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Localized Therapeutic Hypothermia to Preserve Residual Hearing Against Cochlear Implantation: Analytical and Experimental Studies

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LOCALIZED THERAPEUTIC HYPOTHERMIA APPLICATION TO PRESERVE RESIDUAL HEARING AGAINST COCHLEAR IMPLANTATION: ANALYTICAL AND EXPERIMENTAL STUDIES

By

Ilmar Tamames

A DISSERTATION

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Localized Therapeutic Hypothermia to Preserve Residual Hearing Against Cochlear Implantation: Analytical and Experimental Studies (May 2017)

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Objective: Cochlear implants are the most successful neuroprostheses. Unfortunately, the trauma caused during cochlear implant insertion can lead to a loss of residual hair cells in the cochlea. Various therapeutic approaches have been studied to prevent cochlear implantation induced residual hearing loss with limited success. Localized therapeutic hypothermia has the potential to be the standard of clinical care for neuroprotective intervention. This study demonstrates the design, operation and protocols, in an animal and a cadaver temporal bone model, for efficacious delivery of mild therapeutic hypothermia to the cochlea that can be translated to clinical practice in patients undergoing surgical cochlear implantation to preserve residual hearing.

Approach: This thesis developed a custom-designed localized therapeutic hypothermia delivery system that was used in both animal and human cadaver inner ear organs. In chronic experiments, the auditory brainstem responses were recorded from anesthetized rats to assess their hearing function before and after cochlear implantation.

Changes in hearing threshold, for up to a month following electrode alternative insertion trauma, were tested in response to 0.5-32 kHz pure tone pips between three groups: control cochleae, normothermic cochleae that were implanted while at room temperature and hypothermic cochleae that were implanted under hypothermic conditions. At the conclusion of the trials, inner ears were harvested for histology. The results were verified for functional loss in an extended study for up to three months post implantation. Additionally, to achieve clinical translation, the efficacy of the hypothermia probe and resulting heat distribution across human cochlea and surrounding tissues were modeled in 3D in COMSOL. The geometry and dimensions of inner ear and temporal bones were derived from CT and MRI images. Model predictions were compared with experimental observations from human temporal bones.

Main Results: The study found that localized hypothermia protects cochlear hair cells and residual hearing function against surgical and implantation trauma in an in-vivo rat model. A significant loss of residual hearing was observed in the normothermic animal implant group after one month. Comparatively, the residual hearing in the animal cochleae receiving therapeutic hypothermia was significantly conserved. Histology confirmed a significant loss of outer hair cells in normothermic cochleae receiving the surgical trauma when compared to the hypothermia treated group. The results were repeated and extended for an additional two months and the hypothermia induced recovery was still present while the untreated cochleae remained impaired.

To achieve a clinical translation of the hypothermic therapy, we conducted modeling and experimental studies in human cadaver temporal bones. We measured that the cochlear temperature was lowered on the round window, while the overall temperature of the temporal bone did not change significantly during treatment. The model simulations showed uniformly-distributed cooling across the human cochlea.

Significance: Collectively, these results indicate that therapeutic hypothermia during cochlear implantation reduces the traumatic effects of electrode insertion and improve conservation of residual hearing. Overall, the results of the numerical simulations and correlated with experimental observations from animal and human cochlea in the study suggesting that our custom-designed system can effectively provide mild therapeutic hypothermia. The present study demonstrates the efficacy and potential of applying localized mild therapeutic hypothermia for patients to preserve residual hearing after cochlear implantation.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ABR = Auditory Brainstem Responses

ANOVA = Analysis of variance

COMSOL = Multiphysics Modeling Software

CT = Computed Tomography

DAPI = [4,6-Diamidino-2-phenylindole, dihydrochloride]

EIT = Electrode-Induced Trauma

FDA = Food and Drug Administration

FITC = Fluorescein Isothiocyanate

MRI = Magnetic Resonance Imaging

MTH = Mild Therapeutic Hypothermia

NIH = National Institutes of Health

USDA = United States Department of Agriculture

PUBLICATIONS

Refereed Journal Publications

Tamames, I.; King, C.; Bas, E.; Dietrich, W. D.; Telischi, F. F.; Rajguru, S. M. (2016). "A cool approach to reducing electrode-induced trauma: Localized therapeutic hypothermia conserves residual hearing in cochlear implantation." Hear Res 339: 32-39.

To Be Submitted Journal Publications

Tamames, I.; King, C.; Huang, C.; Hoffer, M.; Telischi, F. F.; Rajguru, S. M. (2017). "Theoretical evaluation and experimental validation of localized therapeutic hypothermia application to preserve residual hearing following cochlear implantation "

In Preparation Journal Publications

Tamames, I.; and Rajguru, S. M. (2017). "Localized therapeutic hypothermia conserves residual hearing in cochlear implantation after three months"

Refereed Conference Talks

Tamames, I.; Bas E., King C.; Dietrich, W. D.; Telischi, F. F.; and Rajguru S. M. (2015). "Therapeutic hypothermia for neuroprotection against cochlear implant surgical trauma." 2015 Biomedical Engineering Society Annual Meeting Conference. Tampa, FL. Contribution from: Roberson, E.; Hyunh, S. B.; Marks, V.; Mylavarappu, R.

Conference Posters

Tamames, I.; Pineros, J; King, C.; Huang, C.; Babcock, T.; Hoffer, M.; Telischi, F. F.; Rajguru, S. M. (2017). "Theoretical evaluation and experimental validation of localized therapeutic hypothermia application to preserve residual hearing following cochlear implantation" Association for Research in Otolaryngology 2014 MidWinter Meeting. Baltimore, MD.

Tamames, I.; Truettner, J.; Bas, E.; Dietrich, W. D.; Telischi, F. F.; Prasad, A.; Rajguru, S. M. (2016). "Protective Effects of Mild Therapeutic Hypothermia on Cochlear Implantation Insertion Trauma" Association for Research in Otolaryngology 2016 MidWinter Meeting. San Diego, CA.

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CHAPTER 1: INTRODUCTION

1.1 INTRODUCTORY REMARKS

Inner Ear

The auditory system is essential in human life and development. The visible portion of the ear (pinna) leads to an auditory canal of about 2.5 cm into the tympanic membrane. Sounds that vibrate this membrane are incremented by the pinna and the 3 middle ear bones (ossicles) before affecting the oval window membrane. The oval window vibrates the fluid inside the cochlea, a hollow and spiraled bone structure. Due to the closed environment, movement of the fluid must equalize pressure at the second opening membrane in the cochlea, the round window (Bear, Connors, & Paradiso, 2007).

The cochlea hollow conduit is about 7 mm long in the rat and 32 mm long in the human. It contains two interconnected and consecutive fluid chambers, scala tympani and scala vestibuli, filled with perilymph, a fluid similar to cerebrospinal fluid with low potassium and high sodium. They surround a third chamber, the scala media, which contains endolymph, a fluid similar to intracellular composition with high potassium and low sodium. One of the walls of this chamber is called the basilar membrane and in it resides the organ of Corti. This organ contains the hair cells, which are the auditory receptor cells, easily identifiable due to the stereocilia they possess (Bear et al., 2007).

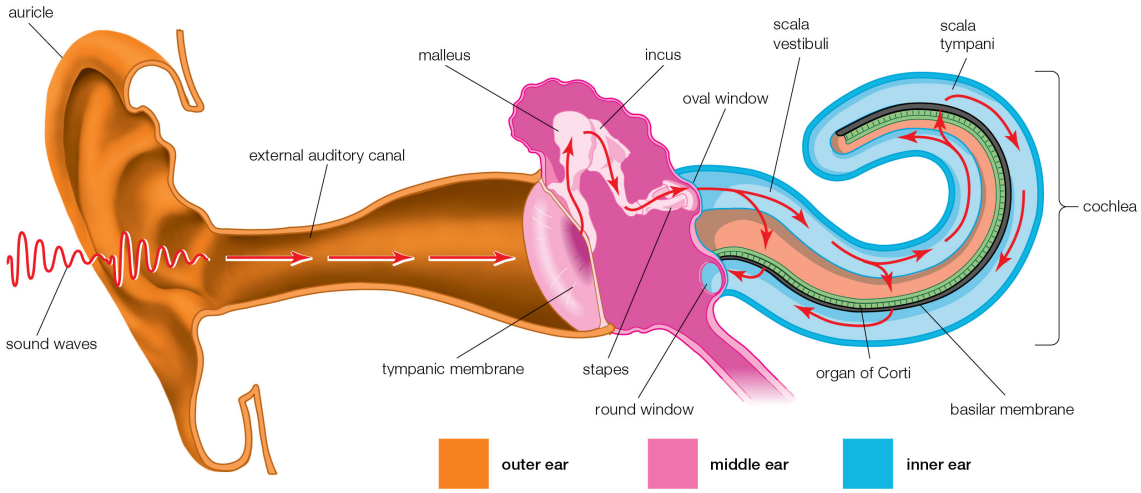

Figure 1.1 shows the cochlea - inner organ structure. (Alamy.com)

The sound waves cause vibration of the basilar membrane, which in turn causes endolymph to move the hair cell stereocilia and transduce the sound waves to action potentials in the spiral ganglion cells. Depending on the position along the length of the basilar membrane, the hair cell transduce a specific frequency of the captured sound (Bear et al., 2007). There are up to 24,000 human hair cells divided into two groups: inner hair cells, which number about 3,500 and are located in a straight line along the basilar membrane, are the actual sound transducers. The remaining hair cells, the outer hair cells, provide amplification of soft sounds and improve acoustic tuning of the cochlea (Bear et al., 2007).

Hearing Loss

Hearing loss, represented by a diminished ability to detect sounds, is a debilitating condition on normal life. In the world, 360 million people have disabling hearing loss (greater than 40dB), of which 32 million are children (WHO, 2017).

Hearing loss can be separated in two originating causes: congenital and acquired. Congenital causes of hearing loss and deafness before birth include: genetic defects; congenital cytomegalovirus, rubella, syphilis or other infections; and inappropriate use of particular drugs. During the neonatal period, additional cause are: low birth weight; birth lack of oxygen; and severe jaundice (WHO, 2017).

Acquired causes encompasses: infectious diseases; chronic ear infections; collection of fluid in the ear (otitis media); use of certain medicines; injury to the head or ear; excessive noise; recreational exposure to loud sounds; ageing; and wax or foreign bodies blocking the ear canal (WHO, 2017).

Cochlear Implants

For patients missing all or part of the sensory mechano-transduction apparatus, cochlear implants have been the solution in place for over 40 years. Cochlear implants are the most successful neuroprostheses in terms of volume. As of 2012, over 324,000 partially or completely deaf individuals have been implanted in the world. Contrary to hearing aids with only amplify existing sounds; cochlear implants restore hearing by stimulating the auditory nerve directly in patients missing the sensory mechano-transduction apparatus. Cochlear implant components include an external microphone, a transmitter, a subdermal receiver and a stimulator leading to the implanted electrode. The electrode is inserted through the natural opening, round window, or through a cochleostomy made in the cochlear wall into the scala tympani.

According to the U.S. FDA, as of December 2012, there are 96,000 cochlear implant patients in the United States of America. Around 60% or 58,000 of the cochlear implants in the United States were implanted in patients who are 18 years of age and older, while the remaining 40%, or 38,000, were implanted in pediatric patients who are 17 years of age and younger. Furthermore, approximately 26% or 25,000 of the cochlear implants were implanted in children aged 5 and younger (FDA, 2015).

Additionally, the FDA-approved indications for cochlear implants now allow implants in younger children (Carlson et al., 2015; Ching et al., 2014), for whom residual hearing levels may be difficult to ascertain (Tharpe & Sladen, 2008). In recent years, new hybrid (acoustic and electric) designs with have greatly increased the range of representation of audible sounds (Irving et al., 2014; Usami et al., 2014). In order to take advantage of this extended range, residual hearing must be preserved.

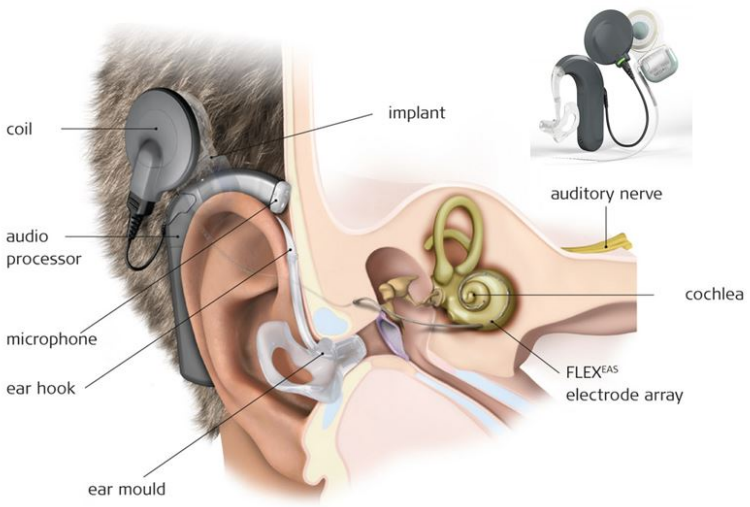

Figure 1.2 shows the hybrid cochlear implant design. Courtesy of www.medel.com.

Mechanisms of cochlear implantation trauma

Trauma to the delicate structures of the inner ear frequently occurs during insertion of cochlear implant electrodes. Such trauma leads to a significant variation in the efficiency of electrode stimulation along the length of the implanted array. This impacts the residual hearing present in patients and prevents them from using the electro-acoustic stimulation devices currently available. In trials for one of the EAS, the FDA reported about 44% of loss of residual hearing after hybrid electrode implantation (FDA, 2013). The loss of residual hearing due to electrode-induced trauma (EIT) can render the additional functionality of the new hybrid electrodes useless. Therefore, the importance of protecting residual hearing during and after implantation has increased in importance.

Cochlear implant insertion can result in inflammation and oxidative stress leading to neural degeneration, hair cell loss and loss of residual hearing (Eshraghi et al., 2005; Wardrop et al., 2005). Research literature has established the relationship between device-specific cochlear implant electrodes, surgical techniques and stimulation with EIT both in animal models and in temporal bone studies (Adunka, Kiefer, Unkelbach, Radeloff, & Gstoettner, 2005; Ahmad et al., 2012; Briggs et al., 2001). Studies in animal models have identified significant increase in hearing thresholds within 7 days of implantation (Balkany et al., 2005). Previous reports have documented a significant loss of residual hair cells caused by either a direct trauma to cochlear structures or by cascading molecular effects (Esperanza Bas, Gupta, & Van De Water, 2012; Eshraghi et al., 2013).

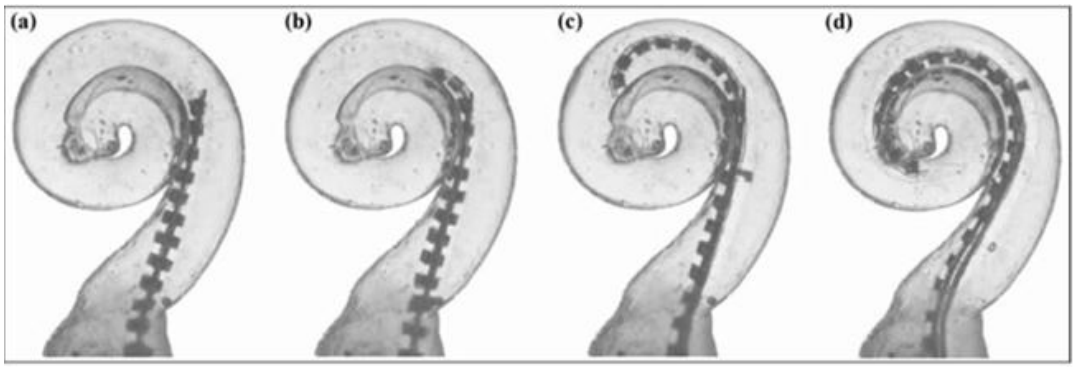

Figure 1.3 shows intended representation of the ideal insertion path of the spiral molded electrode. Adapted from (Rebscher et al., 2008).

Alternative approaches and treatments

To minimize the trauma to the inner ear, a number of refinements into surgical techniques and protective drug-eluting electrodes are currently being researched (James, Eastwood, Richardson, & O'Leary, 2008; Jolly et al., 2010; Van De Water et al., 2010; Vivero et al., 2008). Utilizing soft surgical techniques, such as, the reduction of blood entry into the scala tympani, the prevention of bone dust and perilymph leakage, the use of the round window as a surgery approach and the modification of the shape, flexibility and depth of insertion of the electrode array have reduced the damage caused by EIT (Friedland DR & C, 2009). A previous study by the University of Miami Ear institute tested the otoprotective effect of locally delivered AM-111 of surgically caused EIT (Eshraghi et al., 2013). Van de Water's team has investigated the use of polymer-eluted dexamethasone for otoprotection in a test in vitro system of tumor necrosis factor alpha challenged organ of Corti explants. Their results support the application of polymer containing dexamethasone on the coating of electrode arrays for the conservation of hearing during EIT (Esperanza Bas et al., 2012; Dinh et al., 2008).

Other approaches include the investigation by Dr. Bas and Dr. Bohorquez et al, in testing the otoprotective effect of neuroprotective drug-eluting electrodes. They identified how different levels of dexamethasone affected the surgical insertion trauma when eluted from a custom electrode. The functional hearing threshold was increased with zero drug dosage and with progressively increased dosage, it approached baseline levels. (E. Bas et al., 2016) However, even with the application of these alternative approaches and methods, residual hearing preservation is still a work in progress (Balkany et al., 2006; Kiefer et al., 2004).

Therapeutic hypothermia

A potential alternative could be mild therapeutic hypothermia (MTH). Localized MTH, defined as a temperature decrease of 4 to 6 ºC below body temperature, provides tangible benefits for central nervous system injuries (Cappuccino et al., 2010; Dietrich, Atkins, & Bramlett, 2009; Kawai, Okauchi, Morisaki, & Nagao, 2000; Levi et al., 2010; Purdy, Novakovic, Giles, Miller, & Riegel, 2013; Tzen, Brienza, Karg, & Loughlin, 2013). It has the potential to reduce or prevent oxidative stress (Balkany et al., 2005; K. R. Henry & Chole, 1984; Shintani, Terao, & Ohta, 2010). Previous studies in the literature, have demonstrated the potential protective effects of MTH on ischemic and traumatic injuries to neurons (Levi et al., 2010; Ohta, Terao, Shintani, & Kiyota, 2007; Shintani et al., 2010; Yanamoto et al., 1999; Yokobori et al., 2011).

The mild hypothermia efficacy has been evaluated in both animal and human experimental settings and clinical trials. Cortical infarct volume was significantly decreased by hypothermic treatment. Spinal cord injury assessments demonstrated improvements after intravascular cooling (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010). Nevertheless, systemic hypothermia exhibits multiple side effects on the functioning of organs and organ systems including immune response impairment, higher infection rates, altered effects of other drugs and vascular function deficiencies (Polderman & Herold, 2009).

MTH for cochlear trauma

Modifying the temperature of the cochlea has influenced cochlear responses (Brown, Smith, & Nuttall, 1983; Liberman & Dodds, 1984; Ohlemiller & Siegel, 1992, 1994), predominantly when applying hypothermia (K. R. Henry & Chole, 1984; Watanabe, Koga, Hakuba, & Gyo, 2001). Studies have attempted to localize hypothermia for use in inner ear cochlear EIT models. Balkany et al. in 2005 studied rat cochleae undergoing surgical implantation after receiving hypothermia and showed that they exhibited less functional loss compared to normothermic implanted cochlea (Balkany et al., 2005).

