Detection of electrical activity of pancreatic beta-cells using micro-electrode arrays

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Detection of electrical activity of pancreatic beta-cells using micro-electrode arrays

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Abstract

In this position paper we propose a new approach to provide online in-vivo recordings of organ activity in real time and to overcome three major shortcuts of currently used invasive glucose sensors. In the context of diabetes, standard glucosensors recognize only glucose, whereas circulating lipids as well as amino acids are known to increase the demand in insulin and, under physiological circumstances, its secretion. Furthermore, the integration of other relevant signals such as hormones provides a clear advantage. Consequently, using the β-cell as a signal integrator combined with ASIC technology ensures a physiological signal and its read-out in real time thus avoiding complex algorithms. We describe a closed-loop architecture for exploiting those bio-sensors and potentially able to provide a control feedback for real-time insulin delivery.

Keywords: Biosensors, Micro-electrode arrays, Closed-loop insulin delivery

1 Introduction

Recording the activity of an entire organ represents a considerable challenge but is clearly required to fully understand its function and malfunction that can not simply be derived from the behaviour of its constituents in culture. Imaging techniques have gained considerable popularity and allow physiological imaging at the level of entire living animals such as PET but still lack time resolution [1].
Though such techniques have been used successfully in neurosciences, comparable approaches are lacking for other structures. The endocrine systems formed by the pancreatic islets of Langerhans are one of organs implicated in the pathogenesis of diabetes. Due to its anatomical structure imaging techniques are not feasible in-situ. However, methods allowing high time resolution, long-term recordings and direct measurement of their activity are clearly required to improve our understanding and to go beyond cumbersome and indirect measurements, such as glucose or hormone levels [2]. One of the hallmarks of islets is their electrical activity, on which intracellular signals converge and this activity is closely related to their metabolic state and to insulin secretion. As islets are dispersed throughout the pancreas, direct "wiring" of the islets is impossible. However, culture of islets on a recording device within rodents would already represent a major advance. The major challenge will reside in the relatively small amplitude to be recorded as compared to neurons or (cardio)myocytes [3, 4].

Such a device may also have a major use in the treatment of diabetes in man. As for France, more than 2.5 millions persons or 3.8 % of the population has been diagnosed as diabetic in 2005 with a yearly progression rate of 5% and some 500.000 persons undiagnosed [5].
Multiple daily injections of insulin pose the problem of adaptation to diverse situations in everyday life and the presence of a major nuisance. This point is aggravated by the necessity of repetitive blood controls to ensure adequate therapy and avoid life-threatening hypoglycaemia [6].
Thus, the development of a closed-loop delivery of insulin is not only strongly expected by the patients to improve life-conditions, but also represents a task in view of the challenging increases in financial terms. Though data are still scarce, continuous glucose monitoring seems to improve medical treatment and may represent a valuable tool increasing quality of life and reducing complications as well as costs [7, 8].
Recent progress in technology has allowed using continuous glucose monitoring (CGM) as prototypes in clinical settings [9]. The invasive sensors have a life-time of several days and are mainly based on electrochemical sensors linked to gluco-oxidase. They provide an undeniable progress in the therapy of certain cases of type 1 diabetes [10, 11]. Alternative external, non-invasive approaches have been proposed but are not in clinical use: surface-enhanced Raman spectroscopy, fluorescence, reverse iontophoresis, photoacoustic, thermal or impedance spectroscopy, electromagnetic sensing [12]. The first continuous glucose monitors to display real-time values to patients (Medtronic) only became available in 2006, and is essentially open-loop. Moreover, all current CGMs have a major draw-back. They do only measure one parameter, glucose, and thus do not reflect other important physiological parameters such as circulating hormones as well as the presence of other nutrients, such as lipids or certain amino-acids. They have a considerable lag-time, ascribed to the

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geometry of the electrochemical sensor, or the changes of glucose concentrations in the interstitial tissue as compared to blood levels [13]. Thus, they all rely on complex algorithms to make up for this important draw-back and to accommodate to the rather diverse nutritional situations, encountered especially in the young [14]. Moreover, they are less accurate in hypoglycaemia, a situation that may be life-threatening [15]. Finally, they are unable to provide pulsatile insulin delivery which is thought to be crucial to prevent insulin resistance [16].

