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A SiC microdevice for the minimally invasive monitoring of ischemia in living tissues

Rodrigo Gómez¹, Antoni Ivorra¹, Rosa Villa^{1*}, Anna Solà², Ivan Erill¹, Luís Palacios², Philippe Godignon¹, Georgina Hotter², José Millán¹

¹Biomedical Applications Group, Centro Nacional de Microelectrónica (CNM-CSIC). Campus UAB s/n, 08193 – Bellaterra, Spain. Email: rosa.villa@cnm.es.

²Department of Experimental Pathology, IIBB-CSIC, IDIBAPS. Roselló, 161, 7^a Planta, 08036 – Barcelona, Spain.

ABSTRACT

Monitoring of ischemia in living tissues is a field of increasing interest in many clinical settings. In this work we report for the first time anywhere the development of needle-shaped, minimally-invasive impedance probes based on silicon carbide (SiC) substrates. An in-vitro comparison of these new devices with Si-based impedance probes demonstrates that their effective operation range extends up to the 100 kHz range, thus allowing a wide-spectrum multi-frequency analysis of impedance modulus and phase angle. Furthermore, we show that, when applied to *in-vivo* settings, this kind of analysis yields to an accurate monitoring of ischemia, while making possible the application of more robust mathematical methods for the study of impedance in living tissues.

Keywords

Impedance, probe, silicon carbide, ischemia.

1. Introduction

In many clinical settings a decrease of the blood supply to bodily organs or tissues can have fatal consequences if it is not properly addressed promptly (e.g. mesenteric or myocardial ischemia). Sustained ischemia leads to hypoxia, a stressful condition for cells that is able to trigger programmed cell death (apoptosis) and, consequently, lead to organ failure. In addition, ischemia lies at the heart of several natural and clinically-

* Corresponding author.

induced conditions, like tumor growth, cold-preservation of grafts for transplantation or induced heart-arrest during open-heart surgery. Therefore, the ability to monitor ischemia in clinical and experimental settings is becoming increasingly necessary in order to predict its irreversibility (e.g. in the transplantation setting), to develop drugs that delay its onset or effects, or to diagnose or develop drugs to combat tumor growth.

Unfortunately, most conventional methods for the indirect assessment of ischemia present severe handicaps for their clinical use. Ischemia can be indirectly evaluated by histology or energetic charge measurement (Stubenitsky et al., 1999), but these methods require various tissue biopsies and are often time-consuming, thus imposing heavy restrictions to their use in the clinical and experimental setting for most of the targeted applications. Other methods, such as pH and $[K^+]$ measurements (Warner et al., 1988; Möller et al., 2000) have also been proposed for ischemia assessment when used in a continuous monitoring scheme, but the available sensing systems are markedly invasive and thus also difficult to use in most clinical and experimental settings.

Recently, a minimally invasive system for the continuous and simultaneous monitoring of tissue impedance has been developed (Errachid et al., 2001), and experimental results have shown that ischemia and its effects on tissue can be indirectly but accurately measured by continuous monitoring of tissue impedance. This minimally invasive system consists of a small micro-machined silicon (Si) needle with deposited platinum electrodes for impedance measurement that can be inserted in biological tissues with minimal damage (Ivorra et al., 2003) and experimental results so far have proved its usefulness for detecting structural alterations (Ivorra et al., 2005), anoxic edema and functional impairment in rat kidneys after sustained ischemia (Solà et al., 2003; Genesca et al., 2005). It has been shown that impedance monitoring at low (i.e. 1 kHz) frequencies correlates with the measurement of extracellular space impedance (Gersing, 1998). Extracellular impedance can be used to assess anoxic edema due to ischemic cell swelling (Grimnes et al., 2000), which leads to reduced extracellular space and an increase of the impedance measured at such frequencies. Conversely, high frequency impedance monitoring, based on both the phase and modulus components of impedance, has been correlated to the combined measurement of extracellular and intracellular space and membrane impedance, and can give complementary information on other effects of sustained ischemia. Moreover, multi-frequency monitoring of impedance has the advantage of yielding to more

comprehensive empirical mathematical characterizations (i.e. the Cole model; Cole, 1940) that can provide additional information through the analysis of derived parameters (e.g. α and β dispersions) and improve the reproducibility of results (Raicu et al., 2000).

