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Development of a biocompatible painless microneedle by ion-sputtering deposition

Kazuyoshi TSUCHIYA*1, Satoshi JINNINN*2, Hidetake YAMAMOTO*3, Yasutomo UETSUJI *2 and Eiji NAKAMACHI*2

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Abstract

The purpose of this study is to fabricate and characterize a titanium alloy microneedle, which can be used in a bio-MEMS such as a HMS (health monitoring system) for blood-sugar-level measurement. As an important contribution to bio-MEMS technology, we fabricated a microneedle with an inner diameter of 100 μm and an outer diameter of 200 μm, which mimics the female mosquito's painless blood extraction mechanism. In this study, the microneedle was fabricated through (1) selection of a biocompatible material from titanium alloys by cytotoxic assay, (2) RF magnetron sputtering deposition to generate microtubes with an inner diameter of 100 μm, an outer diameter of 200 μm and a length of 4 mm, and (3) evaluation of the microneedle extraction by vacuum blood-extraction tests. Consequently, the pure titanium and titanium alloy (Ti-15Mo-5Zr-3Al) microneedle deposited by RF magnetron sputtering achieved a flow rate of 6.2 μl/sec.

Keywords: Bio-MEMS, Biomaterials, Microneedle, Biocompatibility, Nonferrous Metal, Titanium, Sputtering Deposition, Blood Extraction

1. Introduction

The selection of material and size for injection needles for medical use, is influenced to QOL(Quality of Life). In the designing and production of the injection needle, therefore, the pain and biocompatibility for the patients must also be important factor. Asakura et al. conducted a questionnaire about the mitigation of pain, slipping, and sharpness with two kinds of injection needles of 0.25mm and 0.3mm in the outer diameter. As the results, he reported that the needle with a smaller outer diameter can mitigate a mental strain[1]. Therefore, microneedles in a smaller diameter are better in order to mitigate a pain for patients so that the injection needle should have stiffness to endure indentation load in order to penetrate through the capillary vessel located under the epidermis and corium for the skin, and the material must be biocompatibility to inject it into in vivo. However, it is difficult to produce an injection needle with smaller diameter by using the existing method such as a drawing process. Therefore, it is not straightforward to create the microneedle in the size allowed the mitigation of pain.

In this paper, we investigates a development of a microneedle production method to create a microneedle in a size mimicking the female mosquito's labium(outer diameter : 50 μm and inner diameter : 30 μm) by using the sputtering deposition method[1],[2] to deposit bombarded particles onto a substrate from the target, which is one of the deposition techniques. Furthermore, we evaluate characteristic of the microneedle through the comparison of the extraction speed by using three microneedles produced by different technique with the various inner diameters such as (1) a deposited microneedle by the sputtering deposition method, (2) a stainless needle, and (3) a Nanopath 33 made by TERUMO Corporation.

2. Design guidelines for the development of the microneedle

In this chapter, the size and materials for the microneedle are discussed. Moreover, the sputtering deposition method as a microneedle production processes is investigated and proposed to produce a microneedle. The mechanical properties such as Young's modulus for the produced microneedle by the method are also evaluated.

2.1 Material candidate for microneedle

In this study, a biocompatibility and a feature of minimally invasion are the necessary and sufficient condition for a development of the microneedle for next generation's medical treatment. Therefore, the material candidate should be selected from the viewpoint of the biocompatibility based on the cell toxicity. The existing materials used for medical fields are generally metal to satisfy high strength and stiffness. Consequently, the nonferrous metals are a powerful candidate for the microneedle.

Table 1 shows biocompatible characteristics of various metals used for medical fields[3]-[7]. Table 2 shows titanium components system used for medical fields. At the present day, Ti-6Al-4V ELI(Extra Low Interstitial) has been commonly used and the development of

*1 Department of Precision Engineering, Tokai University, 1117, Kitakaname, Hiratsuka, Kanagawa, Japan
*2 Department of Mechanical Engineering, Osaka Institute of Technology, 5-16-1, Asahi-ku, Osaka, Japan
*3 Department of Mechanical Engineering, Tohoku Gakuin University, Tagajo, Miyagi, Japan

low toxicity alloy based on titanium has been advanced in Europe. Moreover, the titanium alloys composed by the biocompatible elements such as aluminum, zirconium, molybdenum, and niobium are employed for clinical applications.

Table 1 Biocompatibility of metal elements for medical use.

