Electrochemical Corrosion of STS304 Acupuncture Needles by Electrical Stimulation

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Abstract
We present the first investigation of electrical corrosion in acupuncture needles after electrical stimulation. Using scanning electron microscopy, we observed the occurrence of electrochemical corrosion on the surface of stainless steel 304 acupuncture needles after electrical stimulation in the tibial muscles of rats. Biphasic pulse electrical stimuli with 10-Hz frequency, 1-mA intensity and 1-ms pulse width were applied to the needles. The terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method labels fragmented DNA. Positive staining using this test indicates apoptotic cells in electrically stimulated tissues. The risk of electrical corrosion was found to be less in bipolar, short-duration, low-current or voltage and short-period stimulation than in monopolar, long-duration, high-current or voltage and long-period stimulation. Evaluation with a scanning electron microscope revealed that electrical stimulation can increase the electrical corrosion of stainless steel 304 acupuncture needles. In biocompatibility studies of stainless steel 304 acupuncture needles for electrical stimulation, TUNEL-positive cells were detected in the tibial muscle within 5 days after electrical stimulation. The results of this study demonstrate that the corrosion products of stainless steel 304 acupuncture needles might affect the post-electrical stimulation tissue response.

1. Introduction
Electroacupuncture (EA) is a widely used form of acupuncture. Compared with manual needling, it has the benefit of a stronger therapeutic effect and is more cost effective for the patient [1]. However, the proper application of EA requires appropriate methodology for electrical stimulation at the acupuncture points of the body. Most metal acupuncture needles are made of Type 304 (STS304) or Type 316 stainless steel. Generally, stainless steels are widely employed in medical implants, such as stents and orthopedic replacements, because of their relatively low cost, ease of fabrication, reasonable chemical stability, and good resistance to corrosion. However, it is unavoidable that all metals experience some extent of electrochemical dissolution in the implant environment, which comprised ample body fluids, minerals, amino acids, and proteins [2].

Acupuncture needles composed of STS304 are increasingly being used as implanted electrodes for functional electrical stimulation of acupuncture points, or acupoints, clinically and in experimental research, even though their biocompatibility is poorly understood. The notion of biocompatibility has evolved from the previous concept of an inert
material to a more recent model based on a material's ability to perform with an appropriate host response in a specific environment [3]. The detection of apoptosis is widely used in biocompatibility studies of biomaterials.

Although several studies have assessed the in vitro behavior of these materials [2–5], no in vivo study has been performed to assess the biocompatibility of stainless steel corrosion products for electrical stimulation. The aim of this study was to examine the stability of STS304 acupuncture needles during in vivo electrical stimulation. Thus we investigated the morphological changes in acupuncture needles and acupoints after electrical stimulation.

2. Materials and Methods

2.1. Laboratory animals

All animal use was in accordance with National Institutes of Health guidelines and conformed to the principles specified by the Committee at the Korea Institute of Oriental Medicine, Animal Care and Use Protocol. In all studies, 8–10-week-old male Sprague Dawley rats (Charles River, Wilmington, MA, USA) were housed with two to four rats per cage, on a 12-hour light/dark cycle with free access to chow and water.

2.2. EA stimulation

EA was applied to the tibial muscle for 30 minutes with a pair of bipolar stimulation electrodes after placing the rats under isoflurane anesthesia (flow of oxygen and nitrous oxide mixture; 3% for induction and 1.5% for maintenance). Two stainless steel acupuncture needles (0.25 mm diameter; Dong Bang Acupuncture Inc., Kyunggi-do, Korea) were mounted in a holder with a 1 mm separation between the tips. The needle set was inserted to a depth of 5 mm (cutaneous and muscle) in the tibial muscle. We tested the degradation of the acupuncture needles with two types of electrical stimulation conditions: (1) Grass S88 stimulator and (2) PG-306 pulse generator. The Grass S88 stimulator (Grass Technologies, West Warwick, RI, USA) is typically used in animal studies with 10-Hz frequency, 1-mA, 5-mA or 10-mA intensity, and 1-ms or 10-ms width. The model PG-306 pulse generator (Suzuki Medical, Tokyo, Japan) is widely applied in the clinical treatment of humans with 2-Hz frequency, 0.01-mA, 0.05-mA or 0.09-mA intensity, and 0.25-ms width. The electrical corrosion tests were conducted more than five times under each condition. The delivered current was monitored at all times, and the polarity was reversed every 60 seconds to prevent polarization of the electrodes. The total duration of EA stimulation was 30 minutes. After the termination of EA, anesthesia was immediately discontinued, and rats usually resumed full activity within 5 minutes.

