

IDENTIFYING DIABETIC PARAMETER IN BASILAR MEMBRANE MECHANICS AND MODELS.

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ABSTRACT

The cochlear frequency-place map is believed to be an important determinant of the frequencies that a species can hear. The cochlear frequency-place map is created partially by a stiffness gradient in the basilar membrane (BM) in which stiff regions respond best to high frequencies and more compliant regions respond best to low frequencies. BM mass and stiffness play significant role in frequency-place map. Outer hair cells (OHC) present inside cochlea plays important role for mechanical amplification by introducing active feedback. Mathematical model of active cochlea involves the factor α , the motility factor, which reflects the active feedback mechanism of OHC. Mathematical model of BM shows the presence of stiffness gradient in BM. Both motility factor in cochlear models and stiffness in BM model are affected by prolonged diabetic condition. Diabetic factor in cochlear model is been identified previously. In this paper we present our study of available BM model and identify the diabetic parameter in BM model.

Keywords: Collagen Fibers, Glycation, Ages, Longitudinal Coupling, SEM, TEM.

I. INTRODUCTION

Studies suggest that the basilar membrane (BM) is structurally designed to support a radial tension (Engström, 1955; Henson and Henson, 1988). The BM is composed of a homogeneous, soft ground substance that is traversed radially by fibers, which extend between the spiral lamina and the spiral ligament. The BM can be divided into two regions based on the arrangement of the fibers: The lateral pectinate zone, where the fibers are grouped into bundles; and the arcuate zone, where the bundles separate into individual fibers (Iurato, 1967). The parallel arrangement of fiber bundles in the pectinate zone suggests that the bundles are under a radial tension (Engström, 1955). Studies also suggest that such a tension is maintained by the spiral ligament. It has been shown that the spiral ligament fibers are anchored to the bony cochlear wall by fibroblasts. (Henson *et al* (1984). Fibroblasts contain fibers composed of contractile proteins and have been shown to create tension (Harris *et al.*, 1981). In the spiral ligament, the configurations in which fibroblasts are arranged and oriented suggest that these cells actively maintain a radial tension in the BM (Henson and Henson, 1988).

Our paper is organized in following manner.

Section I give the details of BM and explain that the fibroblast proteins are basically collagen fibers, the basic building blocks of the BM. Latest Laser-confocal microscopy, high resolution scanning (SEM) and transmission electron microscopy (TEM) reveals BM in detail.

Section II explains that the collagen fibers undergo glycation which can affect its mechanical properties prominently its stiffness which has major effect in BM response.

Section III explains the available mathematical model of BM and its solution.

Section IV identifies the diabetic parameter in BM model and explains the need to study the variation of this parameter.

II. SECTION I: HUMAN BM

The human BM consists of four separate layers: (1) epithelial basement membrane positive for laminin- β 2 and collagen IV, (2) BM proper composed of radial fibers expressing collagen II and XI, (3) layer of collagen IV and (4) tympanic covering layer (TCL) expressing collagen IV, fibronectin and integrin[1].

SEM view of the epithelial basement membrane shows it as a carpet-like structure as seen in figure 1. Beneath the basement membrane lies a fibrous layer extending from the tympanic lip of the lamina spiralis to the basilar crest of the spiral ligament which is the proper BM and consisted of various sized radial parallel fiber bundles (10–20 nm in diameter) as seen in figure 2

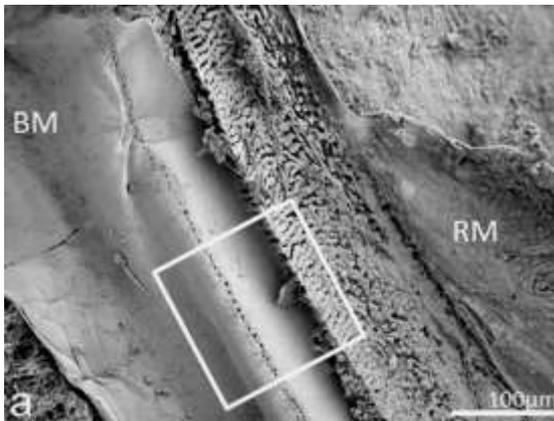


Figure1:SEM view of Basement membrane[1]

