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Silver/silver chloride microneedles can detect penetration through the round window membrane

Joseph M. Wazen,[1] James P. Stevens,[1] Hirobumi Watanabe,[1,2] Jeffrey W. Kysar,[2]* Anil K. Lalwani[1]*

[1]Department of Otolaryngology-Head and Neck Surgery, Columbia University College of Physicians and Surgeons, New York, New York
[2]Department of Mechanical Engineering, Columbia University, New York, New York

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Abstract: Hypothesis: Silver-plated microneedles can be used to confirm penetration of semi-permeable membranes such as the round window membrane (RWM) by detection of voltage change at the moment of perforation. Background: The introduction of microperforations in the RWM can significantly enhance intracochlear delivery of therapeutics. However, the moment of needle penetration through the RWM cannot be reliably detected by visualization or sensation alone. We explore the ability of electrochemical detection of penetration in defining the precise instant a microneedle enters the inner ear. Methods: 0.2 mm diameter stainless steel Minutien pins were electroplated with copper, then silver. Pins were then soaked in bleach for 24 h to complete Ag/AgCl plating. Experiments were performed using a 3 mL Franz cell diffusion system with 1%, 2%, 3%, 4%, and 5% saline solution in the donor chamber and artificial perilymph solution in the receptor chamber separated by 5-μm pore synthetic membrane. Continuous voltage measurements were made throughout the process of membrane penetration by the microneedle ($N = 6$ for each saline concentration). Results: Silver-plated needles were able to detect an instantaneous change in voltage when traversing the membrane from saline solution into artificial perilymph. As calculated, the magnitude of the change in voltage upon penetration increased with increasing saline concentration and was stable across trials. Conclusion: Ag/AgCl coated microneedles are effective in detecting the moment of penetration across semi-permeable membranes. © 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 105B: 307–311, 2017.

Key Words: round window membrane, microneedle, inner ear therapeutics, electrochemical detection, electrochemical plating

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INTRODUCTION

Reliable methods allowing precise delivery of agents into the inner ear for therapeutic purposes while preserving hearing function and maintaining cochlear architecture remain a formidable challenge. Options for intracochlear delivery include systemic administration, transtympanic injection, or direct introduction into the cochlea. Systemic administration of the therapeutic agent has the drawback of systemic toxicity. Transtympanic delivery has rapidly been incorporated into clinical practice but is hampered by variable efficacy and toxicity, as it relies on the diffusion properties of the round window membrane (RWM).[1–5] We believe that an elegant solution to overcome the difficulties of intracochlear delivery is to use microneedles to create temporary perforations, to facilitate reliable and predictable intracochlear delivery across the RWM without anatomic or functional damage.[6–8]

To prevent intracochlear trauma during the process of puncturing the RWM, it is important to limit the depth of microneedle insertion into the scala tympani. However the exact moment of RWM perforation with the microneedle cannot always be visually ascertained, as direct visualization of the RWM can be surgically difficult.

In this manuscript, we exploit the chemical makeup of perilymph solution (that is high in sodium and low in potassium) to test a novel electrochemical method for detecting the precise moment of RWM perforation by a microneedle as it travels from the middle ear space, across the RWM, into the perilymph-containing scala tympani.[9] The concentration of NaCl in perilymph is $\sim 0.125M$, while the cation

*Co-Senior authors.
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Correspondence to: A. K. Lalwani; e-mail: anil.lalwani@columbia.edu
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(Na^+) to anion (Cl^-) mobility ratio is 1.52:1. By filling the RW niche with a known saline concentration, a chloride ion difference across the RWM can be created. Silver chloride plated needles can then be used to measure the voltage change as the needle transitions from saline into perilymph.[10,11] The theoretical voltage difference (diffusion potential plus electrode junction voltage) between two electrolytic solutions, separated by a semi-permeable membrane and measured by the same ion-containing electrodes, can be calculated using the following equation[10]:

$$V_A - V_B = \frac{kB^T}{e}\ln\left(\frac{c_2}{c_1}\right) - \frac{kB^T}{e}\left(\frac{\mu_+ - \mu_-}{\mu_+ - \mu_-}\right)\ln\left(\frac{c_1}{c_2}\right) \qquad (1)$$

Here, $V_A - V_B$ is the voltage difference between solutions, k_B is the Boltzmann constant (8.617×10^{-5} eV/K), T is the temperature in Kelvin, and e is the number of electrons transferred between samples. The three constants simplify to 25.8 mV at room temperature. Thus the equation depends on the ionic concentrations of the two solutions c_1 and c_2, as well as the relative ionic diffusion mobility of the cation and anion, u_+ and u_- respectively. In this study, we explore the feasibility and reliability of measuring voltage change as a means of detecting the precise moment of membrane perforation.

