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A Glucose Sensing Contact Lens: A Non-Invasive Technique for Continuous Physiological Glucose Monitoring

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We have developed a range of glucose sensing contact lenses, using a daily, disposable contact lens embedded with newly developed boronic acid containing fluorophores. Our findings show that our approach may be suitable for the continuous monitoring of tear glucose levels in the range 50–1000 μM, which typically track blood glucose levels, which are ≈5–10 fold higher. Our non-invasive approach may well offer an alternative solution to current invasive glucose monitoring techniques for diabetes, such as "finger pricking."

KEY WORDS: Glucose monitoring; non-invasive; continuous monitoring; contact lens.

INTRODUCTION

Diabetes results in long term health disorders including cardiovascular disease and blindness. One of the major challenges in the management of diabetes is the monitoring of glucose concentrations.

The most commonly used technology for blood glucose determination is an enzyme based method, which requires frequent blood sampling and therefore blood drawing. While "finger pricking" is a relatively painless process, this method does suffer from a few practical problems. The first one is inconvenience and the required compliance by patients, while the second is that this is not a continuous monitoring method. Despite intensive efforts [1–11], no method is currently available for the continuous non-invasive monitoring of blood glucose.

Elevated tear glucose during hyperglycaemia was first demonstrated by Michail *et al*. as early as 1937 [12,13], as tear glucose levels track blood levels, in an analogous manner to the equilibrium that normally exists between blood and tissue fluid [14]. Since that time tear glucose has received very little attention for assessing patients for hyperglycaemia [15–19], and those studies undertaken have employed invasive and non-continuous methodologies.

We have embraced the notion of elevated tear glucose levels during hyperglycaemia and investigated the possibility, for the first time, of monitoring tear glucose, and therefore blood glucose, using a disposable, off-the-shelf contact lens. We chose to adopt commonly used plastic contact lenses,[4] for the primary reason that their physiological compatibility has already been assessed and optimised with regard to vision correction, size and oxygen/analyte permeability etc. However, our ongoing

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[4] The contact lenses were supplied by CIBA Vision, Atlanta, USA, and were stirred in 500 mL water, 20°C for 24 hr before post-doping. The contact lens is a polyvinyl alcohol type polymer which swells slightly in water. Its hydrophilic character readily allows for the diffusion of the aqueous analytes in tears. Doping the contact lenses was undertaken by incubating the lenses in a high concentration of the respective BAF solution for 24 hr, before being rinsed in Millipore water. Doped lenses were then allowed to leach probe for 1 hr into a large volume. Leaching was typically complete after 15–30 min, evident by no further change (loss) in lens fluorescence intensity.

ABBREVIATIONS: BAFs, Boronic acid containing fluorophores.

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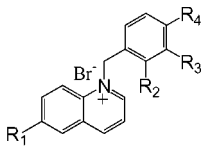

Boronic acid probes

Entry	R_1	R_2	R_3	R_4
1	$-OCH_3$	$-B(OH)_2$	H	H
2	$-OCH_3$	H	$-B(OH)_2$	H
3	$-OCH_3$	H	H	$-B(OH)_2$
4	$-CH_3$	$-B(OH)_2$	H	H
5	$-CH_3$	H	$-B(OH)_2$	H
6	$-CH_3$	H	H	$-B(OH)_2$

Control compounds

7	$-OCH_3$	H	H	H
8	$-CH_3$	H	H	H

Fig. 1. Structural formula of new boronic acid containing fluorophores (BAFs) and their respective control compounds which do not contain the boronic acid moiety.

studies and an intense feasibility study have revealed that published boronic acid containing fluorophores (BAFs), which are well-known to sense monosaccharides [20–26], do not respond well from within the lenses. This is currently though due to the low internal pH and polarity of the contact lens polymers [27], combined with the fact that the pKa's of the BAFs have been mostly designed to operate at physiological blood pH, i.e., pH 7.2 [20–26].

Subsequently, we have designed a range of new BAFs, based on the quaternized form of the quinolinium nucleus, which respond well to glucose within the contact lens and which also display a high monosaccharide affinity, afforded by the stabilization of the anionic glucose bound form (boronate ester form) by the cationic quaternary nitrogen heterocyclic center, Fig. 1. In addition we have synthesized the respective control compounds, which do not contain the boronic acid moiety, and are therefore not sensitive to monosaccharides, but which have allowed us to additionally rationale and correct for potential chloride, oxygen, fluoride, albumin and pH interferences, Fig. 1—bottom.

