Non-invasive Ring Electrode with a Wireless Electrical Recording and Stimulating System for Monitoring Preterm Labor

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***Abstract*— Preterm labor and birth are the primary causes of neonatal morbidities and mortalities. The early detection and treatment of preterm uterine muscular contraction are crucial for the management of preterm labor. In this work, a ring electrode with a wireless electrical recording and stimulating (RE-WERS) system was designed, fabricated, and investigated for the non-invasive monitoring of uterine contraction/relaxation as a diagnostic and therapeutic tool for preterm labor. By using an organ bath system, we confirmed that the uterine contraction force in mice can be decreased by the application of electrical stimulation. Then, the RE-WERS system was inserted non-invasively through the vagina to the cervix of a pregnant minipig, and it successfully recorded the uterine contraction and reflect signals when various electrical stimulating conditions were applied. The difference in the uterine signals before and after the injection of a labor induction drug, such as oxytocin and prostaglandin F2α, was recorded, and the difference was remarkable. In addition, the uterine signal that was recorded was well matched with the signal of the electromyography (EMG) kit during open abdominal surgery. It seemed that the continuous and various electrical stimulations affected the delay or inhibition of childbirth in the pregnant minipig.**

***Index Terms*—Preterm labor, Noninvasive, RE-WERS, Electrical stimulation, Recording**

I. Introduction

The World Health Organization (WHO) defines preterm birth as delivery before the end of 37 weeks of pregnancy [1]. Worldwide, 11.1% of fetuses are born prematurely, and the rates of preterm birth vary from 5 to 18% depending on the country [2]. Preterm birth is the main etiology of neonatal morbidity and mortality, and it is the second-ranked direct cause of death for children under the age of 5 [3]. Various mechanisms can cause preterm labor and birth, such as infection, cervical disease, and the breakdown of maternal-fetal tolerance caused by genetic and environmental factors [4]. However, the definite causes of preterm birth are still unclear.

Current therapies for the prevention of preterm birth, including tocolytic medicines and surgical procedures, such as cervical cerclage during pregnancy, have shown minimal effectiveness, and they can have both maternal and fetal side effects. The cervical cerclage is used in patients who are considered to have cervical insufficiency, which occurs when the cervix starts to shorten and dilates too early [5]. Tocolysis is the administration of tocolytic drugs, such as nifedipine (calcium antagonist), atosiban (oxytocin-receptor antagonist), and ritodrine (β-adrenergic-receptor agonist) to patients who have premature onset of uterine contractions [6]. Although these therapies for the prevention of preterm birth may postpone delivery for a few days, they have not been shown to improve fetal outcomes, and they have limitations, such as minimal efficacy and high incidence of adverse effects for both the mother and the fetus, including cardiovascular, endocrine and skeletal effects. The ideal technique for the prevention of preterm birth would delay delivery without adverse side effects for either the mother or the fetus, and it would do so at a reasonable cost.

Electrical stimulation currently is a novel technique that may become an ideal technique inhibit uterine contraction, and, from the clinical perspective, it may be a cost-effective, user-friendly therapeutic tool if it is constructed as non-invasive system. Recently, there have been active studies of the technique known as functional electrical stimulation (FES) in which an electrical current is applied to a peripheral nerve by a transcutaneous, percutaneous, or implanted electrode. Implantable pacemakers or defibrillators, foot-drop stimulators, cochlear stimulators are examples of the uses of FES, in that the pattern of the stimulation results in the direct generation of a functional movement or sensation [7]. Some research groups have studied the electrical inhibition of preterm birth by inhibiting uterine contractility in rabbit and rat animal models [8-11]. They reported that spontaneous uterine contractions can be inhibited locally and irreversibly in preterm pregnant rabbits and rats by electrical inhibition, and they were convinced that electrical inhibition would be effective in preventing preterm births for women. The previous works concerning such electrical

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inhibition were conducted only in *in vitro* experimental setups, and there have been no cases in which testing was conducted for the practical therapeutics associated with an open abdominal situation.

Herein, we present a newly designed, fabricated, and investigated non-invasive ring electrode with a wireless electrical recording and stimulating (RE-WERS) system for continuous or periodic monitoring of uterine contractions as a therapeutic agent or tool for use when preterm labor occurs. The ring electrode includes a 1-pair stimulating, 1-pair recording electrode, a mini-board with a pre-amplifier in a flexible polydimethylsiloxane (PDMS) cover, and a wireless communication module/battery.

Before applying the ring electrode to the uterus of a mid-sized animal, the efficacy of the electrical stimulation for inhibiting uterine contractions was investigated using a uterus that was extracted from a C57BL/6 pregnant mouse in an organ bath system. Then, the wired ring electrode and the RE-WERS system were inserted non-invasively through the vagina and into the minipig cervix so that the recorded uterine signals associated with the various electrical stimulations could be analyzed. In addition, the contraction and relaxation of the uterus was observed by using a commercial electromyography (EMG) kit and a micro-needle electrode during open abdominal surgery, and, simultaneously, the recorded signals of the RE-WERS system that was inserted at the minipig's cervix were used to evaluate the function. To the best of our knowledge, this work represents one of the rare efforts to develop a personalized therapeutic agent for preterm labor. In addition, the study that was required to develop a non-invasive system that could monitor the contraction of the uterus resulted in a valuable contribution to such research in that it can provide clues or solutions for the early diagnosis and treatment of preterm labor in terms of electroceuticals.

