

Fabrication of TiO₂ Nanowires Using Vapor-Liquid-Solid Process for the Osseointegration

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In order to improve osseointegration for biomedical implants, it is crucial to understand the interactions between nanostructured surfaces and cells. In this study, TiO₂ nanowires were prepared via Vapor-Liquid-Solid (VLS) process with Sn as a metal catalyst in the tube furnace. Nanowires were grown with N₂ heat treatment with their size controlled by the agglomeration of Sn layers in various thicknesses. MC3T3-E1 (pre-osteoblast) were cultured on the TiO₂ nanowires for a week. Preliminary results of the cell culture showed that the cells adhere well on the TiO₂ nanowires.

Keywords : TiO₂ nanowire, Vapor-Liquid-Solid, Sn agglomeration, Pre-osteoblast, Osseointegration

I. Introduction

As medical technology advances especially in the field of implant surgery, the interaction between the body and materials became more interesting. Therefore, the biocompatibility of various materials and the cell adhesion on their surfaces became vigorous area of research [1-5].

In the osseointegration, the cells related with the bone interact with the surface of the implant materials. A cell recognizes and responds to its surrounding environments, extracellular matrix (ECM) [6]. Cells can react to the force and signal (chemical or physical) from the ECM and also sense the topography of the ECM [8]. Thus, for effective inter-

action between cells and the surface of the materials, it is essential to provide an environment similar to ECM for better osseointegration.

Titanium is widely used as an implant material because its surface forms native oxide that provides inertness. It is used for the orthopedic treatment such as fixation of the bone fracture, hip joint replacement, and dental implants [7-10]. There have been many studies about the roughness of the surface of the titanium as implant materials and cell adhesion [3-5,11]. Recent studies indicate that osteoblast adheres better to surface of the implant with micro-scaled structures. However, those methods fabricating the micro scaled structures or increasing roughness on the surface results in the topography

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with randomness. To mimic the ECM and understand the interaction between cells and nanostructures properly, we need to fabricate various nanostructures with controlled factor such as size, diameters, length, pitch, crystallinity, and so on.

Most of nanostructures studied to date for cell adhesion have been nanotubes and nanowires [12]. As a structure that provides sufficient space to transfer fluid such as medium and also allow the control of the size, pitch and other factors for improved understanding, we decide that the nanowire is a proper platform to conduct our studies for the interaction between nanostructures and cells.

There are previous studies of nanowire growth using Vapor–Liquid–Solid (VLS) process with metal catalyst [13–20]. Generally, it is reported that TiO₂ nanowire grows at 800~900°C using VLS process. Previous research by Ha et al. suggests the TiO₂ nanowire growth using Sn layer as catalyst and an inductively heated reactor [17].

We have also synthesized TiO₂ nanowires using VLS process to study cell adhesion and mineralization of osteoblasts following the nanowire morphology. For the control of the size of nanowires, we designed the

10, 30, 50 nm thick Sn thin films to serve as the metal catalyst layer. Following the Sn layer deposition, we fabricated TiO₂ nanowires using VLS process with N₂ heat treatment. Then, the osteoblast was cultured on the nanowires to investigate the effect of the nanowire arrays on cell adhesion.

II. Experimental Procedure

Fig. 1 shows a diagram of tube furnace used in the experiment (Fig. 1(a)) and the process flow of nanowire fabrication (Fig. 1(b)) schematically. First, we prepared a 15×15 mm² Si wafer for the clean and flat surface. For the cleaning process, Si wafer was immersed in Piranha solution (H₂SO₄ : H₂O₂ 3 : 1) for 10 minutes. Then a Sn/Ti bilayer was deposited by evaporator on the substrate. The bottom layer of Ti was deposited with 300 nm thickness as a source material for the growth of the TiO₂ nanowires. The top layer of Sn was deposited on Ti with 10, 30, 50 nm thickness at each sample to use as metal seed after agglomeration. For fabrication of TiO₂ nanowires, the Sn/Ti bilayered substrate was annealed. After the