MATERIALS AND METHODS

In this study, we investigated the use of silver-plated microneedles as silver/silver chloride (Ag/AgCl) electrodes, to detect the moment of penetration through an artificial, semi-permeable membrane.

Copper electroplating

All electroplating was performed in the Columbia University Department of Chemical Engineering, using a three-electrode system and a μAUTOLABIII potentiostat. With Ag/AgCl as the reference electrode and Pt as an auxiliary electrode, 0.2 mm diameter (20-μm tip) Minutien insect pins (Fine Science, Foster City, CA) were submerged in a bath of copper solution consisting of 0.63*M* Cu_2, 80 g/L H_2SO_4, 1.4 m*M* HCl, and 300 ppm polyethylene glycol (PEG). The copper was deposited to achieve a thickness of ~500 nm.

Silver electroplating

After thorough washing of the copper-plated microneedles, the auxiliary electrode was replaced with a 0.999 pure Ag wire, and the bath with a silver nitrate solution (Caswell, Lyons, NY). Silver was deposited to achieve a thickness of 2 μm on the needle surface. Plated microneedles were then soaked in bleach for 24 h, to allow for the formation of a silver chloride layer.[12]

Franz™ cell experiments

Voltage recordings were performed on 30 synthetic membranes using a 3-mL vertical Franz™ Cell-type diffusion system (PermeGear, Inc., Hellertown, PA). The cell consists of three parts: a donor chamber, the artificial membrane, and a receptor chamber (see Figure 1).

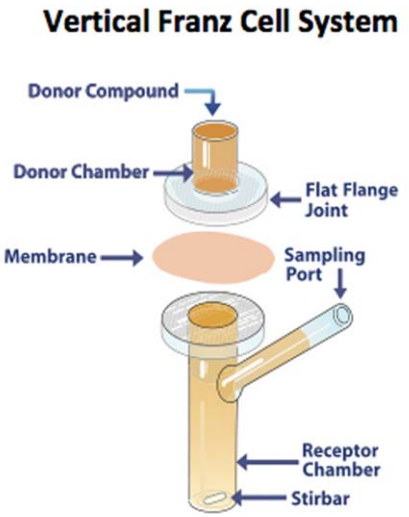

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FIGURE 1. Vertical Franz cell system. The donor chamber at the top was filled with 1–5% saline solution and represented the middle ear space. The artificial membrane separated the donor chamber above from the receptor chamber below akin to the RWM of the inner ear. The receptor chamber on the bottom was filled with artificial perilymph to represent the inner ear space. A stir bar can be used during Franz cell diffusion experiments to enhance mixing, but was not necessary for this experiment. A pinch clamp holds the chambers together to eliminate leaks. (This image is used with the permission of Andrew Wilt of Permegear, Inc.)

Donor chamber. Stock sodium chloride solutions, 1% (0.171*M*), 2% (0.342*M*), 3% (0.513*M*), 4% (0.684*M*), and 5% (0.856*M*) by volume, were prepared using distilled water and solid NaCl. The donor chamber was filled with 0.3 mL of a given concentration saline solution. Six trials were recorded for each concentration, with fresh solution used for each perforation.

Membrane. Synthetic hydrophilic isopore membrane filters, 13 mm diameter with 5-μm pores were used in this study (EMD Millipore, Billerica, MA). An imperforate membrane was used for each trial.

Receptor chamber. A stock of artificial perilymph was prepared using the following recipe: 125 m*M* NaCl, 3.5 m*M* KCl, 25 m*M* $NaHCO_3$, 1.2 m*M* $MgCl_2$, 1.3 m*M* $CaCl_2$, and 0.75 m*M* NaH_2PO_4.[9] The receptor chamber was filled with 3 mL perilymph solution, eliminating bubbles from the system visually and by tilting the Franz cell. The volume filling of the sampling port was adjusted to obtain the same height as the filled donor chamber.

