Original Article

Estimation of Volume of Stria Vascularis and the Length of Its Capillaries in the Human Cochlea

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Abstract

Background: The stria vascularis (SV) is a vascularized epithelium that secretes endolymph and is located on the lateral wall of the membranous cochlea. The capillaries of SV directly influence the composition of the endolymph and hence the generation of impulses by the hair-cells that are auditory receptors and thus affect hearing. Therefore, the real morphology of the SV would be very important for understanding the hearing system. There are few reliable reports of the morphology of the human SV. **Aims and Objectives:** In this research, we have estimated the volume of the SV and total length of strial capillaries in the apical, middle and basal turns of the human cochlea by updated stereological techniques. **Methods:** The point-counting Cavalieri's method and hemispherical volume probes were applied on stained, 40 µm-thick serial sections of five celloidin-embedded, decalcified cochleae. **Results:** The mean age of persons at the time of death was 51 ± 15.25 years, the mean volume of the SV was 0.56 ± 0.054 mm3 and the mean length of the SV capillaries was 289.08 ± 72.96 mm. We also estimated the same parameters with different stereological parameters, probes and in differently stained sections and checked the relationship and limits of agreement between different methods by paired T-test and Bland-Altman plot. We found agreement in our results. **Conclusion:** We provide reliable baseline data on the real morphology of the human SV.

Keywords: Cavalieri estimator, hemisphere volume probe, metabolic presbycusis, morphometry, spiral ligament

Introduction

Stria vascularis (SV) is a highly vascular epithelium found in the lateral wall of the membranous labyrinth that is situated within the bony canal of the cochlea.[1] The SV secretes endolymph through a process of active pumping of ions such as Na^+, K^+, and Cl^- and the passive diffusion of water molecules. Endolymph bathes the hair cells of the organ of Corti, which is the auditory receptor, and helps to maintain the positive endocochlear potential.[1] The secretion of endolymph (in quantity and quality) is directly related to the morphology of the SV, i.e., the total volume of the SV and also the total length of capillaries within the SV.[2] Thus, the morphology of the SV would affect the functioning of the auditory receptors because the hair cells of the organ of Corti are extremely sensitive to ionic changes in their environment, and the composition of the endolymph can be altered by changes in the morphology of the SV.[2] These changes over time can affect the quantity and quality of hearing.[3] Metabolic presbycusis (age-related hearing loss) is associated with strial atrophy and decreases in the number of strial capillaries.[3] Previous investigators have reported changes in the spiral ligament with aging[4] and concluded that its atrophy preceded the degenerative changes in the SV. Another group of investigators[5] using design-based stereology confirmed these findings. However, Jorgensen[5] did not find any correlation between the degree of strial atrophy and degeneration of the organ of Corti. Therefore, there is continuing debate regarding the true morphology of the SV, its capillaries, and the consequences of these changes on hearing. In such a scenario, it becomes important to provide reliable, baseline morphological data on the SV that can be used to compare future studies that may explore the causes of metabolic presbycusis in humans. A reliable morphological tool is a design-based stereology that estimates morphometric parameters independent of considerations of shape and size of the region of interest. We have already used this method

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in our laboratory to study aging and development of different components of the auditory pathway.[6-11] In this study, we have used modern stereological methods to provide reliable, baseline morphological data on the human SV.

Materials and Methods

Specimen collection and tissue preparation

We collected cadaveric, human temporal bones in accordance with the protocol approved by the Institute Ethics Committee (Ref no. IESC/T-438/26.08.2015) and according to the guidelines of the Helsinki Declaration from the mortuary after obtaining written consent from the legal representatives. All the donors were male, died of various causes without any head injury. The donors were 31, 45, 49, 58, and 72 years old at the time of death [Table 1]. The bones were fixed by immersion in 4% buffered paraformaldehyde (0.1 M phosphate buffer, pH 7.4), decalcified in 10% EDTA solution, dehydrated in ascending grades of alcohol, impregnated with celloidin, and embedded in 8% celloidin [Supplementary Figure 1]. The celloidin blocks were sectioned serially on a sliding microtome, at 40 μm thickness, and every tenth section (starting with a random number between 1 and 10), containing the SV, was stained with hematoxylin and eosin (H and E), as per the standard protocol.[12] A series of every tenth section starting with a different random number in the sample obtained from a 31-year-old individual was stained by Masson's trichrome method as per the standard protocol.[12]

Stereological estimates

Every tenth, systematically and randomly selected section (the first section was selected using a random number series between 1 and 10, both numbers included), stained and observed under the Olympus BX61 research microscope attached to a computer, motorized stage controller (LUDL, Germany) and video camera (MBF Biosciences, CX 9000). The live images of the cochlear serial sections were analyzed using the StereoInvestigator software (MicroBrightField Inc., VT, USA), and the volume of SV and the total length of capillaries within it were calculated by the Cavalieri estimator and hemisphere probes, respectively. Before applying the probes, we first identified the turns of the cochlea under the 2X objective lens. The cochlea having the form of a conical helix; in horizontal sections, the basal turn (BT) appeared to be the largest, followed by the middle turn (MT) and apical turn (AT) in size and distance from the base of the cochlea [Figure 1]. The turns of the cochlea appeared as either one or two cross-sections or a single tangential section. Unlike Kurata *et al*.,[3] we did not differentiate between the upper and lower BTs. Next, the boundaries of the SV were identified as described previously[13] [Figure 2].

