Correlation between Histologic and Radiographic Reconstruction of Intracochlear Electrode Position in Human Temporal Bones

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Temporal bone · Cochlear implant · Intracochlear length · Computed tomography · Multiplanar reconstruction · Histology · Celloidin

Abstract
In our laboratory, human temporal bone specimens from patients who in life have undergone cochlear implantation are routinely processed with the implant in situ, embedded in Araldite, sectioned at 20 μm and serially photographed during cutting, stained with toluidine blue and mounted on glass slides. From the images, two-dimensional and three-dimensional reconstructions can be made and a very accurate implant insertion depth can be calculated from the three-dimensional reconstructions. However, this method precludes subsequent special stains and further molecular investigations of the tissue including proteomics and immunostaining, which is now possible with celloidin-embedded tissue. In this study, we correlated measurement of the implant array insertion depth calculated from histologic three-dimensional reconstruction with that measured from three-dimensional radiologic multiplanar reconstruction. Four human temporal bones with cochlear implants underwent postfixation preprocessing CT imaging with a Siemens Somatom Sensation Scanner. The CT scans from these four bones were downloaded into the Voxar software application, reformatted using the multiplanar reconstruction tool, viewed in three dimensions and measurements of intracochlear insertion lengths of the implants were obtained. The bones were processed routinely for in situ Araldite embedding, serial images were made of the block during sectioning, postprocessed using PV-Wave® software, aligned with Amira® software, and used to create histologic three-dimensional reconstructions. From these three-dimensional reconstructions, the insertion depth of the electrode array was mathematically calculated. The range of insertion depths was 15.9 mm (case 1) to 26.6 mm (case 4). The two methods, radiographic multiplanar reconstruction and three-dimensional reconstruction, differed by 0.4–0.9%. This provides confidence that important localization information about the electrode in situ can be gleaned from CT scans, thereby allowing us to extract the implants prior to processing for celloidin embedding and allow further techniques such as special stains and immunostaining to be accomplished in order to evaluate molecular mechanisms involved in cochlear implantation.
Introduction

Histopathologic study of temporal bones from patients who in life had undergone cochlear implantation may be facilitated by a technique developed to section specimens with the electrode remaining in situ [Nadol et al., 1994, 2001; Lee et al., 2011a, b]. This technique allows the electrode to be visualized in approximation to native tissues of the inner ear, and to the biologic responses to the presence of electrodes during life. In addition, the insertion depth of the electrode array can be determined by three-dimensional reconstruction of serial sections [Somdas et al., 2007].

The disadvantages of maintaining the cochlear implant electrode in situ include the requirement to use an embedding material such as Araldite, in turn requiring smaller specimens, and considerable limitations on subsequent histologic processing of the sections. For example, special stains that can be used with celloidin embedding including Luxol fast blue to study the integrity of myelin, or Movat’s pentachrome stain used to evaluate ground substance, elastic fibers, and collagen cannot be used with Araldite sections. More importantly, Araldite embedment precludes immunostaining, which is possible with celloidin sections [Keithley et al., 1995; Ganbo et al., 1997; Markaryan et al., 2008; O’Malley et al., 2009; Balaker et al., 2013].

Celloidin embedment also makes proteomic investigations of temporal bones with cochlear implants possible. This field of study, while still very young, has some proven success with archival celloidin tissue [Aarnisalo et al., 2010; Markaryan et al., 2010; Richard et al., 2013] and would open an entire new avenue of research for temporal bones with cochlear implants. One of our goals for future cochlear implant research is to preserve the ability to obtain a three-dimensional reconstruction of the electrode in situ and be able to accomplish such techniques as immunostaining and special stains. This necessitates the removal of the electrode from the temporal bone prior to processing and embedment in celloidin.

