Lorentz-Force Actuated Needle-Free Injection for Intratympanic Pharmaceutical Delivery

by

Alison Cloutier

Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements for the degree of

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Abstract

Delivery of pharmaceuticals to the inner ear via injection through the tympanic membrane is a method of local drug delivery that provides a non-invasive, outpatient procedure to treat many of the disorders and diseases that plague the inner ear. The real-time controlled linear Lorentz-force actuated jet injector developed in the MIT BioInstrumentation lab was found to be a feasible technology for possible improvement over current intratympanic drug delivery methods. Jet injection holes using a nozzle with a 50 μm orifice were found to be significantly smaller than those made using a standard, 0.31 mm (30-gauge) hypodermic needle. The feasibility of using the jet injector to deliver drug to the inner ear with less tissue damage than seen in standard procedures is shown offering an avenue for improved inner ear drug delivery methods and technology.

Thesis Supervisor: Ian W. Hunter
Title: Hatsopoulos Professor of Mechanical Engineering
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Chapter 1

Introduction

The medical world is constantly evolving as new technology, new devices, and new procedures attempt to keep medicine on the cusp of scientific advancement. One such technology is needleless jet injection. The underlying theory of needleless jet injection is that given a high enough velocity and corresponding pressure a jet of liquid can penetrate a solid. These devices accelerate the fluid through a small orifice to increase the velocity of the jet as it exits the orifice. Injection characteristics have been found to correlate with orifice diameter [1]. Work in the MIT BioInstrumentation lab has led to a jet injection device that provides a controlled pressure profile during injections through use of a linear Lorentz-force motor [2]. Previous work in the lab has proven the technology viable for injection into skin given a variety of animal models, both in vitro [2] and in vivo [3], as well as to the retina at the back of the eye [4]. Intratympanic injections are widely used to treat disorders of the middle ear such as Meniere’s disease [5]. Jet injection provides velocity control, decreased injection time, and an ability to alter jet diameter through nozzle orifice geometry and therefore poses a possible improvement to current drug delivery methods to the inner ear. The study presented in this thesis aims to examine the feasibility of using jet injection technology to deliver drug through the tympanic membrane.

This thesis presents the work done to build an injection ampoule capable of penetrating the tympanic membrane with a hole smaller than that of the current hypodermic needle used for intratympanic delivery of drug. Chapter 2 discusses
relevant background information necessary to advance understanding of intratympanic delivery of pharmaceuticals. Chapters 3 details further characterization of a system developed for intraocular injections and addresses the use of this device and an animal model to explore intratympanic injection feasibility. Chapter 4 discusses the integration of a 50 μm ceramic nozzle for improved delivery and smaller penetration holes. Finally Chapters 5 explores work in other branches of the project and overall conclusions. Appendix A addresses initial work done in the lab.
Chapter 2

Background

2.1 Basic Anatomy and Physiology of the Ear

The ear is essential for conversion of perceived sound from the external environment into electrical signals which the brain can interpret, and is divided into three main sections – the outer, middle, and inner ear as shown in Figure 2-1. The inner ear not only makes up a key portion of the auditory system but also contains the vestibular system allowing for the maintenance of body equilibrium. Though size, shape, and anatomy vary, the auditory system is remarkably similar in mammals. The following sections will discuss the structure of the ear, and more specifically the human ear.

2.1.1 The Outer Ear

The outer ear is most easily recognized by cartilaginous projections seated on both sides of the head. These cartilaginous projections are called the auricles or the pinnae and consist of several distinct prominences. The deepest, bowl-like indentation in the pinnae is the concha which leads to the external auditory meatus. The external auditory meatus, more commonly known as the ear canal, is cartilaginous for approximately one third of its length before changing to an osseous structure medially [7]. The canal has a slightly curved, S-shape and leads to the tympanic membrane which forms the first barrier between the external environment and the
middle and inner ear. The tympanic membrane (eardrum) is situated in the external auditory meatus such that it creates a 45 to 60 degree angle with the inferior side of the canal [8].

2.1.2 The Middle Ear

The medial side of the tympanic membrane forms the lateral wall of the middle ear cavity and serves to transmit pressure waves and fluctuations from the external environment to the middle ear. The middle ear cavity has a volume of approximately 2 mL and is covered with mucous membrane [8]. The medial wall which separates the middle ear from the inner ear houses the oval and round window membranes. The Eustachian tube orifice which connects the middle ear cavity to the nasopharynx is located on the anterior wall [9].

The main function of the middle ear is that of amplification. As such, the middle ear cavity is filled with air maintained at a pressure slightly below atmospheric due to the Eustachian tube [8]. The Eustachian tube opens for short periods of time thus
maintaining equalized pressure on both sides of the tympanic membrane essential to its ability to vibrate freely [10]. Seated within the middle ear are three small bones known as the auditory ossicles – the malleus, incus, and stapes (as shown in Figure 2-2). The manubrium of the malleus is firmly attached to the medial side of the tympanic membrane and the base of the stapes is attached to the oval window. The incus bridges the gap between the malleus and the stapes [7].

Figure 2-2: Model diagraming the human middle ear. Pressure waves travel through the auditory meatus and vibrate the tympanic membrane. The auditory ossicles (malleus, incus, and stapes) connect the tympanic membrane to the oval window at the foot of the stapes. Note that the muscles and ligaments of the middle ear are not shown. Modified from [6].

2.1.3 The Inner Ear

The inner ear is the portion of the ear that is responsible for the translation of mechanical vibrations into a signal which can be interpreted by the human brain. The cochlea, often likened to a snail shell in shape, is known as the organ of hearing. The scala vestibuli, the scala media (cochlear duct), and the scala tympani follow the length of the cochlea and are three, fluid-filled tubes (as shown in Figure 2-3).
Both the scala vestibule and the scala tympani are filled with perilymph while the scala media is filled with endolymph [5]. The organ of Corti is anchored by a highly organized basilar membrane lined with hair cells [8]. These hair cells are the mechano-sensory cells of the hearing system [10]. The blood-labyrinth barrier of the ear serves to prevent components of the blood from entering the inner ear and disrupting the delicate, homeostatic balance [11-13].

![Diagram of the human inner ear](image)

Figure 2-3: Model diagraming the human inner ear. A cross-sectional slice of the cochlea illustrates its internal structure. Modified from [6] [9].

### 2.2 Intratympanic Drug Delivery

The presence of the blood-labyrinth barrier poses a challenge to systemic drug delivery for the treatment of inner ear diseases. As a result, local routes of delivery are being explored, such as intratympanic drug delivery. Intratympanic drug delivery relies on delivery of drug through the tympanic membrane. The drug then travels...
through the air filled space of the middle ear, exposing the round window membrane (RWM) to the drug [14] [5]. Diffusion of drug through the RWM and into the perilymph of the scala tympani relies heavily on passive diffusion [14]. This pathway is shown in Figure 2-4.

![Figure 2-4: Diagram of the pharmacokinetics of the ear. Figure from [15].](image)

Intratympanic injection is a method that had been used for more than 50 years [5] and provides a fairly non-invasive, outpatient procedure that does not require surgery [16] [17]. Applications include treating noise-induced hearing loss, cisplatin ototoxicity, aminoglycoside ototoxicity, radiation ototoxicity, sudden sensorineural hearing loss, Meniere’s disease, and more [5]. Intratympanic injections present an important medical application and research has focused on improving delivery methods.

The most common and least complex method of intratympanic drug delivery is use of a basic needle and syringe. These injections are either done through a small incision in the tympanic membrane (myringotomy) or through the placement of a tympanostomy tube (a small tube placed within the tympanic membrane to maintain an opening often for treatment for the accumulation of fluid) [14]. A 27-gauge needle (0.41 mm outer diameter) is commonly used for these procedures [18–20]. The main challenge that exists with these procedures is loss of drug through the Eustachian
tube. Often patients are required to remain in a position that allows injected drug to collect on the RWM for approximately 30 minutes in order to avoid this drug loss [21]. A key to increased concentration of drug in the perilymph, is increased duration of drug contact with the RWM [15].

More advanced delivery methods have been developed to try to increase drug retention in the middle ear and can be divided into three main categories: stabilizers, catheter systems and pumps, and biodegradable polymers that release drug through diffusion or erosion. Fibrin glue, hyaluronic acid, resorbable gelatin sponges, and hydrogels have been explored as stabilizers [14,22]. The Silverstein MicroWick which travels from the tympanic membrane to the RWM, is used for sustained drug release through a wick (shown in Figure 2-5) [23]. Nanoparticles formulated using the biodegradable polymer, poly-lactic/glycolic acid (PLGA), have also been researched as they can encapsulate drug for sustained release [24].

![Silverstein Microwick](image)

Figure 2-5: Implanted Silverstein Microwick. The device is placed within a ventilation tube in the tympanic membrane. The opposite end of the wick touches the RWM. Figure from [23].

Each of these technologies and mechanisms present advantages and disadvantages to the field of inner ear drug delivery. Any device that requires implantation or
traverses the tympanic membrane for a period of time will present increased expense, increased complexity in device implantation, and increased risk of infection or detrimental perforation of the membrane. The simplicity of a needle and syringe procedure is therefore valuable. Jet injection offers many benefits that a needle and syringe cannot. These include injection velocity control, possible reduction of pain given the interplay between jet diameter and nozzle orifice, more precise delivery, and decreased injection time. In particular, if jet injection technology can be controlled such that drug is delivered directly to the RWM, the likelihood of successful delivery could be increased. Jet injection may therefore prove to be a viable technology for this application.

2.3 The Tympanic Membrane

2.3.1 Anatomy

Because the successful delivery of drug using jet injection technology depends on the interplay between defined jet parameters and the mechanics of the tissue to which the drug is being delivered, understanding the specific structure and mechanics of the tympanic membrane is important. It is, however, important to note that much variability exists in the literature. Some of this variability is due to measurement technique, but some is also due to the inherent variability of the membrane. Kuypers et al. reported measuring the thickness of the tympanic membrane from three fresh human samples using a confocal microscope. Despite obtaining measurements from the same relative location on each sample, thicknesses were measured to be 40, 50, and 120 μm [25].

In terms of structure, the tympanic membrane is described as consisting of three layers – a cutaneous or epidermal layer, a fibrous layer (lamina propria), and a mucous layer as shown in Figure 2-6 A. The epidermal layer is on the lateral side of the membrane, while the mucous layer is on the medial side of the membrane. The membrane is almost entirely surrounded by a fibrocartilaginous annulus attached to
the auditory meatus. The small portion that lacks the outer annulus is known as the pars flaccida and the remainder of the membrane, the pars tensa [7].

First, the epidermal layer ranges in thickness from 5 to 12 μm on average and is a continuation of the epidermal layer that lines the external auditory meatus [26]. The epithelial layer is keratinizing epithelium of stratified squamous and consists of four strata of varying cell types [7, 26]. Vascularization occurs in the subepithelial layer providing blood supply to the tympanic membrane. While the epithelium is void of dermal papillae, hair, epithelial pegs, and hemidesmosomes, there exist many desmosomes and a continuous basil lamina [7]. In addition, the outer circumference of the membrane is thought to have a weakly developed dermal layer which joins the underlying fibrous layer [27].

The fibrous layer accounts for the majority of the membrane’s thickness and is composed of two layers—a radial collagen fiber layer and a circumferential collagen fiber layer (see Figure 2-6 B). Originating from the manubrium of the malleus, the most lateral layer is the radial collagen fiber layer. By nature of being radial, these fibers are more densely packed at the manubrium and become less dense near the fibrous annulus of the membrane [26]. More medially, the circumferential collagen layer, which encircles the umbo, exhibits more densely packed fibers at the periphery which decrease in density closer to the umbo. The collagen filaments are 10 nm in diameter and consist of amino acid compositions thought to be specific to the collagen of the tympanic membrane [7]. Collagen fibrils are known to be Type II and Type III collagen with Type I collagen present in a much lower quantity [28]. In studying the distribution of collagen in the healthy human tympanic membrane, Knutsson et al. found that the radial collagen fiber layer was composed mainly of Type II collagen though both Type I and III were also identified. Contrarily, the circumferential collagen fiber layer was found to be composed primarily of Type III collagen with Types II and I present in lower quantity. Type IV collagen was the major collagen type associated with the continuous basal lamina located at the base of the epithelial layer [29].

Most medially, the mucous layer is the thinnest layer of the tympanic membrane,
Figure 2-6: Schematic showing the anatomy of the tympanic membrane. Layers of the membrane and their approximate thicknesses (A). Fiber orientation in the collagen fiber layers (B). Figure from [26].
measuring on average 1 to 10 μm [26]. The membrane is composed of flat cells, a single layer deep, each of which is tightly bound to the surrounding cells. The mucous layer is continuous with the mucous layer that surrounds the cavity of the middle ear [7].

It is important to note that the above discussion pertains particularly to the pars tensa through which intratympanic injections are administered. Though very similar in structure, the fibrous layers of the pars flaccida are thicker and much more loosely organized. Blood vessels, nerve endings, and mast cells are likewise more abundant in the pars flaccida than they are in the pars tensa [30].

2.3.2 Mechanics

The fibrous layer of the tympanic membrane is responsible for the mechanical integrity of the structure. According to Cheng et al. [31] the mechanical properties of the tympanic membrane were first measured in the 1960's. At that time, the Young’s modulus of the membrane was measured to be 20 MPa by von Bekesy and 40 MPa by Kirikae. Later research by other groups reported modulus values ranging from 23 – 400 MPa or, at low stress (0 – 1 MPa), 0.4 – 22.0 MPa [31]. Overall, values range from 0.4 MPa to 400 MPa [31-33]. It is thought that this variation reflects the variability in experimental setup.