Table 1: Details of the human temporal bones used in this study

Age (years)	Cause of death
31	Poisoning
45	Fall from height (no head injury)
49	Hypothermia
58	Stroke
72	Cardiopulmonary arrest

Time interval between death and postmortem examination was 6-12 h in each case; all samples were derived from male cadavers, and the temporal bones of the left side were processed for examination

Estimation of volume of stria vascularis by Cavalieri probe

Every tenth section of the series was selected, and contours of all the areas of SV were marked under low magnification (×2 objective lens). Each section contained from one to six areas of SV. Under higher magnification (×40 objective lens), differently colored lines were used to mark out the contours of the SV in the BT, MT, and AT [Supplementary Figure 2]. The Cavalieri probe was separately applied to the contours, and the grid points falling within contours were painted using different markers for the three turns of the cochlea [Supplementary Figure 3]. The total volume of SV, in each turn, was calculated using the formula described before.[14,15]

Estimation of total capillary length by hemispherical probe

The hemisphere probe was applied to estimate the length of capillaries within the SV of the cochlea, in every tenth section. After the contours of the SV in the different turns of the cochlea were drawn as described above, a hemispherical probe with a radius of 35 μm was applied within each contour, within a grid of 70 μm by 70 μm. We applied a guard zone of 2 μm on the upper and lower sides of the section. Sampling sites were generated by the software as a part of systematic random sampling workflow. The sections were then scanned through the whole thickness, and markers applied wherever the spline (line passing through the center of the capillary) of the capillaries intersected the probe [Supplementary Figure 4].[15]

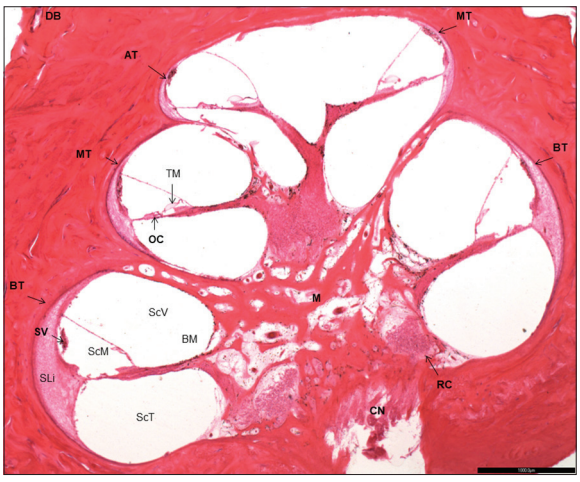

Figure 1: Photomicrograph of hematoxylin and eosin-stained 40 μm thick celloidin section of the human cochlea showing basal turn (BT), middle turn (MT), apical turn (AT), scala media (ScM), scala vestibuli (ScV), scala tympani (ScT), stria vascularis (SV), spiral ligament (SLi), tectorial membrane (TM), basilar membrane (BM), and the organ of Corti (OC). The decalcified temporal bone (DB) shows a central modiolus (M) containing the Rosenthal's canal (RC) in which lies the spiral ganglion neurons from which emerges the cochlear nerve (CN) (scale bar = 1000 μm)

All the intercepts from every tenth section were counted, and the length of capillaries was calculated using the following formula for the hemispherical probe:

$$L = (\sum I_i) \times v / a \times 1 / ssf$$

Where,

n: Number of sections used (I = 1 to n)

I_i: Intersections counted

v: Volume ("grid-X" × "grid-Y" × section thickness)

a: Surface area of the sphere

ssf: Section sampling fraction

The volume of SV was also estimated by the hemispherical probe as a part of the reference volume in estimating the total length of capillaries (calculated from the V_{ref} according to the formula V_{ref} = number of hemispherical probes × grid area × height of the section).

We used the paired *t*-test and the Bland–Altman plots to compare the estimated total length of capillaries visualized by two different staining techniques, i.e., Masson's trichrome and H and E in sections obtained from the 31-year-old individual. We also used these statistical tests to compare the estimates of SV capillary length obtained using slightly different parameters for the hemispherical probe [parameters have been tabulated; Table 2].

The coefficient of error was calculated as follows:

$$CE = \sqrt{TotalVar/S^2}$$

$$TotalVar = s^2 + VAR_{SRS}$$

$$s^2 = \sum_{Qi\,i=1\,to\,n}$$

Variance due to systematic random sampling,

$$VAR_{SRS} = 3\,(A\text{-}s^2)\,\text{-}4B + C/240, m = 1$$

$$A = \sum (Q_i -)^2$$

$$B = \sum Q_i^- \times Q_{i+1} -$$

$$C = \sum Q_i^- \times Q_{i+2}$$

Statistical analysis

The data are represented as individual values in mm for length and mm/mm^3 for length density and mm^3 for volume. A Q-Q plot was made to verify if the data of volume estimation of the SV obtained by the Spaceball and Cavalieri probes distributed normally. Further, paired *t*-test and tests of agreement (Bland–Altman plot) between estimates of volume were obtained from the Cavalieri probe and the hemispherical probe. We used Microsoft Office Excel 2007 (Microsoft Corporation, California, USA) and SPSS version 17 (IBM, New York, USA) for statistical analysis, where required.