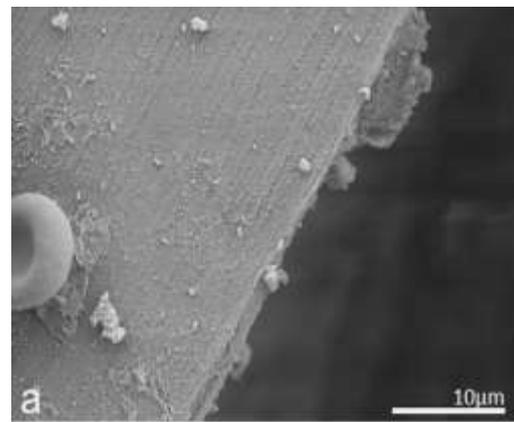


Figure2:SEM view of Proper BM Below Basement membrane [1]

Lateral to the basilar crest, in the spiral ligament, the BM thickened and formed an anchor-like structure from which several fibers emerged. The BM also displayed longitudinally arranged fibrils. The BM merged with the tympanic lip. BM viewed from scala media after rupture of the basement membrane expose collagen fibers as shown in figure 3.

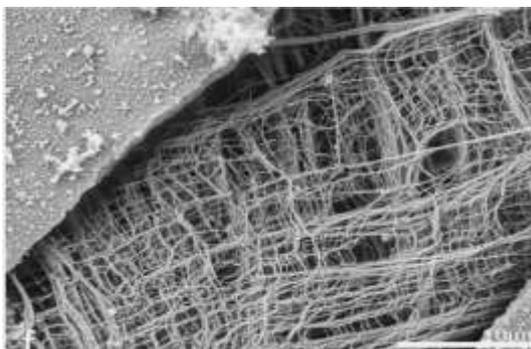


Figure3 SEM view of ruptured proper BM exposing collagen fibers [1]

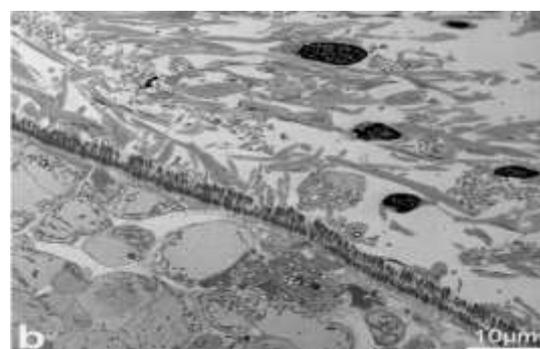


Figure4: TEM view of Proper BM below Basement membrane [1]

Figure 4 shows the SEM view of BM below basement membrane.

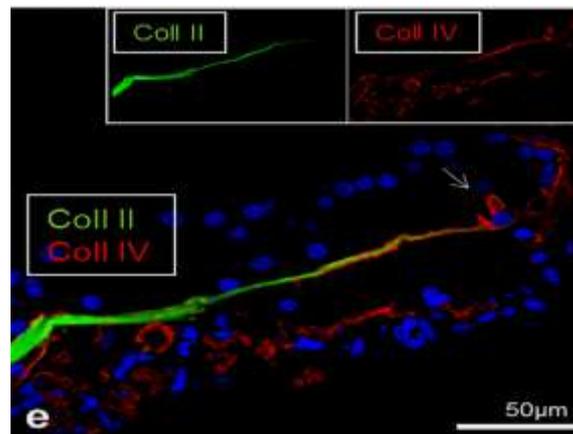


Figure5: TEM View of Proper BM below Basement Membrane [1]

Figure 5 shows Collagen II and IV expression in the BM. Collagen molecules make up 30% of total protein in the body and form the basis of many vital organs (Kadler et al., 2007). The collagen molecule is synthesized as a trimetric molecule containing two $\alpha 1$, and one $\alpha 2$ chains, each of about 1000 amino acids. Upon secretion from the cell, collagen molecules assemble into fibrils and are enzymatically crosslinked (Sweeney et al., 2008).