Allergic element	Co, Cr, V
Low cell compatibility element	Co, Cu, Fe, V
High cell compatibility element	Ti, Mo, Zr, Sn
Strength increase	Sn, Zr
β-stabilization element	Nb, Ta, Mo, V, Cr, Fe

Table 2 Mechanical properties of titanium and its alloys for medical use.

Material	Crystal structure	Young's modulus (GPa)	Tensile strength (MPa)
Ti	α type	102	735
Ti-6Al-4V ELI	α+β type	125	976
Ti-6Al-4V	α+β type	112	860
Ti-6Al-6V-2Sn	α+β type	110	1060
Ti-6Al-7Nb	α+β type	115	900
Ti-6Al-2Nb-1Ta-0.8Mo	α+β type	120	815
Ti-5Al-2.5Fe	α+β type	110	1020
Ti-15Zr-4Nb-2Ta-0.2Pd	α+β type	100	671
Ti-15Sn-4Nb-2Ta-0.2Pd	α+β type	105	883
Ti-15Mo-5Zr-3Al	β type	80	1250
Ti-29Nb-13Ta-4.6Zr	β type	82	975
Ti-29Nb-13Ta-2Sn	β type	78	650
Ti-12Mo-6Zr-2Fe	β type	75	1000

In this study, titanium alloy was selected as the candidate materials for a microneedle. And Ti-15Mo-5Zr-3Al was the best candidate material because the material can be estimated to become more strength after annealing.

2.2 Size of microneedle for flow rate evaluation

When the painless microneedle is designed, the size of microneedles should be mimicking a female mosquito's labium to extract blood almost by painless. Fig. 1 shows SEM images of female mosquito's labium. The inner diameter and the length of the female mosquito's labium are 30 μm and 3.5-4mm, respectively. Here, in the commercial based microneedle for the medical fields, a 100 μm in the inner diameter and 200 μm in outer diameter (Nanopass 33; TERUMO Corporation (2005)) are minimum now.

In this paper, the extraction speeds through the comparison by using various microneedles will be evaluated. However, a female mosquito by using the labium in 30 μm inner diameter extracts 1.9 μl of blood in 2 minutes[8], hence, the flow rate is slow. Therefore, the size of the microneedle should be set from the viewpoint based on the extracting speed to be able to extract 5 μl in the shortest possible time to measure the blood sugar level by a glucose sensor. In this study, the inner diameter of microneedle was defined as 100 μm or less and the length was set at 4mm same as a female mosquito's labium.

Fig. 1 SEM images of female mosquito's labium.

2.3 Fabrication process for a microneedle

We fabricated the microneedle by the thin film deposition process. The RF magnetron sputtering method can rapidly deposit thin films. Fig. 2 shows a schematic diagram of a painless titanium microneedle preparation.

Titanium is well known as a biocompatible material[9],[11], which is important because the microneedle penetrates the skin. The titanium and titanium alloy (Ti-15Mo-5Zr-3Al) were deposited onto a very small diameter (25 or 50 μm) copper wire, which was rotated at 3-5 rpm by a motor in the sputtering chamber. The sputtering time to obtain a microneedle with an outer diameter of 15 μm was 4 hours at an input power of 300 W and total pressure within the sputtering chamber of 2 Pa. The sputtering time is controlled to deposit different wall thickness for the microneedle outer diameter.

First of all, titanium in the thickness of 1-1.5 μm was deposited onto the copper very small wire. Next, titanium alloy (Ti-15Mo-5Zr-3Al) in the thickness of 12.5-50 μm was deposited onto the deposited titanium. Finally titanium in the thickness of 1-1.5μm was deposited onto the deposited titanium alloy again. Here, we need a wet etching treatment to remove the core materials of copper after annealing. However, components materials such as aluminum, zirconium and molybdenum except titanium, are poor against nitric acid. Therefore, we needed titanium as a protective coat on top of the microneedle. After the titanium and titanium alloy were sputtered onto the copper wire, the prescription of solution heat treatment of the microneedle before wet etching of copper wire was processed. The heat treatment procedures are follows; (1) the microneedle with copper wire were heated by the solution treatment method at 735 degrees C in an electric furnace for 20 min, (2) the microneedle with copper wire were aging treated at 500 degrees C in a vacuum furnace for 5 hours. Finally, the copper wire was removed by a wet etching process at ambient temperature. The etching solutions used was 50 % distilled nitric acid.

Fig. 3 shows SEM images of a titanium microneedle (length about 1 mm) compared with a conventional needle (external diameter 900 μm). The titanium microneedle is almost the same size as a female mosquito's labium (50 μm external diameter and 30 μm inner diameter).