2.4 Jet Injection Technology

As discussed briefly in Section 2.2, jet injectors and, more specifically the linear Lorentz-force actuated jet injector developed in the MIT BioInstrumentation lab, may provide a mechanism to improve intratympanic drug delivery. Needle free injection, first termed aquapuncture, was developed in France in 1866. The first commercial device was used in the 1930's and a resurgence of the technology occurred in the 1960's with the development of single dose devices and once again in the 1990's when disposable cartridge jet injectors were implemented [34]. Therefore, these devices have gained significant attention in the research world. Based on the principle that given a high enough pressure, a jet of liquid can penetrate a solid, jet injectors use pressure to
accelerate fluid through a small orifice, thus increasing the velocity at which the jet of fluid exits the orifice. Some of the most highlighted benefits of this technology include elimination of hypodermic needles, accidental needle pricks, disposal expenses, and needle phobias [34].

A variety of devices currently exist on the market today. Many of these devices create driving forces used to accelerate fluid by mechanisms such as compressed springs, compressed gas, and explosive chemicals [35,36]. Injection profiles are described as having an initial peak pressure, a delivery phase, and a final drop-off phase [35]. Today’s market consists mainly of jet injectors which use a disposable cartridge comprised of a clear, plastic nozzle to act as the drug reservoir [34]. One example is the Medi-Jector VISION, a spring-powered device [37]. Applications of jet injectors include, but are not limited to immunization and delivery of insulin, growth hormones, steroids, protein drugs, and other macromolecules [34]. As research and technology expand, so do these applications.

Jet injection presents unique features that separate the technology from needle and syringe not only on the device level, but also in terms of injections. Unlike a needle which punctures the skin and delivers the bolus of drug at the site of the needle orifice, a jet injector uses a high pressure jet, the pressure defining the depth to which drug is delivered. In a study conducted by Schramm-Baxter et al. [38] in 2004, jet injection mechanics were investigated based on injection into polyacrylamide gels (a 2D tissue analogue). Jet injection was and still is described in terms of erosion and dispersion. The depth of the erosion hole is hypothesized to be a function of fluid kinetic energy and backflow and the interplay between these parameters. Once the erosion depth has been reached, a stagnation pressure is created which induces an outward pressure from the original erosion hole, thus resulting in dispersion [38]. These characteristics result in an injection evolution like that shown in Figure 2-7 [2].

In 2004, Schramm-Baxter and Mitragotri investigated the dependence of jet penetration and dispersion on jet power using post mortem pig and cadaver skin [38]. The following equation was used to define jet power:

\[
27
\]
\[ P_0 = \frac{1}{8} \pi \rho D_0^2 u_0^3, \]  

where \( \rho \) is fluid density in kg/m\(^3\), \( D_0 \) is nozzle diameter, and \( u_0 \) is the exit velocity.

Depth of fluid penetration and thus dispersion site along with dispersion shape were found to be dependent on nozzle diameter. Likewise, penetration depth was found to depend on exit velocity. Jet power was determined to be a factor that correlates penetration and dispersion [1].

![Figure 2-7: Evolution of an injection into acrylamide gel as a function of time (A). At 1 ms, the jet is seen as a thin stream creating an erosion hole. This is shortly followed by the development of a bolas of fluid which defines the dispersion of the jet. The graph distinguishes erosion from dispersion as a function of time (B). Figure from [2].](image)

Based on the literature, penetration and dispersion have been found to dominate jet injection characteristics. However, one of the largest drawbacks to the current devices that are on the market is the inability to control the injection profile during the time course of delivery. Therefore, work in the MIT BioInstrumentation lab has focused on developing a jet injection technology that allows for highly controlled injections. By using a linear Lorentz-force motor, electrical control can be used to generate a driving force which varies over the course of a single injection. A high-work
load injector was designed based on a BEI Kimco Magnetics linear Lorentz-force motor and included a position sensor, motor housing, and injection cylinder with a bleed port, autoloader, pressure sensor, and piston. Control of the device was achieved through a linear amplifier, laptop, and computer user interface [39]. Since this device, the jet injector has gone through several modifications.

The current jet injector device in use in the MIT BioInstrumentation lab is designed based on a custom, high power-density voice coil actuator. The voice coil former is made from a high performance polymer which acts to reduce both the moving mass of the coil and to eliminate drag associated with conductive material. Enameled copper wire, gauge 28 is wound six layers deep about the former (a total of approximately 585 turns) to produce a DC resistance that has been measured to be on average 11.6 Ω. A 1026 carbon-steel housing surrounds the coil dictating its linear motion. The magnetic circuit consists of NdFeB magnets fastened to a steel backplate which, together with the steel housing, direct the magnetic flux. This magnetic flux is channeled through the air gap between the coil windings and the inner wall of the housing (see Figure 2-8) and has been measured to be approximately 0.6 T [40] [41]. Further, an ampoule and piston from Injex® (INJEX Pharma Ltd., Miami, FL) is used to hold and drive the injectate. The ampoule is attached to the front plate and the piston to the moving voice coil. In this manner motion of the voice coil moves the piston relative to the ampoule, depressing liquid through the orifice of the ampoule. A 10 kΩ linear potentiometer is mounted to the housing, allowing the slide portion to seat within a notch in the former. The relative motion of the former compared to the housing allows for coil displacement to be monitored.

Control of the device is achieved using a real-time controller and field-programmable gate-array (FPGA, reprogrammable silicon chip) which communicates with a computer via an Ethernet connection. Interchangeable modules which seat within the FPGA chassis provide analog and digital input and output channels. The controller interfaces with the computer through LabVIEW [42]. The controller uses a velocity-based feed-forward model and position-based linear proportional-integral feed-back controller. Feed-back calculations are performed on the FPGA and data is sent to a linear power
Figure 2-8: Depiction of the jet injector voice coil actuator. The NdFeB magnets are surrounded by the steel casing and top plate shown in the image. The voice coil former is depicted in black with small circles representing the copper wire. Thin lines represent the magnetic flux as it travels from one magnetic pole to the other traversing the coil and air gap at the site of the top plate. Figure from [40].

amplifier to then control the jet injector (see Figure 2-10) [2]. Overall, a device that provides active position control is achieved.

Figure 2-9: Depiction of jet injector prototypes built in the BioInstrumentation lab at MIT along with an FPGA chassis and modules (C). A handheld model (A) and benchtop model (B) are both currently in use in the lab. Figure from [2].

The device developed in the MIT BioInstrumentation lab has provided repeatable and reliable injection profiles that have been viable for use in a number of different tissues in vitro [2] and in vivo [3]. The benefits of such a device include control of injection depth, delivery volumes, smooth-controlled movement, bi-directionality, and the ability to tune injection parameters for specific applications. Thus this technology
is a viable option for application to injection through the tympanic membrane.

### 2.5 Current Work

Given an understanding of the anatomy and physiology of the ear, delivery of drug to the middle ear, current advances in drug delivery, and an understanding of jet injection technology, the work presented in this thesis aims to understand the feasibility and implications of using jet injection technology for intratympanic injections. The following chapters explain this process.
Chapter 3

Explanted Tympanic Membrane Injections

This chapter describes the work done to assess the feasibility of using jet injection technology to perform intratympanic injections using an animal model.

3.1 Animal Model

The most common animal models employed in audiology research are the chinchilla, guinea pig, rat, and less commonly rabbits or cats. A comparison between the auditory characteristics of the meatus and tympanic membrane for the chinchilla, guinea pig, rat, and human is provided in Table 3.1.

While the chinchilla is an ideal model for this study, a rat model was chosen. Rats are used by the lab's collaborators and therefore allow comparison between study groups. Rats are also in use in the lab for in vivo, end use studies. Therefore, tympanic membranes are available and can be harvested post euthanasia from these animals in keeping with the 3 R's: replacement, reduction, and refinement.

The frequency range of rat hearing and the modulus of elasticity of the rat tympanic membrane overlap those values in humans. Both tympanic membranes are oval in shape and seated at an angle with respect to the auditory meatus and are comprised of a pars tensa and pars flaccida. Of key importance for injections, the
Table 3.1: Tabulated Auditory System Values

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Chinchilla</th>
<th>Guinea Pig</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency Range (Hz)</strong></td>
<td>20 - 20,000 [43]</td>
<td>20 - 30,000 [44]</td>
<td>50 - 45,000 [45]</td>
<td>1,000 - 50,000 [43]</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td>Oval [26]</td>
<td>Circular [44]</td>
<td>Circular</td>
<td>Oval</td>
</tr>
<tr>
<td><strong>Diameter (mm)</strong></td>
<td>8 - 10 [26]</td>
<td>6 - 9 [46]</td>
<td>9 - 10 [47]</td>
<td>2.2 - 5 [48,49]</td>
</tr>
<tr>
<td><strong>Area ((mm)^2)</strong></td>
<td>85 [50]</td>
<td>60.4 [44]</td>
<td>-</td>
<td>11 [50]</td>
</tr>
<tr>
<td><strong>Conical Depth (mm)</strong></td>
<td>1.42 - 2.0 [26]</td>
<td>1.78 [44]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Thickness (µm)</strong></td>
<td>30 - 120 [26]</td>
<td>7 - 10 [51]</td>
<td>10 [47]</td>
<td>5 [49]</td>
</tr>
<tr>
<td><strong>Young's Modulus (MPa)</strong></td>
<td>0.4 - 90 [49]</td>
<td>-</td>
<td>-</td>
<td>20.5 - 22.9 [52]</td>
</tr>
<tr>
<td><strong>Component Parts</strong></td>
<td>Pars tensa and pars flaccida (pars flaccida is virtually non-existent [53])</td>
<td>Pars flaccida is virtually non-existent [53]</td>
<td>Pars tensa and pars flaccida (pars flaccida occupies one quarter to one third tympanic membrane area) [50]</td>
<td></td>
</tr>
<tr>
<td><strong>Auditory Canal Diameter (mm)</strong></td>
<td>7 - 8 [8]</td>
<td>short, straight, and wide [46]</td>
<td>1 [46]</td>
<td>-</td>
</tr>
<tr>
<td><strong>Visualization of the Tympanic Membrane</strong></td>
<td>Easy</td>
<td>Easy [46]</td>
<td>Difficult due to twisted nature of canal [54]</td>
<td>Easy</td>
</tr>
</tbody>
</table>
3.2 Sample Procurement and Preparation

Animals were euthanized according to a protocol approved by the MIT Institutional Animal Care and Use Committee (IACUC) and in accordance with the National Institute of Health (NIH) Guide for the Use and Care of Laboratory Animals. Briefly, male rats (Wistar or Sprague Dawley, ranging in age from approximately 6.5 - 15.5 months) were euthanized by delivery of an overdose of sodium pentobarbital (120 mg/kg) or slow delivery of compressed, regulated CO$_2$ (20% of the volume of the cage per minute) into a closed container. Once euthanasia was confirmed, the animals were decapitated to allow access to the posterior region of the skull adjacent to the temporal bone.

A scalpel (number 15 blade) was used to cut the skin from the dorsal side of the skull following the mid-sagittal plane in the cranial-caudal direction. The scalpel was further used to gently cut the fascia connecting the skin to the underlying muscle allowing exposure of the posterior end of the skull. The pinnae were removed at this point by severing the auditory meatus and any remaining cervical vertebral bodies were removed.

Using the same scalpel, a portion of the cranial muscle along with the esophagus was removed to better expose the bony processes of the skull. The coronoid process and condyle of the lower jaw which articulate with the zygomatic arch of the skull were released and removed, isolating the lateral side of the posterior skull (see Figure 3.2). Large dissection scissors were used to access the skull cavity. By entering the posterior side of the skull near the occipital bone, the scissors were used to cut the ventral side of the skull along the mid-sagittal plane and to create a channel opening on the dorsal side to facilitate removal of the brain.

The same scissors were used to cut the zygomatic arch and to isolate the auditory bulla. Portions of the parietal and occipital bones were preserved to avoid damaging the auditory bulla during isolation. Only one auditory bulla was addressed at a
time such that the contralateral bulla could remain completely intact within the surrounding musculature. This was done to allow the tympanic membrane to remain \textit{in-situ} and prevent prolonged exposure to the laboratory environment.

Figure 3-1: (A) is an image taken of a rat skull. Of particular interest are the (Z) zygomatic arch, (CP) the coronoid process and (CD) condyle of the lower jaw, (O) the occipital bone, (P) the parietal bone, and (B) the auditory bulla. Figure modified from [54]. (B) is a profile picture of a rat. A red dashed circle is used to give reference between the skull bones and the rat profile. Figure modified from [55].

Care was taken to remove muscle and fascia from the isolated bone. As much of the cartilaginous portion of the auditory meatus was removed as possible, leaving only the osseous portion. This allowed for visualization of the tympanic membrane through the canal. For most injections, the middle ear was exposed prior to injection. A small pair of dissection scissors was used to gently score the thin bony shell of the middle ear cavity on the medial-ventral side of the bulla. Forceps were used to peel the bone away, exposing the medial side of the tympanic membrane, the ossicles, and the middle ear cavity.