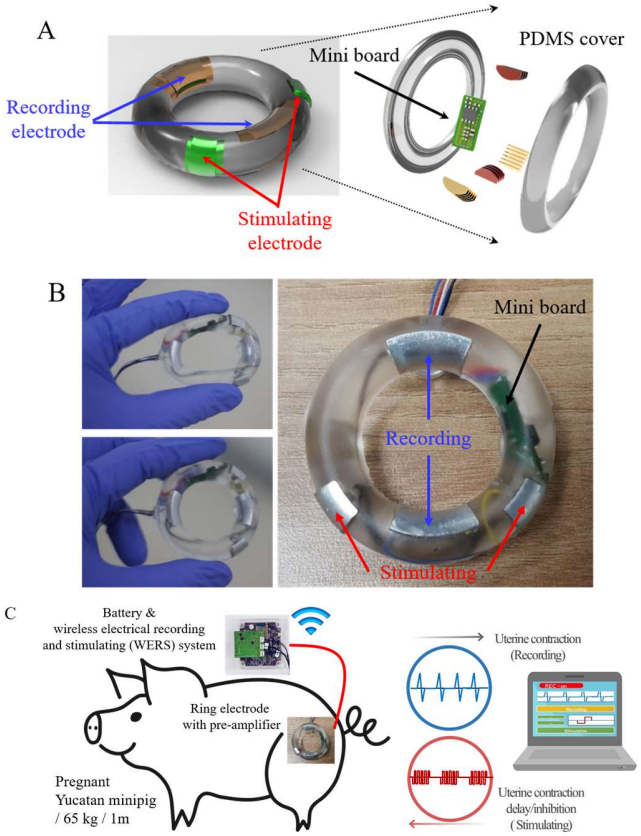

Fig. 1. 3D conceptual drawing for the ring-shaped electrode with stimulating, recording electrodes, and pre-amplifier (A); Photograph images for the fabricated ring electrode (B); Schematic drawing for acute *in-vivo* experimental setup at cervix of pregnant minipig using ring electrode with wireless electrical recording and stimulating (RE-WERS) system (C).

II. Materials and Methods

A. Design and fabrication of the ring electrode

The design of the ring-shaped electrode was a part of our attempt to reduce the risk of invasive surgery and to have various favorable characteristics, such as being user-friendly, allowing self-handling vaginal insertion, flexibility, and wireless electrical stimulation and recording functionalities. The ring electrode consists of a donut-structured PDMS with a 5-cm outer diameter and 3-cm inner diameter, 1 pair of stimulating electrodes and 1 pair of recording electrodes. The ring electrode was constructed in duralumin mold by using PDMS casting after wiring the duralumin electrode pieces and mini-board (pre-amplifier). Fig. 1 shows graphical drawing (A) and the ring electrode that was fabricated (B). It also shows the acute *in-vivo* experimental setup for the RE-WERS system at the cervix of the pregnant minipig (C). As shown in Figs. 1A and B, the stimulating electrodes were located at the 4 o'clock and 8 o'clock positions on the outside of the ring. These positions were based on the anatomical description for direct contact between the electrodes and the cervix connected to the uterosacral ligaments, which contain sympathetic and parasympathetic nerve fibers via pelvic splanchnic nerves and inferior hypogastric plexuses. It is well known that the electrical stimulation of the sympathetic and parasympathetic nerves is directly associated with contraction and relaxation of the uterus [12-15]. The pieces of the recording electrode were located at the 6 o'clock and 12 o'clock positons inside of the ring. This ring electrode was designed and fabricated for non-invasive applications involving the human cervix, as shown in the conceptual drawing in Supplementary Fig. S1.

B. Design and fabrication of the wireless electrical recording and stimulating system

The wireless electrical recording and stimulating (WERS) system (Fig. 2A) has three essential features, i.e., 1) the analog front-end (AFE) circuit that amplifies, filters, and makes analog-to-digital conversion (ADC); 2) the bi-phasic current mode differential neuro-stimulator that utilizes an improved Howland current pump (IHCP) (in Supplementary Fig. S2) [16]; 3) a wireless data acquisition (DAQ) that controls the real-time stimulation pattern to the stimulating electrodes and transmits the data that are recorded from the AFE to a personal computer.

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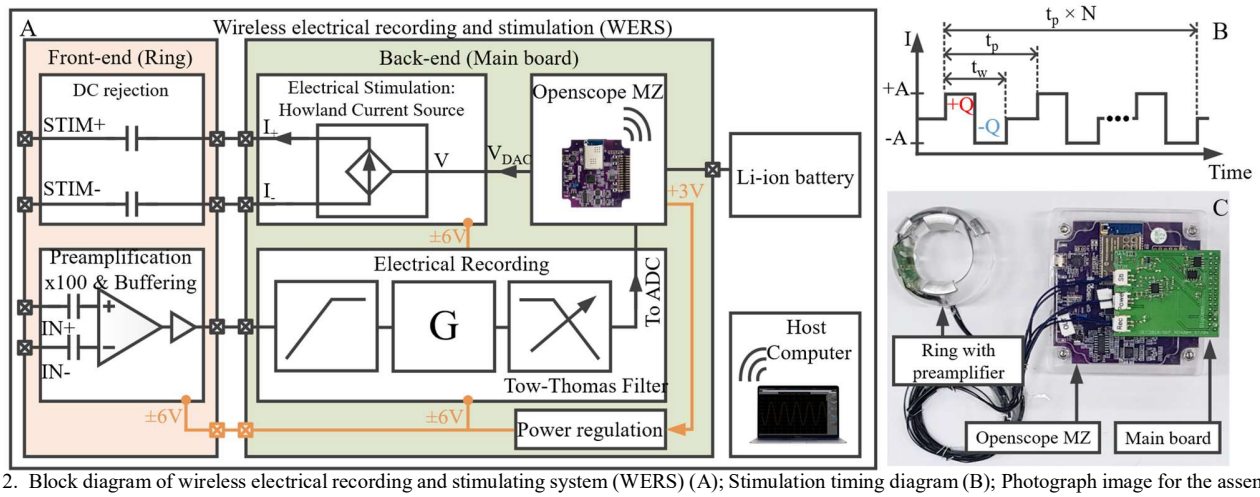

Fig. 2. Block diagram of wireless electrical recording and stimulating system (WERS) (A); Stimulation timing diagram (B); Photograph image for the assembled RE-WERS system (C).

