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High precision pH measurements in biological environments using a portable optical fibre pH sensor

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ABSTRACT

We have demonstrated that an optical fibre-based pH sensor can be utilised to accurately assess pH in a biological environment. Initial measurements were performed on 5 µL drops of culture medium containing individual female mouse reproductive cells (cumulus-oocyte-complexes, COCs), with the goal of obtaining a biomarker of individual cell health during assisted reproductive processes. Improvements to the measurement procedure were found to reduce fluorescence signal variability, enabling improved measurement precision compared to previous studies. Results show the application of treatments which serve to increase lactic acid production by the COC, and thus induce an acidification of the local microenvironment, are detectable by the pH sensor. This optical technology presents a promising platform for the measurement of pH and the detection of other extracellular biomarkers to assess cell health during assisted reproduction.

Keywords: pH, fluorescence, sensing, optical fibre, cumulus-oocyte-complex (COC), microenvironment, lactic acid

INTRODUCTION

Optical fibre-based fluorescent probes have found use in a range of applications, where the fibre geometry (~100-200 µm in diameter) provides access to microvolumes of analyte for non-invasive chemical detection in spatially-hindered biological environments[1]. We recently reported a pH-sensitive optical fibre probe, referred to here as optical fibre probe 1 (**OFP1**), and its measurement of extracellular surface pH in excised human breast cancer tissue samples[2]. The functionalised surface of this probe contains the pH sensitive fluorophore 5(-6)-carboxynapthofluorescein (CNF) embedded within an acrylamide polymer coating on the tip of a 200 µm diameter multimodal optical fibre[3]. **OFP1** successfully detected differences in extracellular acidity, which enabled the margins between healthy and cancerous breast tissue to be discerned.

One promising application for fibre probes is in biosensing, where the local environment makes measurement with conventional pH probes extremely challenging, such as in assisted reproductive technologies, where the limited size of the culture medium drop presents an ideal opportunity to employ **OFP1**. Here we present a new application for **OFP1** measuring the pH in 5 µL drops of culture medium containing unfertilised eggs (oocytes) with their surrounding support cells (the cumulus), which is collectively known as the cumulus-oocyte-complex (COC). Treatments applied to the COC and known to result in the production of lactic acid should decrease the pH of the local COC microenvironment[4-6].

RESULTS AND DISCUSSION

2.1 **OFP1** Pre-bleaching

Initial repeated scans 5 s apart of freshly prepared **OFP1** probes revealed that the CNF response signal exponentially decays over time (Figure 1a), suggesting that a potential method to reduce the impact of photobleaching is to "pre bleach" the probes with 800-100 scans prior to performing biological measurements. After this pre-bleaching process, the decay during experimental measurements is of a smaller magnitude (see Figure 1b); therefore, any observed changes in fluorescence signal after this point may be attributed to meaningful pH changes in the medium being measured.

2.2 *In vitro* Measurements

OFP1 was used to assess the local pH immediately adjacent to the COC, with the probe positioned as shown in Fig. 1c. Trials measured culture medium pH following chemical ($CoCl_2$) or hormonal (FSH) treatment of the cells. COCs treated with 100 µM $CoCl_2$ (chemical treatment) showed a statistically lower calculated pH than untreated COCs, with **OFP1** measuring a clearly distinguishable 0.06 unit drop in pH after treatment.

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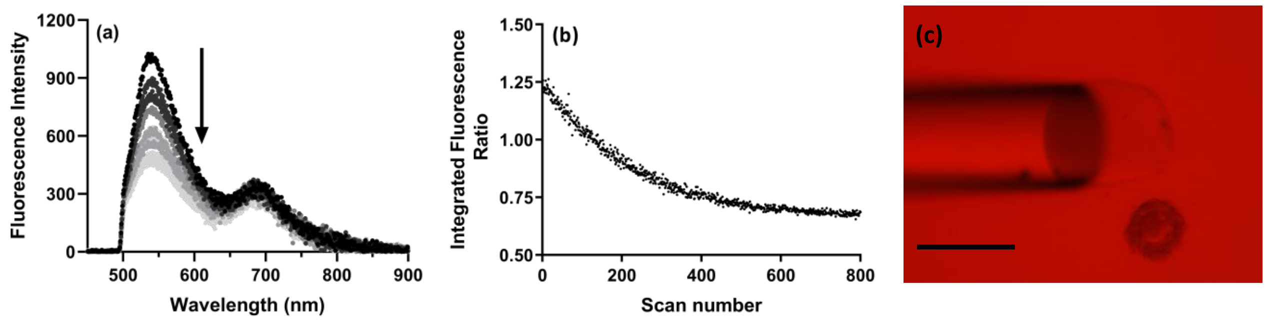

Figure 1. a) Reduction in overall fluorescence signal and b) fluorescence signal ratio change during the CNF pre-bleaching process. c) Position of **OFP1** relative to a COC during measurement. Scale bar represents 200 µm.

Hormonal stimulation of COCs was next explored in order to investigate whether a biological event can trigger a measurable change in pH like the one caused by $CoCl_2$ exposure. In all replicates, FSH stimulation resulted in 0.02-0.03 mean pH unit decrease compared to untreated COCs. As FSH increases lactic acid production by increasing the metabolic activity of cumulus cells[4], the difference detected here by **OFP1** is a direct result of a biological event.

This work presents the first instance of local pH being used as an indirect measure of individual COC metabolism *in vitro*, and confirms that **OFP1** is a suitable technology to detect biological signals in the microenvironment surrounding the COC.

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