The BM consists of a filamentous layer consisting primarily of collagen fibers that run radially from medial to lateral i.e., perpendicular to the length of the cochlear duct.

Collagen is regarded as a main source of basilar membrane stiffness and therefore plays a critical role in establishing its tuned resonant frequency map [2]. Collagen molecules are packed together in a hexagonal manner to form fibrils, which are covalently bound together with groups of other fibrils to form collagen fibers. Ground substance fills in the spaces between fibers.

III. SECTION II: GLYCATION OF COLLAGEN FIBERS

Glycation and Advanced glycation end products (AGEs) are formed in vivo on collagen via non enzymatic reactions that covalently add a sugar moiety onto the protein (Paul and Bailey, 1996) and their accumulation is particularly high in long-lived proteins, such as collagen. The low biological turn over of collagen makes it therefore susceptible to interaction with metabolites, primarily glucose.

Several glycation crosslinks have been proposed (Avery and Bailey, 2006) but are all present in minute quantities except glucosepane (Sell et al., 2005), which is a lysine–arginine crosslinking AGE which could make a significant change to the biomechanics and biological activity of fibrillar collagen as shown in figure 6.

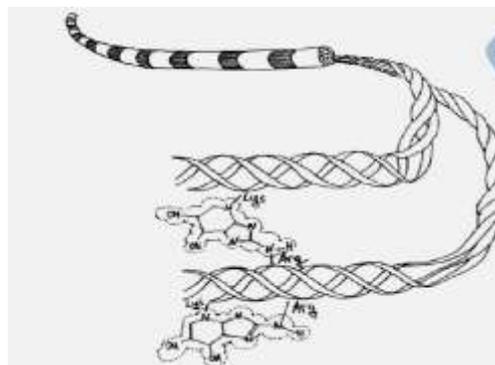


Figure6: Cross Linking in Collagen Fibers [3]

One is the biomechanical effects of non enzymatic inter molecular cross linking: glucose reaction with the amino acid side-chains, and subsequent further reaction to form a crosslink with an adjacent collagen molecule, result in a modification of the physical properties of the collagen (e.g.: elasticity), but the detailed effects of AGEs on collagen nano-mechanics are still unknown. The mechanical effects of AGEs on collagenous tissues are long known and include stiffening, increased failure load and decreased visco elasticity of tendons (Galeski et al., 1977; Andreassen et al., 1981; Daniel sen and Andreassen, 1988; Li et al., 2013). Various studies have shown that already a single non enzymatic crosslink (in addition to the physiological enzymatic ones) is able to drastically change the mechanical properties of collagen tissues, inducing increased stiffness and decreased toughness. Intermolecular crosslinks is likely ≈ 10 per molecule and thus undoubtedly responsible for the observed stiffening of collagen tissues [3].

IV. SECTION III: BM MODEL

Among various BM models we select the BM model proposed in [4]. As per structural mechanics the architecture of the BM is remarkably similar to the architecture of a reinforced slab of concrete [4]. The ground substance may act as the concrete and the fiber bundles can be thought as the reinforcing steel bars as shown in Figure 7. It means that the preferential reinforcement of the BM along its width is designed to particularly enhance the, otherwise poor, ability of the BM ground substance to support large radial forces. This is similar to the manner by which reinforcing steel bars greatly increase the tensile strength of concrete along the direction of reinforcement.

At any given location along the cochlea, the BM is modeled as a plate as shown in Figure 7. The dimensions of the plate change with position along the cochlea. The width of the plate is oriented along the radial direction (x) of the cochlea. The length of the plate is oriented along the length (y) of the cochlear spiral.

