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Histopathological Assessment of Fibrosis and New Bone Formation in Implanted Human Temporal Bones Using 3D-Reconstruction

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Abstract

OBJECTIVE—To evaluate new bone formation and fibrosis in implanted human temporal bones and relate that to neurosensory elements preservation.

STUDY DESIGN—Human temporal bone histopathology study.

SETTING—Temporal bone laboratory.

SUBJECTS AND METHODS—Ten human temporal bones from eight patients with multichannel cochlear implants and one single-electrode implant were examined under light microscopy and reconstructed using AMIRA 4.1 3D reconstruction software. Volumes of new bone formation, fibrosis and patent area were calculated in each bone.

RESULTS—The amount of fibrosis and new bone formation postimplantation varied among bones. There were no statistically significant relationships between age at implantation or duration of implantation and the overall amount of new tissue in the implanted ear. There was a relationship between total amount of new tissue and preservation of neurosensory elements only in Segment I of the cochlea ($Rho = -.75$, $p \leq .013$). Most of the new tissue was located in segments I and II, while segment III had little to no new tissue formation and segment IV was clear in all of the subjects.

CONCLUSION—New tissue formation postimplantation was related to preservation of neurosensory elements primarily in segment I of the cochlea. In an era of hearing preservation surgery and hybrid cochlear implants, soft surgical techniques are advocated as a means to decrease surgical trauma.

INTRODUCTION

With the re-introduction of a short electrode cochlear implant, intended for patients with residual low frequency hearing, there has been a strong emphasis on hearing-preservation surgery, as first championed years ago by Dr. William House.¹ However, residual hearing is lost in the majority of the patients after cochlear implantation with long or standard electrodes.^{2–5}

Immediate intracochlear changes arise from trauma during the surgical insertion of the electrode and include lateral wall trauma, disruption of the spiral ligament and stria vascularis, fracture of the osseous lamina, and basilar membrane and modiolar injuries.^{6–10} Late changes arise from a host response to the electrode, which involves a tissue reaction consisting of inflammation, fibrosis and new bone formation. Trauma at the site of insertion may also

introduce bone dust into the perilymphatic space, which may contribute to the development of fibrosis and osteoneogenesis.^{11,12}

The presence of new bone and fibrosis in the implanted cochlea, which has been found to extend distal to the electrode tip in some cases,¹⁰ has been shown to affect electrode-tissue impedance¹¹ that might increase electrical stimulus threshold and decrease cochlear implant battery life. It has also been thought to alter psychophysical percepts^{13,14} as well as affect the ability to perform subsequent surgeries, such as explantation and reimplantation of an electrode, as well as future therapies such as regeneration of hair cells and neurons.¹⁵

Since it was first determined that cochlear implants work by directly stimulating spiral ganglion cell bodies, there have been many studies on implanted temporal bones attempting to determine the relationship between new tissue formation and preservation of neurosensory elements within the inner ear, and the relationship between neural preservation and performance.^{7-9, 11,13,16-18} In 2007, investigators used an innovative method based on virtual three-dimensional reconstruction of human temporal bones to evaluate the relationship between cochlear osteoneogenesis and fibrosis, duration of implantation, age at implantation, word recognition score, and total number of spiral ganglion cells.^{15,17} In all of the subjects, the greatest amount of new bone and fibrous tissue was localized to the lower basal turn adjacent to the round window, and it decreased as it progressed apically. No significant correlation was found between the new tissue and the above-mentioned variables. However, a significant correlation between damage to the periosteum of the lateral wall and new bone formation was found.^{6,7, 17}

The goal of the current study was to use the same three-dimensional software (AMIRA 4.1) used by the previous investigators^{15, 17} to reconstruct and evaluate the post-implant intracochlear changes of the temporal bones in our collection. The new tissue was quantified and correlated to age of implantation, duration of implantation and remaining neurosensory elements.