The AFE includes two stages, i.e., 1) an in-ring high-pass amplifier, and 2) an ex-body band-pass filter with programmable gain. A fully differential input greatly suppresses common-mode interference (CMI) using the high common-mode rejection ratio (CMRR) of the instrumentational amplifier. The differential input from the recording electrodes connects to 1st order high-pass (0.1~7 Hz) preamplifier with a buffer implemented in a small-footprint, printed circuit board (PCB, 8 mm × 20 mm) to fit in the ring. Then, the single-ended output from the buffer connects through a meter-long wire to a programmable, anti-aliasing, Tow-Thomas filter (TTF) [17] with variable gain and that is low-pass corner programmable in the range of 0 – 26 dB (1 - 20 V/V) and 1.25 - 5 KHz, respectively. The high voltage gain (20/40 dB) of the instrumentation amplifier (AD8429, AD) relaxes the noise requirement of the following stages, where the low output impedance of the line-drive buffer amplifier (BUF634, TI) decouples the capacitive load from the first stage, thereby ensuring stability and reduces the electromagnetic interference (EMI) coupling to the wire. The anti-aliasing Tow-Thomas bandpass filter consists of 3 rail-to-rail amplifiers (TLV2451, TI), and the gain and bandwidth (BW) of the filter are programmable via shunt switches. The maximum gain of AFE measures 66 dB, and it suppresses the input-referred quantization noise (QN) below 5.7 μV_{rms} and 1.4 μV_{rms} for 10 and 12 bits, respectively, giving the ADC an input range of ±20 V. Thanks to the low noise front-end amplifier and Tow-Thomas filter the overall input-referred noise of the AFE is rather dominated by the quantization noise which measures (input Saline shorted) 1.36 μVrms at the bandwidth of 0.7 - 5 KHz and resolution of 12 bit.

The biphasic current stimulation pattern is defined using a user interface that allows a 0.1-μs resolution control of the width of the pulse (t_W), period (t_P), 1 μA amplitude (A), and number of pulses (N) in a single burst. To avoid an irreversible electrolyte reaction, charge-balanced biphasic stimulation pulse pattern is generated that matches the aforementioned amplitude and width setting (Fig. 2B).

The IHCP was implemented using a rail-to-rail amplifier (LM7301, TI). The range of the output current is defined as a function of V_{in}/R_{REF}, where the input voltage (V_{in}) of ±3 V and the R_{REF} of 3 KΩ defines the maximum current amplitude of ±1 mA. For the safety of the user, the minimum amplitude of the current is preferable, so we fine tuned the amplitude of the stimulation with a precision of 1 μA satisfied by the 10-bit (60-dB) dynamic range of the DAQ digital-to-analog converter (DAC). The output of IHCP is AC-coupled via a 0.1 μF ceramic capacitor to the stimulating electrode. A static offset/mismatch current from the manual calibration of the IHCP in conjunction with a big shunt resistor (R= 100 KΩ) removes the unbalanced charges on the output of the IHCP to avoid non-linearity and saturation.

The wireless DAQ was built around a commercial-off-the-shelf (COTS) module (Openscope MZ) that is capable of 12-bit 6.25 MS/s analog sampling resulting in a signal with a bandwidth of 2 MHz and a 10-bit, 10 MS/s arbitrary waveform generator with a full range of ±3 V to provide real-time, closed-loop control through a simple graphic user interface program that is available at the host computer via Wi-Fi.

In order to decouple the power supply noise from the IHCP and DAQ to the AFE circuits and generate a four-quadrant power supply, we used a switched-capacitor voltage converter (MAX1680, MAXIM) to generate ±6 V. A Li-ion battery (3.7 V, 1000 mAh) supplied the Openscope MZ, while the DAQ low-dropout voltage regulator supplied the switched-capacitor voltage converter. Fig. 2C shows the design we used, which consisted of a ring electrode with a preamplifier, a Main board (TTF, IHCP, and power regulation unit), and an Openscope MZ.

The key performance metric of the WERS system is summarized in Table I.

C. Measurement of the wired ring electrode and the RE-WERS system

For the wired ring electrode test, the stimulating electrode was connected to a pulse stimulator (Isolated Pulse Stimulator, Model 2100, A-M Systems, Sequim, WA, USA). Also, the

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Table 1. Summary of WERS characteristics

Parameter	Our work	[18]	[19]	[20]
Gain, dB	20 – 66	0 – 21.5	51.5	50-70
BW, Hz	7 – 1.25K/2.5K/5K	500 – 700K	1-5K	0.59 – 117
IRN[1], μV_{rms}	1.36 (0.7Hz-5KHz)	0.1 (500 – 700KHz)	4.2 (1-1KHz)	2.09 (0.59 – 117Hz)
CMRR, dB	90	86	65	-
ADC Sampling rate, KS/s	2.5 – 10	250	-	-
ADC Resolution, bit	10-12	10	7.1 (ENOB)	10
Stimulation Amp. µA	1 – 1000	<1000	10 – 1000	500 – 3000
DAC res.	7(10)b		8b	-
Pulse Width res.	10b		4b	-

1. IRN – input referred noise

recording electrode was connected to a differential amplifier (Differential AC amplifier, Model 1700, A-M systems, Sequim, WA, USA). The amplified contraction signals of the uterus were collected in a data acquisition (DAQ) device (NI USB-6356, National Instruments, USA). Then, the signals that we collected were processed using LabVIEW software (National Instruments, USA) and displayed on a laptop computer. The electrical stimulation was applied with the amplitude of the pulse ranging from 100 to 500 µA with a width of 100 µs. The differential amplifier gain was a factor of 1000.

The wireless communicated electrical stimulation and uterine signal recording at the cervix of the minipig was performed with the RE-WERS system. The electrical stimulation was applied with various combinations of current pulse of 204 - 1020 µA, pulse widths of 100, 199, 301, 499, and 1000 µs, periods of 52, 99, 200, 496, and 996, pulse repetitions of 10 and 100, and signal gains of 400 and 800. The detailed stimulating conditions for Figs. 6 and 8 are in Supplementary Table S1.

D. Organ bath system using the extracted uterus of a mouse and a cuff electrode for stimulation

All mouse experiments were approved by the Institutional Animal Care and Use Committee for the Care and Use of Laboratory Animals at Korea University (KOREA-2017-0083). Sexually-mature, C57BL/6 mice were used in the study. The pregnant mice were purchased from Koatech (Pyeongtaek, Gyeongi-do, Korea). Water and food were supplied ad libitum. The experimental room was kept under controlled conditions of light (12 h light/12 h dark) and temperature (22 – 24 ℃).

