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Mechanism of an Arched Basilar Membrane in Mammalian Cochlea

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List of Abbreviation

BM	Basilar membrane
IHC	Inner hair cell
OHC	Outer hair cell
SEM	Scanning electron microscope
ANF	Auditory nerve fiber
PMMA	Polymethyl methacrylate
PDMS	Polydimethylsiloxane
CF	Characteristic frequency
TC	Tuning curve
FTC	Frequency tuning curve
FTS	frequency tuning sharpness
SM	Simultaneous-masking
FM	Forward-masking
FTS	Frequency tuning sharpness
CAP	Compound action potential
SFOAE	Stimulus-frequency otoacoustic emissions
ERB	Equivalent rectangular bandwidth
SPL	Sound pressure level
SAW	Surface acoustic wave

Summary

The mammalian cochlea is a highly sensitive transducer which converts acoustic vibration into electrical signal. The acoustic vibration enters via the stapes and travels in the scala fluid along the cochlea from its basal to apical end. Prior to the acoustic-electrical conversion, the acoustic vibration is mapped onto the basilar membrane based on decreasing frequencies from base to apex. The variation of dynamic structural properties of the basilar membrane contributes to the frequency-mapping and sensitivity of the cochlea. The basilar membrane in most species of mammals, including humans, varies in width and thickness. However, in few species of mammals, such as gerbil, their basilar membranes are arched and have a resting radial tension. These mammals retain their cochlear sensitivity despite the lack of varying width and thickness in their basilar membrane. The present research analyses the mechanism of an arched basilar membrane in contributing to the sharp frequency tuning in a gerbil cochlea. The findings provide understanding of an arched membrane in mammalian cochlea and enables development for application of the cochlear mechanics in areas such as microfluidics and artificial cochlear development where limitations on the channel width are critical.

Among the more commonly researched species, the basilar membrane in human cochlea varies significantly in width (300% increase) and thickness (75% decrease) from its basal to apical end. The bandwidth of human cochlear auditory nerve fiber tuning curve is estimated with Yoon et. al.'s 3-Dimensional, push-pull mechanism, two-box model and compared to the bandwidth of gerbil cochlea. The results show comparative sensitivity between gerbil and human cochlea. In order to understand the difference between the two types of basilar membranes, effects of the bending stiffness and radial tension on the acoustic traveling wave in the passive gerbil cochlea is analyzed. The traveling wave number obtained from experimental measurements is compared to that calculated from Steele et. al.'s 3-Dimensional, two-box model which assumed a flat basilar membrane. Significant variation in bending stiffness along the cochlea (1-2 orders in section of 2.2 mm to 3 mm from base) is required in the Steele et. al.'s model in order to match the

wave number obtained from experimental measurements. With knowledge of contributing factors in the mechanism of an arched membrane, the dynamic equation is formulated with experimental measurements of gerbil basilar membrane and substituted to the eikonal equation of the two-box model. The wave number coefficients in the eikonal equation of the present arched basilar membrane model matched Yoon et al.'s verified gerbil cochlear model which used estimated effective basilar membrane properties.

For integration of cochlea mechanics design into microfluidic applications and the development of artificial cochlea, a method of fabricating and bonding the thin, flexible, anisotropic, 3-dimensional basilar membrane is required as the boundary conditions and properties of the basilar membrane are critical to the frequency tuning of the cochlea. Current methodologies of bonding thermo-set elastomer (basilar membrane) and thermoplastic (cochlear walls) is complex. The present ultrasonic bonding method using a thin film of thermoplastic-elastomeric composite is a simple process which provides better flow control by restricting melted thermoplastic with elastomer. The theoretical analysis of the energy absorption and distribution in the thin film composite provides a guideline for selection of thermoplastic and elastomer as well as estimation of the power required for ultrasonic bonding of the film composite. The experiment shows sufficient bonding strength in the bonded samples and verified the feasibility of this methodology. In a mammalian cochlea, the middle ear acts as a force transducer (acoustic-acoustic) which converts high amplitude-low power into low amplitude-high power vibration in order to match the impedance between air and fluid. This research presents an electromagnetic-acoustic transducer which transfer acoustic energy into the fluid with a lower operating voltage. The transducer consists of a voice coil attached to a membrane and a magnet base. The transducer is shown to operate at a similar efficiency (as a micromixer) while requiring a lower voltage input.

Chapter 1

Introduction

1.1 The Mammalian Cochlea

Figure 1.1: Pathway of acoustic vibration in the cochlea.

The mammalian cochlea is a sensitive transducer which converts acoustic vibration into electrical signal. Acoustic vibration captured by the tympanic membrane (ear drum) moves the bones in the middle ear. The middle ear acts as a lever which converts small pressure, large displacement vibration in the tympanic membrane to higher pressure (small displacement) vibration in the stapes. The acoustic vibration enters the mammalian cochlea from via the stapes which is attached to the oval window and travels in the scala vestibuli (fluid) from the base of the cochlea to the apical end, where the helicotrema is located. It then enters the scala tympani and exits through the round window. Along the path towards the helicotrema, some or most of the acoustic energy reaches the scala tympani by passing through the basilar membrane in the organ of corti (Figure1.1 and Figure 1.2). The amount of energy which passes through and excites the basilar membrane (BM) at each cross-section of the cochlea depends on the frequency and amplitude of the acoustic vibration as well as the properties of the basilar membrane at that location.

The frequency at which a location of the basilar membrane vibrates with the highest amplitude, when a pure tone enters the cochlea, is called the characteristic frequency of that position in the cochlea. The characteristic frequencies are higher near the basal end and lower near the apical end of the cochlea. When the amplitude of the basilar membrane vibration at a location exceeds a threshold, the inner hair cell (IHC) at the location sends electrical signals to the auditory cortex in the brain. With higher input amplitude, the location at which the basilar membrane's vibration exceeds the threshold becomes a small section where the inner hair cells are active. As the inner hair cell's signal is non-analogous, the firing of an inner hair cell occurs close to its characteristic frequency and the signal does not contain input amplitude information. The frequency tuning curve is the plot of the minimal amplitude of respective frequencies required for an inner hair cell of a characteristic frequency to fire and the sensitivity of a cochlea at a characteristic frequency is determined from its frequency tuning curve.

Figure 1.2: (a) An overview of the Organ of Corti showing position of various cells (b) Schematic showing the tilt of the OHCs based on SEM image [1]. The outer hair cells (OHC) provide the active amplification of the traveling wave. The walls of the OHC are piezoelectric, so a downward pressure on the basilar membrane at the distance x causes a shear between the reticular lamina and the tectorial membrane. This shear causes the ion channels in the stereocilia to open, change the intracellular electrical potential, which expands the cell, resulting in a downward push on the basilar membrane at $x + \Delta x_1$ through the Deiters rod at the lower end of the cell, and a pull up at the distance $x - \Delta x_2 + \Delta x_1$ through the phalangeal process connected at the upper end of the cell. The motion of the tectorial membrane is also picked up by the inner hair cells which sends signals to the brain.

Other than the input acoustic energy, the basilar membrane also exchanges energy with the chemical potential stored across the cell walls of the outer hair cells. The vibration on the BM causes shear motion of the reticular lamina and tectorial membrane. This motion opens and closes the ionic channels at the stereocilia of the outer hair cell which allows ions to enter the OHC (Figure 1.2(a)). The walls of the OHC are electromotile (piezoelectric). The change in the electrical potential across the OHC walls, due to the flow across the

ionic channel, extends and contracts the OHC and the energy is transferred to the BM via the Deiters' cell. However, the OHC is not in-plane with the cross-section of the cochlea, the BM vibration at x causes a feed forward amplification of the BM vibration at $x + \Delta x_1$. The movement of the reticular lamina at $x + \Delta x_1 + \Delta x_2$ also causes a feed-backward amplification of the BM vibration at $x + \Delta x_1$ through the phalangeal process which links the Deiters' soma to the reticular lamina. Figure 1.2(b) shows an illustration of this feed-forward feedback mechanism.

1.2 Microfluidics

Figure 1.3: Applications of acoustic wave in microfluidics; (a) mass detector¹, (b) micro-mixing [2], (c) micro-pump², (d) fluid flow control³ and (e) micro-particle focusing⁴

Microfluidics deals with the primarily physics in the Lab-On-a-Chip (LOC) or Micro Total Analysis Systems technology, handles and controls the microscale fluids. It has become useful tools in chemical, biomedical, and genomics systems development. Microfluidics systems take advantage of handling small amount of fluids or reagents to realize low-cost applications with fast analysis and minimize the risks of dealing with large-scale hazardous chemical reactions. In general, applications related to this technique require low energy consumption, small design, capability of with-standing high-volume use, and affordable to enable widespread commercial use.

Many of the microfluidic technology rely on the use of acoustic wave. These include micro-particle focusing technique [31] which uses surface acoustic wave (SAW) to induce

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low and high pressure region in the channel and change the concentration distribution of the heavier micro-particles (see figure 1.3(e)). Other uses of SAW are micromixers (see figure 1.3(b)) [2] and micropumps (see figure 1.3(c)) [29] where the acoustic wave induces microstreams which moves the body of fluid. Acoustic wave is also used in other microfluidic applications such as mass detection [32] where the mass changes in the cantilever shifts the resonant frequency of the cantilever (see figure 1.3(a)) and flow control [30] where the pressure changes constricts the channel and allows different flow velocity along the channel (see figure 1.3(d)).

Figure 1.4: Photograph⁵ of piezoelectric acoustic sensor.

There are also works on microfluidic acoustic wave sensing with cochlea mechanics and development of artificial cochlea. Chen et al. [33] fabricated an artificial cochlea with its basilar membrane made by depositing discrete Cu beams on a piezomembrane substrate, simulated the cochlear mechanism and measured the basilar membrane vibration with laser vibrometer. Shintaku et al. [34] developed a piezoelectric sensor to detect the vibrations on the polyvinylidene difluoride (PVDF) membrane of their artificial cochlea (see Figure 1.4) and Inaoko et al. [35] uses the the piezoelectric sensor to generate electric energy and induce auditory brainstem responses in deafened guinea pigs without additional power source.

Figure 1.5: Illustration of ear with cochlea implant [3].

1.3 Present Research

The objective of this research is to build up the technological capability of the development of a passively-powered, microfluidic artificial cochlea. Current cochlea implant picks up acoustic wave via a microphone. The electrical signal is then processed to frequency information and sent via the radio frequency transmitter to the cochlear implant which send the impulse signals to different regions of the auditory nerve via the electrode array (see Figure 1.5). The cochlea implant consumes high power due to the external signal (speech) processing and the radio frequency transmission to the internal receiver [36]. Without the need of electrical power for signal processing (and the need to replace batteries), a passively-powered artificial cochlea is able to be implanted and transmits signal directly to the electrode array. However, there are various technological challenges in the development of the passively-powered artificial cochlea suitable to replace current cochlear implant system. These challenges include fabrication and analysis of the microfluidic artificial cochlear, fabrication of the basilar membrane, study of the fluid mechanics in mammalian cochlea, impedance matching from the tympanic membrane (air) and the oval window (scala fluid). Three areas of research are focused in this work; the mechanism of a relatively constant width and thickness basilar membrane, the development of a bonding methodology for the thin basilar membrane and the development of an electric-acoustic transducer to match the air-to-scala fluid impedance.

The frequency-mapping and sensitivity of the cochlea depend on the variation of dynamic structural properties of the basilar membrane as well as the properties of the OHC. Most of the artificial cochleas require a fish-bone structure [37] to enable a wide hearing

⁵Reprinted from *Sensors and Actuators A: Physical*, 158 (2), Shintaku H., Nakagawa T., Kitagawa D., Tanujaya H., Kawano S. and Ito J., Development of piezoelectric acoustic sensor with frequency selectivity for artificial cochlea, 183-192, Copyright 2010, with permission from Elsevier.

range and frequency selectivity comparative to mammalian cochlea. This limits the size of the artificial cochlea as well as the use for other microfluidics applications. There are two types of basilar membrane in the mammalian cochlea. In most mammals including human, the basilar membrane is an anisotropic flat plate which varies its width and thickness from the basal to apical end of the cochlea. However, in few species of mammals, such as gerbil, their basilar membranes are arched and have resting radial tension. These mammals retain their cochlear sensitivity despite the lack of varying width and thickness in their basilar membrane. In addition, the gerbil cochlea spans about 13 mm which is less than half that of the human cochlea (35 mm).

In most microfluidic chip designs, where the objective is to minimise the fluid sample required, as well as in the design of an artificial implanted cochlea, where there is a limited space for the implant, the size of the microfluidic chip and the width of the channels are critical. The first part of present research on the gerbil arched membrane enables the application of the cochlea mechanism to the microfluidic designs with limited channel size. Firstly, we verify that the gerbil cochlea has a comparative frequency tuning compared to human cochlea (Chapter 2). The theoretical human cochlear model is formulated with Yoon et al.'s 3-dimensional, push-pull mechanism, two-box model and human cochlear experimental parameters [38]. The human basilar membrane velocity tuning curves are obtained by matching the experimental auditory nerve fiber (ANF) threshold for frequencies of 0.5 kHz, 2 kHz and 7.5 kHz. The bandwidth of a human cochlea is calculated from the BM velocity tuning curves and compared to bandwidth of gerbil ANF tuning curves. The result shows a comparative sensitivity for both the gerbil and human cochlea. A parametric study is conducted (Chapter 3) to determine the factors contributing to the sensitivity of a gerbil cochlea. With the previous passive, 3-dimensional, two-box cochlea model and experimental measurements of the gerbil cochlea, the theoretical wave number function is calculated. This theoretical wave number function is compared to the wave number function extracted from basilar membrane velocity measurements with the Shera et. al.'s wave number inversion formula. The results identify important factors which contribute to the differences between an arched membrane and the theoretical flat membrane model. With these findings, the present theoretical model of an arched membrane (Chapter 4) considers the contributing factors and formulates the stress-strain equation of the three components of the arched BM; (i) A soft cell plate with a flat side towards the scala vestibuli and an arched towards the scala tympani, (ii) a straight fiber in the flat side, and (iii) an arched fiber along the BM arch. The present model replaces the BM

force in the eikonal equation of the gerbil cochlear model and the coefficient of the wave number matches Yoon et al.'s results [39] simulated from equivalent BM parameters.

For integration of cochlea mechanics design into microfluidic applications, the second part of the present work develops a method of fabricating and bonding the thin, flexible, anisotropic, 3-dimensional basilar membrane onto a microfluidic chip, as well as an electromagnetic-acoustic transducer for a low impedance transfer of acoustic energy into the microfluidic channels. There are various available methods of fabricating the membrane including the use of 3D thermoplastic printer. However, the bonding of the basilar membrane to channels representing the scala vestibuli and scala tympani is limited as the bonding requirement is stringent due to the edge boundary conditions and properties of the membrane. The present methodology of ultrasonic bonding with a composite thin film of PMMA microspheres in PDMS (Chapter 5) as the fusion layer avoids trapped air and restricts the flow of the melted polymer during welding. The matrix material selection and distribution of PMMA microspheres are determined by the present analysis of the energy absorption and distribution. A welding strength experiment is conducted to determine effect of the concentration of PMMA microspheres on the welding strength and quality of the proposed methodology with the chosen composite. All the samples show no visible sign of trapped air. The present transducer (Chapter 6) uses a voice-coil attached to a thin membrane to convert electromagnetic energy to acoustic energy. When used as a microfluidic mixer, it has similar mixing efficiency compared to other similar mixers while requiring a lower voltage input.

This research will also enable further developments in the use of fluid as the conducting medium in many of the microfluidic applications. The acoustic pressure wave used in the microfluidics field varies from low frequencies in the range of mHz to very high frequencies in the range of MHz and GHz. However, the conducting medium of the acoustic wave in most microfluidic works is solid [40] which resonates in the range of MHz and GHz. The study and analysis of cochlear mechanism will provide further understanding and application of low to medium frequencies (mHz to kHz) in the field of microfluidics. It will also enable the use of frequency (physical location) mapping instead of relying on signal post processing [41, 42] for frequency information which requires computational and electrical power.

1.4 Organisation of Thesis

This thesis consists of two parts. The first part describes the process from comparing the arched and flat basilar membrane cochlea (Chapter 2), analyzing the difference in

Figure 1.6: Organisation of Thesis.

mechanism (Chapter 3) to the modeling the arched basilar membrane (Chapter 4). The second part bridges the technological gap in welding of thin, flexible, anisotropic, 3-dimensional basilar membrane (Chapter 5) and development of a low-voltage acoustic transducer capable of transferring acoustic energy into the fluid (Chapter 6). In each chapter, there is an introduction and detailed literature review specific to the chapter's focus before the description on the methodology, followed by the results and conclusion. Lastly, a conclusion on the research work and future work is given in Chapter 7.

Chapter 2

Frequency Tuning of Mammalian Cochlea

2.1 Introduction

The mammalian cochleas of different species vary significantly in size but most of them have comparative hearing range from approximately 100 Hz to 100k Hz (see Table 2.1). Among the more commonly researched species, the human cochlea (hearing range of 64 Hz to 23 kHz) spans about 35mm when uncoiled [43, 44] and the basilar membrane significantly varies in width (300% increase) and thickness (75% decrease) from its basal to apical end [7] (see Figure 2.1) while the gerbil cochlea (with arched BM and hearing range of 100 Hz to 60 kHz) spans only about 13mm when uncoiled [45] and the basilar membrane width and thickness are relatively constant (see Table 4.1). In order to identify the difference in performance between a flat and arched membrane cochlea, frequency tuning sharpness of the gerbil cochlea, which has relatively fixed width and thickness but is arched, is compared to that of the human cochlea. The sharpness of frequency tuning at each CF is quantified by the Q value where $Q = (CF)/(Bandwidth)$. The bandwidth is measure from the auditory nerve fiber (ANF) tuning curves (TCs) which is easily obtained for experimental animals [6, 5, 46, 4, 47] such as the gerbil, but is impossible to obtain directly in the case for human. Therefore, there are constant efforts to quantify human frequency tuning sharpness (FTS) relative to other mammals.

Species	Gerbil	Human	Cat	Rabbit	Rat	Mouse	Guinea Pig	Chinchilla	Bat
Approx. Min(Hz)	100	64	45	360	200	1000	54	90	2000
Approx. Max(kHz)	60	23	64	42	76	91	50	22.8	110

Table 2.1: Approximate hearing range of various mammals

Figure 2.1: Comparison of mammalian cochlear⁶ (a) basilar membrane width, (b) basilar membrane thickness and (c) scala vestibuli (SV) area with experimental data extracted from Naidu et al. [4], Dallos [5], Cabezudo [6], Wever [7], Kim et al. [8] and Thorne et al. [9]. Only the gerbil basilar membrane thickness is an estimate in Yoon et al.'s model [10].

Yoon et al. [10] presented a process of estimating human ANFTC with a validated three-dimensional model of the human cochlea from physiological measurements. The present chapter reviews this methodology together with the work of Ruggero et al. [11] on SMCAP and Shera et al. [48] with SFOAE, and uses the calculated human cochlear Q-values from iso-velocity tuning curves to compare with gerbil cochlear Q-values from experimental ANF frequency tuning curves. The results show comparative sensitivity of both human and gerbil cochlea which indicate similar function between an arched BM with radial tension and a flat BM which varies in thickness and width along the cochlea.