MATERIAL AND METHODS

Our temporal bone bank contains more than 3 dozen bones from patients who received a cochlear implant, including single- and multi-electrode devices. For this study, we selected 6 unilateral and 2 bilateral cases, a total of 10 implanted temporal bones. The other implanted bones were excluded either because of incomplete medical records or missing sections due to their use in other studies. The bones all belonged to female patients, with the exception of one male and included nine multi-electrode implants and one single-electrode implant in a patient with a multi-electrode implant in the contralateral ear (see Table 1). Institutional Review Board approval for this specific study was not required as approval had already been obtained generically for the temporal bone bank and studies that might ensue from that data (IRB # 06-030).

Temporal bones were prepared in the following way: They were first decalcified in EDTA for 8–12 months. Then they underwent celloidin processing, and were cut with a microtome into 20µm sections. Every 10th section was stained with Hematoxylin & Eosin, placed on a slide and described under light microscopy.

Estimated counts of spiral ganglion cells were obtained per cochlea and per segment using the method described by Otte and Schuknecht (Fig. 1A).¹⁹ The first step in graphic reconstruction of the spiral ganglion neurons is to draw the angulated spiral line that represents the outer margin of the spiral canal. The ganglion is divided into four segments by the vertical (midmodiolar) line. In the anatomical classification (Linthicum classification), the spiral ganglion is divided into basal and upper turns (Fig. 1B). The midmodiolar section is what

defines where the basal and upper turns meet. In this classification, the basal turn includes segments I and II and part of segment III. The upper turn includes the rest of the spiral ganglion. The basal turn can be further divided into proximal and distal portions.

The slides containing spiral ganglion cells were selected and the ganglion cells containing a nucleus were counted using an ocular mounted grid at 200× magnification. The numbers obtained were recorded and multiplied by 10 to account for unmounted sections and then by 0.9 to account for cells that would be doubly counted for their location on the interface between sections. Other histological findings recorded included percentage of remaining hair cells and peripheral processes (dendrites). Spiral ganglion cells were computed as percent of normal based on the assumption of approximately 25,000 ganglion cells in the base and 10,000 in the apex in normal ears.¹⁶

3-D Reconstruction

The slides that contain the cochlea were photographed under a 10× objective and uploaded into the computer. There was an average of 30 images per case. To determine the dimensions of the voxels, we used an image of a scale taken with the same objective, uploaded it with Adobe Photoshop and used the Measurement Scale to determine the distance per pixel.

Once the images were uploaded in order into Amira 4.1, the Alignment tool was used to assess the correct orientation of each slide. This setting was resampled and saved, and the file converted into one channel. Next, we used the Label Tool to identify the different structures we wanted the software to reconstruct in a 3-dimensional manner: blue was used to label healthy cochlear space usually filled with peri- and endolymph, white for neo-osteogenesis, red for fibrosis, green for the electrode array pathway, and yellow for Rosenthal's canal. Once every section was labeled and saved, the SurfaceGen tool generated the 3-dimensional cochlea (Fig. 2). Using the Measure tool, the program was able to determine the volumes of each labeled structure in cubic microns.

The Volume Edit tool allows the user to cut any reconstructed structure in many ways. The Transformer Box let us cut the cochlea using straight lines, which was used for segmentation of the cochlea into the traditional four spiral ganglion segments (Fig.3),¹⁹ and into the less traditional, but anatomical, basal and upper turns (Fig.1B, Fig.4). The Draw tool, which allows us to cut the cochlear segments freehand, was used for a more precise segmentation. The section at the center of the modiolus was used as a reference point for segmentation of the cochlea. The resulting segments were reanalyzed with the Measure tool and the data were recorded.

Since each segment is different in volume, we used percentage of new tissue instead of volume in cubic microns. Statistical analysis included the nonparametric Spearman Rho correlation coefficients between new tissue (fibrosis and osteoneogenesis) and spiral ganglion cell counts by entire cochlea and by segment as well as with other variables. Other variables taken into consideration included patient age at implantation, duration of implantation, and estimation of remaining peripheral processes (dendrites) and hair cells. (The relationship between remaining sensorineural elements and postimplantation performance of these and other bones has been previously presented, and results showed no relationship between auditory performance and surviving sensorineural elements).⁷ For the six unilaterally implanted patients, we compared the implanted to the non-implanted bone using paired t-tests. For all statistical tests, criterion for statistical significance was set at $p \leq 0.05$, two-tailed.

