

## A brief review on microfluidic platforms for hormones detection

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**Abstract** Lab-on-chip technology is attracting great interest due to its potential as miniaturized devices that can automate and integrate many sample-handling steps, minimize consumption of reagent and samples, have short processing time and enable multiplexed analysis. Microfluidic devices have demonstrated their potential for a broad range of applications in life sciences, including point-of-care diagnostics and personalized medicine, based on the routine diagnosis of levels of hormones, cancer markers, and various metabolic products in blood, serum, etc. Microfluidics offers an adaptable platform that can facilitate cell culture as well as monitor their activity and control the cellular environment. Signaling molecules released from cells such as neurotransmitters and hormones are important in assessing the health of cells and the effect of drugs on their functions. In this review, we provide an insight into the state-of-art applications of microfluidics for monitoring of hormones released by cells. In our works, we have demonstrated efficient detection methods for bovine growth hormones using nano and microphotonics integrated microfluidics devices. The bovine growth hormone can be used as a growth promoter in dairy farming to enhance the milk and meat production. In the recent years, a few attempts have been reported on developing very sensitive, fast and low-cost

methods of detection of bovine growth hormone using micro devices. This paper reviews the current state-of-art of detection and analysis of hormone using integrated optical micro and nanofluidics systems. In addition, the paper also focuses on various lab-on-a-chip technologies reported recently, and their benefits for screening growth hormones in milk.

**Keywords** Microfluidics · Lab-on-a-chip · Pancreatic islets · Insulin · Thyroid hormone · Bovine growth hormone

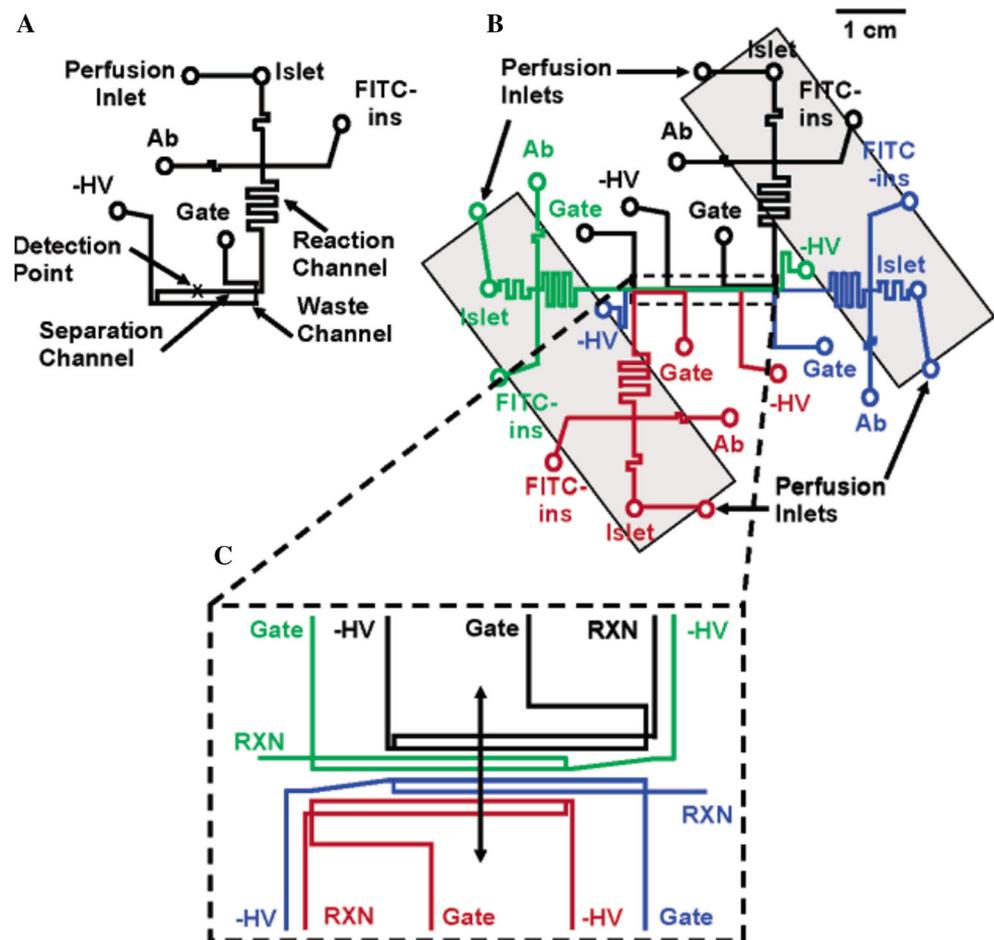
### Abbreviations

AD	Androstene-dione
CWC	Cascaded waveguide coupler
E2	Estradiol
ELISA	Enzyme-linked immunosorbent assay
–HV	Negative high voltage
LIF	Laser induced fluorescence
LOC	Lab-on-a-chip
LSPR	Localized surface plasmon resonance
μELISA	Enzyme-linked immunosorbent assay
μTAS	Micro total analysis systems
PDMS	Polydimethylsiloxane
PG	Progesterone
POC	Point-of-care
PON	Point-of-need
rbST	Recombinant bovine somatotropin
RXN	Reaction
TS	Testosterone
TSH	Thyroid-stimulating hormone
HPLC	High performance liquid chromatography
MS	Mass spectroscopy
FITC	Fluorescein isothiocyanate
SOS	Silica-on-silicon

