博士論文（要約）

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Porous Microneedles Connected to Microfluidic System for Continuous ISF Sampling

(間質液の連続採取のための
多孔質マイクロニードルと流体デバイスの開発)

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Summary

Since co-authors of the publications related to this dissertation do not accept the open-access of the full paper, I summarize my dissertation here.

In this study, the porous PDMS MNs coated with HA were developed using the salt leaching and mold casting for continuous ISF sampling using the microfluidic chip. For the fabrication of the MNs, the salt leaching and mold casting methods were investigated by optimizing the porosity and surface treatment of the mold to avoid breakage during mold release. For the suitable geometrical and mechanical property for the insertion into the dermis layer, the shrunken porous PDMS MNs were coated with HA to realize the intended dimension and buckling force. For the ISF extraction, the porosity and pore size of the MNs were investigated to realize rapid extraction with repeated compressions. The fabricated porous MNs showed the extraction rates of 3.8 μL/min *in vitro* and 0.45 μL/min *in vivo*. The MNs connected to the microfluidic chip, that is designed to interface the randomly distributed pores on the MN array surface and to drive the extracted ISF by the capillary pump, showed the continuous PBS flow with a flow rate of 0.08 μL/min, which is lower the required ISF flow rate of 0.08 μL/min for CGMS due to the HA viscosity and pressure drop at the assay chamber. It is indicated that the flow rate can be increased by optimizing the microchannel design and by extending the porous PDMS MN geometry. This study provides the applicability of the porous MNs to continuous ISF sampling.

ISF is a promising biosample since it contains a wide range of common biomarkers to blood such as glucose. In order to utilize ISF for continuous healthcare monitoring, ISF sampling is a key technology.

However, conventional ISF sampling technologies such as microdialysis and RI have several drawbacks including highly invasive operation and limited measurement methodologies.

On the other hand, MNs have been developed to access the human body in a minimally invasive manner. Among the MNs, porous MNs have advantages such as applicability to fluidic systems and to biocompatible materials. Although the porous MNs were applied to ISF sampling, continuous ISF sampling has not been realized.

For this, a new type of porous MN should be developed to address the challenges of mechanical and fluidic requirements for successful insertion into the skin and ISF extraction continuously. Furthermore, the microfluidic chip should also be realized to interface the porous MNs and realize a continuous flow of ISF, which is ideally at a flow rate of 0.08 μL/min.

In this study, I proposed the integrated fluidic system combining the porous MNs and microfluidic chip

including the capillary pump. The porous MNs are made of PDMS which leads to a flexible MN and results in a lower biological risk. In order to address the mechanical issues and fluidic issues, I proposed HA coating of the porous MNs for enhanced mechanical strength and active extraction of liquid into the porous PDMS matrix by repeatedly compressing the porous matrix. For the microfluidic chip, I proposed the fluidic interface to the porous MNs by distributing the inlet ports, which is connected to the capillary pump for a continuous flow.

In order to realize the proposal above, I investigated the fabrication and the geometrical, mechanical, and fluidic characteristics of the proposed porous PDMS MNs coated with HA. In addition, I also designed, fabricated, and evaluated the microfluidic chip from the perspective of the fluidic characteristics.

The HA-coated porous PDMS MNs connected to the microfluidic system was investigated for continuous ISF sampling. For the minimally invasive ISF sampling, the porous PDMS MNs coated with HA were designed and fabricated using the salt leaching and mold casting according to the designed dimension. This is the first successful fabrication of the porous MNs using the salt leaching and mold casting method. The fabricated porous MNs showed successful extraction by repeated compression at a flow rate of 3.8 μL/min *in vitro* and 0.45 μL/min *in vivo*, which is sufficient rate for glucose monitoring. For the continuous ISF sampling, the microfluidic chip to interface the porous MNs was designed and fabricated including the inlet ports, microchannels, assay chamber, and capillary pump. The integrated porous MNs and microfluidic chip showed a continuous PBS flow rate of 0.08 μL/min, which is lower than the targeted ISF extraction rate. From the experimental results, the proposed and realized fluidic system has capabilities of penetrating the skin *in vivo*, extracting ISF from the skin to the porous matrix *in vivo*, and driving PBS continuously *in vitro*. In addition, it is indicated that the proposed device has the potential to extract ISF at a sufficient rate by improving the assay chamber design. This study provides a potential solution for continuous and minimally invasive ISF sampling.

Summary of each chapter

Here, I conclude the results and discussions in each chapter.