In 2003, Dr. Kenneth studied the influence of 20 minutes of hyperthermia and hypothermia on auditory thresholds prior to acoustic trauma exposure. The study identified that hyperthermic mouse cochleae had significantly elevated thresholds compared to normal temperature cochleae exposed to noise. Additionally, hypothermic cochleae had significantly lowered thresholds than euthermic cochleae (Kenneth R. Henry, 2003).

The glutamate efflux in the perilymph was investigated in hypothermic gerbil cochleae. Occlusion of the vertebral arteries caused drastic increase in the auditory threshold in normothermic cochleae. Hypothermia treated cochleae, with a temperature of 32°C, were less affected and the auditory threshold increase was diminished. In particular, the perilymphatic glutamate concentrations for hypothermic cochleae did not increase as compared to the normothermic cochleae (Hyodo et al., 2001). A different study on gerbils with different hypothermic treatments identified attenuated hearing loss and inner hair cell loss within 3 h after transient ischemia (Takeda et al., 2008).

Based on previous research, localized MTH could potentially be a beneficial treatment and a possible therapy to the loss of residual hearing after cochlear implantation surgery.

Novel device

Before we can begin investigating the therapeutic protection, we have to design a custom system to localize hypothermia. Some of the requirements are that system should be small enough to fit the small surgical approach from a cochlear implant surgeon. Out approach presents a novel delivery method for localized MTH, utilizing a custom-designed, low-cost portable device. When localized delivery is used, the beneficial MTH effects can be achieved for the specific area with no adverse systemic side effects. Due to the large numbers of animal studies and clinical literature, which have already demonstrated the protective effects of hypothermia, it has the potential to be the standard approach to treat EIT related injury in a clinical setting.

Delivering effective drug dosages to the sensory structures in the inner ear remains a significant challenge for the drug-delivery approach of cochlear implantation trauma treatment. In comparison, the hypothermia device is capable of providing a custom range of cooling and warming and is small enough to apply MTH via a biocompatible gold tip of 1.6 mm diameter and 5 mm length. The device consists of two Peltier elements surrounding a copper core, each with its own individual heat pipe system. The core would circulate cooled fluorocarbon to reduce the temperature of the tip. Programmatically it can decrease temperatures by a desired gradient, with continuous feedback from a microthermistor, and apply a similar process for rewarming.

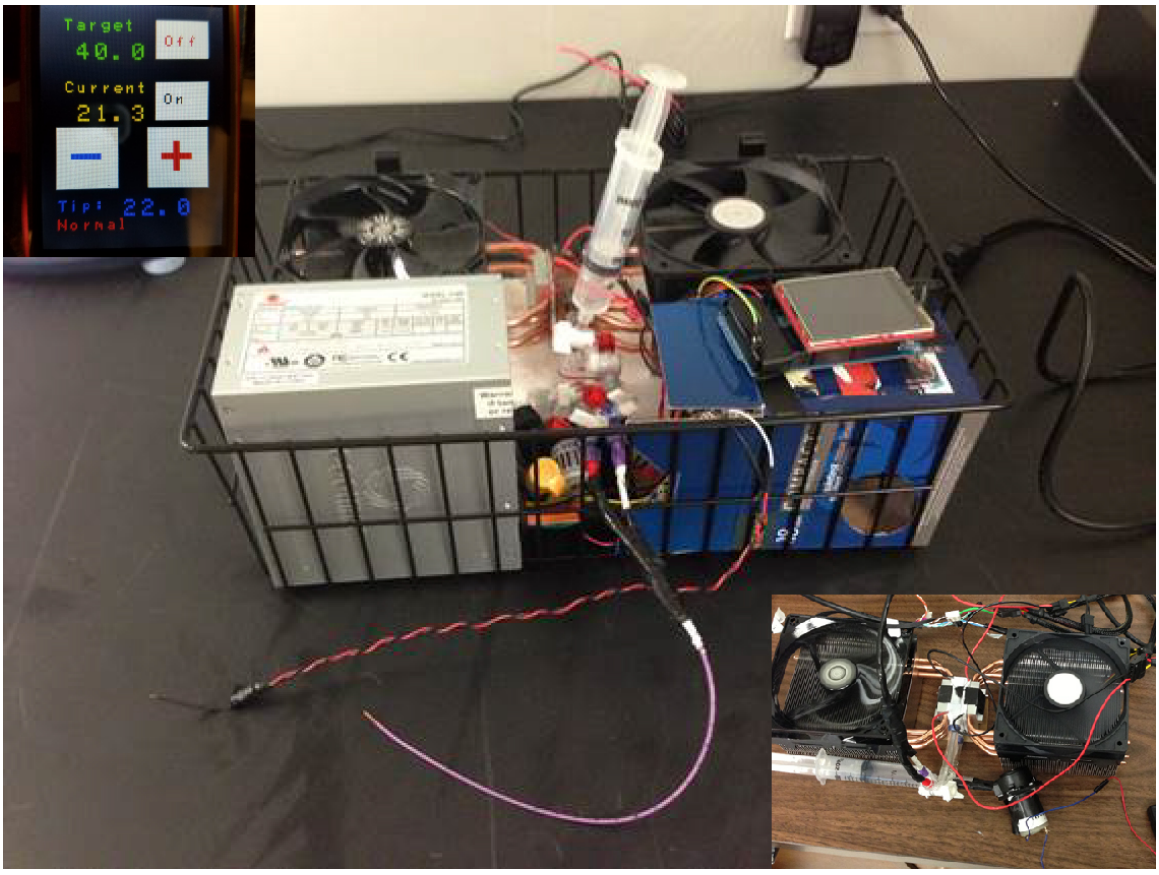

Figure 1.4 shows the custom system to localize hypothermia.

The components (at the total cost of under $500) include: a loop tubing system for cooling the fluorocarbon liquid in a copper chamber and transport it with a peristaltic pump to the gold tip (Rajguru, Roberson, & King, 2015); dual heat pipes and fans to maintain Peltier functionality; an Arduino interface to control the temperature range in the dual Peltier setup; a syringe to allow for refilling of fluorocarbon and for managing of air bubbles; an external temperature sensor; and a standard power supply to provide the power necessary to run all components. The overall weight of the device is less than 1 kg. Temperature capabilities range from of -5 to 50 Celsius. There is no fluorocarbon leakage; therefore it has no harmful residue.

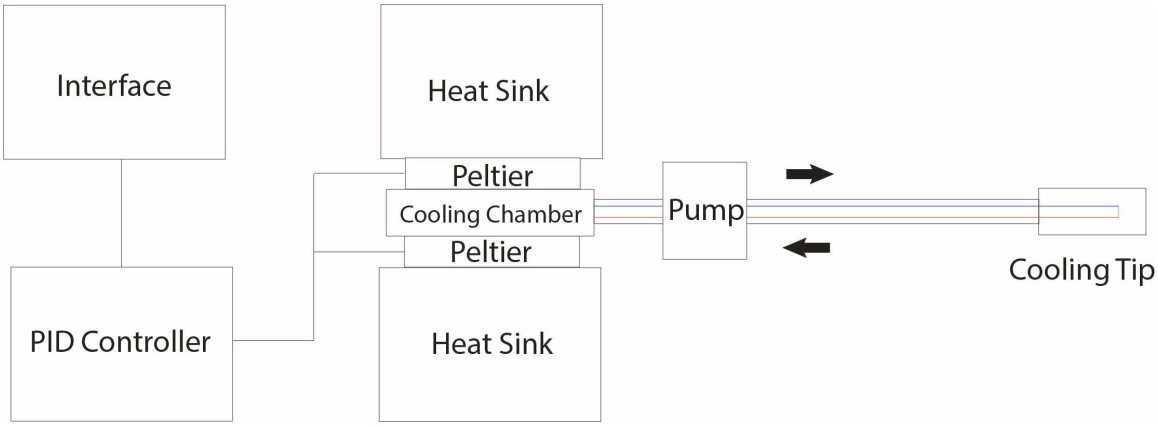

Figure 1.5 shows the schematic and mechanism of the hypothermia device.

Programmatically (see appendix) designed to self-regulate, the touch user interface screen contains on/off and plus/minus buttons for selecting temperature; current readings of equipment temperature; and external microthermistor for reference. If it detects a 5 degree difference, the system switches from warming to cooling to achieve selected temperature, and then maintains it within 0.5 Celsius of the target temperature.

Auditory brainstem responses

To identify the potential benefit of the MTH, auditory brainstem responses (ABR) tests were utilized as a specific marker of distinction using a Smart EP system. ABR is a neurologic test of auditory brainstem function, in which the subject is provided with auditory stimuli produced by headphones (tone bursts in the experiments conducted) and the evoked potential generated is detected to determine auditory threshold. The resulting waveform response is measured by three electrodes set at the vertex of the animal forehead and at both superior post auricular areas. An additional ground electrode at the leg is added.

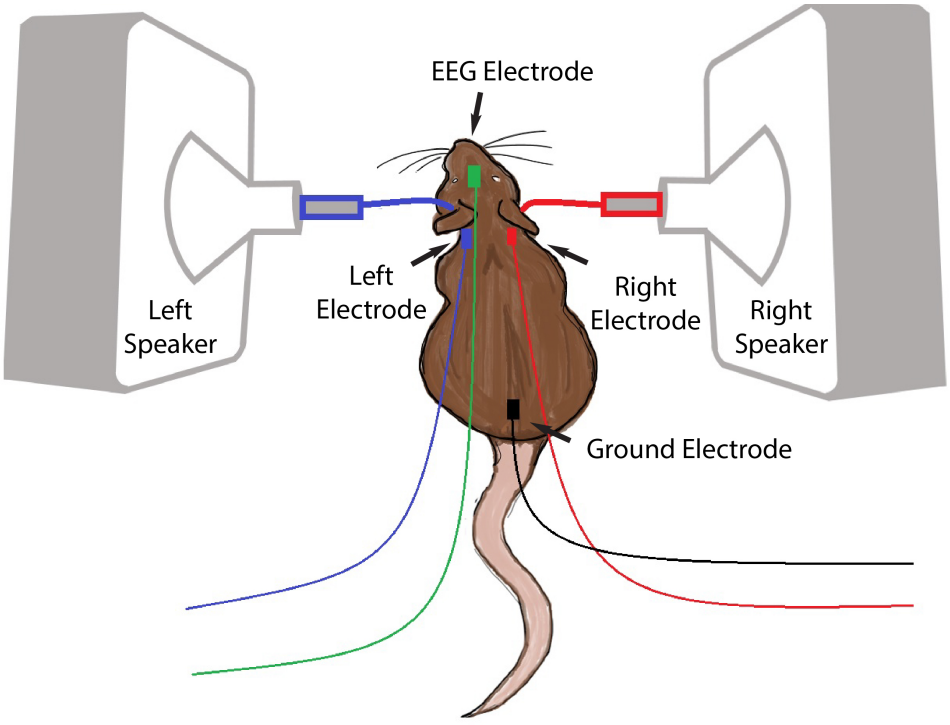

Figure 1.6 shows specimen being readied for an ABR test. (Courtesy of Samantha Rincon)

The frequencies of 0.5, 1, 2, 4, 8, 16, 24, and 32 kHz were tested using 1000 sweeps of tone bursts at a rate of stimulation of 21.1 Hz augmented using an Opti-Amp bioamplifier from Intelligent Hearing Systems (IHS, Miami, FL) and filtered by a low and high frequency filters.

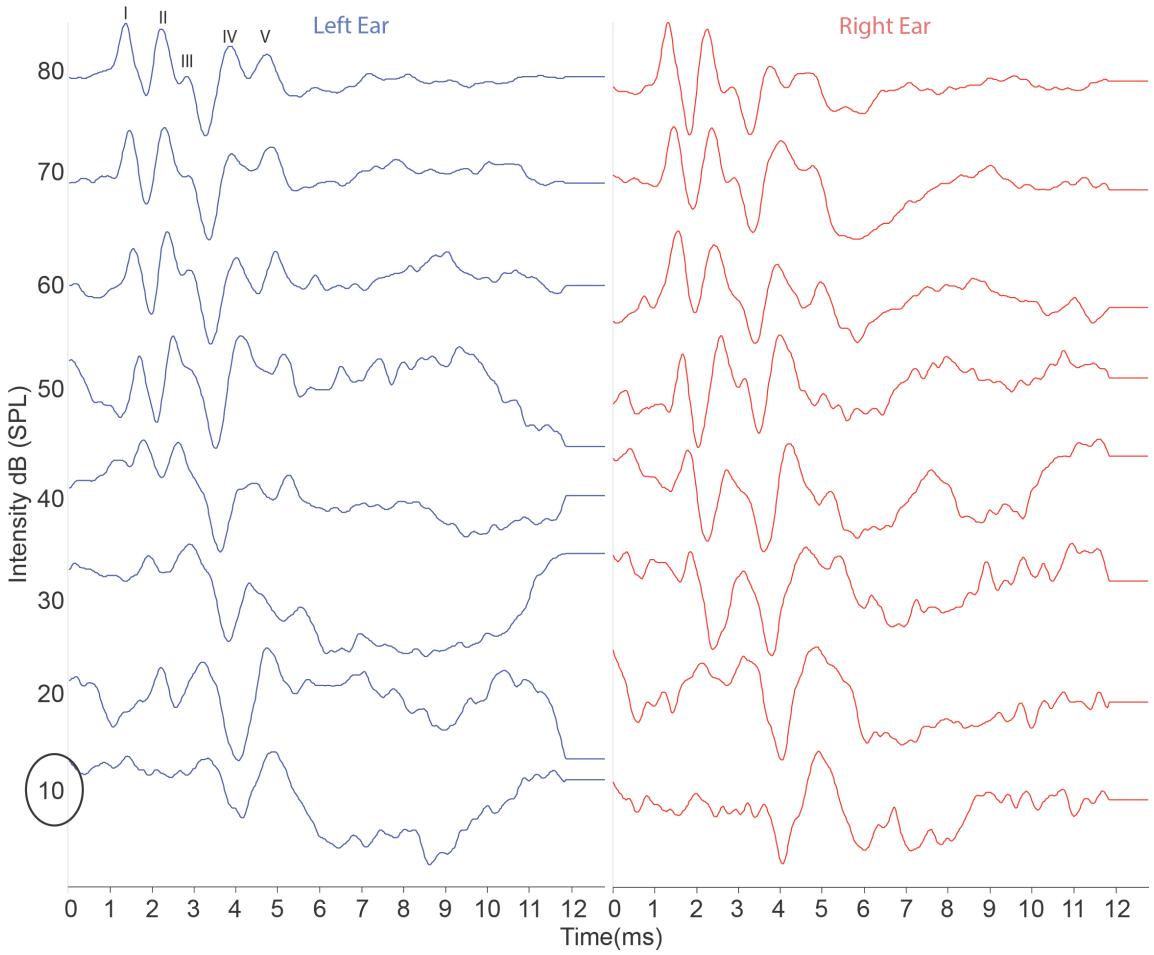

Figure 1.7 shows the auditory brainstem responses to a 16-kilohertz pure tone input in a control, non-implanted cochlea. Blue represents left ear and red the right ear.

The waveform peaks and troughs are labeled I-V. These waveforms normally occur after a 10-millisecond time period after the stimulus is presented. There is a general consensus that peak I is generated by the anteroventral and posteroventral cochlear nucleus (Simha, Paquereau, Cazals, & Aran, 1988) Trough II is assumed to be the contribution of the trapezoid body (Wada & Starr, 1983). Both peak III and trough IV are attributed to superior olivary complex and medial nucleus of the trapezoid body (Gardi & Bledsoe, 1981; Simha et al., 1988) And finally peak V has been found to represent superior olivary complex and the lateral lemniscus or the trapezoid body (Popelar, Grecova, Rybalko, & Syka, 2008; Simha et al., 1988; Wada & Starr, 1983).

The input is gradually lowered by 10 dB SPL to determine the minimum hearing threshold where the most of the ABR signal components are present. Threshold was defined as the minimum stimulating level where an ABR response was both identifiable and repeatable.

1.2 CONTENTS OF THIS DISSERTATION

This study provides insights for design, operation and protocols for efficacious delivery of mild therapeutic hypothermia to the cochlea that may significantly benefit patients undergoing surgical cochlear implantation by preserving residual hearing. To this end, a custom device was designed to locally cool the cochlea without the need for any modifications to the current cochlear implant surgical approach. The auditory thresholds post-surgery were compared in normothermic, hypothermic-treated cochleae and control cochleae. A histological study was carried out at the end of the chronic period and the hair cell loss was quantified.

To verify the possibility of clinical translation, a numerical analysis model was developed and the device was tested on human temporal bones. Proposed research will improve the understanding of the benefits of localized MTH in the inner ear for a potential clinical translation.

In chapter 1, background information regarding inner ear, cochlear implants, cochlear implantation surgery trauma, alternative approaches and treatments, the role of MTH and its uses in cochlea trauma is discussed. Chapter 2 discusses the results of the animal studies that demonstrate the role of MTH in protecting residual hearing loss. In vivo chronic efficacy of therapeutic hypothermia to protect hair cells and minimize hearing loss is presented in chapter 3. Chapter 4 contains a finite element analysis study of the temperature distribution as well as experimental temperature measurements in human temporal bones. To conclude, Chapter 5 outlines the future directions that can extend the findings of this study towards achieving a clinical application.

CHAPTER 2: A COOL APPROACH TO REDUCING ELECTRODE-INDUCED TRAUMA: LOCALIZED THERAPEUTIC HYPOTHERMIA CONSERVES RESIDUAL HEARING IN COCHLEAR IMPLANTATION

2.1 INTRODUCTORY REMARKS

Conservation of residual hearing by reducing cochlear trauma has always been an important goal during inner ear surgeries, especially during cochlear implantation. Patients with residual hearing are now being implanted to take advantage of bimodal electroacoustic stimulation (Irving et al., 2014). The FDA-approved indications for cochlear implants now allow bilateral implants in young children (Carlson et al., 2015; Ching et al., 2014), in whom residual hearing levels may be difficult to ascertain (Tharpe & Sladen, 2008). Furthermore, in recent years, novel cochlear implant electrode designs have improved efficiency and performance by locating stimulation sites closer to spiral ganglion neurons and deeper into the scala tympani. As a result, in the future, the number of implant recipients with some degree of usable hearing is likely to increase. Depending upon the insertion depth and the intracochlear electrode position, however, cochlear implantation can result in inflammation and oxidative stress leading to neural degeneration, hair cell loss and loss of residual hearing (Eshraghi et al., 2005; Wardrop et al., 2005). The nature of cochlear electrode insertion trauma has been characterized both in animal models and in temporal bone studies. Previous research has demonstrated the relationship between device-specific cochlear implant electrodes and surgical techniques with cochlear trauma (Adunka et al., 2005; Ahmad et al., 2012; Briggs et al., 2001).

ABR studies in animal models have observed significant increase in hearing thresholds within 7 days of implantation trauma (Balkany et al., 2005) and can be a result of both the intrinsic and extrinsic cell death signaling pathways (E. Bas et al., 2015).

The loss of residual hearing due to EIT can negatively impact the learning ability and speech recognition in younger patients. As such the importance of protecting residual hearing during and after implantation has grown proportionately. To reduce trauma-associated cellular response, refinements in surgical techniques and protective drug-eluting electrodes are being investigated (Dinh et al., 2008; Friedland DR & C, 2009; James et al., 2008; Jolly et al., 2010; Van De Water et al., 2010; Vivero et al., 2008). While surgical techniques and electrode designs have been advanced, residual hearing prevention has not been achieved (Balkany et al., 2006; Kiefer et al., 2004).

The present study tested the hypothesis that localized MTH applied to the cochlea prior to implantation can protect hair cells and hence may preserve residual hearing. MTH is a promising neuroprotective intervention when induced during or after a central nervous system injury (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010; Matsui, Ishikawa, Takeuchi, Okabayashi, & Maekawa, 2006). When localized and administered prior to trauma, these effects may be enhanced with little adverse effects on other biological phenomena or immune response (Purdy et al., 2013; Tzen et al., 2013). MTH, defined as a temperature decrease of 4 to 6 ºC below body temperature, has the potential to reduce or prevent oxidative stress (Balkany et al., 2005; K. R. Henry & Chole, 1984; Shintani et al., 2010).