Results

Morphology of the stria vascularis

We studied the cochlea and its parts in mid-modiolar sections [Figure 1].

The SV was a stratified cuboidal epithelium in the lateral wall of the scala media [Figures 2 and 3]. It was made of three cellular layers – marginal, basal, and intermediate. Capillaries were seen among the epithelial cells [Figure 4]. Marginal cells were the outermost layer of cuboidal cells [Figure 4]. Basal cells were found at the interface between spiral ligament and SV and had one to three cell layers. Their nuclei were predominantly heterochromatic, were flattened, and were observed to lie parallel to the apical surface of marginal cells. Like the marginal cells, some basal cells contained brown pigment granules in their cytoplasm [Figures 4 and 5]. Intermediate cells were found lying between marginal and basal cell layers. Their nuclei were round, centrally placed within the cells, darker, and smaller than those of the marginal cells. Their cytoplasm formed a thin layer around their nuclei and was thrown into processes that enclosed the surrounding intercellular spaces. Some intermediate cells also contained brown pigment granules in their cytoplasm [Figure 5], which were also seen in their cytoplasmic processes, extending toward neighboring cells. The capillaries of the SV were branches of the blood vessels found in the spiral ligament and the SP. Both longitudinal and cross-sectional profiles of capillaries with a single layer of endothelial cells were found within

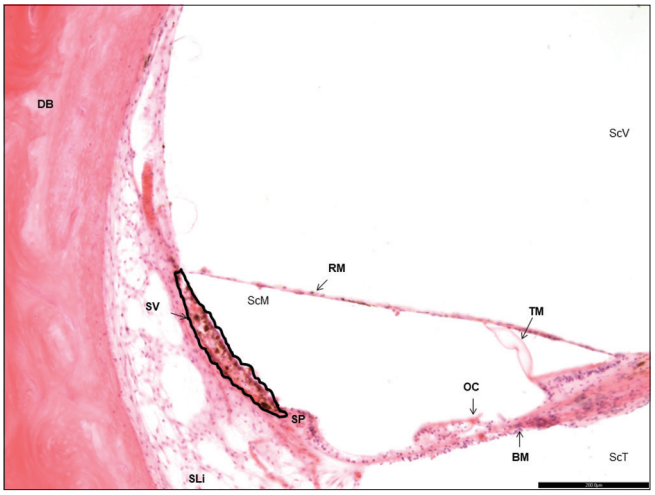

Figure 2: Photomicrograph of hematoxylin and eosin-stained 40 μm thick celloidin section of the human cochlea showing the structures within the scala media (ScM). A contour (black outline) was drawn to show the extent of the stria vascularis (SV), which in this case extends from the spiral prominence (SP) to the attachment of the Reissner's membrane (RM). It rests on the fibrous spiral ligament (SLi). The section also shows the scala tympani (ScT) and the scala vestibuli (ScV). The sensory organ of Corti (OC) lies on the basilar membrane (BM); while the tectorial membrane (TM) that covers it is separated in this section. The spiral ligament rests on the bony wall (decalcified bone-DB) of the turn of the cochlea (scale bar = 200 μm)

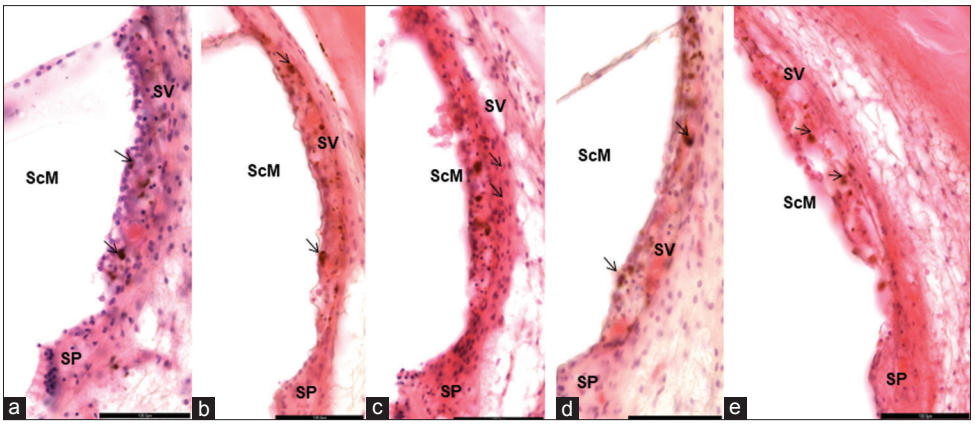

Figure 3: Photomicrograph of hematoxylin and eosin-stained celloidin sections of the middle turn of cochleae of (a) 31-, (b) 45-, (c) 49-, (d) 58-, and (e) 72-year-old samples, respectively, showing the stria vascularis (SV) having some cells that contain brown pigments (arrows). SP, spiral prominence; ScM, scala media (scale bar = 100 μm)

