# Label-free detection of protein molecules secreted from an organ-on-a-chip model for drug toxicity assays

Andres W. Morales[a], Yu Shrike Zhang[b,c,d], Julio Aleman[b,c], and Parissa Alerasool[b,c,e], Mehmet Remzi Dokmeci[b,c,d], Ali Khademhosseini[b,c,d,f,g], and Jing Yong Ye[a]
[a]Department of Biomedical Engineering, the University of Texas at San Antonio, San Antonio, TX 78249, USA; [b]Biomaterials Innovation Research Center, Division of Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston 02139, MA, USA; [c]Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge 02139, MA, USA; [d]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston 02115, USA; [e]Department of Biology, Tufts University, Medford 02155, MA, USA; [f]Department of Physics, King Abdulaziz University, Jeddah 21569, Saudi Arabia; [g]College of Animal Bioscience and Technology, Department of Bioindustrial Technologies, Konkuk University, Hwayang-dong, Kwangjin-gu, Seoul, Republic of Korea.

## ABSTRACT

Clinical attrition is about 30% from failure of drug candidates due to toxic side effects, increasing the drug development costs significantly and slowing down the drug discovery process. This partly originates from the fact that the animal models do not accurately represent human physiology. Hence there is a clear unmet need for developing drug toxicity assays using human-based models that are complementary to traditional animal models before starting expensive clinical trials. Organ-on-a-chip techniques developed in recent years have generated a variety of human organ models mimicking different human physiological conditions. However, it is extremely challenging to monitor the transient and long-term response of the organ models to drug treatments during drug toxicity tests. First, when an organ-on-a-chip model interacts with drugs, a certain amount of protein molecules may be released into the medium due to certain drug effects, but the amount of the the protein molecules is limited, since the organ tissue grown inside microfluidic bioreactors have minimum volume. Second, traditional fluorescence techniques cannot be utilized for real-time monitoring of the concentration of the protein molecules, because the protein molecules are continuously secreted from the tissue and it is practically impossible to achieve fluorescence labeling in the dynamically changing environment. Therefore, direct measurements of the secreted protein molecules with a label-free approach is strongly desired for organs-on-a-chip applications. In this paper, we report the development of a photonic crystal-based biosensor for label-free assays of secreted protein molecules from a liver-on-a-chip model. Ultrahigh detection sensitivity and specificity have been demonstrated.

**Keywords:** Label-free, organ-on-a-chip, PC-TIR, photonic crystal, biosensor, drug toxicity

## 1. INTRODUCTION

Failure of drug candidates during clinical trials, late in the development pipeline, generates immense costs to the pharmaceutical industry and slows down the drug development process. Toxic side effects of the drug candidates result in about 30% of this clinical attrition [1]. These failures partly result from the ineffectiveness of the animal models where they fail to accurately represent human physiology [2, 3, 4]. Hence there is a clear unmet need for human-cells based models to complement traditional animal models for drug toxicity assays before the start of expensive clinical trials.

The latest developments in tissue engineering, biomaterials and microfluidics research have enabled the development of microbioreactors that can grow human tissues in environments mimicking living systems [5, 6]. These organ-on-a-chip platforms present models allowing a more biomimetic human-cells based drug toxicity testing [7, 8, 9]. However, a couple of challenges arise when monitoring the transient and long-term response of tissues to drugs in a microbioreactor. The first challenge lies in the miniaturization of the organ/tissue grown using the organ-on-a-chip technique. The miniaturization of organoids reduces the amount of protein molecules and other biomarkers released from the tissue; resulting in the need for a monitoring technique with a high sensitivity and requiring only a small sample volume. The second challenge

Frontiers in Biological Detection: From Nanosensors to Systems VIII, edited by Benjamin L. Miller, Brian T. Cunningham, Amos Danielli, G. Logan Liu, Sharon M. Weiss Proc. of SPIE Vol. 9725, 972508 · © 2016 SPIE · CCC code: 0277-786X/16/$18 · doi: 10.1117/12.2212971

is the inability to use traditional fluorescence techniques. Since the protein molecules are dynamically and continuously secreted over long periods of time, the ability to obtain quantitative measurements throughout this time is limited by photobleaching. Although fluorescent proteins may be used as reporter biomarkers to provide real-time dynamic monitoring [10, 11, 12], this technique may alter the cells' phenotype and/or the proteins being monitored.