Prior literature, both from animal studies and clinical trials, has shown protective effects of MTH on ischemic and traumatic injuries to neurons (Levi et al., 2010; Ohta et al., 2007; Shintani et al., 2010; Yanamoto et al., 1999; Yokobori et al., 2011) in cases of mild traumatic brain injury (mTBI), seizures (Atkins et al., 2010), ischemia and inflammatory response (Cappuccino et al., 2010; Kawai et al., 2000; Levi et al., 2010; Matsui et al., 2006) after cardiac arrest, and related to spinal cord injuries (Cappuccino et al., 2010; Dietrich et al., 2009). Unfortunately, systemic hypothermia has multiple side effects on functioning of organs and organ systems including impairment to immune response, higher infection, altered effects of other drugs and compromising vascular function. These adverse effects restrict its use in clinical setting, and make it challenging to utilize hypothermia for preserving sensory functions of the inner ear.

That temperature is an important parameter and influences cochlear responses, has been long known (Brown et al., 1983; Liberman & Dodds, 1984; Ohlemiller & Siegel, 1992, 1994) with demonstrated benefit of applied hypothermia (K. R. Henry & Chole, 1984; Watanabe et al., 2001). To test the beneficial effects of localized hypothermia in otoprotection, a custom device was designed to cool the cochlea that does not require any modifications to the current cochlear implantation surgical approach. Direct comparisons were made between hearing thresholds post-surgery in normothermic and hypothermic-treated cochleae. A detailed histological study was also carried out at the end of the long-term implantation and observed significant otoprotection with hypothermia. A power analysis test was conducted to determine the necessary group size based on preliminary studies.

2.2 MATERIALS AND METHODS

Animal preparation

The use of Brown Norway normal hearing rats in this study was approved by the University of Miami Animal Care and Use Committee and was in compliance with USDA and NIH Guidelines for the Care and Use of Laboratory Animals. The animals were anesthetized with an initial intraperitoneal injection of Ketamine and Xylazine (22-60 and 5-10 mg/kg respectively) and maintained with supplemental doses. Lidocaine Hydrogen Chloride 1% was used for local analgesia prior to surgical incision. Withdrawal reflex was measured by extension of one leg and pinching the web of skin between the toes or pinching the ear. A positive reflex was indicated by flexion of the limb with toe, paw pinch or movement of the head or whiskers with ear pinch. Once anesthetized, a bland, sterile ophthalmic base ointment was applied to corneas to prevent eye dryness. The skin over the skull and behind the ears was shaved, and prepped with Betadine scrub. All surgical procedures were performed under aseptic conditions and animals were maintained at 37°C through the procedure.

For the approach to the inner ear organs, a post-auricular incision was made extending from the dorsal skin defect down behind the ear to terminate approximately over the posterior edge of the mandible. The soft tissues were dissected to expose the temporal bone over the bulla. A small defect in the bone of the bulla was created using the tip of a scalpel blade or a hand-held micro-drill to expose both the round window membrane niche and the lateral bony wall of the cochlea adjacent to the niche.

Electrode trauma to the cochlea was accomplished by the insertion of an electrode analog into the scala tympani via the round window to a depth of 5 mm. The electrode analog was 0.28mm monofilament as described previously (E. Bas et al., 2015; Esperanza Bas et al., 2012) to allow for insertion in the smaller scala tympani of the rat. The site around the electrode alternative was secured by packing with a graft of fascia obtained locally from the site of the surgical approach to the bulla. Once the placement and stability of the electrode analog were established, the defect in the ventro-lateral wall of the temporal bone bulla was covered with carboxylate cement, with care taken not to allow the cement to enter into the bulla. A subcuticular closure was made using vicryl absorbable sterile sutures and the skin was closed with vicryl absorbable sutures. A topical antibiotic silver sulfadiazine 1% or Bacitracin Zinc was applied to the wound sites. To prevent post-op dehydration the animals received IP or SC injection of sterile Ringer's solution (up to 1 mL). To prevent post-operative pain, buprenorphine (Buprenex, 0.05 mg/kg) was given once at the time of surgery and sequential dosages were provided for 48 hours to aid recovery. To prevent hypothermia post-op, the animal holding/recovery cages where placed on water-circulating heating pads.

There were three experimental groups: in the first group, the left ear was implanted under normothermic condition and in the second group, MTH (cooling by 3-6 ºC measured at the round window) was provided using the newly developed hypothermia device. The right contralateral ear always served as the intra-animal control.

Delivery of MTH

A novel copper hypothermia probe attached to a custom thermoelectric Peltier device (described on 1.1) was placed in the middle ear adjacent to the cochlea under direct visualization. Fluorocarbon cooled by the Peltier system was used as the refrigerant, and circulated through the metal probe. In acute experiments (n=7), the temperatures at the apex and the basal turn of the cochlea were measured using (QTI Sensing Solutions' T320/E320) microthermistors over time. The temperature of the cochlea was reduced by a 5-6ºC with the device (Figure 2.1) and cooling was maintained within ±0.3ºC, over the duration of the experiment. Using the acute experiments as guide, the following protocol was tested for chronic experiments: localized hypothermia was applied for 20 minutes before, and for 20 minutes after cochlear implantation for total hypothermia duration of 40 minutes.

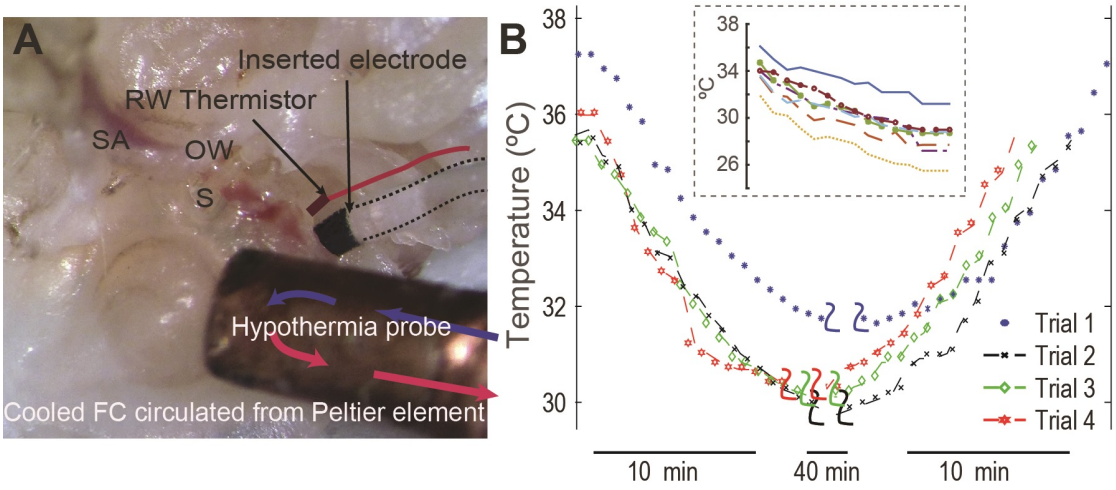

Figure 2.1 (A) shows placement of the hypothermia probe in contact with an excised rat left cochlea. The inserted electrode analog and a thermistor placed at the round window for temperature measurement are highlighted. Fluorocarbon cooled by an external Peltier element is circulated through the copper tip of the probe. RW-round window, SA-stapedial artery, OW-oval window, S-stapes. (B) With the custom-designed thermoelectric cooler and probe positioned anteroinferior to the RW, the cochleae were cooled between 4-6ºC within 10 minutes. The temperature was maintained for 20 minutes prior to insertion of the electrode analog. Hypothermia was maintained for further 20 minutes prior to a gradual rewarming of the cochleae over 10 minutes. Inset shows the level of cooling achieved at the round window measured prior to electrode insertion in animals undergoing chronic insertion (Published)

Measurement of hearing thresholds: ABR

ABR were measured for acoustic tones between 0.5 and 32 kHz before surgery to determine pre-surgical hearing thresholds. All animals selected possessed bilateral normal hearing of approximately 40 dB at .5 kHz, 40 dB at 1 kHz, 30 dB at 2 kHz, 15 dB at 4 kHz, 15 dB at 8 kHz, 15 dB at 16 kHz, 30 dB at 32 kHz and 30 dB at 32 kHz. ABRs were also measured at various time points post-surgery up to 30 days and compared to pre-surgical thresholds.

The changes in residual hearing with electrode analog insertion trauma were tested on the frequencies of 0.5, 1, 2, 4, 8, 16, 24, and 32 kHz using 1000 sweeps of tone bursts of a rate of stimulation of 21.1 Hz amplified using an Opti-Amp bioamplifier from Intelligent Hearing Systems (IHS, Miami, FL) connected to the Smart EP system. Recording negative electrodes were attached to the each ipsilateral superior post auricular area and with a positive reference electrode to the vertex. A ground electrode was inserted subcutaneously on the left leg. The ipsilateral ABR information was assessed bilaterally by averaging 1000 samples at each interval starting from a level of 80 dB SPL tone bursts and decreasing by 10 dB steps until no identifiable ABR response was present at each frequency. Threshold was defined as the minimum stimulating level where an ABR response was both identifiable and repeatable.

The hearing function was compared between the three groups: cochleae that did not receive hypothermia during surgery (normothermic trauma-only group, n=7), cochleae that received localized MTH (hypothermia treated group, n=7) and the contralateral cochleae of each animal (control group, n=14). Two animals did not survive past 14 days and 4 animals were not sampled at day 14. ABR information was additionally reviewed by two blinded investigators for accuracy with no knowledge of the animal group classification.

Histology

At the conclusion of the trials, inner ears were harvested and fixed with 4% paraformaldehyde for histologic evaluation of the organ of Corti. The fixed explants were washed three times in PBS and the organ of Corti was dissected from the bony capsule and subsequently incubated in 5% normal goat serum (Sigma Aldrich, MO) and 1% Triton X-100 (Sigma Aldrich) in PBS for 30 min at 25°C. Specimens were then incubated with FITC-labeled phalloidin (Sigma-Aldrich) for 45 min at 25°C and washed three times. Additionally, they were placed in 600 nM 40, 6-diamidino-2-phenylindole (DAPI) solution to label the nuclei for 5 min and washed three times. After washing, these explants were mounted on a glass slide and viewed under a confocal microscope Zeiss LSM 700. Stereocilia bundles of hair cells stained with phalloidin-FITC were recognized. A hair cell was manually counted if it possessed an intact cuticular plate with an intact stereociliary bundle. Total hair cells were counted for the apex, middle and basal images taken from regions of the Organ of Corti explants and expressed as percentage of hair cells lost on each. Each image count was additionally verified by two blinded investigators individually selecting from a non-descript total set. Counts were then segregated into their respective groups. The hair cell count was compared between the three groups: normothermic, hypothermic and control contralateral cochleae.

Surgical approach in human temporal bone

Experiments (n=6) were conducted on room temperature human temporal bones (n=3) to achieve MTH. The bone was placed on a heat pad to maintain it near room temperature. The facial recess was approached by canal wall up mastoidectomy.

The mucosa and bone of the round window niche were taken down using a 1mm coarse diamond burr and instrument. The round window membrane was visualized and pierced using Rosen needle. The hypothermia probe was placed anteroinferior to the round window via a myringotomy. The bones were perfused via the round window with egress via a cochleostomy on the apical surface with water heated at 45 °C that translated into approximate 36 °C measured near or at the round window and at a hole drilled on the inside of the bone to expose the cochlea apex. The custom device was used to lower the temperature by 4-6 °C at the measuring locations. A microthermistor was placed on the surface of the mastoid to measure temperature over time. Two other microthermistors were placed on the cochleae: one at on the bone covering apical surface (via middle fossa approach) and another near the round window membrane.

Statistical Analysis

The results were assessed by using two-way ANOVA implemented in MATLAB and presented as mean ±S.D. For each frequency, group was the between subject factor, whereas decibels was the within-subject factor. Not significant values are represented with ns, values of $p < 0.05$ are represented by *, values of $p < 0.01$ are represented by ** and values of $p < 0.001$ are represented by ***.

2.3 RESULTS

With the current surgical approach and placement of the hypothermia probe in close proximity to the cochlear wall, the device could successfully achieve 4-6ºC of cooling required for MTH in rat cochleae within 5-8 minutes (Figure 2.1). The design of the device and its efficacy were tested in two acute experiments (Figure 2.1B).

The temperature was maintained within ±0.3ºC in the duration of the experiments. In the present set of experiments, hypothermia was maintained for 20 minutes prior to and post electrode analog insertion for a total of 40 minutes. Contralateral cochleae were used as an intra-animal reference.

Hypothermia prevented a significant elevation in hearing thresholds following Cochlear implantation trauma

In order to investigate the efficacy of MTH, the changes in hearing threshold evoked post cochlear implantation in normothermic and hypothermic-treated implanted cochleae were studied (Figure 2.2A6). The results show the comparison of ABRs between the three groups at one frequency location (mean ±S.D). A significant increase in hearing thresholds was observed at day 1 post-surgery for frequency location of 16 kHz (two-way ANOVA, $p<0.01$, Figure 2.2A6). In the normothermic implanted cochleae, a significant loss of residual hearing post-surgical trauma persisted until 28 days. The hearing threshold increased by 60±20 dB on the day after surgical implantation and it remained elevated for up to 28-days (elevation of 65±27 dB over pre-surgical levels).

In comparison, thresholds in the hypothermic-implanted cochleae increased by 35±26 dB on the day after surgical implantation. Starting day 3 post-implantation, a recovery was observed with the hearing thresholds returning to pre-surgical levels by day 28 (elevation of 4±15 dB). The hearing thresholds of the contralateral cochleae did not change significantly over the duration of the experiments (19±7 dB at day 28 compared to 14±6 dB measured at the day before surgery). All ABR measurements were carried out in euthermic conditions (37ºC body temperature).

Furthermore, the effects of EIT on residual hearing and the efficacy of MTH at multiple frequency locations were investigated. ABRs were measured between 500 Hz (Figure 2.2A1) and 32 kHz (Figure 2.2A8) for changes in hearing threshold evoked post cochlear implantation in normothermic and hypothermic-treated implanted cochleae. These hearing thresholds were repeated on day 1, 3, 7, 14 and 28. The results similarly display increased hearing threshold ($p<0.01$) for normothermic cochleae after surgical implantation with an average increase of 56±12 dB (n=7). The hearing threshold remained elevated with an average increase of 48±24 dB by day 28 from pre-surgical levels. Hypothermic treated cochleae post-surgical increase was 35±24 dB (n=7) with a significant recovery after 28 days to only 7±13 dB elevation over initial hearing threshold. The contralateral control cochleae did not show a significant elevation or progressive loss of residual hearing at any frequency (5±9 dB (n=7) at day 28 compared to 2±7 dB measured at the day before surgery). Figure 2.2B shows the same results as a threshold shift in residual hearing over pre-surgical levels measured at post-implant days 1, 3, 7, 14 and 28.

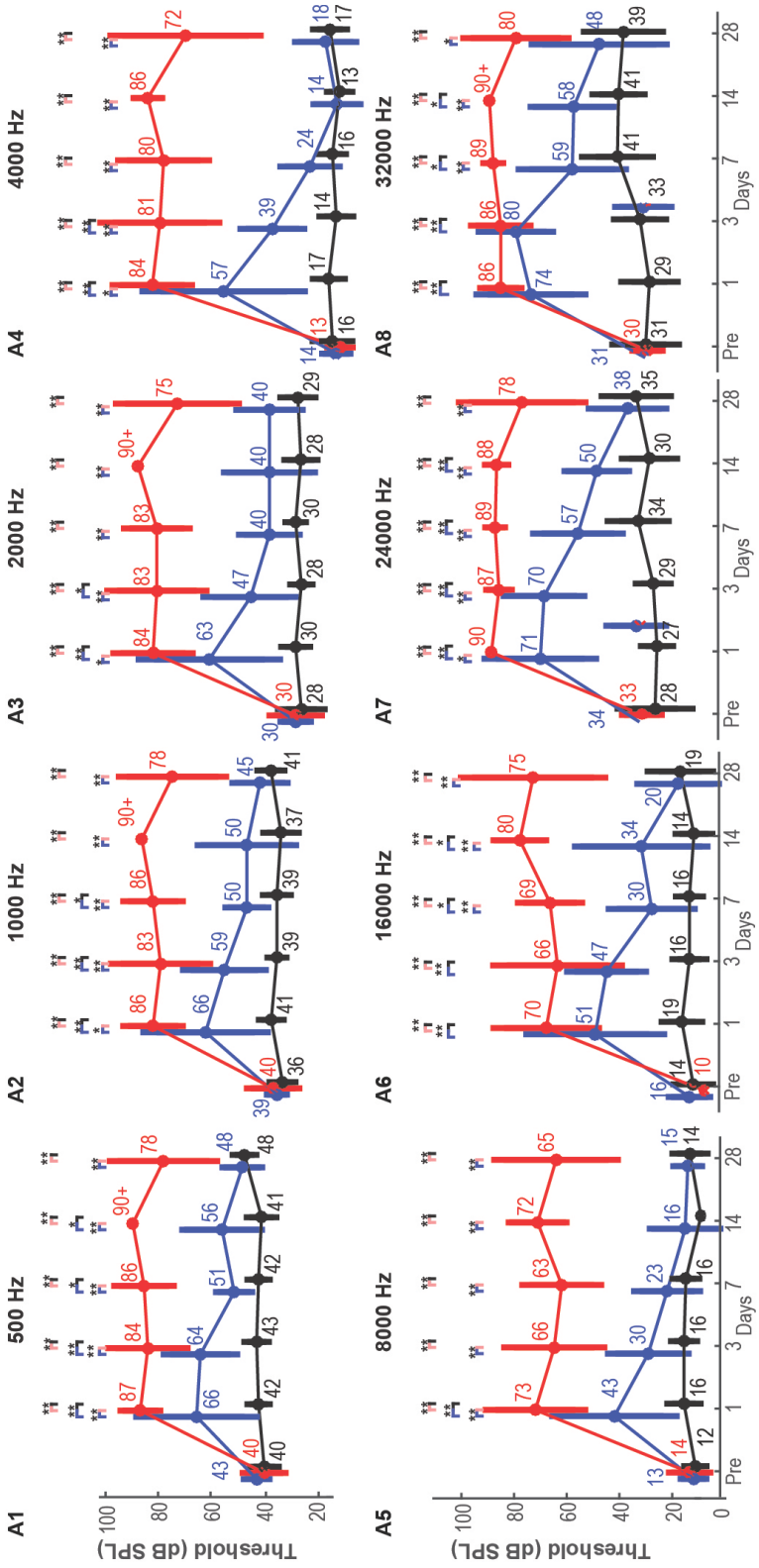

Figure 2.2 shows the mean ±S.D. of the auditory brainstem responses at .5 (A1) -32 (A8) kHz tones were recorded up to 30 days following cochlear implantation surgery (at day 0) in rats. The responses were compared for three groups: normothermic cochleae receiving EIT (red, n=7 except for days 14, where n=5 and 28, where n=6), hypothermic cochleae receiving EIT (blue, n=7 except for days 14, where n=5 and 28, where n=6) and non-operated, contralateral control cochleae (black, n=14 except for days 14, where n=10 and 28, where n=12). The electrode analog was chronically implanted and remained in place throughout the duration. (Published)

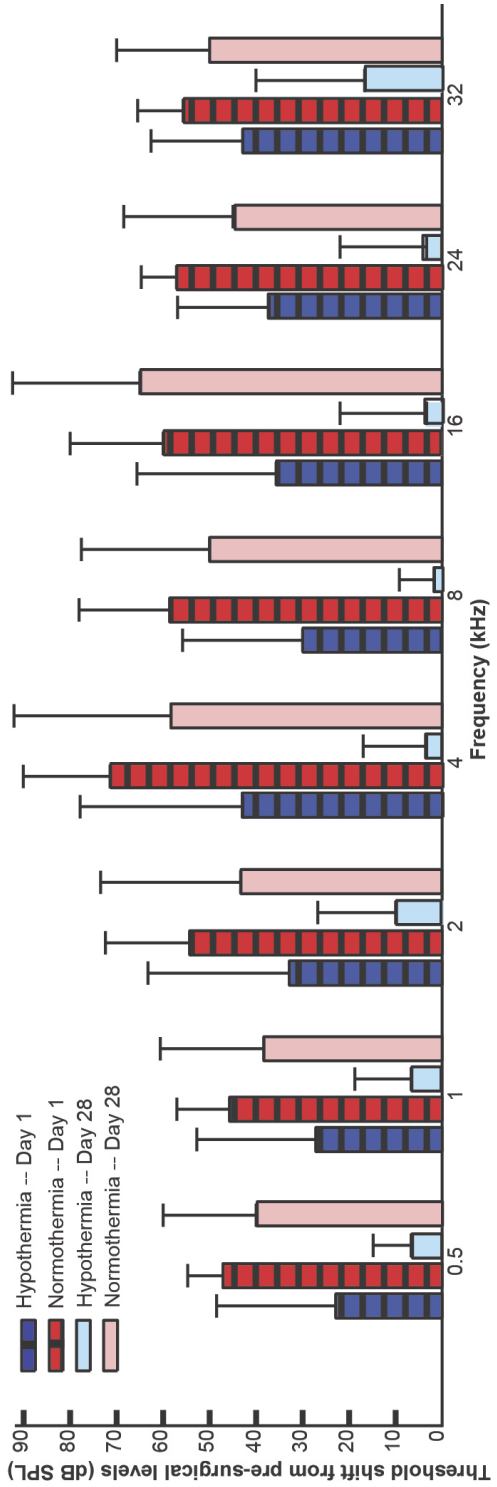

Figure 2.3 shows the results represented as mean ±S.D. of the threshold shift from initial pre-surgery measure recorded prior to surgery on days 1, 3, 7, 14 and 28 following electrode insertion. *(red: normothermic cochleae, blue: hypothermic cochleae, black: control, contralateral cochleae)* (Published)

The ABR results can be observed in contrast using normalized differences with pre-surgery hearing threshold levels. Plotting frequencies tested over the duration of the experiment allow a visual comparison of the tonotopic map. The length of the inserted electrode analog is shown to indicate the locations that receive the insertion damage due to the cochlear implantation. The frequencies affected are estimated up to 8 kHz and is based on the length inserted into the cochleae and frequency regions on the rat cochleae (Muller, 1991).