In an effort to gain more information on each temporal bone specimen containing a cochlear implant, but not lose the ability to obtain a three-dimensional reconstruction, we have compared intracochlear insertion lengths of cochlear implants calculated radiographically and histologically. The radiographic study of cochlear implant arrays has been utilized to study various electrode and cochlear parameters including three-dimensional cochlear lengths, electrode array insertion depths [Ketten et al., 1998; Skinner et al., 2002; Verbist et al., 2010; Kong et al., 2012] as well as electrode migration [van der Marel et al., 2012] postoperatively in living patients. In addition, there have been numerous reports of computed tomography (CT) in cadaveric human temporal bones [Meshik et al., 2010; Schumann et al., 2010; Teymouri et al., 2011]. Correlating histopathology with CT findings has been used to study scala communis defect, facial canal enlargement, and otosclerosis [Makary et al., 2010; Moonis et al., 2011; Quesnel et al., 2013]. If radiologic estimates of the intracochlear implant length in situ are comparable to the histologic estimates, this will allow removal of the electrode in subsequent specimens and embedment in celloidin, in turn allowing such techniques as immunostaining and proteomic analysis.

Materials and Methods

Radiologic Multiplanar Reconstruction

CT (Siemens Somatom Sensation) was performed in the orientation that was estimated to be close to the axial plane (fig. 1a–4a) using external landmarks of the specimen. The scan was performed prior to specimen processing. Scanning was done with 0.6 mm collimation, 120 kV, and 320 mAs. The data set was reformatted with 0.6-mm slices every 0.2 mm using a 512 × 512 matrix and a 65- to 70-mm field of view. The resulting three-dimensional volume data set was loaded into the Voxar imaging system (Toshiba Medical Visualization Systems®) for multiplanar reformattting.

This orientation appears as a mirror image of the histological block (compare a and b for all four figures) because the imaging convention is to display the axial scan as though viewed from inferiorly.

In Voxar, the data from 4 temporal bone specimens with cochlear implants were reformatted into three planes utilizing the multiplanar reconstruction (MPR) tool, viewed in three dimensions, and measurements of intracochlear insertion lengths were made. A specific plane was chosen to align the stacks to closely approximate the plane of section subsequently used in histologic preparation. Once aligned to the desired plane, the multiplanar curved (curved MPR) tool was employed in the coronal plane (fig. 1c, d–4c, d). Curved MPR allows nonplanar structures such as blood vessels, or in our case, in situ cochlear implant arrays, which cannot be viewed in a single plane, to be isolated and examined in a more useful way. A curve direction was chosen for either left or right ears. The curve direction orients the data set and defines how the curved MPR view will be generated. The point of insertion of the cochlear implant was determined by paging through the stack of images to find the cochleostomy site. The midpoint of the implant (mid-width as it passed through the cochleostomy site just inside the cochlea) was determined (fig. 1c, d–4c, d). Using this as the insertion point (the starting point for the measurement), the Curve Tool and Measure Curve Tool were utilized to determine the intracochlear length (length of the cochlear implant array within the cochlea) by clicking the mouse at approximately 1-mm intervals along the midline of the array from the base of the cochlea to the most apical tip (fig. 1d–4d). The curve tool ‘drops’ user-defined control points orthogonal to the desired curve. The curve is a ‘polyline’ consisting of a set of points that are joined together.
by short segments of straight lines. Proprietary Voxar algorithms are employed to calculate coordinates of a curved sheet that is extruded from the user-defined points and then flattened into a two-dimensional curved MPR image on the user interface window (fig. 1e–4e). The length of the curve is the sum of the shorter line segments and is obtained by clicking the Measure Curve Tool icon. The measurement appears on the flattened two-dimensional curved MPR image window (fig. 1e–4e). The points were drawn and measurements were made multiple times and an average was taken. The results appear in table 1, column 3 (radiographic MPR).

For purposes of comparison, measurements were also made along the inner margin and the outer margin of the array in all of the cases. These values were compared to the values obtained when the center or midpoint of the array was used (as described above). Multiple measures were made and averages were determined. The results appear in table 2. Percent differences were calculated for inner spiral versus midpoint, outer spiral versus midpoint, and for inner spiral versus outer spiral.