2.2 Literature Review

2.2.1 Frequency Tuning of Human Cochlea

The earliest research focused on the relationship between psychophysical-tuning measurements, which records human behavioral response to acoustic input, and the ANFTCs [49, 50, 22, 7]. There are many improvements in this methodology for increasing the accuracy of the result. The notch-noise (NN) mask technique minimizes off-frequency listening

⁶Reprinted from Biophysical Journal, 100 (1), Yoon Y.-J.; Steele C. R.; Puria S., Feed-forward and feed-backward amplification model from cochlear cytoarchitecture: an interspecies comparison, 110, Copyright 2011, with permission from Elsevier.

compared to the sinusoidal mask [51] and forward masking (FM) is developed to avoid masker-probe interaction in simultaneous masking (SM) results [52, 23, 53]. Eustaquio-Martin et al. and Lopez-Poveda et al. reported a sharper tuning for iso-response experimental procedures (similar to those used to obtain ANFTCs) compared to isoinput procedures due to the compressive response characteristics of the cochlea [54, 55]. Despite these advances, the results from this methodology are still inconclusive on the subject of human cochlea FTS compared to other mammalian cochleas. The two main subjects of controversy are on the relation between psychophysical-tuning and physiological-tuning measurements as well as that of non-masking (ANFTCs) and masking procedures (FM and SM).

Figure 2.2: Overview of psychophysical auditory response system and tuning curves data extraction.

The wide range of procedures and techniques available in psychophysical-tuning measurements is based on the assumption that the psychophysical auditory frequency selectivity is similar to ANF frequency selectivity [56]. A psychological transformation function is involved during signal transformation from acoustic to psychophysical measurement (Figure 2.2). This transformation function is commonly assumed to be independent of the acoustic input stimulation frequency [48]. In mammals such as guinea pigs, comparisons of psychophysical and physiological measurements show little difference [56]. However, there is no supporting evidence to such assumption in the case of human [48] due to the difficulty in obtaining physiological measurements.

The ANF response is an invasive method which focuses on measuring a single nerve fiber and therefore, there is no need to introduce masking unlike psychophysical-tuning measurements which are affected by off-frequency listening. Ruggero et al. reported a sharper tuning with FM psychophysical procedures compared to SM psychophysical procedures [11] in humans and thus, opens the question on which of the masking procedures is more suitable in estimating the ANFTCs in humans.

Methods of estimating ANFTCs from non-invasive physiological measurements are developed to avoid assuming the relationship between physiological and psychophysical measurements. The first methodology is the measurement of compound action potential (CAP) with masking procedures [57]. The relation between Q values of ANFTCs and CAPTCs is obtained by comparing several species whose both ANFTCs and CAPTCs can be measured. Verschooten et al. used a FMCAP procedure while Ruggero et al. used a SMCAP procedure. Although Verschooten et al.'s work did not provide any species invariant variable or function [58], Ruggero et al. estimated the range of human ANF tuning with three closely varying first-order SMCAP-to-ANF Q-value transformation functions derived from ANF and SMCAP measurements of gerbil, rat, mouse, guinea pig, chinchilla and showed that the human cochlear FTS is similar to these mammals as well as squirrel monkey and cat [11].

Shera et al. developed another method of estimating ANF FTS by using the delay of stimulus-frequency otoacoustic emissions (SFOAEs) [48]. Closely varying SFOAE-delay-to-ANF-Q-value transformation functions are obtained from ANF and SFOAEs measurements of cat, guinea pig and chinchilla [12]. The results from this methodology showed a sharper cochlear frequency tuning in humans [48, 12] and macaque monkeys [59] compared to cat, guinea pig and chinchilla which contradicts Ruggero et al.'s results [11]. In both methodologies, the attempts at defining a species invariant or closely varying CAP/SFOAE-ANF-Q-value transformation function(s) require an extensive number of species for a conclusive evidence of such relationships.

2.2.2 Accuracy of ANFTCs estimation from CAP and SFOAE measurements

There are two masking procedures in estimating the ANFTCs from CAPTCs: SM [11] and FM [58]. As it is an empirical formulation, the masking procedures does not have significant impact on the accuracy other than the relevance of each of the process in estimating the Q-value of ANFTCs. Ruggero et al.'s work [11] which estimates the human ANF FTS from SMCAP measurements reported on the exaggeration of human FM psychophysical tuning curves' sharpness comparing to ANFTCs' and concluded that human cochlea is no sharper in frequency tuning than other mammals. However, the SM CAPTC is sufficient to estimate only lower frequencies where the SM CAPTC Q_{10} is low. The estimates quickly diverge as shown in Figure 2.3(a) when SM CAPTC Q_{10} value increases. Furthermore, the transformation function used is frequency invariant. These limit both

Figure 2.3: Maximum difference of species invariant variable, (a) estimated human ANFTC Q_{10} from human SMCAPTC Q_{10} with Eq. 3,4 and 5 in [11] and (b) Tuning ratio of cat and guinea pig varying with normalized characteristic frequency [12]

conclusions from CAP methodology to lower frequencies where the SMCAPTCs Q_{10} value is < 3.5 .

Shera et al.'s work which estimates the human ANF FTS from SFOAEs delays reported on the underestimation of human psychophysical tuning curves' sharpness comparing to ANFTCs [48] and also concluded that human cochlea is sharper in frequency tuning than other mammals [48, 12]. From Figure 2.3(b), we can see that estimates from tuning ratio (Shera et al.'s species invariant variable) has a high error margin below transition CF for different species (transition CF for human is 1kHz).

Both Ruggero et al.'s and Shera et al.'s works are part of the research in using empirical transformation function to estimate Q-value of human cochlear ANFTCs with physiological measurements [11, 48, 12, 58, 59]. These works use population trends of different species for developing the empirical formulation. However, we have to acknowledge the vast range of different cochleas and their tuning sharpness in a single species population.

⁷Journal of the Association for Research in Otolaryngology, Otoacoustic Estimation of Cochlear Tuning Validation in the Chinchilla, 11, 2010, 343365, C. A. Shera, C. C. Guinan Jr, and A. J. Oxenham. With permission of Springer.

Figure 2.4: ⁷Empirical covariation of cochlear tuning and otoacoustic delay in three species. The three columns of the top row show values of Q_{ERB} computed from auditory-nerve fibers in cat, guinea pig, and chinchilla, respectively (left to right). The bottom row shows corresponding values of N_{SFOAE} , the SFOAE phase-gradient delay in stimulus periods. Loess trend lines (Cleveland 1993) are shown to guide the eye. The auditory-nerve data in cat come from studies by Delgutte and colleagues (e.g., Cedolin and Delgutte 2005), the data in guinea pig from Tsuji and Liberman (1997), and the data in chinchilla from Recio-Spinoso et al. (2005). The otoacoustic data in cat and guinea pig come from Shera and Guinan (2003) and in chinchilla from Siegel et al. (2005).

In Shera et al.’s work (See Figure 2.4) [12] and Ruggero et al.’s work [11], the wide range of Q-values, SFOAE delay and SMCAP in cats, guinea pigs and chinchillas at each CF is shown. For example, the Q_{ERB} value of cats calculated from ANFTCs at 1 kHz can range from 1-9. With such a wide range of values for individual cochleas of the same species, the regression between population trend of two variables becomes less reliable. Works employing empirical transformation function of Q-values should consider the transformation function of individual cochleas of the same species to establish any evidence of a relationship between proposed physiological-tuning measurements and ANFTC Q-value.

2.2.3 Three-dimensional, two-box cochlear model

The three dimensional, two-box cochlear model (Figure 2.5) analyzed by the WKB-numeric method is developed by Steele et al. [27]. For the present derivation of the cochlear model, the origin of the model is taken to be on the x-z plane of symmetry. Although the resultant equations would be the same as Steele et. al.’s cochlear model which defines the origin at the corner as denoted in Figure 2.5, the shift of the origin along y axis would provide easier implementation of the arched BM model in Chapter 4. In this model, we assume the properties of the cochlea duct and basilar membrane to be varying slowly.

Figure 2.5: Schematic drawing of the cochlear box model. The cartesian coordinates x , y , z represent the distance from the stapes, the distance across the scala width, and the height above the partition, respectively. (a) Overview, (b) front, (c) top, and (d) side views of cochlear model. The box is filled with viscous fluid, with properties near those of water. The partition has an elastic portion, the basilar membrane. The input sound is through the piston at the end, the stapes, and the round window connected to the lower fluid region consists of a thin membrane giving essentially a zero pressure condition.

The fluid flow in the model follows the linear Navier-Stokes equation,

$$0 = \ddot{\mathbf{u}} + \frac{1}{\rho_f} \nabla P - \nu \nabla^2 \dot{\mathbf{u}} \quad (2.1)$$

$$\mathbf{u} = \begin{Bmatrix} u \\ v \\ w \end{Bmatrix} \quad (2.2)$$

$$= \nabla \varphi + \nabla \times \boldsymbol{\psi} \quad (2.3)$$

$$\boldsymbol{\psi} = \begin{Bmatrix} \psi_1 \\ \psi_2 \\ \psi_3 \end{Bmatrix} \quad (2.4)$$

where $\mathbf{u}$ is the fluid displacement field, φ is the scalar potential, $\boldsymbol{\psi}$ is the vector potential, ρ_f is the fluid density, P is the fluid pressure and ν is the fluid kinematic viscosity. The boundary conditions are,

1. $v = 0$, at $y = -L_2/2, L_2/2$.
2. $w = 0$, at $z = L_3$.
3. $w = w_p(x, y, t)$, at $z = 0$.

$$4. \quad u = 0 \text{ at } z = 0$$

$$5. \quad v = 0 \text{ at } z = 0$$

Conditions (i), (ii) and (iii) are the wall boundary conditions at the rigid cochlear duct walls, shelves and the basilar membrane, and conditions (iv) and (v) are the non-slip boundary conditions at the shelves and basilar membrane.

Taking consideration of the first mode of vibration, the displacement at the basilar membrane is,

$$w_p(x, y, t) = W\phi(y)e^{i\omega t - i \int n dx} \quad (2.5)$$

$$\phi = \begin{cases} \cos(\frac{\pi y}{b}), & -b/2 < y < b/2 \\ 0, & \text{otherwise} \end{cases} \quad (2.6)$$

where W is a function of x .

Assuming the viscous layer is insignificant at the rigid walls ($y = -L_2/2, y = L_2/2, z = L_3$) and the shelves ($z = 0$ and $|y| > b/2$), the fluid velocity contributed by the vector potential is taken to be 0 at these boundaries. The solution for the scalar potential, with consideration of the symmetrical x - z plane, is in the form,

$$\varphi = W \sum_j \Phi_\varphi(y, z, j) e^{i\omega, t} \quad (2.7)$$

$$\Phi_\varphi(y, z, j) = C_{y(j)} \cos(k_{y(j)} y) \{ C_{z1(j)} \cosh[k_{z(j)}(z - L_3)] \} \quad (2.8)$$

$$= C_{z2(j)} \sinh[k_{z(j)}(z - L_3)] \} \quad (2.9)$$

The coefficient $k_{y(j)}$ can be found by applying the first boundary condition $v = 0$ at $y = -L_2/2, L_2/2$,

$$\frac{\delta \Phi_\varphi}{\delta y} \Big|_{y=-L_2/2} = \frac{\delta \Phi_\varphi}{\delta y} \Big|_{y=L_2/2} = 0 \quad (2.10)$$

$$C_{y(j)} k_{y(j)} \sin(k_{y(j)} \frac{L_2}{2}) = 0 \quad (2.11)$$

$$k_{y(j)} = \frac{2j\pi}{L_2} \quad (2.12)$$

The second boundary condition indicates that,

$$C_{y(j)} \cos(k_{y(j)} y) C_{z2(j)} k_{z(j)} = 0 \quad (2.13)$$

$$C_{z2(j)} = 0 \quad (2.14)$$

Equation 2.7 becomes,

$$\varphi = W e^{i\omega t - i \int n dx} \sum_j C_{y(j)} \cos(\frac{2j\pi y}{L_2}) C_{z1(j)} \cosh[k_{z(j)}(z - L_3)] \quad (2.15)$$

With incompressible fluid in the cochlear duct, the continuity equation is,

$$\nabla \cdot \dot{\mathbf{u}} = 0 \quad (2.16)$$

$$\nabla^2 \dot{\varphi} = 0 \quad (2.17)$$

Substituting Equation 2.15 into Equation 2.17,

$$0 = \frac{\delta^2 \phi}{\delta x^2} + \frac{\delta^2 \phi}{\delta y^2} + \frac{\delta^2 \phi}{\delta z^2} \quad (2.18)$$

$$0 = -n^2 - \left(\frac{2j\pi}{L_2}\right)^2 + k_{z(j)} \quad (2.19)$$

$$k_{z(j)} = \pm \sqrt{n^2 + \left(\frac{2j\pi}{L_2}\right)^2} \quad (2.20)$$

where n is the wave number,

$$n^2 = -\frac{\varphi_{,xx}}{\varphi} \quad (2.21)$$

As $k_{z(j)}$ only appears in even functions, both positive and negative $k_{z(j)}$ can be used. Equation 2.15 becomes,

$$\varphi = W e^{i\omega t - i \int n dx} \sum_j B_{y(j)} \cos\left(\frac{2j\pi y}{L_2}\right) \cosh[k_{z(j)}(z - L_3)] \quad (2.22)$$

We can group variables which are function of x ,

$$\varphi = e^{i\omega t - i \int n dx} \sum_j R_j \cos\left(\frac{2j\pi y}{L_2}\right) \quad (2.23)$$

$$R_j = W B_{y(j)} \cosh[k_{z(j)}(z - L_3)] \quad (2.24)$$

The scalar potential is defined such that the pressure function in the cochlea ducts is,

$$\ddot{\varphi} + \frac{1}{\rho_f} P = 0 \quad (2.25)$$

Substituting the continuity equation (Equation 2.17) and Equation 2.25, the incompressible linear Navier-Stokes equation (from Equation 2.1) becomes,

$$\ddot{\psi} - \nu \nabla^2 \dot{\psi} = 0 \quad (2.26)$$

We consider the symmetrical x - z plane, the viscosity effect to be local (when $z \rightarrow \infty$, $\psi \rightarrow 0$) and use the constraints,

$$\psi_3 = 0 \quad (2.27)$$

The vector potential is in the form,

$$\boldsymbol{\psi} = e^{i\omega t} \sum_j e^{-\gamma_j z} \begin{Bmatrix} \Psi_1(j) \sin(\frac{2j\pi y}{L_2}) \\ \Psi_1(j) \\ 0 \end{Bmatrix} \quad (2.28)$$

Substituting Equation 2.28 into Equation 2.26,

$$\ddot{\boldsymbol{\psi}} = \nu \nabla^2 \dot{\boldsymbol{\psi}} \quad (2.29)$$

$$-\omega^2 \boldsymbol{\psi} = i\omega \nu (-n^2 \boldsymbol{\psi} + \frac{\delta^2 \boldsymbol{\psi}}{\delta y^2} + \frac{\delta^2 \boldsymbol{\psi}}{\delta z^2}) \quad (2.30)$$

$$n^2 + \frac{i\omega}{\nu} = (\frac{2j\pi}{L_2})^2 + \gamma_j^2 \quad (2.31)$$

$$\gamma_j = \pm \sqrt{(\frac{2j\pi}{L_2})^2 + n^2 + \frac{i\omega}{\nu}} \quad (2.32)$$

As the vector potential reduces to 0 as z increases, $k_{z(j)}$ is chosen such that $Re[k_{z(j)}] > 0$.

Next we consider the non-slip boundary condition, $u = 0$ at $z = 0$,

$$u = \frac{\delta \varphi}{\delta x} + \frac{\delta \psi_2}{\delta z} \quad (2.33)$$

$$= 0 \quad (2.34)$$

$$0 = R'_j|_{z=0} \cos(\frac{2j\pi y}{L_2}) + \Psi_2 \gamma_j \cos(\frac{2j\pi y}{L_2}) \quad (2.35)$$

$$\Psi_2 = -\frac{R'_j|_{z=0}}{\gamma_j} \quad (2.36)$$

When we consider the non-slip boundary condition, $v = 0$ at $z = 0$,

$$v = \frac{\delta \varphi}{\delta y} + \frac{\delta \psi_1}{\delta z} \quad (2.37)$$

$$= 0 \quad (2.38)$$

$$0 = -\frac{2j\pi y}{L_2} R_j|_{z=0} \sin(\frac{2j\pi y}{L_2}) - \Psi_1 \gamma_j \sin(\frac{2j\pi y}{L_2}) \quad (2.39)$$

$$\Psi_1 = -\frac{2j\pi}{\gamma_j L_2} R_j|_{z=0} \quad (2.40)$$

Lastly, we expand ϕ using Fourier series of even function,

$$\phi = a_0 + \sum_{j=1}^{\infty} a_j \cos(\frac{2j\pi y}{L_2}) \quad (2.41)$$

$$a_0 = \frac{2}{L_2} \int_0^{b/2} \cos(\frac{\pi y}{b}) \quad (2.42)$$

$$= \frac{b}{L_2} \quad (2.43)$$

$$a_j = \frac{4}{L_2} \int_0^{b/2} \phi \cos(\frac{\pi y}{b}) \quad (2.44)$$

$$= \frac{2b}{2j\pi b + \pi L_2} \sin(\frac{2j\pi b + \pi L_2}{2L_2}) + \frac{2b}{2j\pi b - \pi L_2} \sin(\frac{2j\pi b - \pi L_2}{2L_2}) \quad (2.45)$$

Assuming γ_j is slowly varying and use the boundary equation where the displacement of the fluid equates to the displacement of the membrane at $z = 0$,

$$w = \frac{\delta\varphi}{\delta z} + \frac{\delta\psi_2}{\delta x} - \frac{\delta\psi_1}{\delta y} \quad (2.46)$$

$$= 0 \quad (2.47)$$

$$a_j = -B_j k_{z(j)} \sinh(k_{z(j)} L_3) - \frac{R_j''|_{z=0}}{\gamma_j} \quad (2.48)$$

$$+ \left(\frac{2j\pi}{\gamma_j L_2}\right)^2 \frac{R_j|_{z=0}}{\gamma_j} \quad (2.49)$$

$$B_j = \frac{a_j}{-k_{z(j)} \sinh(k_{z(j)} L_3) + \frac{k_{z(j)}^2}{\gamma_j} \cosh(k_{z(j)} L_3)} \quad (2.50)$$

We approximate the first mode of fluid pressure,

$$P|_{z=0} = e^{i\omega t - i \int n dx} P_1 \cos\left(\frac{\pi y}{b}\right) \quad (2.51)$$

where P_1 is the magnitude of the first mode.

$$P_1 = \frac{\int_{-b/2}^{b/2} \frac{-\rho_f \ddot{\varphi}}{e^{i\omega t - i \int n dx}} dy}{\int_{-b/2}^{b/2} \cos\left(\frac{\pi y}{b}\right) dy} \quad (2.52)$$

$$= -\rho_f \omega^2 W h_f \quad (2.53)$$

$$h_f = \frac{\pi}{4} \sum_j B_j \frac{L_2}{j\pi} \cosh(k_{z(j)} L_3) \sin\left(\frac{j\pi b}{L_2}\right) + B_0 \cosh(k_{z(j)} L_3) b \quad (2.54)$$

where h_f is the effective fluid thickness over the basilar membrane.

For the basilar membrane, the plate equation is used,

$$P = \rho_p h_p \ddot{w}_p + D_{xx} \frac{\delta^4 w_p}{\delta x^4} + 2D_{xy} \frac{\delta^4 w_p}{\delta x^2 \delta y^2} + D_{yy} \frac{\delta^4 w_p}{\delta y^4} \quad (2.55)$$

where P is the pressure on the plate, ρ_p is the plate density, h_p is the plate thickness and D_{ij} is the plate bending stiffness,

$$D_{ij} = \frac{E_{ij}}{1 - \nu_p^2} I \quad (2.56)$$

where E_{ij} is the Young's modulus, ν_p is the Poisson's ratio and I is the moment of inertia ($I = h_p^3/12$ for flat plate).

