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RESEARCH ARTICLE

ELSEVIER

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 acupoints 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

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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, Na_2HPO_4 10 mM, KH_2PO_4 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 washed in buffer and stop solution for 5 minutes at room temperature to stop the reaction. After being washed in Tris buffered saline and incubated in blocking buffer for 10 minutes, the sections were incubated with peroxidase-streptavidin for 30 minutes. Then, 3,3′-diaminobenzidine was used as a chromogen. The sections were counterstained with methyl green. Negative control sections were treated similarly, but incubated in the absence of