Precision Medicine in Ossiculoplasty

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Introduction: Long term results of ossiculoplasty surgery are considered poor with displacement and extrusion amongst the common reasons for failure. Application of 3Dimensional (3D) printing may help overcome some of these barriers, however digital methods to attain accurate 3D morphological studies of ossicular anatomy are lacking, exacerbated by the limitation of resolution of clinical imaging.

Methods: 20 human cadaveric temporal bones were assessed using micro computed tomography (CT) imaging to demonstrate the lowest resolution required for accurate 3D reconstruction. The bones were then scanned using conebeam CT (125 μm) and helical CT (0.6 mm). 3D reconstruction using clinical imaging techniques with microCT imaging (40 μm resolution) as a reference was assessed. The incus was chosen as the focus of study. Two different methods of 3D printing techniques were assessed.

Results: A minimum resolution of 100 μm was needed for adequate 3D reconstruction of the ossicular chain. Conebeam CT gave the most accurate data on 3D analysis, producing the smallest mean variation in surface topography data relative to microCT (mean difference 0.037 mm, $p < 0.001$). Though the incus varied in shape in between people, paired matches were identical. Thus, the contralateral side can be used for 3D printing source data if the ipsilateral incus is missing. Laser based 3D printing was superior to extrusion based printing to achieve the resolution demands for 3D printed ossicles.

Conclusion: Resolution of modern imaging allows 3D reconstructions and 3D printing of human ossicles with good accuracy, though it is important to pay attention to thresholding during this process. **Key Words:** 3D analysis—3D printing—Bioprinting—Conebeam CT—CT—microCT—Ossiculoplasty—Precision medicine.

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3D (three dimensional) printing is having an increasing influence across many domains of Otology (1), yet it has not made substantive translational impact in solving basic clinical challenges in middle ear surgery (1,2,3). Individual ossicles display a great degree of inter-individual variation (4) and reconstructive results are poor in the long term (2). This creates a potential value for the application of 3D printing techniques to produce customized components for use in ossiculoplasty surgery (3).

Outcomes of ossiculoplasty are not limited to surgical factors alone. Patient factors, otological factors and prosthetic factors also play a role (5). Numerous classification systems to define ossiculoplasty type (6) and risk (7) have challenged meaningful pooled outcome analysis (6). Nevertheless, long term outcome of reconstructive surgery remains poor with total ossicular reconstruction prosthesis (TORP) success reported to drop from 53.8% to 39.7% and Partial Ossicular Reconstruction Prosthesis (PORP) results from 73.9% to 58.3% at 6 months and 5 years respectively (8). There has been little improvement in outcomes reported in the last 50 years (9). Displacement and extrusion are considered to be the most common reasons for failure (3). Application of 3D printing science in this field, may assist in improving fit, fixation, retention, surgical planning and potentially also long-term results.

The first step towards achieving better translational results is to attain accurate 3D data of the ossicles. Two Dimensional (2D) morphological studies of ossicular anatomy report variation in study methodologies (4) and measurements are mostly performed manually by a human investigator (10). Inconsistent measurement techniques and inter-observer variability have resulted in a wide range in values of ossicular dimensions (4,11,12). A metanalysis by Noussios et al. reported that mean incus length and breadth ranged from 2.80 to

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6.47 mm and 1.82 to 4.88 mm respectively between studies (4). If anatomical studies can demonstrate such variability in inter-observer data collection, measurements taken intraoperatively during reconstruction, where visibility is more limited, may also demonstrate inter-observer variation affecting reconstructive results. Such methodological errors can be reduced by 3D digital data acquisition and digital planning. Further, such digital data can also be applied to 3D printing.

A major barrier for clinical application of 3D technology to the middle ear had been the resolution of clinical imaging to allow adequate 3D reconstruction of middle ear anatomy. However, with the advent of cone beam CT (computed tomography) achieving resolution as fine as 125 μm (13) and new Helical high resolution CT (HRCT) promising resolution of 150 μm (14), there are new opportunities to improve clinical translation.

The main aim of this study was to identify challenges of 3D printing for ossicular reconstruction. It first addressed the minimum resolution of imaging required to attain 3D reconstructions of the ossicles using microCT imaging. Second, it compared this reference standard to two clinical imaging techniques; conebeam CT (CBCT) and HRCT, to see if 3D reconstructive data was accurate, with a focus on the incus as it is the most common bone to be affected needing reconstruction (15,16). Lastly, it compared two different 3D printing techniques to assess which was more suited for middle ear applications.

METHODS

Specimen Preparation

Ethical approval was attained from the Royal Prince Alfred Hospital Ethics Committee through the Research Ethics and Governance Information System (REGIS) for this study (protocol numbers 2019/ETH13789and X19−0480). Otic capsules of 20 human temporal bones (7 paired and 6 unpaired ears) were dissected with high speed otologic drill. Temporal bones were stained using 1.5% phosphotungstic acid and preserved in 4% paraformaldehyde.

