Biosensors and Bioelectronics 26 (2011) 2890–2896

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

ATP microelectrode biosensor for stable long-term *in vitro* monitoring from gastrointestinal tissue

Bhavik Anil Patel[a,b,*], Michelle Rogers[a], Talia Wieder[a], Danny O'Hare[a], Martyn G. Boutelle[a]

[a] Department of Bioengineering, Imperial College London, London SW7 2AZ, UK
[b] Centre for Biomedical and Health Sciences Research, University of Brighton, Brighton BN2 4GJ, UK

ARTICLE INFO

Article history:
Received 14 August 2010
Received in revised form
22 November 2010
Accepted 23 November 2010
Available online 1 December 2010

Keywords:
Adenosine tri-phosphate
Serotonin
Ileum
Colon
Hexokinase
Biosensor

ABSTRACT

We have developed a stable and selective ATP biosensor for long-term *in vitro* tissue monitoring. The electrode was fabricated by entrapping glucose oxidase (GOx) and hexokinase (HEX) in a poly-phenol film on a Pt microelectrode. The biosensor was stable to a fixed concentration of glucose for over 20 min and had a limit of detection of 9.9 ± 3.2 nM, with a sensitivity of 45.8 ± 1.22 pA μM^{-1}. Most significantly of all, the response on the ATP biosensor did not alter in the presence of 1 mM ascorbic acid, 5 μM dopamine, 5 μM serotonin, 5 μM ADP and 5 μM AMP. The ATP biosensor was also shown to have excellent stability over 7 days, and showed only a 23.92 ± 3.55% loss in sensitivity. The ATP biosensor was utilised for the *in vitro* detection of ATP from gastrointestinal tissue. The ATP biosensor response was stable for 5 h during *in vitro* recordings from ileum tissue. ATP release was shown to be greater from the mucosal surface in the ileum compared to the colon.

1. Introduction

Adenosine triphosphate (ATP) is an important biological molecule with a variety of biological functions. It is well known to play a key role in metabolism, but ATP also influences vascular tone (Burnstock, 2008a; Ralevic, 2009) and functions as a neurotransmitter (Evans et al., 1992). In addition, ATP has an important function within the immune system where, again, it is active as a signalling molecule in the cascade which controls clotting (Bours et al., 2006). ATP is thought to have a significant influence in gastrointestinal motility by regulating serotonin (5-HT) release (Burnstock, 2008b; Christofi et al., 2004; Cooke et al., 2003). ATP is also known to be released from glial cells and may mediate signalling of neurons (Fields and Burnstock, 2006; Abbracchio et al., 2009). There is a clear need for highly stable and selective measurements of ATP that have good temporal and spatial resolution for measurements in complex biological matrixes.

There are various techniques that can provide direct measurements of ATP at physiological concentrations. These include liquid chromatography (Manfredi et al., 2002; Sudo et al., 2000; Deng et al., 2003), fluorescence (Foy and Pacey, 1996; Hou et al., 2005; Zhang et al., 2009), chemiluminescence (Wang et al., 2005; Pérez-Ruiz et al., 2003), bioluminescence (Qiu et al., 2009; Kamidate et al., 2005) and amperometric techniques (Kueng et al., 2004; Llaudet et al., 2005; Masson et al., 2008; Soldatkin et al., 2009). Of all these methods, only microelectrode sensors using constant-potential amperometry have the adequate spatial and temporal resolution and robustness for *in vitro* or *in vivo* measurements.

As ATP is not electroactive, amperometric detection of ATP is carried out using enzymatic reactions on the electrode surface. Two such coupled enzyme schemes are glycerol kinase with glycerol-3-phosphate (Llaudet et al., 2005) and GOx with HEX. In this scheme, ATP is sensed by HEX which then competes with GOx for supplied glucose (Scheller and Pfeiffer, 1980; Kueng et al., 2004; Soldatkin et al., 2009). These enzymes are used to sense ATP, and generate hydrogen peroxide, which is detected at the electrode. The current generated this way can be directly related to the ATP concentration present in the sample as long as the concentration of glycerol/glucose is known.

Many amperometric ATP sensors have been developed, but limited information is known about how these electrodes respond in the presence of key biological interferences. Some single electrode ATP biosensors developed have shown to be selective against ADP (Katsu and Yamanaka, 1993; Soldatkin et al., 2009), but they have shown to be prone to biological interferences (Soldatkin et al., 2009). In some they have overcome this issue by utilising a 'null' electrode to remove interferences (Llaudet et al., 2005; Masson et al., 2008), although this a good strategy the size of the inter-

* Corresponding author at: Centre for Biomedical and Health Sciences Research, University of Brighton, Huxley Building, Brighton BN2 4GJ, UK.
Tel.: +44 0 1273 642148; fax: +44 0 1273 679333.
E-mail address: B.A.Patel@brighton.ac.uk (B.A. Patel).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.11.033