**Histologic Three-Dimensional Reconstruction Measurement**

Temporal bones were fixed in 10% buffered formalin. Decalcification was accomplished in 0.27 M ethylenediaminetetraacetic acid. The temporal bones were rinsed in distilled water and dehydrated in ethanol, passed through several changes of propylene

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**Fig. 1.** a CT scan of case 1 (Nucleus Perimodiolar Contour Advance 22-electrode array) in the axial plane (left ear). The cochleostomy site (circle), implant array (IA), and posterior canal (PC) are noted. b The same specimen embedded in Araldite mounted on a block during cutting. The sectioning is at the level of the cochleostomy site (circle). The IA and PC are noted. c A CT scan of case 1 in the coronal plane. The IA and cochleostomy site (circle) are noted. d The same image as c with a line drawn along the midpoint of the IA to simulate the line that is drawn in Voxar with the ‘curve’ tool. The arrowhead is pointing toward the insertion point of the IA. e The resultant image from the ‘measure curve’ tool using Voxar as the software unwinds the array and measures it linearly. f The Araldite block during sectioning at a level that is superior to b. The IA can be seen hugging the modiolus in the scala vestibuli (SV).
**Table 1.** Average intracochlear insertion length (mm)\(^a\)

<table>
<thead>
<tr>
<th>CI case</th>
<th>Type of implant</th>
<th>Radiographic MPR</th>
<th>3D histologic reconstruction</th>
<th>2D histologic reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CI24RE(CA) – Nucleus Perimodiolar Contour Advance 22-electrode array</td>
<td>15.9</td>
<td>16.0</td>
<td>16.8</td>
</tr>
<tr>
<td>2</td>
<td>Ineraid 6-electrode array</td>
<td>21.0</td>
<td>21.0</td>
<td>15.8</td>
</tr>
<tr>
<td>3</td>
<td>Clarion Enhanced Bipolar 16-electrode array</td>
<td>23.3</td>
<td>23.1</td>
<td>20.4</td>
</tr>
<tr>
<td>4</td>
<td>Ineraid 6-electrode array</td>
<td>26.6</td>
<td>26.5</td>
<td>20.2</td>
</tr>
</tbody>
</table>

\(^a\) Along midpoint of array from cochleostomy to electrode tip.

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**Fig. 2.**  
\(a\) A CT scan of case 2 (Ineraid 6-electrode array) in the axial plane (right ear). The cochleostomy site (circle), implant array (IA), and posterior canal (PC) are noted.  
\(b\) The same specimen embedded in Araldite mounted on a block during cutting. The sectioning is at the level of the cochleostomy site (circle). The IA and PC are noted.  
\(c\) A CT scan of case 2 in the coronal plane. The IA and cochleostomy site (circle) are noted.  
\(d\) The same image as \(c\) with a line drawn along the midpoint of the IA to simulate the line that is drawn in Voxar with the ‘curve’ tool. The arrowhead is pointing toward the insertion point of the IA.  
\(e\) The resultant image from the ‘measure curve’ tool using Voxar as the software unwinds the array and measures it linearly.  
\(f\) The Araldite block during sectioning at a level that is superior to \(b\). Note the IA, electrode balls (EB), and new bone (NB).
oxide and infiltrated with low, then high concentrations of Araldite 502 (EMS, Hatfield, Pa., USA). The specimens were exchanged through several changes of Araldite. A low vacuum was used at this stage to facilitate infiltration. Finally, the bones were hardened in a 45° and then a 60° oven. Sectioning was accomplished in the axial plane on a sliding microtome with a tungsten carbide profile D knife (Leica, Germany).

Two Ineraid 6-electrode arrays, one Clarion Enhanced Bipolar 16-electrode array and one Nucleus Perimodiolar Contour Advance 22-electrode array were examined.

