DEVELOPMENT OF A BLOOD TESTING DEVICE
BASED ON LOCALIZED SURFACE PLASMON RESONANCE
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ABSTRACT
We developed a novel blood testing device which extracts plasma from a minute amount of whole blood using capillary force and detects specific proteins in the extracted plasma using LSPR (localized surface plasmon resonance). The device consists of a main channel and many parallel side channels which were made of PDMS and covered with a glass plate. Also, gold nanoparticles of 100 nm in diameter were immobilized on the glass surface in the side channels. We detected an antigen-antibody reaction by measuring scattering light spectra of the gold nanoparticles, and demonstrated that the present device permits detection of tPA (tissue plasminogen activator).

KEYWORDS
Blood testing, Plasma extraction, Localized surface plasmon resonance, Capillary force

INTRODUCTION
The concern for healthcare and preventive medical care is rising with increase of the lifestyle-related diseases and population aging. There is going to be a greater demand for periodical and on-site blood tests which are carried out at home by nonprofessional people. Then, several research groups have already reported about microfluidic devices having both functions of plasma extraction and biomolecule detection. Fan et al. extracted plasma using the Zweifach-Fung effect [1], and Dimov et al. extracted it using gravitational sedimentation of blood cells [2]. They both succeeded in fluorometric detection of biomolecules in the plasma. However, their devices require a pumping power source for blood sample flow, and also require fluorescent labeling of target molecules for detection. In order to solve these problems, we developed a microfluidic device for automatic plasma extraction using capillary force without external driving sources, and permitted the device to have a function of LSPR-based non-labeling detection of biomolecules with a simple optical setup [3][4]. This will lead to realization of an easy-to-use, compact system that is more suitable for point-of-care blood testing.

METHODOLOGY
The blood testing device consists of a main channel and many parallel side channels which are made of PDMS (polydimethylsiloxane) and covered with a glass plate. The main channel measures 2 mm in width and 100 μm in depth. Each side channel connecting to the upper part of the main channel sidewall has very narrow entrance measuring 3 μm in width and 2 μm in depth, and is extended in a tapered shape. Also, gold nanoparticles of 100 nm in diameter for LSPR detection are immobilized on the glass surface in the downstream areas of the side channels (Fig.1). When a blood sample is placed at the entrance of the main channel, the blood automatically enters the main channel by capillary force. Then, gravitational sedimentation of blood cells permits plasma to flow into the side channels. When the plasma arrives at the area where gold nanoparticles are immobilized, antigen molecules in the plasma will bind to antibody molecules on the gold nanoparticles (Fig.2). Their binding will change a scattering light spectrum of the gold nanoparticles which can be measured using a spectroscope. The PDMS channel surfaces were modified with phosphorylcholine groups in order to improve the performance of automatic sample transportation and prevent non-specific adsorption of sample molecules. Actually, when 3 μl whole blood was injected, plasma extraction was completed within 5 sec (Fig.3).
Gold nanoparticles were immobilized using the following procedure. First, a gold nanoparticle surface was modified with carboxyl groups by dithiodipropionic acid. Next, a glass plate surface was modified with amino groups by APTES (3-aminopropyltriethoxysilane). Finally, the carboxyl groups on the gold nanoparticle surface were combined with the amino groups on the glass plate surface using an amine coupling reaction (Fig. 4). This procedure permitted dense and stable immobilization of gold nanoparticles on a glass plate as shown in Fig. 5.

**EXPERIMENTAL RESULTS**

In order to evaluate the blood testing device, we detected tPA (tissue plasminogen activator) molecule which is a biomarker for blood clot. For this purpose, protein G molecules were combined with the carboxyl groups on the gold nanoparticle, and tPA antibodies were combined with the protein G molecules. Then, a series of tPA solutions of 10, 20, 40, 60, 80 and 100 μg/mL was injected into the LSPR detection area, and scattering light spectra of the gold nanoparticles were measured using a spectroscope to detect reactions between tPA antigens and tPA antibodies (Fig. 6). As a result, it was found that scattering light intensity increases with an increase in tPA concentration (Fig. 7, 8).
CONCLUSIONS
We developed a blood testing device based on LSPR. This device enabled plasma extraction from a whole blood using only a capillary force. Also, dense and stable immobilization of gold nanoparticles on a glass plate surface of the device permitted LSPR-based, single-step, label-free detection of biomolecules without solution exchange. Moreover, we demonstrated tPA molecule detection using the device, and found that the scattering light intensity of gold nanoparticles increases with an increase in tPA concentration. In the near future, we will obtain more experimental data using various biomolecules.

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