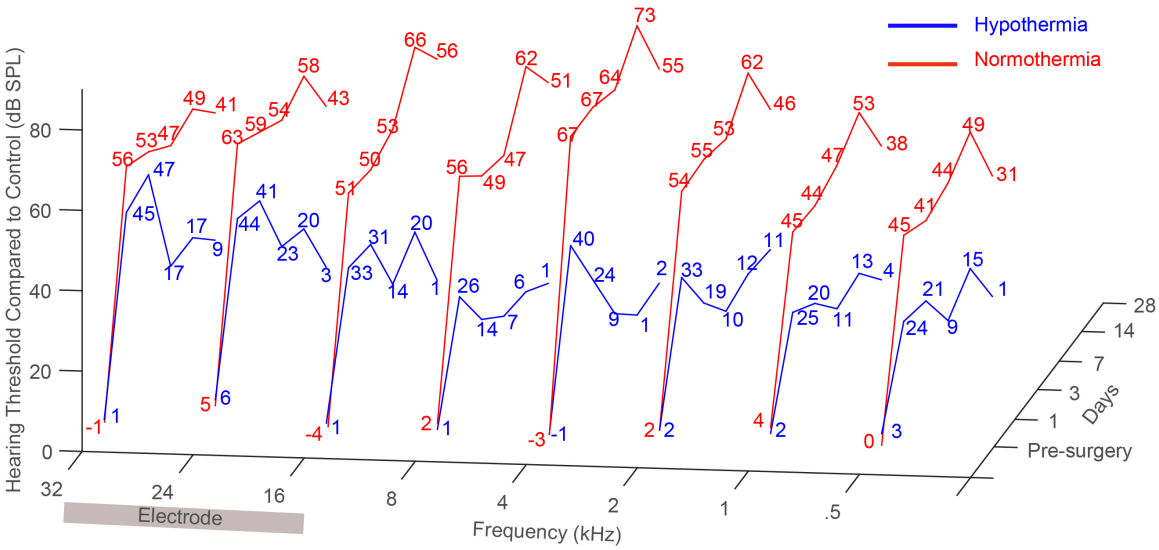

Figure 2.3 shows hypothermic (blue) and normothermic (red) hearing threshold normalized with control hearing threshold levels. The hearing thresholds at all frequencies tested and the experimental timeline represented for visual comparison of the tonotopic map. The approximate site of insertion and length of the inserted electrode analog is shown to indicate the locations that receive the insertion damage due to the cochlear implantation. (Published)

Hypothermia prevented outer hair cell loss following EIT

Cochleae were harvested at day 30 for histologic evaluation of the Organ of Corti. Typical photomicrographs from the middle segment of the cochlea showed stereocilia bundles of hair cells stained with phalloidin-FITC and nuclei stained with ANOVA. Contralateral, control cochlea showed little or no evidence of hair cell loss and normal outer and inner hair cell morphology (Figure 2.4A). As shown in Figure 2.4b, a significant loss of the outer hair cells was observed in the normothermic cochlea when compared with hypothermia-treated cochlea. Hypothermia preserved the outer hair cells as seen in the photomicrographs (Figure 2.4c). No significant inner hair cell loss was present in the all the samples. Quantification analyses of the microphotographs show an average outer hair cell loss of 39.5% of normothermic samples compared to 4.8% loss in hypothermia treated cochleae. Inner hair cell loss was not significant in all samples tested (Figure 2.5).

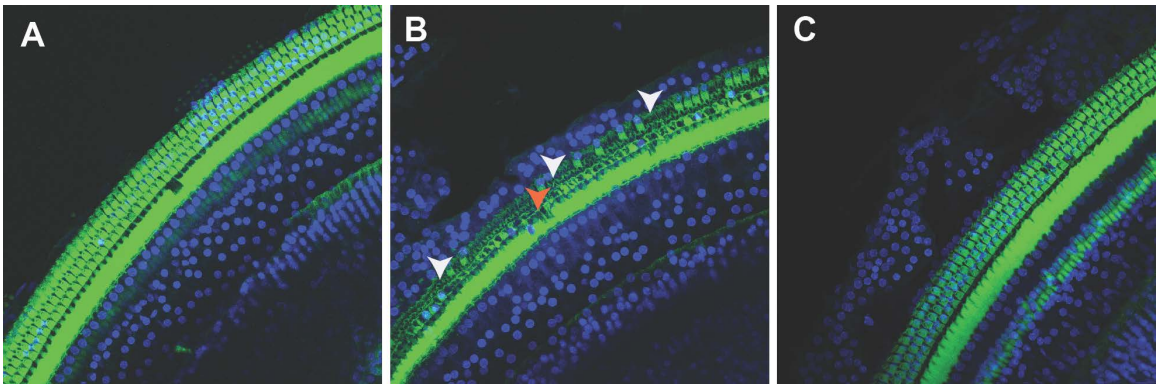

Figure 2.4 shows the organ of Corti of chronically implanted rats harvested at Day 30. Stereocilia bundles of hair cells were stained with phalloidin-FITC (green) and the nuclei with DAPI (blue). A) shows the organ of Corti of a hypothermia-treated cochlea undergoing EIT. B) shows an EIT normothermic cochlea that had significant outer hair cell loss near the electrode analog insertion site indicated by the arrows. C) shows a control, non-operated contralateral cochlea. (Published)

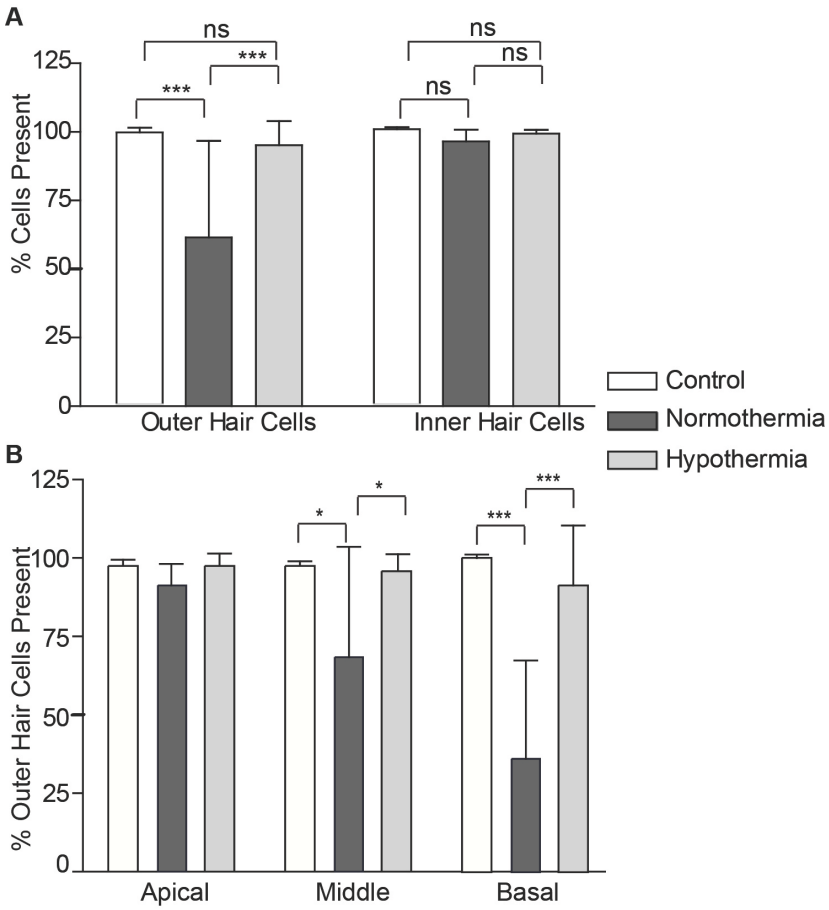

Figure 2.5 shows the hair cell count comparison between outer and inner normothermia treated, hypothermia treated and control cochleae harvested 28 days post implantation. There was no significant inner hair cell loss and pronounced normothermic outer hair cell loss compared to hypothermic outer hair cell loss. B) Quantification of the total number of outer hair cells present at the end of chronic experiment in basal, middle and apical turn regions of the cochleae in control, normothermic and hypothermia-treated implanted cochleae. Note the significant loss of outer hair cells in the middle (*P≤0.05) and in the base (***P≤0.005) of the cochlea. Error bars are S.D. (n=14) (Published)

The extracted cochleae were categorized according to distance from start of basilar membrane. The basal area encompasses the 32 and 24 kHz bands, the middle area includes the 16 kHz and 8 kHz bands, and the apex the remaining 4, 2, 1 and 0.5 kHz bands.

The distribution of missing outer hair cells is delineated in figure 2.5B, and shows the greatest loss in the basal area of the organ of Corti. The more profound loss occurs in normothermic cochleae with an average loss of 64% of all outer hair cells in the basal area, 32.7% in the middle turn and 10% on the apex. Hypothermic implanted cochleae display a much decreased loss as compared to normothermic implanted cochleae.

This correlates to the ABR results, in which the greater post-surgery ABR hearing threshold increase was observed in the frequencies 24 kHz and 32 kHz for both hypothermic and normothermic groups.

Developing a surgical approach for human application

To study the translational potential of this device, temperature measurements in cadaver temporal bones were carried out using the current hypothermia probe designed for the rats (Figure 2.6, n=3). The microthermistor location is shown in Figure 2.6A (control, T1) As can be seen in Figure 2.6B, C, the cochlear temperature was reduced gradually by ~3-6ºC within 10 minutes while the mastoid cooled by less than 1ºC to equilibrate with room temperature. The cochlear temperature was maintained near within ±0.5ºC, following which a slow rewarming protocol was carried out to return the temperature to baseline levels.

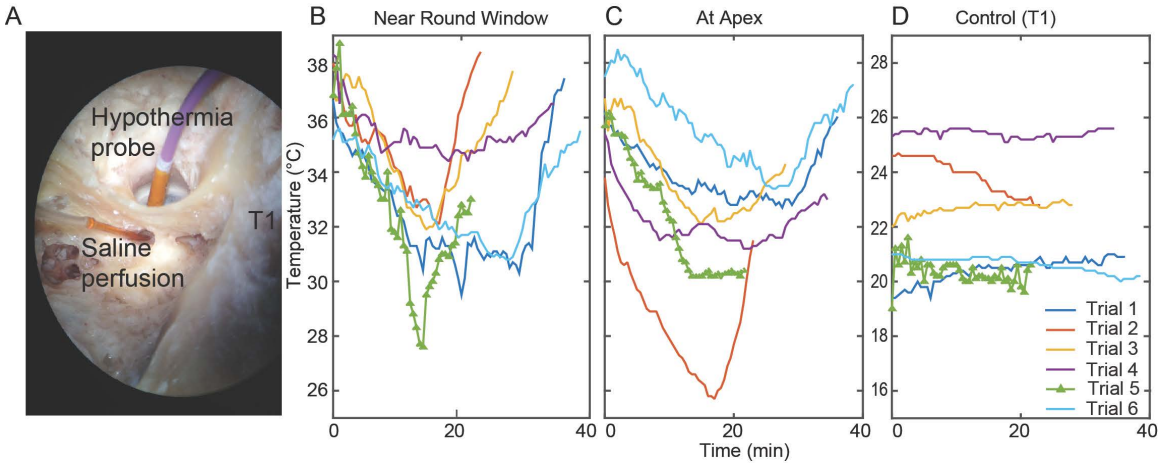

Figure 2.6 shows the measurements of human temporal bone application of mild therapeutic hypothermia. (A) shows the placement of the hypothermia probe anteroinferior to the round window via a myringotomy. The temperature was reduced by 4-6°C in on the bone covering apical surface (via middle fossa approach) (B) and at the round window membrane (C). The mastoid (D) temperature varied less than 1ºC during the experiment. (Published)

2.4 DISCUSSION

Previous reports have documented that EIT results in a significant loss of residual hair cells either by direct trauma to cochlear structures such as basilar membrane, osseous spiral lamina, and modiolus or by cascading molecular effects (Esperanza Bas et al., 2012; Eshraghi et al., 2013). Although the exact mechanisms are still not understood; recent studies have begun to unravel the molecular mechanisms that underlie loss of residual hearing. It has been shown that increased levels of inflammation and oxidative stress following trauma could initiate apoptosis of hair cells and might be associated with the loss of residual hearing. In particular, the mRNA levels of pro-inflammatory cytokines such as TNFα and IL-1β, and pro-inflammatory enzymes, iNOS and COX-2 increased significantly following trauma (Esperanza Bas et al., 2012).

Cytokines, such as interleukin-1 (IL-1) (Touzani, Boutin, Chuquet, & Rothwell, 1999) and tumor necrosis factor alpha (TNF-α) (Dinh et al., 2008; Keithley, Wang, & Barkdull, 2008), are known to contribute to brain damage similarly to free radicals and may cause auditory hair cell and spiral ganglion loss. The apoptotic pathways include the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway, with both involving the activation of caspase-3, which induces chromatin condensation and DNA fragmentation (Elmore, 2007).

The feasibility and safety of MTH is a useful therapeutic method for improving recovery from brain injuries, strokes, and neuron recovery. Its efficacy has been evaluated in experimental settings and clinical trials. Kawai's team assessed that cortical infarct volume was significantly decreased by hypothermic treatment and Levi et al identified improvement of spinal cord injury assessment after intravascular cooling (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010). The neuroprotective mechanisms of hypothermia being investigated include suppression of excitotoxicity, free radical production, neuroinflammation, blood brain barrier (BBB) disruption and regulation of early gene expression (Darwazeh & Yan, 2013; Dietrich, Levi, Wang, & Green, 2011). Studies have shown that hypothermia related protective effects are also correlated with a significant increase in expression of Bcl-2, an important anti-apoptotic gene, in a rat model of transient global ischemia (H. Zhang et al., 2010)

Applications of hypothermia in a clinical setting have varied from systemic hypothermia to localized cooling of surgically exposed tissue. Balkany et al (Balkany et al., 2005) showed that cochleae undergoing surgical implantation in animals receiving MTH had less functional loss compared to normothermic implanted cochlea up to 7 days. While the results were positive, the application of systemic hypothermia for inner ear surgeries has not been clinically feasible. In the present study, the potential of localized hypothermia delivered to the inner ear during cochlear implantation surgery to improve protection of critical structures and preservation of residual function was evaluated. The results of the present study indicate that cooling the inner ear between 4-6ºC protected residual hearing in rats. The hypothermia-treated cochleae showed a similar but significantly smaller shift in the ABR thresholds as the normothermic-implanted cochleae immediately following the cochlear implantation. The ABR thresholds in normothermic-implanted group remained elevated over the next 4 weeks, while those of the hypothermic-implanted group recovered starting week 1. Histological results compared well with the functional data -- hypothermic-cochleae showed significant preservation of outer hair cells and minimal cell death compared to normothermic-cochleae. The approach utilized a novel copper hypothermia probe attached to a thermoelectric Peltier device placed under direct visualization in the surgical cavity adjacent to the site of implant. With continuously circulated refrigerant through the probe tip the temperature of the cochlea could be reduced by 4-6ºC within 5 to 10 minutes. Furthermore, the temperature could be maintained within ±0.3ºC, over the duration of the experiments.

The approach will be feasible for human clinical application as shown here and has significant advantages over systemic or external application of hypothermia. Although the surgical approach and design of the device will need to be optimized in the future to deliver MTH to the human cochleae, these results clearly demonstrated that effective cooling of the cochlea and inner ear structures is possible.

While the current investigation presents the utility of custom-designed probe to deliver localized hypothermia *in vivo* and the efficacy of hypothermia delivered pre-trauma, several limitations should be noted. First, the present study focused on normal hearing animals. The normal functioning cochlea is likely to be different from a cochlea in a hearing-impaired or deafened animal with respect to expression of inflammatory mediators and may present different mechanisms of injury not mitigated by MTH. Second, the auditory function was studied over a relatively short post-implantation period (28 days) and without the potential damage caused by electrical activation of cochlear implants. It is possible that the protective effects of hypothermia may have simply delayed the progressive component of the hearing loss observed following EIT. Additional studies focusing on chronic effects of EIT, electrical activation and potential benefits of MTH are therefore required prior to translating the application to clinical trials. Finally, prior literature has shown that duration of cooling and speed of rewarming can impact the beneficial effects of hypothermia (Polderman & Herold, 2009).

In the present study, hypothermia was delivered for ~60 min intraoperative, with 10 minutes of cooling and rewarming period and 40 minutes of maintained hypothermia. The relatively long duration may restrict widespread adoption of the technique. Future studies will need to focus on determining the critical duration for MTH applied to the cochlea.

2.5 CONCLUSION

In conclusion, the present results are promising and indicate that MTH applied pre-trauma protects residual function in the cochleae following cochlear implantation. In the rat model, localized hypothermia can reduce the post-implant trauma and protect the cochleae against the progressive loss of hearing observed in normothermic implanted cochleae. Additionally, based on the temporal bone studies described, the present approach appears to be applicable to patients receiving cochlear implantation with localized hypothermia achieved during surgery.