The embedded temporal bones with electrode arrays in situ were serially sectioned in the axial plane. The thickness of each section, delta z, was approximately 20 μm. Using a Canon Power Shot Pro1 Camera, a 2,272 × 1,704-pixel jpg image of the block before each section was taken. The serial pictures were then rotated and cropped to 900 × 600 pixels using the PV-Wave® software program. The serial images were aligned using Amira®. The pixel size, delta x, and delta y were obtained using the 'measure and analyze tool' in Image J.

For the Ineraid electrode arrays and Clarion Enhanced Bipolar electrode arrays, the x and y coordinates of the middle points along the length of the carrier were read into a data file using a customized PV-Wave program. The section number was obtained at the same time as the z coordinate. The measurement of the insertion depth (ID) was calculated using the equation:
where $x$ and $y$ are coordinates, delta $x$ ($dx$) and delta $y$ ($dy$) represent pixel size, $z$ is section number, delta $z$ ($dz$) is section thickness, and $n$ is the total number of the middle points. The ‘i’ is a segment variable from 0 to $n-2$, a total of $n-1$ segments.

The Nucleus Perimodiolar Contour array’s design makes it possible to accurately follow the middle points of the electrode’s surface. So rather than the carrier midpoints being measured as for the Ineraid and Enhanced Bipolar arrays, the center point of the half band-shaped electrode surface itself was picked in measuring the insertion length for case 1.

**Histologic Two-Dimensional Reconstruction**

Both types of three-dimensional reconstructions (radiologic and histologic) were compared to the conventional two-dimensional reconstruction technique introduced by Guild [1921]. The method was described in detail by Schuknecht [1993]. Briefly, the cochlea was reconstructed on gridded (0.5-cm) graph paper using the heads of the pillar cells to form the curve representing the cochlea. The histological slides were analyzed and the tangential cuts
through the heads of the pillars were plotted corresponding to their slide number. Semicircles were drawn to connect the appropriate slides and draw the resultant curve. Using a compass and defined distance, millimeter increments were marked along the length of the curve. The established curve was used to determine cochlear length. Hair cells and other cochlear structures can be plotted on the curve as well as the approximate location of a cochlear implant.

Results

Radiologic MPR

The results for the radiologic MPRs appear in table 1, column 3 and in figures 1e–4e. The values in the figures do not match the table because the table values are the result of averaging many measurements and the figures show only a snapshot of one measurement. The range in values for intracochlear insertion length was from 15.9 mm in case 1 to 26.6 mm in case 4.

Table 2. Average intracochlear insertion length (mm) using radiographic MPR

<table>
<thead>
<tr>
<th>As measured along, CI case</th>
<th>Inner spiral of implant array</th>
<th>Midpoint of implant array</th>
<th>Outer spiral of implant array</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.2</td>
<td>15.9</td>
<td>18.6</td>
</tr>
<tr>
<td>2</td>
<td>19.4</td>
<td>21.0</td>
<td>23.3</td>
</tr>
<tr>
<td>3</td>
<td>20.1</td>
<td>23.3</td>
<td>27.2</td>
</tr>
<tr>
<td>4</td>
<td>23.9</td>
<td>26.6</td>
<td>29.4</td>
</tr>
</tbody>
</table>

Radiologic MPR: A Comparison of Length versus Spiral Location

Table 2 contains the insertion lengths using radiologic MPR at different locations along the width of the array. Measurements along the inner margin of the electrode arrays ranged from 14.2 mm in case 1 to 23.9 mm in case 4. Measurements along the midpoint are the same as those that appear in table 1, column 3 and ranged from 15.9 mm in case 1 to 26.6 mm in case 4. Measurements along the outer margin of the electrode arrays ranged from 18.6 mm in case 1 to 29.4 mm in case 4.

Histologic Three-Dimensional Reconstruction Measurement

The results for the three-dimensional histologic reconstruction calculations appear in table 1, column 4. The range for insertion length was from 16.0 mm (case 1) to 26.5 mm (case 4).