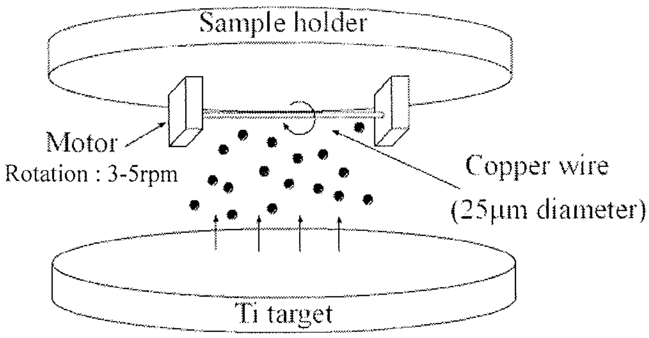

Fig. 2 Schematic diagram of RF magnetron sputtering arrangement.

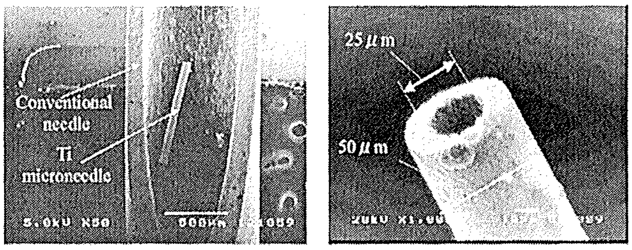

Fig. 3 SEM image of a titanium microneedle compared with a conventional needle (outer diameter 900 μm).

After being produced by the sputtering method, the titanium microneedle was heat-treated and we used a nanoindenter (HYSITRON: Tribo Scope) to measure the Young's modulus and hardness. Before the heat treatment, the Young's modulus and the hardness were about 40 GPa and 0.48 GPa, respectively. After the heat treatment, they increased to 100 GPa and 2.5 GPa respectively. This value was close to the Young's modulus of 115 GPa for bulk titanium.

On the other hand, in order to measure the Young's modulus and a bending strength for the hollow type (microtubes) titanium alloy microneedle, we prepared a bending tester for a microneedle. Fig. 4 shows a schematic diagram of experimental setup for bending of a microneedle. A micro balance and a micro-manipulator were fixed on an anti vibration stage. The microneedle was fixed at the tip of an arm of a micro-manipulator. The micro manipulator operated manually to bend the microneedle at the edge of a cutter, which was mounted on the micro balance. We measured the reaction force produced between the edge of a cutter and the microneedle by touching them each other. Fig. 5 shows boundary conditions for bending of a microneedle and equations of Young's modulus based on Hooke's law.

The slope in linear part of the diagram for a reaction force - deflection corresponds to Young's modulus so that the value for the Young's modulus was calculated by equations shown in Fig. 5. And the bending strength was also calculated from the maximum value of the reaction force. As the results, the Young's modulus and the bending strength were 67.6 GPa and 612 MPa, respectively. We still, however, need to consider whether this has the required stiffness to reach capillary blood vessels.

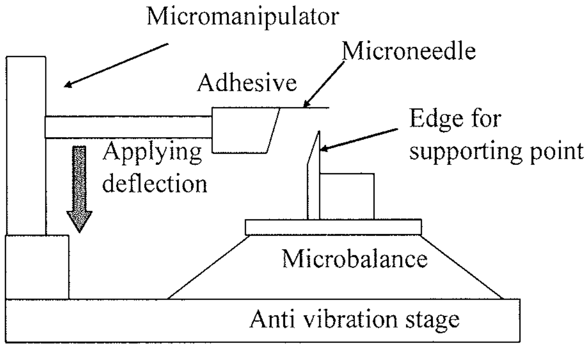

Fig. 4 Experimental setup for bending of a microneedle.

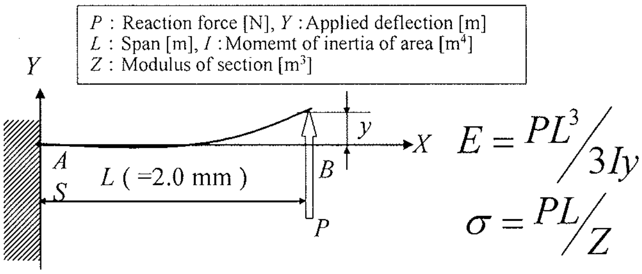

Fig. 5 Boundary conditions for bending of a microneedle.

In order to compare an evaluation for water flow rate through the microneedle in this research, the two sizes of microneedles deposited by the sputtering deposition method were based by 100 and 50 μm in the inner diameter on 4 mm in length.