A main shortcoming of the aforementioned needle-shaped sensor resides on its production using silicon substrates. Although silicon is a highly biocompatible and versatile material that can be easily processed using reliable and standardized technological processes, it does also present some drawbacks for its use in ischemia monitoring. A main handicap of bare Si-based devices in impedance monitoring concerns their limitations due to current leaks across the substrate at high frequencies (i.e. above 5 kHz). At these frequencies, the capacitance of the substrate-solution interface ceases to become a major obstacle for the current injected to measure tissue impedance and the substrate partially short-circuits the system, with injected current leaking across the substrate and generating a false image of impedance decrease. Current leaks across the Si substrate can be partially circumvented through deposition of isolating passivation layers, but these can not be properly applied to the sawed edges of needle-shaped devices. A compromise solution to prevent current leaks across device edges resides in their coating with silicone, Parylene and other organic polymers, although this complicates the technological process and poses questions regarding the safety of devices in the clinical setting, since polymer detachment during operation cannot be disregarded.

Silicon carbide (SiC) is a semiconductor material mostly used in high-power, high-frequency and high-temperature applications that can be processed in clean-room environments and using standard semiconductor processes. In addition, SiC possesses several properties that make it a suitable substrate for the production of biomedical microdevices. SiC is transparent, chemically inert and refractory, and has been shown to be a more hemocompatible material than Si and related biomaterials (Rosenbloom et al., 2004). Moreover, SiC is more resistant to mechanical stress than Si and possesses a wide band gap and a high electrical breakdown constant (Kotzar et al., 2002; Choyke et al., 2003; Neudeck, 2001), making it a suitable candidate for the production of multi-frequency, needle-shaped impedance probes without the need to revert to complex insulation processes. In this paper we examine the feasibility of producing SiC-based needle-shaped impedance probes for continuous monitoring of impedance and temperature in living tissues, we compare their results with previously

reported Si-based probes in *in-vitro* settings and we demonstrate their applicability in *in-vivo* monitoring of ischemia.

2. Materials and methods

2.1. Silicon devices

Silicon micro-needles for impedance measurement (see Figure 1A) were fabricated using previously described methods (Ivorra et al., 2003). Basically, Ti/Pt (30+180 nm) electrodes were deposited and patterned on a 525 μm -thick P-type <100> Si wafer using a lift-off technique, and the device was later passivated through the chemical vapor deposition (CVD) of a standard $\text{SiO}_2\text{-Si}_3\text{N}_4$ (300+700 nm) passivation layer that was also patterned to open windows for electrodes and bonding pads. Ti/Pt electrodes were then electrochemically coated with a porous layer of black platinum through a customized process of platinization to reduce their impedance (Geddes, 1972, Ivorra et al., 2003). Individual needle-shaped devices were sawn from the wafer using a standard microelectronics saw. The completed devices (see Figure 1A) are 14.85 mm-long and 0.83 mm-wide, with two pairs of 300x300 μm^2 Pt electrodes placed 2.8 μm apart along the device length.

2.2. SiC devices

SiC needle-shaped impedance probes (see Figure 1B) were produced in standard clean room conditions using a two-step photolithographic process very similar to that used for Si devices. A 4H-SiC 310 μm -thick silicon carbide wafer was first thermally oxidized to grow a 1.5 μm SiO_2 layer. Over this layer, Ti/Pt (30+180 nm) electrodes were then deposited and patterned through lift-off. Subsequently, a double $\text{SiO}_2\text{-Si}_3\text{N}_4$ (300+700 nm) layer was deposited by CVD to passivate the device. This passivation layer was then patterned to open windows for electrodes and bonding pads. As in the case of Si devices, Ti/Pt electrodes were then electrochemically coated with a porous layer of black platinum to reduce their impedance through a customized process of platinization, and individual needle-shaped devices were sawn from the wafer using a standard microelectronics saw. The finalized devices were 14.85 mm-long and 0.83 mm-wide, presenting the same electrode arrangement of Si devices.

2.3. Electrode characterization

Two-electrode measurements of electrode-electrolyte interface impedance were conducted to validate the stability of the black platinum layer stability in both Si and

SiC devices using a commercial impedance analysis system (SI 1260, *Solartron Analytical*). The sensors were immersed in physiological saline solution (0.9% [NaCl]; nominal resistivity at 298 K= 71.3 Ω -cm) and an impedance spectroscopy was conducted. Independent impedance measurements were carried out for each electrode couple, recording the impedance modulus and phase at several discrete frequencies in the 10 Hz to 1MHz range. These measurements were carried out both before and after the *in-vivo* experiments, in order to validate the stability of black Pt electrodes in *in-vivo* conditions.