RESULTS

The clinical and histopathological characteristics of our subjects are shown in Table 1. There were no statistically significant relationships between age at implantation or duration of

implantation and the overall amount of new tissue in the implanted ear. However, age at implantation did show a significant negative correlation to total new tissue in segment II ($\rho = -.71$, $p \leq .047$), and duration of implantation had a negative correlation with amount of fibrosis ($\rho = -.76$, $p \leq .028$) in segment I, while amount of new bone tended towards a positive correlation in this segment ($.67$, $p \leq .07$).

The volumetric measurements obtained by the 3-D reconstruction program were calculated in μm^3 , but given that cochlear volume decreases towards the apex, we analyzed new tissue formation as a percentage of the total volume. The amount of fibrosis and new bone formation postimplantation varied greatly among studied temporal bones, from an almost histologically normal cochlea (Subject 4) to one that lost more than half of its normal structure (Subject 8R). Across all implanted temporal bone cochlear segments, there was a negative correlation between spiral ganglion cell count and amount of new tissue, although it did not achieve statistical significance ($\rho = -.60$, $p \leq 0.067$). However, this relationship was significant within cochlear segment I ($\rho = -.75$, $p \leq .013$) as was the relationship between new bone and spiral ganglion cell count in segment II ($\rho = -.75$, $p \leq .013$) although total new tissue in segment II was not correlated with spiral ganglion cell count. Most of the new tissue was located in segments I and II, while segment III had little to no new tissue formation and segment IV was clear in all of the subjects in this series (Table 2).

The cochleae were also segmented into basal and upper turns. Almost all fibrosis and osteoneogenesis occur in the basal turn, where the implant is located, but the subjects with the most new tissue had it extend past the tip of the implant (Table 3). Again, the correlation between spiral ganglion cell count and total new tissue was significant for the basal turn ($\rho = -.64$, $p \leq .046$). Segmenting the cochleae in this unconventional way allowed inclusion of other neurosensory elements in the analysis. No bones had any remaining hair cells and only one had peripheral processes in the basal turn, while two bones had a small percentage of hair cells and three had some remaining peripheral processes in the upper turn. Consequently, there was no measurable relationship of these neurosensory elements to the amount of new bone or fibrous tissue present.

Although the subjects differ in a variety of ways in addition to insertion technique, such as etiology, type of device and number of electrodes, as well as whether a revision implant procedure had occurred, we did compare the five round window insertions to the five cochleostomy insertions. There were no statistically significant differences in amount of fibrosis, bone, total new tissue or neurosensory elements remaining overall or for any of the segments.

When comparing overall spiral ganglion cell counts between the implanted and non-implanted sides in the six unilaterally implanted subjects, there was no statistically significant difference in total spiral ganglion cell count (means=13,818 and 16,536), in spite of the difference between ears in presence of new tissue (none in the non-implanted ear). Lack of statistical significance may be at least partially due to the small sample size and large variability. However, on average, segment I of the implanted side had 35% fewer spiral ganglion cells than the non-implanted side (mean=1823, SD=1130 and 2813, SD=1249, respectively; $p \leq 0.037$).

DISCUSSION

In the early years, cochlear implantation used to take place through the round window membrane. Histological studies of a number of temporal bones implanted with a single-electrode device and using this technique show little trauma.¹⁶ Another approach to the basal turn is a cochleostomy anterior to the round window niche, which provides excellent visualization of the scala tympani and modiolus. Temporal bone studies have described a

marked difference in the degree of inflammatory reaction between these surgical approaches, the former being milder. Even though a relationship between intracochlear inflammatory reaction, preservation of neurosensory elements and patient performance has not been found,⁷ there has been a recent interest in emphasizing the round window approach or a soft surgical technique of cochlear implantation for its potential of minimizing insertional injury and therefore maximizing preservation of residual hearing.^{7,12,18}