3. Results and discussions

In order to extract human blood through a microneedle, we prepared a vacuum type extraction system. The system consists of a microneedle, a vacuumed epoxy acrylate tube(extraction case) which was vacuumed at 0.1 kPa, shown in Fig. 6. The pump system was developed to extract blood of 5 μl necessary to measure the blood sugar level within one second. Here, flow rates of water or blood through the various types of microneedle by using the vacuum type system under the conditions shown in Table 3 were investigated. Here, in the vacuum type micropump, the size of the inner diameter for microneedle is an important factor of blood extraction because dissolved oxygen in blood extracted from the inside of the human body expands by rapid decompression. For instance, the inner and outer diameter in Nanopass 33 is designed as a double taper structure and the flow resistance is controlled. In this extraction experiments, we used three types of microneedles as follows, (1) two kinds of the titanium microneedles (100 μm and 200 μm in inner and outer diameter, 50 μm and 100 μm in inner and outer diameter), (2) two kinds of stainless microneedles (100 μm and 200 μm in inner and outer diameter, 50 μm and 100 μm in inner and outer diameter), and (3) a Nanopass33 (the taper type needle : 100 μm and 200 μm in inner and outer diameter). Table 4 shows conditions for various types of microneedle in the flow experiments. Here, at the inside and outside of the titanium alloy microneedle, titanium as a protective coat was deposited and layered by the sputtering deposition method because the components of materials for titanium alloy microneedle are poor against nitric acid. Therefore, it is assumed that the extraction behavior characteristic for titanium alloy microneedle is similar to the pure titanium microneedle. Fig. 7 shows comparison of extracted volume by using vacuum system among various type microneedles. According to Fig. 7, the extracted volume by using vacuum system increased linearly with increase of the extraction time at any inner diameters and any types for microneedles.

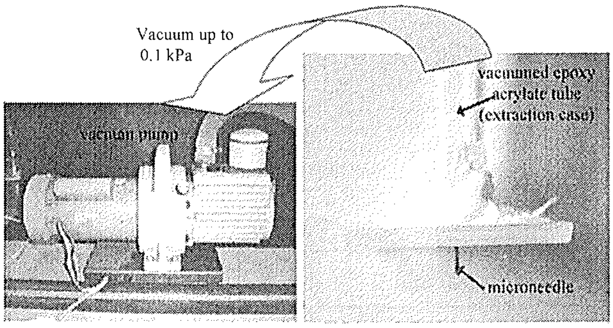

Fig. 6 Pictures of set up for extraction experiments by vacuum type extraction system.

Table 3 Experimental conditions of vacuum extraction.

Initial pressure in a vacuum chamber	100Pa
Inner diameter of a needle	50μm 100μm
Density: ρ	1000 kg/m^3(water) 1050 kg/m^3(blood)
Kinematic viscosity	1.0×10^{-6} m^2/s(water) 3.8×10^{-6} m^2/s(blood)

Fig. 8 shows comparison of liquid extraction performance by various types of microneedle, where numbers correspond to the numbers shown in Table 4. According to Fig. 8, the extracted volumes as a function of extraction time at any conditions except in the case of inner diameter of 50 μm were faster than 5 μl/sec, which value is the target value for the extracting speed in this experiment in order to measure the blood sugar level by a glucose sensor.

Table 4 Conditions for various type of microneedle in the flow experiments.

Sample number	Type	Extracting solution	Inner diameter (μm)	Outer diameter (μm)
1	Nanopath33	blood	100	200
2	Stainless	blood	100	200
3	Ti microneedle	blood	100	200
4	Nanopath33	water	100	200
5	Stainless	water	100	200
6	Ti microneedle	water	100	200
7	Ti microneedle	water	50	100
8	Stainless	water	50	100

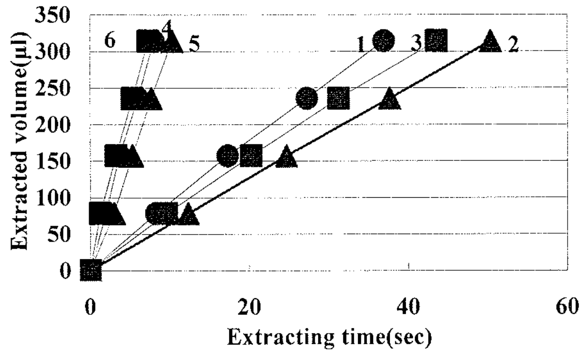

Fig. 7 Comparison of extracted volume by using vacuum system among various type microneedles, where numbers correspond to the numbers 1-6 shown in Table 4.