2.4. Device housing and packaging

To conduct reliable measurements in both *in-vitro* and *in-vivo* settings, and to facilitate an easy handling of the completed needle-shaped devices, both Si and SiC devices were encapsulated in epoxy resin. Prior to encapsulation, each probe was fixed on a 1 mm-thick Printed Circuit Board (PCB) with H77 Epoxy resin (*Epoxy Technology*) and the probe electrodes were wire-bonded to gold contacts on the PCB linked to external contact cables. Packaging then ensued by first encasing the wire-bonding region in H77 Epoxy resin in a glob-top process, and then encapsulating the full PCB region (see Figure 1B) in C8-W795 and H-W796 resins (*Hysol*).

2.5. In-vitro experimental setup

To conduct four-electrode measurements, a front-end was added to the SI 1260 system in order to boost its nominal input impedance (Gersing, 1991). Four-electrode measurements were then conducted by immersing impedance probes at room temperature in either diluted (0.09% [NaCl]) or non-diluted (0.9% [NaCl]) physiological solution. Impedance modulus and phase angle measurements were taken from 10Hz to 1 MHz in order to estimate the acceptable frequency operation range of both Si and SiC impedance probes.

2.6. In-vivo experimental setup

Based on previous work (Genesca et al., 2004), a portable, custom-made instrumentation and its corresponding software system were also developed to carry out the four-electrode measurements of the electrical impedance in *in-vivo* conditions. Basically, the system injects an AC current in the sample through the outer electrodes, while the resulting potential is measured differentially across the inner electrodes. The main features of the custom-developed system are summarized in Table 1. The system

is able to monitor eight separate channels (one channel per probe) in a time-multiplex switch mode. Each channel was programmed to acquire one frequency value per second, sampling discrete frequency values in the 100 Hz to 100 kHz range.

To test the feasibility of system in *in-vivo* conditions an experimental setup protocol of was devised. The study was performed using male Wistar rats (*Ifa Credo*) weighting from 250 to 300 g. Animals were anesthetized with an intravenous injection of sodium pentobarbital (30 mg/kg.). All procedures were conducted under the supervision of the IIBB-CSIC Research Commission and strictly followed the EU guidelines for the handling and care of laboratory animals. The probe was placed close to the cortico-medullar junction, an area that has been found to display the most pronounced effects of ischemia-related injury in ischemia-reperfusion experiments (XXX). In small organs, such as the rat kidney, the applied electric field covers a relative high volume and the measurements may be considered representative of homogeneous tissue properties. For the *in-vivo* experiments here reported, ischemia was induced by clamping of the left kidney artery and sustained for 45 min after which unclamping and blood reperfusion ensued.

3. Results and discussion

3.1. *In-vitro* comparison of SiC- and Si-based impedance probes

To thoroughly evaluate the performance of SiC- and Si-based probes, multi-frequency four-electrode impedance measurements were conducted in an *in-vitro* setting by immersing the probes in 0.9% [NaCl] and 0.09% [NaCl] saline solutions. As it can be seen in Figure 2A, results for silicon probes in physiological serum (0.9 % [NaCl]) reveal a marked distortion with respect to the expected results for saline solution (constant impedance modulus and a zero phase angle). This loss of planarity, evidenced by a continued decrease in impedance modulus above 10 kHz, is also apparent as a distortion in the impedance phase angle above 1 kHz and originates from current leakages across the silicon substrate that result in an apparent decrease of the measured solution impedance. The effects of current leakages across the substrate become only visible above 1 kHz, since at lower frequencies the silicon-solution interface behaves as any metal-electrolyte interface and, consequently, shows a very high interface impedance in comparison to the own solution resistivity. In contrast, results for SiC probes show remarkably planar impedance modulus plots across almost all the frequency range, demonstrating that current leakages across the

substrate are effectively blocked through the use of SiC substrates. This strategy allows extending the use of impedance modulus measurements up to the MHz range, although impedance phase degrades above 10 kHz, when the parasitic capacitance effects of wiring and contact-pad interfaces become manifest.