Image Acquisition - MicroCT

The bones were scanned using MILabs U-CT microCT scanner (Heidelberglaan, Netherlands). Volume reconstruction and visualisation was done using MILabs and Imalytics (Medilumine Inc, Montreal Canada) software. 3D models were rendered and optimised using 3D Slicer (www.slicer.org). MicroCT point cloud data at resolution of 25, 40, 80, 100 and 150 μm was segmented and surface rendered to judge the minimal resolution required to capture important anatomical data relevant to TORP and PORP placement (Fig. 1A).

Image Acquisition-Clinical CT

Each bone was then scanned using CBCT (Newtom Cone Beam CT 3D Imaging Systems, Italy) at resolution of 125 μm and HRCT (GE Revolution CT 256-Slice Scanner, General Electric Company Ltd, Chicago, Illinois, United States) at 625 μm.

Image Segmentation and Presentation

Comparative control included microCT images at 40 μm resolution. The ossicular chain was segmented and reconstructed in 3D using 3D Slicer and Meshlab (www.meshlab.net). Test variables included similar 3D reconstructions of ossicular chain from CBCT and HRCT which were compared to each respective bone's microCT dataset. Image thresholding during segmentation was done using numeric values 3DS Max 2020 (Autodesk Inc. California. United States) which was important as varying the window or threshold range during segmentation was observed to change the accuracy of the final rendered image (Fig. 2).

Segmentation Optimisation

Evaluation of the accuracy of segmentations was performed using Cloud Compare using methods previously described (17) (cloudcompare.org). Measurements from 10,000 surface sampling points on each ossicular chain were taken and the

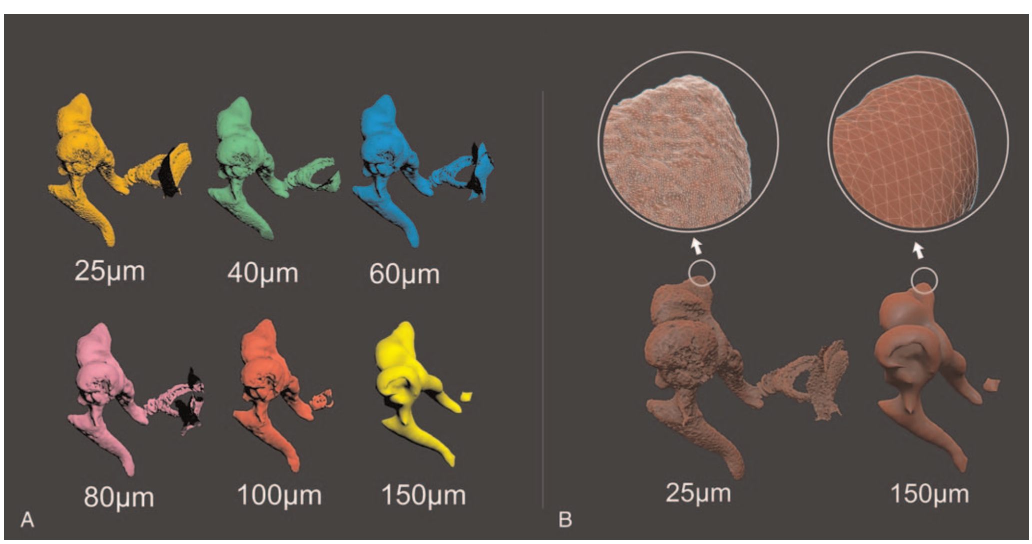

FIG. 1. Image *A* shows 3D reconstruction of the ossicular chain with reducing resolution. Image *B* shows a mesh model of the surface to understand the resulting loss in detail due to volume averaging artefact of surface data points as resolution is reduced.

