Micro electrical impedance spectroscopy on a needle for *ex vivo* discrimination between human normal and cancer renal tissues

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The *ex-vivo* discrimination between human normal and cancer renal tissues was confirmed using μ EoN (micro electrical impedance spectroscopy-on-a-needle) by measuring and comparing the electrical impedances in the frequency domain. To quantify the extent of discrimination between dissimilar tissues and to determine the optimal frequency at which the discrimination capability is at a maximum, discrimination index (DI) was employed for both magnitude and phase. The highest values of DI for the magnitude and phase were 5.15 at 1 MHz and 3.57 at 1 kHz, respectively. The mean magnitude and phase measured at the optimal frequency for normal tissues were $5013.40 \pm 94.39 \Omega$ and $-68.54 \pm 0.72^\circ$, respectively; those for cancer tissues were $4165.19 \pm 70.32 \Omega$ and $-64.10 \pm 0.52^\circ$, respectively. A statistically significant difference ($p < 0.05$) between the two tissues was observed at all the investigated frequencies. To extract the electrical properties (resistance and capacitance) of these bio-tissues through curve fitting with experimental results, an equivalent circuit was proposed based on the μ EoN structure on the condition that the μ EoN was immersed in the bio-tissues. The average and standard deviation of the extracted resistance and capacitance for the normal tissues were $6.22 \pm 0.24 \text{ k}\Omega$ and $280.21 \pm 32.25 \text{ pF}$, respectively, and those for the cancer tissues were $5.45 \pm 0.22 \text{ k}\Omega$ and $376.32 \pm 34.14 \text{ pF}$, respectively. The electrical impedance was higher in the normal tissues compared with the cancer tissues. The μ EoN could clearly discriminate between normal and cancer tissues by comparing the results at the optimal frequency (magnitude and phase) and those of the curve fitting (extracted resistance and capacitance). *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4951702>]

I. INTRODUCTION

The number of kidney cancer cases has increased during the last decade.¹ In 2012, an estimated 338 000 new cases were reported worldwide;² kidney cancer is ranked as the ninth and 14th most common cancer in men and women, respectively.¹ It is also ranked as the 16th most common cause of death from cancer, with an estimated 143 000 deaths reported in 2012 worldwide.¹ Renal cell carcinoma (RCC) accounts for 90% of kidney malignancies, out of which clear cell carcinoma (70%) is the most common histologic type, followed by papillary carcinoma (10%–15%) and chromophobe carcinoma (5%).³ In recent times, the mortality trends are

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found to be stable or have been decreasing in most countries,¹ most likely because of the development of imaging modalities that allow early detection and treatment of tumor.

Partial nephrectomy (PN) is recommended as the standard treatment for small renal masses (SRMs) with size less than 4 cm (T1a renal masses) that are suspected for malignancy.⁴ Along with an excellent oncologic outcome, a lower risk of chronic kidney disease and cardiovascular disease, attributed to preserving renal parenchyma, has been the typical advantage of PN compared with radical nephrectomy (RN).^{5,6} PN has also resulted in a higher overall survival rate⁷ and lower risk of all-cause mortality⁸ than RN. Currently, minimally invasive PN treatments using laparoscopic and robotic approaches are increasingly preferred by both patients and urologists, because of not only shorter hospital stay and improved convalescence but also comparable perioperative and oncologic outcomes to open PN.^{9–11}

During the resection of a tumor from the surrounding normal renal parenchyma, there is a possibility of incomplete tumor resection with PN, resulting in positive surgical margin (PSM). In the cases of endophytic tumors in which the whole or most of the tumor is surrounded by renal parenchyma, it may be difficult to detect the exact depth and location of the tumor during the surgery, leading to higher chances of leaving PSMs. This is a sensitive issue for both urologists and patients, for it may have a negative impact in the postoperative oncologic outcomes such as cancer recurrence and overall cancer specific survival rate. To locate and estimate the depth of endophytic tumors that cannot be observed from the surface of the kidney during the surgery, intraoperative ultrasonography (USG) is applied in most of the situations. However, detecting the exact location and measuring the depth of endophytic SRMs accurately using intraoperative USG for complete tumor resection with maximum parenchymal preservation are still considered a difficult task.