The time-averaged Lagrangian density of the system is,

$$\mathcal{L} = T_f + T_p - V \quad (2.57)$$

$$T_p = \int_{-b/2}^{b/2} \frac{\omega}{2\pi} \int_0^{2\pi/\omega} \frac{1}{2} \rho_p h_p \dot{w}_p^2 dt dy \quad (2.58)$$

$$T_f = 2 \int_{-b/2}^{b/2} \frac{\omega}{2\pi} \int_0^{2\pi/\omega} \frac{1}{2} \rho_p h_f \dot{w}_p^2 dt dy \quad (2.59)$$

$$V = \int_{-b/2}^{b/2} \frac{\omega}{2\pi} \int_0^{2\pi/\omega} [D_{xx}(\frac{\delta^2 w_p}{\delta x^2})^2 + 2D_{xy}(\frac{\delta^2 w_p}{\delta x \delta y})^2 + D_{yy}(\frac{\delta^2 w_p}{\delta y^2})^2] dt dy \quad (2.60)$$

$$(2.61)$$

where T_f and T_p are the time-averaged fluid and plate kinetic energy density, and V is the plate potential energy.

Evaluating Equation 2.57,

$$\mathcal{L} = fW^2 \quad (2.62)$$

$$f = 2F_f - F_{BM} \quad (2.63)$$

where F_{BM} is the effective pressure on the BM, F_f is the effective viscous fluid pressure and the coefficient of 2 with F_f is for the top and bottom of the BM,

$$F_f = \frac{1}{2} \omega^2 \rho_f h_f \quad (2.64)$$

$$F_{BM} = \frac{b}{4} [-\rho_p h_p \omega^2 + D_{xx} n^4 + 2D_{xy} n^2 (\frac{\pi}{b})^2 + D_{yy} (\frac{\pi}{b})^4] \quad (2.65)$$

$$= -\omega^2 M(x) + K(n, x, \omega) \quad (2.66)$$

Equating the time-averaged Lagrangian density to 0 yields eikonal equation,

$$F_{BM} = 2F_f \quad (2.67)$$

The independent variation of n of the time-averaged Lagrangian density yields the transport equation,

$$\frac{d}{dx} \frac{\delta \mathcal{L}}{\delta n} = 0 \quad (2.68)$$

$$\frac{d}{dx} [\frac{\delta f}{\delta n} W^2] = 0 \quad (2.69)$$

$$W(x) = C(\frac{\delta f}{\delta n})^{-1/2} \quad (2.70)$$

For the amplitude coefficient C , we match the displacement of the fluid and stapes at $x=0$,

$$\int u_{st} dA_{st} = \int u|_{x=0} dA \quad (2.71)$$

$$= e^{i\omega t} \left[W \frac{ib}{-n + \frac{n^2}{\gamma \tanh(nL_3)}} \right]_{x=0} \quad (2.72)$$

where A_{st} is the stapes foot-plate area and u_{st} is the stapes displacement.

Let ζ_{st} be the average displacement of the stapes,

$$C = \zeta_{ave,st} A_{st} \left[i \left(\frac{\delta f}{\delta n} \right)^{1/2} \frac{n - \frac{n^2}{\gamma \tanh(nL_3)}}{b} \right]_{x=0} \quad (2.73)$$

For any given harmonic frequency (ω), the eikonal equation is solved to give the wave number (n) by using Newton-Raphson iterative scheme for each cross-section along the cochlear duct. Once n is determined, the amplitude coefficient, W (Equation 2.70), and BM velocity, $\dot{w}_p$ can be determined.

2.2.4 Push-pull mechanism in cochlear model

The general, three dimensional, push-pull mechanism (Figure 1.2), two-box cochlear model (Figure 2.5) developed by Yoon et al. [38] is based on various works from Geisler et al. (1-D model) [60] and Steele et al. (3-D model) [27]. It has shown good agreement with various animal experimental data including gerbil, chinchilla and cat [10, 38]. The formulation includes adding OHC gain to the eikonal equation. The eikonal equation (Equation 2.67) becomes,

$$F_{BM} - 2F_f - F_{BM}^C = 0 \quad (2.74)$$

The equivalent pressure on the BM, F_{BM} , including the dynamic fictitious force, equals to the summation of the viscous fluid pressure on top and below the BM (Figure 2.5), F_f , and the OHC equivalent pressure acting through the Deiters rod, F_{BM}^C (Figure 1.2).

The cell force acting on the BM at point indicated in Figure 1.2(b) is proportionate to the OHC push and the phalangeal process pull, α_1 and α_2 respectively,

$$F_{BM}^C(x + \Delta x_1, t) = \alpha_1 F_{BM}(x, t) - \alpha_2 F_{BM}(x + \Delta x_2, t) \quad (2.75)$$

This is due to linear OHC motility and transduction with small amplitude assumption [61]. The net push and pull are equal due to the small vertical force resistance of the reticular lamina and tectorial membrane and $\alpha_1 = \alpha_2 = \alpha$ is assumed. F_{BM} can then be reduced to,

$$F_{BM} = \frac{2F_f}{1 - \alpha e^{-in\Delta x_1} + \alpha e^{in(\Delta x_2 - \Delta x_1)}} \quad (2.76)$$

2.3 Method

2.3.1 Active human cochlear model

Region	Symbol	Description	Value
Basilar Membrane	ρ_p	Density of the BM plate	$1.0 \times 10^3 \text{ kg/m}^3$
	E_{xx}	Longitudinal Young's Modulus	$1.0 \times 10^{-4} \text{ GPa}$
	E_{yy}	Radial Young's Modulus	1.0 GPa
	E_{xy}	Shear Modulus	0.0 GPa
	ν_p	Poisson's ratio	0.5
Scalar fluid	ρ_f	Density of fluid	$1.0 \times 10^3 \text{ kg/m}^3$
	μ	Fluid dynamic viscosity	$0.7 \times 10^{-3} \text{ Pa s}$

Table 2.2: Material properties for the cochlear model

	Human
Length of cochlea (mm)	35
Stapes footplate area (mm ²)	3.21
Length of outer hair cell (μm)	25-65(from stapes to apex)
Fiber volume fraction (%)	3-0.6(from stapes to apex)

Table 2.3: Dimensions of the human cochlear model Input sound pressure at ear canal (dB SPL)

Input sound pressure (dB SPL)	Human
70-90	-
60-70	0.07
50-60	0.09
0-50	0.11

Table 2.4: Push-Pull Gains (α) vs input SPL at Ear Canal [10]

The human model is established in Yoon et al.'s work [10] and has known properties of the basilar membrane and scalar fluid in Table 2.2 as well as the dimensions of human cochlea in Table 2.3 taken from Bekesy's anatomical measurements [43] and observation of the anatomical data in Voldrich's work [44]. The values from Table 2.4 is a estimated from chinchilla and cat whose model results show the best match with human experimental results [10]. The variation of the BM width and thickness along the length of the cochlea is extracted from Wever's measurement [7] (see Figure 2.1) The scalar vestibuli and scalar tympani cross-sectional area along the length of the cochlea is taken from Thorne et al.'s measurement via magnetic resonance images [9].

The BM velocity thresholds which are impossible to measure experimentally can then be calculated from FM psychophysical-tuning threshold input with the 3-dimensional, push-pull mechanism, two-box cochlear model. The input sound pressure level (SPL) and parameters for a given model were then repeatedly computed for the other frequencies such that these BM velocity threshold values were conserved at the CF. The resulting ensembles of input SPLs are then reported as the BM ‘isovelocity’ tuning curves (iso-response) for the respective CF.

2.4 Results and Discussion

2.4.1 Accuracy of ANFTCs estimation from mechanical model

Figure 2.6: ⁸Left, Actual tuning characteristics of the BM model. **A** Isolevel curves for pure tones from 10 to 90 dB SPL in 10-dB steps. **C** Isoresponse (tuning) curves for different BM velocity criteria from 25 to 1,600 m/s (see legend). Right, Tuning characteristics of the chinchilla BM (case L113 in Ruggero et al. 1997). Note that these data were used to produce the BM model shown on the left panels (Meddis et al. 2001). Matching colors are used to illustrate corresponding model (left panels) and experimental responses (right panels). The tuning curves illustrated by dashed lines in **C** do not have corresponding experimental curves in **D**.

⁸Journal of the Association for Research in Otolaryngology, On the Controversy About the Sharpness of Human Cochlear Tuning, 14, 2013, 673686, E. Lopez-Poveda and A. Eustaquio-Martin. With permission of Springer.

Center Frequency	Model	Moore et. al.	Oxenham et. al.	Other mammals
0.5kHz	2.8	6	-	1.5-2.3
2kHz	5	12	7	2.6-3.8
8kHz	20	11	11	4.2-6

Table 2.5: Rough estimate of Bandwidth Q_{10} values of Human cochlear model and forwardmasking psychophysical tuning curve of Moore et al. [22] and Oxenham et al. [23] as well as the range bandwidth Q_{10} values for other mammals (exact values is found in Fig. 6 in [11])

The human model is formulated from physical governing equations of fluid flow and plate deformation as well as anatomical measurements of the human cochlea with the exception of OHC gain. The OHC gain, contradictory to compressive characteristic of a mammalian cochlea, is assumed linear and the resultant iso-velocity tuning curve can only be considered accurate when the input SPL is small enough for the cochlear response to remain in the linear region. Therefore, one has to exercise care when retrieving Q -value from the tuning curve especially for Q_{ERB} where the whole frequency spectrum has to be integrated. The pure tone iso-level tuning curve in Lopez-Poveda et al.'s work [55] (see Figure 2.6A and B) shows different input SPL in chinchilla cochlea exhibits compressive characteristics at SPL of 50 dB and above. Therefore, the Q_{10} value extracted from the iso-velocity tuning curve in Figure 2.7 can be considered a good estimation of the human cochlear FTS.

In determining the BM velocity threshold, the psychophysical threshold is used. Although there is no assumption of the relation between physiological and psychophysical tuning curves, it is assumed that there is no psychological process involved in the tuning-threshold measurements. This assumption is logical due to the single frequency, low acoustic-input and simple nerve fibre response in these measurements. Moreover, the process of determining such threshold is less complicated than measuring the tuning curve.

2.4.2 Sharpness of human cochlear model frequency tuning

The theoretically estimated ANFTCs at 25.4 mm, 18.2 mm and 9 mm from Yoon et al.'s human cochlear model is compared with Moore's iso-response forwardmasking psychophysical-tuning measurement [22] as shown in Figure 2.7. By matching the threshold SPL level from psychophysical experiments to the model, the BM velocity thresholds for 0.5 kHz, 2 kHz and 7.5 kHz are found to be 100 $\mu\text{m/s}$, 160 $\mu\text{m/s}$ and 330 $\mu\text{m/s}$ respectively (shown in Figure 2.7's legend). The bandwidth, Q_{10} , of the tuning curve in

Figure 2.7: Basilar membrane frequency tuning curve at 0.5 kHz, 2 kHz and 7.5 kHz of the human cochlea from 3-D cochlear model and forward-masking psychophysical-tuning curves of Moore et al. for probes at 10 dB SPL.

Table 2.5 is calculated by,

$$Q_{10} = \frac{\text{center frequency}}{\text{10-dB bandwidth}} \quad (2.77)$$

The human psychophysical frequency tuning curve's sharpness (Figure 2.7) is only slightly exaggerated (40% increased) from the ANFTC (Oxenham et al.'s value) at around middle frequency of 2 kHz (BM vel. 160 $\mu\text{m/s}$) but it is greatly exaggerated at low frequency of 0.5 kHz at twice the value, (BM vel. 100 $\mu\text{m/s}$). However, psychophysical measurements becomes slightly understated at high frequency of 8 kHz (Figure 2.7, BM vel. 330 $\mu\text{m/s}$) at half the value (Table 2.5). When compared to other mammals, the FTS in human cochlea is comparable to other mammals at 0.5 kHz (low frequency) but becomes 3 to 4 times sharper at 8 kHz (high frequency).

2.4.3 Comparing frequency tuning of arched and flat basilar membrane

Figure 2.8 shows the comparability of gerbil arched BM cochlea with other mammals. For the entire spectrum of hearing, the gerbil cochlea has frequency tuning sharpness

Figure 2.8: Q_{10} value comparison between gerbil arched BM cochlea and other mammals with flat BM.

similar to most other mammals of similar cochlea size. Although the human cochlea has exceptionally sharp tuning (Table 2.5) at higher frequencies, the frequency tuning sharpness gerbil cochlea is still quite comparable at below 2kHz considering the difference in the length of the two cochleas.

2.5 Conclusion

In this chapter, we have established the comparative ANF frequency tuning sharpness of gerbil arched BM with various flat BM mammalian cochleas including human cochlea which has one of the most percentage variation in BM width and thickness. In the next chapter, the effect of various parameters and properties of the basilar membrane is analysed to identify significant differences between a flat BM gerbil cochlea model and the measure travelling wave in a real gerbil cochlea.

Chapter 3

Parametric Studies on Gerbil Cochlea

3.1 Introduction

Figure 3.1: (a) Photo of a gerbil cochlea⁹ [13] and (b) simplified basilar membrane in two channel box model [14]. The Cartesian coordinates x, y, z represents the distance from stapes, the distance across the scalar width and the height above the basilar membrane.

In the previous chapter, the relatively similarities in frequency tuning sharpness between cochleas with arched and flat membrane is shown. The theoretical cochlea developed by Yoon et al. [38] shown in Chapter 2 with parameters of flat basilar membrane (chinchilla and cat) matches their respective experimental measurements [10, 38]. However, Yoon et al.'s simulation results showed discrepancies between the push-pull theoretical cochlear model and the experimental measurements of gerbil arched BM cochlea [10]. This deviation is notably apparent in the phase measurements at the entire frequency band and slightly in the basilar membrane velocity ratio, $|V_{bm}/V_{st}|$, at frequencies near the characteristic frequency (CF).

The theoretical cochlear model did not accommodate the uncommon basilar membrane structure of gerbil and is hypothesized to be the cause of the deviation. Figure 3.1(a)

⁹Reprinted from Biophysical Journal, 100(4) , N. Eze and E.S. Olson, Basilar Membrane Velocity in a Cochlea with a Modified Organ of Corti, pages 858-867, 2011, with permission from Elsevier.

and (b) show the photo of the organ of Corti in a gerbil cochlea [13] and the two-channel box model equivalent of the basilar membrane. The basilar membrane of the gerbil is flat on top with an arch towards the scala tympani (Figure 3.1(a)) while the two-channel box model assumes a flat basilar membrane (Figure 3.1(b)). The basilar membrane also varies its radial tension along the cochlea [4]. The organ of Corti is able to support a high resting tension in the basilar membrane without collapsing [62, 63] and Naidu et al. found a significant difference in resting tension of basilar membrane [4] along the gerbil cochlea from its basal to apical end. We hypothesize four differences which significantly affect the basilar membrane interaction with the traveling wave. The arch towards the scalar tympani in the basilar membrane (i) changes the bending stiffness both radial and along the length of the cochlea, (ii) reduces the effective fluid force on the membrane and (iii) increases the basilar membrane inertial. In addition, we also hypothesize that the radial tension in the basilar membrane increases the radial bending stiffness of the BM.

In this chapter, the effects of the hypothesized differences on the acoustic traveling wave are investigated and the contribution of these effects on the discrepancy between previous theoretical model and experimental measurements in gerbil cochlea is examined. We adopted the passive linear cochlea model for comparison with the experimental measurements (90 dB input SPL) of gerbil cochlea to isolate the differences in the basilar membrane and eliminate errors which originate from the dissimilarity between the active model and gerbil outer hair cell motility. The complex wave number function determines the properties of the acoustic traveling wave and its significance is highlighted during the description of the two-channel box, push-pull, theoretical cochlear model [38]. We obtained the experimental, complex wave number function of gerbil cochlea from measurements of gerbil basilar membrane velocity magnitude and phase [15]. The comparison between the theoretical cochlear model and the experimental wave number function of the gerbil cochlea shows the significance of several factors which are neglected by the current two-channel box model [10](Figure 3.5(a) and (b)). Our findings provided insights to the mechanism of frequency selectivity in the gerbil cochlea despite the lack of significant variation in thickness and width of its basilar membrane along the cochlea [64, 39].

Figure 3.2: Illustration of (a) actual basilar membrane modeled as (b) flat plate with uniform fiber density and (c) flat sandwich structure [14].

Figure 3.3: (a) Fiber densities (f_u and f_s) of basilar membrane along cochlea for uniform density and sandwich structure BM model respectively and (b) after normalizing with Young's modulus of fiber, $E_f = 4.0 \times 10^7$ and 5.29×10^6 for uniform density and sandwich structure BM models respectively [14].

3.2 Literature Review

3.2.1 The Two Models of Gerbil Basilar Membrane

Yoon et al. modeled the BM as a flat plate with uniform fiber density through its thickness (in the z-direction) (Figure 3.2(b)). The BM has the bending stiffness,

$$D_{xx} = \frac{E_g h_p^3(x)}{12(1 - \nu_p^2)} \quad (3.1)$$

$$D_{yy} = \frac{f_u(x) E_f h_p^3(x)}{12(1 - \nu_p^2)} \quad (3.2)$$

where E_f and f_u are the Young's modulus of the fiber and the fiber density respectively. The fiber density, assuming uniform through its thickness, is,

$$f_u(x) = \frac{2d_{width}t}{d_{spacing}h_p} \quad (3.3)$$

where d_{width} is the width of the fiber (x-axis), $d_{spacing}$ is the spacing between the fibers and t is the thickness of the fiber measured in Naidu et al.'s work[4] (see Table 4.1 and Figure 4.1).

Naidu et al. [4] proposed a flat sandwich-structure BM (Figure 3.2(c)) to replace the BM of gerbil cochlea. The bending stiffness from Naidu et al.'s model is,

$$D_{xx} = \frac{E_g h_p^3(x)}{12(1 - \nu_p^2)} \quad (3.4)$$

$$D_{yy} = \frac{E_g h_p^3(x)}{12(1 - \nu_p^2)} + \frac{E_f I_f(x)}{d_{spacing}(x)} \quad (3.5)$$

$$I_f \approx \frac{1}{2}t(x)h_p^2(x)d_{width}(x) \quad (3.6)$$

where $I_f(x)$ is the moment of inertia of each set of fiber bundles with respect to the middle axis of the cross section of the plate. We consolidated the fiber variables into a single parameter, $f_s(x)$ (Figure 3.3(a)), similar to the uniform fiber density model,

$$D_{yy} = \frac{f_s(x) E_f h_p^3(x)}{12(1 - \nu_p^2)} \quad (3.7)$$

$$f_s(x) = \frac{E_g}{E_f} + \frac{6t(x)d_{width}(x)(1 - \nu_p^2)}{d_{spacing}(x)h_p(x)} \quad (3.8)$$

As there is limited data on the Young's modulus of the fiber, E_f , we estimated its value by assuming a weakly anisotropic BM at the apex of the gerbil cochlea (13 mm from basal) [65] with a bending stiffness ratio, $D_{yy}/D_{xx} = 1.5$. The Young's modulus of the fiber, E_f , for uniform fiber density through thickness model and sandwich structure model are 4.0×10^7 and 5.29×10^6 respectively and these values, multiplied by their respective fiber densities, are shown in Figure 3.3(b).

3.2.2 Extracting Wave Number Function from Experimental Measurements

Figure 3.4: Real and imaginary parts of velocity of basilar membrane plotted along gerbil cochlea with experimental measurements from Ren [15] (90 dB input SPL) [14].