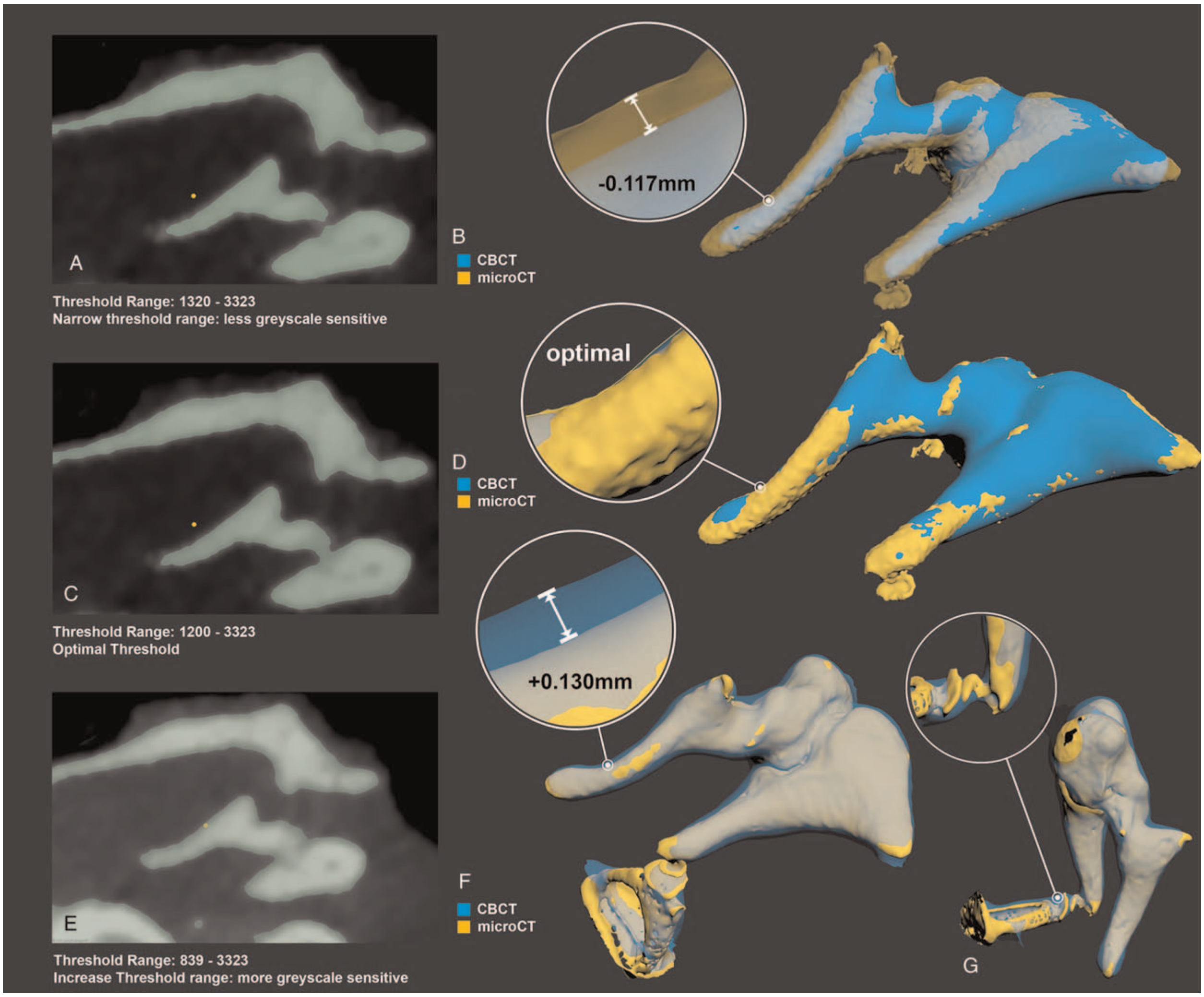

FIG. 2. Image *A*, *C* and *E*, show thresholding range of corresponding CBCT data which is represented in blue in *B*, *D* and *F* respectively. As the threshold range was increased, the stapes and lenticular process could be visualised using CBCT, however, it increased error of accurate dimensions of higher bone density areas. This is best imaged in *F*, where the accurate dimensions of the incudostapedial joint can be reconstructed at the compromise of significant error of malleus handle. Conversely, in *D*, optimal malleus handle compromises reconstruction of the lenticular process.

variation in surface topography data expressed in millimeters (mm). Three comparisons were performed: a) microCT versus CBCT, b) microCT versus HRCT and c) CBCT versus HRCT. A histogram of variation was attained from each study which showed a parametric distribution (Fig. 3). The software calculated the mean, minimum and maximum difference from the 10,000 sampling points. The cumulative mean difference for all 20 specimens was then calculated for each of the 3 comparisons and inspected in order to guide optimal values for analysis (Fig. 3).

Incus Study

Ossicles were then disarticulated under microscope by an otologist and the incus was reimaged using microCT (40 μm resolution). 3D reconstructions of each disarticulated incus from paired bones was visualised. The right incus was mirrored and overlaid on the left incus for each individual patient. Both, inter and intra-individual (between left and right ear) variability in the morphology was assessed in 3D (Fig. 4).

3D Printing

Ossicular chains were 3D printed using scan data from microCT (40 and 100 μm resolution), CBCT and HRCT using 2 different methods of 3D printing. The first, an extrusion based, Fused Deposition Modelling (FDM) 3D printer UpBox (2012–2017 Tiertime, Beijing, China) using PLA (Poly-Lactic-Acid) as print material (print resolution 250 μm). The second was laser based Sterolithography (SLA) printer Form 3 (Formlabs, Massachusetts, United States) with print resolution 100 microns. The print quality was compared (Fig. 5). The key challenges specific for 3D printing and bioprinting pathway was also defined (Fig. 6).