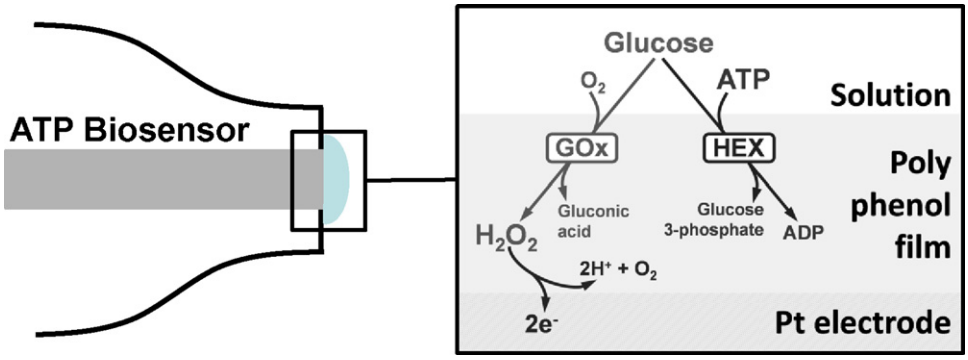

Fig. 1. Schematic diagram showing the operation of the ATP biosensor. The ATP electrode is fabricated using a combination of HEX and GOx entrapped in a poly-phenol film.

ference signal must be small compared to the signal measured for the analyte of interest, and the stability of both the sensor and 'null' electrode must be good during biological recordings. The stability of current electrodes that have excellent biological characteristic is also varied. ATP biosensors vary, with devices shown to lose 70% response after 1 week (Compagnone and Guilbault, 1997) to biosensors loosing 35–60% after 2–3 weeks (Kueng et al., 2004; Liu and Sun, 2007). However no studies using ATP biosensors have looked into the stability of ATP electrodes for long-term biological monitoring.

We have developed a new selective ATP biosensor capable of long-term biological monitoring. The ATP biosensor was composed of GOx and HEX trapped in an electropolymerised poly-phenol film (Fig. 1). The ATP sensor was assessed and characterised to study its sensitivity, selectivity and reproducibility and stability. Following characterisation of the ATP biosensor, the device was assessed for long-term *in vitro* measurement of ATP from ileum and colon tissue.

2. Materials and methods

2.1. Materials and reagents

All experiments were carried out in oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4 (composition: 117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose). For preparation of the biosensor: phenol, glucose oxidase (GOx, 100 U/mg) and hexokinase (HEX, 250 U/mg) were purchased from Sigma–Aldrich. 5-Hydroxytryptamine (5-HT), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), ascorbic acid (AA) and dopamine (DA) were purchased from Sigma–Aldrich and solutions were prepared fresh each day.

2.2. ATP biosensor fabrication

To make the biosensor, a 50 μm Teflon insulated platinum wire (A-M Systems Inc., USA) was threaded through a 27G hypodermic needle. Epoxy resin (Robnor resins, CY1301 & HY1300) was used to fill the internal volume of the needle to secure the wires in place. Once the epoxy had cured, the sharp end of the needle was cut in a 45° angle using a diamond saw (Buehler) to expose the platinum microelectrodes. Alumina slurries (1-, 0.3-, and 0.05 μm) were used sequentially to polish the electrode surface. A schematic diagram of the fabricated ATP biosensor is shown in Fig. 1. The Pt needle electrode was placed in a solution of PBS buffer pH 7.2 containing 50 mM phenol, 40 mg/ml HEX and 10 mg/ml GOx. The pH of the solution was maintained at pH 7.2. The microelectrode along with a stainless steel wire and "no leak" Ag|AgCl (3 M KCl, model EE009, Cypress Systems Inc., USA) was placed in the enzyme solution for 10 min stabilisation time. The electrode was held at 0 V for 20 s, polarised to +0.9 V for 10 min for electropolymerisation of the phenol film and then held at 0 V for 20 s. Following electropolymerisation, the fabricated ATP biosensor was rinsed with deionised water and stored overnight at 4 °C in PBS buffer before use.

2.3. Sensor characterisation studies

The ATP biosensor was assessed in an electrochemical cell with a Ag|AgCl reference and Pt wire auxiliary electrode. Experiments were carried out using a CHI 1030 potentiostat. All measurements were performed at +750 mV vs. Ag|AgCl in Krebs buffer (pH 7.4). For calibrations, aliquots of substrate were added under stirred conditions. Experiments were carried out over 7 days to study the stability of the enzymes in response to varying concentrations of ATP and glucose.

For selectivity measurements, flow injection analysis (FIA) was carried out. An in-house flow cell was produced, using silicone elastomer, as described previously (Anastassiou et al., 2006). The flow cell was connected to a quaternary HPLC pump (HP1050, Aligent), where the flow rate was set at 1 ml min^{-1}. Pump A was used to maintain a constant flow of Krebs buffer pH 7.4 containing 11 mM glucose. The cell was switched to pump B, which contained various interferents, for the duration of 20 s. The responses of an unmodified Pt, ATP and SENT electrode were assessed for selectivity in the presence of 1 mM ascorbic acid, 10 μM serotonin, 10 μM dopamine, 5 μM AMP and 5 μM ADP.