CHAPTER 3: CHRONIC LOCALIZED THERAPEUTIC HYPOTHERMIA APPLICATION PRESERVES RESIDUAL HEARING IN COCHLEAR IMPLANTATION

3.1 INTRODUCTORY REMARKS

Growing environmental and recreational noise has increased the amount of patients with hearing deficiencies in the high frequency region. Bimodal electroacoustic stimulation allows for the possibility to apply both electrical stimulation via cochlear implant to the basal (high frequency) area and acoustic stimulation on the middle and apex of the cochleae (Irving et al., 2014). However, cochlear implantation surgery can result in inflammation and oxidative stress resulting in neural degeneration, hair cell loss and loss of residual hearing (Eshraghi et al., 2005; Wardrop et al., 2005).

Cochlear trauma related to cochlear implantation insertion surgery has been investigated (Adunka et al., 2005; Ahmad et al., 2012; E. Bas et al., 2015; Esperanza Bas et al., 2012; Briggs et al., 2001). Several ameliorating methods have been researched, including refinements in surgical techniques and protective drug-eluting electrodes (Dinh et al., 2008; Friedland DR & C, 2009; James et al., 2008; Jolly et al., 2010; Van De Water et al., 2010; Vivero et al., 2008). The loss of residual hearing impedes the operation of electroacoustic stimulation implants. Recently the FDA reported about 44% of loss of residual hearing after a trial hybrid electrode implantation study (FDA, 2013).

Prior studies conducted at the lab confirmed the hypothesis that the application of localized MTH can protect hair cells in a cochlear implantation trauma model (Tamames et al., 2016). Localized MTH treated cochleae has been used to show less functional loss compared to normothermic cochleae in cases of cochlear implantation (Balkany et al., 2005). Additionally it has been shown that hypothermia treated cochleae had significantly lowered auditory thresholds than euthermic cochleae in acoustic trauma (Kenneth R. Henry, 2003). In cases of occlusion of the vertebral arteries, hypothermia treated cochleae were less affected and the auditory threshold increase was diminished (Hyodo et al., 2001). Takeda's team study with different amounts of hypothermic treatments identified attenuated hearing loss and inner hair cell loss after transient ischemia (Takeda et al., 2008).

While the beneficial effects of localized MTH have been proven in otoprotection, there is a lack of research literature on long term studies after treatment. This study aims to answer the following questions: (1) does MTH protection benefits on hearing thresholds remain past 30 days and (2) do hearing thresholds improve for cochleae missing hypothermic protection past 30 days. For this purpose, the previous study in (Tamames et al., 2016) was extended by an additionally 2 months. As per the previous study, hearing thresholds were analyzed post-surgery in normothermic and hypothermic-treated cochleae.

3.2 MATERIALS AND METHODS

The protocol performed followed Chapter 2 methods for animal preparation, measurement of ABR and MTH delivery (Tamames et al., 2016). There were three experimental groups: in the first group, the left ear was implanted under normothermic condition (normothermic) and in the second group, MTH was provided using the hypothermia device (hypothermia-implant). The right contralateral ear always served as the intra-animal control group.

The animals were anesthetized and prepared for surgery. The bulla on the temporal bone was breached to expose both the round window membrane niche and the lateral bony wall of the cochlea adjacent to the niche. Electrode trauma to the cochlea was accomplished by the insertion of an electrode analog (fishing line) into the scala tympani via the round window to a depth of 5 mm. The defect in the ventro-lateral wall of the temporal bone bulla was covered with carboxylate cement and a subcuticular closure was provided. The animal holding/recovery cages where placed on electric heating pads.

The hypothermia probe used in previous studies was placed in the middle ear adjacent to the cochlea under direct visualization. The temperature of the cochlea was reduced by 4-6ºC with the device and cooling was maintained within a range of ±0.3ºC for the duration of the experiment (Tamames et al., 2016).

ABR were measured for acoustic tones on the frequencies of 0.5, 1, 2, 4, 8, 16, 24, and 32 kHz before surgery to determine pre-surgical hearing thresholds. ABRs were also measured at various time points post-surgery up to three months and compared to pre-surgical thresholds.

The results were assessed by using two-way ANOVA implemented in MATLAB and presented as mean ±S.D. For each frequency, group was the between subject factor, whereas decibels was the within-subject factor. Not significant values are represented with ns, values of $p < 0.05$ are represented by *, values of $p < 0.01$ are represented by ** and values of $p < 0.001$ are represented by ***.

3.3 RESULTS

While in previous work the efficacy of MTH was demonstrated, the present work studies the protection up to three months post implantation. The changes in hearing threshold post cochlear implantation in normothermic, hypothermia-implant, and their respective contralateral control cochleae were studied. The results show the comparison of ABRs between the three groups at one frequency location (mean ±S.D). A significant increase in hearing thresholds was observed at day 1 post-surgery, similar to previous work (Tamames et al., 2016).

Normothermic cochleae hearing thresholds remain elevated up to three months post implantation.

In the normothermic implanted cochleae, a significant loss of residual hearing post-surgical trauma persisted up to three months. The hearing threshold at 8 kHz increased by 60±13 dB on the day after surgical implantation and it remained elevated for up to three months (elevation of 54±7 dB over pre-surgical levels).

Hypothermia treatment protects residual hearing for up to three months following cochlear implantation trauma.

In comparison, thresholds at 8 kHz in the hypothermic-implanted cochleae increased by 43±16 dB on the day after surgical implantation. Starting day 3 post-implantation, a recovery closer to control levels was observed with the hearing thresholds with protection remaining up to three months (elevation of 16±15 dB).

The hearing thresholds of the contralateral control cochleae did not change significantly over the duration of the experiments (2.5±6 dB at day 90 compared to 4±4 dB measured at 8 kHz on the day before surgery). All ABR measurements were carried out in euthermic conditions (37ºC body temperature).

Furthermore, ABRs were measured between 500 Hz (Figure 3.1A) and 32 kHz (Figure 3.1H) before surgery for cochlear implantation. Measurements were repeated on day 1, 3, 7, 14, 28, 56 and 84. The study shows increased hearing threshold (two-way ANOVA, $p<0.01$) for normothermic cochleae remained impaired with an average increase of 50±9 dB by day 84 from pre-surgical levels. Hypothermia-implanted cochleae initial increase was reduced to 23±14 dB elevation over initial hearing threshold after three months. The contralateral control cochleae did not show a significant elevation or progressive loss of residual hearing at any frequency 0.2±7 dB after three months compared to the day before surgery). Figure 3.2 displays same results as a threshold shift in residual hearing over pre-surgical levels.

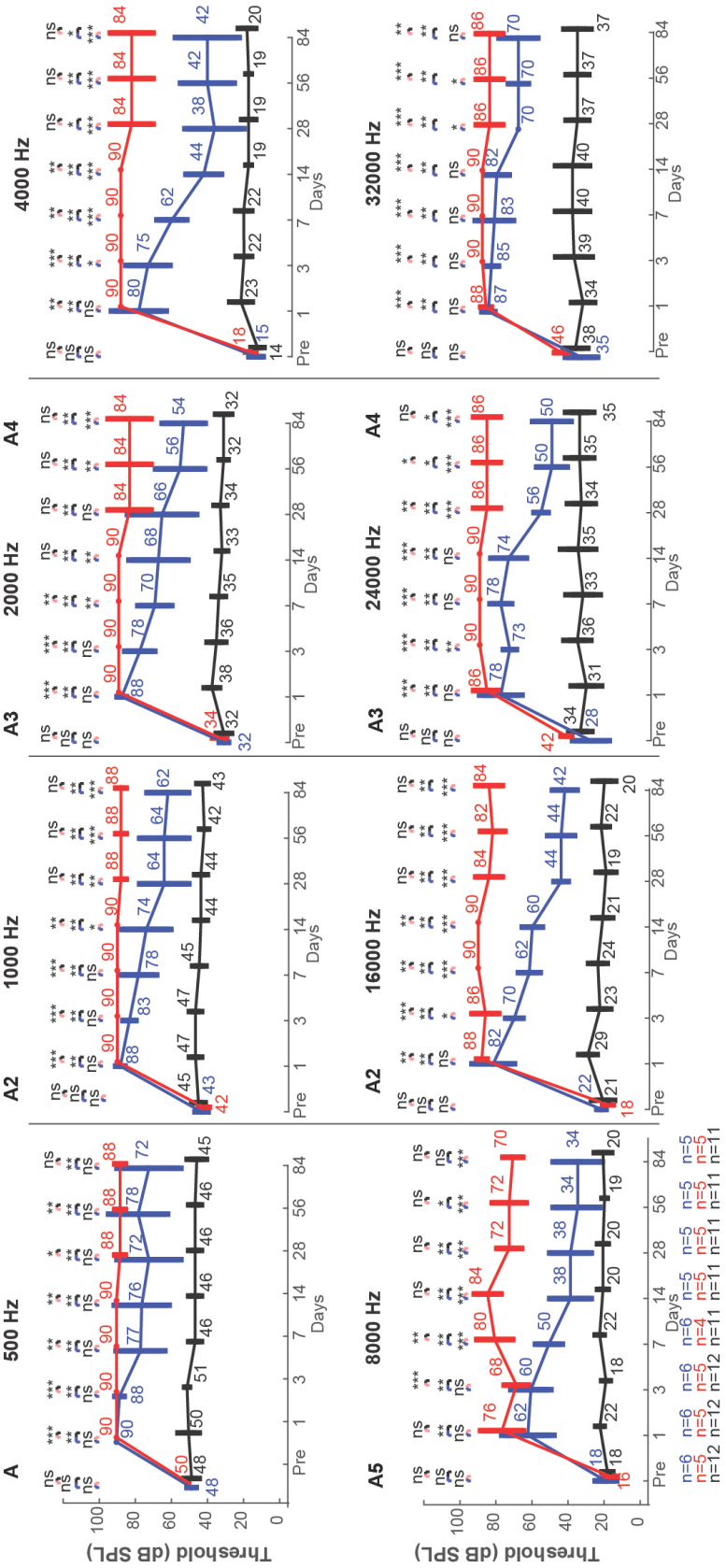

Figure 3.1 shows the mean ±S.D. of the auditory brainstem responses at .5 (A) -32 (H) kHz tones were recorded up to 90 days following cochlear implantation surgery (at day 0) in rats. The responses were compared for three groups: normothermic cochleae receiving EIT (red, n up to 5), hypothermic cochleae receiving EIT (blue, n up to 6) and non-operated, contralateral control cochleae (black, n up to 11). The electrode analog was chronically implanted and remained in place throughout the duration.

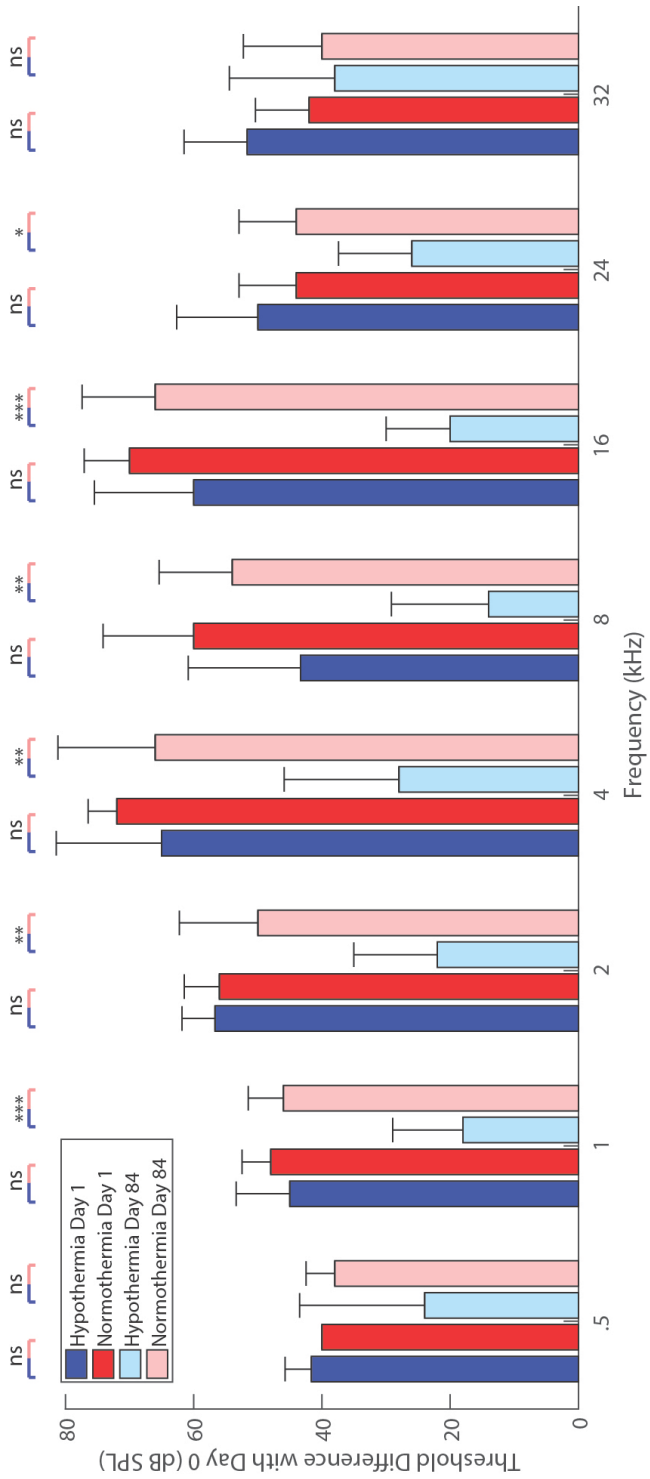

Figure 3.2 shows the results represented as mean ±S.D. of the threshold shift from initial pre-surgery measure recorded prior to surgery on day 84 following electrode analog insertion. (red: normothermic cochleae, blue: hypothermic cochleae)

Plotting the results against their contralateral controls allows for a visual comparison of the tonotopic map. The inserted electrode analog is represented to visualize the locations receiving insertion damage. The length inserted into the cochleae was based on work done by Muller (Muller, 1991).

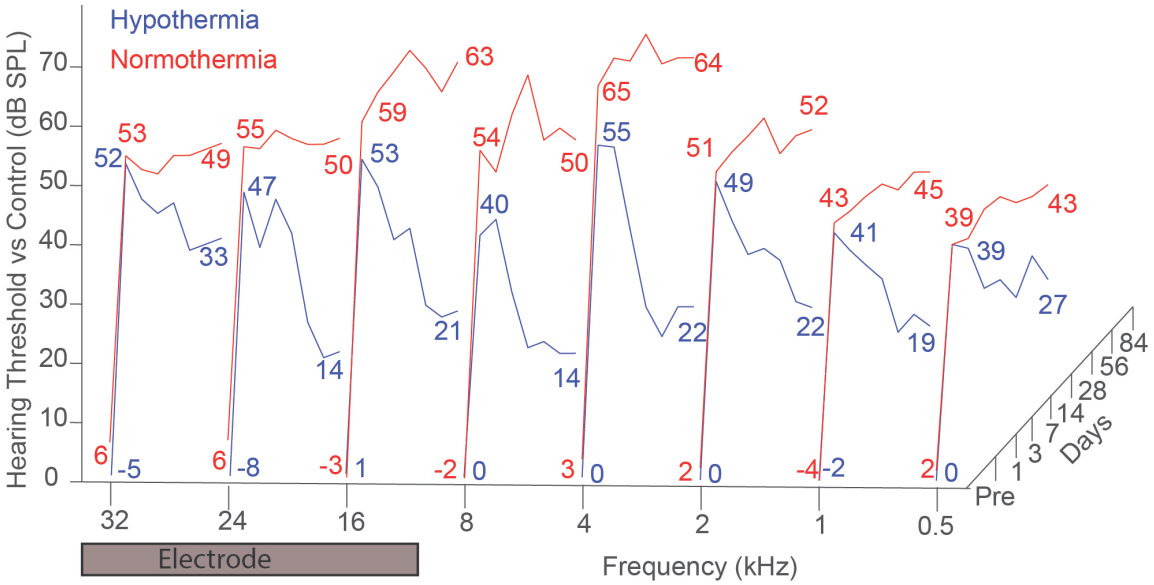

Figure 3.3 shows hypothermic (blue) and normothermic (red) hearing threshold normalized with control hearing threshold levels. The hearing thresholds at all frequencies tested and the experimental timeline represented for visual comparison of the tonotopic map. The approximate site of insertion and length of the inserted electrode alternative is shown up to the frequencies tested.

3.4 DISCUSSION

The feasibility and safety of MTH treatment has been investigate as a useful therapeutic method for improving recovery from brain injuries, strokes, and neuron recovery (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010; Purdy et al., 2013; Tzen et al., 2013).

Its effectiveness has been assessed in numerous previous studies, with generally positive effects (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010). Applications of hypothermia in cochleae trauma have varied from systemic hypothermia to localized cooling of surgically uncovered tissue (Balkany et al., 2005; K. R. Henry & Chole, 1984; Watanabe et al., 2001). Hypothermia treatment has also been investigated for acoustic trauma exposure with shown beneficial effects (Kenneth R. Henry, 2003). Cochlear ischemia effects are ameliorated due to hypothermic treatment (Hyodo et al., 2001; Takeda et al., 2008).

The previous study (Tamames et al., 2016) indicated that cooling the inner ear between 4-6ºC protected residual hearing in rats. The hypothermia-treated cochleae showed smaller shift and greater recuperation in auditory thresholds following cochlear implantation surgery than normothermic cochleae. Histological results matched the functional data, displaying greater preservation of outer hair cells in hypothermia treated as compared to non-treated cochleae up to 28 days after surgery.

The current investigation presents the extended insight into the treatment of localized hypothermia *in vivo* and the efficacy of MTH treatment delivered pre and post trauma. The protocol followed the MTH treatment rationale of the previous study. Additionally, male rats were added to discount the influence of gender. The results show that *localized MTH protects the residual hearing up to three months beyond surgery*. This study identified that the protective effects of MTH *did not delay* a progressive component of the hearing loss observed following EIT. *Auditory thresholds remained elevated in normothermic cochleae up to three months post-surgery*.

In contrast, hypothermia treated and implanted cochlea auditory thresholds decreased and *remained low* over the same time period.

As in the previous study, several limitations should be noted. Additional studies analyzing the electrical activation and potential benefits of MTH in deafened or hearing impaired animals are required prior to translation to clinical trials. First, the present study concentrated on normal hearing animals. The normal cochlea mechanisms differ from a typical candidate for cochlear implantation. MTH might not mitigate conditions, such as inflammatory mediators and mechanisms of injury, occurring during hearing-impaired or deafened animal cochlear implantation surgery. Lastly, the potential damage caused by electrical activation of cochlear implants was not investigated.

3.5 CONCLUSION

The approach presented in previous work has shown to be feasible for human clinical application for long term protection. These results clearly demonstrated that effective cooling of the cochlea and inner ear structures is possible and provides a substantial benefit for otoprotection. In conclusion, the present results indicate that localized MTH applied pre and post trauma protects the residual function in the cochleae following cochlear implantation for up to three months. This reinforces the effectiveness of the therapeutic treatment with a focus on successful clinical translation and improving patient care.