These challenges necessitate a sensitive label-free technique of directly measuring the secreted protein molecules. The label-free detection mechanism discussed in this paper is based on a photonic crystal in a total internal reflection (PC-TIR) configuration with a unique open microcavity structure. Ultrahigh sensitivity and specificity in the measurement of secreted albumin levels from a liver-on-a-chip platform have been demonstrated.

## 2. METHODOLOGY

### 2.1 Design of liver-on-a-chip system

The design and fabrication of the liver-on-a-chip model is described in our recent publication [13]. In short, the bioreactor consisted of the chambers and channels of the bioreactor casted in polydimethylsiloxane (PDMS), a bottom layer of 3-(trimethoxysilyl)propyl methacrylate coated glass, and two layers of PDMS covered poly(methyl methacrylate) (PMMA) sandwiching the other layers (figure 1(a)). Photocrosslinkable gelatin methacryloyl (GelMA) hydrogel containing HepG2/C3A hepatocytes was bioprinted directly into the bioreactor and crosslinked using UV light (figure 1(b)). The bioreactor was continuously perfused with Dulbecco's modified Eagle medium (DMEM) with 10 vol.% fetal bovine serum (FBS) at a flow rate of 200 μL $h^{-1}$ for up to 5 days. The cells were continuously administered with 10 mM of acetaminophen (APAP) and incubated at 37° C with 5% $CO_2$. On days 0, 1, and 5 the media was collected from the bioreactor for measurements.

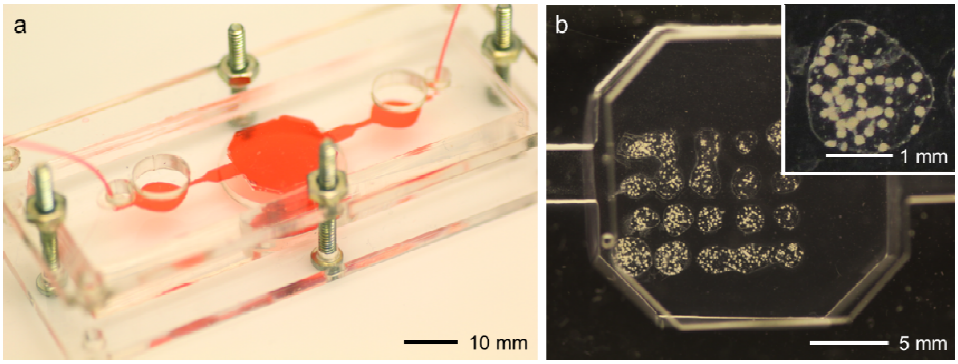


Figure 1. Liver-on-a-chip system: (a) 2 PMMA sheets, tightened by screws, hold the PDMS top layer and glass bottom layer together. (b) HepG2/C3A liver cells embedded in GelMA bioprinted on the microbioreactor.

### 2.2 Design and fabrication of PC-TIR sensor

Fabrication methods and theoretical calculations for the design of the PC-TIR biosensor were discussed in our previous research [14, 15, 16, 17, 18, 19]. To summarize, the sensor consisted of a PC structure under a cavity layer and PDMS was bound to the sensor surface forming microchannels. The PC structure, as shown in figure 2(a), had five alternating layers of titania and silica fabricated on BK7 glass using electron-beam physical vapor deposition. The thickness of the titania and silica layers was 89.8 and 307.2 nm respectively, leading to a 64° incident angle of a probe light into the substrate of the sensor. This PC structure was covered by 382 nm of silica and 10 nm of silicon to form the cavity layer. As shown in figure 2(b), the probe light was an s-polarized Helium-Neon laser which was spatially filtered, expanded to 10 mm, and then focused by a cylindrical lens into a line crossing the microchannels on the sensor surface. The reflected beam was then collimated with another cylindrical lens onto a CCD camera. The resonant angle of the sensor for each channel produces a dark line in the image captured by the CCD camera. Binding events on the sensor surface cause shifts in the positions of these dark lines.