We propose a closed-loop system connecting a biological integrative sensor and a controlled insulin source, it is mandatory to combine: (i) the conditioning of the sensors (here pancreatic beta-cells) signal; (ii) extraction of features from the conditioned signal; (iii) generation of the command signal. The field of computational neurosciences provided recently new insights on efficient techniques for detecting cellular activity (spikes) and computing it as an event-based information with the timing of events being part of the information. Advances in microelectronics during the last decade permit the integration on low cost and low power VLSI circuits of processing functions mixing analog and digital signals. FPGAs (configurable digital processing devices) are used as their very large number of gates and powerful synthesis tools allow the implementation of complex algorithms with an optimized processing architecture. Event-based processing can be translated to hardware using asynchronous computation that is supported by FPGAs due to recent advances. High density analog ASICs (application specific integrated circuits) are developed in CMOS-based technologies for conditioning signals with a low signal-to-noise ratio.

A glucose sensor that reacts in real time, which is capable to take hormones and other nutrients into account and to detect hypoglycaemia, represents an important need and challenge for life expectancy, life quality and medical costs of a growing number of diabetic.

2 Closed-loop system architecture

We propose to record the electrical activity of beta(ß)-cells using multiple and non-invasive extracellular electrodes by the means of micro-electrode arrays (MEAs) [19]. The recording via an array provides signals from multiple cells and is thus closer to the situation in-situ as if only one cell or microrgan (islet) would be recorded. Moreover, it will result in a certain statistical sampling, thus offering robustness and avoiding bias from inherent cell/microorgan heterogeneity. MEAs are used to a considerable extent in neurosciences or cardiac pharmacology as both cell types present action potentials of large amplitudes [4, 20]. Such an approach has, however, never been used previously in the case of endocrine cells: action potentials if present are expected to be much smaller and their character as "excitable cells" is not commonly considered.

Each channel (corresponding to 1 micro-electrode on the MEA) comprises on-line processing units (Fig.1) for conditioning and processing the measured signal, quantification of the insulin need and feedback control for the insulin delivery.

As a first validation step, we built a system to determine the need of insulin in real time. This system relies on three main parts: a set of software tools to interpret ß-cells activity (Software), a hardware

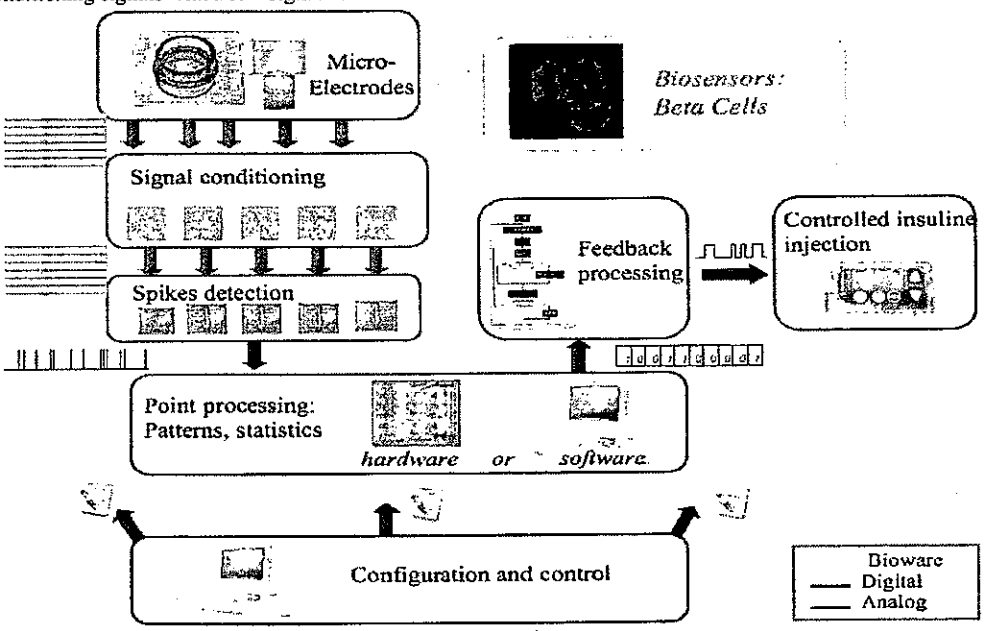

Figure 1: Description of the Closed-loop system architecture

acquisition system designed for low signal levels (Hardware) and the ß-cells on MEA as a sensor (Bioware).

We have a long experience in the design of system dedicated to the connection of integrated circuits to and from biological cells, using intra- and extra-cellular electrodes [3, 4]. We developed closed-loop systems in the context of computational neurosciences [17]. This system processes a closed-loop with a delay <100µs and without data loss [18] thus proving the technical feasibility of such a closed-loop mixing 3 types of data representation in real-time

2.1 Software

The first element of the system is the software layer. It contains four basic parts, three of which are graphical user interfaces (GUIs) that work offline and are visual support to describe the experiment. The last one recovers the information from the cells and pilots the hardware. This part is the most critical one of the system. As the project is still in a prototyping phase, this part is running on the fly without true real-time considerations. It is nevertheless lightweight enough to perform its function as if it was real-time. Most of the software relies in (GUIs) to check the behavior of the system. Analysis functions and methods are still under development as they require calibration experiments to be validated.

