Protein Based Direct Fabrication of Nano-structures On Si Wafer in Aqueous Solution Bio Nano Process, a Wet Nanotechnology

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1. Introduction

Study on the nanometric device is now the hottest research fields and bottom-up technology is believed to play an essential role in fabricating nanodevices. The assessment of the proteins from the nanotechnology points of view reveals that proteins are ideal nano-blocks for fabricating inorganic functional nano-structures of nanodevices.

That is

- 1) the same structure down to atomic scale,
- 2) self-assembly ability,
- 3) selective deposition of inorganic materials (biomineralization)
- 4) vulnerable compared to inorganic materials.

Taking advantage of these characteristics, a biological method to make inorganic nano-functional structures was proposed. Namely proteins are used as scaffolds to fabricate inorganic nanostructures on structures produced by the top-down method, which was named Bio Nano Process (BNP)[1].

2. Experimental results and discussions

The BNP is composed of three steps. One is biomineralization of nano particle (NP) and nano wire (NW) using bio-templates, mainly proteins. The second step is the placement of the biomconjugates, proteins with NPs and proteins with NWs based on the self-assembly of proteins. The final step is the fabrication of nano-devices using the conventional semiconductor process, which means the BNP is a integrated process of bottom-up and top-down technologies. In this paper, the BNP handling NPs is presented.

Fig. 1. Illustration of Some target nano-structures of the Bio Nano Process

2.1 Biomineralization of Nanoparticle in a cage-shaped protein

We have developed a process which produces homogenous nanoparticle (NP) in apoferritins or Lis-Dps. The outer and inner diameters of apoferritin are 12 and 7 nm respectively[2]. LisDps has the outer and inner diameter of 9.5 nm and of 4.5 nm[3]. The inner cavity was used as a spatially restricted chemical chamber for artificial biomineralization of metal, semiconductor, and oxide material NP. Since the cavity size was determined by the DNA information, the shape and size of the cavity is atomically the same. Therefore, NPs synthesized have the same size, which is ideal for the applications. The NP biomineralization process is simple. Solution containing cage-shaped

protein and source ions was incubated for a designated period. Source ions were introduced in and NPs are selectively formed inside the protein shell. The details of the NP biomineralization mechanism are not yet clear. But, it is generally accepted that the collection of negatively charged amino acid residues on the inner surface and three-fold channel play an important roles for sucking in source ions and nucleation. So far, more than 15 kinds of NPs were synthesized in the cage-shaped protein supramolecules[4]. Fig. 2. shows a gallery of TEM images of synthesized NPs.

Fig. 2. Nanoparticle synthesized in the cage-shaped protine, apoferritin

2.2 Placement of two dimensional crystalline cage-shaped proteins, ferritins with NP on a silicon substrate

Genetic modification of ferritin outer surface is a powerful tool to modify the interaction between substrate and ferritin. It is also true that the ferritin surface modification can control the interaction ferritin molecules at the same time. Target-specific peptides which bind to specific material surface selectively are good for such surface modification. We employed the carboneous material binding peptide which Kase et.al., obtained using the phage display method. The peptide was named NHBP1. Its sequence is D-Y-F-S-S-P-Y-E-Q-L-F, which is highly hydrophobic, i.e. insoluble in water [24]. We genteically added the peptide at the N terminus of a ferritin subunit. 24 subunits self-assembled into a protein shell. The recombinat apoferritin displayed 24 carboneous material binding peptides on the outer ferritin surface [5]. The recombinant ferritin not only has the ability to bind to the carboneous material but also attracts other recombinant ferritin through hydrophobic interaction. By experimental exploration, it was found that protein-protein and protein-substrate interactions can be controlled by

buffer solution. Under appropriate conditions, it becomes possible to fabricate a two-dimensional array of ferritins directly on the Si substrate [5,6] (Fig. 3).

Recently, we studied the mechanism of tow dimensional crystalline array formation further and it was found that the combination of spin-coating method and silicon substrate modification by polyethylene glycol (PEG2000) produced two dimensional crystalline recombinant ferritin domains. The silicon substrate was covered by PEG2000 by spin-coating technique and the ferritin molecules in a buffer solution were put on the obtained PEG-substrate. Then the excess solution was removed by spinning, or spin-dried. The obtained substrate was subject to the high resolution SEM. This simple method produced two dimensional recombinant ferritin crystalline domains with sub-micro meter.[** in press] The mechanism of the two dimensional crystal is now under investigation.

Fig.. 3 (a) SEM image of hexagonally packed array of mutant ferritin with peptide which binds specifically carbonaceous surface. White dots represent the inner NPs and protein shell are not visualized by SEM. (b) SEM image of ferritin without target-specific peptides. Both ferritins were applied to the Si surface in the same process.