Our results suggest that a traumatic insertion (the temporal bones available for the current study are from patients implanted prior to the era of ‘softer’ insertion techniques) can produce the formation of new bone and fibrous tissue. This is based on the fact that segment I is the most affected by new tissue formation in 8 of the 10 temporal bones studied, and most of the new tissue is confined to the basal turn of the cochlea, where the electrode was located. These findings are consistent with those of Li et al.¹⁷ The amount of new tissue was found to have a negative correlation with spiral ganglion cell population in segment I in our subjects, and new bone in particular (as versus fibrosis) was related to spiral ganglion cell count in segment II with a trend in this direction in segment I. Interestingly, correlations of duration of implantation with new tissue tended to be negative for fibrosis and positive for bone, suggesting what we would expect, that with a longer time period fibrosis becomes bone.

It is important to point out that even though we found a relationship between new tissue formation and loss of spiral ganglion cells surrounding the site of electrode insertion, it would be premature to conclude that it is simply a cause-effect relationship. There are other variables to consider, such as anatomical variability, severity of the disease, or small sample size. In addition, the bones in this study were from older adults and it is possible that the results may not be applicable to younger patients. Similarly, we had almost all bones from female patients so generalization may be limited in this way. We should also consider that implantation usually takes place in the ear with the most severe hearing loss. In six cases of severe sensorineural hearing loss in our temporal bone bank with one ear worse than 100 dB, poor enough to need an implant, the poorer hearing ear had fewer ganglion cells (1,521) than the contralateral side (2,781) (Linthicum, unpublished data). As the poorer hearing ear is usually the one chosen for implant, we might expect fewer ganglion cells in implanted ears compared to contralateral ears just as a result of the hearing loss. In the current study, there was no statistically significant difference in overall spiral ganglion cell counts between the implanted and non-implanted sides, with a difference only in segment I where most new tissue growth occurs in implanted ears. Thus, it is not clear how much role new tissue growth may play overall in relation to spiral ganglion cell survival. Further data is required for better analysis and more accurate conclusions.

CONCLUSION

Using Amira 3D reconstruction software, we found an inverse relationship between the reaction to the presence of the implant (i.e., new tissue formation) and the preservation of neurosensory elements primarily in Segment I of the cochlea. In an era of hearing preservation surgery and hybrid cochlear implants, soft surgical techniques are advocated as a means to decrease trauma and new tissue formation.

REFERENCES

1. House WF. Cochlear implants: it's time to rethink. *Am J Otol* 1994;15:573–587. [PubMed: 8572056]
2. Boggess WJ, Baker JE, Balkany TJ. Loss of residual hearing after cochlear implantation. *Laryngoscope* 1989;99:1002–1005. [PubMed: 2796546]
3. Rizer FM, Arkis PN, Lippy WH, et al. A postoperative audiometric evaluation of cochlear implant patients. *Otolaryngol Head Neck Surg* 1988;98:203–206. [PubMed: 3127782]

