is repeated several times to find the strongest binder. The binder is replicated and (h) DNA sequence can be extracted and amino acid sequence can be obtained.

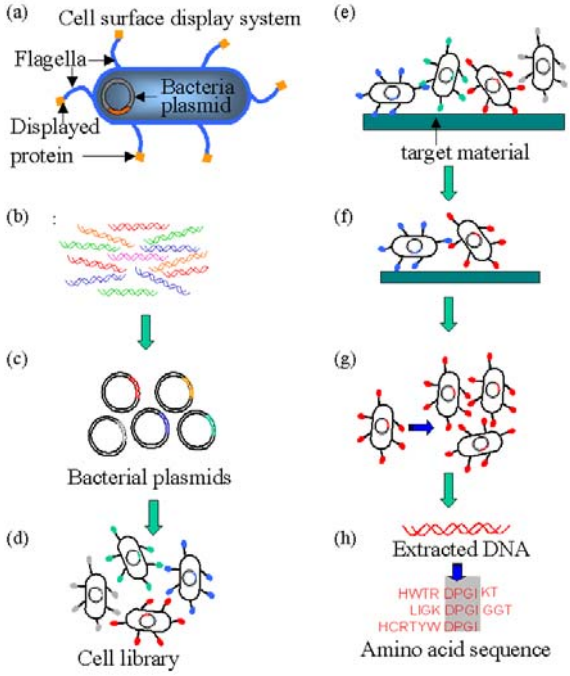

Figure. 2. Genetic selection of Au binding polypeptide by the CSD. (a) The CSD system of a cell. The displayed polypeptide is fused to the flagella protein of the cell. (b) Prior to the selection process, randomized oligonucleotides are generated. (c) They are inserted into the bacterial plasmids. (d) The cell library can be generated by the protein expression of the different plamids. (e) The biopanning of the cell library to the target substrate is performed, followed by (f) the removal of weak binding cell by washing. (g) The biopanning process is repeated several times to find the strongest binder, followed by elution of the bound cell from the surface. The binder is replicated and (h) DNA sequence is extracted and amino acid sequence is obtained.

2.1 Phage display

Figure. 1 shows the schematics of the genetic selection of polypeptide for specific binding materials by the PD.

The PD system of M13 is illustrated in Figure. 1a. The displayed protein is fused to the P8 minor coating protein at the tip of the phage.

The selection process starts with the randomization of a specific segment of DNA, which is then encoded on the phage genome (Figure. 1b). As the result, a

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library of phage genome can be generated (Figure. 1c), and different polypeptide sequences are displayed within the coating proteins of the phage (Figure. 1d). By comparing the binding strength of different proteins to the target inorganic material, a better inorganic-binding phage can be identified, which is illustrated in Figure. 1e, f. The strongest binder is replicated shown in Figure. 1g. Finally, the DNA sequence of the selected phage can be extracted, and the polypeptide sequence can be obtained (Figure. 1h).

2.2 Cell surface display

The selection process by CSD is performed in a similar manner as the PD, which is shown in Figure. 2. Figure. 2a depicts the CSD system of a bacterium. The displayed protein is fused to the flagella protein of the cell.

First, a specific segment of a DNA sequence is randomized (Figure. 2b) and encoded in the bacterial plasmid (Figure. 2c). As the result, different polypeptide sequences are displayed within the flagella proteins of the cells (Figure. 2d). By comparing the binding strength of the polypeptides to the target inorganic surface, a better binding cell can be selected (Figure. 2e-g). The DNA sequence of the cells with a strong binding affinity is extracted, and the polypeptide sequence is determined (Figure. 2h).

As the result, polypeptides binding to different inorganic materials can be obtained. The information of the polypeptides binding to gold (Au) and Pt is listed in **Table I**.

TABLE I.
Examples of Polypeptides Binding to Different Inorganic Materials

Material	Method	Binding polypeptide
Au	CSD	MHGKTQATSGTIQS
Pt	PD	QSVTSTK

3. EXPERIMENT SETUP

After the identification of the peptide sequences that can recognize and specifically bind to the inorganic surfaces of choice via CSD and PD, we use selected polypeptide sequences to demonstrate the specific binding activity to microfabricated structures. Our procedure includes synthesis of the polypeptide, microfabrication of patterned substrates, polypeptide incubation on the substrates, and finally labeling the polypeptide with fluorescent dye and imaging.

3.1. Substrate fabrication

To fabricate the substrate, a silicon wafer is prepared with a thermal silicon dioxide (SiO_2) layer of approximately 25 nm (Figure. 3a). The substrate is then patterned with TiW/Au (10/25 nm) by photolithography, sputtering and a lift-off process (Figure. 3b). The next step is to pattern Ti/Pt (10/35 nm), which can be achieved by photolithography, e-beam evaporation and a lift-off process (Figure. 3c).

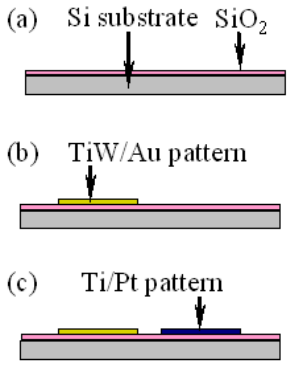

Figure. 3. Substrate fabrication processes: (a) Thermal oxidation of the silicon substrate. (b) TiW/Au is patterned on the substrate by photolithography, sputtering and lift-off process. (c) Ti/Pt is patterned on the substrate by photolithography, e-beam evaporation and lift-off process.