For some studies of selectivity, a sentinel (SENT) or 'null' electrode was used, as others have shown this to be an effective means of achieving selectivity (Llaudet et al., 2005; Masson et al., 2008). The SENT electrode was fabricated in a similar fashion as the ATP biosensor; however the active enzymes were replaced with denatured GOx. We chose thermal denaturing of GOx as this provides a more compact structure than chemical denaturing methods (Zoldák et al., 2004). 200 mg/ml of GOx was heated to 70 °C for 5 min, before being left to cool to ambient temperature. Following this a solution containing 50 mg/ml denatured GOx and 50 mM phenol was prepared in 0.1 M phosphate buffered saline (PBS) pH 7.2 and used to make the SENT electrode.

2.4. Biological tissue preparation

All animal experiments were carried out in compliance with the relevant laws (UK Home Office) and institution guidelines. Male guinea-pigs weighing 300–400 g were euthanized using CO_2 gas. A segment of ileum was removed 15–20 cm proximal to the ileocecal junction and placed in oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4. A 1 cm long segment of ileum was then transferred to a flow bath containing the same oxygenated Krebs'

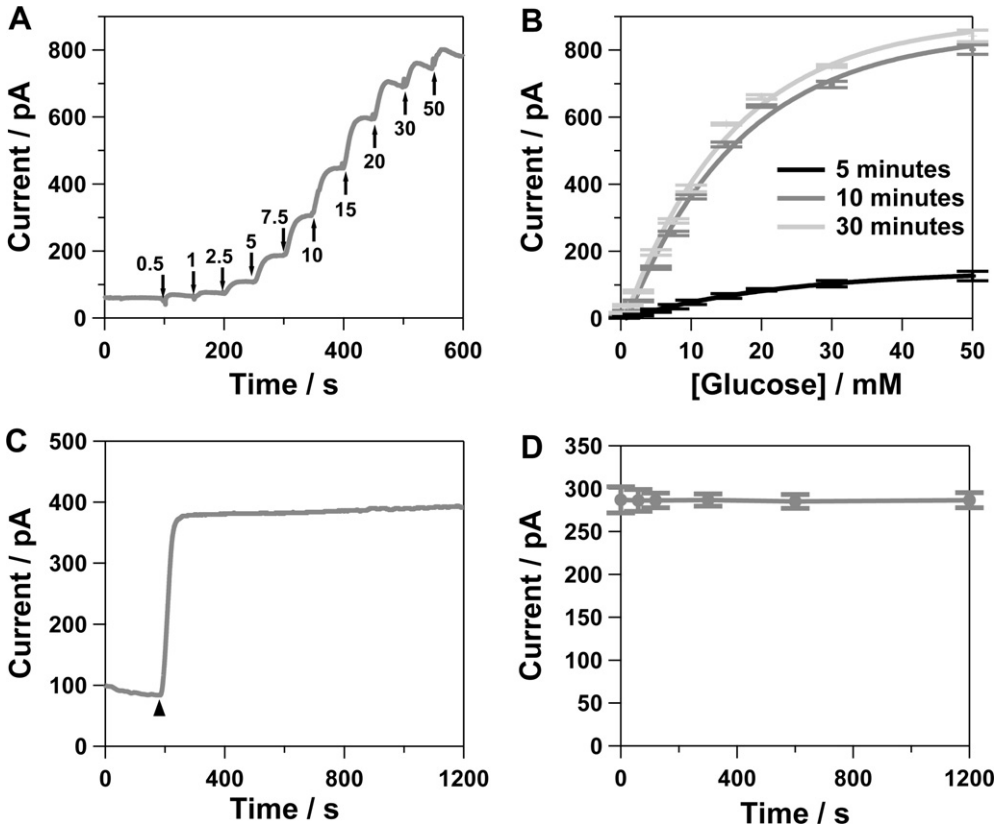

Fig. 2. Investigation of the performance of the GOx component of the ATP biosensor. A typical glucose calibration response on the ATP biosensor is shown in (A), where concentrations are shown in mM. In (B) the influence of stabilisation prior to electropolymerisation of the film coating on the glucose calibration is shown, where responses are shown as mean ± SD and $n = 6$. In (C) a typical experimental response on the ATP biosensor when exposed to 11 mM glucose is shown. The overall drift between 10 sensors is shown in (D), where responses are shown as mean ± SD and $n = 10$ for 11 mM glucose. All experiments are carried out in an amperometric mode at +750 mV vs. Ag|AgCl in Krebs buffer pH 7.4.

solution. The segment of ileum was then cut open along the mesenteric border, lightly stretched and pinned flat in the flow bath using stainless steel pins (50 μm diameter). The mucosal surface was face-up at the bottom of the chamber. This tissue was constantly perfused with warm (~37 °C) oxygenated Krebs' buffer. The tissue was exposed to the flowing solution for approximately 30 min prior to commencing a series of measurements.