2.3. Surface characterization

The surface morphology and composition of the acupuncture needles were assessed with a scanning electron microscope (SEM; Philips 525-M; Royal Philips Electronics Inc., Amsterdam, the Netherlands) and energy dispersive x-ray spectrometry (Philips-EDAX 9100; Royal Philips Electronics Inc.). The dried specimens were mounted on aluminum stubs, cracked with the tip of a fine needle under the microscope, and examined with a SEM at 30 kV.

2.4. Fragmentation of nuclear DNA

Animals were anesthetized with an overdose of sodium pentobarbital (50 mg/kg body weight) by intraperitoneal injection on the fourth day of EA treatment and perfused intracardially with phosphate buffered saline (pH 7.4): NaCl 140 mM, KCl 3 mM, Na₂HPO₄ 10 mM, KH₂PO₄ 2 mM. The skin and muscle were carefully dissected out. Tissues were immediately cryo-embedded and stored at −20°C until processed. Apoptosis was detected via the TUNEL assay using a TdT-FragEL DNA fragmentation detection kit (Cat. No. QIA33; Calbiochem, La Jolla, CA, USA) following the manufacturer’s instructions. The kit contains all materials described below, and each step was performed according to the manufacturer’s recommendations. The tissue was cryo-embedded and 20 μm sections were collected onto SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA, USA). The sections were fixed in 4% paraformaldehyde in phosphate buffered saline (pH 7.4) for 15 minutes at room temperature and subsequently washed with tris-buffered saline. The sections were then permeabilized with proteinase K for 10 minutes to strip off nuclear proteins and then washed with tris-buffered saline. After immersion in equilibration buffer for 20 minutes, sections were incubated with TdT and biotin-labeled deoxynucleotides (dNTP-biotin) in a humidified chamber at room temperature for 1.5 hours. Next, the sections were treated similarly, but incubated with methyl green. Negative control sections were treated similarly, but incubated in the absence of chromogen. The sections were counterstained with methyl green. Negative control sections were treated similarly, but incubated in the absence of
TdT enzyme, dNTP-biotin or peroxidase-streptavidin. We also compared our sections with positive control slides provided in the FragEL Detection Kit.

3. Results

3.1. Acupuncture needle surface characterization

We used an SEM to image a representative surface of an acupuncture needle to evaluate the severity of corrosion. As shown in Figure 1, the damage to the acupuncture needles was severe, considering the level of applied current. No significant differences in the tip shape of the acupuncture needles were observed in needles that had been used for electrical stimulation with a small amount of current. By contrast, the penetrated margin of the STS304 acupuncture needle surface was degraded by atmospheric oxygen after electrical stimulation (2-Hz frequency, 0.05-mA intensity, 0.25-ms pulse width, 30-minute duration; Figure 1C). Electrical stimulation with currents in excess of the capacity of STS304 acupuncture needles resulted in severe corrosion of the tip and the penetrated margin from atmospheric oxygen (10-Hz frequency, 1-mA intensity, 10-ms pulse width, 30-minute duration; Figures 1B and 1D).

The electrical capacity of thin STS304 acupuncture needles in vivo depends on the thickness of the needles and the amount of oxygen in the atmosphere. The surface composition of the acupuncture needles was assessed by energy dispersive x-ray spectrometry. The amount of carbon, nitrogen and oxide in the tissue increased following electrical stimulation, but the amount of iron decreased due to degradation of the acupuncture needle surface (Table 1). The first step of acupuncture needle degradation following electrical stimulation was an increase in the composition of silica at the penetrated margin with air (Figure 1C, Table 1).

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Figure 1  Scanning electron micrograph of STS304 acupuncture needles. (A) and (C) were treated with an electrical current of 2 Hz, 0.05 mA, 0.25-ms pulse width and 30-minute duration using a PG-306 pulse generator. (B) and (D) were treated with an electrical current of 10 Hz, 1 mA, 10-ms pulse width and 30-minute duration using a Grass S88 stimulator. (C) and (D) show the morphology of the acupuncture needle surface inserted into the tibial muscle.
3.2. Detection of fragmentation of nuclear DNA at the EA-stimulated point

We examined apoptotic cell death due to the corrosion products of acupuncture needles that developed following 30 minutes of electrical stimulation with an electrical current of 10 Hz, 1-ms width, and 1-mA intensity (Figures 2B, 2D, and 2F). A normal region (Figures 2A, 2C, and 2E) of the same tibial muscle insertion with an acupuncture needle was used as a control. On the fifth day after electrical stimulation in the tibial muscle and skin, corrosion products of STS304 acupuncture needles were found to remain in the skin (Figure 2B), connective junction of skin and muscle (Figure 2D), and deep muscle (Figure 2F), while no significant change was seen in normal regions (Figures 2A, 2C, and 2E). Furthermore, apoptotic cell death in the skin and muscle layer appeared in the vicinity of acupuncture needle corrosion products on the fifth day after electrical stimulation.