Table 2: Length of strial capillaries in basal, middle, and apical turns of the cochlea in mm

Serial number	Age (years)	Basal turn (mm)	CE (m=1)	Middle turn (mm)	CE (m=1)	Apical turn (mm)	CE (m=1)	Total (mm)	Total CE (m=1)
1	31	197.35	0.06	84.03	0.09	41.25	0.12	322.64	0.04
2	45	165.52	0.06	92.18	0.10	23.43	0.15	270.44	0.05
3	49	268.14	0.05	89.13	0.08	36.15	0.13	393.69	0.04
4	58	173.42	0.06	49.15	0.10	36.16	0.12	258.72	0.05
5	72	120.45	0.07	53.48	0.10	25.97	0.15	199.90	0.05
Mean	51±15.25	184.98±54.19		73.59±20.60		32.59±7.55		289.08±72.96	

CE: Coefficient of error (Gundersen, m=1, to account for biological variability)

Table 3: Comparison of the estimation of total volume of stria vascularis by two stereological probes - Cavalieri estimator and hemispherical probe - in mm^3

Serial number	Age (years)	Cavalieri estimator (mm^3)	Hemispherical probe (mm^3)
1	31	0.58	0.60
2	45	0.58	0.60
3	49	0.63	0.65
4	58	0.51	0.52
5	72	0.50	0.52
Mean	51±15.25	0.56±0.054	0.58±0.057

the intermediate layer. Red and white blood corpuscles were visible within most of the sections of capillaries.

Volume of stria vascularis

The total volume of the SV in the five samples has been tabulated [Table 3]. The volumes of the individual turns of the cochlea in each sample have also been tabulated [Table 4]. The volume of SV was also estimated by the hemispherical probe as a part of the reference volume in estimating the total length of capillaries. The volumes of SV, calculated by these two methods in individual samples, were compared, plotted on a Q-Q plot [Figure 6] which gave a straight line (slope r^2 = 0.99). The limits of agreement were also calculated by Bland–Altman test [Figure 7]. Further, there was no significant difference between the estimated volume of the SV by Cavalieri and the hemispherical volume probe (paired t-test, P = 0.622).

Length of capillaries in stria vascularis

The total length of capillaries in the SV of the five samples was estimated by the hemispherical probe and has been tabulated [Table 2]. The length of capillaries in the individual turns of the cochlea for each sample has also been tabulated [Table 2] and compared. In brief, the length of the SV capillaries decreased from base to apex in all the samples. We observed that the length of the SV capillaries of the BT was approximately 2–3 times that of the MT and 4–7 times that of the AT. We also observed that the ratio of the lengths of the SV capillaries in the MT to AT ranged from 1.3 to 2 times that of the AT. The total length of the capillaries in SV was also calculated in sections stained with Masson's trichrome in the sample obtained from the 31-year-old individual, as described above. The total length of SV capillaries in different turns of the cochlea in these three sets of serial sections of this sample was also estimated and tabulated [Table 2]. There was no significant difference between the length of the SV capillaries estimated in the AT, MT, and BT of the cochlea in the sections stained by either H and E or Masson's trichrome method (Student's paired t-test, P = 0.406) or by different grids (70 μm × 70 μm versus 80 μm × 80 μm, P = 0.304) and different stains and grid sizes (H and E, 70 μm × 70 μm versus Masson's trichrome, 80 μm × 80 μm, P = 0.646) [Table 5]. Bland–Altman plots also revealed good agreement between the mean of the estimates

Table 4: Volume of stria vascularis in basal, middle, and apical turns of the cochlea

Serial number	Age (years)	Basal turn (mm³)	CE (*m*=1)	Middle turn (mm³)	CE (*m*=1)	Apical turn (mm³)	CE (*m*=1)
1	31	0.33	0.029	0.16	0.026	0.09	0.040
2	45	0.34	0.025	0.20	0.046	0.04	0.046
3	49	0.41	0.022	0.14	0.034	0.08	0.042
4	58	0.35	0.014	0.11	0.020	0.05	0.031
5	72	0.30	0.013	0.15	0.024	0.05	0.041

CE: Coefficient of error (Gundersen, *m*=1, to account for biological variability)