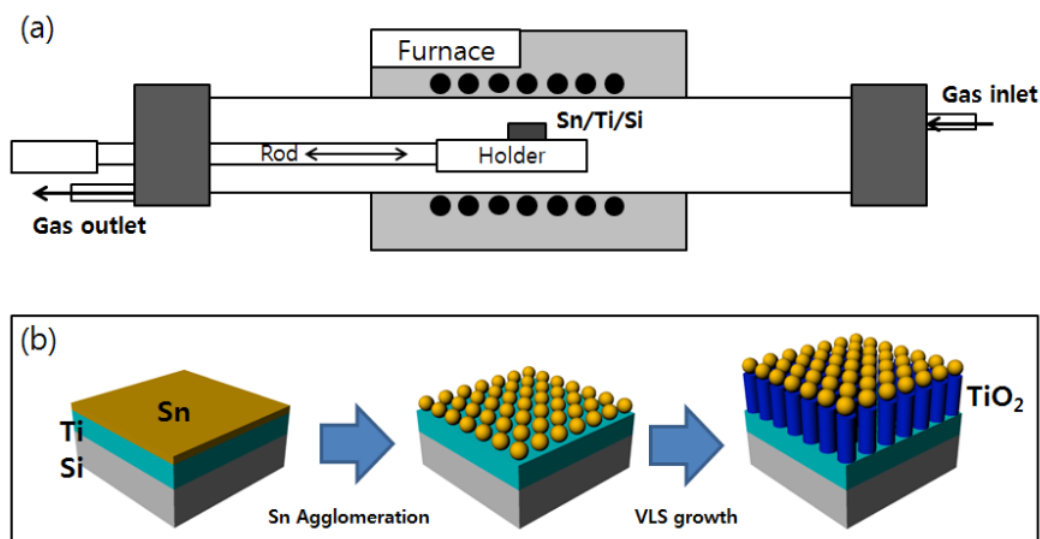


Figure 1. Schematic diagram of (a) the tube furnace we used and (b) the process flow of the experiment.

temperature inside of the tube furnace reached 880°C, we put the samples into holder from one side of the tube furnace and waited for 5 minutes to fill the furnace with N₂ gas. When N₂ gas was filled, the sample was transferred to the middle of the furnace and kept at the process temperature with N₂ flow for 10 minutes. After the heat treatment, the sample was cooled at room temperature. In order to investigate the relationship between the thickness of Sn layer and the size of TiO₂ nanowires, we annealed the other group of sample of Sn/Ti bilayer samples. The samples were placed in the tube furnace at 300°C for 5 minutes and then cooled with its power turned off. We obtained the images of Sn agglomeration and TiO₂ nanowires using FE-SEM (JSM-7100F, JEOL).

To identify the cell adhesion and growth, we cultured MC3T3-E1 on TiO₂ nanowires for a week. Cells were cultured in the incubator to reach the proper amount to apply onto the substrate. After reaching the proper amount, cells were injected on the TiO₂ nanowire substrate and cultured in alpha-MEM (without ascorbic acid) + 10% FBS + 1% penicillin-streptomycin medium for a week. Non-adherent cells were removed after 12 h culture. For observation, cells needed fixing and drying process. The adherent cells were fixed and observed using SEM in 24 h intervals. After fixing, cells were dried with 99.9%, 75%, 50% alcohol in this order. We coated the dried cells with Au to get SEM images.

III. Results and Discussion

Fig. 2 shows TiO₂ nanowire growth mechanism of VLS process using Sn metal catalyst schematically. VLS method is one of the bottom-up process for nanowire growth. At first, the liquid alloy droplet is formed by reaction between the source and catalyst metals on their interface more than critical temperature in the phase diagram. After formation of liquid

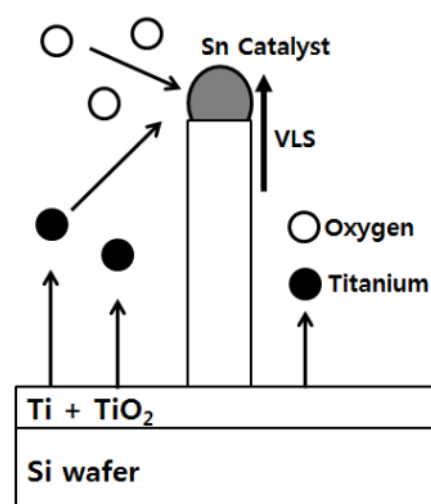


Figure 2. Schematic diagram of TiO₂ nanowire growth via VLS method.

droplet, nanowire growth proceeds by precipitation of source metal which is absorbed from vapor phase. The liquid droplet rides on the tip of nanowire and the nanowire grows by repeating the absorption and the precipitation in the droplet [13].