To accurately locate and estimate the depth of endophytic SRMs, μ EoN (micro electrical impedance spectroscopy-on-a needle), a needle having electrical impedance spectroscopy (EIS) sensor on the needle tip, was developed by us. The EIS is considered suitable to detect different tissue properties and to discriminate between normal and cancer tissues by measuring the electrical impedance of the tissues. Many studies have reported significant differences in electrical impedance, using the EIS devices, between dissimilar cells or tissues from various organs.^{12–17} Despite the availability of EIS probes in tumor detection, it is inappropriate for detecting completely buried endophytic tumors and estimating tumor depth from the surface of the organ. On the other hand, the μ EoN is a needle with EIS incorporated on its tip, which enables the device to penetrate through the tissues and reach the tumor, as well as the deepest margin between the tumor and normal tissues. Thus, the μ EoN can locate and measure the maximum depth of endophytic tumors using electrical impedance change between normal and cancer tissues. Predicting the extent of non-visible tumor accurately using the μ EoN may reduce positive surgical margin ratio along with maximal parenchymal preservation in PN cases.

As a preliminary research, the present study focuses on evaluating whether the μ EoN can effectively discriminate between normal and cancer renal tissues by measuring the electrical impedance in the frequency domain.

II. MATERIALS AND METHODS

A. Device design

Fig. 1 shows the schematic design of the μ EoN which has interdigitated electrodes (IDEs) on the curved surface of a 22-gauge (700 μ m in diameter) hypodermic needle.¹⁸ The IDEs were designed as sensing electrodes, because the small gap (20 μ m) and width (20 μ m) of the IDEs can secure a large sensing area around each pair of IDEs in a limited space. The position of the IDEs should be determined as close as possible to the tip of the needle, in order to minimize the penetration depth for measurements and damage to bio-tissues. The overall length of the IDEs near the tip of the needle was considered to be 300 μ m, which will enable the measurements to be localized at a specific position of the bio-tissues. Considering further clinical trials, the length of connection lines was designed as 20 mm, which allows the μ EoN to measure the electrical impedance of the bio-tissues at deep locations. The structure of the μ EoN is identical

FIG. 1. Schematic design of the μ EoN (micro electrical impedance spectroscopy-on-a-needle).

to the original needle, except for the printed circuit board (PCB) on the plastic hub that facilitates electrical connection with the impedance analyzer. Thus, the primary functions of the needle, such as drug delivery and tissue/blood sampling, are maintained. Although the μ EoN was proposed for discrimination between dissimilar tissues in this study, it can also be widely applied to studies regarding electrical stimulation and recording.

B. Device fabrication

The fabrication process has been introduced in our previous study,¹⁸ employing spray coating method, film photomask, and photolithography processes. The needle was separated from the hub to fabricate the IDEs using the photolithography process. After the electrodes were fabricated on the needle coated with parylene C for insulation (3 μ m in thickness), they were recoated with parylene C (150 nm in thickness) to secure biocompatibility and to prevent the electrodes from peeling off when the needle penetrates the bio-tissues.

As shown in Fig. 2, the IDEs were successfully fabricated at the curved surface of the needle tip. The overall length and width of the fabricated IDEs were 300 μ m and 400 μ m, respectively, and both the width and gap of the IDEs were 20 μ m. The fabrication margin from the

FIG. 2. Images of fabricated IDEs at the tip of the curved surface of the needle: (a) overall length and width of the IDEs were 300 μ m and 400 μ m, respectively; fabrication margin from the tip was 475 μ m; and (b) both width and gap of the IDEs were 20 μ m.

FIG. 3. Images of experimental setup: (a) overall setup, (b) removed cancer tissue, and (c) normal tissue.

tip was as small as $475\ \mu\text{m}$, so that the penetration depth for measurements and the damage to bio-tissues can be minimized during the experiments and clinical trials in the future. To facilitate the ease of using the device, the fabricated μEoN was combined with a plastic hub and a PCB.

C. Experimental setup

One renal tissue specimen was obtained from each of ten RCC patients who underwent RN at Pusan National University Hospital from November 2015 to March 2016. The histologic type of every specimen was clear cell carcinoma with negative surgical margin. The experiment was conducted immediately after the renal tissue was removed from a patient to preserve the initial state of the bio-tissues. The μEoN was fixed on a height controller (resolution: $10\ \mu\text{m}$) and electrically connected to an impedance analyzer (Reference 600, Gamry Instruments) for impedance measurements (Fig. 3). The electrical impedances of the normal and tumor portions of the ten specimens were measured over the frequency range from 100 Hz to 1 MHz. The frequency range was decided by referring to the previous studies that are relevant to the electrode polarization in low frequencies.^{19–23} An operating voltage of $100\ \text{mV}_{\text{rms}}$ was applied to the electrodes considering the induced voltage that can cause damage to the bio-tissues.²⁴ The needle and the sample had a floating potential with respect to the electrodes. The penetration depth of the μEoN was maintained at around 10 mm using the height controller to ensure consistency in the sensor output induced from the connection lines. Each experiment was conducted after performing the cleaning process of the μEoN using acetone, methanol, and deionized water. All the

FIG. 4. Measured electrical impedances of normal and cancer tissues: (a) magnitude and (b) phase.

