Journal of Sensor Science and Technology
Vol. 20, No. 4 (2011) pp. 234-237
DOI:10.5369/JSST.2011.20.4.234
pISSN 1225-5475/eISSN 2093-7563

Micro pH Sensor Using Patterned Hydrogel with pH Indicators

Jisung Jang and Sunghoon Kwon+

Abstract

This paper presents a study into pH Indicator-Embedded hydrogel micro-particles which are encoded various shapes according to the captured indicator. We incorporate various pH indicators into a photo-curable hydrogel, PEG-DA(Poly(ethylene glycol) diacrylate). Using the latest fluidic lithography techniques, we can easily fabricate variously patterned hydrogel particles based on in-situ photo-polymerization of the PEG-DA in a micro-fluidic channel. The shape of the particle is related to the pH indicator inside the particle. We demonstrate that the micro pH sensors change their colors according to the pH levels. The micro pH sensors have various characteristics that are related to the curing time, particle size, etc. By changing these conditions, we can adjust the long term stability and reaction time of the hybrid micro pH sensors.

Keywords : pH sensor, Micro-Particle, Shape Code, Hydrogel

1. INTRODUCTION

When we discover new properties of a cell, it is very important to monitor the cellular states and environmental information around that cell. Particularly, the pH level around the cell is one of the most significant pieces of information when investigating cell properties.

One conventional method to measure the pH level is by using a probe. However, a probe must be used in an open space such as a petri dish. In this case, external factors and the vibrations of the probe can influence the environment around the cells of interest. However, a micro-chip, which is a closed environment, is protected against such disturbances. So, on-chip measurement techniques have become very important in recent years.

There are some conventional on-chip measurement techniques for detecting the properties around cells. The most popular method is using fluorescence observation[1-3]. It is one of the simplest methods, however the bleaching of the fluorescence make it difficult to perform long term observations. An other method is using micro-particles which hold pH-sensitive fluorescent dye[4, 5]. This method is a non-contact and non-destructive method. As such, it can be used for three-dimensional measurement unlike pH sensing microscopes[6]. In spite of these various merits, generating the functional particle is very complicated. In addition, it is difficult to control the shape of the particle. As such, it is important to develop techniques that allow us to generate various coded particles and control the size of these particles freely.

In this paper, we propose a micro pH sensor with a novel fabrication method. In this system, we compose a new mixture by mixing traditional pH indicators and photocuable hydrogel. In the microfluidic channel, the mixture is cured by photo-polymerization for constructing the body of particles[7]. At that time, an OFML(Opto-Fluidic Maskless Lithography) method is used, this can easily generate various shapes and sizes of particles[8]. We use pH indicators, such as Bromothymol blue(BTB), Phenolphthalein, Thymol blue and Methyl red, as indicant materials in the mixture. They are mixed with polyethylene glycol diacrylate(PEG-DA) which is a well known precursor of photocurable hydrogel. These materials, both the indicators and hydrogel, are known to be bio-compatible materials. Using these materials, we produce four kinds of hydrogel indicators, that is, micro pH sensors. Each of these indicators has a specific shape. The shape of the particle represents the identification of the particle. These encoded particles make it easier to perform multiplexing analysis in a single system. These coded micro pH sensors, which are simply fabricated, can be used for quantification of pH level in small systems like microfluidic channels.

School of Electrical Engineering, Seoul National Unversity
SoEECS 104-213, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul 151-744, Korea
+Corresponding author: skwon@snu.ac.kr
(Received : Jun. 30, 2011, Revised : Jul. 19, 2011)

2. FABRICATION METHOD

2.1 Mixing Method

To realize the solid-like indicators in the micro-fluidic channel, we make an indicator-hydrogel hybrid structure. Hydrogel has a net structure, so indicators embedded in the hydrogel do not diffuse out easily. On the other hand, analytes, which are small molecules, easily diffuse into the hydrogel. As such, we can detect the color change of the pH indicators in the hydrogel structure. To make this structure, we dissolve solid-state pH indicators in a photo curable resin, PEG-DA, to a saturation state. After, the mixture is placed in a centrifuge so unmelted solids can be separated from the saturated solution(Fig. 1(a)). Fig. 1(b) is a figure of the pure mixtures of various pH indicators fabricated by this method. Each mixture has its own color.

2.2 Particle Generation

The mixture is injected into a microfluidic channel that was fabricated by conventional methods with PDMS[9]. The height of the channel is 50 um, and it controls the height of the particles.

