MICROFLUIDIC CHIP HAVING MULTI FLUORESCENCE MICROSENSORS FOR SPATIOTEMPORAL SENSING OF CULTURE ENVIRONMENT

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ABSTRACT

We proposed on-chip spatiotemporal sensing for monitoring interactions between cells and environment using fluorescence multi-microsensors. The microfluidic chip integrating fluorescence multi-microsensor is used to construct the harmonious environment like *in vivo* to reconstruct high-quality organs *ex vivo*. Fluorescence sensing is suitable for spatiotemporal sensing with high spatial resolution because wiring to many microsensors limits the number of sensors. First, we classify optical sensing method. Fluorescence and polarization sensing are suitable for spatiotemporal sensing such as physiological and mechanical parameters in culture environment. As a demonstration of spatiotemporal sensing of culture environment, Spatiotemporal variation of pH, Ca^{2+} was measured and during transformation of octacalcium phosphate (OCP) to hydroxyl apatite (HA) inside the microfluidic chip using fluorescence multi-microsensors.

INTRODUCTION

Conventionally, design and assembly of cell structure such as fabrication of spheroid and organ like structure using micro-manipulation system and 3D printing system have been performed. However, quality of the cell structures fabricated *ex vivo* was not enough to transfer into *in vivo*. According to recent research progress in biomedical engineering, mechanical and physiological interactions between cells and environment are essential for bio-emergence of high-order functions. Construction of harmonious culture environment like *in vivo* is one of the most important issues for reconstruction of high-quality organs *ex vivo*. In case of bone regeneration, octacalcium phosphate (OCP) is used widely as biological scaffold combined with bone cells [1-3]. OCP is a precursor of hydroxylapatite (HA), and transform to HA with decrease of pH [4] and change of Ca/P ratio [5] after OCP transformation. To construct the harmonious environment of bone cell in the microfluidic chip, spatiotemporal sensing of pH, Ca2+ concentration, and temperature during OCP transformation is necessary.

MATERIALS AND METHODS

Concept of Microfluidic chip having fluorescence microsensors

Figure 1 and table 1 show a schematic diagram of the microfluidic chip having fluorescence multi-microsensors for spatiotemporal sensing and classification of the optical sensing, respectively. Fluorescence and polarization sensing can measure physiological and mechanical properties [6-9].

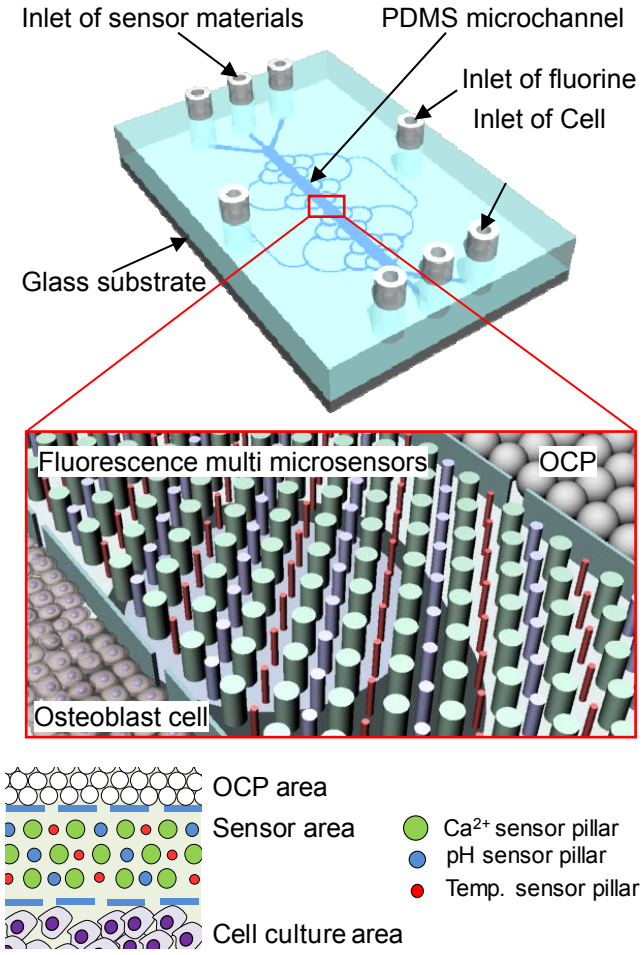


Figure 1: Concept of microfluidic chip having multi fluorescence microsensors for spatiotemporal sensing.

Therefore, design of sensor type and pattern is possible because optical sensing requires no wiring.