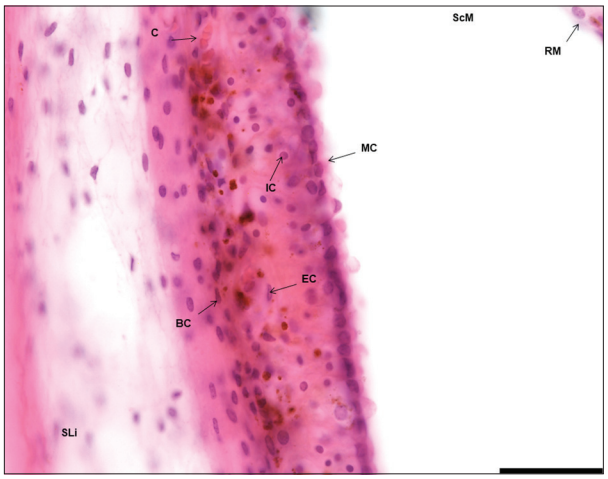

Figure 4: Photomicrograph of hematoxylin and eosin-stained celloidin section of the cochlea showing the stria vascularis (SV) that is made of a stratified epithelium containing marginal cells (MC), basal cells (BC), intermediate cells (IC), and capillaries line with endothelial cells (EC). This figure also shows the spiral ligament (SLi), the scala media (ScM), a small part of the Reissner's membrane (RM), and other capillaries (c) containing red blood cells (RBCs) in the SV (scale bar = 35 μm)

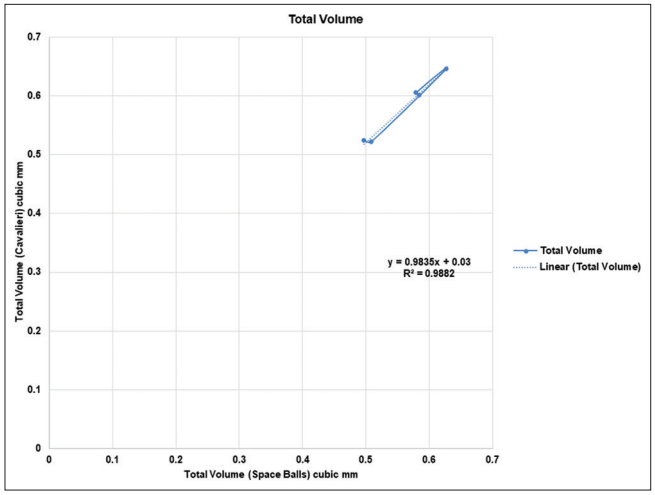

Figure 6: Q-Q plot – the volumes of stria vascularis of five samples estimated by two methods, i.e., Spaceball and Cavalieri estimator were analyzed using a Q-Q plot, where all the values fall on a straight line (of slope ≈ 1) showing that the data are normal in distribution and show little variance between the two methodologies

of the length of capillary of the SV in H and E and Masson's trichrome-stained sections and in H and E-stained sections and

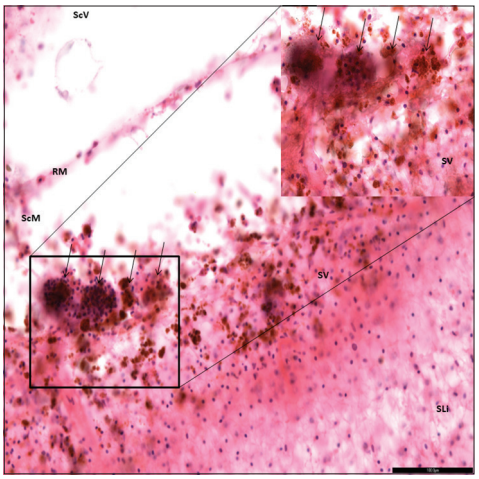

Figure 5: Photomicrograph of hematoxylin and eosin-stained celloidin section of cochlea from a 72-year-old individual showing aggregated brown-colored pigments (arrows) in a tangential section of the stria vascularis (SV) (scale bar = 100 μm). Inset: shows the magnified view of the aggregated pigment depicted by arrows (scale bar = 35 μm)

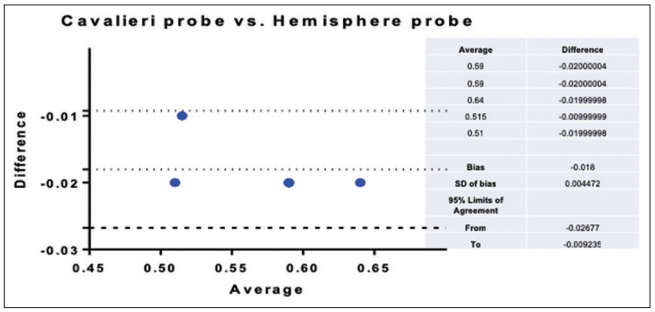

Figure 7: Bland–Altman plot showing the difference of means between the estimation of total volume of the stria vascularis by the grid point-counting Cavalieri probe and the hemispherical probe. The dotted lines indicate the 95% limits of agreement

different grid sizes [Figures 8 and 9]. We have also calculated the length density of the SV capillaries in each turn of the cochlea [Table 6].

Discussion

In this study, we estimated its volume and the total length of capillaries of the human SV in five cochleae and also separately in the AT, MT, and BT.