Figure 2 shows the pH dependence of the emission intensities of probes 1 and 4 in the presence of glucose, fructose and in buffer. Interestingly, the pKa of the glucose bound forms are substantially reduced, pKa $6 \rightarrow 7$, as compared to typical BAFs reported in the literature, pKa's usually $\gg 7$. This reduction in probe-bound sugar pKa affords for a substantial sugar response in the contact lens polymers studied here,[5] which we have shown have an

[5] See FN4.

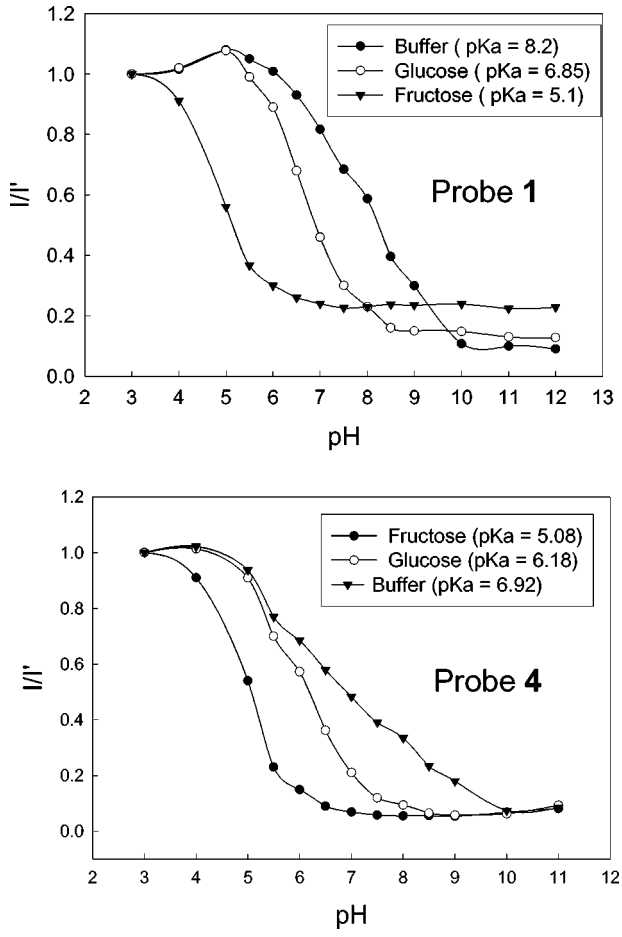

Fig. 2. Emission intensity at $\lambda_{max} = 450$ nm, I, divided by the initial emission intensity, I', as a function of pH for Probe 1 (Top) and Probe 4, $\lambda_{max} = 427$ nm (Bottom) for the fructose-bound, glucose-bound and free probe (buffer) forms.

internal pH in the range 5.5–6.5 and a lens polarity similar to that of methanol [27].

Figure 3 shows the response of the contact lenses doped with both probes 1 and 4, in the tear glucose range of physiological importance, i.e. <1 mM glucose. A similar response can be observed for a probe 1 doped lens towards both glucose and fructose, while a higher fructose affinity can be observed for a probe 4 doped lens, Fig. 3 bottom. Interestingly, the concentration of fructose in blood is typically 10 fold lower than glucose, which suggests that fructose is unlikely to interfere in the glucose response of a probe 1 doped contact lens.

CONCLUSIONS

In this rapid communication we have briefly described a range of boronic acid containing fluorophores