The experiments with the mice were conducted with two models, i.e., 1) normal delivery mice and 2) Preterm Prelabor Rupture of Membrane (PPROM) preterm delivery mice. The PPROM mouse model was made as described previously [21]. The mice were anesthetized on day 15 of gestation by inhaling isoflurane. The mice were cut up the abdomen and the fetal membranes of the uterus were pierced with sterile needles. The rupture of the membranes was confirmed by the flow of amniotic fluid out of the uterus. The subcutaneous tissues of the opened abdomen were sutured with vicryl and the skin was stitched with black silk.

On day 16 of gestation, the mice of the two models were euthanized with CO_2 just prior to the tissue being harvested. The uterine body, which measured 3 mm from the internal os of the cervix, and the attached intact cervix were isolated *en bloc*. The uterine body was sutured with black silk at just above the cervical internal os and the opposite end of the uterine body. The tissue was bathed in Krebs buffer, which was continuously oxygenated with a gas mixture of 95% O_2 and 5% CO_2.

The sutured uterus was mounted on the clamp wires of a myograph (820MS, Danish Myo Technology, Hinnerup, Denmark) and lowered into 7 ml tissue baths that were filled with a standard Krebs buffer maintained at 37 °C. The electrical stimulating was conducted by using cuff electrode, which were used in the uterine cervix implant in our previous work [22-24]. Then, the tissue was placed under a basal tension and left to equilibrate for approximately 20 min. The electrical stimulation was applied with a pulse amplitude that ranged from 300 to 500 µA and a pulse width that ranged from 1 to 100 µs (Supplementary data Table S2 and Fig. S3).

The myograph clamp support was connected to force transducer. The amplified tension signals of the uterus from the force transducers can be shown in gram and were collected in a data acquisition (LabScribe-software, iWORX, USA). Two mice per group were used, and electrical stimulation was performed for five minutes according to stimulating condition. The value of the relative contraction force was the average of values that were acquired three times in 100 s (n = 6).

E. Acute in-vivo pregnant minipig experiment

All minipig experiments were approved by the Institutional Animal Care and Use Committee for the Care and Use of Laboratory Animals at Korea Institute of Toxicology (KIT-B219072-1910-0339 and KIT-B219034-1904-0137). The pregnant minipig were used in the acute *in-vivo* experiment. The pregnant Yucatan minipigs were obtained from Optifarm Solution Inc. (Ochang, Korea). On day 100 and 115 of gestation, the minipig was anesthetized by inhaling isoflurane. The ring electrode was inserted through the vagina to the cervix, and the stimulating electrode of the ring electrode contacted to the uterosacral ligament.

F. Immunohistochemistry stain

The tissues samples were placed in 10% neutral buffered formalin overnight and subsequently embedded in paraffin. Then, the tissue samples were sectioned (5 µm), deparaffinized, processed for antigen retrieval, blocked, incubated with primary antibody [S100 (Cell signaling cat#ab868, 1:50), Tyrosine hydroxylase (Millipore cat#AB152, 1:100), nNOS (Abcam cat#1376, 1:100)], and fluorescence or peroxidase-conjugated secondary antibody. Samples were mounted and photographed using microscopy (Zeiss; LSM800 or Zeiss; AxioVision). For comparison among the groups of experiments, images were

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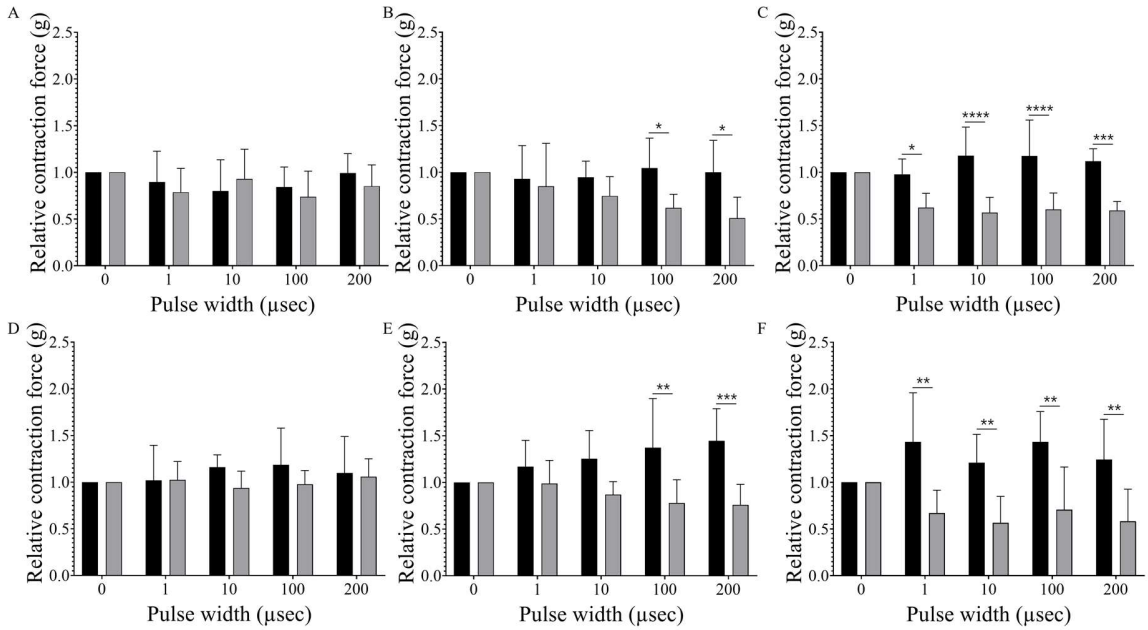

Fig. 3. Relative natural contraction (black bar) and contraction in response to electrical stimulation (gray bar) of uterine body according to the various electrical stimulation to uterine cervix; Normal delivery model pregnancy mice (A-C) and Preterm delivery model pregnancy mice (D-F); Pulse amplitude 500 μA (A, D), 400 μA (B, E), 300 μA (C, F). The differences between the data from 'natural contraction' and 'contraction in response to electrical stimulation' were subjected to GraphPad Prism's (version8.0) two-way analysis of variance (ANOVA; 95% confidence interval). *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.0001$.

acquired with the same exposure time. 3,3'-Diaminobenzidine (DAB) substrate was used for the peroxidase-conjugated secondary antibody, and this was followed by hematoxylin for nuclear counterstaining.