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**Fig. 1** Monitoring of insulin secretion using microfluidic device having four islets. Three different colors indicate each independent channel in the microfluidic network. **a** A single-channel network from the device. *Solid lines* indicate microfluidic channels, and *circles* indicate microfluidic reservoirs and access holes to the channel networks. All channels were 9  $\mu\text{m}$  deep. **b** The chip design (drawn to scale), shaded regions represent heating strips applied to underside of chip. **c** Close view of the gating/detection region (not to scale). The separation channels run parallel to each other, in order to use the scanning laser spot to use for LIF detection. The scanning path of the laser is shown by *arrows*. Reproduced with permission (Dishinger and Kennedy 2007)

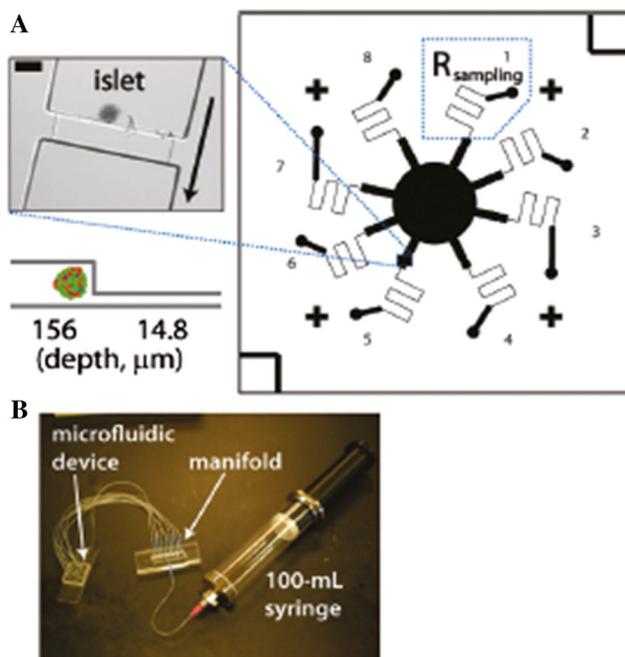


## Introduction

Lab-on-a-chip (LOC), referred also as micro total analysis systems ( $\mu\text{TAS}$ ) are miniaturized systems that can integrate and scale down complex laboratory functions on a device having a very small area of millimeters to a few square centimeters accommodating manipulation of fluids in channels and chambers with dimensions in the order of tens to hundreds of micrometers (Manz et al. 1990; Reyes et al. 2002; Vilknor et al. 2004). The main benefits of miniaturization are the handling of extremely small fluid volumes of reagents and samples, possibility of multiplexing the various operations, portability, disposability, low-cost, high throughput and low power consumption. Analyses can be carried out in LOC more rapidly and the cost of reagents and samples can be reduced considerably, making complex assay protocols more efficient compared with the current laboratory bench-scale methods. This is especially important for biology research where it is necessary for high throughput to maximize the information from very small samples.

LOCs are used in many biomedical applications such as detection of proteins and DNA (Chin et al. 2007), pathogenic organisms (Becker and Hansen-Hagge 2014), hormones (Ozhikandathil 2012), and cell culture (Peterson et al. 2005) and cell manipulation such as trapping of single cells and studying its characteristics under interactions with environment or other cells. These methods will replace gradually the traditional expensive instruments and time-consuming techniques that require trained technicians. They still need to be improved with sensitivity and specificity in order to replace gradually the instruments used in the laboratory. To this end, components at microscopic scales such as micro-pumps, micro-valves, filters, etc., have to be integrated and suitable detection methods have to be developed. This will allow the gradual implementation of point-of-care (POC) and point-of-need (PON) diagnostics that would enable quick and accurate results, leading to improved clinical outcome.

This review paper outlines recent microfluidic-based devices and LOC design strategies recently used for the detection of some of the most important classes of hormones. Examples of recently developed devices are



**Fig. 2** Passively operated microfluidic device for islet secretion sampling and imaging. **a** Device layout, including an image of a trapped islet. **b** Hand-held apparatus for the sampling of secretion without electrical components. Reproduced with permission from (Godwin et al. 2011), Copyright American Society of Chemistry, 2011

presented along with the respective advantages and limitations of each design.