In chapter 3, I investigated the fabrication and mechanical and geometrical properties of the porous PDMS MNs coated with HA. The 169 porous MNs were designed as pyramidal shape, 1200 μm long, 30° tip angle, 1 mm pitch, and aligned on a $1.5 \times 1.5\,\mathrm{cm}^2$ square base with 1.5 mm thickness according to the skin geometry and parametric bucking force calculation. In order to realize the porous MNs, the fabrication using salt leaching and mold casting is investigated and optimized. From the result of prototyping, it is found that the porous PDMS matrix shrinks to 60-65% of the original dimension, which allows HA coating using the same mold as the porous MN molding. The designed geometry of the porous MNs coated with HA was realized using a wire EDM-prepared mold by optimizing the fabrication parameters such as casting pressure, porosity, and curing pressure. The fabricated 60% porous PDMS MNs have average dimensions of 800 μm length and 600 μm bottom width, results in uniform

dimensions of 1200 μm length and 620 μm, as designed, after HA coating. This is sufficient geometric and mechanical characteristics for skin penetration. For the mechanical characteristics, the HA-coated porous MNs show a sufficient buckling force of 0.34 N per needle, which is greater than the general MN insertion force of 0.06 N per needle. In addition, as the mechanical strength is 5 times higher than the required mechanical stability, it is indicated that the amount of the HA coating can be reduced.

In chapter 4, the fluidic characteristics of the porous PDMS matrix and fabricated porous MNs were investigated. From the preliminary study on the relation between the geometrical dimension of the porous structure and extraction of PBS into the porous matrix by repeated compression. From the experimental results and parametric calculation, it is shown that 60% porosity is optimal since the higher porosity leads to a decreased pumping effect and the lower porosity will lead to a decreased permeability. In addition, it is also shown that the bigger pore size results in a higher extraction rate of PBS by compression. From the experimental result using the prototyped porous MNs coated with HA, it was shown that the porous MNs extracts the PBS from a gel with the dissolved HA in the PBS by repeating compressions. This is also demonstrated using the fabricated porous MNs, resulting in a PBS extraction rate of 3.8 μL/min, which is sufficient for a feasible CGMS. The fabricated porous MNs coated with HA were also characterized *in vivo* using mice, showing a successful extraction of mouse ISF at an extraction rate of 0.45 μL/min.

In chapter 5, I investigated the fabrication and fluidic property of the proposed microfluidic chip. By prototyping the microfluidic chip, it is indicated that the microchannel fabrication based on wafer bonding channel sealing is desirable. The microfluidic chip is designed to have the 4096 inlet ports with 100 μm diameter aligned on a square area of $1.5 \times 1.5\,\mathrm{cm}^2$, microchannels to connect the fluidic components, the assay chamber with a capacity of 0.8 μL for glucose sensing, and the open capillary pump that has a capacity of 5 μL and capillary pressure of 1.2×10^5 Pa. The fabrication of the microfluidic chip was based on the standard MEMS process. The microchannels were patterned on a 4 inch Si wafer using photolithography and ICP-RIE, resulting in a 50 μm depth. The microchannels were sealed by room temperature wafer bonding to a 100 μm thick glass substrate using SAB using AlO intermediate layer with a transparent bonding interface. After sealing, the inlet ports, assay chamber, and capillary pump were opened by sandblast etching. As a result, the fluidic structure was realized and showed flow rates of 3.1 μL/min at the assay chamber and 1.8 μL/min at the capillary pump, which were observed by placing a PBS droplet on the inlet ports area.

In chapter 6, the fluidic characteristics of the integrated porous MNs and microfluidic chip were evaluated. From the result of *in vitro* tests, the initial flow rate of PBS from a gel was measured as 0.019 μL/min, which is 100 times lower than the flow rate of the microfluidic chip. However, the PBS flow rate increases to 0.08 μL/min 80 min after insertion to the gel since the concentration of HA decreased by the continuous flow. These results showed that the glucose concentration in the assay chamber reflected that of the added PBS glucose solution on the porous MNs within 5-10 min for 120 min. As the hydraulic resistance gets higher because of the assay chamber, the flow rate can be improved by

the geometrical design. On the other hand, the integrated porous MNs and the microfluidic chip did not demonstrate a successful extraction of rat ISF *in vivo*. As the optical observation showed that the HA coating was not fully dissolved, this can be attributed to a failure of MN insertion.

Comparison with previous studies

This study focuses on the porous PDMS MNs coated with HA and the microfluidic chip including the capillary pump to realize continuous ISF sampling. As an MN-based device for glucose monitoring, this study has several advantages to non-MN-based applications. Compared to invasive devices such as implantable sensors, this study provides a minimally invasive manner to access biological samples without bleeding as shown in chapter 4. At the application level, the commercially available tools to access biological tissues require surgery process or hypodermic needle, which leads to pain, bleeding, a requirement for trained medical skills, and limitation of patients' life. From this perspective, this study enables more desirable biosensor applications.