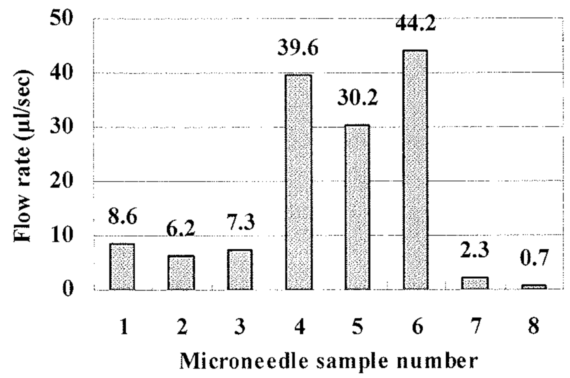

Fig. 8 Comparison of liquid extraction performance among various type microneedles, where numbers correspond to the number shown in Table 4.

In water extraction experiment or blood extraction experiments in Fig. 7 and Fig. 8, the flow rates about Nanopass 33, the stainless needle, and the pure titanium microneedle of 100 μm in the inner diameter showed different value. Especially, in the case of blood extraction by Nanopass 33, the flow rate showed the most excellent rate, and the difference of about 30% was admitted. As for the reasons for different extracting speed, it seems that the shape structure is affected to the extracting speed. Moreover, it was confirmed that the flow rate by titanium microneedle was faster than

that by stainless microneedle.
Here, Equ(1) shows the Hagen-Poiseuille equation. Equ(2) shows continuity equation.

$$P = 32\mu l v / d^2 \cdot\cdot \text{Equ(1)},\quad Q = v \pi d^2 / 4 \cdot\cdot \text{Equ(2)}$$

Table 5 shows factors for pressure loss by pipe friction in Equ.(1) and Equ(2). In an ideal condition that is not with a pressure loss by pipe friction, the pressure difference between atmosphere pressure and vacuum pressure(by vacuum type extraction system) is P_1-P_2 so that the flow rate Q obtained by the conditions shown in Table 5, was 6.2×10^{-8} m^3/s. On the other hands, flow rates for stainless microneedle and titanium microneedle in 100 μm inner diameter were 30.2×10^{-9} m^3/s and 44.2×10^{-9} m^3/s, respectively. This reason is that the pressure loss due to pipe friction for titanium microneedle was smaller than that by stainless microneedle. Difference between flow rate in the ideal condition and flow rate by each microneedle must be due to losing by the pressure losses by pipe friction. Therefore, the pressure losses by pipe friction were calculated from Equ.(1) and Equ(2). As the result, the flow rates by titanium microneedle and the pressure losses by pipe friction by the stainless microneedle were approximately 49 kPa and 72 kPa. The pressure loss by pipe friction for the stainless microneedle was 46 % larger than that by the titanium microneedle. It is clear that the surface properties of inner wall for the titanium microneedle by the sputtering deposition method are better than that by stainless microneedle produced by the drawing process. The extraction speeds showed lower value of 0.5μl/sec so that the blood extractions by stainless microneedle and by titanium microneedle were not achieved in the case of 50 μm inner diameter of microneedle. It was hard for any microneedle in inner diameter of 50 μm to extract blood in the extraction speed of 0.5 μl/sec.

Table 5 Factors for pressure loss by pipe friction.

μ	Coefficient of velocity for water	0.001002Pa・s
P_1	Atmosphere pressure	101325Pa
P_2	Vaccum pressure	100Pa
l	Microneedle length	4mm
d	Microneedle diameter	100μm

4. Conclusions

In this research, the microneedles in which biocompatible pure titanium and titanium alloy were produced by using the sputtering deposition method. And the extraction experiments were evaluated to compare flow rates by water and by blood through the deposited microneedle, a stainless microneedle and a commercial based microneedle in various inner diameters, and drew the following conclusions:

(1) The size of titanium and titanium alloy microneedle in less than 100 μm can be produced by the thin film deposition process.

(2) The vacuum extraction examination shows the flow rate of the titanium microneedle (in the inner and outer diameter of 100 μm and 200 μm) for blood is sufficient to determine the glucose level in the blood by a commercial glucose level monitor.

(3) The blood extraction speed for titanium microneedle produced by the sputtering deposition method shows the better result than that by stainless microneedle produced by drawing process due to different surface properties of inner wall.

5. Acknowledgment

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