Distance measurements were made from the pars tensa (approximate site of desired penetration) to the top of the bone at the entrance to the meatus. The canal was
measured to be approximately 2 mm (across 8 samples). In addition, the rat middle ear has been reported to have a volume of less than 50 µL [56], but preliminary work suggested that a liquid volume of 40 µL filled the middle ear cavity to capacity. A volume of 20 µL was established as a conservative, yet appropriate injection volume for use with the rat model.

3.3 Sample Positioning during Injection

Placement and fixturing of the auditory bulla was important to maintain a rigid position during injections and a consistent standoff distance (estimated to be 3 – 4 mm for the Injex™ ampoule). Initially it was hypothesized that a fixture that would allow the auditory bulla to be suspended but secure would provide the best positioning for intratympanic injections. A simple fixture was designed using laser machined three millimeter thick acrylic, a clear plastic sample box, and M2 set screws (see Figure 3.3). The clear plastic sample box would provide ejectate containment and visualization, while set screws would provide three points of contact and allow adjustment of sample placement.

Implementation of the fixture proved to be difficult. Because of the dissection technique, no two samples were of the same overall size and geometry. In addition, the irregularity of the bulla and the shape of the remaining parietal bone made implementation of the fixture design unfeasible.

A second methodology for positioning the auditory bulla involved seating the explanted bulla in a 0.7% agarose gel. The gel was cast in a petri dish with a diameter of 35 mm and a depth of 10 mm (BD Falcon). The use of an agarose gel rather than a rigid fixture is advantageous because the gel molds to the irregular shape of the explanted auditory bulla. Further, because the gel is pliable, the bulla position can be easily adjusted. This is important in assuring that the trajectory of the jet or needle tracks to the medial side of the tympanic membrane, either on the ventral or dorsal side of the malleus. Also, because the surface of the gel was in line with the surface of the petri dish, the entrance to the auditory meatus was not occluded allowing for
optimal positioning of the ampoule (or needle) relative to the canal opening as seen in Figure 3.3.

In addition to providing a support base, the agarose gel functioned to provide a tracking mechanism for the jet on the medial side of the tympanic membrane as seen in Figure 3-4. This feature was important because it allowed for measurement of the distance travelled by the jet as a function of the jet velocity. In optimizing a waveform for this application, there is a need to balance the ability to penetrate the tympanic membrane while also not allowing the jet to travel so far that it may pose a risk to the ossicles or RWM. Given enough power, the jet could potentially ricochet off the medial-ventral wall of the middle ear cavity and back toward the ossicles or penetrate the RWM.
Figure 3-3: The bony auditory bulla of a rat seated in agarose gel cast in a small petri dish. The Injex™ ampoule is carefully positioned at the entrance of the auditory meatus.

Figure 3-4: Image of the agarose gel taken after injection through the rat tympanic membrane. The auditory bulla has been removed, but the trajectory of the injectate remains in the gel.
3.4 Controller Description

Intratympanic delivery required waveform optimization due to the unique challenges presented by the tympanic membrane. In order to optimize these waveforms the appropriate waveform control was required. A controller was designed for penetration through the sclera for delivery of drug to the retina at the back of the eye [4]. This particular controller was optimized for a waveform that reached a high velocity in a very short period of time (less than 1.5 ms), followed by reversal of the actuator to immediately slow the coil to a slower follow-through velocity for bulk delivery of the injectate. This same waveform was hypothesized to be appropriate for intratympanic injections. The goal for intratympanic injections is to penetrate the tympanic membrane through the creation of an initial hole, but to then back the velocity off quickly and maintain a slower velocity for the remainder of the injection to avoid further damage to the membrane. This controller was used to begin work on intratympanic injections.

As observed by many who use the Injex™ ampoule to perform injections using the current jet injector system in the MIT BioInstrumentation lab, compression of the rubber piston tip occurs as a result of the back pressure from the 193 μm exit orifice [4] [57]. This is especially evident when the system is asked to reach a high velocity in a very short period of time. Work conducted by White [4] focused on creating a feed-forward system that relied on a coil-tip transfer function such that the desired coil waveform would compensate for piston tip compression. Implementation of a second order linear model, an iterative optimization process in MATLAB® [58], and a controller with high gains were used to create a pre-generated coil waveform. Standard parameters specifying desired values for jet velocity ($v_{jet}$), time to reach this jet velocity ($t_{jet}$), follow through jet velocity ($v_{followthrough}$), and desired volume to be delivered ($V_{desired}$) defined the desired piston tip trajectory from which the coil waveform was created. This full state feed-forward coil waveform included a displacement command, velocity command, and compensation command (accounting for coil acceleration, piston tip compression, and coil damping). Position (P), Velocity (D), and a compensation (A) gain were used when outputting the voltage control
signal from the FPGA to the amplifiers (AE Techron 7224 power amplifiers set up in series). Data showed improved agreement between desired and actual piston tip displacement [4]. Figure 3-5 shows a sample desired coil waveform and a LabVIEW generated coil displacement record using the above described controller, along with the coil waveform components that are sent to the FPGA.

3.5 Volume Ejection Study

Testing of the above described controller was performed using a volume of 40 μL. Due to smaller volume requirements for intratympanic injections into the rat middle ear, a volume ejection study was performed. The goal of the study was to test the repeatability and feasibility of using the jet injector to deliver volumes less than 40 μL. The InjexTM ampoule with an average orifice of 193 μm was used.

The standard ejection test procedure as described in [2] was used with water ejected into cotton wool and the difference between pre- and post-injection weight, used to determine ejected volume. Ten trials at $v_{jet}$ velocities of 75, 100, 150, and 200 m/s were performed for delivery volumes of 40, 20, and 10 μL. The average standard deviation across all trials was found to be ±0.28 μL (the range was from ±0.18 – ±0.39 μL). Figure 3-6 shows the results of this study and a representative waveform showing actual coil displacement, command coil displacement, and desired piston tip displacement. Given these positive results, work to determine the feasibility of applying jet injection technology to intratympanic injections was begun.

3.6 Intratympanic Injections Using Jet Injection

In an attempt to evaluate the initial velocity ($v_{jet}$) required to penetrate the tympanic membrane, a constant volume of fluid (20 μL) was delivered using velocities ranging from 100 m/s to 200 m/s. The time to reach $v_{jet}$ was held constant at 1 ms and the follow-through velocity at 5 m/s. The results of these studies are shown in Table 3.2 and Figure 3-7.
Figure 3-5: (A) Plot showing a comparison between a desired coil waveform of the nature: $v_{\text{jet}}$ at 200 m/s, $t_{\text{jet}}$ at 1 ms, $v_{\text{followthrough}}$ at 5 m/s, and a desired delivered $V_{\text{desired}}$ of 40 μL and a LabVIEW generated coil displacement record. (B) Plot showing an example displacement command, velocity command, and compensation command (accounting for coil acceleration, piston tip compression, and coil damping).
Figure 3-6: (A) Plot showing actual coil displacement, command coil displacement, and desired piston tip displacement. The waveform used to construct this plot had the following desired parameters: $v_{\text{jet}}$ at 200 m/s, $t_{\text{jet}}$ at 1 ms, $v_{\text{follousthrough}}$ at 5 m/s, and a desired delivered $V_{\text{desired}}$ of 40 µL. (B) Plot showing the results of the volume ejection study. Each data point represents the average of 10 trials with error bars that represent the standard deviation.
At a $v_{jet}$ of 160, 175, and 200 m/s, the tympanic membrane was penetrated creating a triangular tear pattern, but no residual tearing post injection. Hole dimensions measured using ImageJ® [59] for the 3 tympanic membranes shown in Figure 3-7 were 0.65 mm by 0.20 mm (160 m/s), 0.54 mm by 0.31 mm (175 m/s), and 0.82 mm by 0.23 mm (200 m/s).

### Table 3.2: Waveform ($v_{jet}$) Optimization

<table>
<thead>
<tr>
<th>Jet Velocity (m/s)</th>
<th>Injection Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Did not penetrate</td>
</tr>
<tr>
<td>150</td>
<td>Did not penetrate</td>
</tr>
<tr>
<td>160</td>
<td>Penetrated</td>
</tr>
<tr>
<td>175</td>
<td>Penetrated</td>
</tr>
<tr>
<td>200</td>
<td>Penetrated</td>
</tr>
</tbody>
</table>

### 3.7 Repeatability Study

In order to evaluate the repeatability of intratympanic injection using the Injex™ ampoule versus a 0.31 mm (30-gauge) hypodermic needle, a study was conducted. Data regarding hole characteristics, depth of injection, repeatability of these data, and comparison data were collected.

A total of ten freshly harvested tympanic membranes were included in the study. Each ear was injected using an Injex™ ampoule with an average orifice of 193 μm and an approximate 3 – 4 mm standoff from the tympanic membrane (this was kept as constant as possible given specimen variations). Bromophenol blue at a concentration of 0.25% was used as an injectate. All injections used a $v_{jet}$ of 160 m/s, $t_{jet}$ of 1 ms, $v_{followthrough}$ of 5 m/s, and a desired delivered $V_{desired}$ of 20 μL.

The results of this study are presented in Table 3.3 (holes are characterized by their largest and smallest dimensions). Each injection hole was measured using ImageJ® three times along its largest and smallest dimension. These values were averaged for each sample and further averaged across all ten samples (Appendix B presents a table showing the raw data).
Figure 3-7: (A) is a tympanic membrane penetrated with a $v_{jet}$ of 200 m/s, (B) 175 m/s, and (C) 160 m/s. The holes show a triangular tear pattern, but no post injection tearing.

Table 3.3: Results (Injex™ Ampoule)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jet Injector (Injex™)</th>
<th>0.31 mm (30-Gauge) Needle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large (mm)</td>
<td>Small (mm)</td>
</tr>
<tr>
<td>Average</td>
<td>0.505</td>
<td>0.207</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.163</td>
<td>0.075</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.051</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Jet injection using the Injex™ ampoule yielded injection holes with an average largest dimension of 0.51 ±0.05 mm and an average smallest dimension of 0.21 ± 0.02 mm. Holes from the 0.31 mm (30-gauge) needle were measured to be 0.33 ±0.02 mm by 0.19 ± 0.01 mm on average. Compared to preliminary studies, injection hole shape for the jet injector was found to be more variable across the samples. Similarly, needle injections were less consistent in shape which may be attributed to a larger sample size and better imaging technique. The smallest tremor during a needle injection can cause motion of the needle’s shaft relative to the membrane and thus slight tearing. Figure 3-8 shows several representative images from sample injections.

Figure 3-8: Four representative images of the tympanic membrane post injection via the jet injector with the Injex™ ampoule (J) and a 0.31 mm (30-gauge) needle (N). Injection holes for both the jet injector and the needle exhibit variability. Image A and B show some residual tearing below/above the site of the jet injection hole, while image D shows no residual tearing. Image C shows a jet injection hole that does not appear to exhibit the sharp edge of a crack like those in the other three images.
All measurements were found to have very low standard error across samples. The data suggests that both methods are repeatable, but that jet injection with the parameters used for this study creates an injection hole consistently larger in one dimension than injection holes made with a 0.31 mm (30-gauge) needle. A simple two-tailed, two-sample equal variance Student's t-Test with a cutoff level of $p \leq 0.05$ showed this difference to be significant (the smaller dimension was found to be statistically the same). Test results gave a $p$-value of 0.010 and 0.482 for the larger and smaller dimensions respectively.

The coil velocity across all ten trials was assessed based on the LabVIEW generated coil displacement records. Using MATLAB® to isolate a linear region of the $v_{jet}$ portion of the waveform and the $v_{followthrough}$ portion, a line was fit for each portion (see Figure 3-9). The slope of each portion was taken as the respective average coil velocities. Coil velocities for $v_{jet}$ were found to have very low variability ($\pm 0.03$, ranging from 0.37 – 0.47 m/s) with an average value of 0.42 m/s. The coil velocities for $v_{followthrough}$ had an average of 0.01 m/s.

Despite consistent coil velocities, trajectory depth was found to vary from 7 mm to 13 mm (measured from the entrance to the auditory meatus to the end of the trajectory in the agarose). Trajectories that traveled past 10 mm reached the bottom of the petri dish and suggest that the jet may have traveled even further if given the opportunity. The variability in these measurements is thought to be based on variability in bulla placement in the agarose. Further optimization of the waveform may lead to decreased injection depth while still attaining tympanic membrane penetration.

Pre- and post-injection images were taken to examine any changes in the ossicle chains that may have occurred during injection. Due to the position at which the bulla sits during an injection, the trajectory of the jet was found to entirely miss the portion of the middle ear which contains the ossicles. Therefore, it is difficult to conclude whether damage would occur during in vivo injections. A sample was thus injected with the bulla completely intact. A $v_{jet}$ of 200 m/s, $t_{jet}$ of 1 ms, $v_{followthrough}$ of 5 m/s, and a desired delivered $V_{desired}$ of 20 µL was used for this test. The injectate
Figure 3-9: Example plot showing the $v_{jet}$ portion of the LabVIEW generated coil displacement record (zoomed in on in the inset plot) and the $v_{followthrough}$ portion used to calculate coil velocity. The waveform used to construct this plot had the following desired parameters: $v_{jet}$ at 160 m/s, $t_{jet}$ at 1 ms, $v_{followthrough}$ at 5 m/s, and a desired delivered $V_{desired}$ of 20 µL.
penetrated the membrane and pooled within the middle ear cavity. Post injection, the middle ear was exposed, the dye carefully removed, and the ossicles examined; no apparent damage was observed (Figure 3-10).

![Figure 3-10](image)

Figure 3-10: The auditory ossicles are shown to have been unaffected by the jet during an injection through the tympanic membrane via jet injection.