Design-based stereology provides estimates of number, length, area, and volume of an object of interest without

making assumptions regarding the geometry of the object of interest, and hence, it eliminates potential sources of bias and systematic errors[15,16] that occur commonly with conventional morphometric techniques. In this study, we have used two isotropic probes – Cavalieri estimator and the hemispherical probe – for volume and length estimations, respectively. We have previously used the grid point-counting probe to estimate the volume of the developing inferior colliculus and the adult cochlear nucleus.[6-8]

Capillaries in the SV, which have a regular radial arrangement, as determined in corrosion casts,[17] do not display such features in histological sections of the cochlea because they are tortuous and could be sectioned in a plane independent of the long axis of the capillary, thus behaving as anisotropic elements. Thus, to estimate the total length of the capillaries, we utilized an isotropic hemispherical probe. We had the option of using a two-dimensional probe, like a cycloid, to estimate the length of the capillaries; however, the values obtained would have varied according to the angle of sectioning. This would then require specific mathematical corrections. Therefore, we used the three-dimensional hemispherical probe that is isotropic in nature and thus does not require isotropic sections for its application.[18]

In our study, the volume of SV obtained by Cavalieri method was in agreement to those obtained by hemispherical probe in all the samples [Table 3 and Figure 6]. The length of the strial capillaries also decreased from the BT to the AT. The BT is the largest coil of the helical cochlea and in our study had the maximum volume of SV, and hence, as expected, it also had the maximum length of strial capillaries within it while the AT being the smallest had a lower volume of SV and the least length of capillaries.

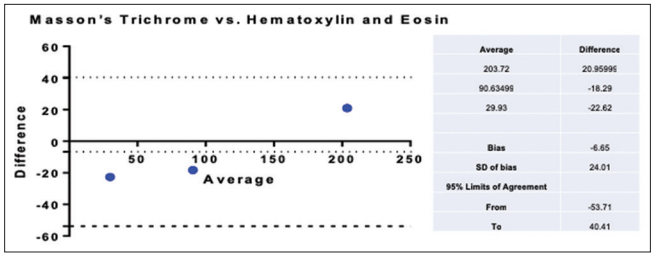

Figure 8: Bland–Altman plot showing the difference of means between the estimation of total length of capillaries of the stria vascularis in hematoxylin and eosin and Masson's trichrome-stained sections. The dotted lines indicate the 95% limits of agreement

It has been seen that the capillary length decreases from the BT to AT in cochleae of gerbils.[19] In this study, we did not use immunohistochemical techniques to identify the capillary endothelial cells. However, we used another staining technique to identify the RBCs and connective tissue – the Masson's trichrome staining technique. We ran the hemispherical probe, with the same parameters, in the sample obtained from the 31-year-old individual and observed that the length of the strial capillaries was almost the same in both methods of identifying capillaries. This, thus, validated our estimates of the total length of the SV capillaries [Table 5] even though neither of the techniques is the gold standard for the identification of a blood vessel. Santi *et al*.[13] reported that the capillary density in the different turns of the cochlea of chinchilla remains constant. We found that the length densities of the BT and MT in 72-year-old samples were 406.85 mm/mm^3 and 363.66 mm/mm^3, respectively, while that of the AT were 484.15 mm/mm^3 [Table 6] and that the length of the strial capillaries and volume of SV were 120.45 mm and 0.30 mm^3, 53.48 mm and 0.15 mm^3, and 25.97 mm and 0.05 mm^3 in the BT, MT, and AT, respectively, of the same sample [Tables 2 and 4]. Hence, on reviewing Tables 2, 4, and 6, we can conclude that length density is not a reliable indicator of the status of strial vasculature. It is better to estimate the total length of the capillaries.

Here, we have standardized a morphometric procedure of studying the SV in human cochlea, and we would like to extend this study in a larger sample set to draw definitive conclusions related to the changes in the SV and its correlation with age-related hearing loss.

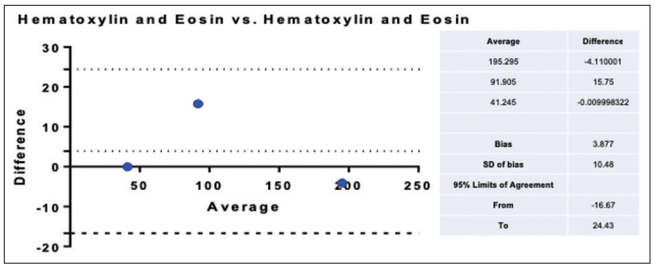

Figure 9: Bland–Altman plot showing the difference of means between the estimation of total length of capillaries of the stria vascularis in hematoxylin and eosin-stained sections of the cochlea, but using different grid sizes in the hemispherical probe (70 μm × 70 μm vs. 80 μm × 80 μm). The dotted lines indicate the 95% limits of agreement

Table 5: Comparison of length estimated by two different staining techniques and stereological parameters for hemispherical probe

Length of capillaries in SV (mm)	Masson's trichrome	Hematoxylin and eosin		Mean (mm)±SD
	Grid 80 μm × 80 μm (mm)	Grid 80 μm × 80 μm (mm)	Grid 70 μm × 70 μm (mm)	
Basal turn	214.2	193.24	197.35	201.60±11.11
Middle turn	81.49	99.78	84.03	88.43±9.91
Apical turn	18.62	41.24	41.25	33.70±13.06
Total length	314.31	334.26	322.64	323.74±10.02