Shera [66] developed a wave number inversion formula for extracting the complex wave number function from experimental measurements of basilar membrane velocity magnitude and phase. Ren's [15] measurements of basilar membrane velocity from gerbil cochlea at the section far from the apex (2.2 mm to 3 mm from base) with 16 kHz input frequency are used to extract the complex wave number function (Figure 3.4). In sections near the apex, radial bending stiffness between the two-channel models has no significant difference (Figure 3.3(a)) due to the assumption of the Young's modulus of the fiber. However, Ren's measurements are limited up to 3 mm from base. For this reason, Shera's wave number inversion formula [66] is modified as,

$$n^2(x, \omega) = \frac{-V_{BM}(x, \omega)}{\int_x^{3mm} \int_x^{3mm} V_{BM}(x'', \omega) dx'' dx' + C} \quad (3.9)$$

$$C \approx \int_{3mm}^L \int_{3mm}^L V_{BM}(x'', \omega) dx'' dx' \quad (3.10)$$

where L is the length of the gerbil cochlea and the constant, C , is an approximated value of the double integral of V_{BM} beyond 3 mm from base.

3.3 Method

3.3.1 Passive Gerbil Cochlear Model

We used the following parameters for gerbil cochlea with the 3-dimensional, two box model (flat BM) shown in Chapter 2.2.3,

Region	Description	Value
Basilar Membrane	Density of the BM plate, ρ_p	$1.0 \times 10^3 \text{ kg/m}^3$
	Young's Modulus, E_g	$1.0 \times 10^5 \text{ Pa}$
	Poisson's ratio, ν_p	0.4
Scalar fluid	Density of fluid, ρ_f	$1.0 \times 10^3 \text{ kg/m}^3$
	Fluid dynamic viscosity, μ	$0.7 \times 10^{-3} \text{ Pa s}$

Table 3.1: Known material properties for gerbil cochlea [24, 25, 26, 27]

Figure 3.5: Comparison of (a) real ($\text{Re}[n]$) and (b) imaginary ($\text{Im}[n]$) parts of wave number function along gerbil cochlea [14] between experimental measurements [15] and theoretical models. We estimate the value of V_{BM} beyond 3 mm via a second order polynomial regression and obtain $\int \int V_{BM} = 4 \times 10^{-13}$. We also adjusted the value of C in Equation 3.9 to obtain the values of the double integral of V_{BM} at $x = 2.9$ mm to be 5×10^{-13} and 3×10^{-13} . The value 5×10^{-13} is the upper limit before $\text{Im}[n]$ becomes positive (negative damping) which is physically impossible. The value 3×10^{-13} is the lower limit where $C = 0$.

Yoon et al. [39, 10] estimated the effective BM thickness and fiber density with anatomical measurements [16] and their results were in good agreement with Greenwood's frequency-to-place mapping [67, 39, 10]. However, Yoon et al. [39, 10] approximate the effective BM thickness which decreases close to an order from base to apex while measurements from Edge et al.'s work revealed slight increment in gerbil BM thickness along the cochlea. For a more experimentally supported model, we used the equivalent BM thickness,

$$h_p = A_{BM}/b \quad (3.11)$$

where A_{BM} is the BM area. We used a quadratic regression (instead of a linear regression which Naidu et al. used [4]) for a more accurate estimation of the variation of BM width (b) [28, 4] and area (A_{BM}) [16] as well as mean fiber bundle width along x-axis (d_{width}) [28], thickness along z-axis (t) [28], and spacing between fiber bundles ($d_{spacing}$) [4] along the gerbil cochlea.

3.4 Results and Discussions

The wave number (n) calculated from the Ren's experimental measurements with the modified inversion formula in Equation 3.9 is accurate from 2.2 mm to 2.7 mm and started to diverge according to the different approximated C value in Equation 3.10. Although the C value is approximated, the real and imaginary values of wave number will not exceed the plot shown in Figure 3.5. This is due to upper limit on the constant C such that the $\text{Im}[n]$ can never be positive in a passive cochlea.

The real part of the wave number ($\text{Re}[n]$) of the sandwich structure BM model is within experimental error of cochleas of different gerbils while the $\text{Re}[n]$ of the uniform fiber density BM model is slightly lower due to the higher estimated Young's modulus of the fiber. However, the variation of the $\text{Re}[n]$ within this section of the cochlea is much higher in the experiment than both the models. The imaginary part of the wave number ($\text{Im}[n]$) represents the damping of the traveling wave and both models show lower damping of 1–2 orders compared to that of the experimental results. We also investigated various shape functions of the membrane arch including circular (Kapur et al.'s estimation [64]), sine and sine square represented respectively (Figure 3.9(a)) as,

$$\left(\frac{b^2}{8h} + \frac{h}{2}\right)^2 = \left(y - \frac{b}{2}\right)^2 + \left(z - \frac{b^2}{8h} + \frac{h}{2}\right)^2 \quad (3.12)$$

$$z = -h \sin\left(\frac{\pi y}{b}\right) \quad (3.13)$$

$$z = -h \sin^2\left(\frac{\pi y}{b}\right) \quad (3.14)$$

Figure 3.6: Cross-sectional area of the BM with width [4] and peak height [16] matching experimental measurements [14]. The description of the circular, sinusoidal and sine square BM arch shapes are shown in Equation 3.12, 3.13 and 3.14 respectively.

where h is the peak of the arched membrane height measured in Edge et al's work [16].

The experimentally measured cross-sectional area of the gerbil BM [16] indicates that the shape of the arch varies significantly from base (closer to a circular arch) to apex (closer to a sin square arch) (Figure 3.6). Due to limited physiological data on the thickness variation along the width (y -direction) of the BM, the shape of the BM arch can only be estimated to simple shape functions. The actual classification of the BM arch shape in gerbil cochlea requires additional experimental measurements.

3.4.1 Effect of Bending Stiffness and Radial Tension on Wave Number

The change in the bending stiffness along cochlea, D_{xx} , has negligible effect on wave number (Figure 3.7) as long as it is more than one order smaller than the radial bending stiffness, D_{yy} (Figure 3.8). When the bending stiffness D_{xx} or D_{yy} is higher, the basilar membrane is expected to greatly reduce in vibration amplitude which reduces the damping on the traveling wave significantly. This can be seen from the drop in $\text{Im}[n]$ in Figure 3.7(b) and Figure 3.8(b).

The variation of $\text{Re}[n]$ with different radial stiffness is similar to that of a Bode's phase plot (Figure 3.8(a)) where the changes in wave length is most prominent when it resonates with the input frequency, 16 kHz. Although the change in radial bending stiffness is able to affect the $\text{Re}[n]$ significantly, it requires variation of at least one order within the short section of the cochlea (from 2.2 mm to 3 mm) in order to match that of the experimental

Figure 3.7: Variation of (a) real ($\text{Re}[n]$) and (b) imaginary ($\text{Im}[n]$) parts of wave number for different bending stiffness values along cochlea, D_{xx} [14]. We referenced other parameters at 2.6 mm from basal end of gerbil cochlea and obtained the maximum and minimum wave number from experimental measurements [15] between 2.2 mm to 3 mm from the basal end of gerbil cochlea (Figure 3.5). The original value of D_{xx} in the models is $1.2 \times 10^{-11} \text{ Nm}^2$. We explore D_{xx} values of one order higher and lower as the change in D_{xx} along the entire length of the gerbil cochlea is less than one order.

(a)

(b)

Figure 3.8: Variation of (a) real ($\text{Re}[n]$) and (b) imaginary ($\text{Im}[n]$) parts of wave number for different bending stiffness values in the radial direction, D_{yy} [14]. We referenced other parameters at 2.6 mm from basal end of gerbil cochlea and obtained the maximum and minimum wave number from experimental measurements [15] between 2.2 mm to 3 mm from the basal end of gerbil cochlea (Figure 3.5). The original values of D_{yy} in the sandwich BM and uniform fiber models are $2.9 \times 10^{-10} \text{ Nm}^2$ and $8.4 \times 10^{-10} \text{ Nm}^2$. We explore the range of D_{yy} where the wave number varies between the maximum and minimum experimental value.

$\text{Re}[n]$. However, the variation of D_{yy} in the models is approximately 2.5% within the section 2.2 mm to 3 mm from base which is insufficient to cause such a large change in $\text{Re}[n]$. The value of $2.9 \times 10^{-10} \text{ Nm}^2$ and $8.4 \times 10^{-10} \text{ Nm}^2$ for the D_{yy} of sandwich BM and uniform fiber models are also much higher than resonating bending stiffness value of 16 kHz.

Although the changes in radial bending stiffness (y-axis) varies the $|\text{Im}[n]|$ significantly, the maximum experimental $|\text{Im}[n]|$ is 1–2 orders higher than the $|\text{Im}[n]|$ of the BM models (Figure 3.8). The $|\text{Im}[n]|$ also indicates that the calculated D_{yy} of both models are significantly higher than the experimental measurements.

Naidu et al. measured and modeled the tension in gerbil cochlea [4]. They estimated the radial tension, $N_r(x)$, along the length of the gerbil cochlea to be,

$$N_r(x) = 1.51e^{-580x} \quad (3.15)$$

where N_r and x are in SI units. The basilar membrane stiffness (Equation 2.66) becomes [4],

$$K(n, x, \omega) = \frac{b}{4} [D_{xx}n^4 + 2D_{xy}n^2(\frac{\pi}{b})^2 + D_{yy}(\frac{\pi}{b})^4 + N_r(\frac{\pi}{b})^2] \quad (3.16)$$

With the effect of the radial tension, the new radial bending stiffness, $D_{yy,eff}$, is,

$$D_{yy,eff} = D_{yy} + N_r(\frac{b}{\pi})^2 \quad (3.17)$$

The radial tension increases the change in radial bending stiffness along x-axis. Although the additional change in radial bending stiffness of the BM is much higher (about 45% decrease from 2.2 mm and 3 mm from base), it is still insufficient compared to the two orders of radial bending stiffness variation required.

3.4.2 Effect of Effective Fluid Force on Wave Number

In the two-channel box model, the calculation of effective fluid mass (or fluid force) (Equation 2.66) is based on the assumption that the basilar membrane is flat. When the basilar membrane has an arch towards the scalar tympani, the effective fluid force (in the z-direction) on the basilar membrane is reduced. We calculated the effective fluid force by integrating pressure across the arched membrane (estimated with Bernoulli theorem of incompressible fluid). With the consideration that the scala vestibuli side of the basilar

Figure 3.9: (a) Variation of fluid force constant, β along the gerbil cochlea with different shaped of the arch membrane assumed [14]. (b) Variation of imaginary parts of wave number with fluid force constant, β [14]. We showed the $\text{Im}[n]$ variation referenced to the original $\text{Im}[n]$ value at 2.6 mm from basal end of gerbil cochlea.

membrane is flat, Equation 2.67 becomes,

$$F_{BM} = (1 + \beta)F_f \quad (3.18)$$

$$\beta = \frac{0.5\rho_f \int V_z^2 \cos^2(\theta) dy}{0.5\rho_f \int V_z^2 dy} \quad (3.19)$$

$$= \frac{4}{b} \int_0^{b/2} \sin^2\left(\frac{\pi y}{b}\right) \left[1 + \left(\frac{\delta z}{\delta y}\right)^2\right]^{-3/2} dy \quad (3.20)$$

$$V_z = V_0 \sin\left(\frac{\pi y}{b}\right) \quad (3.21)$$

where β is the ratio of effective fluid force on an arched membrane over a flat membrane, θ is the angle between the membrane surface tangent and the y-axis, V_z is the velocity of the membrane at y approximated in the cochlea model [68, 69] and V_0 is the peak velocity of the membrane (at $y = 0$). The effective fluid force on an arched membrane (numerator in Equation 3.19) is calculated by integrating the z-axis component of the dynamic pressure across the BM. The $\text{Im}[n]$ of the gerbil arched membrane is reduced up to 70% of the original value (Figure 3.9(a) and (b)) throughout the entire length of the gerbil cochlea. Although this contribution to the $\text{Im}[n]$ is negligible as the value of $\text{Im}[n]$ varies up to an order within a short section near the place of input frequency resonance (Figure 3.5(b)), it is still significant when modeling the entire length of the gerbil cochlea.

3.4.3 Effect of Basilar Membrane Mass Distribution on Wave Number

We also considered the change in inertial force of the basilar membrane due to its non-constant thickness in the y-direction (Figure 3.1(a)). Assuming the velocity (V_z) of the

Figure 3.10: (a) Variation of mass constant, γ along the gerbil cochlea with different shaped of the arch membrane assumed [14]. (b) Variation of imaginary parts of wave number for different mass constant, γ [14]. We showed the $\text{Im}[n]$ variation referenced to the original $\text{Im}[n]$ value at 2.6 mm from basal end of gerbil cochlea.

membrane at y is as approximated in the cochlea model [69, 68] (Equation 3.21), the ratio of inertial force of an arched membrane over a flat membrane is,

$$\gamma = \frac{\int_{-b/2}^{b/2} |z| w_p(y) dy}{|z_{ave}| \int_{-b/2}^{b/2} w_p(y) dy} \quad (3.22)$$

where $w_p(y)$ is the BM displacement profile across y -axis, z of a circular, sinusoidal and sine square arch membranes are defined in Equation 3.12, 3.13 and 3.14, and z_{ave} is the average height of the arched membrane. The inertial force of an arched membrane (numerator in Equation 3.22) is calculated by integrating the the product of the shape function, $z(y)$, and BM displacement across the BM (y -axis) [68]. The BM displacement profile, $w_p(y)$, for simply support at both ends of the y -axis [68, 38, 10] is,

$$w_p(y) = W_p \cos\left(\frac{\pi y}{b}\right) \quad (3.23)$$

where W_p is the maximum displacement and Equation 3.22 becomes,

$$\gamma = \frac{\int_{-b/2}^{b/2} |z| \cos\left(\frac{\pi y}{b}\right) dy}{\frac{1}{b} \int_{-b/2}^{b/2} |z| dy \int_{-b/2}^{b/2} \cos\left(\frac{\pi y}{b}\right) dy} \quad (3.24)$$

The $\text{Im}[n]$ of the arched gerbil BM inertial force varies only less than 6% of the original $\text{Im}[n]$ value calculated from a flat membrane along the gerbil cochlea (Figure 3.10(a) and (b)). Therefore, change in inertial force of the basilar membrane has negligible effect on the accuracy of current two-channel box, theoretical cochlear model.

3.5 Conclusion

In this chapter, it is found that the discrepancies of the BM velocity plot along gerbil cochlea between the two-channel box cochlear model and the experimental measurements

are partly due to significant changes in radial bending stiffness (y-axis) along the length of the gerbil cochlea (x-direction) which is not present in the model [10]. In both uniform fiber density and sandwich structure models, the BM thickness (h_p) which affects the radial bending stiffness in the third order (Equation 3.2 and Equation 3.7), is relatively constant in the section of the cochlea. Although the fiber bundle thickness varies significantly (almost 15%), it affects the radial bending stiffness of both uniform fiber density and sandwich structure model only in the first order (Equation 3.2 and Equation 3.7). This indicates that the fiber bundle thickness contributes to the radial bending stiffness more than the estimates of both models.

Other than the BM bending stiffness, the increase in radial stiffness of the BM and damping of the traveling wave in gerbil cochlea ($\text{Im}[n]$) is also a combination of effects from the radial tension (in the y-axis) and lower fluid force on the BM. The lower fluid force is due to the arch in the BM which pushed the fluid at an angle in the y direction (referenced to z-axis) when the BM vibrates. Contrary to the cochlear mechanics in most mammals which achieves sharp frequency tuning with significant changes in basilar membrane width and thickness along the length of their cochlea, the gerbil cochlea employs a combination of an arched basilar membrane as well as its varying radial tension and fiber bundle thickness along its cochlea. In order to elucidate gerbil cochlea frequency selectivity mechanism, effects of the basilar membrane arch and radial tension in gerbil cochlea need to be included in the current theoretical gerbil cochlear model [10].

In the next chapter, we present an arched BM model which includes significant factors (mainly the arched BM fiber and the radial tension) revealed in this parametric study.

Chapter 4

Arched Membrane Model

4.1 Introduction

In the previous chapter, the radial bending stiffness, D_{yy} , is identified as one of the important factor affecting the traveling wave in the gerbil cochlea. In this chapter, an arched basilar membrane model is formulated, verified for accuracy against the radial bending stiffness variation of Yoon et. al.'s gerbil cochlear model [70, 39] and compared to Naidu et. al.'s sandwich BM model [4]. With the verified model, the stiffness variation with arch shape and fiber bundle thickness is analyzed. The results shows that the arch BM of enable significant variation of the radial bending stiffness with minimal fiber bundle thickness changes.

4.2 Model of an Arched Basilar Membrane

Figure 4.1: (a) Illustration of the present arched basilar membrane model with denotation on parameters of the basilar membrane and (b) a section of the arched plate showing forces on the section.

The present arched BM is modeled (Figure 4.1(a)) with parameters extracted from measurements of gerbil's BM in Edge's work [16] as well as the fiber bundle geometries from Naidu et al.'s measurements[4] and shown in Table 4.1.

Distance from base(mm)	x	3.61	6.86	11.24
BM Area(μm^2)	A	3998	7005	8225
BM height(μm)	h	35	49.7	55.3
BM Width(μm)	b	168	192	207.3
Fiber thickness(μm)	t	1.02	0.57	0.28
Fiber width(μm)	d_{width}	1.13	0.8	0.35
Fiber spacing(μm)	$d_{spacing}$	1.6	1.62	1.67

Table 4.1: Properties of Gerbil arched basilar membrane [4, 16, 28].

4.2.1 Basilar membrane fiber bundle

The arched fiber bundle is approximated to a rectangular cross-sectional beam of thickness t , width d_{width} , with an arch profile w_0 (see Figure 4.1) and a radius of curvature, R . With the following assumptions;

1. The cross-sectional plane cut through the center of curvature and remains in a plane after bending.
2. The radius of curvature is much larger than the thickness, $R \gg t$.
3. The deflection of the arched fiber, w_p , is small.

The strain is,

$$\epsilon_{yy} = \frac{\delta\alpha}{\delta\theta} \left(\frac{R_n}{r} - 1 \right) \quad (4.1)$$

$$\frac{\delta\alpha}{\delta\theta} = - \frac{\delta^2 w_a}{\delta y^2} \frac{R}{\gamma} \quad (4.2)$$

$$R = \frac{\gamma^{3/2}}{\frac{\delta^2 w_0}{\delta y^2}} \quad (4.3)$$

$$\gamma = \sqrt{1 + \left(\frac{\delta w_0}{\delta y} \right)^2} \quad (4.4)$$

where R_n is the radius of the neutral axis, R is the radius of curvature at the centroid axis, w_a is the deflection of the arched fiber due to bending and $\delta\alpha/\delta\theta$ is the change in angle of the cross-sectional plane along a small angular section $\Delta\theta$ of the arch.

The moment and tension per length (x-axis) in the arched fiber are,

$$M_a = -E \frac{\gamma \eta}{R} (\epsilon_a + \frac{\delta \alpha}{\delta \theta}) \quad (4.5)$$

$$N_a = E [R A_m \epsilon_a + \frac{\delta \alpha}{\delta \theta} (R A_m - t)] \quad (4.6)$$

$$E = E_f \frac{d_{width}}{d_{spacing}} \quad (4.7)$$

$$A_m = \int_t^1 \frac{1}{r} dz \quad (4.8)$$

$$= \ln \left(\frac{R + t/2}{R - t/2} \right) \quad (4.9)$$

$$\eta = \frac{R^2}{\gamma} (R_m A_m - t) \quad (4.10)$$

where ϵ_a is the strain at the centroid axis ($r = R$) and η is the equivalent moment of inertia per length of the arched fiber.

The total deflection of the arch fiber can be expressed as,

$$\frac{\delta w_p}{\delta y} = \frac{\delta w_a}{\delta y} + \epsilon_a \frac{\delta w_0}{\delta y} \quad (4.11)$$

For the straight fiber,

$$M_s = EI \frac{\delta^4 w_p}{\delta y^4} \quad (4.12)$$

$$N_s = Et \epsilon_s \quad (4.13)$$

where I is the moment of inertia per length and ϵ_s is the strain of the straight fiber at the centroid.

