DOI 10.5162/EUROSENSORS2025/T6.3.2

DNA-scaffold biosensors for real-time pH tracking in dynamic environments

Gianni Antonelli[1], *Alejandro Chamorro-Garcia*[2], *Paola Spitalieri*[3], *Arianna Mencattini*[1], *Giorgia Curci*[1], *Joanna Filippi*[1], *Alessia Riccardi*[1], *Paola Casti*[1], *Erica Debbi*[1], *Michele D'Orazio*[1], *Federica Sangiuolo*[3], *Andrea Idili*[2], *Eugenio Martinelli*[1]

[1] Department of Electronic Engineering, University of Rome Tor Vergata, Via del Politecnico 1, 00133 Rome Italy
[2] Department of Chemical Sciences and Technologies, University of Rome Tor Vergata, Via della Ricerca Scientifica, Rome 00133, Italy
[3] Department of Biomedicine and Prevention, University of Rome Tor Vergata, Via Montpellier 1, Rome, 00133, Italy
Corresponding Author's e-mail address: g.antonelli@ing.uniroma2.it

Summary:

Lab-on-chips have transformed the approach to studying biological models for drug discovery. However, some information could be misinterpreted without knowing the chemical information near the biological entity. In this work, we exploited DNA scaffolds to realise fluorescent hydrogel pH microsensors that can be easily integrated into lab-on-chips. A machine learning model was trained to read the biosensors' response and predict pH in a dynamic microfluidic environment. The proposed system is highly biocompatible and can be used in many biological applications, such as monitoring organoids' metabolism.

Keywords: DNA scaffold sensors, real-time monitoring, pH sensing, lab-on-chip, machine learning

Background, Motivation, and Objective

In recent years, biological research has advanced significantly, driven by breakthroughs in bioengineering and microfabrication [1]. Organoids and lab-on-chip technologies have enabled researchers to investigate biological mechanisms at unprecedented levels, accurately replicating tissue and organ functions within microscale environments. However, integrating real-time monitoring with high spatiotemporal resolution into these platforms remains a considerable challenge, limiting the ability to capture the intricate dynamics of biological activities, which are fundamental to uncovering new insights into human physiology [2].

Optical sensors play a pivotal role in detecting subtle biological dynamics by translating them into variations in optical properties, offering a powerful approach for real-time analysis near living systems [3]. The data collected from optical biosensors is typically embedded in complex signals (such as spectra or images) and often requires advanced processing to extract meaningful information [4].

This work presents an integrated platform that leverages DNA scaffolding technology to develop hydrogel fluorescent biosensors, combined with computer vision and machine learning, for real-time detection of the target compound. The sensors have been integrated into a lab-on-chip using a custom stereolithographic technique, and a machine learning algorithm was designed to analyse the hydrogel images. To further enhance sensor performance, the system was calibrated for pH monitoring using a ratiometric approach, enabling real-time measurements [5].

We validated this platform by monitoring the pH changes in a microfluidic system. We dynamically imposed the pH using a syringe pump and different buffer solutions, recording real-time fluorescent images while predicting the local pH with the machine learning algorithm. We believe this system can be easily integrated to monitor biological dynamic applications, particularly organoids.

Methods

Lab-on-chips were fabricated using laser ablation of Poly Methyl MethAcrylate and Cyclic Olefin Polymer slabs, making them adhere using adhesives and a hydraulic press.

After that, the biosensors prepolymer was prepared by mixing Poly Ethylene Glycol DiAcrylate, LAP photoinitiator and the DNA scaffold sensing molecule. The solution was introduced in the lab-on-chip chamber and crosslinked using a custom stereolithographic setup. This optical system uses a commercial projector to permit UV light patterning in the lab-on-chip. After rinsing in distilled water, the biosensors were ready for use (see Fig. 1).

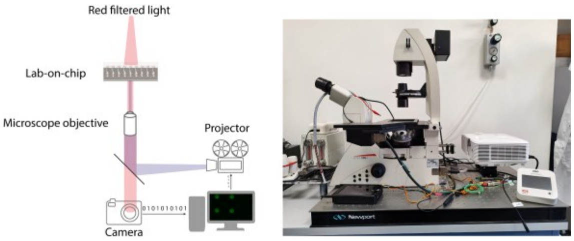

Fig.1: A scheme and a picture of the stereolithographic optical setup used to grow hydrogel biosensors.