III. Results and Discussion

A. Preliminary ex-vivo test using the organ bath system and a cuff electrode

After hanging the uterine body on the myograph and transplanting the stimulator cuff electrodes to the cervix, that was equilibrated for 20 min. Contraction tension was measured at 5 min intervals and electrical stimulation was applied with 4 different pulse widths (1, 10, 100, and 200 μs) and 3 different pulse amplitudes (500, 400, and 300 μA) for 3 s, respectively. The non-stimulating condition was set to 1 to identify the relative contraction force.

When both normal and preterm delivery model mice were stimulated by a 500 μA biphasic current pulse, there was no difference between the natural contractions and the contractions in response to electrical stimulation (Figs. 3A and D).

In normal delivery pregnant mice model stimulated by 400 μA with 100 and 200 μs stimulus, the contraction force was lower than a natural contraction (Fig. 3B). The contraction force was decreased significantly when stimulated with the pulse amplitude of 400 μA and pulse widths from 100 to 200 μs in preterm delivery mice (Fig. 3E). The contraction force was decreased significantly when that was stimulated with the pulse amplitude 300 μA with pulse widths from 1 to 200 μs in the normal delivery pregnant mice model (Fig. 3C). Compared to the natural contractions of preterm delivery mice, the lower contraction force was shown at 300 μA with pulse widths from 1 and 200 μs stimulations (Fig. 3F). The bar graphs in Figs. 3C and 3F show that, compared with 400 and 500 μA, the electrical stimulation of 300 μA significantly decreased the peak contraction force of the uterine body at all pulse widths in normal and preterm delivery model mice.

In previous studies with rabbits and mice, electrodes were implanted directly into the uterine horn to stimulate it, and this approach was effective in inhibiting the contraction of the uterus [8, 9]. In our experiment, when electrical stimulation was applied to the uterine muscle indirectly via the cervix on *ex-vivo*, it was confirmed that the contraction force of the uterus was reduced. Therefore, the indirect electrical stimulation of the uterus through the uterine cervix with pulses of appropriate amplitudes and widths decreases the uterine contractions in the normal and preterm delivery mice models and is a novel method to inhibit uterine contraction. The results showed that proper electrical stimulation of the cervix with a limited range of pulse widths, amplitudes, and durations may prevent preterm delivery.

B. Expression of the sympathetic and parasympathetic nerve of the pregnant minipig

To examine the nerve distribution at the cervix and vagina, we performed immunohistochemistry of S100, which is a peripheral nerve marker. From the vagina to the cervix, whole tissues contained nerves, evenly (Fig. 4A). To determine which type of autonomic nervous system was distributed in the vagina, we detected the tyrosine hydroxylase (TH) sympathetic marker and the nNOS (parasympathetic marker). We found that the cervical and vaginal nerves were consisted of both sympathetic and parasympathetic nerves (Fig. 4B). To identify the localization of the nerves in the vagina, we performed

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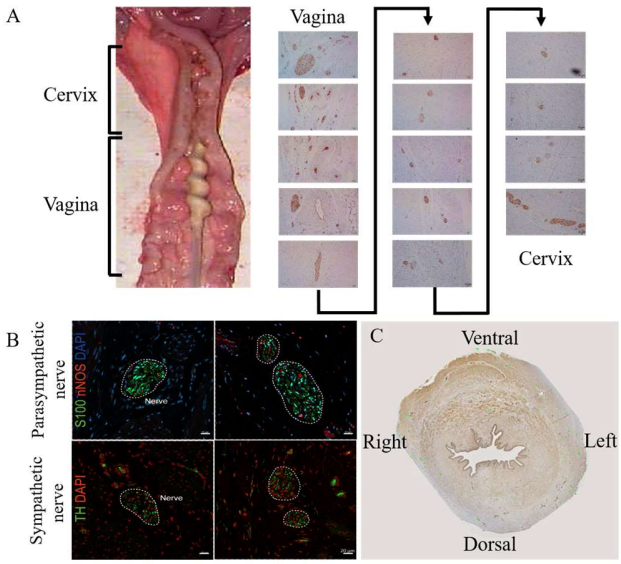

Fig. 4. Distribution of nerve at vagina of minipig; Immunohistochemistry of S100 (peripheral nerve marker) at 14 parts of cervix and vagina (A); Immunohistochemistry of TH (sympathetic) or nNOS (parasympathetic) positive cell at vagina. (B); Cross-section image of S100 at the cervix and surrounding vagina (C). Scale bar = 20 μm.

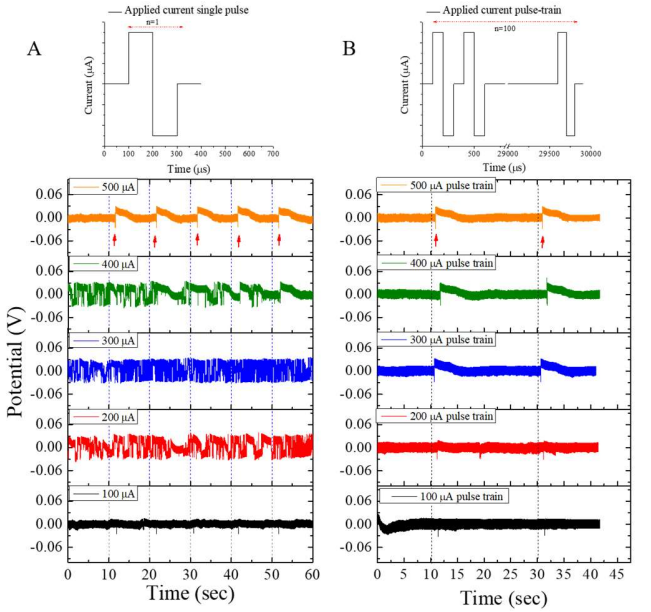

Fig. 5. Applied biphasic current single pulse, pulse-train, and recorded signal of the wired ring electrode at the cervix of minipig to the various biphasic electrical current stimulating (pulse amplitudes of 100, 200, 300, 400, and 500 μA, pulse width of 100 μs); recorded signals to single biphasic current pulse stimulating (A) and current pulse-train (B).

immunohistochemistry of the cross-sectioned cervix. We found that the uterine nerves were located mainly in the directions of the walls on the left and right sides. The nerves around 12 o' clock were considered to be bladder nerves.