By photo-curing with UV light, the PEG-DA monomers in the mixture are polymerized, they become the hydrogel with a net structure. When curing the mixture by UV, we can pattern the hydrogel into various shapes by using various shaped masks. The patterned hydrogel can be identified by their distinct shapes, this makes multiplexing easy. We can control the real-real time patterning by an OFML technique. In this OFML system, a digital micromirror device(DMD, DLP Technology, Texas Instruments) substitutes for the conventional photo mask. The DMD pattern can be changed dynamically, so the patterns of the sensors can be generated easily without changing masks.(Fig. 2(a)) A high-intensity mercury–xenon lamp(200 W bulb) is used for ultraviolet photo-patterning combined with the DMD to dynamically control the shape and size of the polymerized micro-particles. The size of the sensors can be freely controlled from 50 um to 500 um. The exposure patterns of the DMD are controlled by a customized computer program. The UV lamp and DMD was manually equipped with an Olympus IX71 optical microscope. Fig. 2(b) shows microscope images of the fabricated sensors. The size of these sensors are about 200 um.

3. RESULTS AND DISCUSSIONS

3.1 Transition Range

In general, each pH indicators has its own color transition range. However, after curing each mixture by micro-photolithography, the reaction characteristics of each micro pH sensor is slightly changed. Fig. 3 shows how the pH transition ranges of various conventional pH indicators change. As such, a new reaction table is needed and the hybrid structures have to be regarded as novel indicators. In this experiment, various level of commercial pH buffers are used as analytes.

Our results show that the transition pH range of each micro pH sensor shifts to a higher pH level than that of the original pH indicator. The cause of this phenomenon is not clear but our assumption is that the pH level of PEG-DA is about pH 5, which is slightly lower than that of water. So, to change the color of the sensors in PEG-DA, we need higher pH level of analyte than the equivolent analyte in water.

Another cause may be that, when curing the mixtures there is a slight cross-linking between the pH indicator and PEG-DA. Then the density of the pH indicator which reacts with the hydrogen ion becomes lower. So, a higher pH level is needed to change the color of the sensors.

Especially, methyl red cells do not change color for any pH level between pH 1 and pH 12. We supposed that the methyl red cell is well cross-linked with the PEG-DA, so remaining methyl red molecules which can react to pH are almost non-existent.

3.2 Characteristics of Sensor According to Curing Time

We fabricated various shapes of hydrogel particles using in-situ photo-polymerization of the indicator containing PEG-DA in a microfluidic channel. When generating the particles, curing time is an important variable for locking the pH indicators in the PEG-DA polymer. The PEG-DA hydrogel has a polymeric mesh structure, and the mesh size of the polymer is determined by the molecular weight of material and curing energy. If the mesh size of the polymer is larger than the molecular size of the indicator, the captured indicators in the hydrogel can diffuse out of the structure. Then, the particle lacks long term stability because of leakage of the indicator. To prevent diffusion of the indicators, making dense hydrogel structures by

supplying sufficient energy to PEG-DA hydrogels when they are cured is important. We can control the supplied energy to the hydrogel by adjusting the curing time. We fabricated Methyl-Red sensors with different curing times to confirm the relation of curing time with locking stability (Fig. 4). For observing the diffusion characteristics, ethanol, which does not react with the Methyl-Red sensors, is used as the media. The sensors, each with different curing time, are immersed in the media for 15mins. The degree of diffusion is calculated by image processing(Fig. 5). According to our results, higher curing time leads to better long term stability of the micro pH-sensors. I Indicators with a different molecular size need to be cured for different times in order to achieve long term stability. Due to this, the Methyl Red sensors are cured for 2s, while the others are cured for 500 ms.

The reaction time of the hybrid particles also varied with curing time(Fig. 6). The shorter the curing time, the faster the color change. This is because the mesh size is dependent on curing time. When curing time is short, the mesh size is large. So, analytes candiffuse into the hydrogel.

3.3 The Relation between Particle Size and Reaction Time

Since our method is based on micro-photolithography, we can incorporate indicators into very small sized micro-particles(<200 um). The small size of the hydrogel particle decreases reaction time. Fig. 7 shows the color change is based on the diffusion of analytes into the particle. The color change is generated from the edge of the particle, so the smaller the particle is, the faster the color changes. In this demonstration, a BTB sensor of 200 um size needs 10s for a complete reaction. However, the same size's Methyl Red sensor needs 100s for a full color change. This is because of the mesh size of the sensor. We use a 4 times longer curing time for fabricating Methyl Red sensors. As such, the Methyl Red sensor has denser mesh and longer reaction time.