2.5. In vitro tissue measurements

For continuous amperometric *in vitro* recordings from ileum and colon tissue; a three electrode system was also used. A Pt wire counter and a Ag|AgCl reference electrode were also mounted in the bath with the ATP biosensor. Amperometric monitoring was controlled using a Biostat potentiostat (ESA Biosciences). The ATP biosensor was poised at +750 mV vs. Ag|AgCl. The flow bath was mounted on the stage of an inverted microscope (Model 3030, Accu-Scope, USA) and perfused continuously with warm (37 °C) Krebs buffer solution at a flow rate of 2 ml min^{-1}. The ATP biosensor was mounted on one micromanipulator (Model 25033, Fine Scientific Tools, USA) for reproducible placement near the mucosa. For *in vitro* measurements, the electrode was placed within the bulk of the perfusing media (at least 1 cm from the tissue) to establish a stable baseline response on both electrodes. During recordings, the ATP biosensor was carefully moved to 0.5 mm over the tissue for 60 s, before being retracted to the bulk media. This measurement was made over 4 or 5 regions of each tissue section. For measurements of long term stability, this process was conducted over a period of 5 h.

2.6. Data analysis

The ATP signal was analysed as the current difference between the 11 mM glucose response and the ATP response obtained from the ATP biosensor. The *in vitro* calibration for ATP was used to convert the measured currents into ATP concentrations. Results were expressed as the mean current change from the baseline.

3. Results and discussion

3.1. Stability in response to glucose concentrations

Since the detection of ATP on the biosensor is based on a competing reaction of HEX with GOx (Fig. 1), it is essential that the GOx component of the ATP biosensor provides stable and reproducible responses in order to obtain accurate responses of ATP. Therefore the GOx part of the ATP biosensor was assessed for performance. In Fig. 2A a typical calibration response of glucose on the ATP biosensor is shown.

Calibrations were obtained for sensors which had been subjected to different stabilisation times. The stabilisation time allows for solution free enzyme to adsorb to the electrode surface prior to entrapment when electropolymerising with phenol. There was an increase in the current with increasing stabilisation time from 5 to 30 min. The V_{max} (the velocity at maximal concentrations of substrate) for 10 min stabilisation time (853.56 ± 7.8 pA, $n = 6$) was significantly greater than that at 5 min (144.54 ± 30.7 pA, $p < 0.001$, $n = 6$), but there was no difference from that at 30 min (825.69 ± 19.5 pA, $p = 0.24$, $n = 6$). However the increase in the V_{max} correlated with a decrease in K_m (concentration of substrate

that leads to half-maximal velocity) with increasing stabilisation time. K_m values were 16.66 ± 1.63 mM, 12.48 ± 0.57 mM and 10.69 ± 0.18 mM for 5 10 and 30 min stabilisation time, respectively, and are much lower than expected (K_m for free GOx is 33–110 mM). This may be due to the orientation of the enzyme on the electrode surface, as not all of the generated H_2O_2 may be detected. Based on the responses from Fig. 2B, a stabilisation time of 10 min was chosen for the fabrication of ATP biosensor.

In Fig. 2C, a typical response from one electrode to 11 mM glucose is shown on the ATP biosensor. This concentration of glucose was used as it is present in Krebs buffer, which is utilised for *in vitro* biological recordings. The response relative to the background is very stable and the response from multiple ATP biosensors is shown in Fig. 2D. Ten ATP biosensors were assessed for their response to 11 mM glucose over a period of 20 min. There was no significant difference between the currents post stimulus for 20 min (one-way ANOVA), showing excellent stability. In between electrodes there was a percent relative standard deviation (%RSD) of 5.27% to the same stimulus, indicating good reproducibility in the fabrication of the ATP biosensors.

Overall the ATP biosensor showed excellent stability and reproducibility for the detection of glucose, thus making it suitable for the monitoring of ATP. There was minimal drift in the signal which is essential to assure accurate responses of ATP are obtained during *in situ* and *in vitro* measurements.

3.2. Characterisation of ATP biosensor

Following assessment of the GOx component of the ATP biosensor, the ATP biosensor was assessed. The response to the fixed additions of ATP was observed whilst in the presence of 11 mM glucose. The experimental response is observed in Fig. 3A, where responses of the ATP biosensor are shown. The ATP electrode shows a stable current when 11 mM glucose was added alone. There are systematic decreases observed on the ATP electrode when the additions between 0.25 and 2 μM ATP are carried out. Following the addition of ATP, *ca.* between 40 and 50 s is required to resolve the ATP concentration, which is better than some ATP biosensors (Compagnone and Guilbault, 1997). However this is slower than other ATP electrodes where response times in the order of milliseconds have been achieved (Kueng et al., 2004; Masson et al., 2008). However for biological areas of interest within this study the sensor is fit for purpose.

A typical calibration working curve for the ATP responses is shown in Fig. 3B, where there is a good linear relationship between 0.25 and 4 μM. The limit of detection (LOD, defined as the concentration equivalent of 3 times the standard deviation at the *y*-intercept) was 9.9 ± 3.2 nM, with a sensitivity of 45.8 ± 1.22 pA μM^{-1} ($n = 8$). This LOD is at least between 10 and 100 times greater than all other sensors developed utilising the same GOx/HEX scheme as the size of our electrode is far smaller that pervious studies (Scheller and Pfeiffer, 1980; Compagnone and Guilbault, 1997; Kueng et al., 2004; Kueng et al., 2007; Liu and Sun, 2007; Soldatkin et al., 2009) or using other approaches (Llaudet et al., 2005). Our electrode has excellent sensitivity and detection limits and the single layer greatly improves stability (*vide infra*). Such sensitivity is essential to study small fluctuations in ATP release during biological measurements.