That was why our RE-WERS system has the stimulating electrodes at the 4 o'clock and 8 o'clock positions, i.e., to avoid stimulating the bladder.

C. Recording of the uterine signal to the various electrical current stimulations by using a wired ring electrode at the cervix of the minipig

The recorded potential waveforms were investigated with a wired ring electrode at the cervix of a non-pregnant minipig to the various biphasic single current pulse and pulse-train. Fig. 5 shows that transient properties of the voltage were exhibited for the stimulating the electrodes fabricated with a piece of duralumin in response to various current pulses. The upper plots of Fig. 5 indicates the applied biphasic current pulses. In a single biphasic current pulse (5 times) and the application of the current pulse-train (2 times), the recorded pulse seemed to be accumulated due to the effect of the accumulation of a capacitive charge in the tissues by the injection of excessive charge [24-26]. In addition, it seemed that the current stimulation at the condition of 200, 300, and 400 μA could not be transferred smoothly to the tissues due to the unstable contact between the ring electrode and the cervix (Fig. 5A). However, a distinguished response potential was observed from the 300 μA current pulse train stimulation after modification of the contact in Fig. 5B. Therefore, we confirmed that the amplitude of the current for electrical stimulation should be greater than 300 μA.

D. Uterine signal of the RE-WERS system at the cervix of a pregnant minipig before and after the injection of the labor inducing drug

After intact insertion of the ring electrode into the cervix of the minipig, the uterine signal was monitored by the RE-WERS system to the uterine contraction/relaxation before and after intravenous injection of labor induction drugs, which were 10 IU oxytocin (SAMU Median Co., Ltd., Korea) and 2 ml prostaglandin F2α (PGF2-α, Lutalyse, Zoetis). Intravenous injections were administered into a vein in the minipig's ear with a short needle. It is well known that oxytocin has a plasma half-life of about 3 to 5 min. Following parenteral administration, uterine response occurs within 3 to 5 min. Regarding PGF2-α, the elimination half-life is less than 1 min in blood plasma. The uterine contractility upregulates oxytocin receptors and increased response to oxytocin. And the prostaglandin F2α (PGF2-α) is released in response to an increase in oxytocin levels in uterus. So, we injected oxytocin and PGF2-α to mimic the hormonal milieu in labor. Fig. 6A shows that the signal recorded by the RE-WERS system clearly showed the potential for responding to non-periodic electrical stimulation (biphasic current pulse of 254 μA, pulse period of 52/99 ms, and pulse duration of 499 μs), and dense spikes also were shown in the red circle area, which might have been caused by natural uterine contraction. The relatively different peak potential for the same current pulse occurred due to the unstable contact between the ring electrode and the cervix.

However, the signal after the intravenous injection of drugs largely was activated, even without any electrical stimulation, due to the induction of uterine contraction, while the signal to electrical stimulation can be distinguished clearly. These results indicated that our RE-WERS system operated well in both the

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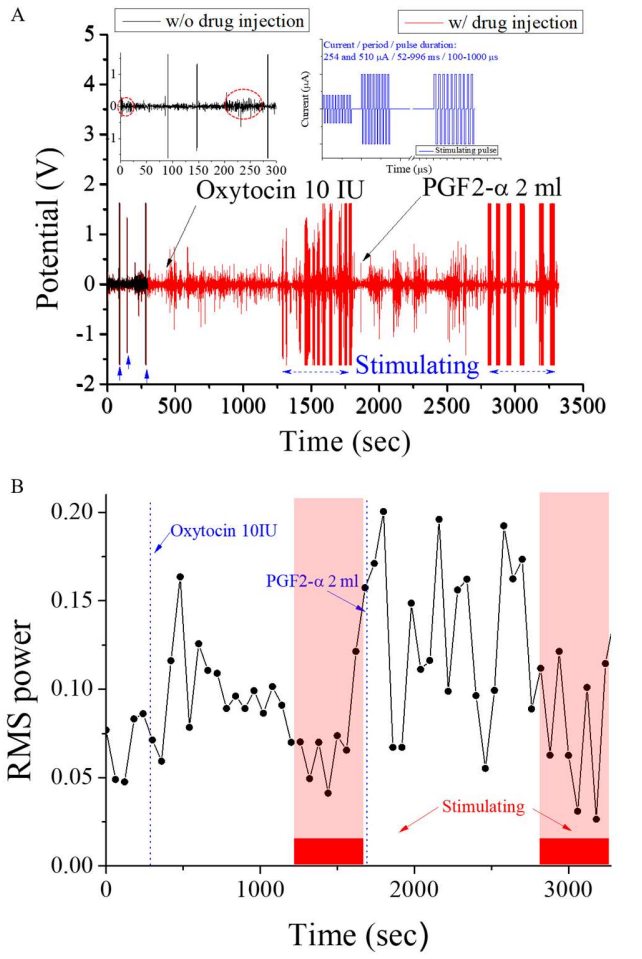

Fig. 6. Recorded signal of the RE-WERS system at pregnant minipig cervix to the various electrical stimulation (current pulses: 254 and 510 μA, pulse periods: 52-996 ms, pulse durations: 100-1000 μs); before injection (block color) of contraction inducing drug and after injection oxytocin and PGF2-α (red color). The insets indicate zoom-in view before injection of drug (left) and stimulating pulse (right) (A) and windowed RMS of the recorded contraction signal as a result of oxytocin, PFG-α, and electrical stimulation after removing artifact by stimulating (B).