### 3.8 Discussion

This data shows that the use of a jet injector to administer intratympanic injections is feasible. Injection holes were consistent with low variability and did not show tearing post injection. Some residual tearing was however seen and is thought to be caused by splash back between the agarose gel and the tympanic membrane during the follow-through portion of the injection. Jet injection holes were found to be larger in one dimension than those produced with a 0.31 mm (30-gauge) needle. Decreases in nozzle orifice diameter and/or standoff distance could decrease jet injection holes.

While Figure 3-10 suggests that the ossicles do not experience damage during injections, the effect of variable follow-through velocities and volumes need to be
explored. Given injection of a larger volume, this may however not be the case. A larger volume may create a fluid induced pressure wave within the middle ear cavity that may threaten the integrity of the ossicles or the RWM.

Tear patterns were visibly different between needle injections and jet injections. While needle injections seem to maintain the general shape of the shaft of the needle, jet injection holes exhibited a much more triangular shape often with at least one sharp pointed corner. In a study conducted by Shergold at al. in 2006 [60], silicone rubber was used to study the penetration mechanics of a high-speed liquid jet. The high-speed liquid jet was found to create a planar mode I crack [61]) (see Figure 3-11) [60]. Figure 3-11 shows a comparison between the results from the 2006 study and the penetration mechanics through the tympanic membrane. The penetration holes are very similar.
Figure 3-11: (A) Depiction of the crack mechanics observed when a sharp object or a high-speed liquid jet punctures silicone rubber. (B) Penetration hole in silicone rubber produced by a high-speed liquid jet. (C) Penetration hole through the tympanic membrane from jet injection. (A) and (B) modified from [60]
Chapter 4

Design of an Intratympanic Injection Ampoule-Nozzle Assembly

Jet injection technology appears to be a viable delivery method for application to intratympanic injections. However, preliminary work discussed in Section 3.7 indicated that the use of the Injex\textsuperscript{TM} ampoule with an average orifice of 193 \(\mu m\) and an approximate 3 – 4 mm standoff from the tympanic membrane yielded injection holes that were significantly larger in one dimension than those produced with a 0.31 mm (30-gauge) needle. Nozzle orifice diameter, standoff distance, and bulla positioning may all be factors that contribute to these results. In order to address both nozzle orifice diameter and standoff distance, a commercially available, 50 \(\mu m\) ceramic nozzle from Small Precision Tools [62] with a nozzle length of 9.53 mm and slim profile was retrofitted to the Injex\textsuperscript{TM} ampoule and used for intratympanic injections and will be discussed in this chapter.

4.1 Nozzle Characterization

The 50 \(\mu m\) ceramic nozzle mimics orifice diameters characteristic of microneedles which typically range in diameter from 40 to 100 \(\mu m\) [63]. Using this nozzle, nozzle

53
orifice diameter was decreased by a factor of 4, decreasing the orifice area by a factor of 16. While the Injex$^TM$ nozzle decreases from a diameter of 3.568 mm to a diameter of 193 μm over an approximate 2 mm distance, the ceramic nozzle decreases in diameter from 2.6 mm to 50 μm in 9.53 mm, creating a much more gradual taper. This taper is a key feature in creating a nozzle that can insert into the rat auditory meatus and decrease standoff distance. Therefore, any comparisons made are between these geometric parameters. Figure 4-1 shows a dimensioned drawing of the micro dispensing, ceramic nozzle and the two designs: taper relief and non-taper relief.

![Dimensioned drawing of the micro dispensing nozzle and the two designs: taper relief and non-taper relief. Figure from [62].](image-url)

Several imaging modalities were used to assess the quality and characteristics of the ceramic nozzles. Initially, the ZEISS Stemi SV II microscope and Canon EOS 50D camera were used to obtain images that could be analyzed using ImageJ® to assess external features.

A sample nozzle was sent to collaborators at the University of Auckland for microcomputed tomography (microCT) analysis. The stack of images generated from
the microCT were rendered using 3D Slicer – a free, open source software used for high performance volume rendering [64]. The microCT images provided an analysis tool to examine the inside contours of the nozzle. Figure 4-2 shows the result of the microCT scans of the ceramic nozzle (the nozzle was positioned slightly crooked and the entire length was not visualized in the scanner region of interest). Scans were conducted at a resolution of 10.7 µm. The inner contour of the nozzle is tapered at two distinct angles along the length of the nozzle – the upper taper covering approximately 2.5 mm and the lower taper, which sits at a more acute angle, spanning the remainder of the nozzle length.

Figure 4-2: MicroCT image of the ceramic nozzle. Image A shows a cross-sectional slice of the middle plane of the nozzle and the two distinct tapers of the inner contour. Image B is a 3D reconstruction of the nozzle in SolidWorks® [65].

Finally, a scanning electron microscope (SEM) was used to obtain high quality surface images that enabled visual analysis of the surface topography of the nozzle. Figure 4-3 shows a compilation of several SEM images of the ceramic nozzle. These images reveal that the nozzle exhibits very precise features. Ceramic injection molding was used to manufacture these nozzles [62]. The orifice diameter was analyzed using ImageJ® and found to be 53.2 µm, while the inlet orifice diameter was measured
to be 2.6 mm. These SEM images indicate that the inner surface of the nozzle is extremely smooth with very few flaws.

Figure 4-3: Compilation of several SEM images of the ceramic nozzle – (A) the exit orifice, (B) the inlet orifice, (C) the inner surface, and (D) the nozzle tip.

### 4.2 Ampoule Alterations

In order to integrate the new ceramic nozzle with the jet injection system, modifications to the Injex™ ampoule were made. Several iterations and design challenges associated with modifying the ampoule led to a final design. This design was tested to ensure that the current jet injector could eject liquid through the 50 μm orifice of the ceramic nozzle. All further iterations focused on the elimination of sharp edges to reduce the
risk of boundary layer separation, loss of energy, and rapid changes in pressure. One of the main challenges was creation of a gradual taper from the 3.568 mm inner diameter of the Injex™ ampoule to the 2.6 mm diameter upper orifice of the ceramic nozzle.

Though an initial design did implement a gradual taper, the use of a luer lock and luer lock adapter created a large volume of dead space within the ampoule (approximately 243 (mm)$^3$). Because the system is required to reach a high velocity over the course of a few milliseconds or less during the initial phase of the waveform ($v_{\text{jet}}$ portion), fluid dynamics play a large role. These dynamics can be described by unsteady flow where the fluid experiences a change in momentum with time. These dynamics are further complicated by the compression of the piston tip during this initial phase as described in Section 3.4. Therefore, despite good waveform following during the steady state follow through, control was very difficult during the initial phase.

The final design, shown in Figure 4-4, relied on allowing the piston tip as much travel range as possible within the ampoule and simplified the transition from the ampoule to the ceramic nozzle. A drill bit of diameter 2.6 mm was used to directly drill a hole through the orifice end of the ampoule, opening the end of the ampoule to a diameter of 2.6 mm which seats flush with the entrance orifice of the ceramic nozzle. The outside diameter of the ampoule was turned down to an appropriate diameter (7.94 mm) so that the metal nozzle housing could be screwed onto the ampoule end (the metal housing is shown in Figure 4-4 surrounding the ceramic nozzle and screwed onto ampoule). A thin, flat o-ring was laser machined from thin rubber (0.38 mm silicone, 10 duro) to create a leak-proof seal between the end of the ampoule and the top of the ceramic nozzle.

### 4.3 Jet Injection Code Alterations

In order to properly implement the ceramic nozzle, changes needed to be made to the control software. While new code had been written and several alterations made
Figure 4-4: Final design for installation of the ceramic nozzle onto the Injex™ ampoule showing the metal housing and ceramic nozzle. (A) Image of the actual nozzle showing the metal housing with the ceramic nozzle seated within. (B) SolidWorks® model showing a cross section of the ceramic nozzle-ampoule assembly.

over the course of working with the Injex™ ampoule for ease of use, data analysis, data interpretation, and troubleshooting purposes, the ceramic nozzle presented new challenges.

Based on the physical constraints of the nozzle orifice and conservation of mass, an injection using the Injex™ nozzle is different from an injection using the ceramic nozzle. First, based on mass flow rate as a function of velocity (assuming a density of 1000 kg/m for water), the Injex™ ampoule with orifice radius 96.5 μm has a mass flow rate of $29v$ (where $v$ is velocity in m/s and the units are, as calculated, mg/m). The ceramic nozzle on the other hand has a mass flow rate of $2v$, making the mass flow rate of the Injex™ ampoule approximately 15 times that of the ceramic nozzle for the same velocity of flow. To make a further comparison, a volume of 40 μL and a velocity of 150 m/s are assumed. The time to reach velocity is assumed to be instantaneous for the purpose of comparison. The time taken to inject the desired volume for the Injex™ ampoule is about 9.1 ms and for the ceramic ampoule, it is approximately 135.8 ms. For a standard waveform with an initial high velocity
impulse and a slower follow-through velocity, an injection using the ceramic nozzle can require more than 1 second.

To account for this increase in time, changes were made to both the waveform generation code and to the LabVIEW FPGA code. First, the desired waveform is generated with a large number of data points, smoothed, and then downsampled to fit in the space on the FPGA. The ability to tune the simulated tip displacement to the desired tip displacement during simulated injections (used to create the pre-generated waveform), is improved given a larger set of data. Therefore, reduction of this data was performed subsequent to generating the desired/command waveform. Two reduction factors were used; one to address the position and velocity command waveforms and one to address the compensation waveform. Reducing the position and velocity commands by a factor twice that used to reduce the Injex™ commands proved sufficient for data to fit on the FPGA. However, because the compensation command provides short duration voltages that give the system needed extra voltage to overcome system dynamics (as shown in Figure 3-5), this degree of data reduction essentially eliminated the compensation spikes. Therefore, the compensation command was reduced by the original factor of 10 and compressed. Sampling time on the FPGA was increased by a factor of two and the allowable volume of data points increased accordingly.

Increased resistance due to the back pressure associated with having a nozzle orifice one sixteenth the size of the Injex™ ampoule posed additional challenges due to the compliance of the piston tip. As described in White [4], a Vision Research Phantom v9.0 high-speed video camera was used to obtain high speed video of the motion of the piston tip during injection. Due to a greater degree of noise in the current videos, a local regression filter was implemented to process the data. Initially a waveform generated for the Injex™ ampoule with a $v_{jet}$ of 75 m/s and a follow-through velocity of 15 m/s was chosen to avoid too high of an impulse and to ensure a follow through velocity that provided enough voltage to overcome back pressure and friction.

After many iterations a reasonable coil-tip transfer function and model parameters
were obtained. However, discrepancy in the quality of waveform following still existed between the $v_{\text{jet}}$ portion of the ejection and the follow-through portion. Changing the FPGA tuning parameters was not an option as the system overshot $v_{\text{jet}}$ slightly, but displayed undershoot in the velocity of the follow-through and total volume delivered. Tuning for the follow-through resulted in dramatic overshoot in the initial $v_{\text{jet}}$ portion. Thus, adjustment of the compensation command was required to allow for better waveform following. Both the coefficients of coil acceleration and tip compression were reduced allowing for modulation of the initial voltage spike. This resulted in much improved piston tip mapping and subsequent waveform following. Figure 4-5 shows an example result from the final iteration. Note that waveform following is not as good as with the Injex$^TM$ ampoule, but that the $v_{\text{followthrough}}$ portion does follow the desired slope and delivers the desired volume. A delay in the transition between the $v_{\text{jet}}$ portion and the $v_{\text{followthrough}}$ portion exists. A method that may be implemented to improve waveform following is discussed in the following section.

### 4.4 Sliding PD Controller

Injections discussed in this thesis utilize a waveform that can be split into two distinct portions. These two portions interplay with the dynamics of the system in very different ways due to the compliant piston tip. Whereas the initial impulse drives the coil forward in a few milliseconds and then slows the velocity down as quickly as possible causing compression and decompression of the piston tip, the follow through velocity is very slow and thus the piston tip tracks to the motion of the coil with negligible compression. Through discussion with James White (a former member of the MIT BioInstrumentation lab), it was hypothesized that the FPGA could be programmed such that one set of P, D, and compensation gains be used for the initial portion and another set for the follow through portion. A set time period and linear change could be used to “slide” from the initial gains to the final gains. Though written and tested (but not optimized) with the Injex$^TM$ ampoule, this system was not implemented with the ceramic code due to the increased need to create space
Figure 4-5: Plot showing a comparison between a desired coil waveform of the nature: $v_{jet}$ at 15 m/s, $t_{jet}$ at 2 ms, $v_{followthrough}$ at 15 m/s, and a desired delivered $V_{desired}$ of 20 µL and a LabVIEW generated coil displacement record.
on the FPGA and the ceramic nozzle code already pushing the capacity limits. This code, however, may greatly improve waveform following for the ceramic nozzle. Figure 4-6 displays pseudo code written in MATLAB® and corresponding plot showing the theory behind the controller. The LabVIEW code follows in Figure 4-7.

```matlab
timess = 0:20e-6:400e-3;
deltaP = 0.0;
deltaD = 1.54;
deltaA = -0.15;

for i = 1:length(timess)
    if timess(i) <= 0.001
        P(i) = 1.0;
        D(i) = 0.01;
        A(i) = 0.7;
    elseif (timess(i) > 0.001) & (timess(i) <= 0.002)
        P(i) = P(i-1) + (deltaP)/50;
        D(i) = D(i-1) + (deltaD)/50;
        A(i) = A(i-1) + (deltaA)/50;
    else
        P(i) = 1.0;
        D(i) = 1.55;
        A(i) = 0.55;
    end
end
```

Figure 4-6: Pseudo code written in MATLAB® and the corresponding plot showing the theory behind the sliding PD controller.