### Detection of insulin and glucagon secretion from pancreatic islets

Most research to date has focused on the microfluidic sampling of hormones secreted by the pancreatic islets, called the islets of Langerhans.

The islets are the endocrine portion of the pancreas and have diameters ranging from tens to hundreds of micrometers, each islet containing 2000–4000 cells. The islets have their own supply of blood through micro capillaries. Each of the five different cell types (R,  $\beta$ ,  $\delta$ PP, and  $\epsilon$ ) is in charge with the secretion of a different hormone. Because diabetic

disease states, obesity and other public health problems are due to impaired function of the islets, the kinetics of insulin secretion was the focus of most research in the field. Following the microfluidic platforms developed by the Kennedy group (Roper et al. 2003; Shackman et al. 2005), several devices have been designed for analyzing the glucose-stimulated insulin secretion by the  $\beta$ -cells by perfusion, especially for understanding islet physiology and evaluation of their quality for transplantation purposes.

The same group further developed a chip that allows serial electrophoresis-based immunoassays to be performed in parallel by multiplexing microfluidics channels (Dishinger and Kennedy 2007; Roper et al. 2003).

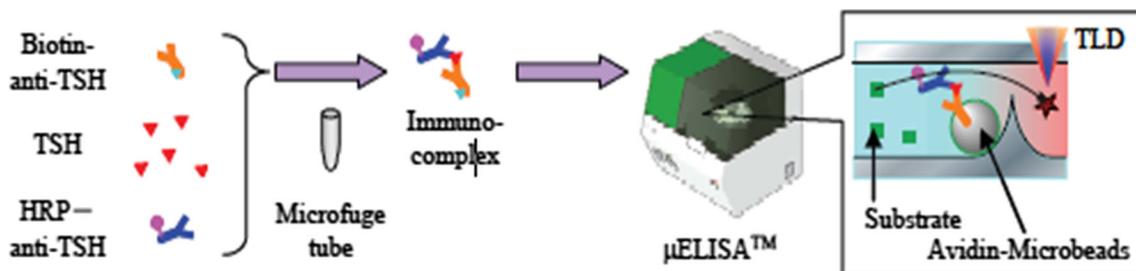
The perfusate containing the insulin is transferred into a reaction chamber by electroosmosis and mixed with labeled insulin and anti-insulin in order to perform the immunoassay. The detection was carried out by laser-induced fluorescence technique by monitoring the insulin secretion at 6.25 s intervals.

As glucose concentration is changed in the perfusing biological media, the perfusate is continuously analyzed for insulin. The new generation microfluidic devices have the capability of performing multiple assays of the secreted products such as electrophoretic analysis and fluorescence measurements.

The microfluidic device developed by Easley's group was designed for sampling of insulin simultaneously from eight individual islets in parallel. The device layout is shown in Fig. 1 (Dishinger and Kennedy 2007).