CHAPTER 4: THEORETICAL EVALUATION AND EXPERIMENTAL VALIDATION OF LOCALIZED THERAPEUTIC HYPOTHERMIA APPLICATION TO PRESERVE RESIDUAL HEARING FOLLOWING COCHLEAR IMPLANTATION

4.1 INTRODUCTORY REMARKS

Cochlear implants restore hearing by providing direct electrical stimulation to the auditory nerve in patients missing all or part of the sensory mechano-sensory apparatus. More than 324,000 partially or completely deaf individuals have already been implanted worldwide (FDA, 2015). With the success of these devices and recent advances in technology, the indications for cochlear implantation have broadened to include patients with single-sided deafness, and those with loss of hearing at high frequencies and residual hearing at lower frequencies. Implantation of new hybrid (acoustic and electric) devices aims to take advantage of the remaining hair cells to provide a more accurate representation of real-world sounds. These implants allow for a wider range of acoustic representation, and may improve child development and providing for a better quality of life (Markman et al., 2011). In particular, preservation of residual hearing during implantation may greatly aid early language development and the later development of speech perception skills in congenital and pre-lingual deaf children (Nikolopoulos, O'Donoghue, & Archbold, 1999; Tobey et al., 2013). However, cochlear implantation surgery has been shown to result in trauma to inner ear structures such as basilar membrane, osseous spiral lamina, and modiolus or by cascading molecular effects such as inflammation and oxidative stress (Eshraghi et al., 2005; Rebscher et al., 2008; Wardrop et al., 2005).

In a recent clinical trial for an electric-acoustic stimulation cochlear implant system, the most prevalent and significant adverse event was the loss of residual hearing (44% of the study population) (FDA, 2013). Therefore, reducing the trauma caused by implantation and preservation of residual hearing could greatly benefit the patients receiving these new electro-acoustic stimulation devices. Further, a reduction of intracochlear damage caused during surgery may also limit fibrosis and ossification, which may be critical in cases where reimplantation may be required.

Localized MTH has been a widely studied method for neuro-protection against secondary injuries as a result of brain trauma, strokes, and spinal cord (Cappuccino et al., 2010; Dietrich et al., 2009; Dietrich & Bramlett, 2010; Dietrich et al., 2011; Eshraghi AA et al., 2007; Kawai et al., 2000; Levi et al., 2010). Modifying the temperature of the cochlea has been shown to influence auditory-evoked responses (Brown et al., 1983; Liberman & Dodds, 1984; Ohlemiller & Siegel, 1992, 1994), predominantly when applying hypothermia (K. R. Henry & Chole, 1984; Watanabe et al., 2001). Studies have attempted to localize hypothermia for use in inner ear cochlear EIT models. Balkany et al. studied rat cochleae undergoing surgical implantation after receiving hypothermia and showed that they exhibited less functional loss compared to normothermic implanted cochlea (Balkany et al., 2005). Henry et al. have studied the influence of 20 minutes of hyperthermia and hypothermia on auditory thresholds (Kenneth R. Henry, 2003).

Hyperthemic mouse cochleae were observed to have significant elevated thresholds compared to normothermic cochleae exposed to noise while hypothermic cochleae had significantly lowered thresholds than euthermic cochleae. The beneficial effects of hypothermia on hearing loss have also been studied in an animal model of transient cochlear ischemia (Hyodo et al., 2001). Hypothermia-treated cochleae, with a temperature of 32°C, were shown to have an improved recovery following ischemia injury along with a significant reduction in perilymphatic glutamate concentrations when compared to normothermic cochleae. Yet another study identified attenuated hearing loss and inner hair cell loss within 3 h after transient ischemia (Takeda et al., 2008).

In a recent study, it was discussed the design and implementation of a custom device to apply localized hypothermia in a rodent experimental model of EIT and highlighted its benefit in preserving hair cells and residual hearing function against surgical and electrode analog implantation trauma (Tamames et al., 2016). A reduction in temperature by 4 to 6 °C from baseline (~37ºC) was measured at the round window of the rat specimens. Our results using functional analysis and histological methods demonstrated that MTH applied during implantation provides significant preservation of residual hearing function and mitigates the adverse effects caused by cochlear implantation surgical trauma. Results showed that the hearing thresholds in implanted cochleae recovered to the pre-surgical levels and matched the contralateral, control cochleae up to 28 days post-implantation (Tamames et al., 2016).

The results from animal studies suggested translational potential of this application since the hypothermia was delivered pre-trauma locally to the inner ear and did not require significant addition to the surgical duration. However, at the present time whether this approach can achieve uniform cooling of the auditory and/or vestibular structures in patients at levels required to achieve MTH remains to be demonstrated.

The objective of the present article is to experimentally determine the feasibility of reducing the temperature of the inner ear sensory structures by 4-6ºC and develop a model of the temperature distribution in the inner ear. This study aims to utilize the model to estimate a cooling protocol that should be applied to provide beneficial MTH while avoiding damaging the inner ear structures. The results show spatial temperature distribution in the inner ear, parameters for cooling and effect of physiological conditions. Such a model may be of value to the clinical and scientific community as the results will directly contribute to improving translational potential of MTH for cochlear implantation and other inner ear surgeries with an aim to preserving residual hearing function.

4.2 MATERIALS AND METHODS

Theoretical model of the inner ear and hypothermia application

For the theoretical model, this study utilized a simplified 3D reconstruction of temporal bone obtained from CT scans. The images and model were obtained from the University Health Network in Toronto by Dr. Eitan Aziza and provided for this study under Creative Commons – Attribution – Share Alike license (https://skfb.ly/OVzX).

The model mesh elements were further reduced to 1k using MeshLab (Cignoni et al., 2008) to improve computational efficiency and imported in COMSOL (Comsol Inc., Burlington, MA). The 3D model of human inner ear (cochlea and vestibular endorgans) derived from MRI images from McGill University was simplified and provided by Dr. Seth Horowitz (http://www.thingiverse.com/thing:27340) under Creative Commons – Attribution – Share Alike license for this study.

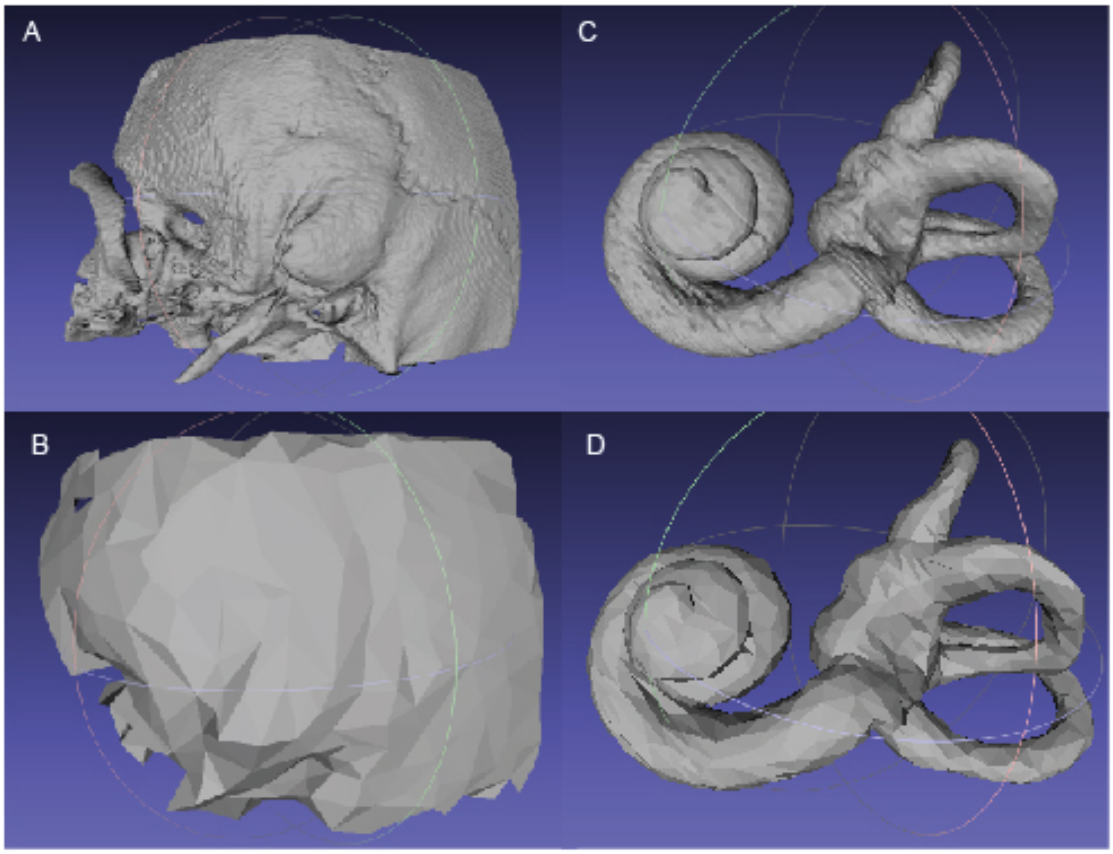

Figure 4.1 shows the model setup used in this study. (A, C) show the original 3D images of a temporal bone and inner ear organs respectively re-constructed from CT and MRI scans. (B, D) show the simplified, reduced mesh versions of the same models prior to importing in COMSOL.

For the computational purposes, this study further reduced the mesh elements of the 3D human inner ear to 3k prior to importing it to COMSOL. The original and reduced models for the temporal bone and human inner ear are shown in Figure 4.1.

To model the application of localized MTH to the temporal bone, a finite element model created using a commercially available software package COMSOL was utilized. Figure 4.2 shows the processed 3D images of the human temporal bone and inner ear organs imported into the numerical analysis tool. The 3D models were sized to scale and merged into a COMSOL mesh. For the present study, brain, skin or muscle tissue was not included since the primary focus was on the inner ear. The temporal bone model size used in this study was ~90 mm in width and 70 mm in height. Inner ear organ model size was 6.2 mm in diameter for the cochlea and 7.1 mm in diameter for the superior semi-circular canal. The probe cylinder tip is 1.6 mm in diameter and 5 mm in length. The values for the inner ear were set based on size assessments in literature (Erixon, Hogstorp, Wadin, & Rask-Andersen, 2009; Klopp-Dutote, Kolski, Biet, Strunski, & Page, 2016). The inner ear fluids were modeled as water for simplicity. The cylindrical hypothermia probe was positioned touching the cochlear bone, approximately 2.5 mm from the round window location. This orientation mimicked the position achievable under facial recess approach and that used for temporal bone temperature experiments.

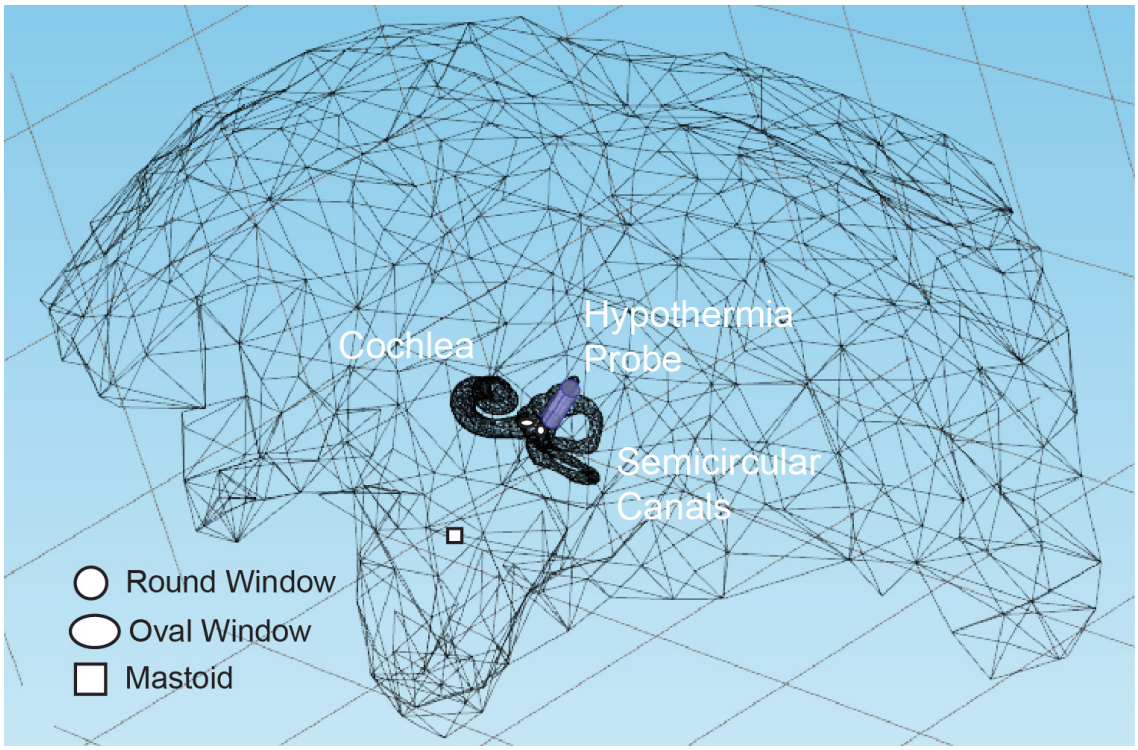

Figure 4.2 shows the COMSOL model mesh of the human temporal bone and inner ear organs along with the hypothermia probe mimicking potential surgical orientation. The locations of round window, oval window and mastoid are highlighted. Temperatures at these three locations, in addition to multiple planes along the inner ear were recorded.

This study modeled a modified bio-heat equation, convection through bone and blood flow, metabolic heat generation and cooling provided by the hypothermia probe to the inner ear organs and temporal bone in COMSOL. A modified bio-heat equation utilizing the Pennes' approximation was used to describe the changes when MTH was applied at the probe. The bio-heat equation (equation 1) was adapted using the bio-heat transfer application mode with time dependency in COMSOL.

$$\rho_m C_m \partial T/\partial t + \nabla \cdot (-k_m \nabla T) = Q + Q_{bio} \quad (1)$$

Where T is the temperature; ρ_m, C_m and k_m, are respectively the density, heat capacity and the thermal conductivity of the material for bone or water. Q is the boundary heat source coefficient of the hypothermia probe. Finally, Q_{bio} is the blood metabolic heat source, derived using the equation:

$$Q_{bio} = \rho_b C_b \omega_b (T_b - T) + Q_{met} \quad (2)$$

Where ρ_b is the density for blood, C_b is the specific heat capacity, ω_b is the perfusion rate, T_b is the arterial temperature, T is temperature of the surrounding tissue and Q_{met} is the metabolic heat source. These properties are listed in Table 4.1. It is assumed that the perfusion is homogeneous and isotropic. The tissues of interest were also assumed to be homogeneous and isotropic. The material properties used in the present study were selected based on literature (K. Zhang, Ma, Zhou, & Wang, 2015) and default properties provided in COMSOL.

Type	Density (kg/m^3)	Specific Heat Capacity (J/kg·K)	Thermal Conductivity (W/m·K)	Blood Perfusion Rate (1/s)	Metabolic Heat Generation (W/m^3)
Temporal Bone	*1,908*	*1,313*	*0.32*	-	-
Perilymph / Endolymph (modeled as water)	*994*	*4,178*	*0.60*	-	-
Blood	*1,050*	*3,840*	*0.52*	*0.00033*	*1,100*

Table 4.1 show the physiological properties used in model computations.

The bone, inner ear and cylindrical probe baseline temperature were initially set at 37ºC. The temporal bone surface mesh, surrounding the sites of mastoid measurement and surgical opening, was assumed to be influenced by room temperature (25ºC). The remaining surface area, in particular to boundary open the brain, was considered an open boundary with temperature set at 37 ºC. Of the three ways of heat transfer in a material, i.e. radiation, convection and conduction, we discounted both radiation and convection. The power radiated through the tissue is given by: $eb = \sigma(T_1^4 - T_2^4)$ where σ is the Stefan-Boltzmann constant (Lienhard, 2013). So the net power radiated, from the probe cooled to ~20.2°C to the round window niche ~31.5°C, was estimated as $e_b \approx 68.5$ W/m^2. For comparison, the heat flux is a function of thermal conductivity and temperature gradient over distance: $q = -k\frac{dT}{dx}$ (Lienhard, 2013). For a temperature difference of 11.3°C at a distance of 2.5 mm with a heat conduction of 0.32 W/m/°C, the heat flux density q is about 1450 W/m^2. Therefore, radiant heat transfer was neglected in our model in comparison with conduction.

For the present discussion, we did not model the fluid movement within the cochlea. The protocol for the MTH followed previously reported experimental and *in vivo* animal studies (Tamames et al., 2016). In addition to the calculated temperature at the three sites (round window, oval window and mastoid), the heat distribution was calculated in modiolar and horizontal planes parallel to the probe starting at the round window and at every 1mm spanning the width and height of the cochlea.

Experimental measurements using human temporal bones

Experimental measurements were carried out using post mortem human temporal bones (n=5, k=8 trials) to mimic the surgical approach, probe location and protocols for MTH application. The bones were warmed to 37ºC by submerging them in a water bath (Thermo Scientific Precision General Purpose Water Bath, model TSGP05) containing metallic beads (Lab Armor Beads, model 42370) set at 50 °C. To maintain the bone temperature during experimental measurements, they remained submerged in the bath. The facial recess was approached by canal wall up mastoidectomy, a common surgical approach for cochlear implantation surgery. The round window membrane was exposed. The hypothermia probe was placed anteroinferior to the round window via the facial recess. The custom device (Tamames et al., 2016) was used to apply cooling to the cochleae. A microthermistor (Omega, 5SC-TT-T-40-36) was placed on the surface of the mastoid to measure temperature changes over time. Two additional microthermistors were placed in the vicinity of the cochleae: one inserted via ear canal near the oval window and another one near the round window membrane via the facial recess approach.

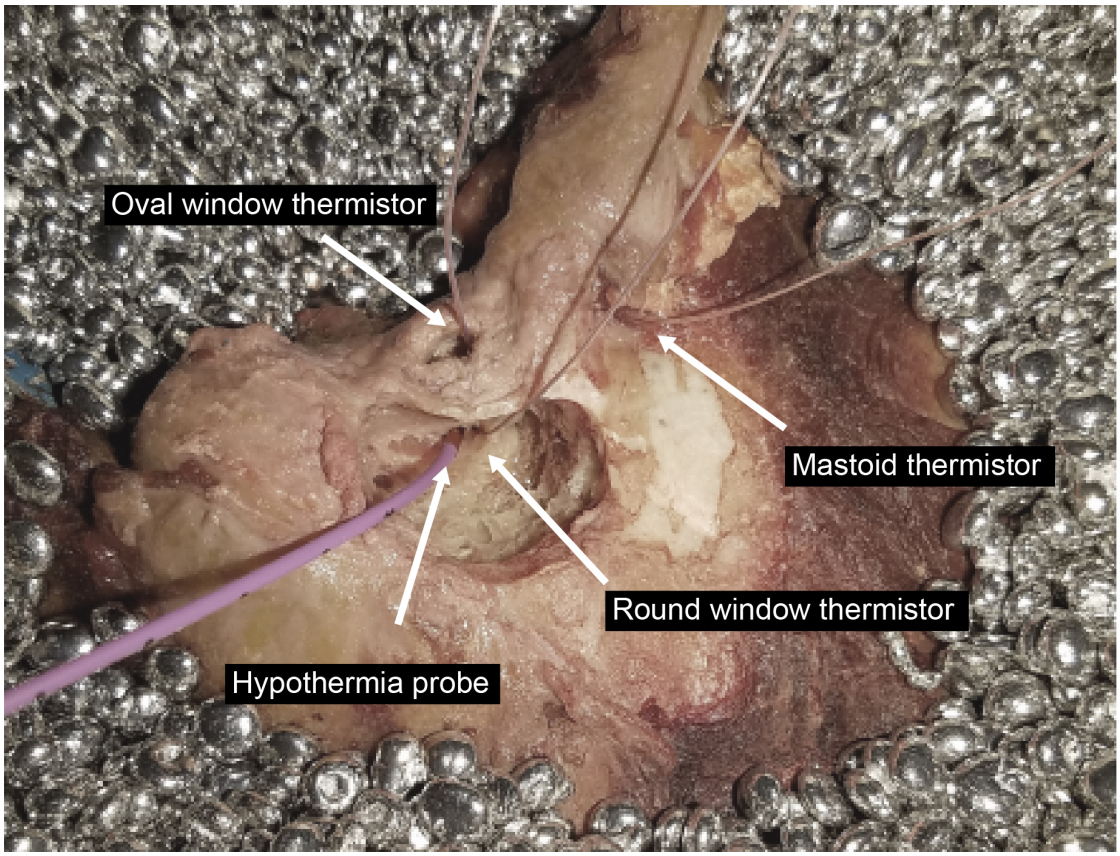

Figure 4.3 shows a human temporal bone submerged in a metallic bead warm bath. A custom-designed hypothermia probe was placed anteroinferior to the round window via a facial recess approach. Three thermistors were placed near the round window, oval window and on mastoid surface for temperature measurements.