Histologic Two-Dimensional Reconstruction

The results for the two-dimensional histologic reconstruction appear in table 1, column 5. The insertion length ranged from 15.8 mm (case 2) to 20.4 mm (case 3).

Discussion

There was essentially no difference between the radiographic MPR and the three-dimensional histologic reconstruction methods. Case 2 measured exactly the same with both methods and cases 1, 3, and 4 differed by 0.4–0.9% with the two methods. Currently, a detailed three-dimensional histological reconstruction created from images of an Araldite block during sectioning affords a precise way to measure the intracochlear insertion length. However, alignment of the images is necessary for an accurate three-dimensional reconstruction. If the block requires reinfiltation, it must be removed from the microtome and camera setup. This makes alignment of the images more difficult after reinfiltation. During the sectioning, if the camera used to photograph the block is moved, the subsequent images are not aligned with the previous images. Our results show that a postfixation pre-processing CT scan, which gives us a stack of images completely aligned, combined with the ability of the MPR to manipulate those images in all three planes (axial, coronal, and sagittal) is equally as good a method to determine the intracochlear insertion length of an implanted human temporal bone. Although the CT scan has lower resolution than histological images (compare fig. 1a–4a with fig. 1f–4f), for purposes of finding the cochleostomy site and the apical tip of the electrode array, the differences in contrast are such that measuring the insertion length is usually straightforward.

There was a large range in the results of the three-dimensional histologic versus the two-dimensional histologic methods. Comparing these methods gave differences ranging from close to 5% up to nearly 25%. The two-dimensional method of reconstruction created by Guild [1921] relies on the position of the pillar cells to create the curve representing the cochlea. The path a cochlear implant takes in the cochlea is intended to be in the scala tympani and does not follow the same path as the pillar cells. Very often an implant, although intended to hug the modiolus, veers outward toward the lateral wall (fig. 1f), quite a distance from the heads of the pillar cells in the organ of Corti. In addition, implant arrays can penetrate the basi-
lar membrane into the scala media or scala vestibuli. In some implant cases, the pillar cells are displaced or destroyed, making two-dimensional histological reconstruction more difficult. Using the two-dimensional histological reconstruction on graph paper is a poor fit for the actual placement of an array in the cochlea. The two-dimensional reconstruction provides a rough estimate of where the implant is inside the cochlea, but does not result in accurate insertion depths. Implant arrays can also bend and kink during insertion, which is difficult to accurately represent and metrically interpret on a two-dimensional plot. In both radiologic and histologic three-dimensional reconstruction, the track an implant takes in space as it traverses the cochlea is more accurately determined.

Using the radiologic MPR method, the array lengths were measured along different portions of the spiral. As would be expected, the inner spiral lengths were shortest and the outer spiral lengths were longest. The percent difference between individual measures varied widely. When values presented in table 2 are compared to each other for inner spiral versus midline, the percent difference ranged from 7.6% (case 2) to 13.7% (case 3) and averaged 10.6%. For outer spiral versus midline, the percent difference ranged from 9.5% (case 4) to 14.5% (case 1) and averaged 12.1%. For inner spiral versus outer spiral, the percent difference ranged from 16.7% (case 2) to 26.1% (case 3) and averaged 21.3%. These numbers are instructive when thinking about the two-dimensional histologic method as well. They demonstrate how different a value can be if the array is actually along the lateral wall of the cochlea instead of beneath the heads of the pillar cells. It is important to measure along the midline of the array with both the radiologic and histologic method in order to determine the most accurate inserted length of an implant.

In summary, the intracochlear length of an implant array as measured by a CT scan of the temporal bone with MPR reliably corresponds to the more laborious three-dimensional histologic reconstruction. This provides confidence that removal of the electrode from the cochlea after CT scanning will preserve important localization information and allow embedment in celloidin and a wide variety of techniques such as immunostaining.

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References