recording and stimulating mode to track the contraction and relaxation of the uterus. In additions, it's not clear whether the apparent temporary decrease in the background noise was due to the effect of the electrical stimulation that lasted for 2800 - 3300 s. In fact, we removed biphasic current stimulation artifact by interpolating measurement points during current stimulation as shown on the left and calculated a 1 min windowed RMS to verify our recording system's capability to record the effect of drug (oxytocin and PGF2-α) and current stimulation (shown in Fig. 6B and Supplementary Fig.S4). A total two batch of current stimulation was applied, where the first (1300-1800 sec) session we swept current amplitude, pulse width, and pulse to pulse duration to evaluate optimal current stimulation parameter. And after injecting PGF2-α we applied fixed current stimulation parameter for multiple cycles to evaluate its' effect. The recording capability of the proposed system is verified by the difference between RMS power during baseline (0-300 sec) and after oxytocin and PGF2-α injection. The suppression of

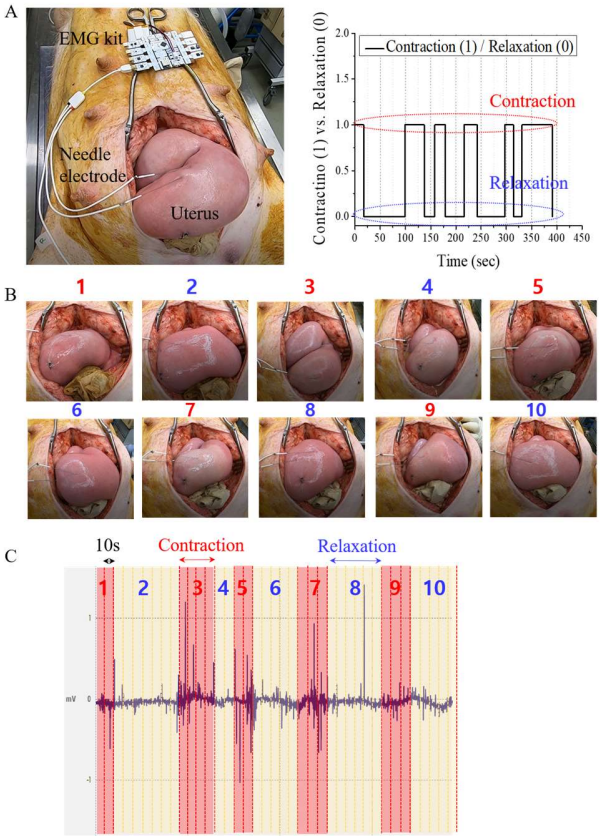

Fig. 7. Open abdominal surgery for the EMG signal recording of the pregnant minipig uterus using the commercial EMG kit and micro-needle electrodes (left) and the duration of the handwriting time plot for contraction (1) and relaxation (0) (right) (A); Photograph images of the uterus by repetitive contraction and relaxation (red color and blue color mean contraction and relaxation, respectively) (B); Recorded EMG signal for the uterus of pregnant minipig (C).

contraction can also be observed while we apply effective stimulation current parameter (1300-1550 sec and 2800-3200 sec). The results are comparable with the results shown in Fig. 3. In order to inhibit uterine contraction, proper electrostimulation conditions, such as pulse amplitude, pulse frequency, etc. are essential.

E. Recording the EMG signal of the uterine contraction and relaxation in a pregnant minipig by open abdominal surgery

In order to gain a more intuitive observation of uterine contraction, the contraction and relaxation of the pregnant minipig's uterus were observed after open abdominal surgery at the gestational age of 115 days. As shown in Fig. 7A, handwriting and a commercial EMG kit with a microneedle electrode were used simultaneously to check the contraction and relaxation of the uterus. In the handwriting, the contraction and relaxation was represented by the values of 1 and 0, respectively. The repetitive change of the volume of the uterus due to the contraction and relaxation was observed clearly by the unaided eye, as described in Fig. 7B. In addition, the simultaneously measured EMG signal also showed a spiked change (< 1 mV peak potential) during the same period as the uterine contraction (Fig. 7C). The time interval between the

contractions of the minipig that was about to give birth ranged between 21 and 82 s. These results are meaningful from a clinical perspective. Practically, if a pregnant woman feels premature regular uterine contractions, labor for childbirth can be diagnosed, and the timing of treatment already may be too late to prevent preterm birth. The current RE-WERS system may make it possible to acquire an early diagnosis of preterm labor and birth.

F. Recording of uterine signals by the RE-WERS system in response to the various electrical stimulation of the cervix of the pregnant minipig

In the same experimental setup, the RE-WERS was inserted into the cervix of the pregnant minipig, and the uterine signal was measured continuously during different levels of electrical stimulation. The measurements were conducted repeatedly after slightly changing the positon of the ring electrode. As was mentioned in the design and fabrication section and as shown in Supplementary Fig. S1, the ring electrode was designed to fit well into a human cervix. Thus, the current design of the ring electrode is believed to have caused some contact problems between the electrode and the cervix of the minipig because it differs slightly from the anatomical cervical structure of minipigs. The obtained uterine signal by the RE-WERS was well matched with the EMG signal and the duration of the handwriting time by uterine contraction/relaxation (Supplementary data Fig. S5). In Fig. 8, the recorded uterine signal was distinguished easily during the non-periodic stimulation, the various electrical stimulations (current pulse of 204-1020 μA), and the system gain (400 and 800). When the ring electrode was positioned or re-positioned into the cervix at 100 s and 1600 s, the response potential was saturated due to an unstable contact. At the gain of 400 and a stimulating current pulse of 508/714 μA in Fig. 8A, the peak-to-peak response potentials were about 0.6 and 0.8 V, respectively. Fig. 8B shows that the response peak-to-peak potentials to the 508 μA current pulse stimulation were increased about factors in the range of 4 to at a gain of 800 compared to a gain of 400. The different response potential to the same electrical stimulating condition might have been caused by an unstable contact between the ring electrode and the cervix. There are some salient points based on these results, i.e., 1) If the ring electrode can more be modified to provide better contact with the cervix of the minipig, it is expected to have a clearer signal resolution; 2) In Fig. 8, although non-periodic and various electrical stimulations were applied to the ring electrode rather than a regular, fixed simulation, it seemed that the levels of the spikes in the background decreased with the passage of time without electrical stimulation after about 900 s. Also, although the pregnant minipig had already given birth to two piglets every 15 min before the experiment was started, she gave birth to two more piglets after the test ended without giving birth to any piglets during the test. We are not sure that the delay in giving birth necessarily was due to the application of electrical stimulation was applied. However, the overall reduced background noise level, including when the electrical