FIG. 5. Discrimination indices of magnitude and phase at all the investigated frequencies.

renal specimens were delivered to the pathologists after the experiment for histologic diagnosis. The present study was approved by the international review board of Pusan National University Hospital (Approval No. PNUHIRB-2015003).

III. RESULTS AND DISCUSSION

A. Electrical impedance of normal and cancer tissues

The measured magnitude and phase of the normal and cancer tissues over the frequency range from 100 Hz to 1 MHz are shown in Fig. 4. With an increase in frequency, the cancer tissues presented drastic decrease in magnitude from 1.03 M Ω to 4.86 k Ω and phase values (with sinusoidal pattern) from -74.69° to -35.58° . In the normal tissues, the ranges of the magnitude and phase were 959.05 k Ω to 4.08 k Ω and -71.50° to -40.75° , respectively. The magnitude decreased with frequency because of the imaginary part of the impedance and dielectric loss that are strongly dependent on the frequency. Higher inductive tendency of the phase was observed at high frequencies, because the tissue conductivity is larger at high frequencies than that at low frequencies.²⁵ This implied that the impedances were mainly contributed by the resistance of the cytoplasm at higher frequencies;²⁶ this was also verified through COMSOL 3D simulation. Prior to in-depth analysis, Mann-Whitney U test was performed using PASW Statistics 18 (SPSS, Inc., Chicago, IL, USA) to compare the difference in the measured electrical impedances between the normal and cancer tissues. Although the magnitudes of the normal and cancer tissues seemed to be similar, statistically significant difference ($p < 0.05$) between the two tissues was observed at all the investigated frequencies. The optimal frequency at which the discrimination capability of the μ EoN is maximized needed to be determined for optimal discrimination between normal and cancer tissues. To obtain the optimal frequency, the discrimination index (DI) that can quantify the discrimination capability of the μ EoN at all the investigated frequencies will be covered in Section III B.

B. Discrimination index for optimal frequency

For faster real-time decisions and clinical trials in the future, the discrimination capability of the μ EoN should be quantified at all the investigated frequencies. Thereby, the optimal frequency at which the discrimination capability is at a maximum can be determined to effectively discriminate between dissimilar tissues.

As expressed in Eq. (1), the DI for two different types of tissues was defined as the ratio of the mean difference and the summation of each standard deviation of the dissimilar tissues.

FIG. 6. Mean values of magnitude and phase measured at the optimal frequencies (magnitude: 1 MHz and phase: 1 kHz).

The variables and subscripts X , D , c , and n denote the mean value, standard deviation, cancer tissue, and normal tissue, respectively. The mean difference in impedance between the cancer and normal tissues should be divided by the summation of each standard deviation of the impedance measured on dissimilar tissues to remove the scale difference in the magnitude between low and high frequencies (normalization factor) and to reflect the repeatability and reproducibility of the experimental results. The DI was calculated at all the investigated frequencies for both magnitude and phase measured from the normal and cancer tissues

$$DI = |X_c - X_n| / (D_c + D_n). \quad (1)$$

The DI will increase as the difference in the mean values in the numerator increases and/or the summation of the standard deviation in the denominator decreases. Herein, a small value of the denominator implies high repeatability and reproducibility of the experimental results. The calculated DIs of the magnitude and phase at all the investigated frequencies are plotted in Fig. 5. The largest DIs for the magnitude and phase were 5.15 at 1 MHz and 3.57 at 1 kHz, respectively.