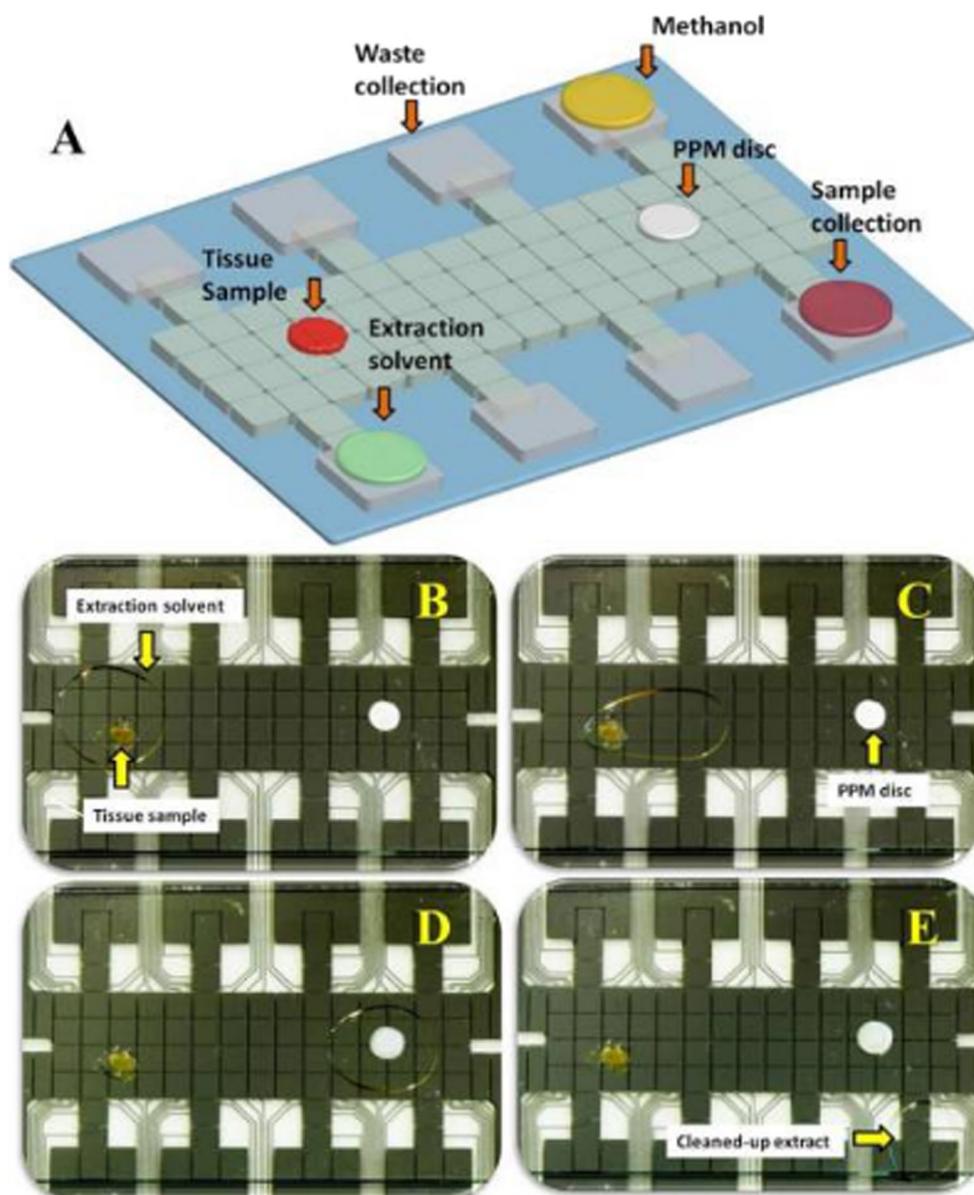
This approach, allowing the sampling over time from individual islets rather than obtaining an ensemble response, is promising for evaluating the response from each islets and gaining invaluable information on single-islet variability. The islets were scanned by confocal reflectance microscopy and their volumes were measured as seen in Fig. 2.

It is important to note that the dimension of the channels matches well with the size of the islets that are in the range of 50–300  $\mu\text{m}$ , making microfluidics a versatile tool for sampling and studying the complex kinetics of insulin secretion. The insulin secretion over one hour was analyzed by using enzyme-linked immunosorbent assay (ELISA). The device is easy to use and secretion sampling is done using a hand-



**Fig. 3** Schematic of the sandwich immunoassay used for detecting TSH. Reproduced with permission from Ohashi et al. (2010), Copyright 2010

**Fig. 4** Digital microfluidic extraction/cleanup device from tissue samples. Schematic representation of the device (a), and frames from a movie describing extraction from tissue sample (b, c) and cleanup on a porous polymer (PPM) disc for solid-phase extraction (SPE) (d, e) (Kim et al. 2015)



held syringe. The authors found only a limited correlation between the volume of the islets and the amount of the insulin secretion and suggested that the cellular architecture may be relevant. The assessment of dynamic insulin secretion by using microfluidic islet perfusion devices has also been reported in ref (Adewola et al. 2010; Mohammed et al. 2009).