4. Discussion

The aim of this study was to examine the stability of STS304 acupuncture needles by in vivo electrical stimulation. We found that electrical stimulation changed the morphology of the acupuncture needles and stimulated acupoints.

Little information is available to guide the acupuncturist in the placement of intradermal electrodes for EA. Generally, the needles are inserted as deeply as possible into the target acupoints, with or without concomitant electrical stimulation. We demonstrated the stability of implanted acupuncture needles used for electrical stimulation. We also studied the effects of STS304 acupuncture needle corrosion products, which formed following electrical stimulation, on the morphology of intradermal acupoints.

In the electrochemical study of STS304 in simulated body fluids over a one-week period, STS304 became more susceptible to pitting corrosion when it was covered with cells [2]. The corrosion resistance of stainless steel 316 decreased in the presence of cultured murine fibroblast L929 cells, and a decrease in diffusivity at the interface was indicated by a decrease in the cathodic current density. In the cytotoxicity test of stainless steel 316 corrosion products on vascular smooth muscle cells, growth inhibition, altered cell morphology, and induced cell necrosis correlated well with an increased level of nickel ions in the corrosion products [3]. It is well known that the presence of microorganisms on a metal surface can alter its local physical and chemical properties and lead to microbiologically influenced corrosion [6]. Although these
Figure 2  Apoptotic nuclei are present (brown stained TUNEL reaction) on the fifth day following electrical stimulations. (A, B) Skin. (C, D) Connective junction of skin and muscle. (E, F) Deep muscle. The arrow shows the corrosion product of acupuncture needles. All of the tissues were stained with methyl green counterstaining solution. (A, C, E) Acupuncture needles inserted without electrical stimulus (B, D, F) Treated with an electrical current of 10Hz, 1-ms width and 30-minute duration with the Grass S88 stimulator.
in Oriental medicine, without any safety guidelines being used as electrodes for electrical stimulation. A previous study found that techniques for the selective activation of small axons require long-duration stimulation pulses (>500μs) and large stimulation amplitudes, which may lead to electrode corrosion [7]. Popovic et al [8] reported that bipolar intramuscular electrodes are more efficient and more selective than monopolar electrodes, but only if they are placed as far apart within the muscle as possible. They also noted that anodic corrosion may rule out the bipolar configuration for electrode materials such as stainless steel [8]. With a balanced bipolar current, it is assumed that the chemical products of electrolysis are neutralized with each polarity reversal. However, this may not be the case at low frequencies, or if the pulse duration or interphase interval is long [1]. Furthermore, Cargo et al [9] reported that the optimal pulse duration for reducing the possibility of tissue damage is less than or equal to 0.01 ms for intramuscular stimulation.

In this study, two acupuncture needles were inserted within specific acupoints, as close as possible, to determine the effects of bipolar currents on specific acupoints. Electrode acupuncture needles for electrical stimulation were inserted percutaneously via needles, even into deep muscles. The risk of tissue damage and acupuncture needle corrosion was studied as a function of the conditions of electrical stimulations being used in clinical and animal studies.

The penetrated margin of the STS304 acupuncture needle surface was particularly easily degraded by atmospheric oxygen after electrical stimulation (2-Hz frequency, 0.2-ms pulse width, 30-minute duration). The duration of EA stimulation is typically 15 to 30 minutes, but the duration may be longer in cases of dental and surgical analgesia, withdrawal or paralysis [10]. The intensity of electrical stimulation in animal studies is generally stronger than in clinical studies because of the use of anesthesia. This means that the electrical conditions of acupuncture in animal studies make the STS304 acupuncture needles more susceptible to corrosive change than in a clinical setting.

Noninsulated STS304 acupuncture needles are being used as electrodes for electrical stimulation in Oriental medicine, without any safety guidelines for electrical stimulation. Therefore, we recommend that the possibility of STS304 acupuncture needle corrosion should be announced on the packaging of acupuncture needles to protect both patients and operators from acupuncture needle corrosion, which may occur during electrical stimulation. This recommendation is particularly important when discussing the standards of acupuncture needles due to the broad use of stainless steel acupuncture needles as electrodes for electrical stimulation in Oriental medicine.

In conclusion, the biocompatibility of corrosion products of STS304 acupuncture needles might affect the tissue response following electrical stimulation. Further study is needed to protect both patients and practitioners from the detrimental effects of acupuncture needle corrosion during electrical stimulation.

References