The chip has three areas such as OCP area, sensor area and cell culture area to analyze the interaction between osteoblast cells and OCP. The sensor layer has fluorescence sensor arrays of Ca^{2+}, pH and temperature sensors. In this study, Fluo-3, FITC, and CdSe/ZnS were used as mixed with photo-crosslinkable polymer, polyethylene(glycol) - diacrylate (PEG-DA), respectively to fabricate Ca^{2+}, pH and temperature sensor. These microsensors were different sizes in diameter: Ca^{2+} sensor (ϕ20 μm), pH sensor (ϕ15 μm), temperature sensor (ϕ10 μm) for exciting 488 nm laser. Simultaneous multi-sensing is possible by discriminating each parameter from the microsensor size.

Table 1. Methods of optical environment sensing

Sensing parameter	pH	Temp.	Ion (Ca^{2+})	Oxygen	Glucose	Stress
Sensing method	Fluorescence (Intensity, Lifetime, wavelength)			Polarization (Retardance)		
Example of sensing material	FITC	Lumidot 480	Fluo-3	$Ru(bpy)_2Cl$	Glucose	PU/ PVA
Excitation	488 nm	488 nm	488 nm	560 nm	UV to	UV to
Emission	510 nm	515 nm	527 nm	580 nm	IR	IR

Fabrication process of the microfluidic chip having fluorescence multi-microsensors

Figure 2 shows fabrication process of the microfluidic chip having fluorescence sensors. The chip was fabricated by replica molding using polydimethylsiloxane (PDMS). Then, PEG-DA solution with each fluorescence dye were injected and patterned by photolithography to fabricate fluorescent microsensors. Fig. 3 shows the fabricated microfluidic chip and OCP and sensors in the chip. From upper side, fluorine solution was flowed and OCP starts to transform to HA. After the microsensors were fabricated in microchannel, OCP slurry was delivered into OCP microchannel. OCP was injected into the chip by micro syringe pump. As shown in Fig. 3(b), OCP was successfully injected into the upper microchannel, and fluorescent microsensors in the middle microchannel.

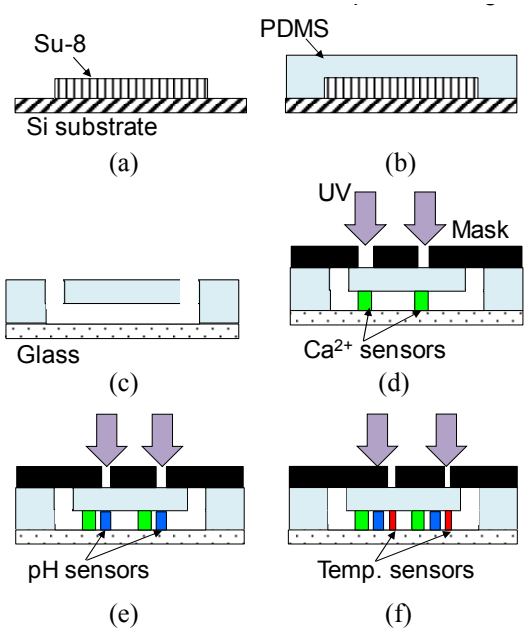


Figure 2: Fabrication process of sensor integrated microfluidic chip. (a) Fabrication of Su-8 mold, (b) Replica molding of the chip. (c) Bond of the chip and glass, (d) Fabrication of Ca^{2+} sensor, (e) Fabrication of pH sensor, (f) Fabrication of temperature sensor.

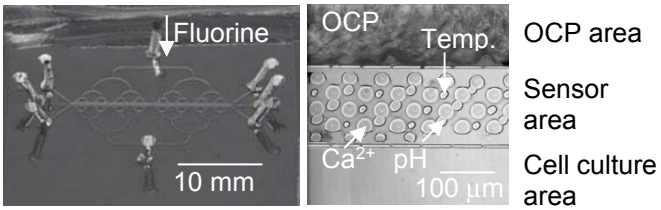


Figure 3: Photo of the microfluidic and fluorescence multi-microsensors in the microchannel. (a) Overview of the microfluidic chip, (b) photo of the fluorescence multi-sensors and OCP in the microchannel.