Although the 0.9% [NaCl] is a valid reference concentration to assess the performance of impedance probes, previous reports of impedance measurements with inserted needle-shaped in *in-vivo* settings suggested that measured tissue resistivity was substantially higher than the nominal resistivity of 0.9% [NaCl] physiological serum (~200 Ω). Therefore, and in an effort to reproduce *in-vivo* conditions more accurately, a second round of *in-vitro* experiments was carried out with diluted physiological serum (0.09% [NaCl]). As expected, the results in Figure 2B show that effects of current leakages across the Si substrate become noticeably aggravated by the ten-fold increase in solution resistivity (~2 k Ω). In this setup, the decrease in impedance modulus for Si probes is apparent and more accused above 1 kHz, while phase angle distortions can be already appreciated above 100 Hz. This severely limits the range of efficient operation for Si probes in *in-vivo* environments, where the rise in tissue resistivity lowers the frequency threshold at which current leakages become detrimental. Therefore, these results show that the distortion caused by current leakages across the substrate is not a systematic error, as it depends heavily on the resistivity of the measured medium. In this respect, it should be stressed that current leakages across the substrate do not only compromise the accuracy of experiments, but also their reproducibility in slightly different settings. As in the previous setup, however, SiC probes present a quasi-planar impedance modulus up to 100 kHz range and no substantial phase angle distortions up to 10 kHz. Thus, even though the increase in solution resistivity does also have an effect upon SiC devices, as current leakages across the substrate do also take place in this kind of devices at very high frequencies, their effective range of operation extends up to the 10 kHz range. This is substantially greater than the effective operation range for Si probes, and it is significant since it allows the use of impedance phase angle readings in a multi-frequency analysis up to 10 kHz, thus improving the accuracy of generated Cole models and enabling a more detailed analysis of impedance in *in-vivo*-like conditions.

3.2. *In-vivo* evaluation of SiC-based impedance probes

In-vivo evaluation of SiC-based impedance probes was conducted on rat kidneys undergoing warm ischemia by clamping of the XXX artery. The results, shown in

Figure 3, reflect clearly the evolution of impedance modulus (A) as a consequence of arterial clamping-induced ischemia and validate the use of 0.09% [NaCl] solutions (~ 2 k Ω) as a model of the *in-vivo* setting. The substantial rise in impedance modulus observed in Figure 3A (XXX to XXX min)) can be attributed to cell swelling due to ischemia (XXX), which decreases extracellular space. Upon unclamping of the XXX artery (XXX min), impedance modulus can be seen to return to its basal value, a fact that can be attributed in this experimental setting to a reversion from a short period of ischemia without substantial structural damage to the tissue. A fall in impedance modulus at low frequencies, however, has also been reported as a consequence of cell death and membrane disruption due to sustained ischemia (XXX). It is in this respect that the multi-frequency analysis of the phase component of impedance made possible by the use of SiC-based probes conveys useful complementary information. As seen in Figure 3B, the phase component becomes informative of ischemia-induced changes above 1 kHz. At these frequencies, both the departure from and the sudden return of impedance phase angle to its basal levels reflect clearly the period of arterial clamping-induced ischemia. Most importantly, the return of impedance phase angle values to their basal levels upon reperfusion contributes to discard cell death and membrane disruption, since such events should not lead to basal impedance phase values, complementing and confirming thus the results inferred from impedance modulus readings at low frequencies.

4. Conclusions

In this work we report for the first time the fabrication and experimental evaluation of needle-shaped impedance probes based on SiC substrates. *In-vitro* results obtained with SiC-based impedance probes are compared with those obtained with Si-based probes, and we demonstrate that the use of SiC substrates is mandatory to extend the effective operation range of impedance probes beyond the 1 kHz range. Moreover, we show that this range extension allows conducting multi-frequency impedance analyses using both the modulus and phase angle components of impedance and yielding to both an improved monitoring of ischemia and the possibility of creating more accurate frequency-dependent analytical models of impedance.

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TABLES

Number of channels	8
Frequency range	1 Hz $\Rightarrow$ 1 MHz
Frequency resolution	11,64 mHz
Current amplitude	(5 μ Ap, 50 μ Ap, and 0,5mAp)
CMRR	>70dB
Input differential impedance	>50 M Ω // 5pF
Voltage differential gain	1, 10, 100, 1000
Current gain	1, 10, 100, 1000
PC rate communications	115200 bps, without parity, 8 data of bits , 1 Stop bit.

Table 1 – Impedance measurement equipment features.

FIGURES AND LEGENDS

Figure 1 – (A) Needle-shaped Si probe for impedance and temperature monitoring. (B) Needle-shaped SiC probe for impedance and temperature monitoring. Encapsulated devices are shown in the top-right boxes.

Figure 2 – (A) *In-vitro* results for SiC- and Si-based impedance probes immersed in a 0.9% [NaCl] saline solution and (B) in a 0.09% [NaCl] saline solution.

Figure 3 – Impedance modulus (A) and phase (B) *in-vivo* results for SiC-based impedance probes in rat kidney warm ischemia experiments.