### 4.5 Nozzle Ejection Comparison

Once the results shown in Figure 4-5 were obtained, an ejection comparison was made between the Injex™ ampoule (orifice diameter of 193 μm and taper length of 2 mm) and the ampoule-ceramic nozzle assembly (orifice diameter of 50 μm and taper length of 9.53 mm). A waveform with identical desired parameters was created using the appropriate waveform generation code for each. These parameters were: $v_{jet}$ of 75 m/s, $t_{jet}$ of 2 ms, $v_{followthrough}$ at 10 m/s, and a desired delivered $V_{desired}$ of 20 μL. A $t_{jet}$ of 2 ms was chosen because it proved to be a sufficient amount of time to allow the ceramic nozzle to reach the desired $v_{jet}$. This is consistent with a quick study
Figure 4-7: LabVIEW code to implement a sliding PD controller. One set of P, D, and compensation gains define the gains for the $v_{jet}$ portion; a set time period and linear change allows the controller to “slide” from these initial gains to the final, Vfollowthroug portion gains.

conducted using the Injex$^{TM}$ ampoule which suggested that $v_{jet}$ was not reached until between 1 and 1.5 ms.

Figures 4-8 and 4-9 show an ejection using the above described waveform for the Injex$^{TM}$ ampoule and the ceramic nozzle respectively. Images were obtained using high-speed video and stills were captured that corresponded to interesting points for each ejection. The graph displayed below the images shows the LabVIEW generated record of the coil displacement as a function of time. Red lines indicate the chronological time stamps for each of the still images. Note that ringing occurs in the ceramic nozzle waveform when pulling back from the initial impulse due to compression of the piston tip. The ejected water from the ceramic nozzle is notably thinner than that for the Injex$^{TM}$ ampoule. Using ImageJ® and taking the standoff from the tympanic membrane to be 4 mm for the Injex$^{TM}$ ampoule, the liquid spread during the $v_{jet}$ phase is approximately 0.41 mm. If a predicted 1 mm standoff is made for the ceramic nozzle, a spread of about 0.25 mm exists. Therefore, these ejections predict a thinner jet upon impact with the tympanic membrane when using
the ceramic nozzle, which is hypothesized to lead to a smaller injection hole.
Figure 4-8: Ejection of water into air using the Injex\textsuperscript{TM} nozzle. Each still image corresponds to an interesting time point on the LabVIEW generated coil displacement record shown in the graph. These time points are marked with red lines. The waveform used to construct this plot had the following desired parameters: \( v_{jet} \) at 75 m/s, \( t_{jet} \) at 2 ms, \( v_{followthrough} \) at 10 m/s, and a desired delivered \( V_{desired} \) of 20 \( \mu \)L.
Figure 4-9: Ejection of water into air using the Ceramic nozzle. Each still image corresponds to an interesting time point on the LabVIEW generated coil displacement record shown in the graph. These time points are marked with red lines. The waveform used to construct this plot had the following desired parameters: \( v_{\text{jet}} \) at 75 m/s, \( t_{\text{jet}} \) at 2 ms, \( v_{\text{followthrough}} \) at 10 m/s, and a desired delivered \( V_{\text{desired}} \) of 20 \( \mu \)L.
As discussed previously, the ceramic nozzle creates a higher back pressure due to orifice diameter. Pressure during the $v_{jet}$ portion of the waveform can be compared using the data from these two ejection trials to estimate the pressure at the piston tip. A comparison is made based on the fact that the ejections have the same desired waveform parameters. The relationship between pressure and force is applied. The force at the piston tip can be estimated as the Lorentz-force generated by the coil as described by the following equation:

\[ F = K I, \]

(4.1)

where $K$ is motor force constant in N/A and $I$ is current in A. In order to calculate the motor force constant, the motor constant is taken to be 3.21 N/√W [41] and the resistance of the coil, 11.5 Ω. First, from the ejection data using the Injex$^TM$ ampoule, the peak current was found to be 0.76 A. The force at the piston tip was calculated to be 8.27 N. For the ceramic nozzle with a peak current of 2.52 A, the force is 27.43 N. Therefore the pressure for the Injex$^TM$ ampoule is 0.83 MPa and the pressure for the ceramic nozzle is approximately 3 times higher at 2.74 MPa. This higher pressure creates a higher hoop stress in the ampoule when injecting with the ceramic nozzle.

### 4.6 Application to Intratympanic Injections

In order to determine appropriate waveform parameters for the ceramic nozzle, the waveform described in Section 4.5 was initially used. Despite a clear trajectory into the agarose (produced by $v_{jet}$), injectate was found to pool on the lateral side of the tympanic membrane indicating that the follow-through velocity was insufficient. Therefore, the follow-through velocity was increased over a range from 10 to 30 m/s. $v_{jet}$ was held constant at 75 m/s, $t_{jet}$ at 2 ms, and $V_{desired}$ at 20 μL. The results are shown in Table 4.1.

Though a $V_{followthrough}$ of 15, 20, and 30 m/s were all sufficient, 15 m/s was found to be most appropriate as the pressure at higher velocities yielded splash back
through the erosion hole. Two successful injections at a $V_{\text{followthrough}}$ of 15 m/s resulted in injection holes measured to be 0.19 by 0.07 mm and 0.30 by 0.20 mm (using ImageJ®).

Table 4.1: Waveform ($V_{\text{followthrough}}$) Optimization

<table>
<thead>
<tr>
<th>Follow-through Velocity (m/s)</th>
<th>Injection Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Did not deliver</td>
</tr>
<tr>
<td>15</td>
<td>Delivered</td>
</tr>
<tr>
<td>20</td>
<td>Delivered</td>
</tr>
<tr>
<td>30</td>
<td>Delivered</td>
</tr>
</tbody>
</table>

4.7 Repeatability Study

A repeatability study was carried out to access injection holes made using the ceramic nozzle versus those made using a 0.31 mm (30-gauge) needle. A total of eight fresh harvested tympanic membranes were used for the study. Each ear was injected using the ceramic nozzle with an approximate standoff of 1 mm from the tympanic membrane. A waveform with $v_{\text{jet}}$ at 75 m/s, $t_{\text{jet}}$ at 2 ms, $v_{\text{followthrough}}$ at 15 m/s, and a desired delivered $V_{d_{\text{esired}}}$ at 20 μL was used to deliver Bromophenol blue at a concentration of 0.25%.

Table 4.2 shows the data from this study. Like with the Injex™ repeatability study, each injection hole was measured using ImageJ® three times along its largest and smallest dimension, these values were averaged for each sample, and finally, averaged across all eight samples.

Injection holes were measured on average to be 0.20 ±0.03 mm by 0.13 ±0.02 mm using the jet injector and ceramic nozzle. Holes from the 0.31 mm (30-gauge) needle were measured to be 0.33 ±0.02 mm by 0.19 ±0.01 mm on average. Similar to previous finding, injection holes created both with the jet injector and the needle proved to be variable in shape. However, use of the ceramic nozzle seemed to yield more rounded injection holes with less prominent crack characteristics than those produced by the
Table 4.2: Overall Results (Ceramic Nozzle)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jet Injector (Ceramic)</th>
<th>0.31 mm (30-Gauge) Needle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large (mm)</td>
<td>Small (mm)</td>
</tr>
<tr>
<td>Average</td>
<td>0.201</td>
<td>0.127</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.088</td>
<td>0.067</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.031</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Injex™ ampoule. No post injection tearing was noted during this study, but some residual tearing which was thought to be caused by the follow-through was observed. Figure 4-10 shows several representative images from these injections.

Measurements made in this study were found to have very low variability. A simple two-tailed, two-sample equal variance Student’s t-Test with a cutoff level of \( p \leq 0.05 \) was performed to determine statistical significance. The larger dimension was found to have a \( p \)-value of 0.002, while the smaller dimension had a \( p \)-value of 0.03. Therefore, using the jet injector with the ceramic nozzle yields injection holes that are significantly smaller than those made when injecting with a 0.31 mm (30-gauge) needle.

Coil velocities for \( v_{jet} \) were found to be 0.73 ±0.03 m/s and coil velocities for \( v_{followthrough} \) had an average of 0.0029 m/s (±0.0001 m/s). A higher coil velocity is required using the ceramic nozzle given higher back pressure and piston tip compression despite a lower desired \( v_{jet} \). In terms of tracking the trajectory depth, results from the agarose gel yielded trajectories ranging from about 6 mm to about 12 mm. Again, trajectories that traveled past 10 mm were found to reach the bottom of the petri dish.

4.7.1 Discussion

The data from the repeatability study suggests that the combination of the ceramic nozzle, a standoff estimated to be only 1 mm or less, and a slower desired \( v_{jet} \) does yield injection holes that are smaller in size than those created with the 0.31 mm (30-gauge)
Figure 4-10: Six representative images of the tympanic membrane post injection via the jet injector with the ceramic nozzle (J) and a 0.31 mm (30-gauge) needle (N). Injection holes for both the jet injector and the needle exhibit variability. Image C shows some residual tearing above the site of the jet injection hole. Image E shows an example of a very small injection hole made by penetration with the jet injector.
needle. In addition, jet injection holes using the ceramic nozzle are similar in shape to those created with a needle (more rounded). These results provide improved support that using jet injection to deliver drug through the tympanic membrane is a feasible option.
Chapter 5

Conclusions and Future Directions

5.1 Intratympanic Injection Summary

A summary of the data obtained using a 0.31 mm (30-gauge) needle, a jet injector with an Injex\textsuperscript{TM} ampoule, and a jet injector with an Injex\textsuperscript{TM} ampoule retrofitted with a 50 \textmu m ceramic nozzle for intratympanic injections is presented in Figure 5-1 and Table 5.1. From Figure 5-1, a side-by-side comparison of the data from the Injex\textsuperscript{TM} ampoule versus the needle and the ceramic nozzle versus the needle can be made. Table 5.1 shows that while the Injex\textsuperscript{TM} ampoule produced injection holes that are larger than holes using a 0.31 mm (30-gauge) needle in the largest dimension, the ceramic nozzle created injection holes that are significantly smaller than those created with a 0.31 mm (30-gauge) needle. Given use of a more standardly used 0.41 mm (27-gauge) needle which is larger than the 0.31 mm (30-gauge) needle, these results become more significant. Because the waveform used to generate the data for the Injex\textsuperscript{TM} ampoule and the ceramic nozzle rely on different parameters, these data can not be directly compared.

5.2 Future Directions

Many different avenues offer opportunities to further explore and improve the data presented in this thesis. The following sections will discuss three of these avenues –
Figure 5-1: Bar graph summarizing the compiled injection data for the largest and smallest dimensions from each penetration hole. Each bar represents the average and the error bars represent the standard deviation from the mean.

Table 5.1: Summary Statistical Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Large $p$-value</th>
<th>Small $p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t$-Test (Injex$^M$ vs 0.31 mm (30-Gauge) Needle)</td>
<td>0.0011</td>
<td>0.4441</td>
</tr>
<tr>
<td>$t$-Test (Ceramic vs 0.31 mm (30-Gauge) Needle)</td>
<td>0.0019</td>
<td>0.0278</td>
</tr>
</tbody>
</table>
the development of a tissue analogue, delivery of hyaluronic acid using jet injection, and hardware and control.

5.2.1 Tissue Analogues

One aspect of the project at hand is to explore injection through a thin membrane designed to mimic the acoustic and mechanical properties of the human tympanic membrane. Because the tympanic membrane is mainly composed of collagen as discussed in Section 2.3, a collagen gel was used for preliminary work (done by former undergraduate researchers in the lab). Gels cross linked with glutaraldehyde were found to mimic the Young's modulus of the human tympanic membrane. Despite many attempts to obtain data similar to that obtained previously, several setbacks occurred in developing more analogues. Progress on the forefront of data analysis and a literature review to explore different cross-linking substances was instead made.

In terms of data analysis, both a fixture for mechanical testing was machined and MATLAB® code written for post processing. The tissue analogues that are generated in the MIT BioInstrumentation lab are mechanically tested on a custom Dynamic Mechanical Analyzer (DMA). Due to issues aligning the fixtures as well as difficulty fixturing the tissue analogues, a new fixture was designed in SolidWorks® [65] and machined on the wire EDM. One of the biggest issues with the current clamping system was vertical alignment of the clamps. The fixture that attaches to the DMA load cell (Figure 5-2 E) was designed to be stationary and the opposite fixture was designed with a drive screw and linear shafts to allow vertical motion in a constrained space (Figure 5-2 A – D). In terms of clamping mechanisms, in hopes to create a “quick-clamp” system, bulldog clamps were modified to try to decrease the force with which they would make contact with the collagen gel. Additional clamps were designed which act on the principle of a more even distribution of the clamping force and may prove more useful given the delicate nature of these gels.