4. CONCLUSIONS

We demonstrated a micro pH sensor using an indicator-hydrogel hybrid structure. This structure can be easily patterned by the latest micro photo-lithography method called OFML. As this method uses DMD, we can easily control various patterns of UV light without masks unlike other conventional lithography techniques. Using this fabrication method we can easily make an appropriate size of particle for various applications. The combination of a colorimetric pH indicator and photo curable hydrogel allows us to fabricate various encoded probes. As these sensors are encoded, they can be used in a multiplexed analysis system. We can control the reaction time and long term stability of the micro pH sensors by adjusting the curing time. The materials used in the sensor particle are bio-compatible. As such, we hope that the sensors can be used for various bio applications.

ACKNOWLEDGMENT

This work was partly supported by the NSI-NCRC project of KOSEF, the KOSEF grant funded by the Korea government(MEST)(2008-05446)

REFERENCES

[1] Kyosuke Shinohara, Yasuhiko Sugii, et al., "Measurement of pH field of chemically reacting flow in microfluidic devices by laser-induced fluorescence", *Meas. Sci. Technol,* vol. 15, no. 5, pp. 955-960, 2004.

[2] Go Nishimura, Yasuhiro Shiraishi, and Takayuki Hirai , "A fluorescent chemosensor for wide-range pH detection", *Chem. Commun.*, no. 42, pp. 5313-5315, 2005.

[3] Norbert Klauke, Paul Monaghan, et al., "Characterisation of spatial and temporal changes in pH gradients in microfluidic channels using optically trapped fluorescent sensors", *Lab Chip*, vol. 6, no. 6, pp. 788-793, 2006.

[4] Raoul Kopelman, Hiroshi Masuhara, Keiji Sakaki, and Zhong-Yon Shi, "Three-dimensional pH microprobing with an optically-manipulated fluorescent particle", *Chem. Lett.*, vol. 25, no. 2, pp.141-142, 1996.

[5] H. Maruyama, T. Hukuda, et al., "On-chip pH measurement using functionalized gel-microbeads positioned by optical tweezers", *Lab Chip*, vol. 8, no. 2, pp. 346–351, 2008.

[6] M. Nakao, S. Inoue, R. Oishi, T. Yoshinobu, and H. Iwasaki, "Observation of microorganism colonies using a scanning-laser-beam pH-sensing microscope", *Journal of Fermentation and Bioengineering*, vol. 79, no. 2, pp. 163-166, 1995.

[7] D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, and P. S. Doyle, "Continuous-flow lithography for high-throughput microparticle synthesis," *Nature Materials*, vol. 5, no. 5, pp . 365-369, 2006.
[8] S. E. Chung, W. Park, H. Park, K. Yu, N. Park, and S. Kwon, "Optofluidic maskless lithography system for real-time synthesis of photopolymerized microstructures in microfluidic channels", *Applied Physics Letters*, vol. 91, no. 4, 2007.
[9] G. M. Whitesides, E. Ostuni, S. Takayama, X. Y. Jiang, and D. E. Ingber, "Soft lithography in biology and biochemistry," *Annual Review of Biomedical Engineering*, vol. 3, pp. 335-373, 2001.

Jisung Jang graduated and received bachelor's degree in Department of Electrical Engineering from KAIST, Daejeon, Korea in 2008, and the M.S. degree in Electrical Engineering from Seoul National University, Seoul, Korea in 2010. He is currently pursuing the Ph.D degree in Electrical Engineering from Seoul National University, Seoul, Korea. His current research interests are in the fields of microfluidic technology for chemical and biological sensors.

Sunghoon Kwon received the BS degree in electronic engineering from Seoul National University, Seoul, Korea, in 1998 and his M.S. degree in Biomedical Engineering from Seoul National University, Seoul, Korea, in 2000. In 2004, he received Ph.D. degree in Bioengineering from University of California, Berkeley, with his thesis work concerning micromachined conforcal microscope. He was a Postdoctoral Researcher in Molecular Foundry at Lawrence Berkeley National Laboratory from 2004 to 2006. Since August 2006, he has been with the School of Electrical Engineering, Seoul National University, Korea, and currently working as an assistant professor at Biophotonics and Nano Engineering Labratory.