Spectrochimica Acta Part A 85 (2012) 61–65

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Infrared spectroscopic analysis of human interstitial fluid *in vitro* and *in vivo* using FT-IR spectroscopy and pulsed quantum cascade lasers (QCL): Establishing a new approach to non invasive glucose measurement

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ARTICLE INFO

Article history:
Received 7 June 2011
Accepted 4 September 2011

Keywords:
Infrared spectroscopy
FT-IR
Quantum cascade lasers (QCL)
Attenuated total reflection (ATR)
Blood glucose
Non-invasive glucose measurement
Interstitial fluid
Diabetes

ABSTRACT

Interstitial fluid, *i.e.* the liquid present in the outermost layer of living cells of the skin between the *Stratum corneum* and the *Stratum spinosum*, was analyzed by Fourier transform infrared spectroscopy and by infrared spectroscopy using pulsed quantum cascade infrared lasers with photoacoustic detection. IR spectra of simulated interstitial fluid samples and of real samples from volunteers in the 850–1800 cm^{-1} range revealed that the major components of interstitial fluid are albumin and glucose within the physiological range, with only traces of sodium lactate if at all. The IR absorbance of glucose in interstitial fluid *in vivo* was probed in healthy volunteers using a setup with quantum cascade lasers and photoacoustic detection previously described [11]. A variation of blood glucose between approx. 80 mg/dl and 250 mg/dl in the volunteers was obtained using the standard oral glucose tolerance test (OGT). At two IR wavelengths, 1054 cm^{-1} and 1084 cm^{-1}, a reasonable correlation between the photoacoustic signal from the skin and the blood glucose value as determined by conventional glucose test sticks using blood from the finger tip was obtained. The infrared photoacoustic glucose signal (PAGS) may serve as the key for a non-invasive glucose measurement, since the glucose content in interstitial fluid closely follows blood glucose in the time course and in the level (a delay of some minutes and a level of approx. 80–90% of the glucose level in blood). Interstitial fluid is present in skin layers at a depth of only 15–50 μm and is thus within the reach of mid-IR energy in an absorbance measurement. A non-invasive glucose measurement for diabetes patients based on mid-infrared quantum cascade lasers and photoacoustic detection could replace the conventional measurement using enzymatic test stripes and a drop of blood from the finger tip, thus reducing pain and being a cost-efficient alternative for millions of diabetes patients.

1. Introduction

Perhaps the most frequently performed bioanalytical measurement is the determination of glucose in blood determined by diabetes patients from a drop of blood taken from the finger tip; approx. 6 million type II diabetes patients perform around 10–15 million measurements per day only in Germany. This "invasive" measurement is considered painful by patients and is costly for the health insurances because of the enzymatic test stripes used. A true "non-invasive" device to measure spot blood glucose which could replace the finger prick is not available to the patient, in spite of the many different approaches by various research groups. None of the techniques has ever managed to successfully reach the market, presumably because of the low specificity of most of the physical or chemical principles used [1,2]. Among these are near infrared (NIR) spectroscopy of the glucose overtones [3,4], skin impedance spectroscopy [1] or optical coherence tomography, OCT [1,5]. Semi-invasive technologies have been proposed and possibly offer more specific information on blood glucose [6,7]. However, they require frequent surgical intervention, which may be the reason that they are not easily accepted by patients.

Mid-infrared spectroscopy (MIR) in the range of approx. 800–1300 cm^{-1} is very specific in the identification and quantification of glucose and other body fluids [8,9]. The absorption of glucose in aqueous solution within this range exhibits maxima and shoulders near 1152, 1106, 1080, 1036 and 992 cm^{-1}, all of them arising from coupled ring–C–O–H stretching and bending modes. This infrared glucose fingerprint can be used to

Abbreviations: ISF, interstitial fluid; QCL, quantum cascade lasers; IR, infrared; FT-IR, Fourier transform infrared; ATR, attenuated total reflection; OGT, oral glucose tolerance test; PBS, physiological buffer solution; PA, photoacoustic; PAGS, photoacoustic glucose signal.

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doi:10.1016/j.saa.2011.09.007

quantify glucose concentration in blood or in blood plasma samples and has been successfully developed by us towards a reagent-free point-of-care measuring system for glucose and for several other blood parameters [9]. Infrared spectroscopy in this wavelength range, although highly specific for glucose, has the drawback that it reaches only the upper layers of skin, *i.e.* the *Stratum corneum* and the *Stratum spinosum*, with most of the IR energy deposited in a depth less than 50 μm. Within this range, blood vessels are not in reach.

The liquid present in the compartment within the epidermal layers containing living cells, termed epidermal interstitial fluid, presents an alternative. This fluid compartment probably represents a significant proportion of 20% of the volume in the epidermal layer [1]. Although hardly characterized with respect to its constituents, it is known that the glucose content of interstitial fluid approximates that of blood (around 80–90% of blood glucose) [10]. Moreover, it is know that the glucose variation in interstitial fluid follows that of blood with only minimal delay (around 5–10 min, depending on the skin part probed), which is a necessary prerequisite for a reliable measurement required by the diabetes patient. Indeed, a long delay time between glucose concentrations appearing in a body fluid which may be easily accessible, such as tears, and blood glucose, presents a knock-out argument for a measuring method.