Fig. 3 depicts the SEM image of representative Sn nanosphere by agglomeration of Sn layer from the Sn/Ti bilayered substrate. The SEM images show that Sn is agglomerated relatively well at 300°C. We set the temperature of annealing because there is a temperature gradient and we measured about 300°C at the end of the tube furnace when the Sn/Ti bilayered substrate is located in one side of tube waiting for changing ambient gas. SEM images provide the average diameters of the nanospheres 44 nm (Fig. 3(a)), 90 nm (Fig. 3(b)), and 150 nm (Fig. 3(c)) respectively varying with the initial thickness of Sn layer. Comparing among Fig. 3(a) to Fig. 3(c), the smaller agglomeration from thinner layer provides more uniformity. It is owing to amount of Sn and annealing time. If agglomeration occurs with sufficient time, there will be fewer number of smaller spheres on the surface of substrate due to the Ostwald ripening.

TiO₂ nanowires we fabricated had different sizes according to the thickness of Sn layer. Fig. 4 shows

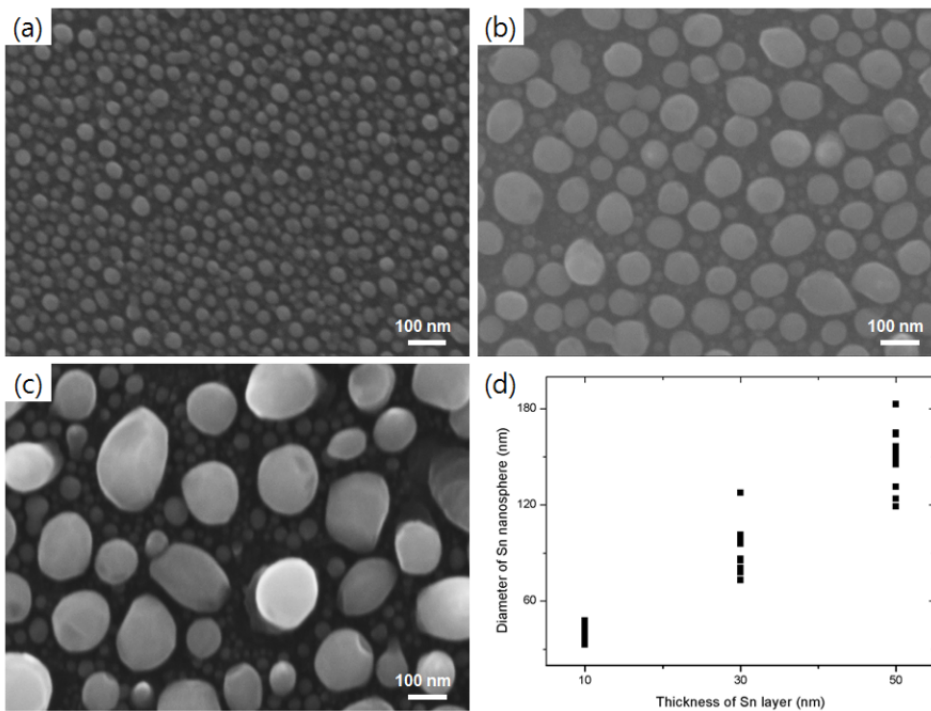


Figure 3. SEM images of agglomeration of Sn layer. The thickness of Sn layer were 10 nm for (a), 30 nm for (b), 50 nm for (c).