4.2.2 soft-cell of basilar membrane

In order to formulate the bending equation for the soft-cell in the BM, we simplify the problem with the following assumption,

1. The cross-sectional plane (x-z-axis plane) remains in a plane after bending.
2. The Young's modulus of the soft-cell is much smaller than that of the fiber bundle,
 $E_f \gg E_g$.
3. The deflection of the soft-cell, w_p , is small.

The deflection of the soft-cell can be separate into bending and shearing deflection,

$$w_p(y) = w_b(y) + w_s(y) \quad (4.14)$$

where w_s and w_b are the deflection of the soft-cell due to shearing and bending respectively.

We equate the strain of the soft-cell and the fiber bundle,

$$\epsilon_s - \epsilon_a = -|w_0| \frac{\delta^2 w_b}{\delta y^2} \quad (4.15)$$

$$\frac{N_s}{Et} - \epsilon_a = w_0 \frac{\delta^2 w_b}{\delta y^2} \quad (4.16)$$

Using the continuity of traction at the straight fiber,

$$G(\epsilon_{yz}) = \frac{\delta N_s}{\delta y} \quad (4.17)$$

$$G\left(\frac{\delta w_s}{\delta y}\right) = \frac{\delta N_s}{\delta y} \quad (4.18)$$

$$Gw_s = N_s + C_s \quad (4.19)$$

where ϵ_{yz} is the engineering shear strain, G is the shear modulus and the constant, C_s , can be found by applying the boundary condition.

As the Young's modulus of the soft-cell is much smaller than that of the fiber bundles, the change in total tension can be expressed as,

$$\frac{\delta}{\delta y}[N_s + N_{ay}] = p_y \quad (4.20)$$

where N_{ay} is the y-axis component of the arched fiber tension and p_y is the y-axis component of the external pressure.

The pressure on the BM (see Figure 4.1) can be expressed as coefficients of the wave number n . Equating the integral of pressure along the y-axis of the BM would yield the same eikonal equation (formulation in [68]),

$$q = P_{xx}n^4 + P_{xy}n^2 + P_{yy} \quad (4.21)$$

$$P_{yy} = \frac{\delta^2 M_{yy}}{\delta y^2} \quad (4.22)$$

$$M_{yy} = \frac{\delta M_a}{\delta y} + \frac{\delta M_s}{\delta y} - |w_0|N_s \quad (4.23)$$

The pressure causing radial bending, P_{yy} is a function of the shearing and bending deflection. The ratio of shearing and bending deflection can be found with Equations 4.19, 4.6, 4.20, 4.11 and the boundary conditions of the arched BM.

4.2.3 Arched basilar membrane model

In the present model for arched BM, the boundary conditions at the shelves are taken to be hinged and the total deflection of only the first harmonics is considered,

$$w_p(y) = W_p \cos\left(\frac{\pi y}{b}\right) \quad (4.24)$$

The profile of the arch is approximated to be,

$$w_0(y) = c_0 + c_2 y_2 + c_4 y_4 \quad (4.25)$$

$$w_0|_{y=b/2} = h/10 \quad (4.26)$$

where the coefficients c_0 , c_2 and c_4 are found by substituting the BM area, A , width, b , and height, h .

We also approximated that there is no y -direction motion of the BM and therefore, $p_y = 0$ and Equation 4.20 becomes,

$$N_s + N_{ay} = N_e \quad (4.27)$$

where N_e is the external tension in the BM. However, as the profile shape, w_0 and the deflection w_p is reference to the rest state of the BM with the presence of the radial tension, N_e does not include the radial tension and is approximate to be 0.

From Equation 4.19, using the hinged boundary condition where $M_s = 0$ and $w_s = 0$ at the shelves,

$$C_s = 0 \quad (4.28)$$

The solution to w_s is found by discretizing the y -axis (500 elements) and solving the simultaneous equation matrix. The matrix is filled via automatic differentiation, thus, there is no need to evaluate the coefficients of different orders of differentiation of w_s manually [?].

4.3 Results and Discussion

As there are limited measurement data on the Young's modulus of the fiber bundle and the estimations of different model differs significantly. Yoon et. al. estimates the Young's modulus of the fiber bundle to be in the order of 1 GPa [39] while Naidu et al. estimate it to be around 14 MPa [4]. In order to remove the error in such estimation, we compare

Figure 4.2: Plot of scaled force per unit length for comparison of Yoon et al.'s, Naidu et al.'s and present model. The scaling factors are $\lambda = 1, 0.03, 47$ and 52 respectively.

the BM integral of pressure, $\int P_{yy}$, variation along the gerbil cochlea after scaling to Yoon et al.'s model simulation for comparison in Figure 4.2. The present arched BM model matches Yoon et al.'s gerbil cochlear model in term of variation along the cochlear length while Naidu et al.'s sandwich BM model does not vary significantly. This result is in agreement with the parametric study in the previous model (see Chapter 3) where the insufficient variation of, D_{yy} is shown. In Figure 4.2, the present model excluding the radial tension is also shown and the data is much closer to that of Yoon et al.'s simulation. Naidu et al. [4] measured the point-load deflection of the gerbil BM. The radial tension is calculated with the deflection measurement and a sandwich model without shearing. Therefore, the results might deviate from the actual radial tension values due to the presence of shearing in the soft-cell.

One of the significant assumption is that only the first harmonics is considered for the deflection. In reality, the deflection is,

$$w_p(y) = \sum_n W_n \cos\left(\frac{n\pi y}{b}\right) \quad (4.29)$$

where W_n is the amplitude associated with the mode, n . The pressure profile and the deflection must match as the deflection causes the fluid pressure (a first mode deflection profile does not necessary indicate a first mode pressure profile), therefore, the quadratic

Figure 4.3: Ratio of pressure integral to its quadratic mean

mean of the these different modes must be in the same order,

$$\text{quadratic mean} = \sqrt{\int P_{yy}^2 dy} \quad (4.30)$$

Figure 4.3 shows ratio of the integral of pressure in different mode. The ratio of the first mode is more than 2.5 times higher than that of other modes. This indicates that the first mode assumption has a good enough accuracy to estimate the pressure integral.

4.3.1 Simplification of model

In this subsection, we determine the possible simplification of current model. Figure 4.4(a) shows the radius of curvature across the BM at three different location. Although, we can approximate the radius (at $y = 0$) of the arched membrane as constant for regions near the apical end of the BM, the radius needs to be discretized and calculated at the basal end due to the large changes near the shelves. However, for the value of η the changes from the center to the shelves of the BM along the y-axis is around 50% (see Figure 4.4(b)) and too significant to make any constant approximation.

It is common in many works [39, 4] to estimate the BM as a flat, with,

$$\frac{\delta w_0}{\delta y} \ll 1 \quad (4.31)$$

Figure 4.4: Plot of (a) radius of curvature and (b) equivalent second moment of area per length of the arched membrane at $x= 3.61$ mm, 6.86 mm and 11.24 mm from the basal end of the BM.

Figure 4.5: (a) Arch profile w_0 and (b) changes in arch profile along the y-axis of the BM at $x= 3.61$ mm, 6.86 mm and 11.24 mm from the basal end of the BM.

 Figure 4.6: Plot of calculated pressure integral with different ratio of E/G

However, such assumption is invalid as the change in height of the BM is in the order of 1 near the shelves (see Figure 4.5(b)).

We also simulated and calculated the pressure integral (without radial tension) for a finite value to E/G (see Figure 4.6). The pressure integral variation along the BM approaches the simulation results of Yoon et. al.'s result as the ratio of G/E tends to 0. In the present model, the shear modulus, G , is approximate to 0. However, any approximation in the order of $G/E = 10^{-7}$ is quite accurate with less than an order difference.

4.3.2 Parametric study of the arched membrane

 Figure 4.7: Ratio of pressure integral of different height at the shelves to that of $w_0|_{y=b/2} = -h/10$ for (a) $G = 0$ and (b) $G/E = 10^{-7}$.

Figure 4.8: Variation of pressure integral with fiber bundle thickness for (a) $G = 0$ and (b) $G/E = 10^{-7}$. The dotted line estimates the pressure integral for a pure shearing, flat sandwich beam

In this subsection, we study the effect of different parameters of the arched BM model on the resultant pressure integral. We varied the height at $y = b/2$ in order to change the shape of the arch without changing the BM area, width and height. When G is close to 0, the change in arch shape has insignificant effect on the calculated pressure integral (the changes is $< 5\%$). When G increases, the region near the basal end remains insensitive to the change in arch shape. However, the pressure integral near the apical end decreases $> 50\%$ when the height at the shelves decreases and the arch becomes more curved. With this decrease the variation of pressure integral along the BM becomes more in agreement to Yoon et. al.'s model (see Figure 4.2).

Figure 4.8 shows the variation of pressure integral with fiber bundle thickness. The pressure on a pure shearing flat beam is,

$$P_{yy} = 2EI \frac{\delta^4 w_p}{\delta y^4} \quad (4.32)$$

$$I = \frac{t^3}{12} \quad (4.33)$$

For both cases ($G = 0$ and $E/G = 10^7$), the pressure integral is roughly a 2–3 order increase with fiber thickness which supports the conclusion in Chapter 3 where previous flat BM models are insufficient to model the gerbil cochlea.

4.4 Conclusion

In this chapter, an arched BM model is presented. The effect of the arch shape is studied as well as the relationship between the fiber bundle thickness and the radial bending

stiffness is established. The arch shape and the low Young's modulus of the soft cell enable the radial bending stiffness to vary with the fiber bundle thickness in higher order of dependency. This allows the gerbil cochlea to achieve comparative frequency tuning (Chapter 2) despite having a basilar membrane with relatively constant width and height (equivalent BM thickness). This concludes the first part of the research work on the mechanism of an arched basilar membrane in a mammalian cochlea. In the next part, we attempt to bridge some technological gaps for implementing cochlea mechanics into the field of microfluidic as well as contribute to the development of a passively-powered artificial cochlea.

Chapter 5

A thermoplastic-elastomeric composite ultrasonic welding methodology for cochlear biomimetic microfluidic mixer fabrication

5.1 Introduction

In the first part of this work, the cochlear mechanics of an arched BM is analyzed and the model for an arched BM cochlea is formulated. In this part of the present work, the mechanism of the arched BM, which enables a fully functional cochlea without requirements for a large varying membrane width and height, is applied to the field of microfluidics where there are strict limitations on the width of channels and properties of the membrane.

One of the critical factors found the gerbil cochlear mechanism is a membrane with very low Young's modulus. For the integration of cochlea mechanics design into microfluidic applications and the development of a passively-powered artificial cochlea, a method of fabricating and welding the thin, flexible (low Young's modulus), anisotropic, 3-dimensional basilar membrane is required as the boundary conditions of the basilar membrane is critical to the frequency tuning of the cochlea.

In this chapter, an ultrasonic welding method using a thin film of thermoplastic-elastomeric composite is presented. This present work explores the feasibility of using a composite film made of distributed thermoplastic particles in an elastomeric matrix to weld two layers of themoplastic together and acts as a clamp on the elastomeric layer. The elastomer also restricts the flow of melted energy directors and eliminate problems of trapped air, thus reducing its impact on the shapes of flow channels of microfluidic devices. The design process includes the matrix material selection, energy director distribution

and optimizing the ultrasonic welding process parameters. Experiments on the welding strength of PMMA samples welded using the present methodology are also conducted to determine the limitations of this methodology.

5.2 Literature Review

There has been tremendous interest in microfluidics for applications in biomedical due to their ability to provide fast response and low cost production with only trace amounts of chemical bioreceptors [71]. The microfluidic devices are usually fabricated using glass or silicon with chemical and plasma etching and photolithography. Other materials include polymethyl methacrylate (PMMA), polydimethylsiloxane (PDMS) and polycarbonate (PC) [72]. Industries commonly prefer PDMS and PMMA as cheaper alternatives to glass due to low cost and the ease to mass produce [73]. They have the potential to create complex, stable geometries using simple fabrication methods while being able to accommodate errors in terms of reversible change upon softening and melting [74].

In the production of microfluidic devices, the welding process plays a critical role in sealing open microstructures and allowing integration into a closed fluidic paths [74]. There are numerous welding techniques including capillary adhesive welding, chamber adhesive welding, adhesive film welding, laser welding, solvent diffusion welding, conventional thermal welding and plasma assisted (thermal) welding. There are also other techniques such as UV degradation supported thermal welding [75] which applies to a few types of polymers only. The main welding research on these techniques focused on finding the ideal process that has short process time and retains the channel geometrical structure while still produces high strength welds. The stability of design geometry and strength of welding are important criterion the manufacturing of microfluidic devices. Hence the welding has to be tight sealing at joint interface, void of contaminants and creates minimal deformation of the existing microstructures [76, 77]. However, the process of welding PDMS and PMMA are currently limited to adhesive [78], surface treatment [79] and clamping. The surface treatment process is complex (see Figure 5.1(a)) with several steps of surface modifications. While using adhesive (see Figure 5.1(b), process **a-c**) and clamping (applying pressure on the top and bottom of the chip) are simple processes, using adhesive adds to the membrane stiffness and/or modify the boundary conditions, and clamping is not permanent and requires additional clamping jig.

We combined ultrasonic welding (adhesive) and clamping methodology to develop an ultrasonic welding methodology for thermoplastic-elastomer-thermoplastic (PMMA-PDMS-PMMA) joining where the welded thermoplastic layers permanently clamps the elastomeric layer. Ultrasonic welding is a technique in which the specimens are connected by the melting of plastic with ultrasonic acoustic energy source. This method is highly efficient due to its stability and ability to produce strong joints [80] with automated compact equipment, thus, it enables rapid process time (in the order of seconds) and economical mass production [81]. The ultrasonic welding process utilizes an ultrasonic energy source (usually with a frequency of 20–50 kHz) and low amplitude (15–60 μm) mechanical vibration to induce localized frictional heating through viscoelastic dissipation by intermolecular friction [82, 83]. The generated heat melts contacting surfaces of the polymer and joins the two surfaces.

Figure 5.1: (a) The surface modification¹⁰ process for bonding PMMA and PDMS. (b) Fabrication process of the PMMA/adhesive/PDMS¹¹ part and the peristaltic micropump.

The present ultrasonic welding of composite film also reduce problems due to the flow of melted thermoplastic and air trapped in-between layers. Truckenmuller et al. fabricated the first ultrasonically welded microfluidic device by concentrating energy with convex energy directors [75]. The time for energy director to melt is in the order of seconds.

¹⁰BioChip Journal, The optimization of PDMS-PMMA bonding process using silane primer, 4 (2), 2010, 148-154, Kim K., Park S. W. and Yang S. S.. With permission of Springer.

¹¹Reprinted from Sensors and Actuators B: Chemical, 151 (1), Tan H. Y., Loke W. K. and Nguyen N.-T., A reliable method for bonding polydimethylsiloxane (PDMS) to polymethylmethacrylate (PMMA) and its application in micropumps, 133-139, Copyright 2010, with permission from Elsevier.

Therefore, it is difficult to precisely control the flow of the melted polymer. Although, overflow and underflow of molten polymer is acceptable for welding of most macro devices, it may clog (overflow) or leave small gaps (underflow) in the micro structures and cause a microfluidic device to be unusable [77]. Sun et al. used micro energy directors to allow more space for the flow of molten PMMA [84]. However, this does not eliminate problems of overflow and underflow entirely and it also causes air trapped when the energy directors are tightly arranged [84].

5.3 Method

The fusion layer of the ultrasonic process in this work is a thin composite film consisting of micro-energy directors and a elastomeric matrix. The elastomeric matrix would constrict the flow of PMMA energy directors when they are in viscous state as well as replace the air which might be otherwise trapped during the process. Figure 5.2 shows the ultrasonic welding using air (conventional) and elastomeric polymer as matrix. Other than the presence of trapped air and uncontrolled molten polymer flow, the two main notable differences of the welded products are the change in separation distance (shown in Figure 5.2) and welding strength. The change in separation distance of conventional ultrasonic welding is,

$$h_s = h_{ed}Vol_{ed}/A \quad (5.1)$$

where h_{ed} is the initial height of the energy directors, Vol_{ed} is the total volume of the energy directors and A is the area of material welding (excluding channels etc.). The change in separation distance using composite film is negligible due to incompressibility of the matrix material.

In this section, a methodology of composite material selection and design with a theoretical model for initial feasibility analysis followed by an experimental verification of such design is presented. The 1-dimensional theoretical model assesses the suitability of different matrix material as well as distribution of energy directors on the composite welding film. For this work, we chose PDMS as the matrix material based on studies from the theoretical model after comparing some common elastomeric materials. The composite is fabricated by spin coating PMMA-microspheresmixed-PDMS-base. Following so, the experiment presents the effect of different composite ratio on the welding strength of the proposed method. The experimental result also elucidates limitation of the fabrication method.

Figure 5.2: Illustration of polymer using (a) conventional and (b) composite film ultrasonic welding. In conventional ultrasonic welding, the energy directors melts and flow across the surface of the samples, resulting in a thinner fusion layer. In ultrasonic welding using composite film, the energy directors melts but maintains its height due flow restriction by the matrix material.

5.3.1 Ultrasonic Welding Model

Figure 5.3: (a) Maxwell Standard Linear Solid and (b) Wiechert Model[17]. The viscoelastic materials of the thermoplastic-elastomeric composite is modelled as a system of springs (with spring constant k) and Newton dashpots (with damping coefficient η). In a small range of temperature and vibration frequency, we approximate the Wiechert Model to the Maxwell Standard Linear Solid with equivalent spring and damping constants.

The mechanism of ultrasonic welding is categorized into mechanics and vibrations, viscoelastic heating of thermoplastic, heat transfer process, flow and wetting, and inter-molecular diffusion [80]. In this model, we consider the mechanics and viscoelastic heating of thermoplastic for the purpose of material choosing and energy director distribution. The Maxwell form of the standard linear solid (SLS) (Figure 5.3(a)) is adopted to estimate the strain response with material parameters.

Figure 5.4: Design of composite film to prevent overflow of PMMA during Ultrasonic Welding. (a) Cross-section of the composite model where h_b and h_{ta} are the effective height of the base PMMA and target PMMA respectively. h_{ed} and h_{mm} are the height of the PMMA energy directors and matrix material thickness respectively. (b) White light interferometry analysis of the PMMA-microspheres-mixed-PDMS composite on a PMMA substrate [18].

The strain response for input stress, $\sigma(t) = \sigma_0 \cos(\omega t) + \sigma_c$, in SLS is,

$$\epsilon(t) = \sigma_0 R \cos(\omega t \delta) + \frac{\sigma_c}{k_e + k_1} \quad (5.2)$$

$$R^2 = U^2 + V^2 \quad (5.3)$$

$$U = \frac{(k_e + k_1)(\tau\omega)^2 + k_e}{(k_e + k_1)[(\tau\omega)^2 + k_e^2]} \quad (5.4)$$

$$V^2 = \frac{k_1(\tau\omega)}{[(k_e + k_1)^2(\tau\omega)^2 + k_e^2]} \quad (5.5)$$

$$\delta = \tan^{-1}\left(\frac{V}{U}\right) \quad (5.6)$$

where $\tau_i = \eta_i/k_i$.

Due to the time scale difference in the ultrasonic frequency and change in temperature, a constant temperature is assumed within one cycle of oscillation and the averaged net power dissipation per volume (PDPV) is,

$$P = \frac{\sigma_0^2 \omega}{2} V \quad (5.7)$$

During ultrasonic welding, the total change in length of the sample at areas with the energy directors and with the matrix material are,

$$L_{ed}(t) = \epsilon_b(t)h_b + \epsilon_{ta}(t)h_{ta} + \epsilon_{ed}(t)h_{ed} - (h_{mm} - h_{ed}) \quad (5.8)$$

$$L_{mm}(t) = \epsilon_b(t)h_b + \epsilon_{ta}(t)h_{ta} + \epsilon_{mm}(t)h_{mm} \quad (5.9)$$

where Figure 5.4(a) shows the denotations of the equation, and h_b and h_{ta} are the effective length due to the non-uniformity and coarse distribution of the energy directors where

beyond the effective length, there is an uniform distribution of stress. The effective length also depends on the stiffness of the PMMA and matrix material.