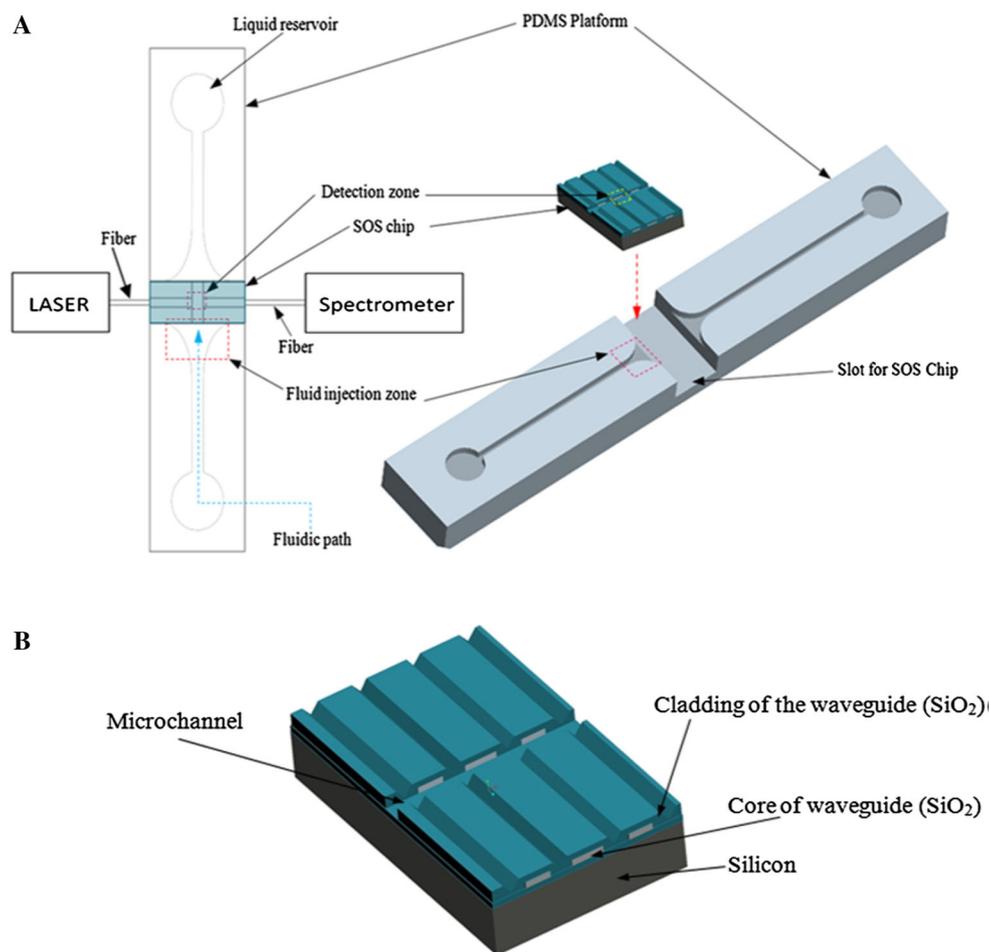
### Thyroid hormone detection

The thyroid-stimulating hormone (TSH) is a significant marker for the diagnosis of thyroid dysfunctions such as hyperthyroidism or hypothyroidism.

The miniaturization of the well-known enzyme-linked immunosorbent assay (ELISA) by trapping microbeads in

a microfluidic device, managed to reduce considerably the required volumes of reagents as well as the time of the assay to only 15 min (Ohashi et al. 2010) (Fig. 3). The principle of the micro-ELISA assay developed by the authors and the portable unit integrating all the components (micro-autosampler, syringe pump, micro-valves and thermal lens detector) is shown in Fig. 3.

The method makes use of a sandwich assay, with the anti-TSH linked to HRP (horseradish peroxide) and biotin-labeled anti-TSH while the analyte is the antigen, that is, the thyroid-stimulating hormone (TSH). The method proved to be highly sensitive ( $0.1\text{--}10\ \mu\text{IU mL}^{-1}$ ) and the portable system can be used as a point-of-care clinical diagnostic tool.



**Fig. 5** Silica-on-silicon-PDMS lab-on-a-chip used for the fluorescence detection of rbST. Figure reproduced from Ozhikandathil and Packirisamy (2012). **a** Schematic of microfluidic chip fabricated in

PDMS material integrated with optical waveguides, **b** the enlarged view of optical waveguides for clarity

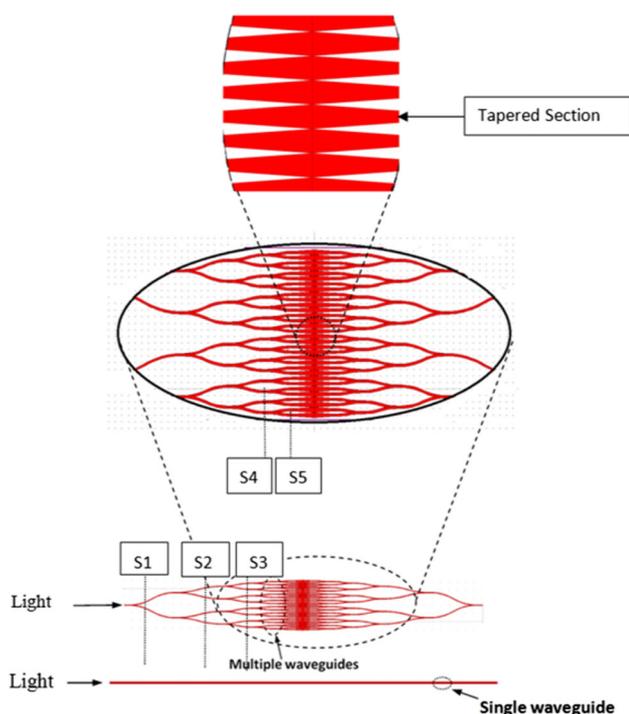
Recently, a new method, for the detection of the thyroid-stimulating hormone (TSH), based on electrical impedance measurements, was reported (Tashtoush 2014). This method, which is a label-free impedimetric immunoassay, has shown a high sensitivity and selectivity towards thyroxine and triiodothyronine secreted by the thyroid stimulated by the TSH. The results demonstrated the advantages of electrochemical methods for the detection of thyroid hormones. Magnetic particle labels have also been used to detect parathyroid hormone by a sensitive and rapid immunoassay (Dittmer et al. 2008).