The Ag/AgCl microneedles and reference electrode wires were painted with enameled nail polish for insulation, sparing the tip, then soldered to Teflon-insulated 20 gauge stranded steel wires to enhance conductivity. The electrodes were connected to a low noise, precision instrumentation amplifier with the gain set to 100 (model AMP01; Analog

Devices, Norwood, MA). Voltages were recorded using a DI-718B Data Logger and WinDaq®/Lite acquisition software (DATAQ Instruments, Inc., Akron, OH). For each trial, one microneedle and one AgCl wire reference electrode were placed in the donor chamber solution, and the voltage was measured until a reading of ~0 mV was achieved. The data logger then recorded the voltage as the microneedle was manually lowered to penetrate through the synthetic membrane and contact perilymph solution in the receptor chamber. A spike in voltage illustrated on the acquisition software confirmed penetration. The microneedle was removed at the end of the 60 s.

Analysis

Voltage readings were recorded by the data logger every 0.083 s over the course of 60 s for each trial. Voltage was determined by measuring the differences in potential just prior to and immediately following perforation, to the moment at which the sharp spike decreased in slope or read a new constant level. Statistical analysis was performed with Microsoft Excel. All data are presented as their mean ± standard deviation (SD).

RESULTS

Electroplating

The techniques described resulted in a qualitatively even, visible deposition of copper and silver layers on microneedles, while the pins maintained a pointed tip. The copper was an effective base layer for the silver-plating, as previous attempts at directly applying silver chloride to stainless steel were unsuccessful. Soaking samples in bleach for 24 h led to a visible darkening/graying of the silver needles from chloride deposition. This effect was not seen upon soaking in bleach for 1–4 h, while submersion for 72 h led to brittleness in the needles and a propensity for breaking during experimental set-up. Upon covering needle shafts in enamel, soldering to wire, and connection to an amplifier, silver chloride plating was confirmed by a zeroed voltage upon dipping both electrode tips in a saline solution. After the voltage reached ~0 mV, it remained within a close range of this value. Noise was minimal during this process, with oscillations of typically <1 mV prior to membrane perforation (around the range of the standard deviations below).

Membrane Perforation

For all trials ($n = 30$), there was a visible voltage change seen at the instant of RWM perforation. The spike was seen within milliseconds of the experimenter's visual and tactile confirmation of artificial membrane perforation, by both feeling the force needed for perforation compared to movement through solution as well as having both the vertical Franz cell membrane and computer screen within one's field of vision. An example of such a spike can be seen in Figure 2. In this example, it is noted that after the initial spike, the voltage continues to increase gradually over the next few seconds and then levels off, but does not surpass the calculated voltage.

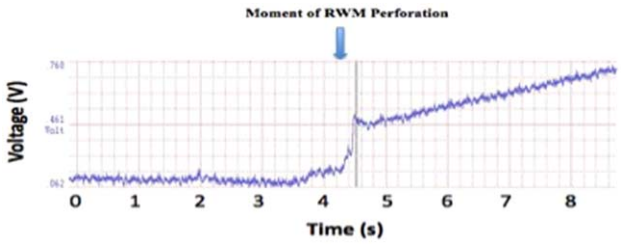

FIGURE 2. Voltage vs. time during the process of membrane perforation. The initial "zeroed" part of the graph is while both electrodes rest in the donor chamber, reaching approximately 0.62 V (after gain of 100). The spike occurs at the instant of membrane perforation via the silver chloride microneedle. The initial spike levels off but still gradually increases for a few seconds as the microneedle enters the receptor chamber. The voltage was measured with WinDaq voltage software.

The voltage changes in the instant of penetrating into artificial perliymph solution were compiled into Figure 3 and Table I, showing the results from the trials of each saline concentration. The new voltage was measured as the value at which the steep spike changed slopes to level off, right near the 0.461 point in Figure 2. As the table reflects, for each of the trials the experimental voltage change was less than the calculated value. The voltage spike was between 41.0% and 84.0% of the predicted value, with no trend in percentage versus saline concentration. However, the absolute voltage difference between experimental and theoretical values increased as the predicted (and measured) voltage changes increased, from 3.78 mV at 1% saline to 10.51 mV at 5% saline. The standard deviations of the six trials for 1–5% saline were 0.933, 0.923, 1.713, 0.757, and 0.422 mV, respectively.