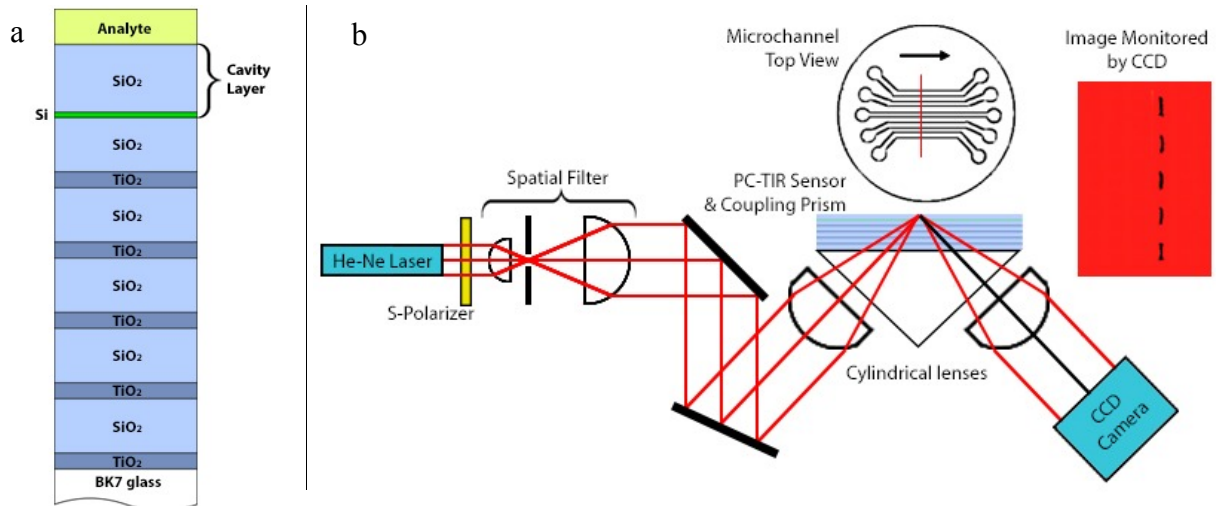


Figure 2. PC-TIR sensor: (a) Photonic crystal structure with open cavity. (b) Schematic diagram of a PC-TIR experimental setup with image of resonant dark lines captured by the CCD camera.

### 2.3 Functionalization of the PC-TIR sensor

To allow biomolecular immobilization, 2% (v/v) 3-aminopropyltiethoxysilane (APTES) in ethanol was flowed across the sensor surface for 25 minutes, followed by a 40 minute ethanol wash and drying overnight. The sensor was then functionalized for 20 minutes with 50 mM goat anti-human albumin in phosphate buffered saline (PBS). This was followed by 20 minutes of 3x diluted steelhead salmon serum (SEA) in PBS to block possible nonspecific binding sites.

### 2.4 Binding assays with the PC-TIR sensor

Samples were injected into the channels for 20 minutes followed by a PBS wash, while images were recorded using a CCD camera every 40 seconds.

## 3. MEASUREMENTS

An image was taken as a reference from an empty channel for the purpose of removing the background noise. To determine the position of each dark line, each channel was split into 3 horizontal segments, whose intensities were vertically summed and then fitted to a Lorentzian function. For each channel, the displacement of the dark line in each segment was used to find the dark line's average displacement and standard deviations. The resonance shift was then measured as the difference in displacement of the dark line before and after the sample was flowed across the sensor. We flowed water through one of the channels and subtracted its resonance shift from the others to account for shifts due to changes in environmental factors like temperature.

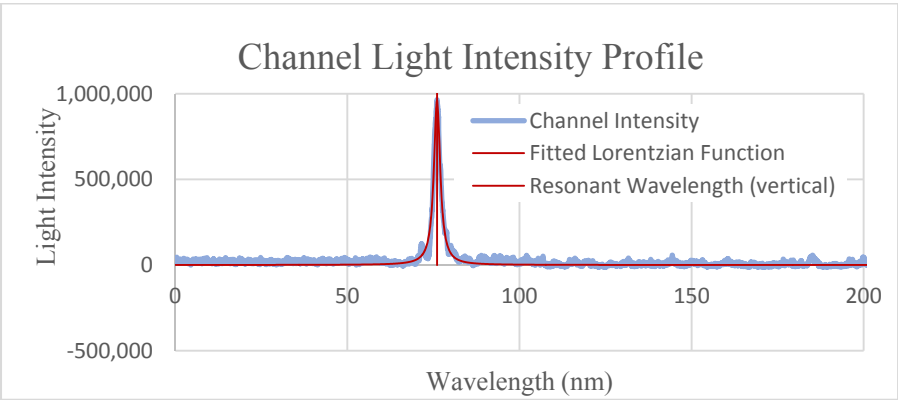


Figure 3. Light intensity profile of a channel after subtraction from the reference channel. The peak was the dark line in the channel image. To find the center of the dark line, the profile was fit to the Lorentzian function (equation 1).