RESULTS

MicroCT Data

Images of the ossicular chain were obtained at 25, 40, 80, 100 and 150 μm resolution settings and these were compared next to each other. The main difference in the

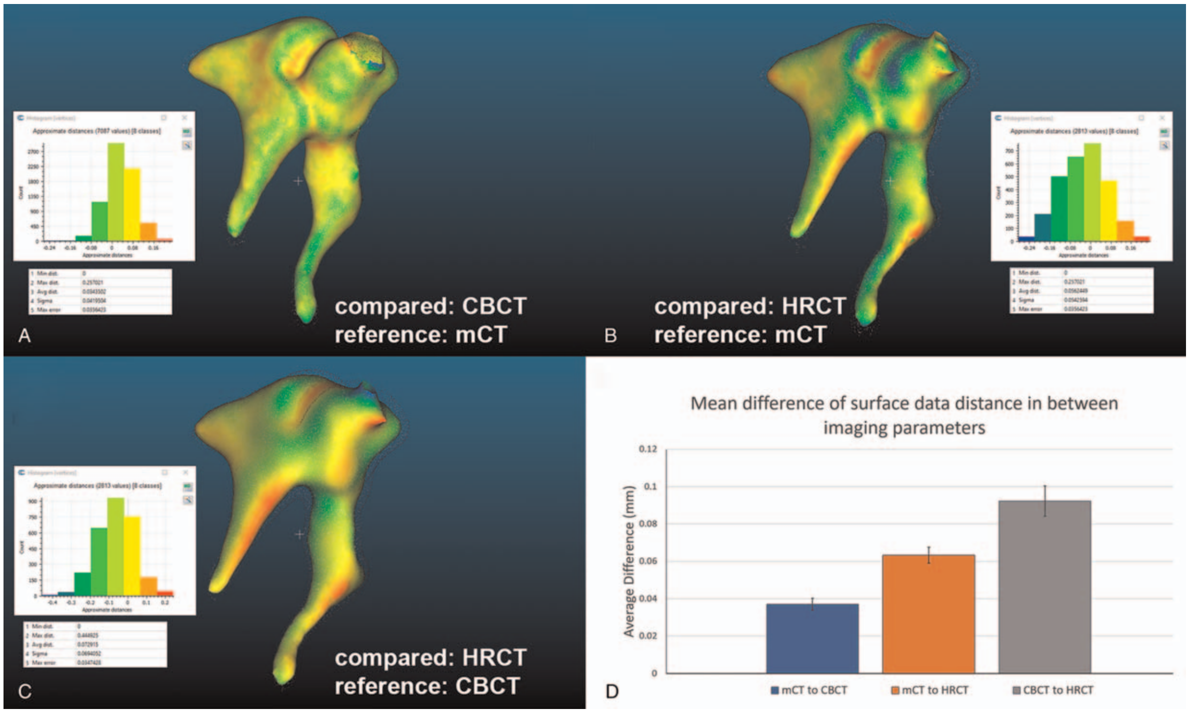

FIG. 3. Difference in 10,000 surface data points between 2 imaging techniques. Image *A*, *B* and *C* show study of one typical bone with CBCT and MicroCT (*A*), HRCT and MicroCT or mCT (*B*), and CBCT and HRCT (*C*) with individual histograms attained for each study. This was repeated 20 times for each bone and mean difference calculated along with 95% confidence interval (error bars) as shown in Image *D*.

