GROWTH OF CARBON NANOTUBES DIRECTLY FROM NATURAL PROTEINS

Hao-Chieh Hsieh¹, Md. Mahbubur Rahman¹, T.C. Shen² and Hanseup Kim¹

¹Electrical and Computer Engineering, University of Utah, Salt Lake City, Utah, USA

²Department of Physics, Utah State University, Logan, Utah, USA

ABSTRACT

This paper presents a new class of a nano manufacturing method: the synthesis of carbon nanotubes (CNTs) directly from natural proteins, particularly native ferritin, tyrosinase, and urease molecules. This manufacturing method enables controlled nano-manufacturing by taking advantages of unique biological properties including nano-scale sizes, self-assembly, self-alignment, large-area coverage, low cost, and diverse metal element availability. In particular, this paper presents the first 'proof-of-concept' results proving its feasibility: single and multiple CNTs were successfully grown from immobilized proteins at the desired spots in predictable lengths up to 10 μm in height.

INTRODUCTION

Carbon nanotubes have many superior properties and have been widely applied to various applications including biosensor electrodes, explosive detectors, super-capacitors, and others. For example, CNTs have tensile strength up to 63 GPa, which is much stronger than steel (1200 MPa), and demonstrate a wide range of conductivities from metal to semiconductor levels, which is potentially useful for next generation electronic devices.

CNTs can be produced by several methods (Table 1); however, the major trade-off between controllability and manufacture sizes has prevented their practical uses for mass-production. For example, some previous technologies are applicable to either only a small area (nanolithography, field-assisted nanowire growth), serial and slow (nanolithography); or cover a large area with random control over the numbers and locations of the nanostructures (CVD). Thus, the development of a practical nano-manufacturing technology is clearly necessary that is capable of both accurate control of locations and mass-production over a large area. Such capabilities could be simultaneously achieved by utilizing a biological subject as a CNT growth seed that (1) naturally controls the spacing through the self-assembly and self-alignment, (2) easily grows over an extended area of surface, and (3) provides various metal types after lysis.

Metalloproteins can be an excellent candidate for such a biological seed that provides the foundation for the controlled and wide-area CNT growth. Metalloproteins are specific proteins that contain metal ions inside the structure, such as ferritin, tyrosinase, and urease, and are excellent candidates for the purpose (Table 2). Most of the metalloproteins utilize metal ions as catalyst in specific metabolic functions; some are specially used to maintain body ion concentrations. They also have been providing a wide range of choices for electronic manufacture materials and applications such as biosensors. Recently, a few research groups have reported the use of metalloproteins as catalysts for CNT growth. However, these previous methods involve genetic modification of proteins, which might cause degraded activity or loss of metal ions. Here, we report our first attempt in utilizing natural metalloprotein as the catalysts to provide variety metal sources for massively-parallel and controlled CNT growth.

METALLOPROTEIN

Metalloproteins play important roles in various chemical reactions using their metal cores from basic chemical reaction

(electron transfer and hydrolysis) to complex signal transduction and ion channel control. Several different metalloproteins exist, and each contains specific metal elements as listed in Table 2. Note that these metalloproteins provide a specific number of metal ions per molecule and a specific volume shape. The dimensions of metalloproteins range from several nanometers to a few micrometers, while each metal atom holds the size of 100-200 picometers. A ferritin protein, for example, (Fig. 2) is a sphere shell molecule composed with 24 polypeptide subunits and has a diameter about 9 nm. The inner space contains up to 4500 iron ions. In some research ferritin shells have been utilized as a mold to form metal nanoparticles. Specifically, ferritin, tyrosinase, and urease have been utilized in this work.

Process Flow

Analyzed Results

1. Surface Modification (3% APTES in acetone)

2. Metalloproteins Immobilization

3. Metal Seeding (metal atoms remains after Enzyme lysis)

4. Carbon nanotubes induction

Figure 1: The main concept of the protein-based CNT growth: (Left) the fabrication procedure and (Right) the measurements at each stage.

Table 1: Advantage comparison of CNT growth methods.

Fabrication method	Controllability	CNT Growth Area
Optical Lithography [5]	High	Low
Fabrication by Electric Field [6]	Medium	High
Chemical Vapor Deposition [7]	Low	High
Vectorial Growth by CVD [20]	High	Low
Plasma Enhanced CVD [21]	Medium	Medium
Pyrolysis by HydroCarbon [22]	Medium	Medium
*Bio platform-based CNT growth	High	High
*Approach in this paper.		

Table 2: Comparison of metalloproteins.

