Bio-sensing on a chip with compact discs and nanofibers†

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This paper describes a novel, sensitive detection system for biomolecules (DNA and proteins etc.) that is integrated in a lab-on-a-chip utilizing optical compact discs (CDs) and bio-nanofibers. The new method comprises a microchannel containing CD grating that confines fragments of unique bacterial cellulose fibrils (BC), which have nanometre scale fibers and holes. A maximum of six times higher sensitivity to detect DNA was obtained with this CD and BC system compared to a conventional method. We also demonstrate an effective light-confining effect for biological application with the new method.

Introduction

Various types of optical devices, such as photodiodes and charge-coupled devices (CCDs), have been used to sense and analyze minute changes in biological information from organisms. 1 Our new approach for sensitive detection of biomolecules employing Compact Discs (CDs)2 and bio-nanofibers3–7 is unique and surprisingly effective. Bacterial cellulose (BC),3–6 which has nanometre scale fibers and holes (Fig. 1a) was used as an effective light scattering medium. A combination of CD grating, which is easily recognizable as a rainbow pattern of colors on the CD surface (Fig. 1b), and the BC fibrils led to an effective light-amplifier in the microchannel.

Recently, nanofibers such as carbon nanotubes (CNTs)7 have attracted considerable attention as new nanomaterials. There is, however, a different type of nanofiber, the BC fibril, which is produced by some strains of Acetobacter. 3 In general, cellulose derivatives are used as the polymer solution in DNA separation media for capillary or microchip electrophoresis, a popular detection system for biomolecules. On the other hand, the BC is composed of self-entangled ultra-fine fibrils, 50–80 nm in width and 3–8 nm in thickness, that have a three dimensional nano-network structure3–6 (Fig. 1a), in which the scales are quite different from plant cellulose (e.g., wood). Many unique characteristics and applications of BC have been exploited. For example, it is a useful food additive (it is well known as a component of the dessert “Nata de Coco”), and it is also utilized in construction of a commercially available vibration membrane in a speaker phone. 5 The unique BC structure of fine fibrils would be expected to collect scattered light beams effectively, such as a textured structure in a solar cell, 3 which may differ from the conventional grains (1.6 μm in diameter) that Wiersma used as a random laser. 3 In addition, the diffraction from CD grating promotes a “light-confining effect”, 10,11 similar to that in a solar cell. A schematic diagram of the proposed light-confining effect in our high detection method is shown in Fig. 1c.

Experimental

Bacterial cellulose (BC)

The BC buffer was prepared in the following manner. BC was produced from a static culture of Acetobacter hansenii (ATCC 35959T Sumisho Pharma International, Japan). The wet BC pellicle contained about 1 wt% of cellulose fibrils. Fragments of bacterial cellulose pellicle with dimensions of approximately several μm were prepared from wet pellicles under wet conditions using a sterile mill (SM-1 Labcat, USA). These BC pellicle fragments are comprised of a nanometre scale thickness network frame with a 10 nm–3 μm pore size (Fig. 1a). The BC fibrils are difficult to dissolve in media because of their high molecular weight (>10 000 DPw), stiffness (the Young’s modulus of a dry sheet is 33.3 GPa), 6 and hydrogen-bond network. This BC fragment suspension was used as an additive to the buffer. Contamination of bacterial DNA from the BC medium was not detected.

Control medium and additional media in the channel

A polymer solution (hydroxy propyl methyl cellulose (HPMC, Hitachi Chemical, Hitachi, Japan)) was utilized as the control medium. To investigate the effect of additional media, a BC buffer (0.5 wt% BC fragments), as mentioned above, a CNT buffer (0.1 wt% CNT; carbon single-wall nanotube, SES Research, Houston, TX, USA) or latex buffer (0.22 wt% 10 μm spheres (LX; Latex, Interfacial Dynamics Corporation)) was prepared by adding each material to the control buffer. These media were confined in a microchannel (100 μm in width and 30 μm in depth; i-chip3 or i-chip12, Hitachi Chemical, Hitachi, Japan).
The following materials were placed on the channel: CD, compact disc (fragment of CD-R disc, Taiyo Yuden, Tokyo, Japan, thickness \( t = 1.17 \) mm, refraction index (RI) = 1.59, having a track without reflective mirror); GS, slide glass (Matsunami, Tokyo, Japan, \( t = 1.28 \) mm); PMMA, microchip (i-chip 3, Hitachi Chemical, \( t = 1.1 \) mm, RI = 1.49); PS, polystyrene slide glass (Nalge Nunc, IL, \( t = 1.0 \) mm, RI = 1.59); and PF, adhesive polymer film (ABgene, UK, \( t = 56 \) \( \mu \)m). As a control, one channel contained the control buffer with no materials on it. The raw experimental values were divided by the mean of the control (relative intensity of control = 1, \( n = 50 \)).

### Fabricated grating chip

The parallel-type or cross-type grating was fabricated on the channel of an i-chip3 or i-chip12 (Hitachi Chemical) by a hot-emboss method (Fig. 3a–e; fabricated by ASTY, Hamamatsu, Japan). To investigate the effect of refraction, a mirror was covered with aluminium on the surface of the grating (Fig. 3f). The average fluorescent intensity of the dsDNA (500 bp in 100 bp DNA ladder, TakaraBio, Siga, Japan) was compared between the control, grating chip and grating with mirror chips (\( n = 12; 3 \) channels \( \times 4 \) chips). The DNA intensity degradation curve was also compared between the control, grating, grating with BC, and grating and mirror with BC. Fluorescent intensity was pulsed at each 100 s interval excitation.