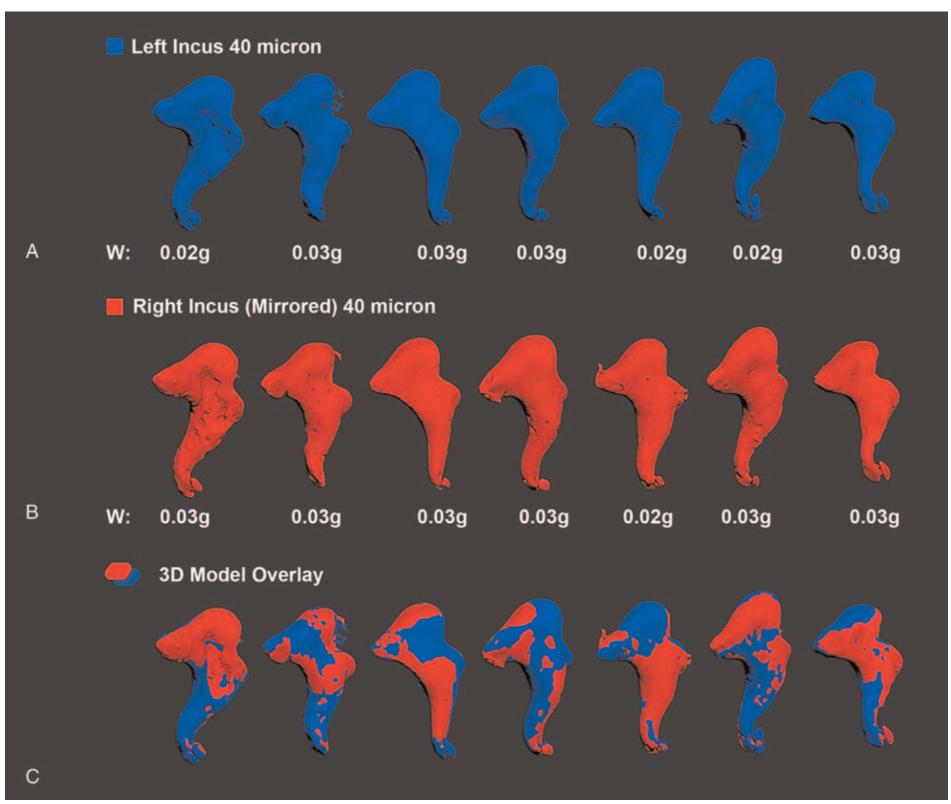

FIG. 4. *A* and *B* show paired incus bones. Image *A* shows all left bones. In Image *B*, each paired right bone is mirrored using computer aided design. *C* shows an image when *A* and *B* are overlaid. There appeared to be significant variation in the shape of the incus in between patients. However, when the right incus in each patient was mirrored and overlaid onto the respective left incus in *C*, the shape appeared identical.