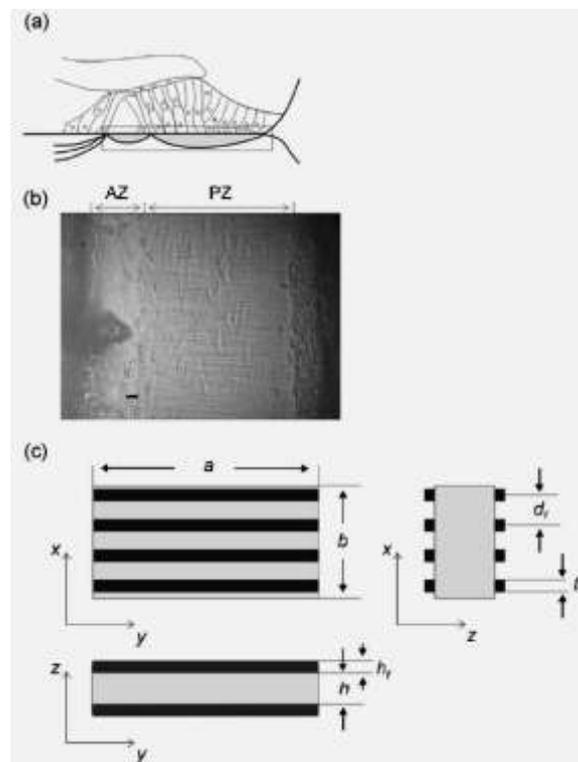


Figure 7 BM Plate Approximations for Model [4]

The BM plate is composed of two components: (a) Anisotropic plate of BM ground substance; and (b) two sets of fiber bundles, which travel along the top and bottom of the plate in order to preferentially reinforce the plate along its width. Due to the preferential reinforcement by the fiber bundles along the y direction, the plate is an isotropic, whereby it has different mechanical properties along its width and length.

4.1 Determination of BM Plate Dimensions

At a given position, x , along the cochlea, the dimensions of the plate are determined as follows. The width of the plate, a , is equal to the width of the BM, $w(x)$. The length of the plate, b , is calculated as

$$b(x) = 5\lambda_c(x), \quad (1)$$

where $\lambda_c(x)$ is the space constant that describes the amount of BM longitudinal coupling[5]. As shown in the figure 8 the plate length is taken as per longitudinal coupling involved which means relevant length affected by deflection for model estimation

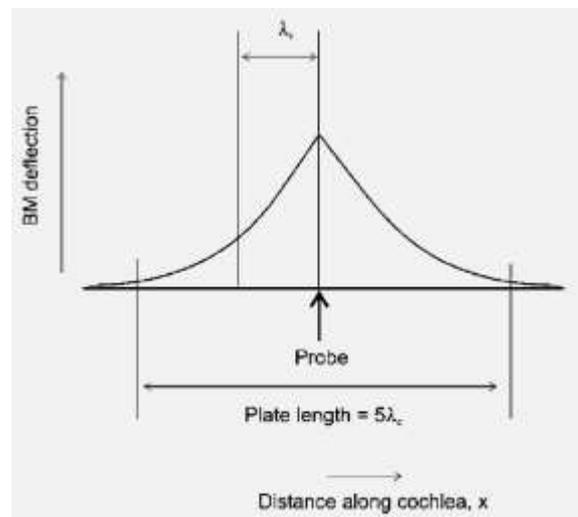


Figure 8: Deflection Profile from Excitation [4]

The effective thickness, $h(x)$ of the plate is calculated as the cross-sectional area of the BM divided by its width.

4.2 Determination of BM Plate Material Properties

The deflection equation of a plate is determined by its flexural rigidities, D_x , D_y , and H , which are functions of the plate architecture, the elasticity of the fibers, and the elasticity of the ground substance. Given the preferential reinforcement of the plate along the radial (x) direction, the flexural rigidities, D_x , D_y , and H , of the plate can be calculated (Timoshenko and Woinowsky- Kreiger, 1959) to be

$$D_x(x) = \frac{E_g h^3(x)}{12(1-\nu^2)} \quad (2)$$

$$D_y(x) = \frac{E_g h^3(x) + E_f I_f(x)}{12(1-\nu^2) d_f(x)} \quad (3)$$

$$H(x) = D_x(x) \quad (4)$$

Where E_g and E_f represent the elasticity moduli of the ground substance and fiber bundles, respectively, ν is Poisson's ratio of the ground substance, $h(x)$ is the thickness of the BM plate, $d_f(x)$ is the spacing between fiber bundles, and