In this work, superparamagnetic particle labels were used, combined with actuating electromagnets below and above the sensor chip. The antibody of the parathyroid hormone is coupled directly to the magnetic particle (1-step assay format) allowing the capture of the parathyroid hormone molecules from the solution.

### Detection of steroids in core needle biopsies

Kim et al. (2015) reported recently a digital microfluidic technique for the quantification of steroid hormones in core needle biopsy samples (in tissues) as an alternative to surgical biopsy. The microfluidic sample processing is performed by a custom HPLC MS/MS (high performance liquid chromatography, mass spectroscopy) method allowing the simultaneous quantification of estradiol (E2), androstene-dione (AD), testosterone (TS), and progesterone (PG). The microfluidic extraction of the sample is shown in Fig. 4.

Milligram-size tissue sample is enough for the microfluidic extraction. The authors suggest the use of this method for personalized approaches to diagnosing hormone-sensitive cancers.



**Fig. 6** Cascaded waveguide coupler for the detection of rbST (Ozhikandathil and Packirisamy 2013)

### Detection of bovine growth hormone

Detection of growth hormone is demonstrated with optical lab-on-chip devices (Ozhikandathil 2012). Two different types of approaches are used for the detection of recombinant and nature form of bovine growth hormone, namely fluorescence labeled (Ozhikandathil et al. 2012a) and label-free (Ozhikandathil et al. 2012b). Both the labeled and label-free techniques have been used in the microfluidic devices and hence the detection growth hormone is achieved with greater sensitivity as low as few ng/ml.

The recombinant growth hormone was labeled with Alexa-647 and Fluorescein isothiocyanate (FITC) and the performance of a hybrid-integrated lab-on-a-chip is evaluated for the fluorescence detection (Ozhikandathil and Packirisamy 2010). The LOC was fabricated by integrating a silica-on-silicon (SOS) waveguide with polydimethylsiloxane (PDMS) microfluidic chip. The fabrication detail of the SOS-PDMS LOC is explained in reference (Ozhikandathil and Packirisamy 2010, 2012). The excitation of the tagged-rbST was carried out by exciting the tagged-spices through the top window of the microchip. The detection limit of the LOC was investigated and found that the sensor is capable of detecting rbST as low as 240 ng/ml. Figure 5 shows the integration process of SOS-PDMS lab-on-a-chip.

A monolithically integrated sensor platform using a cascaded waveguide coupler (CWC) is used for an

enhanced detection of recombinant growth hormone (Ozhikandathil and Packirisamy 2013) as shown in Fig. 6. In the cascaded waveguide coupler configuration helped to increase surface area to bind the antibody with an enhanced penetration depth of evanescent wave in order to excite the tagged-rbST. The sensor is demonstrated for the detection of fluorescently tagged recombinant growth hormone with the detection limit as low as 25 ng/ml.

An LOC was integrated with gold nanoislands formed on the glass substrate. Device was integrated with the PDMS microfluidic chip, and sensing is carried out by injecting the rbST solution to the microfluidic chip. The chip was placed in a spectrophotometer and the localized plasmon resonance spectrum of the gold nano-islands was measured. The shift of the plasmon response is used to detect the growth hormone. The device showed great potential for screening of rbST with sensitivity as low as 5 ng/ml (Ozhikandathil et al. 2012b). Figure 7 is the schematic of the LOC used for the detection of bST. The LOC was integrated with gold-PDMS nanocomposite also with the detection limit in the range of 3 ng/ml (SadAbadi et al. 2013).

In a recent paper, a portable device is report for the detection of rbST in milk. Herein the milk was spiked with rbST and detection was carried out in a microfluidic device with the help of a portable device (Ozhikandathil et al. 2014).