$$I = \frac{max\ height * width^2}{((x-center)^2 + width^2) + base\ height} \quad (1)$$

## 4. RESULTS

To carry out our measurements, samples obtained from bioreactors on various days were dispensed onto the sensors. As expected, the resonance shift due to albumin binding significantly increased starting from day 1 after culture and continued to increase throughout the rest of the period tested in our liver-on-a-chip devices (figure 4). This indicated that the liver organoids remained alive and were metabolically active. It was further observed that the cells treated with 10 mM acetaminophen showed slightly lower albumin production at day 5, indicating reduced cell survival due to drug treatment. This was also confirmed by a live/dead cytotoxicity assay that qualitatively showed similar cell viabilities between the two cell groups (figure 5). However, no significant difference in drug-induced cell death was observed at this dosage of APAP.

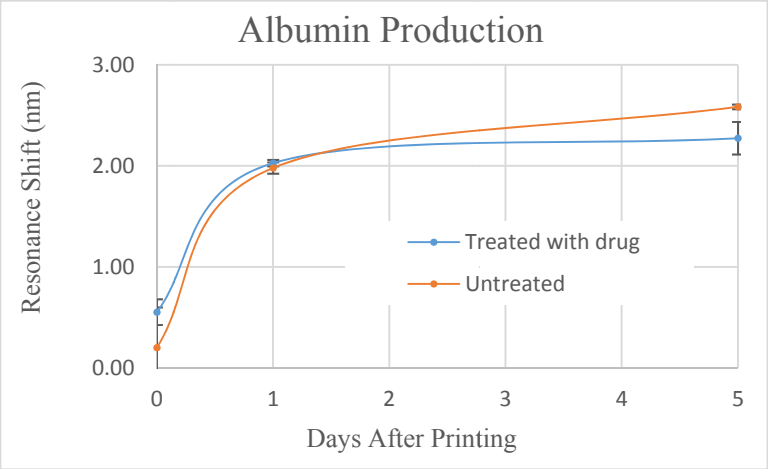


Figure 4. Albumin levels in media from liver-on-a-chip on different days after bioprinting cells onto the chip. Day 0 was from media flowing through the organ-on-a-chip system before printing the liver tissue inside.

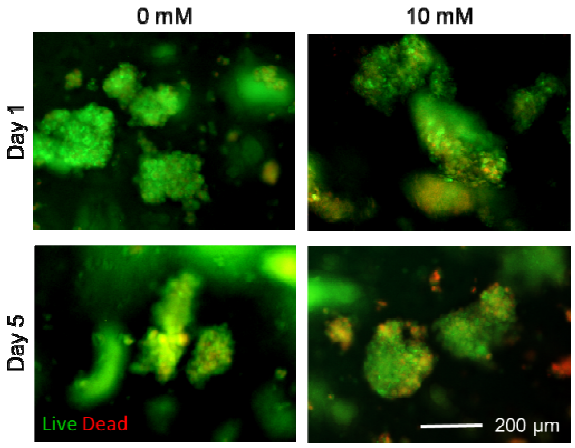


Figure 5. Live/dead assay showing the viability of HepG2 cells in the liver bioreactor at day 1 and day 5 following drug treatment.

## 5. CONCLUSIONS

A label-free biosensor for organ-on-a-chip drug toxicity assays has been successfully developed using a photonic crystal structure in a total internal reflection configuration. The open microcavity formed by this configuration allowed the sensor surface to be easily functionalized, was easily accessible by analytes, and simplified the fabrication of microfluidic channels. The label-free biosensor was able to monitor the transient and long-term response of microbioreactor tissues to drugs. The experimental results confirmed the possible use of a PC-TIR sensor in human-cells based organ-on-a-chip drug toxicity models in conjunction with the current animal models.

## REFERENCES


[1] J. R. Empfield and M. P. Clark, [Reducing Drug Attrition], Springer Berlin Heidelberg, (2014).

[2] Hackam, D. G. and Redelmeier, D. A., "Translation of Research Evidence From Animals to Humans," *JAMA,* 296(14), 1727 (2006).

[3] van Midwoud, P. M., Verpoorte, E. and Groothuis, G. M. M., "Microfluidic devices for in vitro studies on liver drug metabolism and toxicity," *Integr. Biol.,* 3(5), 509 (2011).