$I_f(x)$ is the moment of inertia of each set of fiber bundles (top and bottom) with respect to the middle axis of the cross section of the plate calculated as

$$I_f(x) = \frac{1}{12} t_f(x) h^3(x) [1 - (1 - 2h_f(x))^{-3}]$$

This can be approximated as

$$I_f(x) = \frac{1}{12} t_f(x) h^2(x) h_f(x) \quad (5)$$

Where $h_f(x)$ is the height of fiber bundles and $t_f(x)$ is the thickness of fiber bundles.

4.3 Parameter Estimation

The model was used to determine BM tension at six locations along the cochlea [4]. Table 1 lists the experimental measurements which were used to calculate the model dimensions and mechanical properties.

Parameter	Symbol	Value						Reference	mm
Location from base	x	1.3	3.61°	4.55	6.86°	7.54	11.26°	1, 2	mm
BM width	a	149	168	194	211	231	259	A, 2	μm
Coupled length	b	54	67	83	96	110	136	3	μm
BM height	h	8.54	12.5	13.1	15.0	15.9	17.7	1	μm
Bundle thickness	h_b	1.5	1.02	0.77	0.57	0.59	0.28	2	μm
Bundle width	t_f	1.37	1.13	1.03	0.80	0.73	0.35	2	μm
Bundle spacing	d_f	1.58	1.60	1.61	1.62	1.63	1.67	A	μm
Point stiffness	k_p	1.98	1.26	0.72	0.46	0.28	0.12	4	N/m
Poisson's ratio	ν	0.4	0.4	0.4	0.4	0.4	0.4	5	N/m

Anatomical dimensions of the fiber bundles were obtained in following manner. The thickness and height of the fiber bundles were determined from table 1 [6]. At each location of interest, the average thickness of the fiber bundles was calculated as the mean of the measured thickness of the upper and lower fiber bundles. The spacing of the fiber bundles at locations in the three turns were obtained using digitized images of the BM in excised turns of gerbil cochlea.

The stiffness at the center of the BM was estimated from the measurements of Naidu and Mountain (1998b) as being equal to one-half the stiffness of the OC as measured under the outer hair cell region. The stiffness was experimentally measured at a BM deflection of z_0 approximately equal to $5\mu\text{m}$. Measurements of longitudinal coupling of the BM were obtained from Naidu and Mountain (2001). Poisson's ratio of the ground substance of the BM was estimated to be 0.4 from measurements on dog lung tissue by Lai-Fook *et al.* (1976).

The ratio of the elasticity of the fibers to the elasticity of the ground substance was estimated to be 100. This ratio value makes the BM weakly an isotropic in the apex, which is consistent with the experimental measurements of Naidu and Mountain (1998a).

4.4 Model Solutions

The general equation describing the deflection, $z(x, y)$, of the anisotropic BM plate in response to a generalized load, $q(x, y)$, and tension, N_y , along the y direction is given by

$$D_x \frac{\partial^4 z(x, y)}{\partial x^4} + 2H \frac{\partial^4 z(x, y)}{\partial x^2 \partial y^2} + D_y \frac{\partial^4 z(x, y)}{\partial y^4} - N_y \frac{\partial^2 z(x, y)}{\partial y^2} = q(x, y), \quad (A1)$$

where the flexural rigidities D_x , D_y , and H are given by Eqs (2)–(4).

The deflection in response to a concentrated force, P , applied at the center of the plate may be then obtained from Eq(A1) in the form of a trigonometric series (Timoshenko and Woinowsky-Krieger, 1959).