In addition, MATLAB code was written to facilitate the ease of post processing data obtained from the DMA. Code was written to be easily integrated with the current method of data storage used by the DMA. Three functions comprise the
Figure 5-2: SolidWorks® model showing the DMA fixture design machined on the wire EDM. The left fixture ((A) backplate, (B) moving fixture stage) is composed of a drive screw (D) and linear shafts (C) to allow vertical movement while the right fixture (E) is stationary when fixed to the DMA load cell.

post processing code – the main interface, dataprocessor.m, and two subfunctions, tensile_test_getfiles.m and tensile_test_processdata.m (code in Appendix C). The main features include the ability for files to be chosen from any and multiple directories if necessary, and user obtained input to define the linear region and the failure point of a sample based on a force-displacement plot.

Tissue analogues offer a unique opportunity to develop a membrane with specific mechanical properties. In addition, they also pose a model where the Injex\textsuperscript{TM} ampoule and the ceramic nozzle can be more directly compared. It is thought that the decreased standoff distance in combination with a smaller orifice diameter when using the ceramic nozzle attributed to the ability to use a lower $v_{jet}$ and still obtain penetration through the membrane. Because the tissue analogue does not have the restriction of an auditory meatus, it can be used to compare injections with the same parameters (standoff, waveform) for each nozzle in the future.
5.2.2 Hyaluronic Acid Injections

Another aspect is that of drug delivery. As inner ear drug delivery methods advance, so does investigation into the use of viscous substances as delivery mechanisms [14] [22]. One such solution is hyaluronic acid (HA). HA is a glycosaminoglycan that is readily found throughout the body in the lubricating proteoglycans of the synovial fluid, vitreous humor, and other tissues where it forms a gel in the intercellular space [66]. HA has been used in the treatment of inner ear disorders [14]. For example, in a study conducted in 2005 by Gouveris et al., HA was used as a delivery mechanism for dexamethasone for the treatment of idiopathic sudden sensorineural hearing loss [67] and in a study conducted in 2012 by Shibata et al., HA was found to be a clinically feasible method that enhanced the delivery of gene therapy to the cochlea [68]. In both studies, HA was found to enhance the permeability of drug through the RWM.

As use of such substances are explored, the technology used must be capable of placing/delivering these substances. Often, the more viscous a solution (like HA), the lower the gauge needle required to deliver the solution [69, 70]. This limits the ability to deliver viscous solutions to the middle ear via traditional needle and syringe as a lower gauge creates a larger perforation, increases risk of infection, and extends healing time. Jet injection technology has been shown in this thesis to be a viable technology for intratympanic injections and has likewise been shown to be feasible for delivery of HA (See Appendix D for data from a pilot study using the jet injector and the Injex™ ampoule to deliver HA to pig skin). Future work will focus to integrate these two feasible options in order to explore delivery of drug to the inner ear using the jet injector and the ceramic nozzle.

5.2.3 Hardware and Controller

In addition to the avenues addressed above, the work discussed in Appendix A may prove to be invaluable in future iterations of the jet injector as implementation of the linear encoder may provide more precise control of the device. Combining more precise
control with reduced discrepancy between the coil velocity and the piston tip velocity due to tip compression, would lay ground for more conclusive comparisons between different nozzle geometries. Additionally, implementation of the sliding PD controller discussed in Section 4.4 may provide improved waveform control and following in the current device. The ability to better predict and control the jet injector could increase reliable delivery of drug to the RWM and greatly impact the efficacy of drug delivery to the inner ear.

5.3 Conclusion

In conclusion, the data presented in this thesis shows that jet injection is a plausible technology for the delivery of pharmaceuticals to the inner ear via injection through the tympanic membrane. A nozzle with a 50 μm orifice in conjunction with a highly-controllable jet injection system yields injection holes that are significantly smaller than those created by a 0.31 mm (30-gauge) hypodermic needle. Future work directions offer opportunities to improve injection parameters, drug delivery, and injection control.
Appendix A

Alterations to the Jet Injector System

In light of creating a device that is both portable and self-monitoring, it was desired that several sensors be integrated with the body of the jet injector. In the process of creating another iteration of the desktop jet injector device it was reasonable to work to implement changes in the design that would allow for sensor integration. Of key interest was the ability to monitor changes in the magnetic field over time and coil temperature. In addition, improved resolution in position sensing was desired. Suggestions and previous work from Adam Wahab, a PhD candidate in the MIT BioInstrumentation lab and Dr. Bryan Ruddy, a PhD who did his PhD work in the lab, led to the choice of a Hall effect sensor for magnetic field sensing, a thermopile for temperature monitoring, and a linear encoder for position sensing.

A.1 Magnetic Field Sensing

A.1.1 Hall Effect Theory

The Hall effect was first discovered by E. H. Hall in 1879. Governed by the Lorentz force described in Equation A.1, if current is forced to flow through a conductive material placed in the presence of a magnetic field, a force perpendicular and proportional
to both the electric and the magnetic field is generated.

\[ F = q (E + v \times B), \]  

(A.1)

where \( q \) is the particle charge, \( E \) is the electric field, \( v \) is the velocity of the charged particle, and \( B \) is the magnetic field.

As charged particles are forced to one side of the conductive material due to the Lorentz force, a voltage difference is produced across the material. The electric field associated with this voltage difference is known as the Hall Effect and is described by the following equation:

\[ V_H = - \left( \frac{1}{nq} \right) \frac{I_x B_z}{L}, \]  

(A.2)

where \( n \) is the charge carrier number density, \( I_x \) is the current in the x-direction, \( B_z \) is the magnetic field in the z-direction, and \( L \) is the length of the conductive material.

### A.1.2 Hall Effect Sensor

A continuous-time ratiometric linear Hall Effect sensor was chosen for magnetic field sensing. The sensor is supplied by Allegro MicroSystems Incorporated and has the ability to supply an output voltage that is proportional to the magnetic field to which the chip is exposed. The chip offers a low voltage operation between 4.5 and 6.0 V, low noise, and high precision output due to internal circuitry in a package measuring 3.0 mm in width, 2.0 mm in length, and 1.0 mm in height. The A1302 was chosen due to a higher sensitivity range than the A1301 [71].

### A.1.3 Preliminary Testing and Calibration

Preliminary testing and calibration of the Hall Effect sensor was conducted using a Gaussmeter (F.W. Bell, Gauss/Teslameter, Model 5080) and the standard NdFeB magnet used in the jet injector (measured to have magnetic flux density of 0.6 T). A calibration curve was developed and the linear relationship was found to have an
R-squared value of 0.994 and a sensitivity of -0.038 T/T. Using this data to calibrate the raw data output by the Hall Effect sensor, the relationship between the magnetic field strength and the distance from the sensor was determined. Figure A-1 shows this data. A relationship described by \( y = 0.0579e^{-0.049x} \) fits the calibrated data generated from the sensor well with an R-squared value equal to 0.996.

Figure A-1: Magnetic field strength as a function of sensor distance. With increased strength the magnetic field strength decreases by the function \( y = 0.0579e^{-0.049x} \).
A.2 Temperature Sensing

A.2.1 Infrared Temperature Sensing Theory

Infrared temperature (IR) sensors are based on the theory that objects emit infrared radiation based on temperature. This radiation can be detected and converted to a voltage signal which in turn can be interpreted as the temperature of the object of interest. Thermal radiation depends on the emissivity of the surface of the object of interest. Emissivity is defined as an object’s ability to radiate energy from its surface. Black bodies, which are defined to have an emissivity of one are used as a reference from which the emissivity of other materials is defined.

A.2.2 Thermopile

Developed for MEMS applications, a single-chip digital infrared temperature sensor was chosen for implementation with the jet injector technology. Provided by Texas Instruments, the TMP006 provides non-contact temperature sensing in a package measuring 1.6 mm in both width and length and 0.625 mm in height and is capable of measuring temperature over a range from -40 – 125 °C. The chip requires a minimum supply current of 240 μA and a minimum supply voltage of 2.2 V and is thus advertised to be appropriate for use with batteries. The chip uses three pull-up resistors for the communication bus and a bypass capacitor for the power supply [72]. The small package size, temperature range, and low power consumption of the TMP006 are all key features for this application.

A.2.3 Preliminary Testing and Calibration

A Fluke 61 infrared thermometer served as a standard by which to create a calibration curve for the TMP006. In order to safely test and obtain data from a range of temperature values obtained from heating the coil, short duration current was delivered to the coil at the bottom of its stroke (the coil was not moving, but instead pushing on the front plate). Tests were repeated consistently in order to
cause the coil temperature to rise. Though this methodology was not ideal as the voltage delivered to the coil during a single test would ultimately not correspond with the thermopile output, it did allow for better monitoring of coil temperature and successful data collection without overheating the coil or demagnetizing the magnet.

In accordance with the TMP006 User's Guide, a series of equations were required to calculate the temperature of the target object, or in this case, the coil. Equation A.3 allows the user to correct for the changes in the sensitivity of the thermopile given changes in temperature. To account for the self-heating of the thermopile as an artifact of its operating temperature, Equation A.4 is used. The Seebeck coefficients are calculated using Equation A.5 and Equation A.6 draws a relation between the transfer of IR energy from the object to the thermopile and the conducted heat in the chip itself. The key parameters used in these equations can be found on page 10 of the TMP006 User's Guide [73].

\[
S = S_0 \left[ 1 + a_1 (T_{DIE} - T_{REF}) + a_2 (T_{DIE} - T_{REF})^2 \right], \quad (A.3)
\]

\[
V_{OS} = b_0 + b_1 (T_{DIE} - T_{REF}) + b_2 (T_{DIE} - T_{REF})^2, \quad (A.4)
\]

\[
f(V_{OBJ}) = (V_{OBJ} - V_{OS}) + c_2 (V_{OBJ} - V_{OS})^2, \quad (A.5)
\]

\[
T_{OBJ} = \sqrt{\frac{T_{DIE}^4 + \left( \frac{f(V_{OBJ})}{S} \right)}{c}}, \quad (A.6)
\]

The relationship was found to be linear with an R-squared value of 0.977 and a sensitivity of 0.924 °C/°C.
A.3 Position Sensing

A.3.1 Linear Encoder Theory

Many instruments which utilize fine position motion such as CNC lathes, coordinate measuring machines, and laser machining tools utilize linear encoders to sense motion. A linear encoder is an electromechanical device used to monitor position by way of electrical signals in the form of a square wave. While a variety of linear encoder technologies exist, optical encoders are very common. Optical encoders work through interfacing with an encoder strip that has both opaque and transparent sections that either serve to block or transmit light, creating a square wave pattern. Provided two channels, channel A and channel B, both position and direction of motion may be determined. For example, if channel A detects a transparent portion of the encoder strip, it will return a one logical signal. At the same time, channel B detects an opaque portion of the encoder strip and returns a zero logical signal [74]. This pattern is indicative of forward motion.

The above assumes quadrature incremental encoding where the channels are “coded ninety electrical degrees out of phase.” Many encoders have a third channel, or index, which is used to determine location. The index is particularly useful for rotary encoders to indicate one complete revolution. By monitoring the state of one channel in regard to the other, quadrature encoding increases the reliability of incremental encoders. Quadrature encoders also have the ability to achieve increased resolution given higher degrees of interpolation. To achieve only 1× resolution, the counter is required to only count the rising edge of the square wave for one channel. To increase resolution to 2×, the same channel’s rising and falling edges are counted. If both the rising and falling edges of both channels are counted, the interpolation is increased to 4× [75].

Due to the ability to achieve increased resolution by using a linear encoder rather than the currently used potentiometer, this technology was a very favorable option. Fortunately, headway had already been made in light of such desire by Wahab. Integration of the smallest three channel optical encoder chip on the market with a
custom linear encoder strip designed by Wahab posed a very compact and inexpensive method of integrating linear encoder technology with the existing jet injector. This technology was explored in moving forward.

A.3.2 Linear Encoder Chip

Like with the thermopile, the smallest available three channel encoder chip was chosen for this application. Provided by Avago Technologies, the AEDR-8500 reflective encoder chip houses both a light emitting diode and a photodiode detector in a surface mount, leadless package that measure 3.95 mm in length, 3.40 mm in width, and 0.96 mm in depth. The chip has both an A and a B channel, as well as an index channel and can perform quadrature encoding at interpolation factors of 1x, 2x, and 4x. The chip requires only a 5 volt supply and a resistor rated at 180 Ω to limit the current drawn by the LED and provides digital output. The encoding resolution ranges from 294 to 304 lines per inch, or approximately twelve lines per millimeter [76].

A.3.3 Linear Encoder Strip

The linear encoder strip designed by Wahab consisted of black ink printed onto transparency paper. While the strips themselves were designed using AutoCAD® 2011 [77], the design was printed onto transparency paper locally by PageWorks. A spacing of 42 μm was used to create the alternating pattern between opaque and translucent stripes allowing for approximately 12 opaque stripes per millimeter and likewise, approximately 12 translucent stripes per millimeter.

Because Avago AEDR-8500 is a reflective encoder chip and the translucent portions of the encoder strip were not reflective enough to reflect the light from the LED back toward the detector, the strips required a reflective backing. The design of such a backing was far more challenging than anticipated and required many test iterations before a final method for generating a reflective cover for the translucent portions of the encoder strip was determined.

Initial encoder strip iterations using metallic film proved very promising when
tested using a test bench setup and yielded 0.02 mm/mm of sensitivity, but the strip was found to be too thick once the coil was seated in the housing. Other iterations involved the use of metallic foil paper as a backing for the transparent strip and the possibility of printing the encoder strips directly onto the metallic foil paper. Tested methods either proved to generate impurities that affected the ability to accurately read ticks from the encoder strip or inconsistent construction. Though printing the strips directly onto metallic paper was a viable option, the benefit to the transparency paper was its ability to provide resistance to moisture damage. Because this feature is essential to a device that interacts with fluids, further designs involving the transparency strips were explored.