3.2. Platinum binding phage incubation

The experiments can be carrier out either with the labeled polypeptides or with peptides as displayed on the surface of a phage. We have previously studied the binding of a three-repeat custom synthesized gold-binding polypeptide [10]. Here, we discuss the validation experiments with polypeptide as expressed on phase surface.

Prior to incubation, we clean the samples by sonicating them in ethanol and isopropanol for ten minutes respectively, followed by vacuum dry and ten-minute exposure ultra violet (UV) decontamination.

For incubation of the phage with the Pt binder polypeptide, the substrate is immersed in the phage (Ph.D.-7, New England Biolab Inc.) solution, with the

aqueous buffer of KH_2PO_4 (0.75 %wt.), Na_2CO_3 (0.476 %wt.) and NaCl (1.168 %wt.) overnight (9~10 hours). The Pt binding polypeptide is displayed at the tip of the phage.

3.3. Labeling with florescent reagent

To label the phage with Pt binding protein, we mix antibody IgG and Zenon labeling reagent (Alexa Fluor 488, Molecular Probes) with the ratio of 1:5 in volume for thirty minutes (Figure. 4a, b). The mixture is then diluted with the buffered solution with the ratio of 3:100 in volume. Finally, the substrate is immersed in the dye solution for forty-five minutes, and the labeled IgG is bound to the phage. Therefore, the florescent dye is attached to the phage (Figure. 4).

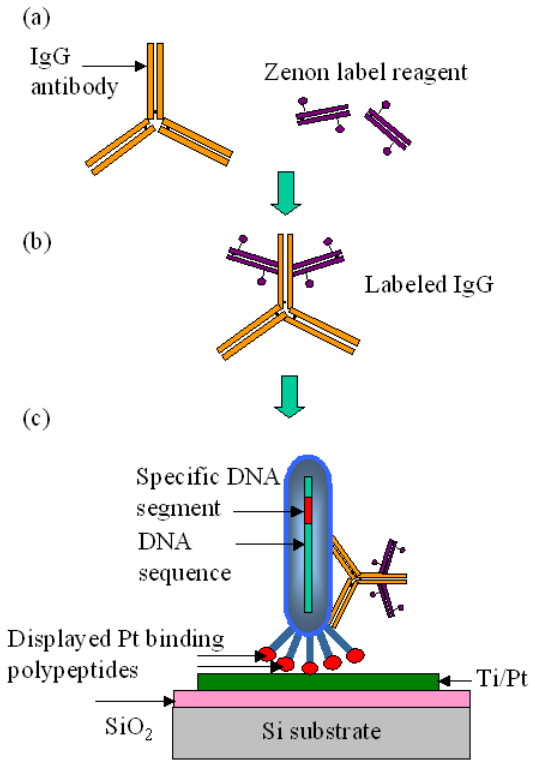

Figure. 4. Schematic plot of labeling the phage with florescent dye. (a) Antibody IgG is incubated with a fluorophore-labeled Fab fragment, and (b) the Fab fragment binds to the IgG. (c) When incubated with the phage, the labeled IgG binds to the phage.

4. RESUTLS AND DISCUSSIONS

4.1. Selective Pt binding

The results of phage binding on Pt are shown in 5, with 5a being a bright field image, and 5b being a florescent image of a substrate with Au and Pt squares on the SiO_2 background. Comparing these two pictures, we can see that the phage binds only to Pt patterns on the substrate and can readily distinguish between Pt, Au, and silicon dioxide areas.

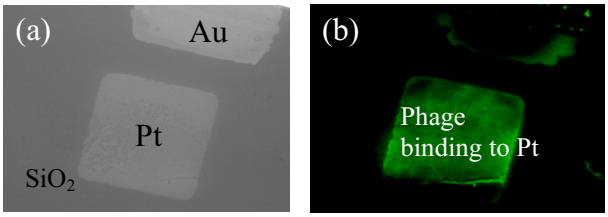

Figure. 5. Images of substrates patterned with Au and Pt squares on a SiO_2 background after Pt binding phage incubation: (a) the bright field and (d) fluorescent images of a substrate with overnight phage incubation. The squares are 100×100μm^2. The darker background in the images is SiO_2 area.

4.2. Discussions

In this paper, we present a methodology to identify polypeptides that can recognize and specifically bind to inorganic materials. These polypeptides provide the basis for constructing a tool kit that merges the biological and abiotic domains and can be used for the self-assembly of complex functional structures. We have discussed how the identification process could be achieved via phage display or cell surface display. We have employed the genetic tools to identify a variety of polypeptides. As an example, we have used a polypeptide that specifically recognizes and binds to Pt. We demonstrate that the polypeptide, being expressed on the tip of a phage, can distinguish between different inorganic surfaces such as Pt, Au, and SiO_2 and selectively bind only to the Pt regions of a microfabricated structures.

These genetically engineered polypeptides with unique recognition ability can be used to program self-assembly across the nano and micro scales. They can also guide the material synthesis and device placement onto microfabricated structures for novel applications.

5. Acknowledgements

The author thanks the National Institutes of Health for providing the partial support for this work through the Center of Excellence in Genomics at the University of Washington and the University of Washington-DURINT Project via Army Research Office. They also appreciate help from the Washington Nanotechnology Center User Facility and the Washington Technology Center Microfabrication Facility.

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