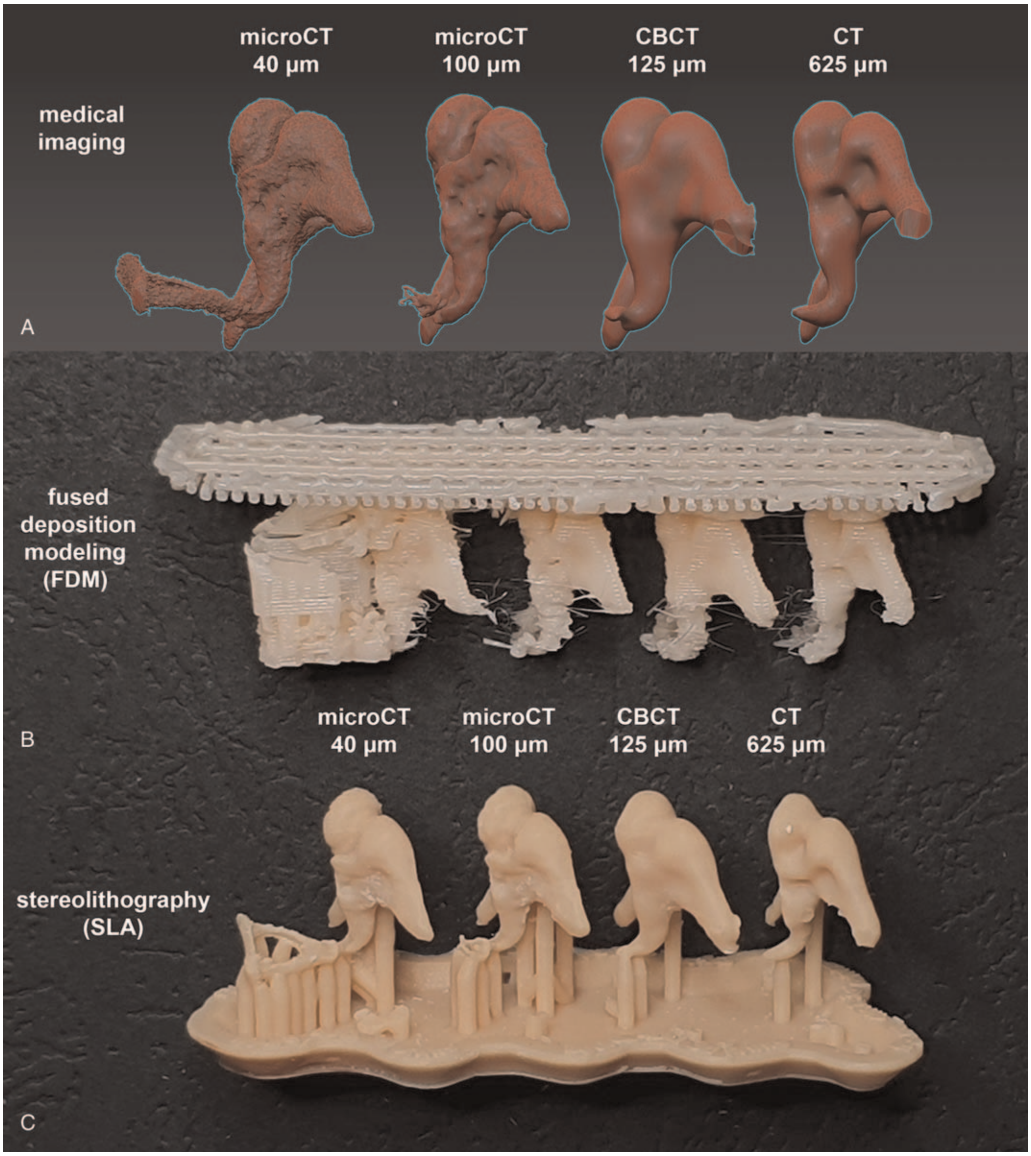

FIG. 5. Image *A* shows the 3D data file used in the printing process and the individual resolution. *B* shows results attained using FDM print technique (printer resolution 250 μm), *C* shows, print quality using SLA technique (print resolution 100 μm). Importantly, use of laser in the SLA technique also causes lack of visibility of print layers. This figure displays that it's not only the resolution of the imaging, but the resolution and the technique of the printer that is also key to the final product.

3D models with reducing resolution was volume averaging artefact that reduced fine details of the ossicular surface topography (Fig. 1B). The minimal degree of resolution required to contain major data points for ossicular reconstruction was 100 microns (Fig. 1A).

The ossicle most affected by reducing resolution was the stapes (Fig. 1A). At 25 μm the whole of the footplate and superstructure could be visualised but at 100 μm, only the stapes head was visible on 3D reconstruction. Given this is a joint area, the stapes head being visible is a relative advantage as it forms the fixation points for incus replacement prosthesis. The incus lenticular process was the next most affected region. When the incus alone was studied, there appeared to be significant inter-individual variability in the incus (Fig. 4) which is consistent with data present in the literature (4). However, digital overlay of paired left and right incus bones showed that there is very little intra-individual variability in normal ears across all 7 paired bones (Fig. 4).

Comparison of microCT to Clinical Imaging Modalities

Incus reconstruction from microCT analysis at 40 μm satisfactorily demonstrated all relevant anatomical features, and was therefore considered the gold Standard for this comparison. Cloudcompare analysis showed the least difference in surface point variation between the

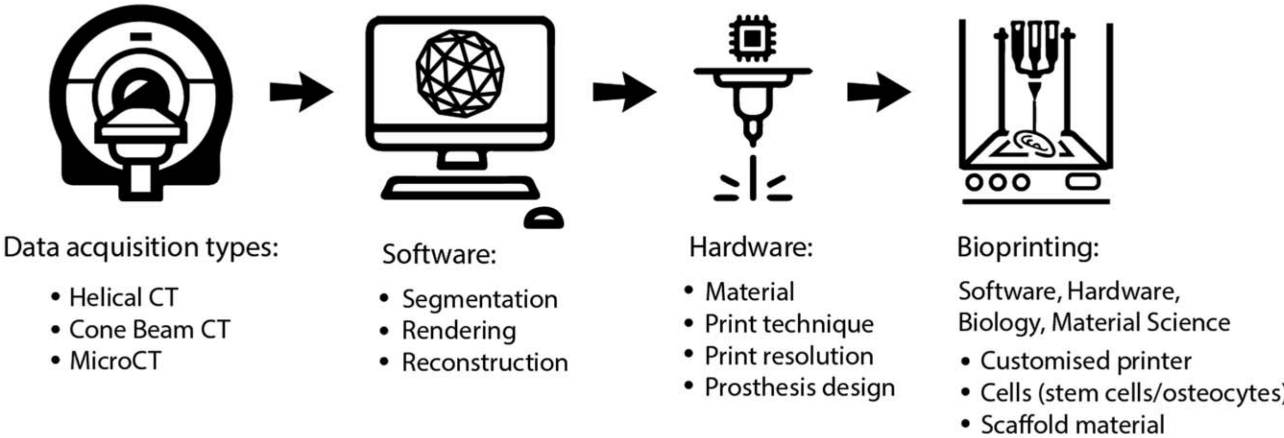

FIG. 6. Pathway of 3D printing from data acquisition to introducing stem cells. At each step, resolution, material, software and hardware challenges may affect the translation of the final product.

CBCT and microCT data (Fig. 3D) (mean difference 0.037 mm). There was an increased mean difference between HRCT and microCT of 0.063 mm. The difference between the 2 means was statistically significant ($p < 0.001$). Further, CBCT versus HRCT analysis showed the highest mean difference in surface data variation (0.092 mm, $p < 0.001$), indicating that these 2 data sets should not be used interchangeably to achieve 3D reconstructions.