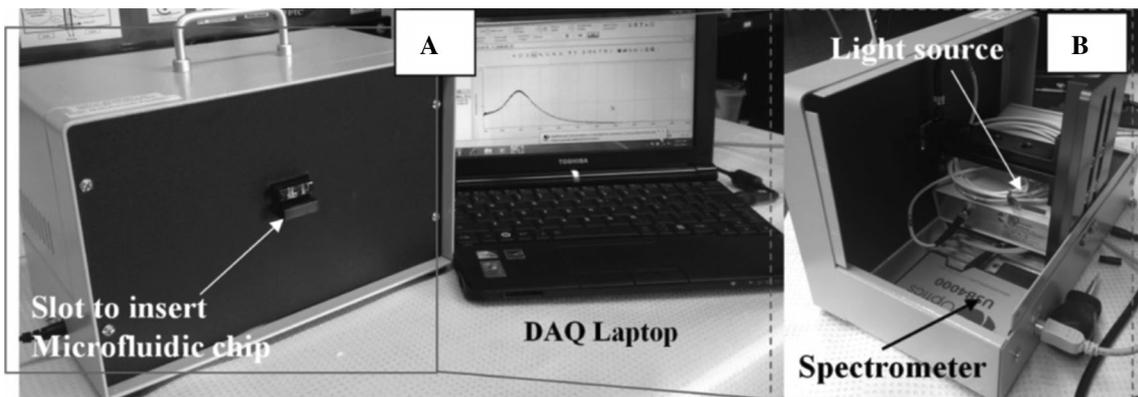
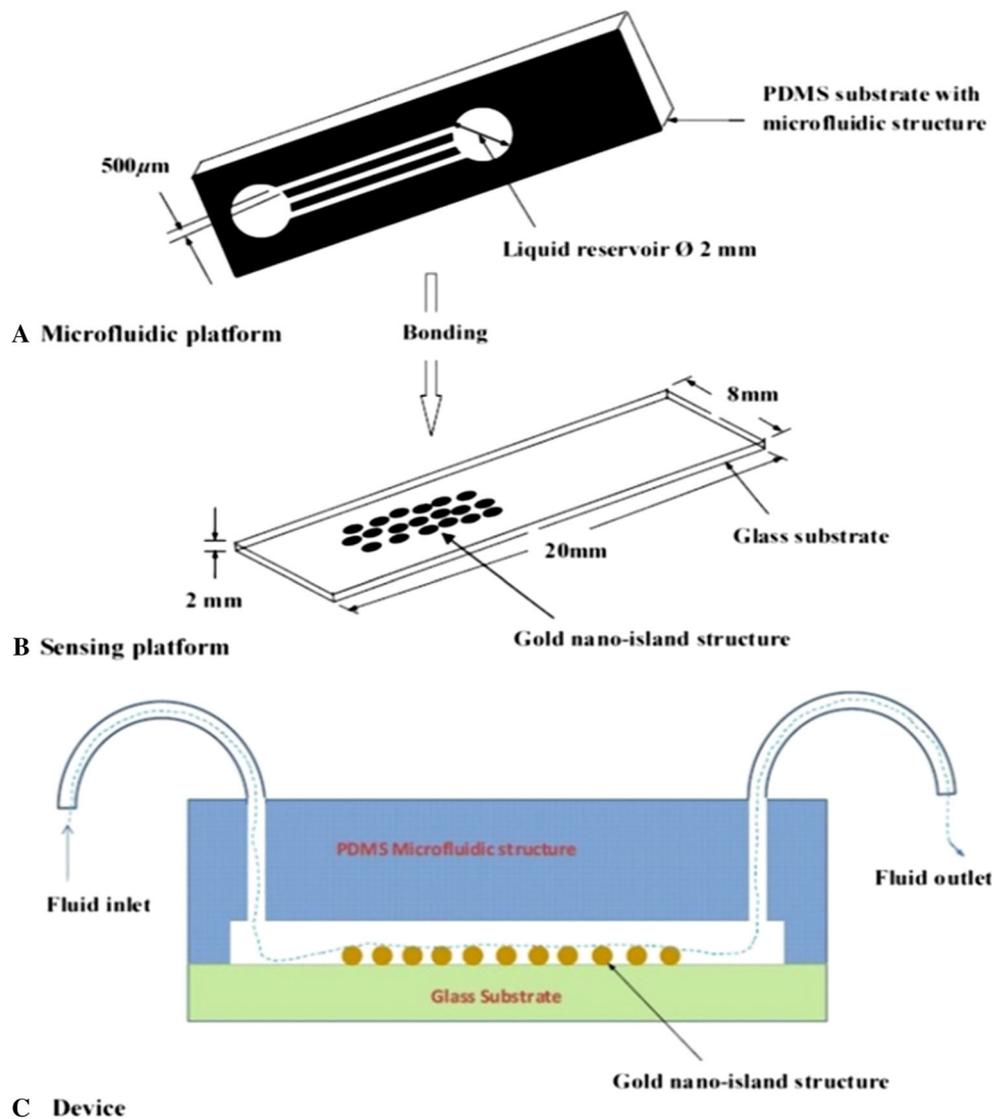
A monolithically integrated lab-on-a-chip was fabricated on a silica-on-silicon platform for the detection of fluorescence detection of rbST (Ozhikandathil and Packirisamy 2014) (Fig. 8). The chip includes multiple waveguides and microfluidic channel on the SOS waveguides, and the integration of LOC is carried out in single step lithography and etching and a simplified fabrication approach is explained. The rbST was tagged with Alexa-647 and FITC and detection is demonstrated in the LOC. Figure 9 shows the monolithically integrated LOC.