3.3. Selectivity of the ATP biosensor

We investigated the selectivity of our ATP biosensor against expected biological interferences. Fig. 4 shows experimental traces for various biological analytes on a bare Pt electrode, the SENT or 'null' electrode and the ATP biosensor. Responses from multiple measurements are shown in Supplementary Table 1. Interferent

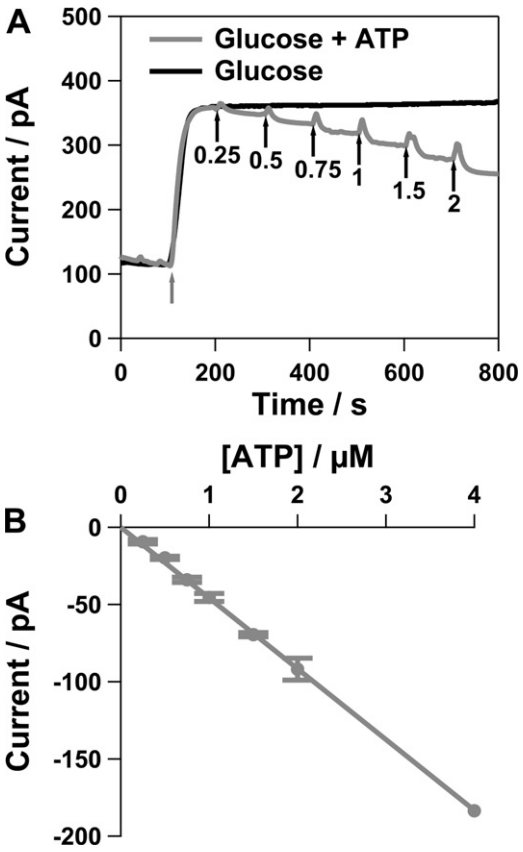

Fig. 3. Calibration response of ATP biosensor. In (A) responses are shown from ATP biosensor. All sensors were exposed to 11 mM glucose (grey arrow) and ATP concentrations in the μM were added. The difference in the current (Δ current) from the 11 mM glucose response (black trace) and the ATP response (dark grey trace) was obtained and plotted as function of ATP concentration in (B). All experiments are carried out in an amperometric mode at +750 mV vs. Ag|AgCl in Krebs buffer pH 7.4. Data shown as mean ± SD, $n = 8$.

concentrations were based on the highest concentrations observed during physiological measurements (Rice, 2000; Robinson et al., 2008; Patel, 2008; Bertrand and Bertrand, 2010). No responses for ADP and AMP were observed on all electrodes ($n = 6$). This is not surprising for bare Pt and SENT as AMP and ADP are not electroactive. However surprisingly, no interferences are observed on the ATP biosensor. HEX is known to phosphorylate a six-carbon sugar, such as glucose to form glucose-6-phosphate. The tertiary phosphate is able to reach deep into the active site of HEX to be cleaved and attached to the glucose. The same process theoretically can occur for ADP and AMP, however the phosphate moieties in these molecules may be unable to reach deep into the active site, thus preventing interference to ATP monitoring.

No interference was observed on the ATP biosensor (Fig. 4) from AA, DA and 5-HT. This is most likely due to the partial selectivity of the electroplymerised film and adsorbed enzymes. Compared to other ATP electrodes, the electrode surface is most likely covered with a tight layer of enzyme, which is trapped in a polymeric film, which limits the extent of exposed electrode available.

Although the ATP biosensor is selective against all common analytes, responses were observed on the unmodified Pt and SENT electrode for AA, DA and 5-HT. The responses on the SENT electrode were significantly lower than that on the unmodified Pt electrode (Suppl. Table 1, $n = 6$, $p < 0.001$) for all analytes. Although the SENT electrode was designed to be utilised within the biosensor to act as a control for the ATP biosensor for our measurements *in vitro*, it is not required as our ATP biosensor has sufficient selectivity. However in other biological matrixes and where there is a need to

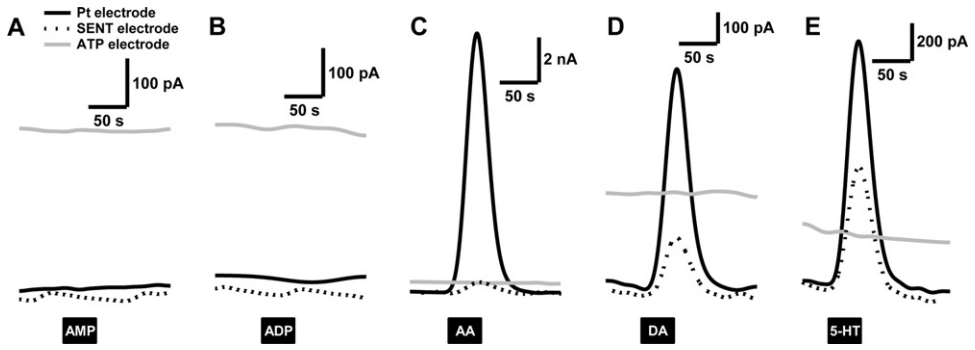

Fig. 4. Influence of biological interferences on ATP biosensor. The ATP biosensor, a SENT electrode and unmodified Pt electrode were assessed using FIA to study the influence of 5 μM AMP (A), 5 μM ADP (B), 1 mM AA (C), 5 μM DA (D) and 5 μM 5-HT (E). All experiments were carried out in Krebs buffer pH 7.4 at 1 ml min^{-1} flow rate, with analytes exposed to the background flow for a duration of 20 s ($n = 6$).

monitor other neurochemicals the SENT electrode may be necessary.