SD: Standard deviation, SV: Stria vascularis

Table 6: Length density of the stria vascularis capillaries in the basal, middle, and apical turns of the cochlea in mm^2

Serial number	Age (years)	Basal turn	Middle turn	Apical turn
1	31	591.76	529.18	447.43
2	45	480.22	468.35	622.23
3	49	657.13	625.89	474.46
4	58	495.41	456.15	718.89
5	72	406.85	363.66	484.14

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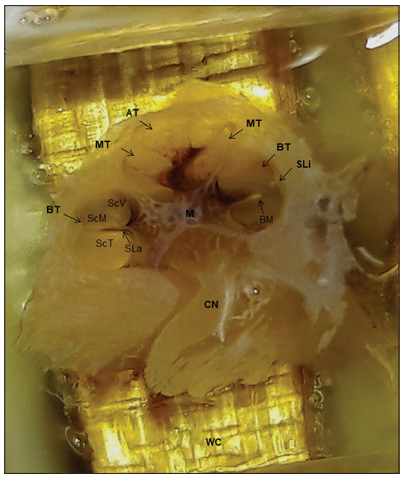
Conflicts of interest

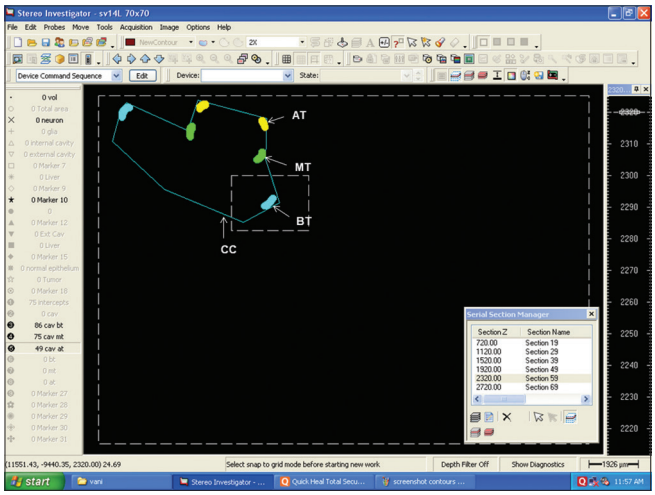
There are no conflicts of interest.

References

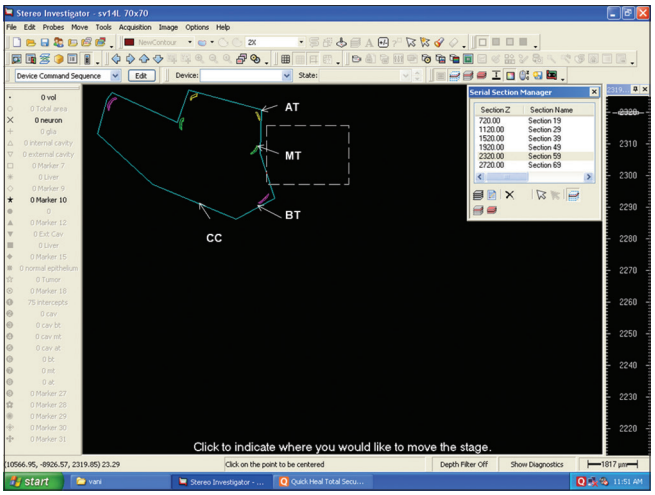
1. Standring S, Anand N, Birch R, Collins P, Crossman AR, Gleeson M, *et al*., editors. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 41st ed. London, UK: Churchill Livingstone/Elsevier; 2016. p. 641-57.
2. Liu H, Li Y, Chen L, Zhang Q, Pan N, Nichols DH, *et al.* Organ of corti and stria vascularis: Is there an interdependence for survival? PLoS One 2016;11:e0168953.
3. Kurata N, Schachern PA, Paparella MM, Cureoglu S. Histopathologic evaluation of vascular findings in the cochlea in patients with presbycusis. JAMA Otolaryngol Head Neck Surg 2016;142:173-8.
4. Kusunoki T, Cureoglu S, Schachern PA, Baba K, Kariya S, Paparella MM. Age-related histopathologic changes in the human cochlea: A temporal bone study. Otolaryngol Head Neck Surg 2004;131:897-903.
5. Jorgensen MB. Changes of aging in the inner ear. Histological studies. Arch Otolaryngol 1961;74:164-70.
6. Sharma V, Nag TC, Wadhwa S, Roy TS. Stereological investigation and expression of calcium-binding proteins in developing human inferior colliculus. J Chem Neuroanat 2009;37:78-86.
7. Sharma S, Nag TC, Thakar A, Bhardwaj DN, Roy TS. The aging human cochlear nucleus: Changes in the glial fibrillary acidic protein, intracellular calcium regulatory proteins, GABA neurotransmitter and cholinergic receptor. J Chem Neuroanat 2014;56:1-2.
8. Sharma S, Nag TC, Thakar A, Bhardwaj DN, Roy TS. Age associated changes in the human cochlear nucleus – A three-dimensional modelling and its potential application for brainstem implants. J Anat Soc India 2014b; 63:12-8.
9. Sharma S, Jacob TG, Nag TC, Thakar A, Bhardwaj DN, Roy TS. Changing population of neurons and glia in the human cochlear nucleus during aging. In: Rath PC, Sharma R, Prasad S, editors. Topics in Biomedical Gerontology. Singapore: Springer; 2017. p. 245-51.
10. Kaur C, Pal I, Saini S, Jacob TG, Nag TC, Thakar A, *et al.* Comparison of unbiased stereological estimation of total number of cresyl violet stained neurons and parvalbumin positive neurons in the adult human spiral ganglion. J Chem Neuroanat 2018;93:30-7.
11. Pal I, Paltati CR, Kaur C, Shubhi Saini, Kumar P, Jacob TG, *et al.* Morphological and neurochemical changes in GABAergic neurons of the aging human inferior colliculus. Hear Res 2019;377:318-29.
12. Bancroft JD. Theory and Practice of Histological Techniques. 6th ed. Philadelphia PA: Churchill Livingstone Elsevier; 2008.
13. Santi PA, Lakhani B, Bingham C. The volume density of cells and capillaries of the normal stria vascularis. Hear Res 1983;11:7-22.
14. Ishiyama G, Tokita J, Lopez I, Tang Y, Ishiyama A. Unbiased stereological estimation of the spiral ligament and stria vascularis volumes in aging and Ménière's disease using archival human temporal bones. J Assoc Res Otolaryngol 2007;8:8-17.
15. West MJ. Basic Stereology for Biologists and Neuroscientists. 1st ed. New York: Cold Spring Harbor Laboratory Press; 2012.
16. Schmitz C, Hof PR. Design-based stereology in neuroscience. Neuroscience 2005;130:813-31.
17. Carraro M, Park AH, Harrison RV. Partial corrosion casting to assess cochlear vasculature in mouse models of presbycusis and CMV infection. Hear Res 2016;332:95-103.
18. Mouton PR, Gokhale AM, Ward NL, West MJ. Stereological length estimation using spherical probes. J Microsc 2002;206:54-64.
19. Prazma J, Carrasco VN, Butler B, Waters G, Anderson T, Pillsbury HC. Cochlear microcirculation in young and old gerbils. Arch Otolaryngol Head Neck Surg 1990;116:932-6.