Metalloproteins	Metal ion	Metal ions per molecule	Atom radius
*Ferritin	Fe	4500	126 pm
*Tyrosinase	Cu	2	128 pm
*Urease	Ni	2~12	124 pm
Amylase	Ca	1	197 pm
Catalase	Fe	1	126 pm
Arginase	Mn	2	127 pm
Nitrogenase	Mo	1	139 pm
Thermolysin	Zn	1	139 pm

*Proteins have been tested in this paper.

Figure 2: Left: Ferritin molecule contains 24 subunits to form a sphere with inner space to contain iron ions. Right: Tyrosinase molecule with copper atoms.

FABRICATION

The ‘proof-of-concept’ CNT growth was performed in three stages: metalloprotein immobilization, metal seeding, and CNT induction (in parallel to Fig. 1). The immobilization step overcomes random catalyst distribution, and avoids losing metalloprotein while removing impurities during cleaning process and secures metalloproteins onto the wafer for reliable CNT growth. If not secured, CNT growth is degraded because the impurities, mostly salt, form the crystal cover the surface and cause contamination. The metal seeding step secures only metal atoms by removing the other parts of the metalloproteins including shells thus expose the metal catalysts to feeding gases and also increase the contact area. The CNT induction step grows CNTs from the secured metal atoms in single or multiple CNT configurations.

Surface Modification and Metalloproteins immobilization

Metalloprotein immobilization was performed by modifying the surface of the target wafer diced into 1 cm x 1 cm utilizing 3% (v/v%) 3-Aminopropyltriethoxysilane (APTES, A3648, Sigma-Aldrich, St. Louis, MO) in acetone. Unbound APTES was removed with acetone in sonicator for 1 minute. For example, ferritin molecules (F4503, Sigma-Aldrich, St. Louis, MO) were secured on the wafer surface by immersing the wafer into a ferritin solution later diluted with DI water for 12 hours on orbital shaker at room temperature. Then the wafer surface with ferritin molecules was washed with static DI water in a beaker to gently remove unbound ferritin molecules.

Metal seeding

From the immobilized metalloproteins, only metal atoms are needed for CNT growth. Thus this step preserves only metal atoms by removing the protein shell. In order to remove the shell and expose the metal core, the seeded wafers were first dried and air burned by elevating the temperature up to 500°C in a furnace (Thermolyne 79400, Thermo Fisher Scientific, Massachusetts) for 10 minutes. Such heating process decomposed the protein structure and left only metal atoms on the substrate. For example, ferritin exposed a certain number (4500) of metal ions (iron) once their protein shell was removed. These iron atoms may start to diffuse on the surface or into the wafer and form bigger cluster with each other, thus the temperature control and time were very critical.

Carbon nanotubes induction

CNTs were induced to vertically grow by utilizing the standard thermal CVD method that is the most widely accepted. Because the previous seeding process involved air and heat to remove protein shell, it oxidized the metal irons and resulted in the loss of their catalyst activity. Thus, before inducing CNTs, we first chemically reduced iron catalysts by feeding Ar/H₂ gas mixture (50 sccm/45 sccm) in the furnace for 10 minutes to assist their activity recovery. Next, the feeding gas, Ar/H₂/C₂H₄ gas mixture, as a carbon source (50 sccm/45 sccm/5 sccm), was introduced to induce CNT growth at 700°C for 10 minutes. Once the C₂H₄ was decomposed on the surface of the catalyst, carbon atoms had a chance to connect to each other around the catalyst and started growing and forming carbon tubes. Finally, the samples were cooled down to 50°C under Ar/H₂ gas mixture and analyzed by scanning electron microscopes (SEM, Quanta 600 FEG, FEI, Oregon).

TESTING METHODOLOGY

Surface Modification and Metalloprotein immobilization

To ensure the ferritin molecules were immobilized on the wafer surface through APTES bonding. Samples were analyzed with Fourier Transform Infrared Spectroscopy (FT-IR) to confirm the existence of ferritin protein shell. In addition, surface profile was measured by utilizing the AFM-based physical profiler.

Metal seeding

To ensure the protein shell was completely removed, Fourier Transform Infrared Spectroscopy (FT-IR) was performed to compare samples before and after heat treatment. Immobilized ferritin samples were placed on hot plate (PC-420D, CORNING, Massachusetts) at 500°C, and then analyzed with FT-IR (Varian 3100, Varian, Inc. California).

To confirm the iron seeds were still remained on the wafer surface after heat treatment, Energy-dispersive X-ray spectroscopy (EDAX™ Genesis system, EDAX Inc, New Jersey) was

performed to identify metal types on the wafer surface by focusing X-ray beams of 20kV at 8.6-mm working distance.