The measured magnitude and phase at each optimal frequency (magnitude: 1 MHz and phase: 1 kHz) are depicted in Fig. 6. In the case of normal tissues, the magnitude and phase were $5013.40 \pm 94.39 \, \Omega$ and $-68.54 \pm 0.72^\circ$, respectively. In the case of cancer tissues, the magnitude and phase were $4165.19 \pm 70.32 \, \Omega$ and $-64.10 \pm 0.52^\circ$, respectively. The experimental results showed that the electrical impedance was higher in the normal tissues compared with the cancer tissues, which was in good agreement with the previously reported study on renal tissues measured over the frequency range from 200 kHz to 5 MHz.²⁷ Although the discrimination at each optimal frequency seemed to be clear, it should be noted that the above magnitude and phase values included not only the tissue properties but also the device characteristics, which can hide the behavior due to sample-device interaction. To exclude the electrical characteristics of the device through curve fitting method, an equivalent circuit needs to be proposed based on the μ EoN structure on the condition that the μ EoN is immersed in the bio-tissues.

FIG. 7. Electrical equivalent circuit to extract the resistance and capacitance of sample tissues (normal or cancer).

FIG. 8. Comparison of curve fitting and experimental results in terms of magnitude and phase for (a) normal tissues and (b) cancer tissues.

C. Curve fitting for tissue resistance and capacitance

Although the proposed μEoN can discriminate between normal and cancer tissues clearly, the experimental results include not only the resistance and capacitance of normal and cancer tissues but also the device characteristics, such as resistance and capacitance induced by parylene passivation, double layer capacitance, and non-uniform electric field caused by non-uniform surface of the needle. Thus, the extraction of the tissue-only properties from the total sensor output was required to obtain more informative and accurate properties of normal and cancer tissues. As depicted in Fig. 7, an equivalent circuit was proposed based on the μEoN structure on the condition that the μEoN was immersed in the bio-tissues.^{28–34} The total sensor output is a combination of the resistance (R) and capacitance (C) of parylene C and tissue samples, double layer capacitance, and constant phase element (CPE).

Subscripts p, dl, and t indicate parylene C, double layer, and tissues (cancer or normal), respectively. The CPE represents the non-ideal capacitive behavior induced by the fractal nature of the electrode surface, surface roughness, and non-uniform current distribution.³⁵ In reality, as shown in Fig. 2, the needle has randomly distributed surface defects such as scratches and craters; thus, the CPE was placed facing the electrodes in the equivalent circuit. C_t and C_p are connected in parallel with R_t and R_p , respectively, and in series with C_{dl} (double layer capacitance) and CPE .

Curve fitting was performed using Echem Analyst (Gamry Instruments) software to extract the electrical components (R_t and C_t) of the normal and cancer tissues. The curve fitting results were compared with the experimental results, as shown in Fig. 8.

For both cases (normal and cancer tissues), the fitting results nearly matched the averaged experimental results. As shown in Fig. 9, the average and standard deviation of the extracted resistance and capacitance for the normal tissues were $6.22 \pm 0.24 \text{ k}\Omega$ and $280.21 \pm 32.25 \text{ pF}$,

FIG. 9. The average and standard deviation of the extracted resistance and capacitance of normal and cancer tissues by curve fitting with experimental results.

respectively, and those for the cancer tissues were $5.45 \pm 0.22 \text{ k}\Omega$ and $376.32 \pm 34.14 \text{ pF}$, respectively. While the resistance of the cancer tissues was lower than that of the normal tissues, the capacitance of the cancer tissues was larger than that of the normal tissues. The magnitude decreases as the resistance decreases and/or the capacitance increases, which implies that cancer tissues have less electrical impedance than normal tissues. It can be explained by the fact that the malignant tissues are lack of a tight junction between the cells compared with the normal tissues, and that the malignant cells have higher water content than normal cells.^{22,36–38} Therefore, electrical current could flow intracellularly through a malignant cell with less resistance. The suggested μEoN could discriminate between normal and cancer renal tissues clearly, and it can be considered that the μEoN can locate and measure the maximum depth of endophytic tumors during PN, thus reducing the positive surgical margin ratio.

IV. CONCLUSIONS

The electrical impedance of ten renal cancer and normal tissue specimens was measured using μEoN over the frequency range from 100 Hz to 1 MHz. The experimental results indicate that the cancer tissues have less electrical impedance than normal tissues. Considering sensor output, the magnitude at 1 MHz is recommended as the optimal parameter to discriminate between normal and cancer tissues, because the DI of magnitude (5.15 at 1 MHz) was higher than that of phase (3.57 at 1 kHz). Through curve fitting, it was confirmed that the cancer tissues have less electrical impedance than normal tissues. The μEoN could clearly discriminate between normal and cancer tissues based on the sensor outputs (magnitude and phase) and tissue properties (extracted resistance and capacitance). It can be considered that the μEoN will be able to locate and measure the maximum depth of endophytic tumors during PN to reduce positive surgical margin ratio.

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