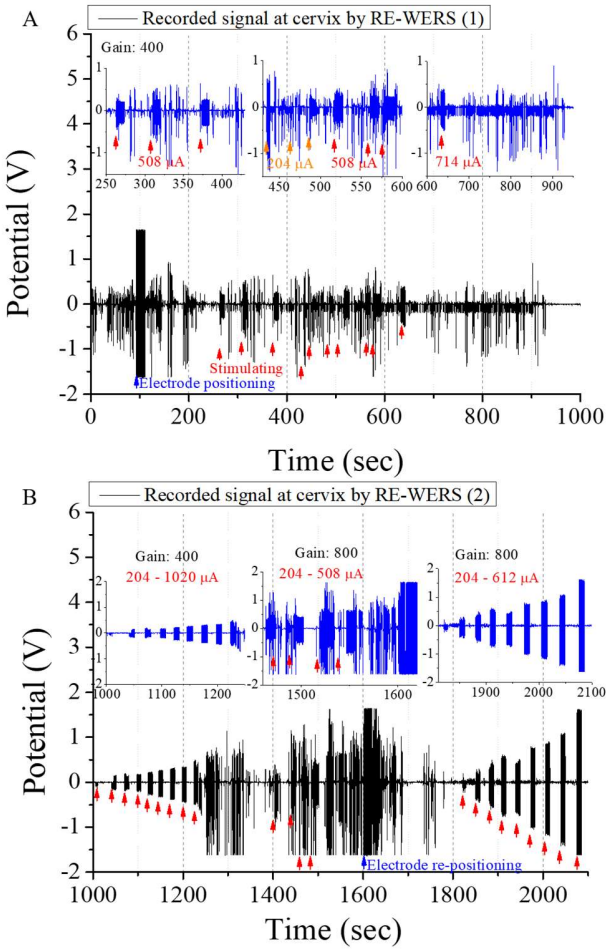

Fig. 8. Recording of uterine signal of the pregnant minipig's cervix by the RE-WERS system according to the various electrical stimulation conditions: Stimulation conditions are gain of 400 and current pulse amplitudes of 204, 508, and 714 μA (Inset plots indicate zoom-in view) (A); Stimulation conditions are gains of 400 and 800, current pulse amplitude of 204, 308, 406, 508, 612, 714, 814, 916, and 1020 μA, pulse width of 400 μs, and repetition of 100, respectively. (Inset plots indicate zoom-in view) (B).

stimulation was applied, means that the stimulation of the ring electrodes had an effect on the rhythmical contraction/relaxation cycle.

In previous reports, the nerves in the vagina and cervix were densely innervated [27, 28]. The relative difference in the density of the adrenergic and cholinergic nerves in the vagina of a rat was identified, and the density of the cholinergic nerve was higher in the proximal site, and the density of distal rat's vagina was similar [29]. Contraction of the smooth uterine muscle can be produced by activation of both parasympathetic and sympathetic nerves via the muscarinic cholinergic receptors [15]. When nerve stimulation via the RE-WERS system was applied to the cervix and the surrounding upper vagina, where the nerve-contained uterosacral-cardinal complex is densely attached, the electrical stimulation may release various muscle relaxing substances, such as low-dose norepinephrine, vasoactive intestinal polypeptide, and interstitial free radicals [8]. The contraction-inhibitory effect might be from stimulation of the neural reflex like the sacral neuro-modulation for bladder

control, which, as pelvic organs, share the concept of autonomic neural control [30]. For now, it is uncertain what the exact mechanisms are that affect the uterine inhibitory role.

IV. Conclusion

First, we investigated, designed, and fabricated a non-invasive ring electrode with a wireless electrical recording and stimulating (RE-WERS) system in order to monitor the contraction and relaxation of the uterus, and we checked the feasibility of using the device to conduct *ex-vivo* mouse tests and acute *in-vivo* minipig tests. First, the preliminary check for the change in the force of uterine contraction due to electrical stimulation was observed in an *ex-vivo* test using a cuff electrode and a uterus extracted from a pregnant rat in an organ bath system. After the electrical stimulation was applied, the contraction force of the extracted uterus was decreased significantly. Then, we designed and fabricated the RE-WERS system, which was inserted into the cervix of a pregnant minipig. One pair of stimulating electrodes were located at the 4 o'clock and 8 positions to contact the uterosacral ligament, which are inflow route of sympathetic and parasympathetic nerve fibers to the uterus. For the intuitive observation, the contraction/relaxation of the uterus was monitored with a commercial EMG kit and a micro-needle electrode after open abdominal surgery.

The experimental results indicated that the RE-WERS could record the uterine contraction/relaxation at the cervix as well as the uterine signal to the various electrical stimulation conditions. The remarkable difference in the uterine signal before and after the injection of contraction inducing drug was recorded. In addition, the recorded uterus signal was well matched with the signal from the EMG kit and the duration of the handwriting. The continuous and various electrical stimulations might delay the time at which a pregnant minipig will give birth.

We expect that an ergonomically designed system in secure contact with the ring electrode and the cervix can be used as a therapeutic agent for the early detection and inhibition of preterm labor. Subsequently, the long-term recording signal analysis of the preterm labor model to the non-periodic and unstable contraction/relaxation pattern is needed using a pregnant minipig.

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