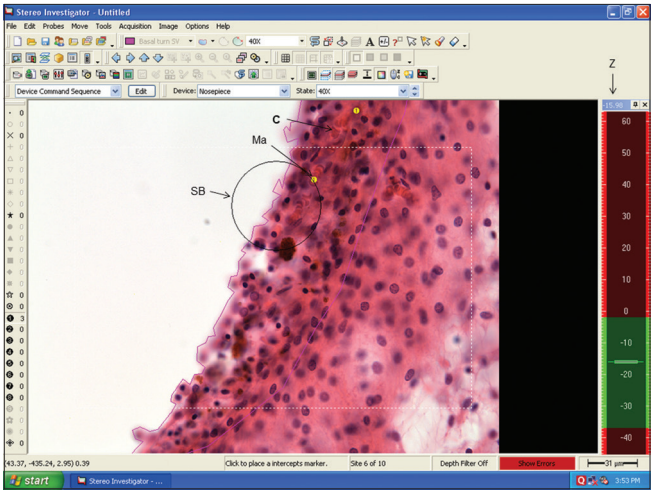
Supplementary Figure 1: Top end view of a celloidin block, containing a cochlea, showing the cutting surface for sectioning. Also seen is the wooden chuck (WC) on which the block was held. The sectioned surface of the cochlea reveals that the cochlear nerve (CN) and the modiolus (M) are parallel to the surface of the wooden block. The section also shows the spiral ligament (SLi), scala vestibuli (ScV), scala media (ScM), scala tympani (ScT), basilar membrane (BM), and the three turns of the cochlea – basal (BT), middle (MT), and apical (AT)



Supplementary Figure 3: Screenshot showing the application of the Cavalieri estimator to measure the volume of the stria vascularis (SV). The grid points of the probe have been painted in with different markers individually for the different turns of the cochlea. The contour of the cochlea is blue (CC); the Cavalieri markers within the contours of the SV of basal turn (BT) were painted in blue, of the middle turn (MT) in green, and that of the apical turn (AT) in yellow



Supplementary Figure 2: Screenshot of the software used to estimate the volume of the stria vascularis (SV), showing the various contours used to outline cochlea (CC – blue), SV of the basal turn (BT – pink), SV of the middle turn (MT –green), and the SV of the apical turn (AT – yellow)



Supplementary Figure 4: Screenshot showing the application of the hemispherical probe (black circle) within the contour of the stria vascularis (SV) of the BT (pink). In the figure, the probe is at a depth 16 μm, indicated by the Z-axis (Z) within the inclusion depth (green part) of the hemispherical Spaceball of radius 35 μm. The intersection of the spline of the capillary with the Spaceball was marked by a yellow marker (Ma). A capillary (C) containing RBCs is also indicated