[4] Kaplowitz, N., "Idiosyncratic drug hepatotoxicity," *Nature Reviews Drug Discovery,* 4(6), 489-499 (2005).

[5] Whitesides, G. M., "The origins and the future of microfluidics," *Nature,* 442(7101), 368-373 (2006).

[6] Annabi, N., Selimović, Š., Acevedo Cox, J. P., Ribas, J., Afshar Bakooshli, M., Heintze, D., Weiss, A. S., Cropek, D. and Khademhosseini, A., "Hydrogel-coated microfluidic channels for cardiomyocyte culture," *Lab on a Chip,* 13(18), 3569 (2013).

[7] Huh, D., Matthews, B. D., Mammoto, A., Montoya-Zavala, M., Hsin, H. Y. and Ingber, D. E., "Reconstituting Organ-Level Lung Functions on a Chip," *Science,* 328(5986), 1662-1668 (2010).

[8] Polini, A., Prodanov, L., Bhise, N. S., Manoharan, V., Dokmeci, M. R. and Khademhosseini, A., "Organs-on-a-chip: a new tool for drug discovery," *Expert Opinion on Drug Discovery,* 9(4), 335-352 (2014).

[9] Bhise, N. S., Ribas, J., Manoharan, V., Zhang, Y. S., Polini, A., Massa, S., Dokmeci, M. R. and Khademhosseini, A., "Organ-on-a-chip platforms for studying drug delivery systems," *Journal of Controlled Release,* 190, 82-93 (2014).

[10] Wheeler, D. B., Carpenter, A. E. and Sabatini, D. M., "Cell microarrays and RNA interference chip away at gene function," *Nature Genetics,* 37(6s), S25-S30 (2005).

[11] Bailey, S. N., Ali, S., Carpenter, A., Higgins, C. O. and Sabatini, D. M., "Microarrays of lentiviruses for gene function screens in immortalized and primary cells," *Nature Methods,* 3(2), 117-122 (2006).

[12] Kepp, O., Galluzzi, L., Lipinski, M., Yuan, J. and Kroemer, G., "Cell death assays for drug discovery," *Nature Reviews Drug Discovery,* 10(3), 221-237 (2011).

[13] Bhise, N. S., Manoharan, V., Massa, S., Tamayol, A., Ghaderi, M., Miscuglio, M., Lang, Q., Zhang, Y. S., Shin, S. R., Calzone, G., Annabi, N., Shupe, T. D., Bishop, C. E., Atala, A., Dokmeci, M. R. and Khademhosseini, A., "A liver-on-a-chip platform with bioprinted hepatic spheroids," *Biofabrication,* 8(1), 014101 (2016).

[14] Zhang, B., Morales, A. W., Peterson, R., Tang, L. and Ye, J., "Label-free detection of cardiac troponin I with a photonic crystal biosensor," *Biosensors and Bioelectronics,* 58, 107-113 (2014).

[15] Guo, Y., Divin, C., Myc, A., Terry, F. L., Baker, J. R., Norris, T. B. and Ye, J. Y., "Sensitive molecular binding assay using a photonic crystal structure in total internal reflection," *Opt. Express,* 16(16), 11741-11749 (2008).

[16] Guo, Y., Ye, J. Y., Divin, C., Huang, B., Thomas, T. P., Baker, J. J. R. and Norris, T. B., "Real-Time Biomolecular Binding Detection Using a Sensitive Photonic Crystal Biosensor," *Analytical Chemistry,* 82(12), 5211-5218 (2010).

[17] Ye, J. Y. and Ishikawa, M., "Enhancing fluorescence detection with a photonic crystal structure in a total-internal-reflection configuration," *Opt. Lett.,* 33(15), 1729-1731 (2008).

[18] Zhang, B., Dallo, S., Peterson, R., Hussain, S., Weitao, T. and Ye, J. Y., "Detection of anthrax lef with DNA-based photonic crystal sensors," *Journal of Biomedical Optics,* 16(12), 127006 (2011).

[19] Zhang, B., Tamez-Vela, J. M., Solis, S., Bustamante, G., Peterson, R., Rahman, S., Morales, A., Tang, L. and Ye, J. Y., "Detection of Myoglobin with an Open-Cavity-Based Label-Free Photonic Crystal Biosensor," *Journal of Medical Engineering,* 2013, 1-7 (2013).