$$z(x,y) = \frac{4P}{ab\pi^4} \sum_{n=1,3,\dots} \sum_{m=1,3,\dots} \frac{\sin \frac{m\pi}{2} \sin \frac{n\pi}{2}}{\left[D_x \frac{m^4}{b^4} + 2D_x \frac{m^2 n^2}{a^2 b^2} + D_y \frac{n^4}{a^4} + \frac{N_y m^2}{\pi^2 a^2} \right]} \times \sin \frac{m\pi x}{b} \sin \frac{n\pi y}{a}, \quad (A2)$$

where H has been substituted by D_x using Eq (4). After approximation the deflection profile is then given by the expression

$$z(x,y) = \frac{4Pab}{K\pi^4 D_x} \frac{1}{(1+\alpha)} \sin \frac{\pi x}{a} \sin \frac{\pi y}{b}, \quad (A3)$$

where

$$K = \left(\frac{D_x b^2}{D_x a^2} + 2 + \frac{a^2}{b^2} \right) \quad (A4)$$

and

$$\alpha = \frac{N_y b^2}{\pi^2 D_x K} \quad (A5)$$

Two constraints are then applied to calculate the BM tension. First, the physiological stiffness, kp , is measured experimentally at the center of the BM at a deflection, z_0 in response to a force, P . The measured stiffness must be equal to the stiffness at the center of the plate calculated from the deflection profile using Eq (A3) as follows:

$$\frac{1}{k_p} = \frac{dz(a/2, b/2)}{dP} \Big|_{z_p} = \frac{z_0(a/2, b/2)}{P} = \frac{4ab}{K\pi^4 D_x} \frac{1}{(1+\alpha)} \quad (A6)$$

Second, during deflection, the tensile strain must balance the strain due to deflection. The strain, ϵ_y , generated in the plate by the tensile stress, σ_y

$$\epsilon_y = \frac{\sigma_y}{E_y} = \frac{N_y(1-\nu^2)}{hE_y}, \quad (A7)$$

where E_y represents the equivalent elasticity modulus along the y direction, which is related to the flexural rigidity D_y by

$$D_y = \frac{E_y h^3(x)}{12} \quad (A8)$$

The strain, ϵ_d due to deflection is given by

$$\epsilon_d = \frac{1}{2a} \int_0^a \left(\frac{dz(b/2, y)}{dy} \right)^2 dy, \quad (A9)$$

where the right hand side of the equation is the ratio of the plate extension produced by deflection to the width a of the plate (Timoshenko, 1955). The plate extension is approximated by the difference between the arc length of the deflection profile and a . Equating the tensile strain and strain due to deflection, we get

$$\frac{N_y(1-\nu^2)}{hE_y} = \frac{1}{2a} \int_0^a \left(\frac{dz(b/2, y)}{dy} \right)^2 dy. \quad (A10)$$

Substituting for $z(b/2, y)$ using Eq. (A3), and substituting for E_y using Eq. (A8), Eq. (A10) becomes

$$\frac{N_y h^2}{3D_y} = \left[\left(\frac{4Pab}{k\pi^4 D_x} \right) \left(\frac{1}{1+\alpha} \right) \right]^2 \left(\frac{\pi}{a} \right)^2. \quad (\text{A11})$$

By dividing Eq. (A11) by Eq.(A6) and substituting for N_y using Eq. (A5), the value of α is calculated as

$$\alpha = \frac{3}{Kh^2} \frac{b^2}{a^2} \left(\frac{D_y}{D_x} \right). \quad (\text{A12})$$

The tension, N_y is then be obtained from α by dividing Eq. (A5) by Eq. (A6)

$$N_y = \frac{4a}{\pi^2 b} \frac{\alpha}{(1+\alpha)} k_p. \quad (\text{A13})$$

D_x and D_y may then be calculated from Eqs. (A5),(3), and (4).

4.5 Model Solution

The model is used to calculate BM tension by simulating the experimental measurements of BM stiffness (Naidu and Mountain, 1998b). An analytical expression is derived for the deflection profile of the plate in response to a concentrated force, which is applied at the center of the BM. The deflection profile is a function of the elasticity of the fibers and ground substance, and the radial tension. Since the ratio of the elasticity of the fibers and ground substance is assumed to be 100, the deflection profile is only a function of two unknown variables, the elasticity of the ground substance and the radial tension. Therefore, only two equations, instead of three, are required to solve the tension of the BM. The two equations are determined as follows. The first equation is obtained by equating the calculated stiffness to the stiffness as measured experimentally. The second equation is derived from the constraint that edges of the BM are not observed to move towards each other when the BM is deflected during the experimental stiffness measurement.