Overall, the ATP biosensor is selective against common biological interferents. This is an improvement on the limited selectivity data presented for other ATP biosensors. A similar HEX/GOx biosensor was prone to AA interference (Soldatkin et al., 2009). The ATP biosensor based on the GK/G3POX enzymes is affected by interferences, thus a null electrode was required to remove the influence of interferences (Llaudet et al., 2005). This in principle works well, however is not ideal for biological measurements as the ATP and null sensor are located in different spatial locations over the tissue and cell, where release of signalling molecules will be different. For other ATP biosensors, limited data has been shown for the selectivity of the electrode against known or expected interferences (Scheller and Pfeiffer, 1980; Compagnone and Guilbault, 1997; Kueng et al., 2004; Liu and Sun, 2007).

3.4. Long-term stability of the ATP biosensor

Fig. 5 shows the stability data for the ATP biosensor response over 7 days. In Fig. 5A, normalised current response over 7 days for both responses to glucose (11 mM) and ATP (1 μM) is shown. The responses are normalised from experiments carried out during day 1. There is a significant decrease in current response after 1 day for both ATP and glucose, however there is no significant difference in the response of ATP and glucose between days 2 and 7. After 7 days, there is a 15.87 ± 1.34% loss in the glucose response ($n = 6$), whilst after 7 days there is a 33.41 ± 3.52% loss in the ATP response ($n = 6$). However monitoring stability of biosensors is subject to sensor variability, as single concentration point recordings are utilised to study current losses over time. A better measure is to consider the stability of the calibration response. In Fig. 5B, the sensitivity of the ATP calibration response between 0.25 and 4 μM was plotted over 7 days, which provided a better insight into the activity of the electrode. There is a 23.9 ± 3.6% loss in sensitivity of the ATP biosensor after 7 days ($n = 6$). Overall this stability is a marked improvement on all other ATP biosensors published. No such data is provided for GK/G3POX ATP biosensors (Llaudet et al., 2005); however for other HEX/GOx ATP biosensor the normalised current is decreased by 35–55% for ATP after 7 days (Liu and Sun, 2007; Kueng et al., 2004). Compared to our sensor, this is a significantly greater loss in response, as these are all based on single point responses.

3.5. Long term biological recordings of ATP release

After the development of the ATP biosensor, the sensor was utilised for the detection of mucosal released ATP from ileum tissue. Reproducible responses of ATP were observed when the electrode was moved over the tissue surface (Fig. 6A). When the electrode is placed far from the tissue surface, the ATP electrode current is similar to that observed for 11 mM glucose from characterisation measurements. When the electrodes are brought over the tissue, a decrease in current is observed on the ATP electrode. Responses varied as measurements were carried out from 5 regions to reflect the tissue variations. There was also a good stability of the 11 mM glucose response which was constantly perfused during the duration of the recordings as shown from measurements from dead tissue. This suggests that there are no alterations in the perfusion of the glucose and that decreases in the current are due to ATP

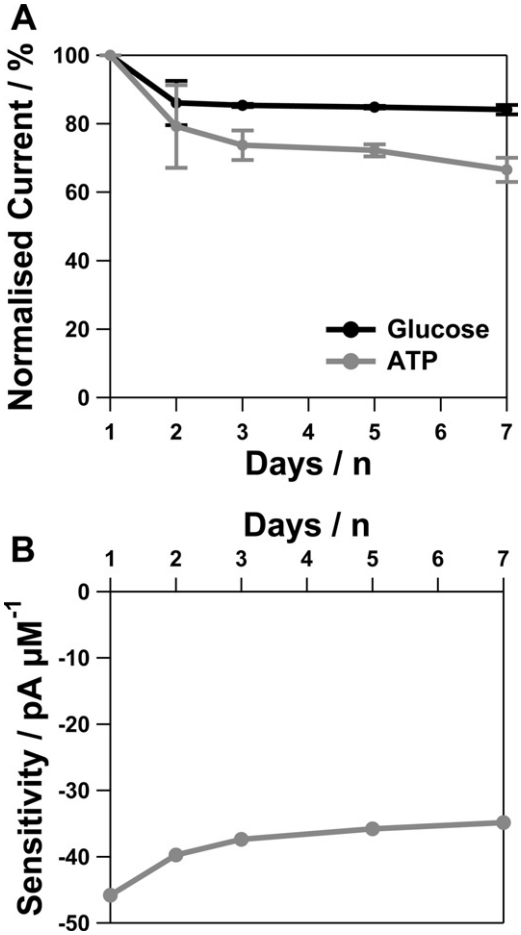

Fig. 5. Stability of ATP biosensor. In (A) the change in the normalised current response from 11 mM glucose and 1 μM ATP is shown after 7 days. In (B) the loss in the sensitivity of the ATP biosensor calibration plot is shown after 7 days. Data shown as mean ± SD, $n = 6$.