Subsequently, fluorescence microscopy was used to capture images of the biosensors at different pH levels. This image dataset was then processed and utilised to train a custom machine-learning algorithm.

After the training, the lab-on-chips were connected to a microfluidic setup to impose the flow of different buffer solutions in the chambers. In the meanwhile, the device was monitored using fluorescent time lapse microscopy.

Results

The proposed system was calibrated using a series of buffer solutions, enabling the integrated machine-learning algorithm to estimate pH values based on fluorescence images. The platform demonstrated a rapid response time, making it suitable for integration into dynamic microfluidic environments.

To assess its performance under changing conditions, we used a syringe pump connected to a microfluidic setup to modulate the pH in real time while continuously acquiring fluorescence images of the hydrogel-based biosensors. This approach allowed for continuous monitoring and estimation of local pH within the microfluidic chamber throughout the whole experiment. An example of pH estimation under dynamic conditions is shown in Figure 2, where changes in pH were precisely tracked within the microfluidic system.

As an additional validation step, the Lab-on-Chip device was tested using embryoid bodies, one of several possible biological models such as organoids or microtissues, that can be monitored during experiments. In this case, we observed that biosensors positioned closer to the biological structures exhibited greater sensitivity, detecting localised decreases in pH associated with cellular metabolism (detailed data will be shown at the conference).

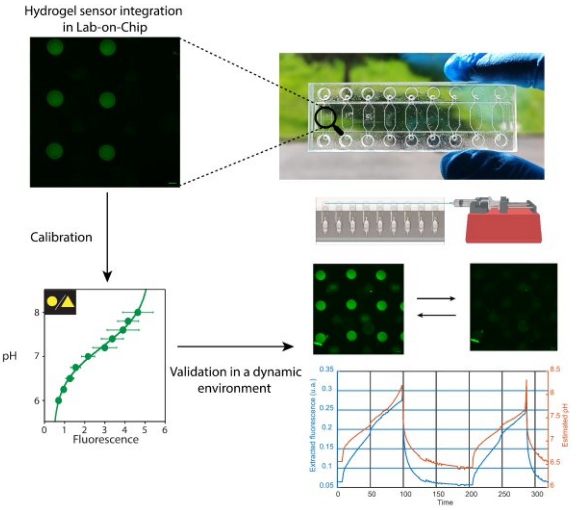

Fig 2: pH hydrogel biosensors are integrated into Lab-on-Chip, and a machine learning algorithm is calibrated to predict pH. This platform is then validated in different dynamic environments.

Ultimately, the system provides a straightforward and efficient method for real-time pH measurement in Lab-on-Chip environments. Furthermore, the platform can be extended with appropriate modifications to accommodate multiple hydrogel-based sensors, allowing simultaneous detection of various analytes within a single experiment.

References

[1] S. E. Park, et al., “Organoids-on-a-chip”, *Science* 634, 960-965 (2019); doi: 10.1126/science.aaw7894

[2] E. Gayibov et al., “The use of patient-derived xenografts and patient-derived organoids in the search for new therapeutic regimens for pancreatic carcinoma. A review”, *Biomedicine & Pharmacotherapy* 182 (2025), 182, 117750; doi: 10.1016/j.biopha.2024.117750.

[3] Z. Liao et al., “Microfluidic chip coupled with optical biosensors for simultaneous detection of multiple analytes: A review”, *Biosensors and Bioelectronics* 126 (2019), 697-706, doi: 10.1016/j.bios.2018.11.032

[4] G. Antonelli et al., “Integrating machine learning and biosensors in microfluidic devices: A review”, *Biosensors and Bioelectronics* 263 (2024), 116632, doi: 10.1016/j.bios.2024.116632

[5] G. Antonelli et al., “Continuous pH Monitoring in Organ-on-Chip devices with Single-step Fabricated DNA Scaffold Sensors Conjugated Hydrogels and Machine learning”, *Submitted*