Mathematically, the constraint is equivalent to balancing the strain developed in the BM due to deflection by the strain developed in the BM due to tension. The two equations formulated as described above are solved simultaneously to calculate the tension in the BM.

4.6 Model Results

Figure 9(a) shows the calculated BM tension $N_y(x)$ plotted as a function of position along the length of the cochlea. The tension decreases by about two orders of magnitude from a value of about 0.76 N/m at the base to about 0.001 N/m at the apex. The continuous variation in tension, in units of N/m, with distance x along the cochlea is described by a regression fit to the predicted values, which is given by the function

$$N_y(x) = 1.51 e^{-0.58x},$$

where x is specified in units of mm. The corresponding tensile stress $\sigma_y(x)$ acting on the BM was calculated by dividing $N_y(x)$ by the effective height, $h(x)$, of the BM plate. Figure 9(b) shows the calculated tensile stress as a function of position along the length of the cochlea. The continuous variation in tensile stress, in units of N/m², with distance x along the cochlea is described by a regression fit to the predicted values, which is given by the function

$$\sigma_y(x) = 1.67166 \times 10^5 e^{-0.65x},$$

where x is specified in units of mm. The predicted flexural rigidities of the plate, D_x and D_y , are plotted as a function of position along the cochlea in Fig.4. The continuous variation in D_x and D_y , in units of Nm, with distance x along the cochlea are described by the regression fit to the predicted values, which are given by the functions

$$D_x(x) = 3 \times 10^{-11} e^{-0.01x}$$

$$D_y(x) = 3.3 \times 10^{-9} e^{-0.33x}$$

where x is specified in units of mm.

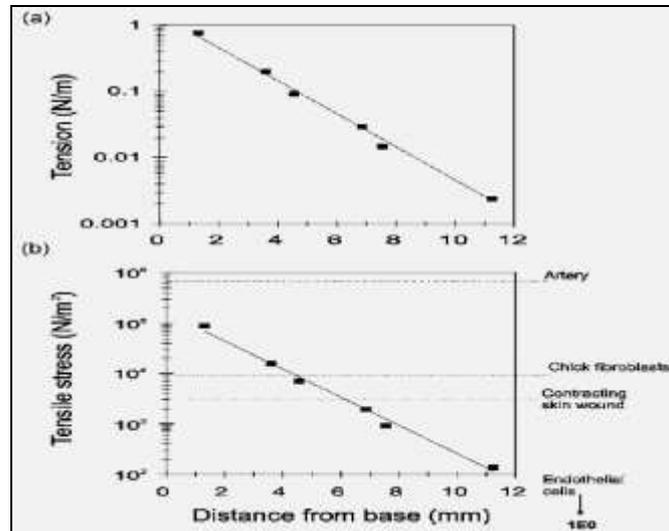


Figure 9: a) BM tension profile and b) BM tensile stress profile [4]

V. SECTION IV: EFFECTS OF DIABETES

Besides the elderly, people who suffer with type II diabetes are particularly badly affected by AGE cross-linking (Andreassen et al., 1981; Schneider and Kohn, 1982). Whilst elder people have abundance of long-lived proteins that have slowly accumulated AGE crosslinks, type II diabetics have abnormally high levels of glucose in their system leaving a surplus which is available to glycate proteins.

The deflection profile equation (A3) in BM mathematical model contains K which is function of D_x and D_y is a diabetic parameter.

As D_x and D_y are dependent E_f which represent the elasticity moduli of fiber bundles, which is altered by prolonged diabetic condition.

Cochlear active model proposed in [7] involves the motility factor α . Motility factor α varies due to loss of hair cells under prolonged diabetic condition [8]

BM model proposed in [4] involves K which varies due to glycation of collagen fibers involved in BM structure

VI. CONCLUSION

After identifying diabetic parameter K , we propose that the realistic variations of K should be imposed on the mathematical model to investigate the impairments caused by diabetes.

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