SINGLE-STEP AND MULTIPLE BIOASSAY
BASED ON COMBINABLE PDMS CAPILLARY (CPC) SENSOR ARRAY
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ABSTRACT
Combinable poly(dimethyl siloxane) (PDMS) capillary (CPC) sensor array allowing single step and multiple bioanalysis by simply introducing a sample solution by capillary action, followed by spontaneous mixing and reactions for fluorescence readout was developed. Since the CPC is fabricated by combining "Convex"-shaped PDMS plate immobilizing an insoluble membrane containing analytical reagents and "Concave"-shaped PDMS plate immobilizing a soluble membrane containing other kind of analytical reagents, it is very useful for designing various types of "single-step" bioassay. Here, enzyme inhibitor assay and glucose assay are presented as examples of single step assay. Furthermore, doping of PDMS-poly(ethyleneglycol) (PEG) copolymer into "concave"-shaped bulk PDMS gave fully hydrophilic surface, which solved the problems of capillary action-based sample introduction and homogeneous coating of highly hydrophilic PEG membrane on PDMS.

KEYWORDS: combinable-PDMS capillary(CPC), enzyme inhibitor assay, enzyme / substrate immobilization

INTRODUCTION
Single-step and multiple bioassay is one important research direction of bioanalysis for acceleration of drug development and medical diagnosis speed. In order to demonstrate this, fused-silica capillary modified with reagent layer is one ideal tool. Because the capillary action makes sample introduction much easier and subsequent spontaneous reaction with introduced sample and reagent within the capillary gives certain optical response, eliminating the complicated step-by-step operations required for some conventional bioassays, leading to the realization of "Sample-in & Signal-out" type biosensing. Furthermore, simply arraying required capillaries would lead to the development of "Order-made" multianalyte-sensing device which can be applied for various important bioanalyses in life science field.

On the other hand, we have been working on the development of capillary-assembled microchip (CAs-CHIP) fabricated by embedding the chemically-modified square capillaries into PDMS channels having same size to square capillary. Among many techniques on the development of CAs-CHIPs, development of capillaries possessing various types of chemical sensing functions played important roles. We have already developed some single-step chemical sensing capillaries such as enzyme activity-sensing, electrolyte sensing, or biosensing for integration on a CAs-CHIP. However, in order to make the enzyme inhibitor assay single step, immobilization of both fluorescent substrate and enzymes, which can react each other during the immobilization procedure, is required. Thus it is technically difficult for making it single step by employing available capillary.

In order to solve the problem, we proposed the use of CPC that assembles two independent PDMS structures immobilizing different analytical reagents to form the "capillary". CPC is fabricated by combining "Convex"-shaped PDMS plate immobilizing an insoluble membrane containing analytical reagents and "Concave"-shaped PDMS plate immobilizing a soluble membrane containing other kind of analytical reagents. Therefore, the above mentioned problem can be solved. Furthermore, since the PDMS is formed by curing the liquid prepolymer, simply mixing a certain additives to prepolymer may lead to the control of bulk and surface functions of the capillary device.

In the present work, enzyme inhibitor assay and glucose assay are demonstrated as examples of single step assay. In addition, doping of PDMS-PEG copolymer into "concave"-shaped bulk PDMS to improve the utility of CPC is also described.

Figure 1: General concept of CPC sensor for single-step assay
EXPERIMENTAL

Fabrication of CPC:

Convex- and concave-shaped PDMS structures illustrated in Figure 1 were prepared by standard PDMS molding procedure using glass mold. In general, CPC was fabricated by simply combining these PDMS without permanent bonding.

Preparation of sensing capillaries for enzyme inhibitor and glucose:

For enzyme inhibitor assay, water-soluble polyethylene glycol membrane containing resorufin-β-D-galactopyranoside, fluorescent substrate of β-galactosidase, was immobilized at the two corners of concave-shaped PDMS along the channel length. In this case, constant amount of tris buffer containing resorufin-β-D-galactopyranoside, PEG, and PDMS-PEG was introduced into flow channel of concave-shaped PDMS plate. Then, it was dried under vacuum for 2 hours to form dissolvable membrane containing fluorescence substrate. On the other hand, enzyme (β-galactosidase) was immobilized on a convex-shaped PDMS by layer-by-layer deposition method. In this case, convex-shaped PDMS plate was dipped in poly-anion solution [poly(acrylic acid, sodium salt)] for 1 min, washed using ultra pure water and dried by N₂ gas. Subsequently, the PDMS plate was similarly dipped in poly-cation solution [poly(dimethylammonium chloride)] containing enzyme for 1 min, washed using ultra pure water and dried by N₂ gas. These processes were repeated 10 times to form enzyme-immobilized membrane at the surface of convex-shaped PDMS plate. These PDMS plates were combined to obtain the capillary for enzyme inhibitor assay.