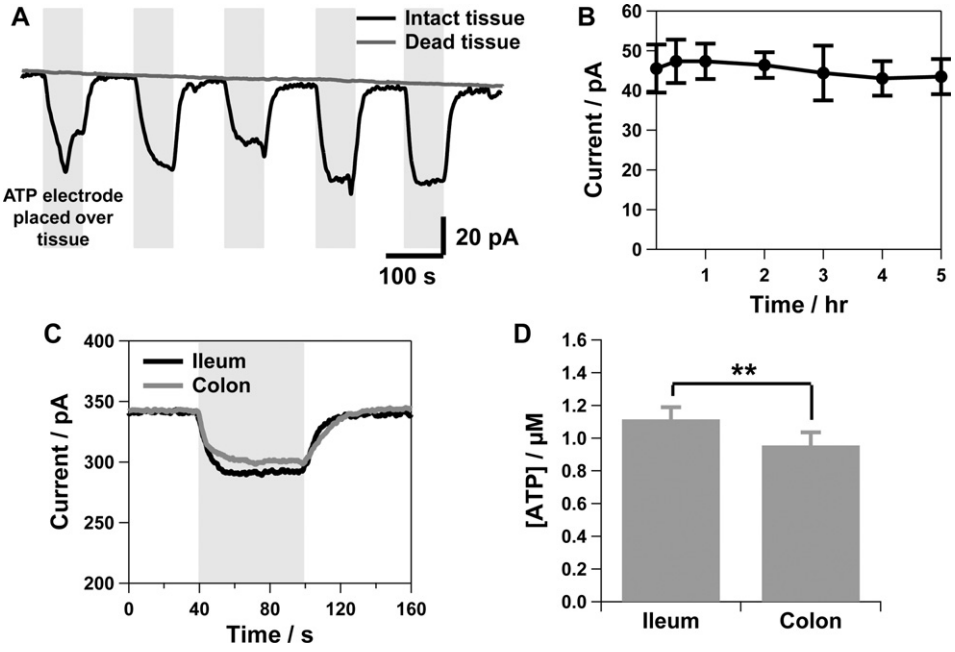

Fig. 6. Long-term measurements of ATP from gastrointestinal tissue. In (A) experimental responses obtained from ileum tissue on the ATP biosensor are shown, where measurements are carried out from multiple regions over the tissue. In (B) the Δ current responses of ATP recordings over ileum tissue for 5 h are shown. In (C) experimental responses of ATP are shown from ileum and colon tissue. In (D) the overall changes in ATP release from mucosal surface from ileum and colon are shown as a function of concentration. In (A) and (C), the grey box indicates when the electrodes are positioned over the tissue surface. Data presented as mean ± SD, $n = 5–10$.

release. The sensor was kept over the tissue to study the stability of the device for long-term tissue recordings. In Fig. 6B, recordings were carried out over the tissue for a duration of 5 h. There was no significant difference in the response over the 5 h period. The sensor was shown to be stable in a complex biological matrix that contains common fouling agents such as proteins and mucus. This is the first data to show the long-term *in vitro* stability of an ATP biosensor.

3.6. *In vitro measurements from ileum and colon tissue*

As the ATP biosensor shows excellent selectivity, ATP release can be measured *in vitro* in ileum and colon tissue without the need for a SENT or 'null' electrode. We have previously shown that 5-HT is released from the mucosal surface (Patel et al., 2007; Patel, 2008; Chau and Patel, 2009), however 5-HT does not interfere with the ATP signal on the biosensor. Experimental responses for the ileum and colon are shown in Fig. 6C. When the electrodes are brought over the tissue, a decrease in current is observed on the ATP electrode. There is a greater decrease in the current response from ileum tissue compared to colon tissue.

In Fig. 6D, an experimental trace is reported as a function of concentration of the ATP. There was a lower amount of ATP released in the colon compared to the ileum ($p < 0.05$, $n = 10$). This may important as ATP is felt to mediate 5-HT release in the gastrointestinal tract (Cooke et al., 2003). Endogenous application of ATP levels on BON cells (Christofi et al., 2004) and *in vitro* ileum (Lwin and Patel, 2010) tissue sections have been shown to elevate 5-HT levels. The stable ATP biosensor will provide a means of studying the regulation of mucosal release mechanisms.

4. Conclusion

In this manuscript, we present an ATP biosensor with exceptional sensitivity, long-term stability and selectivity from common biological interferents. The biosensor was also shown to have sensitivity and limit of detection, suitable for *in vitro* biological monitoring. The ATP biosensor was stable under biological conditions and was able to conduct long term biological recordings from *in vitro* ileum tissue without any signal attenuation. The biosensor was used to detect variations in ATP release from isolated ileum and colon tissue. The device provides a means of selectively monitoring ATP in other biological systems for a long duration.