2.3 Placement of a single ferritin with NP.

Several methods could make ferritins selectively adsorb on a specific position on a substrate, however, it is hard to place single ferritin, the diameter of which is only 12nm, at the designated position. We employed electrostatic interaction to perform single ferritin placement. Ferritin is negatively charged and the Si surface was highly negatively charged around a neutral pH. Nanometric positively charged pattern on the Si surface could potentially attract an ferritin in aqueous solution. However, electrostatic interaction is a long distance force and, in genral,

is thought to be inappropriate for handling nanometric materials. Yoshii and Kumagai solved this issue by carrying out simulation of ferritin-substrate electrostatic interaction and showed that ferritin molecules can be placed on the substrate one by one [7-9].

We adopted a 45nm circular pattern of 3-Aminopropyl-triethoxysilane (APTES) on a Si substrate for the single ferritin placement. For larger electrostatic attractive interaction between APTES pattern and ferritin, ferritin surface was so modified to have more negative charges. The positive charged amino acids, Lysines, were genetically replaced by negatively charged amino acid glutamic acids. Poisson-Boltzmann equation was numerically calculated to obtain three-dimensional electrostatic potential distribution in solution. When the ferritin molecule moves toward the substrate, mobile ions in the buffer solution accumulate between the ferritin and the substrate to generate osmotic pressure. The potential barrier for the ferritin to reach the APTES disk was evaluated as a change in the total free energy that includes electrostatic energy, entropic effects of mobile ions (osmotic pressure), and van der Waals interaction. A simulation of the ferritin adsorption carried out and the best conditions for single ferritin placement was obtained. Once first ferritin adsorbed on the APTES, the negative charge of the first adsorbed ferritin repelled the second ferritin, which made it possible that one ferritin was placed onto one APTES circular pattern.

Fig. 4. SEM image of single ferritin placement. on 45 nm APTES disk. Each APTES disk has one ferritin with Fe NP. The charge enhanced performed much better single ferritin placement[9].

We placed a 0.5 mg/ml solution of ferritin with an iron oxide core in a MES/Tris pH 7 buffer with a designed ionic strength of 0.01 mM on a

substrate with 45nm APTES for 1 min at room temperature. The samples were then rinsed with pure water and spin dried. Then, surfaces were observed with a scanning electron microscope SEM, JSM-7400F at 5.0 kV (Fig. 4). Each 45 nm APTES disks arranged in a grid pattern had one mutant ferritin even though the disk is seven times larger than ferritin molecule. No adsorption on the SiO₂ surface was observed.

2.4 Nanoetching with NP formed and placed by ferritin

NPs placed on a substrate were used as masks for neutral beam etching (NB) which is a new technique developed by Dr. Samukawa. A multilayer, 1-2nm poly-silicon layer sandwiched by thin SiO₂ layer was fabricated on a Si substrate. NPs which were synthesized in the ferritin cavity were placed above the layer and the protein shells were selectively eliminated, which left only iron-oxide cores. Then, the sample was etched by NB (Fig. 5(a)).

The cross section TEM images confirmed successful production of a Si nanodisk [10,11]. Electron energy-loss spectroscopy was carried out. Fig. 5(b) clearly showed the iron-oxide core worked as a nano-etching mask and a Si nanodisk was produced and buried in the silicon dioxide layer. The separation between nanodisk and Si substrate was narrow enough for electron tunneling.

Fig. 5. (a) Schematic drawing of the nano-etching by neutral beam etching using ferritin core (NP) as etching mask. (b) Upper : a STEM-EELS elemental mapping of a nanodisk for and Lower : cross-section TEM image of the obtained nanodisk.

It was shown that the nanodisk worked as a quantum well component. The diameter (about 8 to 10 nm) of the nanodisk was controlled by changing the surface-oxide-removal conditions, and the nanodisk thickness (about 2 to 4 nm) was controlled by deposition thickness of poly-silicon.

Current–voltage measurements of the nanodisk showed staircase characteristics at room temperature (RT). The dependence of stair case widths are shown in Fig. 6. The staircase width depends strongly on nanodisk thickness, which is reasonable based on quantum theory, but it does not depend strongly on the nanodisk diameter, which are very useful from application points of view. The nanodisk diameter is hard to be controlled but we can neglect the effect of the diameter dispersion because of its low dependence on the staircase width. On the other hand, the thickness is easily controlled and the stair case width can be defined precisely. The result suggests that the nanodisk can be used as an easy controllable quantum-well at RT.

The two dimensional Si nanodisk array is now under investigation for applications in novel quantum solar cells.

Fig. 6. Staircase width in I–V curves as a function of (a) nanodisk diameter and (b) nanodisk thickness.

3. Conclusion

Making NPs using the cage-shaped protein apoferritin, has been proven effective and is promising for the production of homogeneous NPs. The number of materials that can be synthesized in the cavity has rapidly increased as a result of successful research. Considering the progress being made, it will soon become feasible to produce a variety of inorganic nano-material shapes, other than spheres, using protein supramolecules. Moreover, the outer surface of the protein can be used to make nanostructures by self-assembly for various applications. Combined with protein self-assembly, it will become possible to build complex functional inorganic nanostructures on the substrate including Si wafer, glass and plastic substrates in the near future. This newly emerging biological approach will open up a

new path to the fabrication of functional nanostructures based on inorganic NPs.

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