Interstitial fluid (ISF) thus provides a potential access for mid-infrared spectroscopy. It emerges from skin upon surface abrasions. When a lesion is produced in the skin by cooling, heating or by friction, the binding among the cells between the *S. corneum* and the *S. spinosum* is weakened provoking a local depression area around the lesion zone. The relatively high pressure of the surrounding causes accumulation of interstitial fluid under the lesion; then a bulla is produced on the skin.

In order to obtain a solid basis for a true non-invasive glucose measurement, we have analyzed here the composition of ISF using FT-IR spectroscopy of model interstitial fluid samples mixed from albumin, glucose and lactate, up to now considered the significant constituents of ISF. We have compared these model spectra with the IR spectra of real ISF samples obtained from volunteers. In a second attempt, we have studied the IR absorption of interstitial fluid *in vivo* at volunteers. Pulsed IR radiation at two wavelengths relevant for glucose, near the spectral maxima of glucose reported above, was obtained from quantum cascade lasers. The detection of IR absorbance in skin was performed with a photoacoustic cell in contact with the skin. In order to obtain variable glucose concentrations for the volunteers, standardized oral glucose tolerance tests (OGTs) were performed.

The results help to identify and quantify the constituents of interstitial fluid and demonstrate the glucose-dependent absorbance of skin for specific mid-IR wavelengths as a basis for a non-invasive yet specific glucose measurement.

2. Materials and methods

2.1. Models for epidermal interstitial fluid

Model samples (63) for interstitial fluids were produced by mixing different potential components of interstitial fluid, such as glucose, albumin and sodium lactate in physiological buffer solution (PBS). For each component, three concentrations within the physiological range were chosen. The concentration values for each component are shown in Table 1. The 63 simulated interstitial samples are combinations of these concentration values for each component.

Table 1
Basic concentration values of glucose, albumin and sodium lactate combined to produce the 63 simulated interstitial fluid samples.

Albumin (mg/dl)	Glucose (mg/dl)	Sodium lactate (mg/dl)
1500 (A1)	80 (G1)	5 (L1)
2750 (A2)	110 (G2)	20 (L2)
5000 (A3)	300 (G3)	50 (L3)

2.2. Real interstitial fluid samples

The real interstitial fluid samples from three volunteers were collected after producing a bulla on their skin. Two of the volunteers had developed the bulla during a walk in the mountains for several hours, the third after a 15 s application of liquid nitrogen to a small portion of skin, approx. 5 mm of diameter. A syringe was use to extract approximately 12 μl of clear light yellow interstitial fluid from each bulla, a volume sufficient for IR analysis. These samples were kept frozen at −20 °C until use.

2.3. Fourier transform infrared (FT-IR) spectroscopy

Infrared spectra of model and real ISF samples in the range between 850 cm^{-1} and 1800 cm^{-1} were recorded using a BRUKER FT-IR spectrometer type Vector 22 (BRUKER Optics, Ettlingen, Germany) equipped with a MCT detector. Spectra were recorded from 128 interferograms at a resolution of 4 cm^{-1}. An attenuated total reflection unit with a diamond internal reflection element and 5 reflections was used. Samples were kept at 22 °C during the measurement. For all measurements, physiological buffer solution served as a reference.

2.4. UV/VIS spectroscopy and gel electrophoresis of ISF samples

UV absorption spectra of the real ISF samples were recorded using a Nanodrop 2000c spectrometer from Thermo Scientific. The protein fraction in the interstitial fluid samples was estimated by polyacrylamide gel electrophoresis using standard procedures.

2.5. Photoacoustic spectroscopy of the skin

Photoacoustic signals of the skin were recorded using a setup previously described in [11]. Up to six quantum cascade lasers can be used. Each laser output is formed to a parallel beam using an off-axis parabolic mirror. A confocal set of parabolic mirrors is then used to focus the lasers onto the skin. The two strongest QCL lasers with single-mode emission at 1054 cm^{-1} and 1084 cm^{-1} were selected due to their proximity to glucose absorption maxima. As a reference wavelength, a third QCL emitting at 1100 cm^{-1} was selected. All QCL were provided by Alpes Lasers, Neuchatel, Switzerland. The photoacoustic cell was designed as a twin Helmholtz photoacoustic gas cell with a broad resonance around 2 kHz. Consequently, this frequency was selected for the laser pulse sequence. One Helmholtz cell served as a sample cell with the laser beam focus on the skin, the other as a reference cell without laser pulse to compensate for environmental noise. The holes of the cell were approx. 5 mm in diameter and adjacent to each other. The measurement of the photoacoustic PA signals from the skin was initiated by mildly pressing the palm of the thumb to the sample and reference cells. The differential microphone signal from the cells (sample – reference) was amplified and processed in a sound processor card to obtain the PA signal.