Considering the balance of force and comparing the time varying component of $L_{mm}(t) = L_{ed}(t)$, the ratio of PDPV (from Equation 5.7), α , is,

$$\begin{aligned} & \frac{P_{PMMAed}}{P_{PMMAmm}} \\ &= \frac{(h_{efr})^2 + R_m^2 \left(\frac{h_{mm}}{h_{ed}}\right)^2 + 2R_m R_d \cos(\delta_M) h_{efr} \frac{h_{mm}}{h_{ed}}}{\frac{\sin(\delta_{PMMAmm})}{\sin(\delta_{PMMAed})} R_d [h_{efr}^2 + 1 + 2h_{efr}]} \end{aligned} \quad (5.10)$$

where $h_{efr} = (h_b + h_{ta})/h_{ed}$, $R_d = R_{PMMAmm}/R_{PMMAed}$, $R_m = R_{MM}/R_{PMMAmm}$ and $\delta_M = \delta_{PMMAmm} - \delta_{MM}$. The subscripts MM' and PMMA' denotes the matrix and PMMA materials respectively, and the subscripts mm' and ed' denotes the area with PMMA–matrix material and PMMA–PMMA contact respectively.

The power dissipation in the energy director as a ratio of the total power dissipation is,

$$\begin{aligned} & \left(\frac{Power_{PMMAed}}{Power_{Total}} \right) \\ &= \frac{\alpha A}{\alpha A_r (1 + h_{efr}) + h_{efr} + \frac{V_{MM}}{V_{PMMAmm}} \left(\frac{h_{mm}}{h_{ed}}\right)} \end{aligned} \quad (5.11)$$

where $A_r = A_{ed}/A_{mm}$.

5.3.1.1 Effect of Matrix Material Properties and Distribution of Energy Directors

The terms in the numerator and denominator in Equation 5.10 are compared to determine the significant factors affecting the PDPV ratio. The ratio of sine terms and the term R_d in the denominator represent the contribution of the change of PMMA viscoelastic damping and dynamic modulus with temperature on the PDPV ratio.

Considering the small differences in other factors, $R_m(h_{mm}/h_{ed})$ is the determining factor on the feasibility of using any material as the matrix. Matrix material selection has to satisfy the condition, $\min(R_{MM})h_{mm} > R_{PMMA}h_{ed}$ to ensure that the PDPV is always greater at the energy directors contact area regardless of the temperature of the matrix material. The effective length ratio in the first term reduces the effect of R_m in the PDPV ratio.

The third term indicates that the ratio of PDPV at energy director contact area reduces with the increased difference between the viscoelasticity of the materials ($\delta_{PMMA} -$

δ_{MM}) and the extent of the effect is determined by the effective length ratio, $(h_b + h_{ta})/h_{ed}$. This particular conclusion corroborates with the experiment in Sun et al.'s work [84] where widening the dimension of the energy directors (making the distribution coarser) reduces the percentage area of fusion.

5.3.1.2 Composite Material Selection and Design

Figure 5.5: (a) Inverse Dynamic Modulus, $|R|$, and (b) damping, $\tan(\delta) = V/U$ of PMMA and LDPE using broadband viscoelasticity spectrometer [19, 20] as well as 3–4 mm PDMS and 0.13 mm SU8 [21] using a commercial BOSE Electroforce 3200 machine. Measurements at temperature, $T = 23^{\circ}\text{C}$. The temperature-time shift is approximated with Arrhenius form.

In actual materials, the Wiechert Model (Figure 5.3(b)) represents the dynamics of the viscoelasticity more closely than the SLS model (Figure 5.3(a)). We can reduce the Wiechert model to the SLS model with equivalent k_e and k_1 where the viscoelastic effect is the greatest ($\delta V / \delta(\tau\omega) = 0$) by grouping together relaxation time, τ_i , of the same order.

Capodagli et al.'s work concluded that Arrhenius form is more accurate in predicting the temperature shift in PMMA[85],

$$\frac{\tau(T)}{\tau(T_{ref})} = e^{\frac{\Delta H}{R}(\frac{1}{T} - \frac{1}{T_{ref}})} \quad (5.12)$$

where ΔH is the activation energy of mechanism of internal friction taken to be 7.1kJ/mol [86] and R is the gas constant. With this estimations, we obtain the properties graph $1/R[\tau(T), \omega_{ref}]$ (Figure 5.5(a)) from measurements of dynamic modulus in the frequency domain [19, 20].

Capodagli et al. measures the changes in the inverse damping peak of PMMA experimentally and approximate it to [85],

$$\frac{1}{\tan(\delta_{peak})} = -0.1108(T - 273.150) + 17.410 \quad (5.13)$$

where T is the temperature in Kelvin and the damping, $\tan(\delta_{peak})$, approaches infinity at melting temperature of PMMA. This property of PMMA, together with Equation 5.10, reveals two mechanism in the ultrasonic welding of PMMA. At the start where temperatures are similar, the concentration of energy is achieved by the term $R_m(h_{mm}/h_{ed})$. When the temperatures difference increases, the PDPV ratio increase exponentially due to the increase damping of PMMA (ratio of sine terms in Equation 5.10).

PDMS is an excellent choice for matrix material for PMMA with its R value approximately 2 orders higher than PMMA (Figure 5.5(a)). With such large value R_m , the PDPV ratio can be approximate to R_m^2 . Although the difference in damping, V/U , of PMMA and PDMS is relatively large compared to other polymers (Figure 5.5(b)), the value of cosine term in Equation 5.10 varies between 0.95 to 1 only.

5.3.2 Experiment

5.3.2.1 Preparation of Composite Film

We used PMMA microspheres in conduction with PDMS to study its effect on welding and controlled melting. The use of PMMA microspheres enables slightly non-uniform distribution to show a minimal welding strength in the experiment so that the conditions would be closer to practical microfluidic devices with multiple channels. It also shows the possibility for future fabrications of ready-to-use composite films for ultrasonic welding. Using an optical microscope, we found that the PMMA microspheres had a distribution mean of $260.2\mu m$ by $271.8\mu m$ and variance of $88.6\mu m$ and $87.2\mu m$ respectively. We

Figure 5.6: PDMS spin coated thickness with different rotating speed and PMMA microspheres concentration [18]. The error of the thickness value to any trend is caused by the large variance of the PMMA microspheres size.

introduced the PMMA microspheres with precise concentration into a PDMS mixture of a fixed recipe of 184 silicone elastomer base and curing agent at a ratio of 10:1, spin coated the mixture onto a PMMA substrate and cured it at 80°C for 30 minutes. We established that spin coating parameters of 1000 rpm for 30 s (Figure 5.6) provides the best control over the distribution of PMMA microspheres.

5.3.2.2 Ultrasonic Welding Process and Pull Test

We welded the PMMA welding plate (32 mm × 32 mm) to a blank PMMA base plate (40 mm × 40 mm) using the Hermann Ultrasonic Welding Machine with base fixture and platform leveled to ensure balanced and equal welding across the welding plate (see Figure 5.7). The frequency, maximum power, weld force and displacement amplitude were 35 kHz, 1 kW, 100 N and 16.25 μm respectively. We set the trigger height at values between 15–20 μm and adjusted the ultrasonic horn to control to trigger height within the trigger window.

Two settings were used in the experiment. The optimal parameters (welding time of 1.5 s) is reference from results on non-coated PMMA with energy director hot embossed onto the plate surface. We also used alternative parameters which reduces the welding time to 1s to prevent over-welding as there are high amount of melt and shattering present in samples welded with optimum parameters (Figure 5.8(a)). This pattern of melt could be caused partially by the difference in sizes of the PMMA powder. Since ultrasonic welding at such a micro level requires precision leveling, this deviation in sizes presents a

Figure 5.7: Ultrasonic welding setup. The fixture (shown on right) is attached and balanced on the Hermann Ultrasonic Welding Machine, it serves to align the two samples to be welded.

Figure 5.8: Ultrasonic welding of composite film coated PMMA samples with (a) 100% amplitude (1 kW), 100 N weld force, 1.5 s weld time (optimum parameters) and (b) 100% amplitude (1 kW), 100 N weld force, 1 s weld time (adapted parameters) [18].

Figure 5.9: Pull test setup. The sample is glued to the structure on the right bottom, slided into the slot, assembled and place in the Instron MicroTester for tensile pull test.

certain level of difficulty in obtaining a good weld. However, in the adapted parameters which the weld time is 1 s, the resulting weld produced a much better weld (Figure 5.8(b)).

Welding strength is determined by a tensile pull test using the Instron Microtester (see Figure 5.9). Pull test to break is executed at a rate of 5 mm/min after calibrating the tester with fine positioning, load balancing and gauge length zeroing.

5.4 Results and Discussion

The samples prepared with different concentration of PMMA microspheres are used in the pull test to determine the welding strength with the objective of,

1. determining welding strength of present welding methodology
2. determining the optimum process of this methodology
3. finding any limitations in using this methodology.

and the results is shown in Figure 5.10.

When using the alternative parameters, the concentration of 0.24 is optimum where the energy input is able to melt all the PMMA microspheres. Any further increase in concentration of PMMA microspheres requires a longer welding time. However, with

Figure 5.10: PMMA plate welding strength with spin coated PDMS with PMMA microspheres mixture at different concentrations (mass of PMMA microspheres / total mass of PDMS with curing agent). The model (Equation 5.13) estimates of optimum parameters curve (without over-welding) from alternative parameters curve is also plotted. The horizontal lines depict the limit of welding strength of the samples with the respective ultrasonic welding parameters.

a longer welding time (such as that of the optimum parameters), Figure 5.2(a) show undesirable over-welding at the edge on one side of the samples. This over welding is due to the diverse distribution of the PMMA microspheres diameter, which cause PDMS mixture coating to be highly non level at high concentrations of PMMA microspheres. The overwelding can also be seen from the optimum parameters trend line which is much higher than that of the alternative parameters and the model estimates of optimum parameters, and it does not appear to have any maximum at any concentration of PMMA microspheres (Figure 5.10).

While there are no visible signs of overflow, underflow and trapped air in all specimens, the resultant welding strength in this methodology is limited by the dispersity of the PMMA microspheres. Composite with more dispersed microspheres has a lower maximum ultrasonic energy which it can be subjected to before over melting occurs. This limits the maximum welding strength at 35kPa (Figure 5.10) for composite film with concentration of 0.24 when utilizing PMMA microspheres with a variance over mean ratio of 0.34 and 0.32. Reducing the variance of the PMMA microsphere diameter distribution would allow increased welding time without risk of over-welding. The local welding strength, σ_{LWS} , can be approximated with,

$$\sigma_{LWS} \approx \frac{\delta\sigma_T}{\delta c} \times \frac{\rho_{PMMA}}{\rho_{PDMS}} \quad (5.14)$$

where σ_T is the total welding strength of the sample. The local welding strength is approximated to be 188 kPa.

With matrix material of large α wrt PMMA such as PDMS, the total power dissipation ratio (Equation 5.11) can be approximated to $1/(1 + h_{efr}) = \text{constant}$, which is similar to a fusion layer without the matrix material (Sun et al.'s micro energy directors). This effect of a constant total power dissipation ratio regardless of energy director height and concentration can be seen from the straight line trend of the alternative parameters in Figure 5.10. Taking reference from the experimental results of the alternative parameters, the energy input required can be calculated,

$$\text{Energy input} = 1.53 \times 10^{10} c A h \quad (5.15)$$

where c is the PMMA microsphere concentration, A is the area of the sample and h is the mean diameter of the PMMA microspheres (all parameters in SI unit).

5.5 Conclusion

A composite layer fabricated with PMMA microspheres mixed in matrix material limits overflow and underflow of the melted PMMA during ultrasonic welding. The elastomeric matrix material also prevents air from trapping in the fusion layer which may result in leakage in the microfluidic device. The matrix material selection is significantly determined by the ratio of dynamic modulus of the matrix material over that of the PMMA. With materials of much lower dynamic modulus such as the PDMS, the response of the composite layer to the ultrasonic welding process is similar to one without the matrix material. However, a PMMA microspheres mixed in PDMS composite film has its welding strength (after ultrasonic welding) limited by the dispersity of the PMMA microspheres. This problem may be alleviated by spin coating matrix material onto surface with uniform micro-energy directors such as those in Sun et al.'s work [84]. In this present methodology of ultrasonic welding, the welding strength is also smaller compared to conventional ultrasonic welding. However, it provides an option to compromise welding strength for better control of molten polymer flow and problems of trapped air in welded samples especially for applications where the boundary conditions are critical.

In the next chapter, a low-power acoustic transducer capable of transferring acoustic energy into the fluid with low supply voltage is developed.

Chapter 6

An Electromagnetic-Acoustic Transducer for Microfluidic Application

6.1 Introduction

In the previous chapter, a method of welding thermoplastics while maintaining the boundary conditions of channels is presented. In this chapter, an electromagnetic-acoustic transducer is presented. This transducer replaces the middle ear by supplying high pressure acoustic waves into the fluid without the need for the lever like middle ear. The development of this low-power, hearing frequency operated transducer will enable the use of cochlea-inspired designs into the field of microfluidics. As many of the acoustic transducer for microfluidics is more commonly used as a mixer, this chapter will focus on micromixing. In order to show the performance of this transducer, a mixing experiment is conducted with the transducer as the input source and compared to other similar mixers.

6.2 Literature Review

There are several methods to introduce acoustic wave into microfluidic chamber or channel for various applications such as pumps, sensors and mixers. There are various mediums, such as trapped micro air bubbles [87], a thin solid plate [88], or a piezoelectric membrane [89, 90, 91] which can be used to transfer the acoustic energy. There are also possibilities of manipulating an elastic magnetic membrane using the electromagnetic principle [92]. An affordable and elastic, permanent magnetic piece is embedded in the membrane and when the magnetic field is generated from the mixer base (coil), the generated magnetic field vicinity of the magnetic piece causes the membrane to vibrate (an up-and-down

motion). Pulsing fluids with a micropump to lead the pressure field disturbance is another simpler way to introduce acoustic energy and it is used to implement active micro mixer in Glasglow et. al.'s work [93]. Petkovic-duran et al. [94] demonstrated a transducer using two commercial loudspeakers mounted firmly on the base of the microfluidic chip. They transfer audio frequencies to mix fluids in a small acrylic open-well chamber (ensuring the liquid-air interface). However, the energy transferred to the fluid (which is indicated in the mixing efficiency) is small.

Figure 6.1: ¹² Measured quasi-static relationships between applied voltage and endpoint displacement and between applied force and endpoint displacement.

Although the piezoelectric actuator/transducer is commonly used [95, 96, 97] for microfluidics applications, the output obtained from the piezoelectric actuator is low (compared to the input), and thus, an external amplified must be connected to compensate for its high impedance. Therefore, it becomes difficult to scale down the entire system. Figure 6.1 [98] shows the order of displacement for an applied voltage and force. In order for a similar displacement compared to the stapes of a mammalian cochlea (see Figure 6.3(a) [99]), it requires an applied voltage in the order of 10 to 20 V and a corresponding force of 100 to 200 N. Thus, a low voltage input transducer is required for an artificial cochlea as well as other microfluidic applications.

Voice coils are thin, and lightweight wires are typically used in a dynamic speaker. The commercial voice coils come in a variety of sizes in terms of American wire gauge (AWG), but AWG 30 (cross section area= 0.0510 mm^2 , resistance= $338 \Omega/\text{km}$) or larger types of coils are commonly used in the loudspeaker industries. A higher AWG number indicates thinner wire. When a speaker is operating, a current drives through the voice

¹²©1997 IEEE. Reprinted, with permission, from M. Goldfarb and N. Celanovic, Modeling piezoelectric stack actuators for control of micromanipulation, IEEE Control Systems, 17 (3), 69-79, Jun 1997.

coil, and then AC magnetic fields are generated around the coil. Due to a permanent magnet in the speaker (with a DC magnetic field), the voice coil moves as it alternates its AC magnetic polarities. As a moving part in the system is the coil, the coil must be as light as possible. The usual material for the voice coil is copper with an insulation coating (i.e., enamel). The coil is wound to a higher packing density and glued to fix its coiling shape. The characteristics of the voice coils (i.e., thin, lightweight, low-power) make them suitable for microfluidic applications and it can be utilized as a safe, low-cost, and portable microfluidic application.

6.2.1 Micromixer

Micro-mixer is a device which mixes multiple samples in micro-scale. The Reynold's Number ($Re = \rho v L / \mu$) of the fluid flow is significantly reduced due to the small characteristic length, L , and flow velocity, v . The Low Reynold's number flow implies that there is predominantly diffusion mixing which occurs slower than turbulent mixing. In order to reduce the micro-fluidic channel size as well as increase the throughput of the micro-fluidic system, micro-mixers are used to achieve the required mixing in a shorter channel length and time. The efficiency and performance of the micro mixer is determined by the mixing time, length and mixing index [90, 100, 101, 102]. Although there is a lack of standardize parameter to compare mixing performance, the most commonly used method is to record the image of mixing two fluids with different intensity. The mixing index, $I(x)$, is [102],

$$I = \frac{\sigma}{C} \quad (6.1)$$

where σ is the standard deviation (STD) and C is a constant defined differently in different works [103].

Micro-mixers can be classified into two types; passive and active. Active methods mainly involve transferring some form of energy to the flow, passive methods uses the flow's own energy to achieve mixing. Active methods includes using acoustic, piezoelectric membrane, pressure perturbation dielectrophoretic, electrokinetic time-pulsed force, electrohydrodynamic force, thermal bubble pump, magneto-hydrodynamic flow, electrokinetic instability, and micro-impellers, valves, pumps and centrifuge [100, 104, 105]. The passive methods which are mainly mechanical include using lamination, zigzag channels, 3-D serpentine structure, embedded barriers, twisted channels, surface-chemistry, and flow-induced oscillation. Most of the passive methods of mixing involve altering the channel geometry and do not require an additional device, which is relatively larger, to provide

energy for mixing. However, the main fallback of passive mixers is the inability to control mixing and they require large input flow pressure (or energy) as these methodologies extract energy from the main fluid flow for mixing. Some of the active mixing methods such as dielectrophoretic [106], electrohydrodynamic and magneto-hydrodynamic [107] requires specific properties of the mixing fluid(s) or injection and extraction of additional particles into the stream (such as Tsai et. al.'s thermal bubble pump [108]). Mixers such as Electrokinetic time-pulsed [109] and Electrokinetic instability [110] mixers requires electrolytic fluid transported via Electro-osmosis effect [111]. Installation of micro impellers, stirrers, valves or pumps in the main channel to disturb the laminar flow are challenging to fabricate. Pressure perturbation or the periodical variation of pumping capacity uses pulses of additional velocity flow from side channel to the main channel (or entire channel such as La et al's centrifugal force-based mixing device [112]) to disturb the laminar flow of the fluid can be used for most fluid and does not requires additional installations. However, such methodologies which affects the entire channel flow has limited applications on integrated lab-on-chips.

6.3 Methods: Design, Fabrication, and Experimentation of the Electromagnetic-acoustic Transducer

We designed and fabricated a simple electromagnetic-acoustic (EMA) transducer to be used in a micro-chamber mixing system. Mixing experiments and simulations were also performed to test the mixing efficiency of the developed system.