Final iterations involved the use of thin metallic shim. Initially steel shim with a thickness of 0.04 mm was tested. On average, a discrepancy of approximately three ticks was noted between travel over a thirty millimeter length in both the positive and negative direction. Given these positive results, aluminum shim with a thickness of 0.0254 mm was ordered from McMaster-Carr. Aluminum shim provided the benefit of being both a more reflective material than the steel shim and non-magnetic. The final design consisted of the encoder strip attached to the aluminum shim via spray-on adhesive and the use of Loctite to adhere the encoder strip assembly to the former. Results proved to be encouraging with an average of 48 μm calculated per tick and a standard deviation of ±0.02 μm. Note that a further iteration was made, but will not be discussed as part of this thesis as they were worked on solely by Ashin Modak, a Master’s student in the MIT BioInstrumentation lab.

### A.3.4 Preliminary Testing and Calibration

Preliminary data was obtained using a test bench setup which consisted of a former mounted to an optical stage and the linear encoder chip mounted to a stationary platform such that the encoder chip and encoder strip (adhered to the coil) could be moved at micrometer increments relative to one another. Initially a National Instruments (NI) USB-6215 data acquisition system in combination with simple LabVIEW code was used to test the linear encoder, but transition to a compact
RIO was preferred for integration with the current jet injector system. Thus, an
NI 9411 compact-RIO, 6-channel differential digital input module was obtained and
integrated with code written in LabVIEW for preliminary testing. A calibration curve
obtained when drawing a comparison between distance measurements obtained using
LabVIEW to count linear encoder ticks and the actual known distance covered by
movement of the former-linear encoder assembly relative to the encoder chip using
a micrometer stage yielded a linear relationship with an R-squared value of greater
than 0.99 and a sensitivity of 0.02 mm/mm. In addition, the linear encoder was able
to accurately detect direction of motion.

A.3.5 Velocity calibration

Once assembled within the jet injector shell, another calibration was performed to
determine the relationship between steady state voltage and the resulting steady state
jet velocity like that described in [2]. Using water ejected into air, tests were run for
a period of 20 ms (15 ms once a voltage of 100 V was reached to decrease the chance
of causing damage to the coil if exposed to high voltage for too long) with an input
of 10 V. Note that these were open-loop tests and did not rely on any feedback from
the system. Using an AE Techron LVC 5050 linear amplifier, the gain was initially
set to a value of 3 and subsequently increased by unity until a gain of 25 was reached.
MATLAB® was used to determine the steady state voltage of the system and the
corresponding steady state velocity of the coil. Jet velocity was further determined
using conservation of mass and mass flow rate to relate the velocity at the piston
tip (assumed to be the same as the coil velocity) to the velocity at the orifice in
the ampoule. Figure A-2 shows the data obtained during testing. A second order
polynomial relationship was determined with an R-squared value of 0.9749 and the
following equation:

\[ y = 0.0181x^2 + 0.5896x - 8.9304. \] (A.7)
Figure A-2: A second order polynomial relationship was found to relate the steady state voltage of the jet injector system to the resulting steady state jet velocity.

A.4 Sensor Printed Circuit Board Design

In order to integrate all three sensors in a space efficient manner with the current design of the jet injector, a printed circuit board (PCB) was designed to seat onto the steel casing/housing (using EAGLE 5.11.0 Light® [78]). In designing the board, positioning of each of the sensors was a key consideration. While the Hall Effect sensor provided the most flexibility in terms of placement, both the linear encoder chip and the thermopile were restricted to certain locations relative to the jet injector assembly.

First, because proper functioning of the linear encoder and accurate encoder reading rely on the linear encoder strip being within “sight” of both the encoder emitter and detector at all times, the encoder chip had to be positioned accordingly. The current design of the steel casing incorporates four large windows that decrease the overall volume of steel and thus the weight of the shell, allow for air flow, allow visualization of portions of the coil, and ease in piston and ampoule instillation. Fortunately, due to the height of the field guide-magnet assembly, the coil former has restricted travel such that the lip of the former which forms the top edge of the coil
gap can travel no further than the base of these windows. As a result, there exists a 7 mm portion of the former to which the linear encoder strip can begin to be adhered to that is always visible through one of the four windows. Thus the encoder chip was placed on the PCB such that when mounted to the steel shell, it was aligned with this 7 mm region.

The thermopile posed the most challenging design issues because of restrictions based on the design of the chip and its function. According to layout guidelines set by Texas Instruments for the TMP006, a large keep-out region measuring approximately 8 mm by 8 mm is ideal for thermal isolation of the chip from other components on the PCB. Thus, though the chip itself only measured 1.6 mm by 1.6 mm, it required far more space on the board. In addition, though the thermopile provides non-contact sensing, it does require a field of view in which the target object is "visible" at all times for temperature reading. Unlike the linear encoder which could be exposed to a linear encoder strip mounted along the entire length of the coil former, the thermopile needed to be in line with the actual coil windings which, at the upper extreme of the motor stroke, are not visible.

Because of the need for coil visibility, a portion of the steel casing needed to be removed. In order to minimize material removal, the PCB was designed so that the thermopile was isolated on a narrower region of the board. Maintaining the keep-out region at the advised dimensions, the portion of the board with the thermopile was made only slightly wider than these dimensions.

The Hall Effect sensor was placed as close to the magnetic field as was possible given the other two chips, but still within one of the windows on the shell. All resistors were placed in an effort to optimize space efficiency and thus some were placed on the top layer of the board while others were placed on the bottom layer. Bypass capacitors where placed close (if possible) to the power or VCC pad of each of the chips. Wires supplying power were designed with a width of 0.3 mm and signal wires were designed with a width of 0.18 mm. Vias were dimensioned with an outer layer diameter of 0.8128 mm and a drill size of 0.4064 mm. A simple 16-pin, female ribbon connector was placed on the bottom of the circuit board for powering and
communicating with the chips. Drill holes sized for M2 screws were placed on the board as well for connection to the jet injector shell. Board assembly was conducted in house using Chip Quik solder paste, a ZEISS Stemi SV8 microscope, and a LPKF Laser & Electroncis, Proto Flow S reflow oven.

Overall, the PCB has a total length of 31.5 mm with the wider portion 21.0 by 15.0 mm and the thinner portion, 10.5 by 11.0 mm. Figure A-3 shows the populated board.

![Populated PCB](image)

Figure A-3: The populated jet injector printed circuit board. (A) Location of 16-pin, female ribbon connector, (B) Hall Effect sensor, (C) Linear Encoder chip, (D) Thermopile.

### A.5 Jet Injector Shell Design Alterations

In light of the PCB design, alterations to the jet injector shell were required. Despite the already existing presence of the windows in the upper shell, due to mounting requirements an additional window needed to be created. Much of the material removal for this window was taken from non-critical areas of the shell (areas that did not contribute to magnetic shielding). Also, the least possible number of alterations were made to maintain continuity with the jet injector designs already present in the MIT BioInstrumentation lab.

The design criteria followed for the redesign of the shell involved transecting the outer radius of the shell to create a flat mounting plate for the PCB and removing
material to allow the three sensors to “see” the necessary jet injector components. Initially the design was optimized to remove as little material as possible, but due to machining capabilities of the Mazak this design needed to be altered. With only x and z axes, the motion of the tooling relative to the stock material was limited.

An inset was milled out of the shell with a 16 mm endmill to create a mounting surface for the PCB. To allow the PCB to lie properly, 201.7 (mm)$^3$ of material was removed from the critical portion of the shell. Note that the depth of cut was only 1.5 mm thus leaving behind 1.4 mm of steel to encase the coil at the location of the milling. Because the top layer of the PCB was directly mounted to the shell, a clear plastic sheath was laser machined to ensure that the steel casing would not cause the circuitry on the board to short.

A through slot was then created using an 8 mm endmill in the non-critical region of the shell. This area served as a window for both the linear encoder and the Hall Effect sensor. A square cut-out 4.0 by 4.0 mm was calculated to be a sufficient size so that coil windings would be “visible” to the thermopile yet the edges of the shell casing would not pose any interference with the sensor field of view. Thus, the 8 mm slot was extended using a 4 mm endmill and removing 18.9 (mm)$^3$ of additional material from the critical region of the shell. The total material removal was determined to not have an effect on the injector capabilities and magnetic circuitry. Figure A-4 shows the PCB slot on the shell and provides a comparison to the traditional jet injector shell design.

All shell features were modeled in SolidWorks® and imported into FeatureCAM® [79]. Using the Machiner’s Handbook to calculate appropriate feeds and speeds for each tool along with the appropriate post processor, G-code was written and the shell constructed on the Mazak.

Because the linear encoder strip was mounted on the outside of the former and thus gave the coil extra dimension, additional material was removed from the inside of the shell to allow the encoder strip to freely move. G-code was written for use with the wire EDM and a square slot 0.69 by 7.0 mm aligned with the PCB slot was machined to complete the shell.
A.6 Contact Sensor

In addition to the PCB discussed above, an additional board was designed. Unlike the traditional linear potentiometer used in the jet injector which works in absolute position, the linear encoder works in relative position. It was desired to have a simple, yet highly repeatable method for zeroing the position of the linear encoder each time the LabVIEW controller code was initialized. A PCB was thus designed such that contact made between a probe and the top of the travel guide would cause completion of an electrical circuit. Logic was used to determine when contact was made and thus at which point to zero the linear encoder position reading. The IC OPAMP unity gain 8SOIC chip (ISL55002IBZ-T7) was chosen as the unity gain buffer in the circuit. Figure A-5 shows the PCB design.

A.7 Discussion

Despite potential to improve control resolution through use of the linear encoder, much work was still required before this particular device could be proven as reliable as previous devices for use in animal studies. With much interest in moving forward
with the application of the jet injector to auditory applications, a previous iteration of the jet injector was implemented for the work discussed previously in this thesis. Ashin Modak, however, continued to work on the alterations to implement the above described work into the jet injector system.
Appendix B

Raw Injection Data
<table>
<thead>
<tr>
<th>Sample</th>
<th>0.31 mm (30-Gauge) Needle</th>
<th>0.31 mm (30-Gauge) Needle</th>
<th>0.31 mm (30-Gauge) Needle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Largest Dimension (mm)</td>
<td>Smallest Dimension (mm)</td>
<td>Largest Dimension (mm)</td>
</tr>
<tr>
<td>1*</td>
<td>0.589 0.585 0.577 0.584</td>
<td>0.279 0.276 0.274 0.276</td>
<td>0.370 0.366 0.360 0.365</td>
</tr>
<tr>
<td>2</td>
<td>0.657 0.651 0.645 0.651</td>
<td>0.359 0.357 0.358 0.358</td>
<td>0.261 0.261 0.260 0.261</td>
</tr>
<tr>
<td>3</td>
<td>0.493 0.495 0.491 0.493</td>
<td>0.220 0.218 0.217 0.218</td>
<td>0.251 0.249 0.252 0.251</td>
</tr>
<tr>
<td>4</td>
<td>0.278 0.280 0.276 0.278</td>
<td>0.131 0.134 0.133 0.133</td>
<td>0.533 0.530 0.522 0.528</td>
</tr>
<tr>
<td>5</td>
<td>0.564 0.561 0.560 0.562</td>
<td>0.167 0.167 0.166 0.167</td>
<td>0.274 0.272 0.272 0.273</td>
</tr>
<tr>
<td>6</td>
<td>0.419 0.413 0.419 0.417</td>
<td>0.151 0.149 0.151 0.150</td>
<td>0.219 0.217 0.211 0.216</td>
</tr>
<tr>
<td>7</td>
<td>0.789 0.785 0.778 0.784</td>
<td>0.267 0.267 0.267 0.267</td>
<td>0.319 0.322 0.321 0.321</td>
</tr>
<tr>
<td>8*</td>
<td>0.313 0.317 0.318 0.316</td>
<td>0.190 0.189 0.188 0.189</td>
<td>0.356 0.354 0.353 0.354</td>
</tr>
<tr>
<td>9</td>
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<td>0.118 0.116 0.119 0.118</td>
<td>0.438 0.447 0.449 0.445</td>
</tr>
<tr>
<td>10</td>
<td>0.650 0.647 0.642 0.646</td>
<td>0.193 0.194 0.200 0.196</td>
<td>0.302 0.301 0.299 0.301</td>
</tr>
</tbody>
</table>
Table B.2: Intratympanic Injection via Ceramic Nozzle

<table>
<thead>
<tr>
<th>Sample</th>
<th>Jet Injector with Ceramic Nozzle</th>
<th>0.31 mm (30-Gauge) Needle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Largest Dimension (mm)</td>
<td>Smallest Dimension (mm)</td>
</tr>
<tr>
<td>1</td>
<td>0.190 0.194 0.193 0.071 0.077 0.073 0.493 0.488 0.490 0.490 0.197 0.197 0.195 0.196</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.296 0.296 0.296 0.206 0.203 0.204 0.351 0.352 0.350 0.351 0.150 0.149 0.147 0.149</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.251 0.252 0.251 0.213 0.211 0.212 0.278 0.273 0.271 0.274 0.167 0.169 0.169 0.168</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.097 0.098 0.099 0.058 0.056 0.057 0.258 0.262 0.264 0.275 0.205 0.206 0.202 0.204</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.344 0.345 0.344 0.196 0.196 0.195 0.250 0.256 0.254 0.253 0.162 0.162 0.162 0.162</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.166 0.162 0.162 0.127 0.126 0.126 0.301 0.298 0.291 0.297 0.077 0.078 0.075 0.077</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.112 0.114 0.144 0.113 0.057 0.058 0.060 0.058 0.325 0.319 0.321 0.322 0.144 0.148 0.145 0.146</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.152 0.152 0.149 0.151 0.092 0.092 0.095 0.095 0.335 0.339 0.352 0.342 0.271 0.283 0.275 0.276</td>
<td></td>
</tr>
</tbody>
</table>
Appendix C