For glucose assay, only the concave-shaped PDMS was coated by water-soluble polyethylene glycol membrane containing the reagents necessary for glucose assay. PEG membrane containing glucose oxidase, peroxidase, and thymine (fluorescent substrate) was coated in the same manner mentioned above. Then convex-shaped PDMS prepared by native PDMS was combined to obtain the capillary for glucose assay.

Chemical modification of PDMS:

In order to hydrophilize the PDMS surface, doping of PDMS-PEG into bulk PDMS was carried out. In this case, known amount of PDMS-PEG (1%, 5%, 10% (w/w)) was doped in PDMS prepolymer, then cured at 90 degree Celsius for 2 hours.

RESULTS AND DISCUSSION

In order to improve the capillary action-based sample introduction behavior and homogeneous surface modification of hydrophilic PEG membrane on a highly-hydrophobic PDMS surface, doping of commercially available PDMS-PEG copolymer into the bulk of "Concave"-shaped PDMS was carried out and surface properties were evaluated by contact angle measurements.

When the PDMS-PEG was added into PDMS, contact angle was reduced from ca. 100 degree to 60 degree, indicating that the surface became fully hydrophilic. In addition, this hydrophilic property lasted at least one month. This might be attributed to the achievement of equilibrium of the bulk PDMS-PEG and surface PDMS-PEG in PDMS. By using the PDMS-PEG-doped "Concave"-shaped PDMS, fully homogeneous coating of dissolvable PEG membrane was successfully confirmed by fluorescence microscope observation. Since the color of PDMS-PEG-doped "Concave"-shaped PDMS became white, native clear PDMS plate was used as a "Convex"-shaped PDMS for optical detection. Concerning the sample introduction using this CPC, smooth introduction of sample solution was successfully demonstrated since the three-fourth of inner surface of CPC became fully hydrophilic.

Figure 2: Enzyme inhibitor assay and glucose assay based on CPC sensors
Finally, this CPC was applied for the enzyme inhibitor assay (β-galactosidase), and glucose bioassay. Figure 2 shows the results. For enzyme inhibitor assay, fluorescence response for buffer solution showed strong fluorescence response, while that for sample solution containing lactose (inhibitor) showed suppressed fluorescence intensity. Concerning the glucose assay, introduction of glucose solution gave fluorescence response and calibration curve for glucose at submillimolar concentrations was obtained. These results indicated that the CPC sensors presented here successfully worked as "Single-Step" inhibitor sensor or glucose sensor.

By using CPC sensor, simply arraying various CPCs would lead to the development of single-step multianalyte sensing device. Figure 3 shows a typical demonstration of stack& slice concept for parallelization and mass production of CPC sensor array. Since the CPC is fabricated by PDMS, simply slicing the CPC array gave mass production of multianalyte sensing "pieces" having exactly the same compositions of immobilized reagents and additives in bulk PDMS, which would lead to the inexpensive mass production of multianalyte sensing device.

CONCLUSIONS

Here we demonstrated the simple modification of concave-shaped PDMS by adding PDMS-PEG copolymer into PDMS prepolymer to give hydrophilic PDMS. Homogeneous surface coating of hydrophilic PEG membrane was quite successful by using this hydrophilic PDMS. Bonding with native convex-shaped PDMS gave CPC sensor allowing various types of single-step chemical sensing such as enzyme inhibitor assay and glucose assay. Especially, in an enzyme inhibitor assay, complicated step-by-step mixing procedure was successfully switched into single step by using capillary-action based sample introduction. Finally, stack & slice concept was shown as an implication to demonstrate inexpensive mass production of multianalyte sensing device. This type of multiple biosensing device is expected to be applied for various multiple bioassays including drug screening, medical diagnosis, and many other life science fields.

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