The copper probe used to deliver localized MTH was connected to a custom thermoelectric Peltier system that circulated cooled fluorocarbon (Tamames et al., 2016). The experimental protocol was based on our previous work, where the temperature of the circulating fluorocarbon was reduced every 2 minutes. Measurements were taken at the round window, oval window and mastoid surface for the duration of the experiment.

Statistical analysis

Two-way ANOVA implemented in MATLAB (MathWorks, MA) was used to assess the changes in temperatures obtained from the experimental study. Values, that did not reach significance are represented with "ns", values with $p < 0.05$ are represented by *, values with $p < 0.01$ are represented by ** and values with $p < 0.001$ are represented by ***.

4.3 RESULTS

Numerical model of MTH

Figure 4.4 shows a volumetric mesh of the temporal bone and inner ear used in the numerical analysis. The temperature contours showing heat distribution at the probe, across the inner ear and at the surface of the temporal bone at the final time point in cooling protocol are shown. The simulated cooling was calculated at three sites: round window, oval window and mastoid. The volume nearest to the probe reached temperatures near 30ºC, while the cochlear temperature varied between 31ºC at the round window and 33ºC at the oval window. The surface of the temporal bone did not cool significantly in comparison.

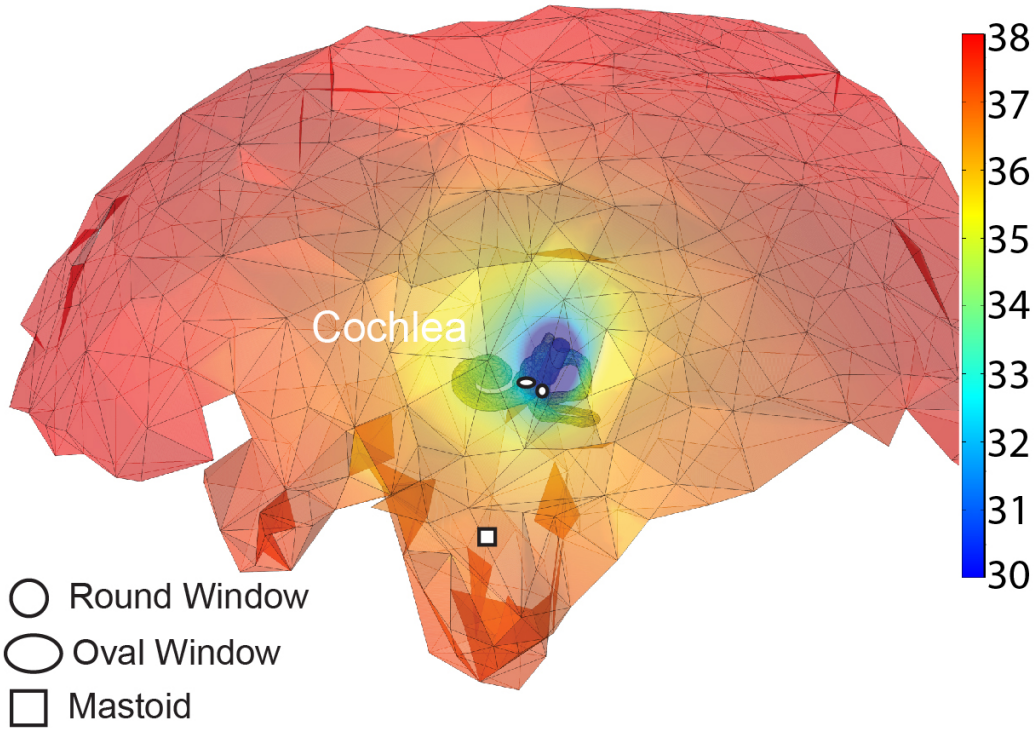

Figure 4.4 shows the numerical 3D model of the temporal bone with the temperature distribution achieved from localized mild hypothermia. Locations of round window, oval window via ear canal approach and mastoid thermistors are represented. Temperature contour was obtained at 18 minutes into the hypothermia protocol. The scale was fixed between 30-38ºC.

Figure 4.5 shows temperature contours in 2D at six modiolar isothermal slices across the temporal bone including the cochlea, starting from the round window (A1, nearest to the probe) and traversing the cochlea every 1 mm (A2-A6). The bottom part of the contours representing the inside face of the temporal bone did not achieve significant cooling. However, the volume that includes the cochlea cooled between 31-33ºC uniformly.

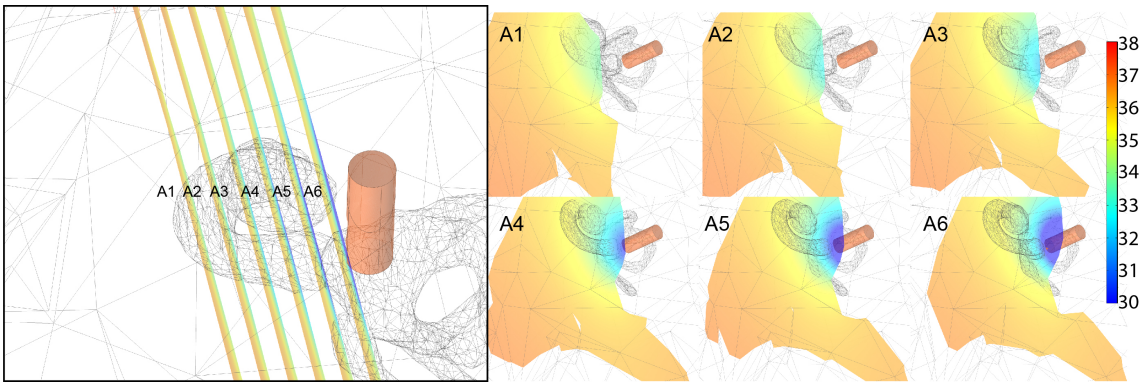

Figure 4.5 shows the calculated temperature contours across the temporal bone and cochlea. The contours surfaces represent modiolar slices parallel to the probe every 1 mm. The inset shows the same surfaces rotated in the z-plane for ease of visualization. The temperature scale was fixed between 30-38ºC.

Figure 4.6 shows temperature contours in 2D at four horizontal isothermal slices across the temporal bone including the cochlea, starting from the basal turn and traversing the cochlea every 1 mm until the apical turn (A1-A4). The slices include the hypothermia probe and also show that the volume that includes the cochlea cooled between 31-33ºC uniformly.

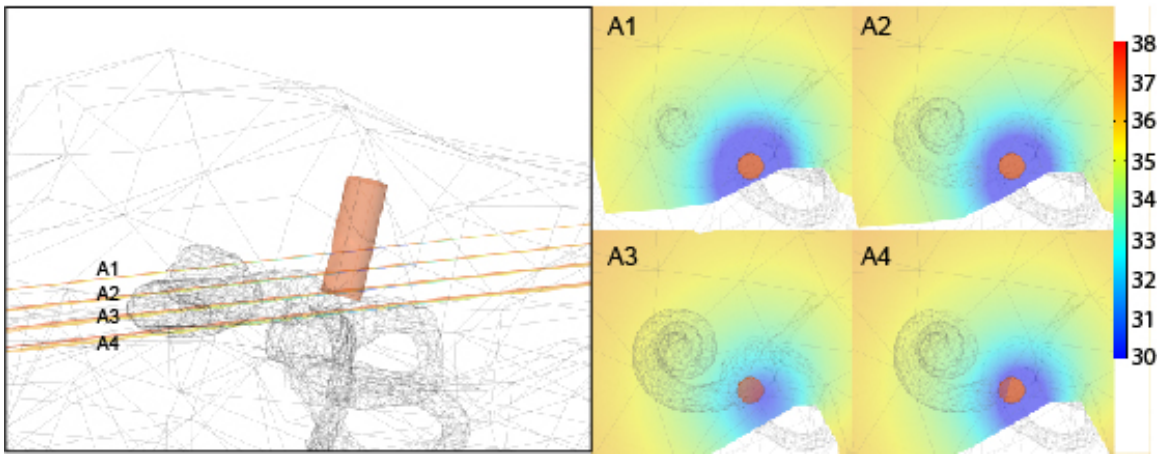

Figure 4.6 shows the calculated temperature contours across the temporal bone and cochlea. The contours surfaces represent horizontal slices parallel traversing the cochlea every 1 mm. The inset shows the same surfaces in the x,y-plane for ease of visualization. The temperature scale was fixed between 30-38ºC.

The specific temperatures during hypothermia protocol were calculated at the round window, oval window via ear canal approach and mastoid (Figure 4.7). The set temperature for the hypothermia probe over the same duration is shown on the right vertical axis. Over a period of 18 minutes, the temperature drop was calculated to be ~6ºC (starting temperature was selected at 37ºC) at the round window. The temperature at the oval window was reduced by ~3.5 ºC from the baseline, while the mastoid temperature did not reduce significantly (0.5 ºC). The calculated cooling results closely correlate with probe temperature, and reach levels that will be required for in vivo application.

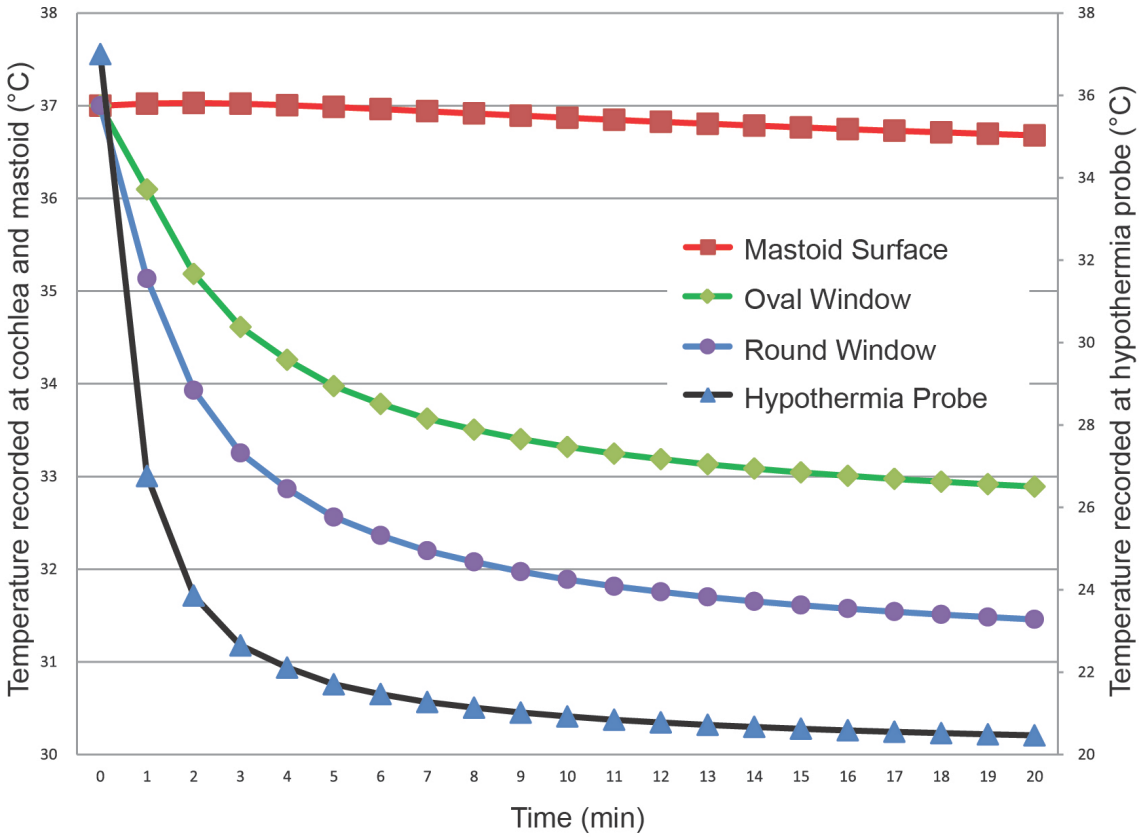

Figure 4.7 shows the numerically obtained temperatures at the round window, oval window and mastoid surface achieved with localized mild hypothermia. With the hypothermia protocol, the round window (blue) and oval window (green) temperatures reduced by an average of 4-6 ºC while the surface temperature of the temporal bones remained near baseline.

Experimental validation in cadaver temporal bones

Temperature measurements were carried out in 5 cadaver temporal bones over 8 trials to analyze the translational potential of this device. The locations of microthermistors, the orientation of the probe and facial recess are identified in Figure 4.3. Figure 4.8 shows the measured temperatures at the round window, oval window and mastoid surface. The temporal bones were warmed in a bath to achieve a baseline between 36 to 38ºC in the cochleae and approximately 35ºC at the mastoid surface.

The mastoid surface was affected only by the room temperature and as a result, its temperature did not reduce significantly (~1ºC). With the present system and placement of the hypothermia probe through the facial recess, the temperature at the round window reduced by an average of 6ºC following a hypothermia protocol lasting 18 minutes. Additionally, the temperature at the oval window was reduced by an average of 4ºC from baseline.

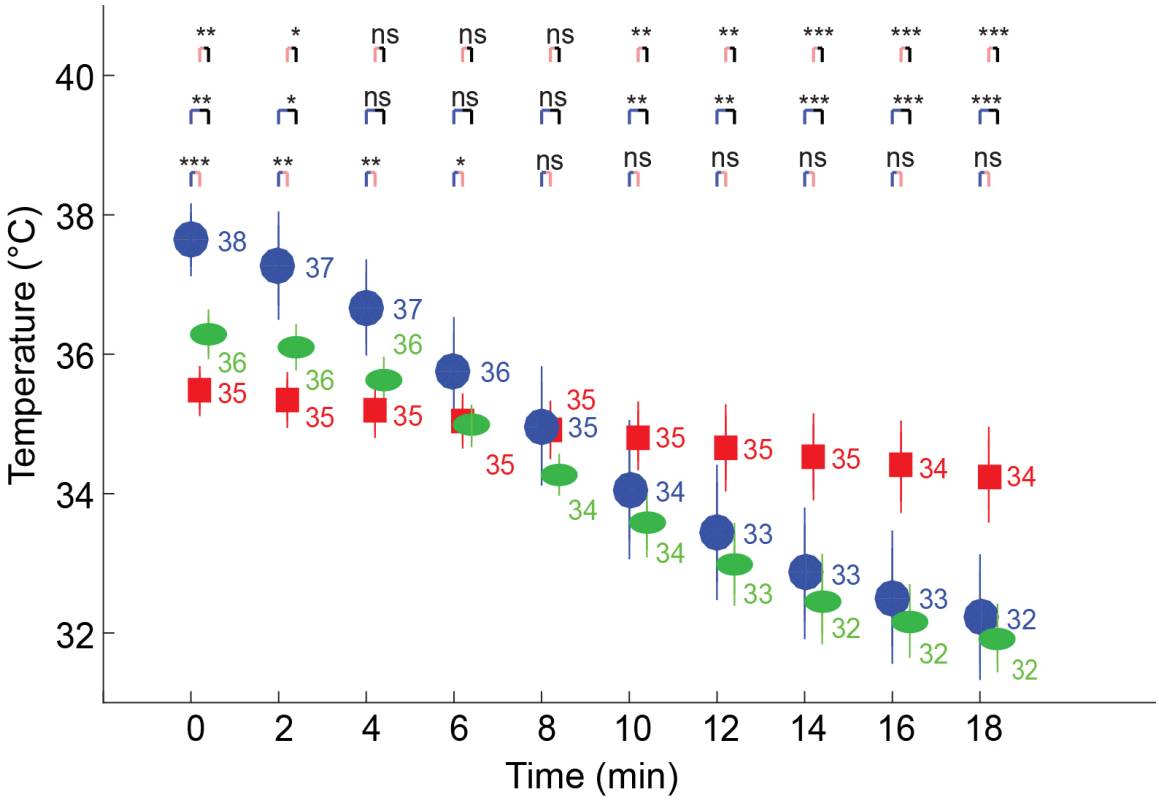

Figure 4.8 shows the experimentally obtained temperature measurements taken in cadaveric temporal bones. With the hypothermia protocol, the round window temperature (blue) reduced by an average of 6 ºC whereas the temperature at the oval window (green) reduced by an average of 4 ºC from baseline. The temporal bones were warmed to achieve a baseline between 36-38ºC. The mastoid temperature (red) reduced only by an average of 1 ºC and remained steady throughout the experiments. The placement of the hypothermia probe and three microthermistors are shown in companion Figure 4.3

4.4 DISCUSSION

The distribution of temperatures across an anatomically accurate 3D human cochlear geometry in response to the placement of hypothermia delivery system near the round window niche was investigated as a function of anatomical location and compared with experimental observations in a cadaveric temporal bone. This study built upon previous studies that utilized simplified models and experimental approaches to investigate translational potential of MTH application. Keller *et al*. employed simulated hypothermia treatments to lower the brain temperature and identified a neckband, covering the carotid triangles of the neck, as the most successful hypothermia device (Keller et al., 2009). Theoretical and in-vivo experiments have been successfully completed to test the effectiveness of a torso-cooling pad to reduce the temperature in the spinal cord and brain in rats (Smith, 2011). Following these studies, a modified Pennes bioheat equation and finite element analysis to simulate the temperature distribution in the cochlea during MTH was utilized. Studies of heat distribution in the cochlea during infrared stimulation utilized simplified geometries to effectively identify an increase of the cochlear temperature (De Paolis et al., 2017; Liljemalm, Nyberg, & von Holst, 2013; Manoussaki & Chadwick, 2000; K. Zhang et al., 2015). The present study produced a novel numerical analysis model using a 3D inner ear organ and a temporal bone, derived from MRI and CT scans. The model calculations provide insight into the localized effects of the temperature distribution and allow visualization of temperature gradients in otherwise inaccessible locations.

Our simulations show that the temperature at the round window reaches MTH (32-33 °C) in approximately 16-18 minutes from baseline levels. The temperature at the oval window reduced by 4ºC, settling at 33ºC during the hypothermia treatment. The detailed distribution profiles along modiolar and horizontal planes (Figures 4.5, 4.6) clearly show that the mild hypothermic treatment permeates from the basal turn volume closest to the probe to the apex region of the cochlea.

To work towards a clinical translation of our device for MTH treatment, the model results were validated experimentally. For this purpose, efficacy of the hypothermia device was tested in cadaver temporal bones. The experimental measurements showed similar temperate decrease and rate of change at the three locations compared: the round window, oval window and mastoid surface. Long-duration thermal equilibrium of the temporal bones (near 37ºC) was achieved by immersing the bones in a heated metallic-bead bath. (Keck & Thoma, 1988a, 1988b; Kozin et al., 2014) Utilizing the custom hypothermic device placed next to the round window niche via the facial recess, the temperature at the round window was reduced to 32-33 °C (by 6ºC on average) within an 18 minutes cooling period. In comparison, the temperature at the oval window reduced to 32ºC (by 4ºC on average) and the mastoid surface temperature remained unchanged, affected mostly by room temperature. In a previous study, it was demonstrated the hypothermia application as a tool in otoprotection and established that functional capacity of the cochlea was restored in an animal model (Tamames et al., 2016). Results showed that the hearing thresholds in hypothermic-treated and implanted cochleae recovered to the pre-surgical levels.