Figure 10 shows sensitivity comparison chart for various lab-on-a-chip detection methods reviewed in this paper for the detection of bovine growth hormone. The label-free method, that is the LSPR integrated LOC gives the detection limit in the rage of 1–5 ng/ml.

### Conclusion

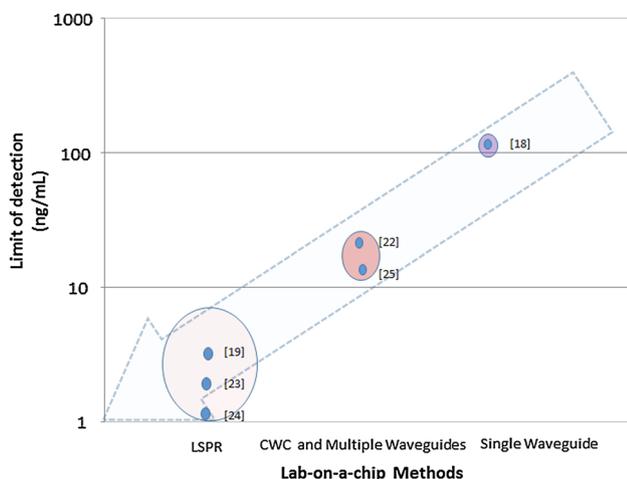
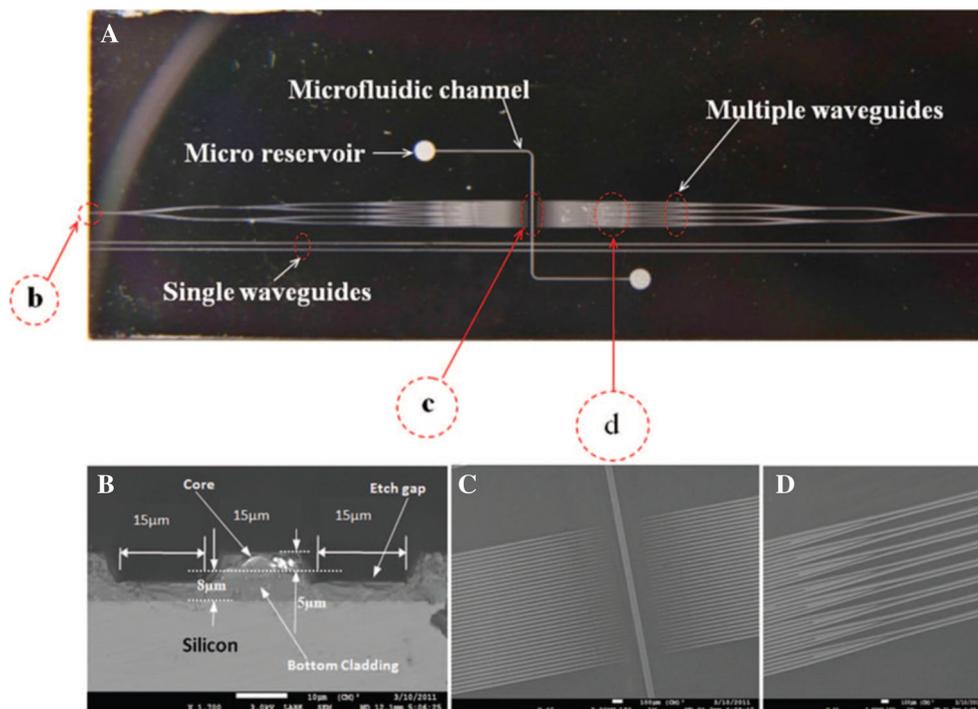
Application of microfluidics for hormone study and detection is an active multidisciplinary field. This paper reviews some the recent developments in the area of lab-on-a-chip devices for the detection of hormones. The comparison of the various lab-on-a-chips devices, integration and detection methods are reviewed. A comprehensive overview of the fluorophore-labeled and label-free

**Fig. 7** Gold nano-islands integrated LOC used for the detection of growth hormone. Figure reproduced with permission from Ozhikandathil et al. (2012b)



**Fig. 8** LOC with portable device for the detection of rbST in milk. Figure reproduced with permission from Ozhikandathil et al. (2014)

**Fig. 9** Monolithically integrated optical LOC for the detection of rbST (Ozhikandathil and Packirisamy 2014)



**Fig. 10** Sensitivity of various LOC detection methods to bovine growth hormone

detection methods of bovine growth hormone using lab-on-a-chip carried out in authors' laboratory is presented. As it can be seen, microfluidics with its significant advantages of low sample and reagent volumes and possibility of multiplexing creates new opportunities for detecting hormones in the microenvironment of individual cells, with an excellent spatial and temporal resolution. In spite of the progress achieved in this particular field, a great deal of work has to be done by biologists and people working in microfabrication, before the field will take full advantage of the advanced capabilities of microfluidics.

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