DISCUSSION

In this study, we demonstrate that voltage measurement utilizing silver-chloride plated microneedle is an effective means to detect the exact moment of RWM perforation with

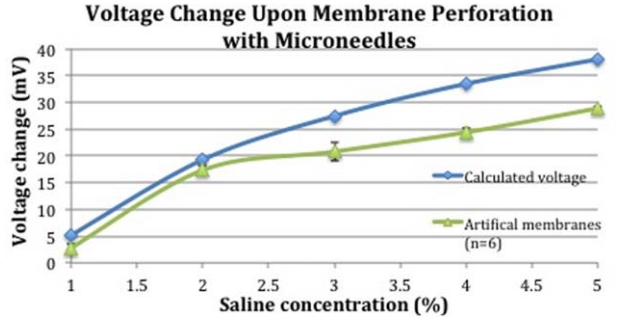

FIGURE 3. Voltage change vs. saline concentration. There is a direct relationship between saline concentration gradient and voltage. The artificial membrane trials in green are less than the calculated voltages for all saline concentrations, from 1 to 5%.

TABLE I. Predicted and Measured Voltage Change Upon Membrane Perforation with Silver-Plated Microneedles

	1% Saline	2% Saline	3% Saline	4% Saline	5% Saline
Calculated value (mV)	6.41	20.62	28.79	34.8	39.4
Ag plated microneedles ($n = 6$) (mV)	2.63 ± 0.933	17.33 ± 0.923	20.79 ± 1.713	24.40 ± 0.757	28.89 ± 0.422

a needle. The magnitude of the voltage change was related to the concentration of the saline solution; while the measured values were smaller than predicted, the moment of perforation could be electrically demonstrated across a wide range of saline concentration. Even with amplification, the background noise produced by the wires and the experimental environment was small relative to the voltages being measured during experimentation. Instantaneous notification of voltage change with perforation would provide the clinician with ample temporal warning to not insert the needle any deeper and thus avoiding inner ear trauma. The voltage software could also be equipped with an audible "beep" signaling membrane perforation without requiring the clinician's eyes to leave the surgical field.

The experimental setup can easily be adapted for use clinically by simply placing saline solution adjacent to the RWM. The anatomy of the human middle ear and RW niche should allow saline solution to rest in the space surrounding the RWM during electrode equilibration. As perforation of RWM with needles should only take a few seconds, this time frame is sufficient to minimize inner ear electrolyte changes. Hisashi et al. have previously measured the changes in K^+ and Na^+ activity within the scala tympani of guinea pigs in response to the middle ear space being flushed with saturated NaCl solution and found that increase in activity peaked at 30 min.[13]

The magnitude of the voltage change was related to the saline concentration. However, the experimental voltage spike was less than the calculated value using Eq. (1). The semipermeable membrane between the donor and receptor chamber likely allowed for diffusion of Na^+ and Cl^- ions during the time electrodes are being equilibrated prior to perforation. Mixing of the saline with the artificial perilymph solution upon puncture may further reduce the electrolyte differences between the two solutions and reduce the magnitude of voltage spike. The mixing of solutions is also be reflected in Figure 2. The gradual increase in voltage after an initial voltage spike may be a reflection of the electrode sampling a purer portion of artificial perilymph as it delves deeper into the receptor chamber. Other reasons for a decrease in voltage measurements include small imperfections in the AgCl plating on the needle surface that are not visible to the human eye. There may also be a gradual loss of the needle Cl^- over time once placed in the donor chamber.

Despite not matching the predicted values, change in voltage was seen at all concentrations tested. The exact voltage measurement is not as critical as being able to detect a change denoting membrane perforation. Thus, voltage measurement reliably addressed the binary question of whether the membrane was "perforated or not perforated." Our method should also be able to overcome inter- and intraperson variability in perilymph composition and changes seen with infection or prior treatment.[9,14–16]

Further work is needed to repeat and refine our methods *in vitro*, for both guinea pig animal models and human cadaveric RWM's. The success of such trials would lead to survival surgery and *in vivo* guinea pig studies testing electrochemical efficacy. The ultimate goal of such work is the application of this technology to human clinical trials, with sterile, commercially manufactured microneedles whose designs are based on the mechanical properties of the RWM.

CONCLUSION

Voltage measurements utilizing silver-chloride plated microneedles are an effective means to note the exact moment of RWM microperforation during clinical intervention. This information can help reduce potential trauma to the cochlea, and avoid the possibility of incomplete perforations during the treatment of inner ear diseases such as Ménière's disease or sudden sensorineural hearing loss (SSNHL) via the RWM.

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