Histological results demonstrated survival of hypothermia-treated outer hair cells as compared to untreated outer hair cells. These results combined suggest that using our approach, a localized MTH treatment may be successfully provided to the human inner ear during cochlear implantation and other neurotologic surgeries.

Several limitations need consideration and further study prior to transferring the results of the model and temperature recordings into a clinical application. While performing the experiments, this study identified that the facial recess may need minor modification for placement of the probe. In several of the bones it was possible to create a small facial recess, and to place the hypothermia probe while simultaneously visualizing the round window. In clinical practice, it may be preferable to open the facial recess more widely to improve visualization of the round window niche and to better allow for optimal placement of the probe. This may also facilitate creation of a cochleostomy for insertion of the electrode array, if necessary. An alternative approach for the placement of probe may be through a myringotomy, which will be explored in a future study. Experimental observations and simulations suggest that 16-18 minutes may be necessary to achieve the optimal temperature required for MTH. In comparison, the *in vivo* rat model cooling period was shorter in the previous study (Tamames et al., 2016) and may need to be reduced for the clinical application. There are several factors that may explain the longer durations required in the current application. In the present study, it was not considered the movement of the inner ear fluid or blood perfusion within the cochlea.

Numerical results have confirmed the presence of local and temporal pressure gradients that may be important factors contributing to heat transfer (De Paolis et al., 2017; Olson, 1999; Salt & Ma, 2001; Salt, Thalmann, Marcus, & Bohne, 1986). Additionally, it is likely that the closer proximity of the hypothermic probe to the cochlea in the animal model influences the results. In a human application, the space available is more limited and may require a modification in the surgical approach as discussed above.

The present model also does not take into account differences in soft and hard tissues in the human ear and may benefit from higher resolution micro-computer tomography (CT) geometric model (De Paolis et al., 2017; Manoussaki & Chadwick, 2000).

4.5 CONCLUSION

Overall, there is a close correlation between the results of the numerical simulations and experimental observations in this study. The custom-designed hypothermia system is capable of effectively providing MTH locally to the human cochlea. When combined with results from in vivo animal experiments, the present study suggests that the application of localized MTH may hold potential for patients with an aim to preserve residual hearing after cochlear implantation.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

This study investigated the feasibility of localized MTH utilizing a novel device for protection of residual hearing in cochlear implantation surgery. Such surgery can result in inflammation and oxidative stress, and subsequently neural degeneration, hair cell loss and loss of residual hearing (Eshraghi et al., 2005; Wardrop et al., 2005). The significant loss of residual hair cells is caused by either a direct trauma to cochlear structures or by cascading molecular effects (Esperanza Bas et al., 2012; Eshraghi et al., 2013).

MTH has been shown to provide benefits for central nervous system injuries (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010; Purdy et al., 2013; Tzen et al., 2013), prevention of oxidative stress (Balkany et al., 2005; K. R. Henry & Chole, 1984; Shintani et al., 2010), protection on ischemic and traumatic injuries to neurons (Levi et al., 2010; Ohta et al., 2007; Shintani et al., 2010; Yanamoto et al., 1999; Yokobori et al., 2011), and improvement to spinal cord injury assessment (Cappuccino et al., 2010; Dietrich et al., 2009; Kawai et al., 2000; Levi et al., 2010).

MTH treated cochleae shown benefit in reducing functional loss in cases of cochlear implantation, (Balkany et al., 2005), acoustic trauma, (Kenneth R. Henry, 2003) and transient ischemia (Hyodo et al., 2001; Takeda et al., 2008). However, systemic hypothermia can affect the functioning of organs and organ systems including immune response impairment, higher infection rates, altered effects of other drugs and other vascular function deficiencies (Polderman & Herold, 2009).

For this purpose, this study designed and invented a novel device to deliver the MTH therapy in a localized and controlled manner. The hypothermia device provides a custom range of cooling and warming and is compact enough to apply MTH in an ambulatory setting. A temperature reduction of the cochlea by 4-6 ºC is possible utilizing the device's Peltier elements and innocuous liquid transport system to cool a copper tip. Programmatically it can decrease temperatures by a gradient, with continuous feedback from a microthermistor, and apply rewarming to the selected area.

In the present study, it was determined that *MTH is beneficial to the protection of outer hair cells in cochlear implantation surgery.* Analysis of long term MTH treatment confirmed this hypothesis for up to 3 months from surgery. In particular, basal hair cell survival rate was increased. Additionally, *long term normothermic cochleae trauma does not improve greatly with time* as compared to hypothermic treatment and undergo a functional loss after cochlear implantation trauma.

The results of this study moreover identified the temperature distribution of utilizing the novel device on a human temporal bone. *Localized MTH can be applied to human subjects without changing the current surgical approach*. A workable numerical analysis model of the inner ear located in the temporal bone was created. This model allows for precise observation of the temperature gradients from hypothermic therapy.

Future directions of this research include the study of the cellular mechanisms involved in MTH protection. Recent studies have begun to investigate the molecular mechanisms that trigger loss of residual hearing. Previous research has observed the increase following trauma of the mRNA levels of pro-inflammatory cytokines (TNFα and IL-1β), pro-inflammatory enzymes (iNOS and COX-2) (Esperanza Bas et al., 2012), cytokines, such as interleukin-1 (IL-1) (Touzani et al., 1999), tumor necrosis factor alpha (TNF-α) (Dinh et al., 2008; Keithley et al., 2008), and the activation of caspase-3 (Elmore, 2007). If a specific gene or protein could be identified as the main culprit of the hair cell survival, it could be isolated and be applied exclusively. Additionally, we could investigate different target temperatures, such as 30°C, or different therapy durations for application in a hypothermia treatment. Other trauma models could benefit from therapeutic hypothermia, such as acoustic trauma and vestibular microsurgery.

I believe that the use of therapeutic hypothermia, combined with refinements in surgical implantation and the use of neuroprotective drug-eluting electrodes could result in complete preservation of residual hearing against cochlear implantation trauma.

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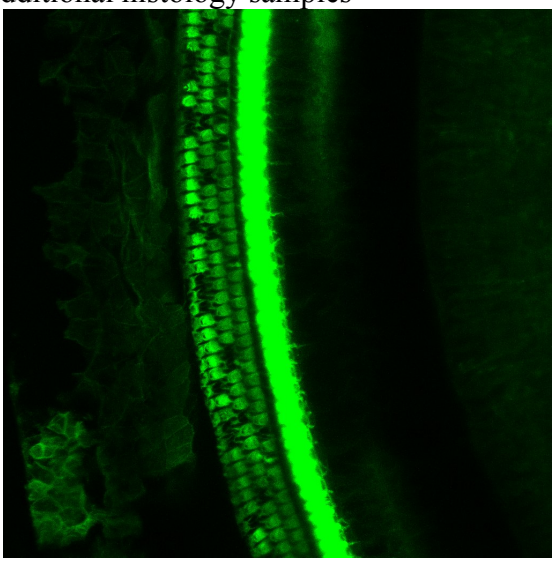
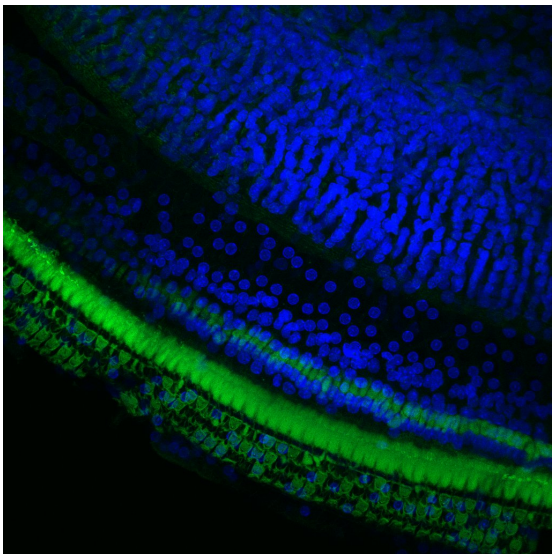
APPENDIX

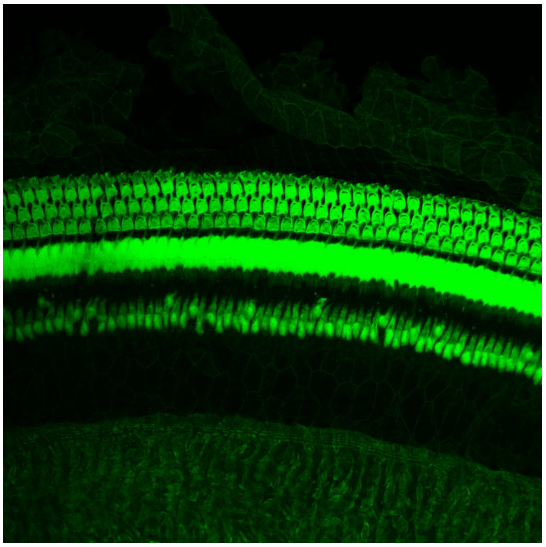
Additional histology samples



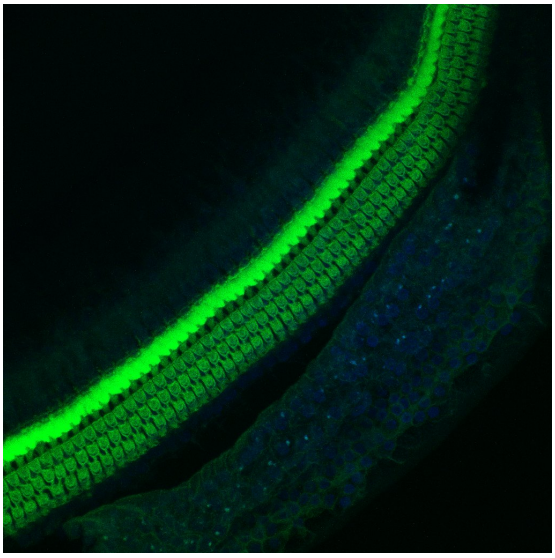
Normothermia Trauma Basal Image



Normothermia Trauma Middle Image



Hypothermia Trauma Basal Image



Hypothermia Trauma Middle Image

Arduino Program

```
// Arduino controlling Program by Ilmar Tamames
// Version 6.3
    // Library loading
    #include<stdlib.h>
    #include <stdint.h>
    #include <TouchScreen.h>
    #include <TFT.h>

    #include <math.h>
    #ifdef MEGA
      #define YP A2   // must be an analog pin, use "An" notation!
      #define XM A1   // must be an analog pin, use "An" notation!
      #define YM 54   // can be a digital pin, this is A0
      #define XP 57   // can be a digital pin, this is A3
    #endif

    // Declaring variables
    char temp1[8];
       char temp2[8];
       char temp3[8];
       char temp4[8];
       char buffer[10];
       String tem;
       int Rate = 20;
       float TargetTemp = 40;
       char Temp0[8];
       boolean PeltiersOn;
       float TempDiff;
       int Cooling;
       float ripple =.5;
       int maximum = 5;
       int PowerLevel;
       float STa = 0.001131625067266;
       float STb = 0.000233724373244;
       float STc = 0.000000089107569;
       float e   = 2.71828182846;
    // enumerating 3 major temperature scales
    enum {
      T_KELVIN=0,
      T_CELSIUS,
      T_FAHRENHEIT
    };
```

```
#define TS_MINX 140
#define TS_MAXX 900
#define TS_MINY 120
#define TS_MAXY 940

TouchScreen ts = TouchScreen(XP, YP, XM, YM, 300); //init TouchScreen port pins
 double TempHistory;
double TempHistory2;
double TempHistory3;
double TempHistory4;
int counter;
int counter2;
int RPWM = 46; // H-bridge leg 2 ->RPWM
//int enR = 34; // H-bridge enable pin 2 -> R_EN
int Relay1 = 26; // relay ground  nc3 nc4
int Relay2 = 27; // relay positive no3 no4

// Temperature function
float Temperature(int AnalogInputNumber)//,int OutputUnit,float B,float C,float T0,float R0,float R_Balance)
{
 float R,T,LG,V;
   V=float(analogRead(AnalogInputNumber));
 R=10000.0f*V/(1023.0f-V);
 LG = log(R)/log(e);
 //T=1.0f/(1.0f/T0+(1.0f/B)*log(R/R0) +(1.0f/C)*log(R/R0)*log(R/R0)*log(R/R0));
 T=1.00f/ (STa+STb*LG +STc*LG*LG*LG);
 return T;
}

/////Setup initial conditions
void setup()
{
 Serial.begin (9600);

 pinMode(Relay1, OUTPUT);
 pinMode(Relay2, OUTPUT);

// set board
 Tft.init();
 TempHistory=0;
 TargetTemp=40.0;

 tem = dtostrf(TargetTemp , 5, 1, buffer);
```

```
  tem.toCharArray(Temp0, 8) ;
  Tft.drawString(Temp0,20,50,3,GREEN);
  PeltiersOn=false;
  Cooling=0;/////"OFF";
  Tft.drawString("Current",30,100,2,YELLOW);
  Tft.drawString("Target",30,20,2,GREEN);
  Tft.fillRectangle(20,160,80,80,WHITE);
  Tft.fillRectangle(140,160,80,80,WHITE);
  Tft.drawString("-",30,170,8,BLUE);
  Tft.drawString("+",150,170,8,RED);
  Tft.drawString("Normal",20,280,2,RED);
  Tft.drawString("Tip:",20,255,2,BLUE);
  Tft.fillRectangle(150,20,70,50,WHITE);
  Tft.drawString("Off",160,40,2,RED);
  Tft.fillRectangle(150,100,70,50,WHITE);
  Tft.drawString("On",160,115,2,BLACK);
  digitalWrite(Relay1,LOW); //Disconnect Peltier
  delay(10);

}

//// Main programing loop
void loop()
{
  counter++;
 if (counter % 50 ==0 )
  {
   counter2++;

// Calculate temperature at core and tip
   TempHistory = TempHistory +
Temperature(8);//,T_CELSIUS,4300.0f,9000.0f,298.15f,10000.0f,10000.0f);
   TempHistory3 = TempHistory3 +
Temperature(11);//,T_CELSIUS,4300.0f,9000.0f,298.15f,10000.0f,10000.0f);

   if (counter2 % 10 ==0 )
   {
    counter2=0;
   Tft.drawString(temp1,20,130,3,BLACK);

   tem = dtostrf(TempHistory/10 , 5, 1, buffer);
   tem.toCharArray(temp1, 8) ;
   Tft.drawString(temp1,20,130,3,YELLOW); //temp1
   TempHistory2=TempHistory/10;
```

```
   TempHistory=0;

    /// Display temperature at core and tip
   Tft.drawString(temp2,80,255,3,BLACK);
   tem = dtostrf(TempHistory3/10 , 5, 1, buffer);
   tem.toCharArray(temp2, 8) ;
   Tft.drawString(temp2,80,255,3,BLUE); //temp1
   TempHistory4=TempHistory3/10;
   TempHistory3=0;
   }

// Main decision area

 // Activate cooling
 if ( counter % 500 ==0)
{
 if (PeltiersOn==true  )
 {

  TempDiff=TargetTemp- TempHistory2;

 // Activate the heating
  if(  TempDiff>maximum && Cooling != 2  || TempDiff>0 && Cooling==0)
  {
    if (TempHistory4<60)
  {
   Cooling = 2; /// Heating
   Tft.drawString("Cooling",20,280,2,BLACK);
   Tft.drawString("Normal",20,280,2,BLACK);
   Tft.drawString("Heating",20,280,2,RED);
  digitalWrite(Relay1,LOW); //Disconnect Peltier
    delay(50);
    digitalWrite(Relay2,LOW);// NC4 and COM4 Connected; //Heating
    delay(50);
     digitalWrite(Relay1,HIGH);  //Connect Peltier
     Tft.drawString("Off",180,280,2,BLACK);
     Tft.drawString("On",180,280,2,RED);
   PowerLevel=255;
  }
 }

  // Initiate cooling
 if(  TempDiff<-maximum && Cooling != 3 ||  TempDiff<0 && Cooling==0 )
 {
```

```
  Cooling = 3; /// Cooling
  Tft.drawString("Heating",20,280,2,BLACK);
  Tft.drawString("Normal",20,280,2,BLACK);
  Tft.drawString("Cooling",20,280,2,RED);
   digitalWrite(Relay1,LOW); //Disconnect Peltier
   delay(50);
   digitalWrite(Relay2,HIGH);// NC4 and COM4 Connected; //Cooling
   delay(50);
    digitalWrite(Relay1,HIGH);  //Connect Peltier
    Tft.drawString("Off",180,280,2,BLACK);
    Tft.drawString("On",180,280,2,RED);
 PowerLevel=255;
 }

    if(TempDiff>ripple && Cooling==2  || TempDiff<-ripple && Cooling==3  )
    {
     //heat at inccreasing speed if less than ripple degrees
      if( PowerLevel==0 && TempHistory4<60)
      {
       digitalWrite(Relay1,HIGH); //Connect Peltier
       PowerLevel=255;
        Tft.drawString("Off",180,280,2,BLACK);
        Tft.drawString("On",180,280,2,RED);
      }

    }

     //Decreasing control
    if(TempDiff<ripple && Cooling==2 || TempDiff>-ripple && Cooling==3  )
    {
     if( PowerLevel==255)
      {
     //heat at decreasing speed if less than ripple degrees
      digitalWrite(Relay1,LOW); //Disconnect Peltier
      PowerLevel=0;
       Tft.drawString("On",180,280,2,BLACK);
       Tft.drawString("Off",180,280,2,RED);
      }
    }
 }

else
{
 if (Cooling>0)
```

```
          {
           PowerLevel=0;
           Cooling=0;
           digitalWrite(Relay1,LOW); //Disconnect Peltier
          Tft.drawString("On",180,280,2,BLACK);
          Tft.drawString("Off",180,280,2,RED);
           //set peltier voltage to zero
          Tft.drawString("Heating",20,280,2,BLACK);
          Tft.drawString("Cooling",20,280,2,BLACK);
          Tft.drawString("Normal",20,280,2,RED);
          }
         }

         }
         }
        // add or increase temp
         Point p = ts.getPoint();

       // turn device on or off
       if (p.z > ts.pressureThreshhold)
        {
         counter=1;
         p.x = map(p.x, TS_MINX, TS_MAXX, 240, 0);
         p.y = map(p.y, TS_MINY, TS_MAXY, 320, 0);

       if ( p.x >= 145 && p.x <=225 && p.y >= 15 && p.y <=75 && PeltiersOn==true )
          {
           PeltiersOn=false;
           Tft.drawString("On",160,115,2,BLACK);
           Tft.drawString("Off",160,40,2,RED);
          }
          if ( p.x >= 145 && p.x <=225 && p.y >= 95 && p.y <=175 && PeltiersOn==false
)
          {
           PeltiersOn=true;
           Tft.drawString("Off",160,40,2,BLACK);
           Tft.drawString("On",160,115,2,RED);
          }

       /// Increase or decrease target temperature
         if ( p.x >= 20 && p.x <=100 && p.y >= 160 && p.y <=240 ) {
            TargetTemp=TargetTemp-1;

          tem = dtostrf(TargetTemp , 5, 1, buffer);
```

```
    Tft.drawString(Temp0,20,50,3,BLACK);
    tem.toCharArray(Temp0, 8) ;
    Tft.drawString(Temp0,20,50,3,GREEN); //temp1
  }
if ( p.x >= 140 && p.x <=220 && p.y >= 160 && p.y <=240 ) {
    TargetTemp=TargetTemp+1;
    tem = dtostrf(TargetTemp , 5, 1, buffer);
    Tft.drawString(Temp0,20,50,3,BLACK);
    tem.toCharArray(Temp0, 8) ;
    Tft.drawString(Temp0,20,50,3,GREEN); //temp1

  } } }
```