Carbon nanotubes induction

The CNTs growth pattern under immobilization effects was analyzed with SEM at 30kV in a high vacuum mode. The influence of CNTs growth with or without immobilization and growth pattern were compared from SEM images.

RESULTS

Surface Modification and Metalloproteins immobilization

The measured FT-IR spectrum (Fig. 3) indicates that the metal proteins are immobilized on the target wafer surface. The spectrum clearly showed a peak of amide bond (A) from protein shell at 1150 cm^{-1} . This peak indicated that the ferritin outer shell was immobilized in the wafer surface. Note that peaks of protein molecules typically appear at 1650 and 1540 cm^{-1} . We believe that this difference may be caused by the molecular bonding onto wafer surface instead of free molecular status in conventional tests.

Metal seeding

The combined results of FT-IR and EDX detection confirmed that protein shells were successfully removed and the target metal seeds were exposed on the wafer surface (Fig. 3). As shown in the FT-IR results in Figure 3, the amide bond peak at 1150 cm^{-1} dramatically shifted between before (A) and after (B) heat treatment. Such reduction indicates the protein shell was removed after the heat treatment. In parallel to FT-IR results, the EDX results (Fig. 3, inset table) clearly showed the FeKa and FeKb X-rays emitted from Fe element and the existence of 29.79 Wt% of Fe element on the analyzed area, verifying the successful metal seeding process.

Carbon nanotubes induction

Figure 4 shows the top view of the wafer sample surface after CNT growth, indicating the influence of immobilization on the CNT growth uniformity. Specifically, immobilization increased the growth uniformity. Without immobilization process, ferritin molecules, which were initially preserved in saline solution, started forming precipitation on the wafer surface during air-dry process and produced a rough surface (A1). The resultant rough surface led to random CNTs growth. In contrast, when the ferritin molecules were immobilized, the CNT grown surface (B1) turned out to be smoother, and ferritin was distributed more evenly. Resultantly, the CNTs were evenly located on the post-immobilization wafer surface (B2).

Figure 5 shows that the higher concentration of seeds led to the higher density of CNT growth. The left photo shows the CNT growth produced from a higher ferritin molecules concentration (C1: 0.44 mg/mL), and the right photos shown the lowly concentrated CNT growth from a lower ferritin concentration (C2: $0.44 \times 10^{-2}\text{ mg/mL}$) as expected. It was also observed that the CNT distribution was not so even on the wafer especially at low concentration. This suggested that ferritin molecules form some self-aggregation during immobilization step, causing some areas to have more ferritin molecules than other areas and resulting in non-uniform CNTs distribution. It might be also possible that the iron catalysts may be randomly diffused onto the wafer surface resulting in differently-sized aggregated catalyst. It was also observed that all of higher temperature, heating with longer time and higher ferritin concentration led to forming more severely-aggregated catalysts.

Figure 6 shows the successfully grown CNTs from the natural metalloproteins. The diameters of CNTs were determined by the

catalysts sizes and the length was by the period of gas feeding as well as the lifetime of catalyst. The measured height of a single CNT was about $10\text{ }\mu\text{m}$ in length and 120 nm in diameter. By changing the seeding densities of iron catalysts, different types of CNT growth were observed including single carbon nanotubes and CNT forests with the dimension of $5.19\text{ }\mu\text{m}$ in height and $9.42\text{ }\mu\text{m}$ in width. We believe that the iron catalysts density on the surface and the self-aggregation of ferritin molecules influenced these variations.

Figure 3: FT-IR spectrum of ferritin molecules (A) before and (B) after heat treatment.

Figure 4: SEMs: A1: wafer with free ferritin. A2: CNTs growth from free ferritin. B1: wafer with immobilized ferritin. B2: CNTs growth from immobilized ferritin.

Figure 5: SEMs: C1: wafer with ferritin 0.44 mg/mL . C2: wafer with ferritin $0.44 \times 10^{-2}\text{ mg/mL}$.

Figure 6: SEMs of (Left) single carbon nanotube and (Right) carbon nanotube forest.

DISCUSSIONS AND CONCLUSIONS

We have successfully proven the feasibility of producing CNT growth directly from native proteins (ferritin, urease, tyrosinase in this proof-of-concept tests). We have also demonstrated metalloprotein immobilization, metal seeding, and CNT induction. Despite the remaining challenges of regulating the precise location of metal catalysts, the results provide sufficient verification of the proof-of-concept methods as a new class of nanomanufacturing to enable controllable, patternable and massively-parallel CNT production.

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CONTACT

*H.C. Hsieh, tel: +1-801-808-0948; hchsieh1982@gmail.com