4. Rubinstein JT, Parkinson WS, Tyler RS, et al. Residual speech recognition and cochlear implant performance: effects of implantation criteria. *Am J Otol* 1999;20:445–452. [PubMed: 10431885]
5. Gomaa NA, Rubinstein JT, Lowder MW, et al. Residual speech perception and cochlear implant performance in postlingually deafened adults. *Ear Hear* 2003;24:539–544. [PubMed: 14663353]
6. Cervera-Paz FJ, Linthicum FH Jr. Cochlear wall erosion after cochlear implantation. *Ann Otol Rhinol Laryngol* 2005;114:543–546. [PubMed: 16134351]
7. Fayad JN, Linthicum FH Jr. Multichannel cochlear implants: relation of histopathology to performance. *Laryngoscope* 2006;116:1310–1320. [PubMed: 16885730]
8. Choi CH, Oghalai JS. Predicting the effect of post-implant cochlear fibrosis on residual hearing. *Hear Res* 2005;205:193–200. [PubMed: 15953528]
9. Nadol JB Jr. Patterns of neural degeneration in the human cochlea and auditory nerve: implications for cochlear implantation. *Otolaryngol Head Neck Surg* 1997;117:220–228. [PubMed: 9334769]
10. Nadol JB Jr, Shiao JY, Burgess BJ, et al. Histopathology of cochlear implants in humans. *Ann Otol Rhinol Laryngol* 2001;110:883–891. [PubMed: 11558767]
11. Clark GM, Shute SA, Shepherd RK, et al. Cochlear implantation: osteoneogenesis, electrode-tissue impedance, and residual hearing. *Ann Otol Rhinol Laryngol Suppl* 1995;166:40–42. [PubMed: 7668722]
12. Roland PS, Wright CG. Surgical aspects of cochlear implantation: mechanisms of insertional trauma. *Adv Otorhinolaryngol* 2006;64:11–30. [PubMed: 16891834]
13. Kawano A, Seldon HL, Clark GM, et al. Intracochlear factors contributing to psychophysical percepts following cochlear implantation. *Acta Otolaryngol* 1998;118:313–326. [PubMed: 9655204]
14. Shiroma M, Honda K, Yamanaka N, et al. Factors contributing to phoneme recognition ability of users of the 22-channel cochlear implant system. *Ann Otol Rhinol Laryngol* 1992;101:32–37. [PubMed: 1728882]
15. Somdas MA, Li PM, Whiten DM, et al. Quantitative evaluation of new bone and fibrous tissue in the cochlea following cochlear implantation in the human. *Audiol Neurotol* 2007;12:277–284. [PubMed: 17536196]
16. Linthicum FH Jr, Fayad J, Otto SR, et al. Cochlear implant histopathology. *Am J Otol* 1991;12:245–311. [PubMed: 1928309]
17. Li PM, Somdas MA, Eddington DK, et al. Analysis of intracochlear new bone and fibrous tissue formation in human subjects with cochlear implants. *Ann Otol Rhinol Laryngol* 2007;116:731–738. [PubMed: 17987778]
18. O'Leary MJ, Fayad J, House WF, et al. Electrode insertion trauma in cochlear implantation. *Ann Otol Rhinol Laryngol* 1991;100:695–699. [PubMed: 1952658]
19. Schuknecht, HF. Pathology of the ear. Vol. 2nd ed. Philadelphia: Lea & Febiger; 1993.

Fig.1. (A) Segmentation according to Otte and Schuknecht.¹⁹ The midmodiolar line (broken line) is perpendicular to the section plane and divides the spiral ganglion into four segments. (B) Segmentation according to Linthicum. The midmodiolar section (broken line) divides the spiral ganglion into basal and upper turns.

Fig.2. Virtual three-dimensional reconstruction of one subject built using Amira 4.1. (blue) Normal tissue, represented by space filled with fluid. (white) Osteoneogenesis. (orange) Fibrosis. (green) Cochlear implant electrode.

Fig.3. Virtual cochlea sectioned into the four traditional segments of the spiral ganglion. (blue) Normal tissue, represented by space filled with fluid. (white) Osteoneogenesis. (orange) Fibrosis. (green) Cochlear implant electrode. Note that the scala vestibuli is healthy in segment I, yet in segment II it is filled with new tissue. This is due to the electrode being inserted in the scala tympani and breaking through to the scala vestibuli as it was moved into position.

Fig.4. Virtual cochlea sectioned into basal and apical turns. (blue) Normal tissue, represented by space filled with fluid. (white) Osteoneogenesis. (orange) Fibrosis. (green) Cochlear implant electrode. Note that most of the new tissue is located in the basal turn, where the implant is located.

Table 1

Clinical and histopathological characteristics of implanted temporal bones.