DMA Mechanical Analysis Code

```matlab
function dataprocessor

% This function serves as an interface to allow the user to choose
% one or multiple m-files (produced by the Dynamic Mechanical
% Analyzer) to process. The files are temporarily copied into
% the current directory so that the variables in the m-file become
% a part of the current workspace. In addition, because the files
% are copied into the workspace before processing, files can be chosen
% from any directory and do not have to already exist in the current
% directory.

% Usage: data processor tool for data produced by the DMA
% Input: none
% Output: Excel spreadsheet with compiled data
% Returned values: none
% Written by Alison Cloutier, BioInstrumentation Lab, January 2013

clc
close all;

% Initialize variables
data = struct([]);
```

99
% Initialize variables as persistent so that values are retained in memory between function calls
persistent SampleLength;
persistent SampleWidth;
persistent SampleThickness;
persistent SampleArea;
persistent SamplingFrequency;
persistent TotalTime;
persistent StrainRate;
persistent NPerV;
persistent ClampOffset;
persistent InitialPosition;
persistent PositionResolution;
persistent RawData;

% Call tensile_test_getfiles to obtain file(s) to be analyzed and choose output location
[filename, pathname, output, nofiles] = tensile_test_getfiles;

currdir = pwd;

% Create Excel file for data output and populate column labels
[Y, M, D, H, MI, S] = datevec(datestr(now, 0));
calcdatal_file = ['TensileTestOutput-', num2str(Y, 4), num2str(M, 2), num2str(D, 2), '_', num2str(H, 2), num2str(MI, 2), num2str(S, 2), '.xls'];
complete_calcdatal_file = fullfile(output, calcdatal_file);
column_labels = [{'File Name'},{'Ultimate Force (N)'},
                 {'Displacement at Ultimate Force (mm)'},
                 {'Structural Stiffness (N/mm)'},
                 {'Energy to Failure (mJ)'},{'Ultimate Stress (MPa)'},
                 {'Ultimate Strain (%)'},{'Youngs Modulus (MPa)'},
                 {'Strain Energy Density (mJ/mm^3)'}];
xlswrite(complete_calcdatal_file, column_labels, 'Sheet1', 'A1');

% Use for-loop to process each file to be analyzed - copy files into
% current directory, run each file, populate the structure data with
% variables, and process the data
for i = 1 : nofiles
    fname = filename{i};
    full_filename = fullfile(pathname, fname);
    copyfile(full_filename, currdir);
    complete_filename = fullfile(currdir, fname);
    run(complete_filename);
    data(i).ClampOffset = ClampOffset;
    data(i).InitialPosition = InitialPosition;
    data(i).NPerV = NPerV;
    data(i).PositionResolution = PositionResolution;
    data(i).RawData = RawData;
    data(i).SampleArea = SampleArea;
    data(i).SampleLength = SampleLength;
    data(i).SampleThickness = SampleThickness;
    data(i).SampleWidth = SampleWidth;
    data(i).SamplingFrequency = SamplingFrequency;
    data(i).StrainRate = StrainRate;
    data(i).TotalTime = TotalTime;
    [calcdata] = tensile.test.processdata(data, i, fname, output,
                                          complete_calcdata_file);
end

% Delete all copied files
for i = 1 : nofiles
    delete(filename{i});
end
function [filename, pathname, output, nofiles] = tensile_test_getfiles

% This function serves to allow the user to choose one or multiple
% m-files (produced by the Dynamic Mechanical Analyzer) to process.
% The user is also asked to choose a directory in which to store the
% output data.
% Usage: file selection
% Input: m-file(s), output directory
% Output: selected files, chosen output directory
% Returned values: filename, pathname, output, nofiles
% Written by Alison Cloutier, BioInstrumentation Lab, January 2013

% Choose the m-files to be processed and analyzed
[filename, pathname, filter_index] = uigetfile
('*.m', 'Select the MATLAB data file(s) of interest:', 'MultiSelect',
 'on');

% Check that files were properly chosen and display chosen files
if isequal(filename, 0) || isequal(pathname, 0)
    disp('Cancel was selected.')
    return;
else
    fprintf('\n');
    disp([\'Selected file(s): '', filename])
end

% Determine whether one or more files were chosen so each file is
% properly counted
if ischar(filename);
    filename = {filename};
    nofiles = numel(filename);
else
    nofiles = numel(filename);
end
% Choose an output directory
output = uigetdir(pathname, 'Choose the directory you wish to store the output data:');

% Check that a directory was properly chosen and display output
if isequal(output, 0)
    disp('No directory was chosen.')
    return
else
    fprintf('

');
    disp(['Output directory: ', output])
end
function [calcdata] = tensile_test_processdata(data, i, fname, output, complete_calcdata_file)

% This function serves as the main data analyzer and processor as it
% processes the raw data from the m-file that the DMA creates and
% allows the user to select points on a force-displacement plot that
% define the linear region and the failure point. A PDF of the
% equivalent stress-strain data is created for future reference
% indicating where the user-selected points lie on the plot.
% All relevant structural and material properties are also calculated.
% Usage: data processor/analyzer
% Input: linear region on force-displacement plot and failure point
% Output: PDF stress-strain plot
% Returned values: calcdata
% Written by Alison Cloutier, BioInstrumentation Lab, January 2013

% Initialize variables
calcdata = [];

% Process raw data (as done in original DMA m-file)
Time = [0:1/data(i).SamplingFrequency:(length(data(i).RawData)-1)/data(i).SamplingFrequency];
Force = (data(i).RawData(:,1)-data(i).RawData(1,1))*data(i).NPerV;
Displacement = (data(i).RawData(:,2)-data(i).RawData(1,2))*data(i).PositionResolution;
Stress = Force/data(i).SampleArea;
Strain = (Displacement/data(i).SampleLength)*100;

% Display instructions for selecting key points on stress-strain plot
fprintf('
Please select three points on figure %i.
', i)
fprintf('The first point should indicate the lower bound
')
fprintf('of the linear range, the second, the upper bound of
')
fprintf('the linear range, and the third, the failure point.
')
fprintf('Double click when choosing the last point to indicate such.
')
fprintf('Press ENTER in the command window to continue.

');
% Ensure instructions are read
pause

% Allow user to select lower and upper bounds for the linear range to define stiffness and Young's Modulus, also allow user to choose failure point
figure(i);
scrsz = get(0,'ScreenSize');
set(gcf,'position',scrsz);
hold on;
box on;
grid on;
orient landscape;
plot(Displacement, Force);
set(gca, 'FontSize', 20);
title('Force versus Displacement');
set(get(gca,'Xlabel'),'fontsize', 20, 'String','Displacement (mm)');
set(get(gca,'Ylabel'),'fontsize', 20, 'String', 'Force (N)');
[x,y] = getpts(i);

% Determine actual data points closest to selected points
for j = 1:length(Displacement)
    if x(1) < Displacement(j)
        lower_index = j - 1;
        disp_lower = Displacement(lower_index);
        break
    else
        j = j + 1;
    end
end

for j = 1:length(Displacement)
    if x(2) < Displacement(j)
        upper_index = j;
        disp_upper = Displacement(upper_index);
break
else
    j = j + 1;
end
end

for j = 1:length(Displacement)
    if x(3) ≤ Displacement(j)
        if abs(Displacement(j) - x(3)) ≤ abs(x(3) -
            Displacement(j - 1))
            failure.index = j;
            disp.failure = Displacement(failure.index);
        else
            failure.index = j - 1;
            disp.failure = Displacement(failure.index);
            break
        end
    else
        j = j + 1;
    end
end

% Close figure
hold off;
close(i);

% Determine structural stiffness
linear.range.x = Displacement(lower.index:upper.index);
linear.range.y = Force(lower.index:upper.index);
p = polyfit(linear.range.x, linear.range.y, 1);
slope = p(1);
y_intercept = p(2);

Structural_Stiffness = slope;
% Determine Young's Modulus
linear_range_x_strain = Strain(lower_index:upper_index);
linear_range_y_stress = Stress(lower_index:upper_index);
pp = polyfit(linear_range_x_strain, linear_range_y_stress, 1);
f = polyval(pp, linear_range_x_strain);
slope_ss = pp(1);
y_intercept_ss = pp(2);

Youngs_Modulus = slope_ss*100;

% Plot stress-strain data, user selected points, and linear fit line
% and save to a PDF in output directory for later reference
% Pause for 6 seconds to allow user to look over plot
% Display files that have been created
figure(i*100);
set(gcf,'position', scrsz);
hold on;
box on;
grid on;
orient landscape;
commandlinewidth = 2;
plot(Strain, Stress);
hold on
plot(linear_range_x_strain, linear_range_y_stress, 'g')
hold on
plot(linear_range_x_strain, f, 'm')
hold on
plot(Strain(lower_index), Stress(lower_index), 'o', 'MarkerEdgeColor',
'k', 'MarkerFaceColor','k','MarkerSize',8)
hold on
plot(Strain(upper_index), Stress(upper_index), 'o', 'MarkerEdgeColor',
'k', 'MarkerFaceColor','k','MarkerSize',8)
hold on
plot(Strain(failure_index), Stress(failure_index), 'p',
'MarkerEdgeColor', 'k', 'MarkerFaceColor','r','MarkerSize',12)
set(gca, 'FontSize', 20);
title('Stress versus Strain');
set(get(gca,'Xlabel'),'fontsize', 20, 'String','Strain (%));
set(get(gca,'Ylabel'),'fontsize', 20, 'String','Stress (MPa)');
legend('Stress-Strain Data', 'Linear Region Data', 'Linear Fit Line',
     'Lower Fit Point', 'Upper Fit Point', 'Failure Point', 'Location',
     'Northwest');

long_filename = fullfile(output, fname);
pdf_name = [long_filename(1:length(long_filename)-1), 'pdf'];
fprintf('

')
disp(['Created files: ', pdf_name])
fprintf('

')
print(gcf, '-dpdf', pdf_name);
pause(6);
close;

% Determine ultimate force, extension at ultimate force, failure load,
% ultimate stress, and ultimate strain
[UltimateForce, ultimate_force_index] = max(Force);
Displacement_at_Ultimate = Displacement(ultimate_force_index);
UltimateStress = Stress(ultimate_force_index);
UltimateStrain = Strain(ultimate_force_index);

% Determine energy to failure and strain energy density
Force_to_Failure = Force(1:failure_index);
Displacement_to_Failure = Displacement(1:failure_index);
Energy_to_Failure = trapz(Displacement_to_Failure, Force_to_Failure);

Stress_to_Ultimate = Stress(1:ultimate_force_index);
Strain_to_Ultimate = Strain(1:ultimate_force_index);
Strain_Energy_Density = trapz(Strain_to_Ultimate, Stress_to_Ultimate);

% Create array of variables
calcdata = {{fname}, {Ultimate_Force}, {Displacement_at_Ultimate},
             {Structural_Stiffness}, {Energy_to_Failure}, {Ultimate_Stress},
{Ultimate Strain}, {Young's Modulus}, {Strain Energy Density});

% Write data to Excel file created in dataprocessor.m
row = ['A', int2str(i+1)];
xlswrite(complete.calcdatalfile, calcdatal, 'Sheet1', row);
Appendix D

Hyaluronic Acid Injections

Previous work in the MIT BioInstrumentation lab showed promise for use of the jet injector to deliver hyaluronic acid. A further feasibility/pilot study was conducted using the same jet injection system as described for the intratympanic injections along with the Injex™ ampoule. Pig skin was chosen as the injection media to first address feasibility for more broader injection applications. Solutions of HA at concentration of 0.1, 0.25, 0.5, 0.75, and 1.0 percent by volume mixed with tissue marking dye were injected. A mixture of water and tissue marking dye was used for the 0% HA control. Five or more injections were executed at each of the concentrations. A waveform with desired parameters: $v_{jet}$ of 200 m/s, $t_{jet}$ of 1.5 ms, $v_{followthrough}$ of 5 m/s, and a desired delivered $V_{desired}$ of 40 μL was used. At the higher HA concentrations of 0.75 and 1.0 percent, a jet $t_{jet}$ of 2.0 ms was required for penetration. Figure D-1 shows an image of a sliced sample of a successful injection where the injectate (0.75% HA) traveled to the subcutaneous adipose tissue. Figure D-1 also shows the average injection depth in millimeters as a function of HA concentration. The data is separated according to the desired waveform used to inject. Injection depths ranged from shallow, but in the viable dermal tissue, to deep within the muscle underlying the subcutaneous adipose tissue. This data shows that jet injection technology can be used to deliver HA to viable depths within pig skin and forms a basis upon which to explore application for intratympanic injections and possible use of the ceramic nozzle.
Figure D-1: Two plots showing average injection depth in millimeters as a function of HA concentration. Each average represents at least 5 samples and the error bars represent standard deviation for both plots. The plots are divided according to desired waveform. Image of a sliced sample of pig skin where the injectate (0.75% HA) traveled into the subcutaneous adipose tissue.
Bibliography