Furthermore, from the aspect of measurement reliability, MN-based device has advantages. From the perspective of targeted biosample, this study focuses on ISF, which has similar compositions to blood, while tear, sweat, saliva, and other non-invasive biosamples do not. Moreover, other approaches to extract ISF by minimally or non-invasive ways such as RI or sonophoresis have drawbacks such as unstable extraction depending on the skin surface condition and hair pore distribution. In this view, the porous MNs investigated in this study have the capability to access inside skin through successfully punctuated pores, as shown in chapter 4. In addition, the flow rate is controlled by the fluidic system, leading to a reliable ISF extraction.

From the aspect of MN-based devices, this study also shows some advantages. First, compared to the hollow MN-based applications, the porous MNs in this study are fabricated more simple process, in other words, the combination of the salt leaching and mold casting. In addition, the porous PDMS MNs coated with HA provide less biological risks because of the flexibility.

Second, compared to the solid MN-based applications, this study focuses on the biological sensing outside the body, which enables a wide range of biosensing not only for glucose but also other biomarkers using μTAS technology.

Third, compared to the swellable MN-based application, the device concept provided in this study enables more rapid and simple biosensing by directly connecting to fluidic systems, as the swellable MNs need an additional process to separate extracted biosamples from the MN body.

Among the porous MN-based applications, this study has two originalities. One is the direct connection to the microfluidic system including the capillary pump. This enables direct and continuous transportation of the extracted ISF through the microchannels, leading to potential applications for μTAS technology.

Another point is the extraction mechanism. The porous MNs studied in this study show a new mecha-

nism to extract ISF by repeated compression. This is enabled by combining the HA coating and flexible PDMS porous matrix. However, it is indicated that the extraction rate can be improved by modifying the porous geometry and coating material. This exhibits a new potential use of the porous MN array for the ISF sampling.

Future perspective

Insertion of the porous MNs into the skin

The fabricated porous MNs were demonstrated to penetrate the mouse skins as shown in chapter 4 and Appendix. However, the continuous ISF sampling by the integrated porous MNs and the microfluidic chip has not been demonstrated *in vivo*. One reason was indicated that the amount of effused ISF from the rat skin depends on the MN insertion including the depth of the inserted MNs and the number of MNs inserted.

In this study, the MN insertion was evaluated by observing the penetrated mice skins and the extraction of ISF. Therefore, the insertion characteristics of the proposed MNs should be investigated.

This is also related to the continuousness of ISF sampling. As continuous ISF sampling requires the fluidic connection through the skin, the insertion of MNs should also be investigated from a fluidic perspective.

Investigation on fluidic characteristics

As discussed in chapter 6, there are three main factors to reduce the hydraulic resistance of the integrated porous MNs and the microfluidic chip. One is the interface between the pores of the porous MNs and the inlet ports of the microfluidic chip. The randomly distributed 30-60 μm pores of the porous MNs are connected to the uniformly aligned 100 μm inlet ports in the current design, which causes a pressure drop at the interface. In order to reduce the hydraulic resistance at the interface, the fluidic interface should be optimized using computer-aided parametric simulation.

The second factor is the hydraulic resistance at the assay chamber. The experimental and calculated results show that the hydraulic resistance at the assay chamber rises due to the connection of the microchannel and the 4 ×4 mm assay chamber. As the current design is for colorimetric detection of glucose at the assay chamber, the assay chamber can be optimized depending on the measurement method in the future.

The third factor is the viscosity of the HA coating. It is also clear that the viscosity of the dissolved HA coating in the extracted ISF increases the hydraulic resistance. As the coating material is dissolved in the sampled ISF and measured together in the assay chamber, the coating material should not affect the sensing process. Therefore, an optimized material selection for the MN coating will lead to a lower viscosity keeping the mechanical characteristics.

Sensor packaging

In this study, I aimed for continuous ISF sampling using the porous MNs integrated with the microfluidic chip. However, in order to realize the ISF-based continuous health care monitoring, the packaging of the sensor in the fluidic system is necessary. The microfluidic chip fabricated in this study has an assay chamber, which is potentially for sensor packaging. As this study investigated the fluidic function for continuous ISF sampling, the sensor packaging for continuous measurement has not been achieved using the proposed device concept. In the next step of this study, the design and fabrication process should be investigated in terms of sensor packaging.