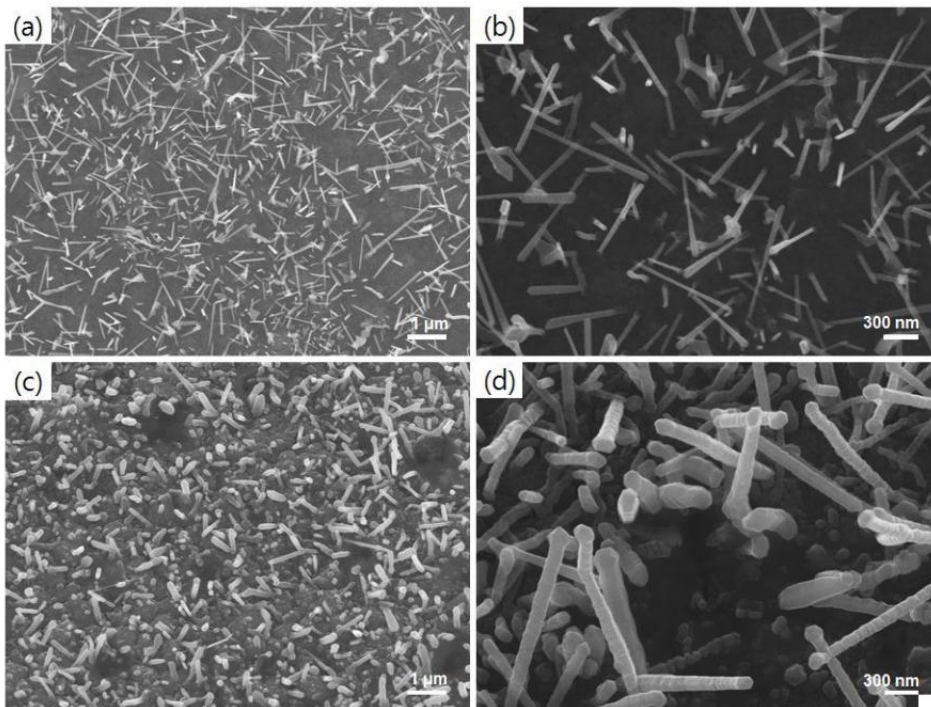


Figure 4. SEM images of TiO_2 nanowires. The thickness of Sn layer as metal catalyst were 10 nm for (a, b), 50 nm for (c, d).

the TiO_2 nanowires fabricated by VLS process. In the case that the thickness of Sn layer is 10 nm, the diameter of nanowires is 44 nm on average and the shapes of them are generally smooth. According the

Ha et al., the shapes are induced from their growth and crystallinity, usually with single crystal TiO_2 nanowires [17]. On the contrast, the shapes of the nanowire fabricated with 50 nm thick Sn layer have

rough surfaces most likely originated from the stacked structure of rutile TiO_2 [20].

The length of the nanowires is about $1\ \mu\text{m}$. It is due to the thickness of Ti layer we deposited. According to the result of Ha et al., they got $3\sim 8\ \mu\text{m}$ long TiO_2 nanowires on Ti layer [17]. They used Ti foil and deposited Ti thin film as sufficient source of nanowire growth. Since we do not need such a long nanowire for the cell adhesion and growth in order to observe the cell adhesion on the surface of nanowire arrays, we designed that the Ti source layer thickness of 300 nm for growing nanowires of desirable length.

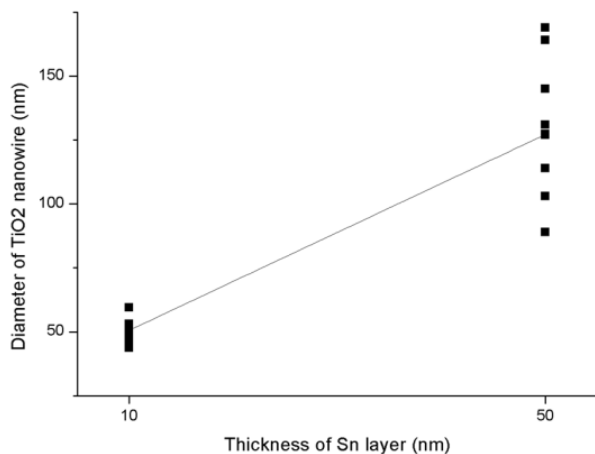


Figure 5. Diameters of TiO_2 nanowires along the thickness of Sn layers.