2.2 Hardware

The second stage of the system is implemented on hardware, and represents the bridge between the software and the bioware. The different hardware elements are commercial or custom. Our objective is to provide an entirely configurable system to the user. We use two commercial elements and a dedicated system in our acquisition loop. Commercial elements are dedicated to the layers transitions. The first one is a National Instrument (NI) lab board to get easy access to hardware from the software layer. The second one is a MEA device set from MCS. It includes mechanical connection to the MEA and preamplifiers to reduce influence of wires on the SNR. Hardware elements are a series of boards plugged in a modular and autonomous rack, which convey buses of shared data. All boards are configurable to investigate the best architecture on the definitive system.

2.3 Bioware

The last component of the system is the biological material that is being cultured and which provides the signal for acquisition. The B-cells culture exhibits spontaneous trains of spikes. The cells are routinely kept healthy and active to perform a long lasting sensor.

3 Preliminary data

Biological signals measured on the MEAs are voltages resulting from the coupling of the electrical activity of cells with the metal electrodes. The resulting voltages present a low amplitude (less than 10 µV) and a low signal-to-noise ratio (S/N) processed at different levels (Fig 2). Action potentials and bursts can be discriminated and analyzed with ad-hoc signal conditioning and processing, computed by specifically designed integrated circuits.

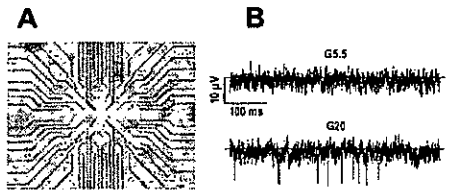

Figure 2: Culture microphotograph (A) and recording (B) of pancreatic B-cells in MEA

Experiments show that the firing rate results from the glucose concentration (Fig. 3) and is modulated by agents such as the GLP-1 (Glucagon-like Peptide-1). This new model will be suited to understand the electrical code used by these cells to translate glucose/nutrient/hormone signals into precisely adapted secretion of insulin

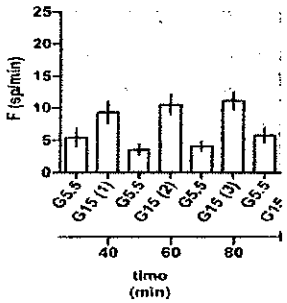

Figure 3: Beta cells firing rate F (number of spikes/minute) follow patterns of glucose stimulations. G5.5: 5.5mM of glucose (hypoglycemia); G15: 15mM of glucose (hyperglycemia)

4 Conclusion

We presented in this paper the principle and preliminary results of a biosensor using MEAs for contextual glucose detection using ß-cells electrical activity. We also propose the architecture of a closed-loop system for the control of insulin release in the context of the treatment of diabetes. The system is inspired from closed-loop systems used in neural engineering and brain-machine interface. The initial prototypes combine hardware and software computing supports and will be used for defining a standardized response of the ß-cells to defined glucose concentrations.

Current state of the art permits to combine these different hardware on a unique substrate as an embedded and integrated system thus building a closed-loop architecture with a negligible computation delay (<1ms) as compared to the physiological kinetics (at least for parameters such as glucose). Microelectronics provide the optimal support for such a system considering its integration density and its low power characteristics, minimizing battery issues and ensuring the absence of heating stress deleterious for the biological environment and components.

5 References

[1] D.J. Rowland, and S.R. Cherry, "Small-animal preclinical nuclear medicine instrumentation and methodology", *Semin Nucl Med*, 2008, 38(3), pp 209-22 (2008).

[2] A. Mari, A. Tura, G. Pacini, A. Kautzky-Willer, and E. Ferrannini, "Relationships between insulin secretion after intravenous and oral glucose administration in subjects with glucose tolerance ranging from normal to overt diabetes", *Diabet Med*, 2008, 25(6) pp 671-7 (2008).

[3] G. Le Masson, S. Renaud-Le Masson, D. Debay, and T. Bal, "Feedback inhibition controls spike transfer in hybrid thalamic circuits", *Nature*, 2002, 417(6891), pp 854-8 (2002).

[4] G. Bontorin, C. Lopez, Y. Bornat, N. Lewis, S. Renaud, A. Garenne, M. Chanaud, and G.L. Masson, "A Real-time Setup for Multisite Signal Recording and Processing in Living Neural Networks", *Proceedings of the IEEE 2008 International Symposium on Circuits And Systems, (ISCAS'08)*, 2008. Seattle, USA (2008).