Acknowledgements

BAP acknowledges support provided by an EPSRC LSI fellowship grant (EP/C532058/1). BBSRC for funding studentship for MR (BB/E528401).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.bios.2010.11.033.

References

Abbracchio, M.P., Burnstock, G., Verkhratsky, A., Zimmermann, H., 2009. Trends Neurosci. 32 (1), 19–29.
Anastassiou, C.A., Patel, B.A., Arundell, M., Yeoman, M.S., Parker, K.H., O'Hare, D., 2006. Anal. Chem. 78 (19), 6990–6998.
Bertrand, P.P., Bertrand, R.L., 2010. Auton. Neurosci. 153 (1–2), 47–57.
Bours, M.J., Swennen, E.L., Di Virgilio, F., Cronstein, B.N., Dagnelie, P.C., 2006. Pharmacol. Ther. 112 (2), 358–404.
Burnstock, G., 2008a. Pharmacol. Rep. 60 (1), 12–20.
Burnstock, G., 2008b. Neurogastroenterol. Motil. 20 (s1), 8–19.
Chau, R.M.W., Patel, B.A., 2009. Biomed. Chromatogr. 23 (2), 175–181.
Christofi, F.L., Kim, M., Wunderlich, J.E., Xue, J., Suntres, Z., Cardounel, A., Javed, N.H., Yu, J.G., Grants, I., Cooke, H.J., 2004. Gastroenterology 127 (1), 188–202.
Compagnone, D., Guilbault, G.G., 1997. Anal. Chim. Acta 340 (1–3), 109–113.
Cooke, H.J., Wunderlich, J., Christofi, F.L., 2003. News Physiol. Sci. 18 (2), 43–49.
Deng, Q., Kauri, L.M., Qian, W.J., Dahlgren, G.M., Kennedy, R.T., 2003. Analyst 128 (8), 1013–1018.
Evans, R.J., Derkach, V., Surprenant, A., 1992. Nature 357 (6378), 503–505.
Fields, R.D., Burnstock, G., 2006. Nat. Rev. Neurosci. 7 (6), 423–436.
Foy, G.P., Pacey, G.E., 1996. Talanta 43 (2), 225–232.
Hou, F., Wang, X., Jiang, C., 2005. Anal. Sci. 21 (3), 231–234.
Kamidate, T., Yanashita, K., Tani, H., Ishida, A., Notani, M., 2005. Anal. Chem. 78 (1), 337–342.
Katsu, T., Yamanaka, K., 1993. Anal. Chim. Acta 276 (2), 373–376.

Kueng, A., Kranz, C., Mizaikoff, B., 2004. Biosens. Bioelectron. 19 (10), 1301–1307.
Kueng, A., Kranz, C., Mizaikoff, B., 2007. Biosens. Bioelectron. 22 (11), 2774–12774.
Liu, S., Sun, Y., 2007. Biosens. Bioelectron. 22 (6), 905–911.
Llaudet, E., Hatz, S., Droniou, M., Dale, N., 2005. Anal. Chem. 77 (10), 3267–3273.
Lwin, A., Patel, B.A., 2010. J. Sep. Sci. 33 (11), 1538–1545.
Manfredi, G., Yang, L., Gajewski, C.D., Mattiazzi, M., 2002. Methods 26 (4), 317–326.
Masson, J.-F., Kranz, C., Mizaikoff, B., Gauda, E.B., 2008. Anal. Chem. 80 (11), 3991–3998.
Patel, B.A., 2008. Analyst 133 (4), 516–524.
Patel, B.A., Bian, X., Quaiserova-Mocko, V., Galligan, J.J., Swain, G.M., 2007. Analyst 132 (1), 41–47.
Pérez-Ruiz, T., Martínez-Lozano, C., Tomás, V., Martín, J., 2003. Anal. Bioanal. Chem. 377 (1), 189–194.
Qiu, J., Zhou, Y., Chen, H., Lin, J.-M., 2009. Talanta 79 (3), 787–795.
Ralevic, V., 2009. Curr. Vasc. Pharmacol. 7 (1), 3–14.
Rice, M.E., 2000. Trends Neurosci. 23 (5), 209–216.
Robinson, D.L., Hermans, A., Seipel, A.T., Wightman, R.M., 2008. Chem. Rev. 108, 2554–2584.
Scheller, F., Pfeiffer, D., 1980. Anal. Chim. Acta 117, 383–386.
Soldatkin, O.O., Schuvailo, O.M., Marinesco, S., Cespuglio, R., Soldatkin, A.P., 2009. Talanta 78 (3), 1023–1028.
Sudo, J.-i., Terui, J., Iwase, H., Kakuno, K., 2000. J. Chromatogr. B 744 (1), 19–23.
Wang, J., Jiang, Y., Zhou, C., Fang, X., 2005. Anal. Chem. 77 (11), 3542–3546.
Zhang, S., Yan, Y., Bi, S., 2009. Anal. Chem. 81 (21), 8695–8701.
Zoldák, G., Zubrik, A., Musatov, A., Stupák, M., Sedlák, E., 2004. J. Biol. Chem. 279, 47601–47609.