With respect to CBCT imaging, threshold impacted on visualization of different structures. If the threshold range was optimized for accuracy of the malleus handle, then the lenticular process and stapes could not be visualized even on CBCT (Fig. 2D). If the threshold range was set to accurately image the dimensions of incudostapedial joint and stapes on CBCT (Fig. 2F), an error of more than 0.25 mm could be expected in the total width of the malleus handle: an error if not corrected could cause significant instability in interposition prosthesis designs.

3D Printing

SLA (Sterolithography) printing was superior to FDM printing due to higher print resolution and less visible layers (Fig. 5). Support structures were easier to remove using SLA reducing post processing artefact. When higher resolution data (microCT at 40 μm) was printed using SLA (100 μm) the majority of details was present, though the surface texture was expectedly smoother compared to the raw data due to resolution loss during the printing process. However, the print quality was far worse with FDM (250 μm) with obvious layers visible. In addition, the large amount of support material obscured the visibility of the stapes and removal of the support would destroy the overall anatomy.

DISCUSSION

This study evaluated the accuracy of clinical imaging to provide individualized 3D data for use in customized solutions for middle ear surgery. Point cloud comparison showed that CBCT performed well compared to a gold standard of 40 μm microCT data, with the caveat that close attention must be given to appropriate thresholding of structures.

Advantages of Precision Medicine for Middle Ear Applications

The pathway of 3D printing an ossicle includes a) attaining digital data of adequate resolution, b) accurate software to segment, threshold and render that data in 3D and c) adequate print resolution to print a small and complex 3D shape (Fig. 6). Perceived advantages of 3D data sets include digital analysis, digital planning and 3D printing. Digital analysis removes perspective error of 2D analysis reducing interobserver variability (4) and facilitates 3D surface analysis. For instance, in this study 10,000 data points could be analyzed digitally, compared to 5 to 10 data points if done manually (2,18), and different imaging parameters could be digitally compared. 3D shapes could be mirrored and overlaid to perform shape analysis, a technique that was demonstrated in Figures 2 and 3.

Digital planning is a more complex process which is another advantage. 3D datasets allow significant flexibility in prosthesis design. Since the malleus handle is not directly in line with the stapes head, interposition prosthesis that attempt to connect the stapes head to the malleus handle can be unstable while columellar reconstruction that make a direct connection between the stapes and the tympanic membrane risk extrusion. The most common ossicle in need for ossiculoplasty is the incus (15,16), a potential focus area for digital planning. This study showed inter-individual variation in the anatomy of the incus but intra-individual homogeneity in between left and right ears of each patient. Thus, data from the contralateral incus could be used for digital planning in prosthesis design if the incus is absent in one ear in some patients either due to disease or previous surgery. Both articular surfaces of the incus are complex 3D shapes. Since the incus bridges the malleus and stapes and has a non-linear relationship between the 2 bones, a customized prosthesis not only needs to match its dimensions but also needs to be customized to the patient's middle ear anatomy. For instance, in chronic ears with a retracted malleus handle, eroded incus and a small middle ear volume, a 3D printed prosthesis based on the incus dimensions of a normal ear for that patient (such as in the contralateral non diseased ear) may not be an appropriate fit in the current (diseased) ear. Further, the planned surgery that needs to be undertaken to treat the disease may change the pre-operative anatomy significantly. Therefore, prosthesis design needs to be customized to the diseased ossicular chain and middle ear volume (3). Digital data allows complex planning and potentially more predictable outcomes for prosthesis design (19) whilst navigating these individual challenges. This can be attained through current clinical imaging parameters at least through CBCT.

Data Acquisition

MicroCT data showed that 100 μm resolution gave adequate data resolution for 3D printing of the incus. Clinical CT scans are improving in resolution with CBCT at this stage coming closest to this resolution requirement. With CBCT reducing radiation dose to patients by almost one sixth that of standard CT (20), yet delivering, comparable resolution to microCT, there is a promising future in this area. HRCT, despite wider slice width (625 μm in this study), through helical scanning, allow image processing algorithms for slice interval volume averaging of data, which may improve the resolution. Thus, images of better quality maybe expected using HRCT of 150 μm resolution than if microCT data was reconstructed at 150 μm. This needs to be subjected to future study.

Currently, CBCT could be used for both PORP and TORP design, however for accurate PORP design, the thresholding needs to be done twice: one to get data of the stapes fixation point and a second study to attain accurate data of the malleus fixation point. The bone density varies in different areas of the ossicular chain (21), with the lenticular process and stapes showing relatively lower bone density than the body or short process of the incus and malleus which was noted in the microCT images in this study. This is a barrier that challenges accuracy of 3D reconstruction from clinical imaging tools which have lower resolution than microCT. Therefore, lowering the threshold to make the voxel greyscale more sensitive to one structure of lower bone density captured artefact in other structures of higher bone density: e.g. the shape of the lenticular process maybe captured but it overestimated the thickness of the malleus handle which may create inaccuracy in dimensions of the malleus fixation points of a PORP (Fig. 2). In this study microCT data was an important control parameter to monitor this, but this is not practical to apply in clinical practice. To clinically translate this, perhaps machine learning algorithms can be applied in the future to both bridge this gap of resolution, and set optimal thresholds so 3D reconstruction from clinical imaging minimizes thresholding artefact.