[5] O. Kusnik-Joinville, A. Weill, B. Salanave, P. Ricordeau, and H. Allemand, "Diabète traité : quelles évolutions entre 2000 et 2005 ?" *Pratiques et Organisation des Soins, 2007*, 38(1) pp 1-12 (2007).

[6] L.C. Deeb, "Diabetes Technology During the Past 30 Years: A Lot of Changes and Mostly for the Better", *Diabetes Spectrum*, 2008. 21, pp 78-83 (2008).

[7] I.M. Wentholt, J.B. Hoekstra, and J.H. Devries, "Continuous glucose monitors: the long-awaited watch dogs ?", Diabetes Technol Ther, 2007, 9(5), pp 399-409 (2007).

[8] I.B. Hirsch, J. Abelseth, B.W. Bode, J.S. Fischer, F.R. Kaufman, J. Mastrototaro, C.G. Parkin, H.A. Wolpert, and B.A. Buckingham, "Sensor-augmented insulin pump therapy: results of the first randomized treat-to-target study", *Diabetes Technol Ther*, 2008, 10(5) pp 377-83 (2008).

[9] H. Hanaire, "Continuous glucose monitoring and external insulin pump: towards a subcutaneous closed loop", *Diabetes Metab*, 2006, 32(5 Pt 2), pp 534-8 (2006).

[10] W.V. Tamborlane, R.W. Beck, B.W. Bode, B. Buckingham, H.P. Chase, R. Clemons, R. Fiallo-Scharer, L.A. Fox, L.K. Gilliam, I.B. Hirsch, E.S. Huang, C. Kollman, A.J. Kowalski, L. Laffel, J.M. Lawrence, J. Lee, N. Mauras, M. O'Grady, K.J. Ruedy, M. Tansey, E. Tsalikian, S. Weinzimer, D.M. Wilson, H. Wolpert, T. Wysocki, and D. Xing, "Continuous glucose monitoring and intensive treatment of type 1 diabetes", *N Engl J Med*, 2008, 359(14), pp 1464-76 2008).

[11] M.R. Burge, S. Mitchell, A. Alison Sawyer, and D.S. Schade, "Continuous Glucose Monitoring: The Future of Diabetes Management", *Diabetes Spectrum*, 2008, 21, pp 112-119 (2008).

[12] A. Tura, "Noninvasive glycaemia monitoring: background, traditional findings, and novelties in the recent clinical trials", *Curr Opin Clin Nutr Metab Care*, 2008, 11(5), pp 607-12 (2008).

[13] T.r.P. Meeting, "Real-time continuous glucose monitoring", *ECRI*, (2007).

[14] G. Steil, K. Rebrin, and J.J. Mastrototaro, "Metabolic modelling and the closed-loop insulin delivery problem", *Diabetes Res Clin Pract*, 2006, 74 Suppl 2, pp S183-6 (2006).

[15] V. Melki, F. Ayon, M. Fernandez, and H. Hanaire-Broutin, "Value and limitations of the Continuous Glucose Monitoring System in the management of type 1 diabetes", *Diabetes Metab*, 2006, 32(2), pp 123-9 (2006).

[16] P. Gilon, M.A. Ravier, J.-C. Jonas, and J.-C. Henquin, "Control Mechanisms of the Oscillations of Insulin Secretion In Vitro and In Vivo", *Diabetes*, 2002, 51(90001), pp S144-151 (2002).

[17] S. Renaud, J. Tomas, Y. Bornat, A. Daouzli, and S. Saïghi, "Neuromimetic ICs with analog cores: an alternative for simulating spiking neural networks", *Proceedings of the IEEE 2007 International Symposium on Circuits And Systems, (ISCAS'07)*, New-Orleans, USA (2007).

[18] B. Belhadj, J. Tomas, O. Malot, G. N'Kaoua, Y. Bornat, and S. Renaud, "FPGA-based architecture for real-time synaptic plasticity computation", in *International Conference on Electronics, Circuits and Systems*, Malta (2008).

[19] K.-H. Boven, M. Fejtl, A. Möller, W. Nisch, and A. Stett, "On Micro-Electrode Array Revival", *Advances in Network Electrophysiology Using Multi-Electrode Arrays*, T.M. Baudry M, Editor, 2006, New York, Springer Press, pp 24-37 (2006).

[20] A. Natarajan, P. Molnar, K. Sieverdes, A. Jamshidi, and J.J. Hickman, "Microelectrode array recordings of cardiac action potentials as a high throughput method to evaluate pesticide toxicity", *Toxicol In Vitro*, 2006, 20(3) pp 375-81 (2006).

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