EXPERIMENTS

Confirmation of response of multi-microsensors

First, the function of the fluorescence microsensor was examined as shown in Fig. 4. The calcium solution with a concentration of 200 nmol/l was injected into chip in which the original Ca^{2+} concentration was 0 nmol/l. The result shows the fluorescence intensities of Ca^{2+} sensor increase with time after calcium solution injection and become stable in less than 1 min. The response of the pH sensor was also confirmed using the solution with a pH value of 8.0. The solution of pH 8.0 was injected into chip in which the original pH value was 5.0. The fluorescence intensities of pH sensor increased with time after new pH solution (pH=8) injection and become stable in less than 1 min. From these results, we confirmed the detection of the local environment change using fabricated micorsensors.

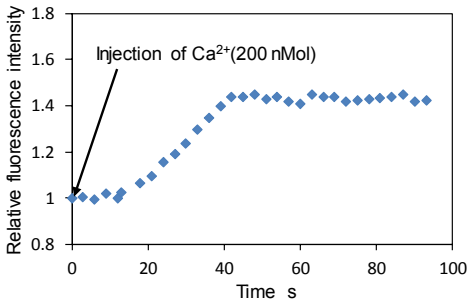


Figure 4: Response of the Ca^{2+} sensor after variation of concentration of Ca^{2+} in the microchannel.

Calibration of fluorescence in multi-microsensors

The calibration results of the sensitivities of different microsensors are shown in Fig. 5. Fig. 5(a) shows the fluorescence intensities of Ca^{2+} sensor under different Ca^{2+} concentrations. The fluorescence intensities of pH and temperature sensor do not change with the Ca^{2+}. Fig. 5(b) shows the fluorescence intensities of pH sensor under different pH values. The fluorescence intensities of pH sensor changed under different pH values, while no changes are observed in the fluorescence intensities of Ca^{2+} and temperature sensor under different pH values. Fig. 5(c) shows the fluorescence intensities of temperature sensor under different temperature. It should be noted that the pH sensor also show fluorescence responses to temperature change. It is because FITC is sensitive to both pH and temperature. The temperature change can also lead to fluorescence changes of FITC. In order to eliminate interference from temperature, temperature compensation is needed for pH calibration.

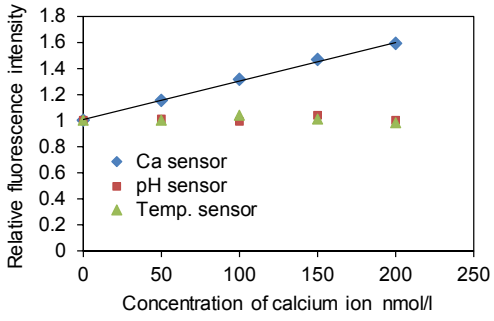
For temperature compensation, fluorescence responses of pH sensor to temperature changes in different pH values was calibrated. The fluorescence change of pH sensor induced by temperature change ($\Delta F_{(FITC)}$) was described as equation 1. The fluorescence change of pH sensor induced by pH change ($\Delta F_R'$) could be calculated by equation 2.

$$\Delta F_{R(FITC)} = f(pH) \times \Delta T \quad (1)$$

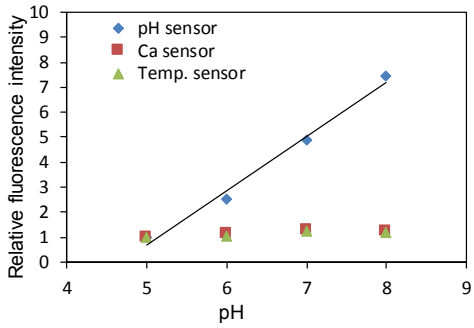
$$\Delta F_R^{'} = f(pH) \times \Delta T + \Delta F_R \quad (2)$$

$\Delta F_{(FITC)}$ is the fluorescence change of pH sensor induced by temperature change, $\Delta F_R'$ is the fluorescence change of pH sensor induced by pH change, ΔFR is the total fluorescence change of pH sensor induced by surrounding changes.

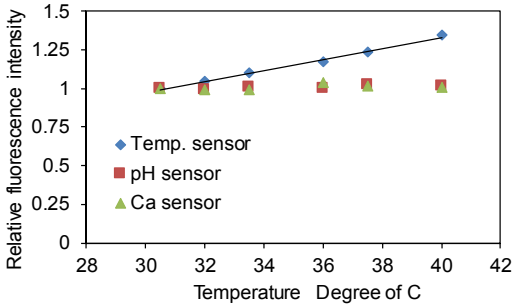
Fluorescence responses of different sensors to temperature change with temperature compensation were shown in Fig. 5(b). Through the calibration results in Fig. 5, it is clear that the sensors can respond to Ca^{2+}, pH, and temperature changes of the surrounding.