ID#	Ear	Sex	Age CI	Yrs CI	Implant Device	Insertion [*]	Fibrous Tissue ^{**}	New Bone ^{**}	Total New Tissue ^{**}	SGC Count [*]
1	L	F	60.4	0.9	Nucleus 22	Cochleost-R	23.19	0.67	23.86	24,399
2	R	F	74.4	2.7	Nucleus 22	Cochleost	26.48	1.55	28.03	4,869
3	L	F	48.3	12.9	Ineraid	RW	9.24	39.37	48.61	6,408
4	R	F	-	-	Nucleus 22	Cochleost	0.38	0.07	0.45	19,746
5	R	M	83.8	3.3	Nucleus 22	Cochleost	18.14	9.53	27.68	14,004
6R	R	F	77.4	9.5	Nucleus 22	RW	4.16	6.06	10.23	18,486
6L	L	F	-	-	House single	RW-R	2.25	6.75	9.00	13,765
7	L	F	66.9	6.4	Nucleus 22	RW	21.87	9.54	31.41	6,075
8R	R	F	66.5	9.9	Nucleus 22	RW	19.17	37.77	56.94	2,286
8L	L	F	74.0	2.4	Nucleus 24	Cochleost-R	10.48	1.50	11.98	3,276

* Cochleost=Cochleostomy, RW=Round Window, -R=revision

** Values shown reflect percentage of total cochlear tissue, based on volumes measured in μm^3

Table 2

Histopathological variables by cochlear segment[#] in implanted temporal bones

Subject	SEGMENT I				SEGMENT II				SEGMENT III			
	FT*	NB*	Total NT*	SGC Count	FT*	NB*	Total NT*	SGC Count	FT*	NB*	Total NT*	SGC Count
1	39.28	2.56	41.84	3,105	35.77	0.00	35.77	8,937	10.45	0.00	10.45	5,859
2	46.61	3.08	49.70	216	30.64	2.30	32.94	1,215	27.03	0.25	27.28	1,089
3	6.44	58.31	64.75	513	12.92	67.20	80.12	2,025	15.77	3.53	19.30	1,755
4	0.87	0.23	1.10	2,682	0.27	0.00	0.27	5,661	0.14	0.00	0.14	4,104
5	30.26	30.68	60.94	1,575	28.83	4.35	33.18	6,354	0.99	0.00	0.99	2,241
6R	13.41	24.04	37.45	2,547	2.71	0.64	3.35	7,560	0.00	0.00	0.00	4,266
6L	10.68	34.26	44.94	2,122	0.57	0.56	1.13	7,542	0.00	0.00	0.00	4,320
7	36.72	30.56	67.28	1,539	34.97	4.41	39.38	2,457	4.12	0.00	4.12	909
8R	23.96	47.29	71.24	405	34.29	55.14	89.43	729	3.71	31.49	35.20	576
8L	24.04	40.44	64.47	999	7.44	4.45	11.89	918	0.00	0.00	0.00	477

[#] Segment IV is 100% healthy in all temporal bones studied; FT=Fibrous Tissue, NB=New Bone, NT= New tissue, SGC=Spiral ganglion cell

* Values shown reflect percentage of total cochlear tissue, based on volumes measured in μm^3

Table 3

Histopathological variables by cochlear turn in implanted temporal bones

Subject	BASAL TURN			UPPER TURNS		
	Fibrous * Tissue	Osteoneq- * genesis	Total New * Tissue	Fibrous * Tissue	Osteoneq- * genesis	Total New * Tissue
1	33.52	0.97	34.50	0.80	0.00	0.80
2	37.66	2.23	39.89	0.01	0.00	0.01
3	12.11	55.53	67.65	3.48	0.67	4.16
4	0.51	0.09	0.60	0.00	0.00	0.00
5	25.66	13.48	39.14	0.00	0.00	0.00
6R	6.13	8.92	15.05	0.00	0.00	0.00
6L	3.39	10.14	13.52	0.00	0.00	0.00
7	30.70	13.46	44.17	0.46	0.00	0.46
8R	26.59	52.26	78.85	0.00	0.87	0.87
8L	13.95	19.93	33.88	0.00	0.00	0.00

* Values shown represent percentage of total cochlear tissue, based on volumes measured in μm^3