### Effect of optical grating

Of the media examined, only the CD had a grating structure, a property that may account for its ability to amplify light. Therefore, in order to investigate the effect of the optical grating on the peak intensity, a grating was directly fabricated on the surface of the microchannel. Two types of optical grating with a 1.6 \( \mu \)m spacing (Fig. 3c), parallel-type (Fig. 3a and d), and cross-type (Fig. 3b and e) relative to the channel direction, were fabricated by a hot-emboss method. DNA fragments were analyzed using the grating chips without BC. Fig. 3g shows that approximately 1.5–2 times higher intensity was obtained by using the grating chip. This result indicates that light amplification occurs with grating in the chip itself as well as with the CD grating. The intensity was further improved by grating with a reflective mirror on the chip (2.5–3.0 times; Fig. 3f and g). The parallel type grating was somewhat better at amplification than the vertical type, but the difference was not statistically significant. In addition,

### Results and discussion

#### Media on or in the channel

First, we investigated the effectiveness of light amplifying media that are utilized on or in the channel. Fig. 2 shows the effects of the media on the fluorescent intensity of DNA. Some of the media placed on the channel, i.e. polyfilm (PF), polystyrene film (PS), and polymethylmethacrylate (PMMA), gave decreased intensity values, while others, including glass plate (GS), gave the same values, whereas the relative intensity with the CD fragment increased. Utilizing latex (LX) in the channel as an additive to the medium decreased the intensity, whereas BC or CNT increased the intensity. The intensity was amplified further, by an average of 2–3 fold with a maximum of six-fold, by the combination of CD with BC or CD with CNT. Thus, the characteristics of the CD grating and the nanostructure of the nanofibers seemed to have significant effects on light amplification.

### Detection system

A detection system, SV1100, Hitachi Electronics, Hitachi, Japan, with a blue LED (470 nm) light source and a photodiode detector or SV1210, Hitachi Electronics, with a diode laser emitting at 635 nm and a cool CCD camera system, was used. The fluorescent intensity was investigated using dsDNA fragments (2 ng \( \mu L^{-1}, 500 \) bp) stained by ethidium bromide (Hitachi chemical).
although the control showed a steep slope in the degradation curve of fluorescent intensity, grating with and without BC showed a one-dimensional gentle slope (Fig. 3h). In the case of the grating and mirror with BC the intensity was shown to persist. These results indicate that the optical grating, nanofibers, and mirror have a light amplification effect. This apparent effect of the grating was supported strongly by the results obtained using fabricated grating on the channel. In addition, the difference in degradation curves between chips with no grating, and those with grating or grating (+mirror) with BC, indicated the possibility of a light-confining effect.

**Why CD with BC?**

In Fig. 2, nanofibers (BC fibrils or CNTs) in a channel showed an improved intensity, which may come from balancing of the dual effects of transmittance and refraction by the characteristic nanofiber (Electronic Supplementary Information, Supporting Fig. 2). Usually emission light disperses spherically; however, with additives such as LX, incident light is interrupted and therefore the intensity decreases. On the other hand, in the case of nanofibers, addition of BC results in some incident light travelling uninterrupted through pores in the fine BC fibrils. Convincing evidence for such an effect was the finding that transmittance in the BC buffer was higher than in the LX buffer (BC: 46%, LX: 21%) at an optimal concentration of BC (0.5 wt%). In addition, the interior of the nanotube is known to have decreased water conduction (relative dielectric constant), associated with a decreased refraction index (RI), which might have led to effective refraction on the BC. Thus, BC can function uniquely as an element of an effective light-amplifier. Combined with the CD grating, the multiple refractions, reflections, and diffractions resulted in an increased amount of incident light striking the DNA (Fig. 1c). It is well known that a solar cell increases absorption of light by increasing the path length of the incident light through reflection and diffraction arising from textured structures of transparent conductive electrodes. Finally, the amplified-light can reach the detector through the characteristic BC nanopores, again with only a little light-scattering loss.

**Light-confining effect for biology**

A commercial application of the light-confining effect is a solar cell, and we are now in the golden days of photonic crystal applications. Optical microcavities are effective in confining light to small volumes by resonant recirculation, and this improves the quality factor ($Q$). In particular, in the field of electronics, a perfect confinement system is necessary to convey light through long optical cables. However, in a
biological detection system the detection point is a spot, and light does not need to be conveyed through a long channel. Thus, the design of a concentrated light amplifier method will be important for practical applications such as improving the detection sensitivity for biomolecules. The dual effects of transmittance and refraction of BC could provide the solution as an effective light scattering medium.

The features described above could find more practical applications, for example, the detection of single nucleotide polymorphisms (SNPs) in DNA electrophoresis using a CD chip in which the microchannel is fabricated directly onto the surface of the CD. The characteristic peaks derived from SNPs were well identified by a CD with BC, and this required only half the time of the usual PCR amplification conditions (data not shown). Further applications may include protein expression in early stages of diseases, especially cancer.

In conclusion, a lab-on-a-chip using CD with BC functions as a highly bio-sensitive detection system. The multiple combination of diffraction on the CD grating, reflection on the CD mirror, and refraction on the fine BC fibril led to an effective light-confining effect. This is the first time, to our knowledge, that the BC and CD technologies have been designed for applications besides electronics, such as photonics or solar cells, and this method shows great promise as a sensitive detection method for various biological applications.

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