6.3.1 The design and fabrication process

The design and picture of the voice-coil transducer with a mixing chamber are described in Fig. 6.2. The system includes two parts: a mixing chamber and a EMA transducer. The EMA transducer consists of a voice coil attached to the bottom membrane, a Polymethylmethacrylate (PMMA or acrylic) housing, a permanent magnet, a metal cap, and an iron case.

The mixing chamber with the voice coil membrane is fabricated as follows:

1. PMMA mould as a negative shape of the designed chamber (a cylindrical shape PDMS chamber) is prepared.

Figure 6.2: The developed mixing system with its design details is shown. The mixing system consists of two parts: the EMA transducer (a magnet and a metal piece, the left picture, right part) and the mixing chamber (the left picture, left part). The picture on the right shows the assembled shape of the EMA mixer system, the chamber is plugged onto the base. Due to the delicate characteristic of the voice coil, a small space is considered as a wire release gap.

2. We cast PDMS mixture (Polydimethylsiloxane, Sylgard 184 : curing agent = 10 : 1) with the PMMA mould and cure it in the oven (Eyela VOS-301SD) at 65°C for 12 hours after degassing the mixture using a vacuum machine.
3. The membrane is fabricated on a PMMA wafer with the Spin Coater (Laurell WS-400BZ-GNPP). The spin coater is set to three steps. The details of each step are followed; (i) 500 rpm, 560 rpm/s, 15 s (ii) 3245 rpm, 896 rpm/s 30 sec (iii) 500 rpm, 560 rpm/s, 30 sec. The membrane is cured in the Oven (Eyela VOS-301SD) at 65°C for 2-3 hours.
4. The cylinder is bonded to the membrane using stamp-and-stick technique [113] and cured in the oven for 1 hour (65°C).
5. A cleaned voice-coil is Dipped into PDMS mixture (using a tweezer) place underneath of the chamber, on the membrane and left to cure in the oven for 1 hour (65°C).

A voice coil, a magnet, and a metal piece are used for the EMA transducer fabrication (Fig. 6.2):

1. A round shape magnet (bottom) and a metal ring (top) with the same diameter are attached. Their diameter must be smaller than the voice coil diameter as the voice coil is surrounding the magnet-metal stack.

¹³Reprinted with permission from MiddleEar Characteristics of Anesthetized Cats, Guinan, J. J. and Peake, W. T., The Journal of the Acoustical Society of America, 41, 1237-1261. Copyright 1967, Acoustic Society of America.

Figure 6.3: (a) Stapes displacement versus SPL for Cat¹³. Each point represents the average of 5–10 measurements. Range of measurements is indicated by the vertical line. Stimulus frequency: 315 Hz. (b) Membrane displacement of present micromixer calculated with measured data from laser vibrometer.

2. The product is placed in a metal case to secure their electromagnetic fields inward.
3. A PMMA cylinder whose inner diameter is similar to the outer diameter of the mixing chamber is cut, sand and placed as shown in Fig. 6.2 (right). This PMMA piece holds the chamber while testing the device.

Once the chamber and the EMA transducer base are prepared, the chamber is placed on top of the base and the voice coil terminals are connected to a input generator. For the stability purpose, the coil ends are soldered onto the copper tape then connected to the driving source such as a function generator. The mixer top is left intentionally open in order to enable large pressure gradient to facilitate faster mixing [94].

The vibrational displacement of the mixer is measured Figure 6.3(b) to ensure the operation of the transducer and verify that it has a lower operational voltage than a piezoelectric transducer while giving a displacement amplitude in the order of 1 μ m.

6.3.2 Mixing experiments: the mixing efficiency

The mixing tests were performed using a high speed camera (Photron, Fastcam APX RX) set at 50 frames/s; 0.2 ml glycerol (Ajax Finechem 242) and florescence dye (Polysciences Inc. Cat #15702) were mixed with various amplitude and frequency input via a function generator (Agilent 33250A). The details of the experimental setting are shown in Fig. 6.4.

Figure 6.4: The left picture explains the experiment setup (equipments and materials). On the right side of this figure, images from two test cases are displayed, diffusion and 2 V at 100 Hz. In case of the diffusion, the dye is floated on the top of the chamber, does not mix with the glycerol at all while mixing was completed within 6 s in the 2 V at 100 Hz case. The selected Region Of Interest (ROI) from the original image file is cropped and used to calculate mixing efficiency of the developed mixer based on the mixing index described in Eq. 6.1

After adjusting the focus of high speed camera and light source to obtain a clear view of the side well of the mixing chamber, two terminals of the EMA mixer are connected to the function generator, then 0.2 ml of glycerol was loaded into the chamber carefully. Immediately after dropping the dye (2 drops, using a 3 ml pipette) in the chamber, the speed camera begins to record with the 50 frame/sec speed and the function generator was started. The camera recording was stopped once the two fluids were sufficiently mixed or the time taken was more than two minutes.

Originally 13 sets of input conditions, including the diffusion as a reference case, were tested (input voltage strengths of 1 V, 2 V and 3 V, and frequencies of 50 Hz, 100 Hz, 200 Hz and 300 Hz). There are 3 cases where the mixing was not completed (diffusion, 1 V at 50 Hz and 1 V at 300 Hz), therefore in this study, we consider only 10 cases to evaluate the mixing efficiency of the EM mixer. The best mixing efficiency is at 3V, 200 Hz case where glycerol mixing was completed within 2.5–3 s. We crop the Region Of Interest (ROI) from the raw image file with MATLAB and compute the mixing efficiencies.

Glycerol is chosen for our test which is a relatively viscous fluid and relatively difficult to mix [91]. The reported viscosity of Glycerol is 1.412 Pa·s while the viscosity of water

at 25 °C is 8.90×10^4 Pa.s. Glycerol viscosity table is also available in Sheely *et. al*'s work [114]. We use the low frequency EMA transducer to input acoustic energy into the mixing chamber at the system resonant frequency.

6.4 Results and Discussion

In this section, we characterize the developed EM mixer compared to other microfluid mixers (chamber types) in terms of mixing efficiency. The efficiency and performance of the micro mixer are both determined by mixing time, length and mixing index [101, 102, 90, 100].

We use Equation 6.1 to calculate the mixing index. However, in this experiment, the standard deviation is unreliable and the mean intensity across the test set is unstable due to low penetrating power of the light source (see Figure 6.4). We normalize the σ using the mean value for a relative comparison across the image set (Eq. 6.2).

The the mean normalized standard deviation (σ_{norm} in Eq. 6.3) is a popular statistical method called ‘coefficient of variation’ or ‘relative standard deviation.’ It is a dimensionless number that gives understanding of the standard deviation outside of the mean context of the data,

$$I_{norm} = \frac{\sigma_{norm}}{C}, \quad (6.2)$$

$$\sigma_{norm} = \frac{\sigma}{\text{mean}}. \quad (6.3)$$

In order to compare across different mixers, we use the mixing time scale, τ , where the change in normalized mixing index along time is fitted in the curve,

$$I_{norm} = e^{-\frac{1}{\tau}t} \quad (6.4)$$

where I_{norm} is the mixing index which has the value of 1 when unmixed and 0 when mixed completely which can be estimated by adjusting C in Equation 6.1.

6.4.1 Mixing efficiency of present transducer

In microfluidics, sources which use $< 30 V_{pp}$ is classified as low voltage [115]. The present EMA mixer requires AC voltage, < 3 V ($2-6 V_{pp}$) and is able to operate without power amplification. The present EMA mixer uses less power than any of the micromixer shown in Figure 6.5 where the mixing time-scale is compared with other acoustic mixers. Due

Figure 6.5: Mixing time scale (τ) of the various acoustic mixers plotted against characteristic length/viscosity. A rough trend of decreasing time scale is observed as the L/ν increases.

to the difference in viscosity of the fluid used and the characteristic length of the mixer, we compared the mixers' efficiencies with the time scale trend line of the variable K ,

$$K = \frac{L}{\nu} \quad (6.5)$$

where L is the characteristic length (the smallest width or the diameter of the mixer) and ν is the kinematic viscosity of the mixing fluid. The variable K is similar to the Reynolds number except it does not consider the velocity of the fluid as some mixers are chamber design.

6.5 Conclusion

An electromagnetic-acoustic transducer-based, low frequency, low cost, and low-power high-efficiency micro-fluid mixer system has been developed by utilizing properties the low frequency, non-contacting force transmission to blend microfluids. The present transducer works in the range of human hearing frequency signal and has been shown to be useful in one of the microfluidics application; micromixer. This EMA transducer enables the transfer of acoustic energy to a microfluidic system with low voltage input.

Chapter 7

Conclusions and Future Work

7.1 Conclusions

This thesis contributes to the technological capability build up of the development of a passively-powered, microfluidic artificial cochlea. We studied the gerbil arched basilar membrane in order to reduce the size of the artificial cochlea and developed a method of welding soft elastomeric basilar membrane to hard thermoplastic channel walls as well as an electromagnetic-acoustic (voice coil) transducer to match the impedance of air-fluid acoustic vibration, replacing the function of the middle ear.

The performance of the arched basilar membrane in the gerbil cochlea is evaluated with experimental measurements from other mammalian cochlea including the human cochlea (Chapter 2). It is found that the arch in the basilar membrane, the radial tension and the varying fiber bundle stiffness provide similar mechanism as a membrane with significant varying thickness and width (Chapter 3). The arched basilar membrane model developed in this research shows a second to third order dependency of the bending stiffness on the fiber bundle thickness (Chapter 4). With the arched basilar membrane model, mechanism of a mammalian cochlea can be integrated into microfluidics applications as the arched membrane does not require varying channel width which expands the type of applications into microfluidics. This research further contributes in applying cochlea mechanics into the field of microfluidics and the development of a passively-powered artificial cochlea with the thermoplastics ultrasonic welding methodology developed in Chapter 5 which enables bonding of flexible, thin membrane onto microfluidic chip without the loss of flexibility near the edge. An electromagnetic transducer is also developed to enable the transfer of acoustic energy into microfluidic channel or chamber without the need of expensive devices nor voltage amplifier and it is shown to be efficient in one of the microfluidic application; micromixer (Chapter 6).

This research work also contributed to the estimations of human ANFTC (Chapter 2). The work in the past provides various contradicting results to the actual ANFTC of human cochlea. The present work provides a new approach to estimating the human cochlear ANFTC with the theoretical cochlea model.

7.2 Future Work

Several improvements can be made to the gerbil cochlea model and the cochlea-inspired mixer in future research. The current fluid potential flow solution does not take into account of the arched membrane in gerbil cochlea. The work in Chapter 3 has estimated a fluid pressure decrease of up to 30% difference between the arched membrane and the flat plate. However, The calculation of the fluid force is a rough approximation to determine the need to do a detailed model of the actual fluid force. The equation involves is estimated with Bernoulli theorem (without damping nor 3-D flow considerations). Therefore, reformulation of the actual potential flow is required for a more detailed understanding of the arched membrane effect on the traveling wave. Another improvement to the cochlea model is the consideration of wall damping into the wave number calculation. In the current model, the viscous vector potential flow is assumed to be zero at the rigid walls. An analysis on the effect of cochlea duct size on the damping of traveling wave would improve the accuracy of the theoretical cochlea model.

Figure 7.1: Design of a passively-powered artificial cochlea. The voice-coil transducers converts large vibrational amplitude at the tympanic membrane to a high pressure acoustic vibration and poweres the microfluidic chip which maps the frequency information onto the artificial basilar membrane. Small piezo-transducers picks up the signal and stimulate the auditory nerve via the electrode array.

For the cochlear-inspired application in the field of microfluidics and development of a passively powered artificial cochlea (see Figure 7.1), future work would include other

fabrication technology development such as optimization of 3-D printing process for thin membranes as well as processes for embedding sensors in the arched membrane. Methodology of fabricating a smaller electromagnetic-acoustic (voice coil) transducer is also required before an attempt on integrating the contributions of this work into fabrication of the passively-powered artificial cochlea.

Publication

1. W. X. Chan, H.S. Son and Y.-J. Yoon, Computational efficiency of meshfree methods with local-coordinates algorithm, *International Journal of Precision Engineering and Manufacturing*, 16(3), 547-556, 2015.
2. W. X. Chan, N. Kim and Y.-J. Yoon, Empirical and biophysical estimations of human cochleas psychophysical tuning curve sharpness, *AIP Advances*, 6, 015205 (2016)
3. W. X. Chan, H.S. Son and Y.-J. Yoon, Effects of basilar membrane arch and radial tension on the traveling wave in gerbil cochlea, *Hearing Research*, 327, 136-142, 2015.
4. W. X. Chan, S. H. Ng, K. H. H. Li, W.-T. Park, Micro-ultrasonic Welding using Thermoplastic-Elastomeric Composite Film. (Under review in *JMM*)
5. W. X. Chan, Y.-J. Yoon, An analytical study on the Stiffness Gerbil Arched Basilar Membrane (in preparation)
6. N. Kim, W. X. Chan, S. H. Ng, Y.-J. Yoon, An Acoustic Micromixer Using Voice Coil Actuation (in preparation)

References

- [1] Y. Raphael, M. Lenoir, R. Wroblewski, and R. Pujol, “The sensory epithelium and its innervation in the mole rat cochlea,” *The Journal of Comparative Neurology*, vol. 314, no. 2, 1991.
- [2] T.-D. Luong, V.-N. Phan, and N.-T. Nguyen, “High-throughput micromixers based on acoustic streaming induced by surface acoustic wave,” *Microfluidics and Nanofluidics*, vol. 10, pp. 619–625, Mar. 2011.
- [3] National Institutes of Health, “Cochlear implant,” 2005.
- [4] R. C. Naidu and D. C. Mountain, “Basilar membrane tension calculations for the gerbil cochlea,” *The Journal of the Acoustical Society of America*, vol. 121, no. 2, pp. 994–1002, 2007.
- [5] P. Dallos, “Low-Frequency Auditory Characteristics: Species Dependence,” *The Journal of the Acoustical Society of America*, vol. 48, no. 2B, pp. 489–499, 1970.
- [6] L. M. Cabezudo, “The ultrastructure of the basilar membrane in the cat,” *Acta Otolaryngol*, vol. 86, pp. 160–175, 1978.
- [7] G. V. Wever, *Theory of Hearing*. New York: Wiley, 1949.
- [8] N. Kim, Y. Yoon, C. Steele, and S. Puria, “Cochlear anatomy using micro computed tomography (μ CT) imaging,” pp. 68421A–68421A–10, Feb. 2008.
- [9] M. Thorne, A. N. Salt, J. E. DeMott, M. M. Henson, O. W. J. Henson, and S. L. Gewalt, “Cochlear fluid space dimensions for six species derived from reconstructions of three-dimensional magnetic resonance images,” *Laryngoscope*, vol. 109, pp. 1661–1668, 1999.
- [10] Y.-J. Yoon, C. R. Steele, and S. Puria, “Feed-forward and feed-backward amplification model from cochlear cytoarchitecture: an interspecies comparison,” *Biophysical Journal*, vol. 100, no. 1, pp. 1–10, 2011.

REFERENCES

- [11] M. A. Ruggero and A. N. Temchin, “Unexceptional sharpness of frequency tuning in the human cochlea,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, pp. 18614–18619, Dec. 2005.
- [12] C. A. Shera, C. C. Guinan Jr, and A. J. Oxenham, “Otoacoustic Estimation of Cochlear Tuning Validation in the Chinchilla,” *JARO*, vol. 11, pp. 343–365, 2010.
- [13] N. Eze and E. S. Olson, “Basilar membrane velocity in a cochlea with a modified organ of corti,” *Biophysical Journal*, vol. 100, no. 4, pp. 858–867, 2011.
- [14] W. X. Chan and Y.-J. Yoon, “Effects of basilar membrane arch and radial tension on the travelling wave in gerbil cochlea,” *Hearing Research*, vol. 327, no. 0, pp. 136–142, 2015.
- [15] T. Ren, “Longitudinal pattern of basilar membrane vibration in the sensitive cochlea,” *Proceedings of the National Academy of Sciences*, vol. 99, pp. 17101–17106, Dec. 2002.
- [16] R. M. Edge, B. N. Evans, M. Pearce, C. P. Richter, X. Hu, and P. Dallos, “Morphology of the unfixed cochlea,” *Hearing Research*, vol. 124, no. 1–2, pp. 1–16, 1998.
- [17] A. S. Wineman and K. R. Rajagopal, *Mechanical response of polymers : an introduction*. Cambridge: Cambridge University Press, 2000.
- [18] W. K. L. Chow, “Microfluidic chip development for medical application,” interim report, Nanyang Technological University, 2014.
- [19] M. Brodt, L. S. Cook, and R. S. Lakes, “Apparatus for measuring viscoelastic properties over ten decades: refinements,” *Review of Scientific Instruments*, vol. 66, no. 11, pp. 5292–5297, 1995.
- [20] T. Lee, R. S. Lakes, and A. Lal, “Resonant ultrasound spectroscopy for measurement of mechanical damping: comparison with broadband viscoelastic spectroscopy,” *Review of Scientific Instruments*, vol. 71, no. 7, pp. 2855–2861, 2000.
- [21] J. Le Rouzic, P. Delobelle, P. Vairac, and B. Cretin, “Comparison of three different scales techniques for the dynamic mechanical characterization of two polymers (PDMS and SU8),” *The European Physical Journal - Applied Physics*, vol. 48, no. 01, pp. 1–14, 2009.

REFERENCES

- [22] B. C. J. Moore, “Psychophysical tuning curves measured in simultaneous and forward masking,” *J. Acoust. Soc. Am.*, vol. 63, pp. 524–532, 1978.
- [23] A. Oxenham and C. Snera, “Estimates of Human Cochlear Tuning at Low Levels Using Forward and Simultaneous Masking,” *Journal of the Association for Research in Otolaryngology*, vol. 4, pp. 541–554, Dec. 2003.
- [24] D. J. Lim, “Cochlear anatomy related to cochlear micromechanics. a review,” *Journal of the Acoustical Society of America*, vol. 67, no. 5, p. 1686, 1980.
- [25] C. E. Miller, “Structural implications of basilar membrane compliance measurements,” *Journal of the Acoustical Society of America*, vol. 77, no. 4, p. 1465, 1985.
- [26] K. D. Karavitaki, *Measurements and models of electrically-evoked motion in the gerbil organ of Corti*. PhD thesis, MIT, Cambridge, 2002.
- [27] C. R. Steele and K. M. Lim, “Cochlear Model with Three-Dimensional Fluid, Inner Sulcus and Feed-Forward Mechanism,” *Audiology and Neurotology*, vol. 4, no. 3-4, pp. 197–203, 1999.
- [28] L. Schweitzer, C. Lutz, M. Hobbs, and S. P. Weaver, “Anatomical correlates of the passive properties underlying the developmental shift in the frequency map of the mammalian cochlea,” *Hearing Research*, vol. 97, no. 1–2, pp. 84–94, 1996.
- [29] S. Girardo, M. Cecchini, F. Beltram, R. Cingolani, and D. Pisignano, “Polydimethylsiloxane–LiNbO₃ surface acoustic wave micropump devices for fluid control into microchannels,” *Lab on a Chip*, vol. 8, pp. 1557–1563, Aug. 2008.
- [30] D. C. Leslie, C. J. Easley, E. Seker, J. M. Karlinsey, M. Utz, M. R. Begley, and J. P. Landers, “Frequency-specific flow control in microfluidic circuits with passive elastomeric features,” *Nature Physics*, vol. 5, pp. 231–235, Mar. 2009.
- [31] J. Shi, X. Mao, D. Ahmed, A. Colletti, and T. J. Huang, “Focusing microparticles in a microfluidic channel with standing surface acoustic waves (SSAW),” *Lab on a Chip*, vol. 8, pp. 221–223, Jan. 2008.
- [32] T. P. Burg and S. R. Manalis, “Suspended microchannel resonators for biomolecular detection,” *Applied Physics Letters*, vol. 83, pp. 2698–2700, Sept. 2003.