Technique and Resolution Consideration for 3D Printing

Printing methods include nozzle based (extrusion), laser based or droplet based (inkjet) printing techniques (22). Extrusion based printers are the most common methods of low-cost 3D printers printing layer by layer filament material and have been used in temporal bone printing for application for mastoid surgery (23). Although printers may have the capacity to print in high resolution, the final resolution of extrusion printing is often dictated by ink properties, nozzle size, height of deposited layers, temperature and printing speed. This was visible in this study (Fig. 5), with the best resolution of 250 μm possible through the extrusion based printing method. This created an additive loss of resolution from data acquisition to printing. Thus, inkjet or laser-based printing methods maybe more suitable for printing protocols for ossicles. While inkjet printing was not tested in this study, laser-based printing techniques used in this study could achieve print resolution as low as 40 μm. However, 100 μm resolution of the laser based printing was effective in preserving the features of the incudostapedial joint if the source data captured the information (Fig. 5). Extrusion based printing, however, may be used if combined with alternate fabrication techniques such as extrusion printing of the cast of the ossicle followed by injection molding of the ossicle shape.

Translation to the Clinical Practice

Given the strengths and limitations of the current resolution and fabrication technology, how can this 3D data be translated to the clinical practice at the present time? Firstly, there is an obvious role in surgical training and simulation using more accurate patient specific data of the ossicles. Secondly, 3D digital planning can be applied to increase accuracy of reconstruction which has demonstrated benefit in reconstructive challenges in Otolaryngology such as mandibular reconstruction (24). Thirdly, contralateral ossicular data may be used as normal template for that patient though that needs to be customized to fit the 3D dimensions of the diseased ear and the operative interventions performed on the ear which may alter that anatomy. Finally, to avoid the challenges of thresholding artefact (which requires further research), an alternative is to simply stage ossicular reconstruction. It is acceptable in several conditions such as cholesteatoma to remove disease and perform ossiculoplasty at a second stage when the middle ear conditions are more hospitable. At this time, the incus is often removed and discarded. However, an alternative, prior to disposing the incus, is to attain ex-vivo microCT data of the incus to assist in developing a customize a 3D printed prosthesis for implantation at a second stage. In some ears, the remnant incus may be significantly eroded by disease or even missing limiting the practicality of this method. Here digital planning using methods previously proposed may assist. The ultimate effect on such customized designs on extrusion and sound conduction (3,25) is subject to future research, but prosthesis design also needs to consider other variables such as weight and mechanical properties of the material that may play a role in sound conduction.

Future Directions

Despite the advent of 3D printing, complications of middle ear prosthesis such as extrusion or erosion are especially troublesome given more than 80% of ossicular injuries are due to cholesteatoma or chronic suppurative otitis media (26), a hostile environment for foreign bodies. Therefore, autologous grafts are preferred to alloplastic materials (27) which opens a clinical need ultimately for tissue engineered solutions in this area. Bioprinting, a combination of 3D printing and tissue engineering technology has been applied in chondrogenesis for microtia reconstruction (28) with the first clinical application of this technology (29) recently reported. There has been a global interest in the advent of tissue engineering for outer ear reconstruction. While the technology is being applied in a different region of the ear, it is possible that it may have a future role in middle ear surgery. However, for future feasibility experiments of this technology in the middle ear, the findings of this study are important as it sets the resolution requirements for the printer of at least 100 μm to capture the anatomical detail of the incus. This is an important initial parameter to establish and it may allow better visibility for researchers for middle ear specific needs of bioprinter and scaffold technology that need further development.

CONCLUSION

Ossicular anatomy varies in between patients along with the spatial relationships of the ossicular chain. The current practice of assessing this intraoperatively and customizing a prosthesis on table crafted from cartilage or bone or sizing a prefabricated prosthesis is challenging. Displacement and extrusion are the most common reasons of failure. Long term results of ossiculoplasty may be improved with the application of 3D printing technology if solutions are customized for the ear. A major challenge to attain this goal has been limitations in the resolution of imaging and printing technology. However, improved resolution of clinical imaging is making this now a reality. Digital data set creation allows the ability for more accurate analysis of shape and dimensions and digital planning of surgery, reducing human or perspective error. Customized prosthesis may reduce rates of prosthesis displacement. 3D printing technology may help in achieving customized solutions but printing technique needs to be adopted to the resolution challenges of the middle ear which appears to be set minimally at 100 μm. Laser based techniques proved superior in matching such resolution requirements compared to extrusion based 3D printing techniques for middle ear application.

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