(a)



(b)



(c)

Figure 5: Calibration results of fluorescence multi-microsensors. (a)Calibration of Ca^{2+}, (b) Calibration of pH, (c) Calibration of temperature

Measurement of Ca^{2+}, pH changed during OCP conversion

After sensor fabrication and OCP delivery in the microfluidic chip, fluorine solution was introduced into the microfluidic chip for spatiotemporal sensing of the surrounding environment of OCP during transformation of OCP to HA. Many researches have proved that fluorine ion can promote the transformation of OCP to HA [10-12]. The transformation products are related with the concentration of fluorine ion. The fluorine solution used in our experiment was 100 ppm. After the fluorine solution was injected by syringe pump, the relative fluorescent intensity of Ca^{2+} and pH microsensors were measured for 30 minutes by laser confocal microscope. The results are shown in Fig. 6. The exposure time for one time is 200 ms and the time interval of images is 60 s. Then, the Ca^{2+} changes and pH changes during OCP transformation at the presence of 100 ppm F^- could be calculated from the fluorescence changes in Figs. 6(b) and 6(c) using the sensor sensitivities that is shown in Fig. 5. Figs. 5 shows the spatiotemporal variation of Ca2+ (C1-C6) and pH (P1-P6) was also changed spatiotemporally. In other experiment, temperature didn't change during OCP transformation.

From these results, effectiveness of spatiotemporal sensing was confirmed.

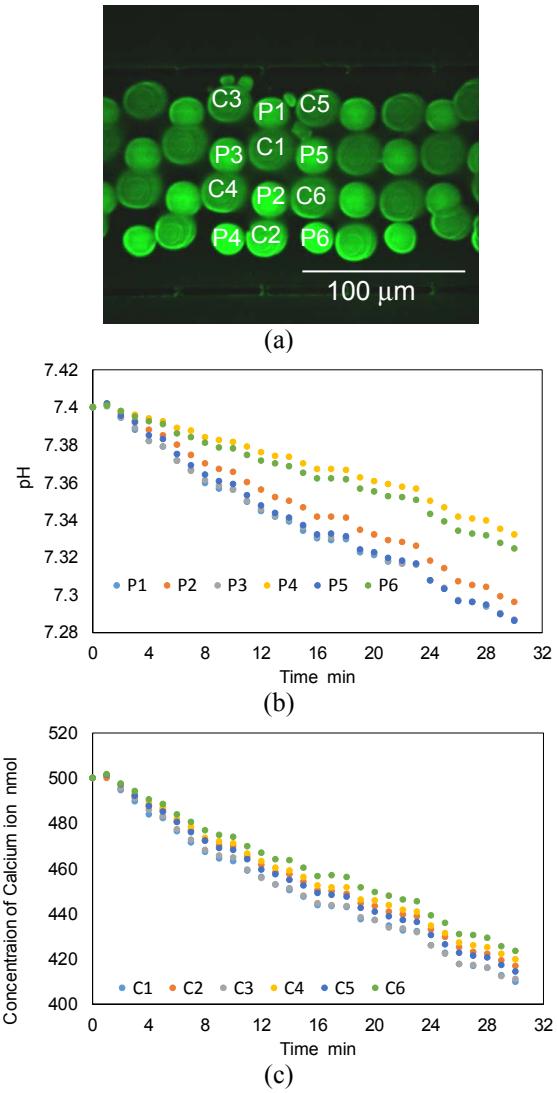


Figure 4: Results of spatiotemporal multi-sensing. (a) Fluorescence image. (b) Ca^{2+} and pH sensing.

CONCLUSIONS

We succeeded in the fabrication of OCP analysis chip and multi fluorescence multi-microsensors with different sizes for Ca^{2+}, pH and temperature sensing respectively in microfluidic chip. The different microsensors show fluorescence under same laser wavelength and can be recognized by their different sizes. The fluorescent multi-microsensors were proved to be responsive to Ca^{2+}, pH and temperature, respectively. And their sensitivities have been detected. OCP was also delivered into the microfluidic chip successfully. The microsensors were used to detect the Ca^{2+} and pH changes during transformation of OCP t at the presence of F^-. The results showed that spatiotemporal distribution of Ca^{2+} concentration and pH was measured during OCP conversion at the presence of 100 ppm F^- using fluorescence multi-microsensors. This spatiotemporal sensing of culture environment will make great contribution to reconstruct high-quality organ in ex vivo.

ACKNOWLEDGEMENTS

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