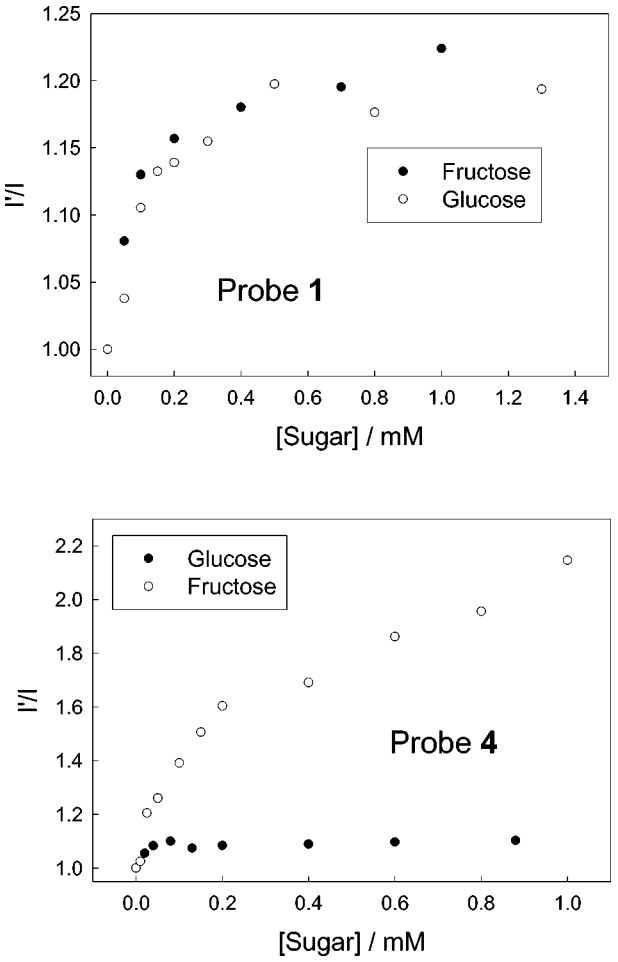

Fig. 3. Response of a Probe 1 (Top) and a Probe 4 (Bottom) doped contact lens towards both glucose and fructose. I—emission intensity in the presence of sugar and I' in the absence of sugar.

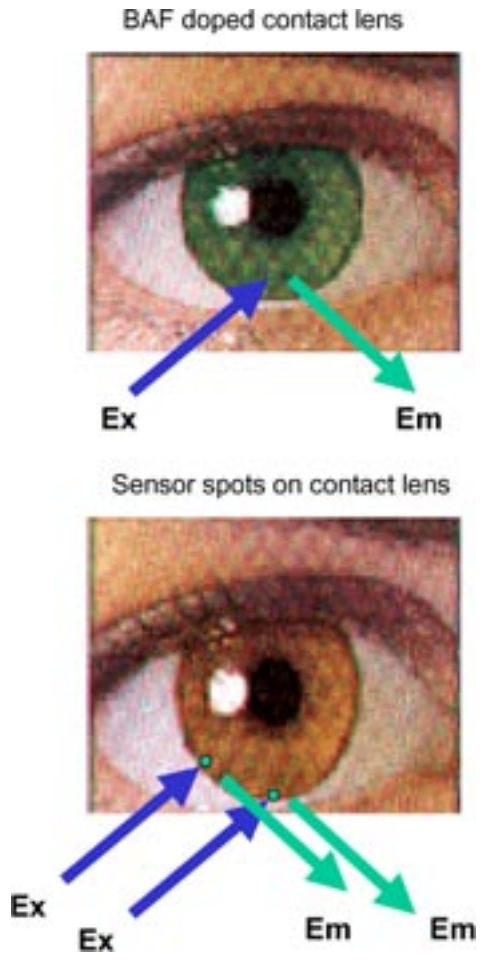

Fig. 4. Potential methods for non-invasive continuous tear glucose monitoring. (Top)—BAF doped contact lens as described here, and (Bottom)—Sensor spots on the surface of the lens to additionally monitor other analytes in addition to glucose, such as chloride or oxygen. Sensor spot regions may also allow for ratiometric, lifetime or polarization based fluorescence glucose sensing.

which have been both designed and synthesised to respond to physiological tear glucose in a disposable, plastic, off-the-shelf contact lens. The probes have been designed to be compatible with both the internal pH and polarity within the lens, as well as to respond well too physiological tear glucose, which is typically present at a 10-fold lower concentration in tears, than in blood. The reversible nature of our glucose sensing contact lens, their sensitivity, and their requirement for non-UV excitation, strongly suggests that our approach could be used for the continuous and non-invasive monitoring of physiological glucose, Fig. 4. We envisage that multiple modes of sensing can be undertaken in the contact lens, such as ratiometric, lifetime and (visual) polarisation based glucose fluorescence sensing [28], Fig. 4, for both doped lenses and lenses containing unique sensing regions (spots). Further details will be reported in a full paper in due course.

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