REFERENCES

- [33] F. Chen, H. I. Cohen, T. G. Bifano, J. Castle, J. Fortin, C. Kapusta, D. C. Mountain, A. Zosuls, and A. E. Hubbard, “A hydromechanical biomimetic cochlea: Experiments and models,” *The Journal of the Acoustical Society of America*, vol. 119, pp. 394–405, Jan. 2006.
- [34] H. Shintaku, T. Nakagawa, D. Kitagawa, H. Tanujaya, S. Kawano, and J. Ito, “Development of piezoelectric acoustic sensor with frequency selectivity for artificial cochlea,” *Sensors and Actuators A: Physical*, vol. 158, pp. 183–192, Mar. 2010.
- [35] T. Inaoka, H. Shintaku, T. Nakagawa, S. Kawano, H. Ogita, T. Sakamoto, S. Hamanishi, H. Wada, and J. Ito, “Piezoelectric materials mimic the function of the cochlear sensory epithelium,” *Proceedings of the National Academy of Sciences*, vol. 108, pp. 18390–18395, Aug. 2011.
- [36] F.-G. Zeng, S. Rebscher, W. V. Harrison, X. Sun, and H. Feng, “Cochlear Implants: System Design, Integration and Evaluation,” *IEEE reviews in biomedical engineering*, vol. 1, pp. 115–142, Jan. 2008.
- [37] D. Haronian and N. C. MacDonald, “A Microelectromechanics Based Artificial Cochlea (membac),” in *The 8th International Conference on Solid-State Sensors and Actuators, 1995 and Eurosensors IX.. Transducers '95*, vol. 2, pp. 708–711, June 1995.
- [38] Y. Yoon, S. Puria, and C. R. Steele, “A cochlear model using the time-averaged lagrangian and the push-pull mechanism in the organ of corti,” *Journal of Mechanics of Materials and Structures*, vol. 4, no. 5, pp. 977–986, 2009.
- [39] Y.-J. Yoon, S. Puria, and C. R. Steele, “Intracochlear pressure and derived quantities from a three-dimensional model,” *The Journal of the Acoustical Society of America*, vol. 122, no. 2, pp. 952–966, 2007.
- [40] T. Dung Luong and N. Trung Nguyen, “Surface Acoustic Wave Driven Microfluidics – A Review,” *Micro and Nanosystemse*, vol. 2, pp. 217–225, Sept. 2010.
- [41] I. Daubechies, “The wavelet transform, time-frequency localization and signal analysis,” *IEEE Transactions on Information Theory*, vol. 36, pp. 961–1005, Sept. 1990.
- [42] L. B. Almeida, “The fractional Fourier transform and time-frequency representations,” *IEEE Transactions on Signal Processing*, vol. 42, pp. 3084–3091, Nov. 1994.

REFERENCES

- [43] G. V. Békésy, *Theory of Hearing*. New York: AIP Press, 1960.
- [44] L. Voldřich, “Experimental and Topographic Morphology in Cochlear Mechanics,” in *Mechanics of Hearing* (E. de Boer and M. A. Viergever, eds.), pp. 163–167, Springer Netherlands, 1983.
- [45] W. Plassmann, W. Peetz, and M. Schmidt, “The Cochlea in Gerbilline Rodents,” *Brain, Behavior and Evolution*, vol. 30, no. 1-2, pp. 82–102, 1987.
- [46] G. A. Manley, O. Gleich, H. J. Leppelsack, and H. Oeckinghaus, “Activity patterns of cochlear ganglion neurones in the starling,” *Journal of Comparative Physiology A*, vol. 157, pp. 161–181, Mar. 1985.
- [47] M. B. Sachs, E. D. Young, and R. H. Lewis, “Discharge patterns of single fibers in the pigeon auditory nerve,” *Brain Research*, vol. 70, no. 3, pp. 431–447, 1974.
- [48] C. A. Shera, J. J. Guinan, and A. J. Oxenham, “Revised estimates of human cochlear tuning from otoacoustic and behavioral measurements,” *Proceedings- National Academy of Science USA*, vol. 99, pp. 3318–3345, 2002.
- [49] H. Fletcher, “The Mechanism of Hearing as Revealed Through Experiment on the Masking Effect of Thermal Noise,” *Proceedings of the National Academy of Sciences*, vol. 24, pp. 265–274, July 1938.
- [50] B. C. Moore, “Parallels between frequency selectivity measured psychophysically and in cochlear mechanics,” *Scand Audiol Suppl.*, vol. 25, pp. 139–52, 1986.
- [51] D. Johnson-Davis and R. D. Patterson, “Psychophysical tuning curves: restricting the listening band to the signal region,” *Acoust Soc Am*, vol. 65, pp. 765–770, 1979.
- [52] B. C. Moore and B. R. Glasberg, “Auditory filter shapes derived in simultaneous and forward masking,” *Acoust Soc Am*, vol. 70, pp. 1003–1014, 1981.
- [53] M. G. Heinz, H. S. Colburn, and L. H. Carney, “Quantifying the implications of non-linear cochlear tuning for auditory-filter estimates,” *The Journal of the Acoustical Society of America*, vol. 111, no. 2, pp. 996–1011, 2002.
- [54] A. Eustaquio-Martin and E. A. Lopez-Poveda, “Isoresponse versus isoinput estimates of cochlear filter tuning,” *JARO*, vol. 12, pp. 281–299, 2011.

REFERENCES

- [55] E. Lopez-Poveda and A. Eustaquio-Martin, “On the Controversy About the Sharpness of Human Cochlear Tuning,” *Journal of the Association for Research in Otolaryngology*, vol. 14, pp. 673–686, Oct. 2013.
- [56] E. F. Evans, “Latest comparisons between physiological and behavioral frequency selectivity,” in *Physiological and behavioral bases of auditory function* (J. Breebaart, A. J. M. Houtsma, A. Kohlrausch, R. Prijs, and R. Schoonhoven, eds.), pp. 382–387, Maastricht: Shaker, 2001.
- [57] P. Dallos and M. A. Cheatham, “Compound action potential (AP) tuning curves,” *The Journal of the Acoustical Society of America*, vol. 59, no. 3, pp. 591–597, 1976.
- [58] E. Verschooten, L. Robles, D. Kovacic, and P. X. Joris, “Auditory Nerve Frequency Tuning Measured with Forward-Masked Compound Action Potentials,” *JARO*, vol. 13, pp. 799–817, 2012.
- [59] P. X. Joris, C. Bergevinb, R. Kalluric, M. Mc Laughlina, P. Micheleta, M. Heijdena, and C. A. Shera, “Frequency selectivity in Old-World monkeys corroborates sharp cochlear tuning in humans,” *PNAS*, vol. 108, no. 42, 2011.
- [60] C. D. Geisler and C. Sang, “A cochlear model using feed-forward outer-hair-cell forces,” *Hearing Research*, vol. 86, no. 1–2, pp. 132–146, 1995.
- [61] K.-M. Lim and C. R. Steele, “A three-dimensional nonlinear active cochlear model analyzed by the WKB-numeric method,” *Hearing Research*, vol. 170, no. 1–2, pp. 190–205, 2002.
- [62] H. Engstrom, “The structure of the basilar membrane,” *Acta Oto-Rhino-Laryngologica Belgica*, vol. 9, pp. 531–542, 1955.
- [63] M. M. Henson and O. W. J. Henson, “Tension fibroblasts and the connective tissue matrix of the spiral ligament,” *Hearing Research*, vol. 35, pp. 237–258, 1988.
- [64] S. Kapuria, C. R. Steele, and S. Puria, “Mechanics of the unusual basilar membrane in gerbil,” *AIP Conference Proceedings*, vol. 1403, no. 1, pp. 333–339, 2011.
- [65] R. C. Naidu and D. C. Mountain, “Can longitudinal coupling within the organ of corti be neglected?,” in *Association for research in Otolaryngology, 21st Midwinter research meeting*, vol. 21, p. 718, 1998.

REFERENCES

- [66] C. A. Shera, “Wave interference in the generation of reflection- and distortion-source emissions,” in *Biophysics of the Cochlea: From Molecules to Models* (A. W. Gummer, ed.), pp. 439–453, World Scientific, 2005.
- [67] D. D. Greenwood, “A cochlear frequency-position function for several species-29 years later,” *The Journal of the Acoustical Society of America*, vol. 87, pp. 2592–2605, 1990.
- [68] K.-M. Lim, *Physical and mathematical cochlear models*. Ph.d., Stanford University, Ann Arbor, 2000.
- [69] C. R. Steele and L. A. Taber, “Comparison of WKB calculations and experimental results for three-dimensional cochlear models,” *The Journal of the Acoustical Society of America*, vol. 65, no. 4, pp. 1007–1018, 1979.
- [70] Y.-J. Yoon, S. Puria, and C. R. Steele, “Frequency and spatial response of basilar membrane vibration in a three-dimensional gerbil cochlear model,” *Journal of Mechanics of Materials and Structures*, vol. 2, no. 8, pp. 1449–1458, 2007.
- [71] H. Chang and L. Y. Yeo, *Electrokinetically driven microfluidics and nanofluidics*. New York: Cambridge University Press, 2010.
- [72] H. Becker and L. E. Locascio, “Polymer microfluidic devices,” *Talanta*, vol. 56, no. 2, p. 267, 2002.
- [73] K. Kalkandjiev, L. Riegger, D. Kosse, M. Welsche, L. Gutzweiler, R. Zengerle, and P. Koltay, “Microfluidics in silicon/polymer technology as a cost-efficient alternative to silicon/glass,” *Journal of Micromechanics and Microengineering*, vol. 21, no. 2, 2011.
- [74] Y. Luo, Z. B. Zhang, X. D. Wang, and Y. S. Zheng, “Ultrasonic bonding for thermoplastic microfluidic devices without energy director,” *Microelectronic Engineering Analysis with Boundary Elements*, vol. 87, no. 11, 2010.
- [75] R. Truckenmuller, R. Ahrens, Y. Cheng, G. Fischer, and V. Saile, “An ultrasonic welding based process for building up a new class of inert fluidic microsensors and -actuators from polymers,” *Sensors and Actuators: A. Physical*, no. 1, p. 385, 2006.

REFERENCES

- [76] Z. B. Zhang, X. D. Wang, Y. Luo, S. Q. He, and L. D. Wang, "Thermal assisted ultrasonic bonding method for poly(methyl methacrylate) (PMMA) microfluidic devices," *Talanta*, vol. 81, no. 4, pp. 1331–1338, 2010.
- [77] Z. Zhang, Y. Luo, X. Wang, Y. Zheng, Y. Zhang, and L. Wang, "A low temperature ultrasonic bonding method for PMMA microfluidic chips," *Microsystem Technologies*, no. 4, p. 533, 2010.
- [78] H. Y. Tan, W. K. Loke, and N.-T. Nguyen, "A reliable method for bonding polydimethylsiloxane (PDMS) to polymethylmethacrylate (PMMA) and its application in micropumps," *Sensors and Actuators B: Chemical*, vol. 151, pp. 133–139, Nov. 2010.
- [79] K. Kim, S. W. Park, and S. S. Yang, "The optimization of PDMS-PMMA bonding process using silane primer," *BioChip Journal*, vol. 4, pp. 148–154, June 2010.
- [80] A. Benatar and T. G. Gutowski, "Ultrasonic welding of peek graphite apc-2 composites," *Polymer Engineering and Science*, vol. 29, no. 23, pp. 1705–1721, 1989.
- [81] S. H. Ng, Z. F. Wang, and N. F. de Rooij, "Microfluidic connectors by ultrasonic welding," *Microelectronic Engineering*, vol. 86, no. 4-6, pp. 1354–1357, 2009.
- [82] D. A. Grewell, "A prototype 'expert' system for ultrasonic welding of plastics," *Plastics Engineering*, no. 2, p. 33, 1999.
- [83] R. M. Rani, K. S. Suresh, K. Prakasan, and R. Rudramoorthy, "A statistical study of parameters in ultrasonic welding of plastics," *Experimental Techniques*, no. 5, p. 53, 2007.
- [84] Y. Sun, Y. Luo, and X. Wang, "Micro energy director array in ultrasonic precise bonding for thermoplastic micro assembly," *Journal of Materials Processing Technology*, vol. 212, no. 6, pp. 1331–1337, 2012.
- [85] J. Capodagli and R. Lakes, "Isothermal viscoelastic properties of PMMA and LDPE over 11 decades of frequency and time: a test of time-temperature superposition," *Rheologica Acta*, vol. 47, pp. 777–786, Sept. 2008.
- [86] N. McCrum, C. Buckley, and C. Bucknall, *Principles of polymer engineering*. Oxford University Press, 2nd edition ed., 1997.

REFERENCES

- [87] R. H. Liu, R. Lenigk, S. R. L. Druyor, J. Yang, and P. Grodzinski, "Hybridization enhancement using cavitation microstreaming," *Anal. Chem.*, vol. 75, pp. 1911–1917, 2003.
- [88] T. R. Tsao, R. M. Moroney, B. A. Martin, and R. M. White, "Electrochemical detection of localized mixing produced by ultrasonic flexural waves," in *Ultrasonics Symposium, 1991. Proceedings., IEEE 1991*, pp. 937–940 vol.2, 8–11 Dec 1991.
- [89] P. Woias, K. Hauser, and E. Yacoub-George, "An active silicon micromixer for microTAS applications," in *Micro Total Analysis Systems*, pp. 277–282, 2000.
- [90] D. Ahmed, X. Mao, B. Juluri, and T. Huang, "A fast microfluidic mixer based on acoustically driven sidewall-trapped microbubbles," *Microfluidics and Nanofluidics*, vol. 7, pp. 727–731, Nov. 2009.
- [91] A. Ozcelik, D. Ahmed, Y. Xie, N. Nama, Z. Qu, A. A. Nawaz, and T. J. Huang, "An Acoustofluidic Micromixer via Bubble Inception and Cavitation from Microchannel Sidewalls," *Analytical Chemistry*, vol. 86, pp. 5083–5088, May 2014.
- [92] W. J. S. Zhuang Zhi Chong, "Elastic Magnetic Membrane for Improved Mixing in Microwells," *Micro and Nanosystems*, vol. 6, no. 4, pp. 232–236, 2014.
- [93] I. Glasgow and N. Aubry, "Enhancement of microfluidic mixing using time pulsing," *Lab on a Chip*, vol. 3, pp. 114–120, May 2003.
- [94] K. Petkovic-Duran, R. Manasseh, Y. Zhu, and A. Ooi, "Chaotic micromixing in open wells using audio-frequency acoustic microstreaming," *BioTechniques*, vol. 47, pp. 827–834, Oct. 2009.
- [95] K. Ward and Z. H. Fan, "Mixing in microfluidic devices and enhancement methods," *Journal of Micromechanics and Microengineering*, vol. 25, no. 9, p. 094001, 2015.
- [96] Z. Yang, S. Matsumoto, H. Goto, M. Matsumoto, and R. Maeda, "Ultrasonic micromixer for microfluidic systems," *Sensors and Actuators A: Physical*, vol. 93, no. 3, pp. 266–272, 2001.
- [97] S. O. Catarino, L. R. Silva, P. M. Mendes, J. M. Miranda, S. Lanceros-Mendez, and G. Minas, "Piezoelectric actuators for acoustic mixing in microfluidic devices—Numerical prediction and experimental validation of heat and mass transport," *Sensors and Actuators B: Chemical*, vol. 205, pp. 206–214, Dec. 2014.

REFERENCES

- [98] M. Goldfarb and N. Celanovic, “Modeling piezoelectric stack actuators for control of micromanipulation,” *IEEE Control Systems*, vol. 17, pp. 69–79, June 1997.
- [99] J. J. G. Jr and W. T. Peake, “Middle-Ear Characteristics of Anesthetized Cats,” *The Journal of the Acoustical Society of America*, vol. 41, pp. 1237–1261, May 1967.
- [100] C.-Y. Lee, C.-L. Chang, Y.-N. Wang, and L.-M. Fu, “Microfluidic Mixing: A Review,” *International Journal of Molecular Sciences*, vol. 12, pp. 3263–3287, May 2011.
- [101] Y. K. Lee, P. Tabeling, C. Shih, and C. M. Ho, “Characterization of a MEMS-fabricated mixing device,” in *ASME International Mechanical Engineering Congress & Exposition*, 2000.
- [102] R. H. Liu, M. A. Stremler, K. V. Sharp, M. G. Olsen, J. G. Santiago, R. J. Adrian, H. Aref, and D. J. Beebe, “Passive mixing in a three-dimensional serpentine microchannel,” *Microelectromechanical Systems, Journal of*, vol. 9, no. 2, pp. 190–197, 2000.
- [103] S. Balasuriya, “Dynamical systems techniques for enhancing microfluidic mixing [TOPICAL REVIEW],” *Journal of Micromechanics and Microengineering*, vol. 25, p. 094005, 2015.
- [104] V. Hessel, H. Löwe, and F. Schönfeld, “Micromixers—a review on passive and active mixing principles,” *Chemical Engineering Science*, vol. 60, no. 8–9, pp. 2479–2501, 2005.
- [105] E. A. Mansur, M. X. Ye, Y. D. Wang, and Y. Y. Dai, “A state-of-the-art review of mixing in microfluidic mixers,” *Chinese Journal of Chemical Engineering*, vol. 16, no. 4, pp. 503–516, 2008.
- [106] C. Eunpyo, K. Byungkyu, and P. Jungyul, “High-throughput microparticle separation using gradient traveling wave dielectrophoresis,” *Journal of Micromechanics and Microengineering*, vol. 19, no. 12, p. 125014, 2009.
- [107] H. Bau, J. Zhong, and M. Yi, “A minute magneto hydro dynamic (MHD) mixer,” *Sens. Actuators B*, vol. 79, pp. 207–215, 2001.

REFERENCES

- [108] J.-H. Tsai and L. Lin, “Active microfluidic mixer and gas bubble filter driven by thermal bubble micropump,” *Sensors and Actuators A: Physical*, vol. 97–98, no. 0, pp. 665–671, 2002.
- [109] L. Chia-Yen, L. Gwo-Bin, F. Lung-Ming, L. Kuo-Hoong, and Y. Ruey-Jen, “Electrokinetically driven active micro-mixers utilizing zeta potential variation induced by field effect,” *Journal of Micromechanics and Microengineering*, vol. 14, no. 10, p. 1390, 2004.
- [110] L. Fu, T. Hong, C. Wen, C. Tsai, and C. Lin, “Electrokinetic instability effects in microchannels with and without nanofilm coatings,” *Electrophoresis*, vol. 29, pp. 4871–4879, 2008.
- [111] A. Ajdari, “Electro-Osmosis on Inhomogeneously Charged Surfaces,” *Physical Review Letters*, vol. 75, no. 4, pp. 755–758, 1995.
- [112] M. La, S. Park, H. Kim, J. Park, K. Ahn, S. Ryew, and D. Kim, “A centrifugal force-based serpentine micromixer (CSM) on a plastic lab-on-a-disk for biochemical assays,” *Microfluidics and Nanofluidics*, vol. 15, pp. 87–98, July 2013.
- [113] S. Satyanarayana, R. Karnik, and A. Majumdar, “Stamp-and-stick room-temperature bonding technique for microdevices,” *Journal of Microelectromechanical Systems*, vol. 14, pp. 392–399, Apr. 2005.
- [114] M. L. sheely, “Glycerol Viscosity Tables,” *Industrial & Engineering Chemistry*, vol. 24, pp. 1060–1064, Sept. 1932.
- [115] W. Y. Ng, S. Goh, Y. C. Lam, C. Yang, and I. Rodríguez, “DC-biased AC-electroosmotic and AC-electrothermal flow mixing in microchannels,” *Lab on a Chip*, vol. 9, pp. 802–809, Mar. 2009.