The diameters of the resultant nanowires depend strongly on the thickness of Sn layer. It is because of VLS process as described previously. In the VLS process, liquid alloy droplet plays an important role in growth mechanism. In our experiment, Sn nanospheres were agglomerated by annealing Sn layer. As shown in Fig. 3(d), the size of nanosphere and its variation increased with the thicker Sn layer. Similarly, Fig. 5 also depicted that the diameter of nanowires and its variation increased with the thicker layer because they were induced from Sn nanosphere using VLS process. With this result, we identified a method of controlling the size of nanowires so that we can apply to the study for improved osseointegration.

In order to evaluate the differentiation and proliferation of cells on the nanowires and eventually the osseointegration, we cultured the MC3T3-E1 (pre-osteoblast) on the sample for a week to examine cell adhesion and growth. Fig. 6(a) shows that cell adhered well on the nanowire surface derived from 50 nm thick Sn film, but the shape of the cell became distorted over time (Fig. 6). We believe that Sn catalyst may have toxicity to the cells. Thus, we are going to conduct further experiments on the VLS growth of TiO_2 nanowire using other seed materials that provide more compatible and benign interactions with the cells.

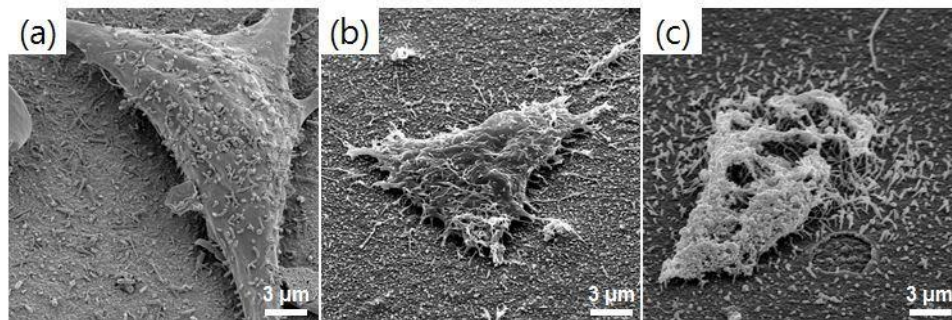


Figure 6. SEM images of MC3T3-E1 on TiO_2 nanowires. The shape of cell was distorted with time; (a) cell adhered after 12 h; (b) cell was distorted after 60 h; (c) necrosis occurred after 108 h.

IV. Conclusions

We fabricated TiO₂ nanowires via VLS method with Sn as catalyst and cultured MC3T3–E1 on it, as a first step, for the osseointegration. The size of nanowires was controlled by varying the thickness of Sn layer. From the SEM imaging of the nanowires and cells, we found that the size of nanowires were varied with different thickness of Sn layer. Our preliminary results indicate that the cells initially adhered well onto the nanowires became distorted and scattered earlier than we anticipated. We believe that it is indebted to the incompatibility of the Sn catalyst with the cells and currently plan future work on using inert catalyst materials.

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골융합을 위한 Vapor-Liquid-Solid 법을 이용한 TiO₂ 나노와이어의 합성

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임플란트와의 골융합을 향상시키기 위해서 세포와 임플란트 표면의 나노구조의 상호작용에 대한 이해가 중요하다. 본 연구에서는 Sn를 촉매로 이용하여 Vapor-Liquid-Solid 법을 이용하여 TiO₂ 나노와이어를 튜브 전기로 안에서 질소기체 조건하에서 합성하였다. 이 때 촉매로 사용된 Sn 박막의 두께에 따라 응집된 나노스피어를 이용하여 TiO₂ 나노와이어의 크기를 조절하였다. 골융합을 위한 예비 실험으로써, 만들어진 TiO₂ 나노와이어 샘플 위에서 조골전구세포(pre-osteoblast)를 1주일간 배양하였고, 세포가 TiO₂ 나노와이어에 잘 결합함을 볼 수 있었다.

주제어 : TiO₂ 나노와이어, Vapor-Liquid-Solid 법, Sn 응집, 조골전구세포, 골융합

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